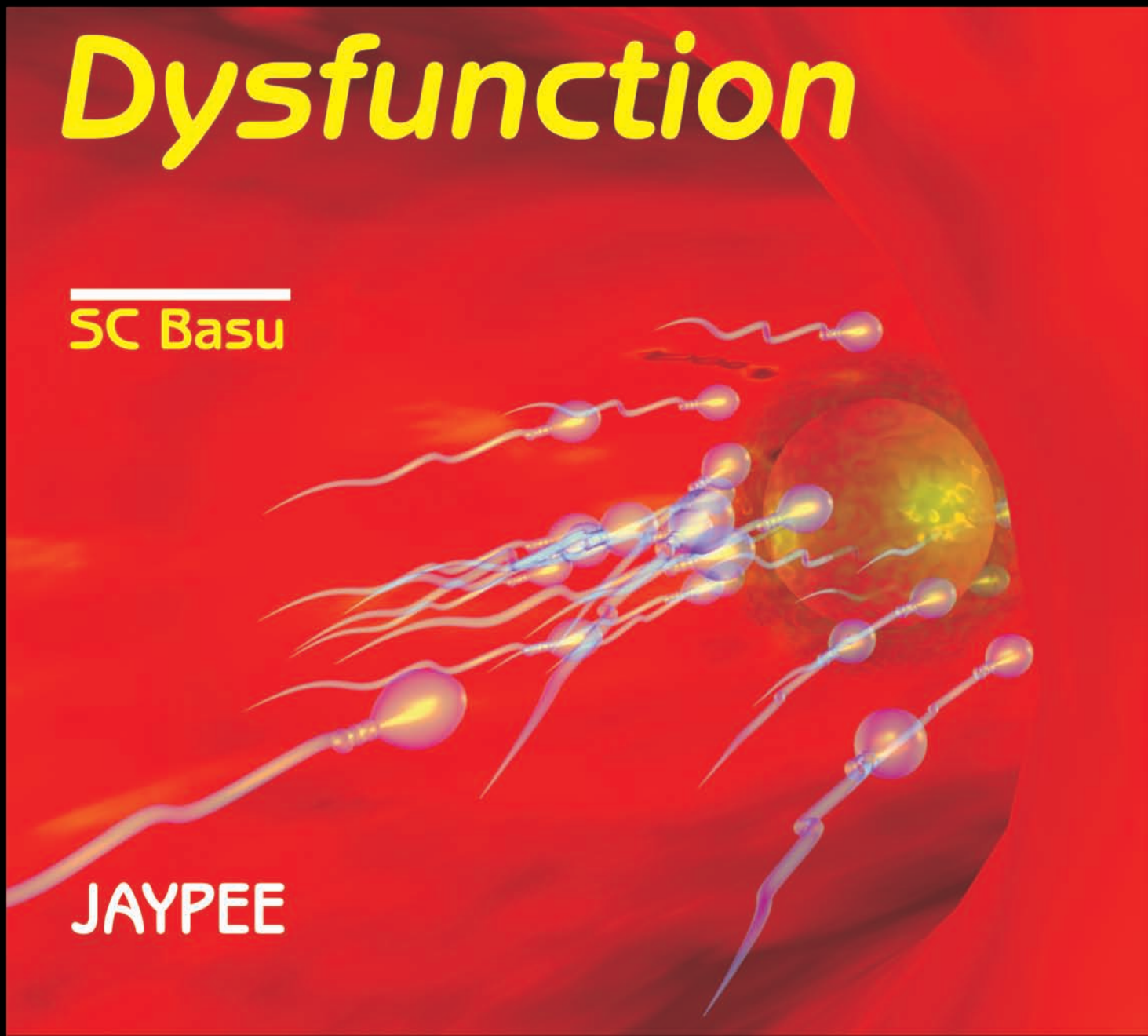
Male Reproductive Dysfunction

SC Basu



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Male Reproductive Dysfunction

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The book is dedicated to
my mother
Mrs Baruna Basu

And

to the memory of my late father
Dr Suresh Chandra Basu

Preface

Reproductive function of a male broadly encompasses three interrelated and synergistic steps, the spermatogenesis (formation of sperms), and regulation of the male reproductive functions and performance of the male sexual act. Various hormones and other factors regulate the primary reproductive functions. The male sex hormones also exert their influence on the accessory sexual organs, cellular metabolism and on other functions of the body.

Fertilisation– a union normally of a single out of many a million sperms of a male with the egg or ovum of a female remains an intriguing phenomenon. A comprehensive explanation of each of these steps, especially the necessity of so many millions of sperms to create an environment for the effective fertilisation still eludes us. For the said union a male sperm has to be formed in the male reproductive system and be deposited to the female reproductive system. Broadly, there is a *manufacturing unit* that is provided by the testes, a *storage unit* provided mainly by the vas and its dilated part or the ampulla and a *delivery unit* that involves the ejaculatory ducts and urethra, which in a male is a common urinary and genital passage. Each of these units has some overlapping functions.

Function of the primary sex gland in the males (testis) is controlled by a complex neuro-humoral mechanism with its epicentre at the hypothalamic-pituitary axis. Erection and ejaculation, which constitute the delivery system, are controlled by the lumbo-sacral part of the spinal cord with complementary actions of sympathetic ($L_{-1, 2}$) and parasympathetic nervous systems ($S_{-2, 3 \& 4}$). Successful delivery of the sperms from testes to the vagina naturally presupposes its unhindered and safe passage through vas deferens (commonly known as vas running from the testes to the seminal vesicles), and through the ejaculatory ducts passing through another secondary sex gland or prostate to the prostatic part of the male urethra.

However, successful union of ovum and the sperm does not guarantee normal development of the zygote. The sperms have two different constituents- one with "X" and another with "Y" chromosomes and chromosomal abnormalities could lead to faulty development of the unborn child in the forms of Klinefelter's and Down's syndromes. Thus, there is the proverbial many a slip between the cup and the lip- each slip in the absence of synergy of these functional units would cause a diseased condition.

This book deals with the abnormalities or dysfunctions of the male reproductive system with emphasis on common conditions in the sub-continental perspective. Outlines of the basic anatomy and physiology including the endocrinology have been included, so that the pathology of dysfunctional or diseased conditions is easily understood. The management, which includes diagnosis and treatment of these conditions, has been dealt with based on my own experience spreading over three decades and collating other experts' knowledge.

I am aware of the stupendous task of writing a monograph on any subject single-handedly. The book without doubt shows a bias of an andrologist or physician dealing with male factors in infertility. Some of us over the years have developed special skill in the management of many of these conditions. They justifiably could have put their authoritative stamp in the respective chapters of a multi-author publication. But I believe that a single-author monograph could ensure a free flow and lucidity in dealing with the subject, as the writing styles of different authors could not be the same. Moreover, it is often difficult to avoid repetition in some chapters, a task that is never easy to mend for the editor of a multi-author monograph.

All said and done, even in the first decade of the twenty first century, the treatment of male infertility because of its innate nature, still has a long way to go to achieve the same level of excellence and success of medicine in other fields. The results of surgery or other therapies at times are capricious, and the success is

never guaranteed. Assisted reproductive technology especially the intracytoplasmic sperm injection has indeed made great promise in recent times. Unfortunately, in the subcontinent and perhaps in many developing countries, its accessibility is limited to the big cities, and only to a very few rich. Hopefully, future would unearth newer methods and open new vistas to solve the vexed problem of reproductive dysfunction, and they would be cost-effective and universally affordable.

I have deliberately mentioned the websites and e-mail numbers in the reference. I sincerely believe that with the astounding progress of the internet, these should now form part of the reference as much as the journals.

SC Basu

Acknowledgements

Firstly, I am really grateful to my long time friend Prof PK Basu, who went through the manuscript more than once, and made invaluable suggestions.

I must also acknowledge inspiration I derived from Prof FH Comhaire, whom I never met. Yet his edited book on Male Infertility published by Chapman and Hall, London provided me with a lot of data, which I have referenced frequently in many chapters. I am also grateful to Prof Shafik of Cairo, who communicated me through electronic mail, and permitted me to use his diagrams.

I take this opportunity to thank Prof Sima Mukherjee and Dr Sanjay Thulkar, Department of Radio-diagnosis of AIIMS for making available published data and diagrams in the book. I also thank Dr R Rattan, Bahrain and Dr R Sachdeva, New Delhi for the diagrams they provided.

I am also indebted to my numerous patients, who suffered silently, yet enriched my knowledge and insight to tackle the subject of infertility. It would be rewarding if the book helps health care providers to ameliorate sufferings of future patients.

The list of acknowledgement would be incomplete without the mention of the publisher of the book, M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, particularly Mr JP Vij, Chairman and Managing Director, who reposed faith in me, and Mr Tarun Duneja, General Manager (Publishing), who helped me at every step during publication of the book.

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Contents

CHAPTER 1

Brief Historical Preview of Reproductive Science

Normal functioning of reproductive systems of male and female, no doubt is expected of all individuals. But one of the vagaries of nature is aberration of this function. While fertility is an essential ingredient for survival and continuity of species, not all couples are capable of furthering their families. So the problem of infertility finds its place in the recorded history of ancient civilisations of Babylonia, Persia and Greece.[1] Goddesses of fertility, fertility rites, and superstitions encompassing the process of birth are mentioned in the history of these civilisations. Even in the Hindu pantheon of Gods, there is a Goddess (*Shashthi*) responsible for fertility. History also reveals that the ancient Western physicians were aware of the mechanical barriers to conception as a possible cause of infertility.

Roman and Byzantine gynaecologists recognised obesity as one of the factors in infertility, and they are also credited to have used vaginal pessaries to treat infertility associated with retroverted uterus. In the first century, during the reigns of the Emperors "Trajan" and "Hadrian", Soranus of Ephesus, a physician in Rome, contributed greatly to our knowledge. He described the pelvic organs, the process of labour, the uses of vaginal specula and even methods for contraception. Interestingly, he mentioned that the most favourable time for conception was shortly after the menstrual period, and felt that staying in bed after coitus would improve fertility.[2] Celsus, in his *De Medicina* written in the first century AD credited the Greeks with the first description of a varicocele and recorded his own observations "the veins are swollen and twisted over the testicle, which becomes smaller than its fellow, in as much as its nutrition has become defective".[3]

Not much is known about the progress of knowledge of reproductive dysfunction or infertility in particular, in the Middle Ages. But it is possible that the ancient manuscripts preserved in the monasteries were not looked into or studied in details. There is, however, a record that Ambrose Par'e in 1585 gave an accurate description of penile anatomy and erection.[4] But the human quest for an in-depth knowledge of the tissues continued and the credit for developing the first useful tool for the purpose like compound microscope for such studies goes to Hans Jansen and his two sons Zacharias, and Hans Lippershey from the Netherlands in 1595.[5] Anton van Leeuwenhoek, another Dutch (1632-1723) was one of the first scientists to record observation of life in biological tissues and used the light microscope for the purpose in 1674. Leeuwenhoek studied several animal sperms and wrote his observations on the algae and protozoa in 1677.[5, 6]

Around the same time in England, the 17th-century scientist, Robert Hooke, used it to look at sections of cork and coined the word 'cell'. But not much progress in the understanding of the problems of infertility was evident in that century. However, Peyronie's disease causing thickening of penis was mentioned in the contemporary writings in 1561 by Fallopius and by other workers in 1687.[7] François de

la Peyronie, surgeon to Louis XIV of France, described the disease in details in 1743. In the 18th century, John Hunter's (1728-1793) use of insemination with semen from a husband with hypospadias, now known as AIH (artificial insemination with husband's semen), stands out as an outstanding achievement in the treatment of male reproductive dysfunction. John Hunter made an enormous contribution to the development of urology by his research and books concerning this discipline, which until then, simply constituted a branch of surgery.[8] In 1787, he also postulated a venous spasm factor that prevented exit of blood during erection. Prior to Hunter, earlier in 1718, Dionis, for the first time described the possibility of an underlying vascular factor in erection. The contemporary work of Lazzaro Spallanzani, who showed after studying amphibian sperms that these were essential for fertilisation, was equally commendable.

The speciality of urology was really not established till the beginning of the twentieth century, but one of the first few to investigate the "hormonal" action of internal secretions was Theophile de Bordeu, who died in 1776. Most medical doctors in Paris challenged his views but at the University of Leuven, his concept on the hormonal activity of semen was appreciated in 1780 on the occasion of a public debate (*disputatio*) conducted by the medical student, Gregorius-Josephus Jacquelart. He later was considered as the first andrologist at the University of Leuven.[9]

Carl Ernst Von Baer first described the mammalian ovum in 1827. There was rapid widening of the horizon in the field of infertility in the middle of the 19th century with gathering of knowledge of the scientific background of embryology, physiology and cellular pathology. Varicocele was mentioned as a cause of male infertility by a British surgeon named Barwell[3],[10] in 1885. Bennett [11] in 1889 is known to note the change in the semen characteristics associated with it.

In real terms, the modern era of advanced knowledge in the field of infertility has begun only in the last century with the studies of Huhner[12] on sperm survival in the cervical mucus in 1913, the test for tubal patency described by Rubin[13] in 1920, the development of the modern concepts of menstruation by Alien and Doisy[14] in 1924, and a description by Moench[15] in 1931 of semen characteristics associated with infertility and fertility. Knowledge collated from these studies on the cervix, endometrium, ovulatory factor in a female, and the male factors in reproduction, set the diagnosis and therapy of infertility to its logical and scientific course.

Meaker[16] in 1934 recognised the complex nature of diagnosis and treatment of infertility. He wrote about the "multiplex nature of causation" and "division of responsibility of male and female partners". Even in the 21st century, these principles and their utilisation in practice form the basis for investigation of infertile couples. Intricate knowledge of the cells and the tissues was only possible with the advent of the electron microscope[5] in 1946 by Porter, Claude and Fullam from New York, and development of its first working model later by Ruschka. Very recent addition of the electron-probe microanalyzers to scan as well as to correlate the structure and composition of tissues, has put the foundation of the modern medicine of infertility on a firm scientific footing.

In 1929, Macomber and Saunders[17] reported restoration of fertility in men following bilateral varicocele surgery. They were the first to publish works on sperm counts. However, the significance of varicocele in spermatogenesis was not taken seriously till 1950, when Tulloch[3,10] reported restoration of spermatogenesis in an azoospermic man after treatment of varicocele. It is worth mentioning the pioneering works of Macleod,[18] who first set the standards for semen analysis, and described the sperm pathology in varicocele in 1965. Mann published his works on seminal biochemistry[19] in 1964. Later, there were valuable contributions from Zorginetti, Dubin, Amelar, Comhaire and Goldstein in the last three decades towards better understanding and management of male infertility.

Last one hundred year has seen a quantum jump of medicine starting with the use of improved light and electron microscopes, and the X-rays in the later half of the nineteenth century facilitating diagnosis of diseased conditions. Radioactive materials for analysis of various biochemical substances (radioimmune assay or RIA), and extensive use of computers for the analysis of data have made immense contribution towards the management of various disorders. Treatment of infertility naturally shared the spoils of these medical advancements.

In the middle of the twentieth century, improvement of the imaging techniques starting with ultrasonography in the sixties, advancement of

Doppler studies (first discovered by Professor Johann Christian Doppler[20] of Prague in 1842), and later CT scan and MRI in the seventies revolutionised the diagnostic aids for the precise anatomical localisation of infertile conditions. Recent advance of using endorectal coil with MRI has been a great improvement to demonstrate anomalies of the genitourinary anatomy. Other biomedical aids like hormone assay, chromosome studies, etc. are no longer the exclusive domain of a few research scientists. They are now being used very frequently for the etiological diagnosis of reproductive dysfunctions in many centres.

Appropriate hormonal replacement therapy has now been made accessible to andrologists and endocrinologists for relatively easier diagnosis through hormone assay. Success in treating varicocele, an eminently treatable cause of infertility, improved with Palemo[21] (1949), Ivanissevich[22] (1960) and later by Bernardi, Dubin and Amelear (1970)[3, 23] advocating the inguinal and the supra-inguinal high ligation instead of the scrotal approach. In the last decade, use of laparoscope for varicocele has also been added as an alternative procedure. Laparoscope, endoscope[24] and operating microscope have now vastly improved the prognosis for obstructive lesions. Operating microscope, which helped surgeons to do away with using naked eyes or a magnifying loop, has ensured that the anastomosis of epididymis and the vas can now be performed with precision with little or no chance of leakage of sperms at the site of anastomosis to cause a sperm granuloma.

A great stride was evidenced in the *in vitro* fertilisation technique in the seventies and the first "test-tube" baby, Elizabeth Brown, was born in 1978. The results of experimental studies for several decades by Lillie (1962), Hiramoto, Lin (1966), Uehera and Yanagimachi (1976)[25-29] were able to establish the fact that the activation of the oocyte and formation of male pronuclei are possible in an artificial set up without the normal sperm-ova fusion inside a species. Mitha et al[30] first reported clinical application of microinjection in 1985. In human beings Lanzendorf [31] and co-workers in 1988 made the first successful attempt of single sperm injection into the cytoplasm of ovum following similar attempts made by Uhera et al[28] using hampter eggs in 1976. Since then the technique made rapid strides and different micromanipulation techniques were developed. The technique of intracytoplasmic sperm injection (ICSI) was developed in Belgium, and Palermo et al[32] reported the first pregnancy with ICSI in 1992. This technique involves injecting a single sperm into the egg at the time of *in vitro* fertilisation or IVF (process, whereby an egg is removed from the mother, fertilised by one sperm in a laboratory, and then returned to the mother).

With almost unlimited potential to achieve pregnancy regardless of sperm quality, it seems that ICSI has opened up an immense possibility for men, who possess no sperms in their ejaculated semen (azoospermia), to further their prospects of having their own biological children instead of depending on the artificial insemination of donor sperms. Research is on for the use of the germ cell implantation (GCI) and ROSNI (round cell spermatid nuclear injection)—two latest methods suggested for the treatment for males with sperm defects following chemotherapy and for nonobstructive azoospermia.

Many aspects of male fertility are influenced by genetics and the important role of genetic abnormalities in the causation of human male infertility is now increasingly taken note of. With powerful modern technologies such as ICSI and ROSNI now being able to bypass severe male-factor infertility, the diagnosis of genetic infertility is gaining added importance to do away with the conditions potentially transmissible to offspring.[33-35] Gene manipulation, which is still in its infancy very much like the drawing board stage of a building, offers an astounding solution for the chromosomal anomalies causing reproductive dysfunction.

The medical and surgical treatments of infertile couple, without doubt, have made great strides with tremendous advancement in X-ray, endoscopy and microsurgery, electron microscope, chromosome studies and radioimmune assay of hormones.

However, the problem of infertility still has not been lifted completely out of the realm of magic and superstition. Ancient rituals persist even today and the fertility rites are practised in many developed and developing countries of the world including the subcontinent. Statuettes of pregnant females or of males with outsized phalluses are used as fertility fetishes and symbols in Africa, Central America, Indonesia and Polynesia. History of endocrinology still quotes the folkloric practices of Hungarian peasant women, who bite their own placenta or "afterbirth" for enhancing their fertility, very similar to Chinese women,[2] who are still given dried placenta

to eat. We continue to hear about these "old midwives' tales" of administering placenta that really is the source of chorionic gonadotrophin—one of the hormones prescribed in the present-day treatment for failure of ovulation.

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CHAPTER 2

Male Reproductive Anatomy and Physiology

OUTLINE OF BASIC ANATOMY

Testis

The testis is the primary male sex gland. An adult human testis weighs between 30 to 45 gm. A careful examination of the testes is an essential part of any andrological examination. Normal adult testis is approximately 4.5 cm long and 2.5 cm wide with a mean volume of about 20 cc or ml (see Chapter 6). If its internal structures such as seminiferous tubules are damaged before puberty, the testes are small and firm; but with postpubertal damage, they are usually small and soft.[1]

A sac derived from the peritoneum acquired during its descent during foetal development covers each testis. This sac or tunica vaginalis has an outer parietal and an inner visceral layers. A thick capsule of collagenous connective tissue called tunica albuginia surrounds each testis under the visceral layer of the tunica vaginalis. An orchidometer or a caliper is used by the andrologists to measure testicular volume (Fig. 2.1, Plate 1).

Microscopically, the testis is composed of up to 900-coiled seminiferous tubules (up to 60 cm long and 0.2 mm in diameter) in which the sperms or spermatozoa are formed.These tubules lead to the epididymis. The glandular part of the adult testis is composed of 200 to 300 lobules, each containing two or three coiled seminiferous tubules, which are joined together at the apices of lobules to form 20 to 30 straight tubules anastomosing with one another through a meshwork of ducts called rete testis. From the rete testis, 12 to 20 efferent highly coiled ductules emerge to form the head of the epididymis (Figs 2.2 and 2.3, Plate 1).[1,2]

The epididymis is located posterolateral to the testis and appears like a drape over the top of the testis. It has three anatomical parts caput or head, corpus or body and the cauda or tail. The tail leads to the vas deferens. The epididymis consists of a narrow tightly coiled-up tube and these coils, when stretched out measure approximately 20 feet (6-7 meters) in length. The name epididymis is the Greek for "upon the twins."

Human epididymis is 4 to 5 cm long and is attached to the testis through epididymal ligaments. The vasal ligament attaches the vas to the tail of epididymis and maintains the acute epididymal-vasal angle. Anomalies of epididymis include appendix epididymis, superior and inferior aberrant ducts and paradidymis. Failed congenital connections between individual efferent ducts and the epididymis may lead to the formation of simple cysts of the head of the epididymis.

The vas deferens is a muscular duct 30 to 35 cm (15 inches), long and enlarges into the ampulla, immediately before it enters into the substance of the prostate gland. Latter is considered as the secondary male sexual gland.[1,2] A seminal vesicle, each located on each side and above the prostate

gland, empties into the prostatic end of the ampulla. The contents of both the ampulla and the ducts of the seminal vesicles from each side join to form the ejaculatory ducts passing through the body of the prostate to empty into the urethra (Figs 2.2; 2.3, Plate 1; and 2.4). The prostatic ducts in turn empty into the ejaculatory ducts. Finally, the urethra drains the semen to the exterior.[1-3]

Numerous mucous glands line the urethra. There are two relatively big ones known as bulbourethral or Cowper's glands situated just below the prostatic portion of the urethra. In patients with a genetic defect causing cystic fibrosis, the vas deferens or epididymis and seminal vesicles are usually absent. The persistence of efferent ducts, but absence of epididymis proper and vas in cystic fibrosis, reflects different embryological origins of the epididymis, vas and efferent ductules. The epididymis and vas develop from the wolffian or mesonephric ducts and the efferent ductules from the mesonephric tubules.

Development of Testis and Male Reproductive System

The testis develops from the developing mesonephros at the posterior part of the coelome at the level of T_{10} segment. This explains the autonomic supply of the testis from the corresponding spinal segment. The mesonephros plays a fundamental role in the process of gonad formation. The nephrotome, a stalk of the somites, is the precursor of mesonephros. The blastemal somatic cells that originate from differentiation of the mesonephros contribute to the formation of the genital ridge. The testis develops from the medulla of bipotential human gonad (See Figs 2.5a and b).

Differentiation of the primitive bipotential gonadal ridge into primitive testis is mediated by various factors. SRY gene (*sex - determining region - Y*) diverts the ovarian (female) to the testicular (male) pathway. It alters the fates of different cell types to three gonad-specific lines—the supporting cell, steroid cells (Sertoli and Leydig) and germ cells together with vascularised connective tissues. Role of *testis-determining factor* (TDF) has been debated for years and in 1987 a gene named ZFY (*zinc finger protein-Y*) was identified, and it was encoded by a gene from the TDF region on the Y-chromosome. It appeared that ZFY expression correlated with the colonisation of the testis by primordial germ cells. In 1990, SRY gene was isolated. It is expressed in the genital ridge where testicular cords originate.[3-6]

Primitive gonad is bipotential till the 6th week of the intrauterine life (IUL). TDF and mullerian inhibitory substance (MIS) then determine its subsequent fate around the 7th week. At this stage, the TDF helps the primitive gonad to differentiate into the primitive testis, which gets transformed into foetal or primitive germ, Sertoli and Leydig cells, while the MIS suppresses the development of Mullerian system. Foetal

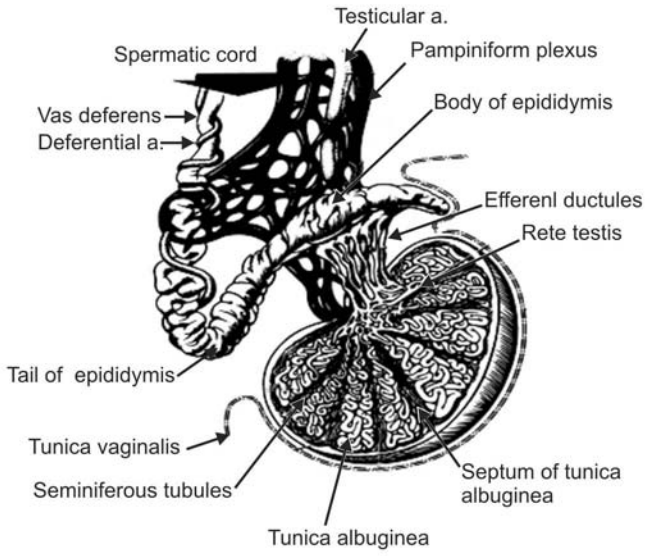
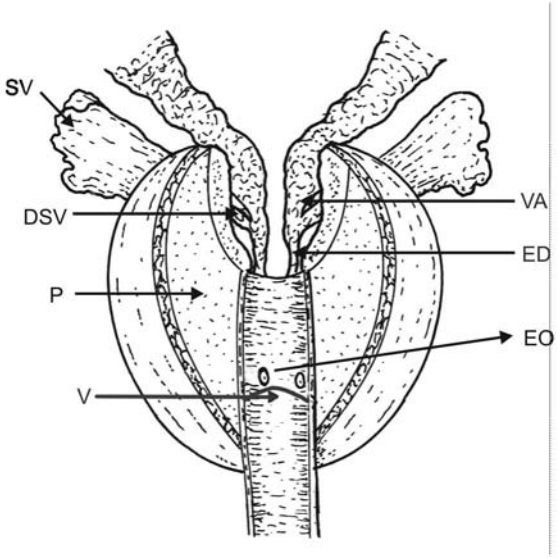


Fig. 2.2: Anatomy of testis



vasal ampulla; SV, seminal vesicle; P, prostate; DSV, duct of seminal vesicle; ED, ejaculatory duct; V, verumontanum; EO, ejaculatory duct opening.

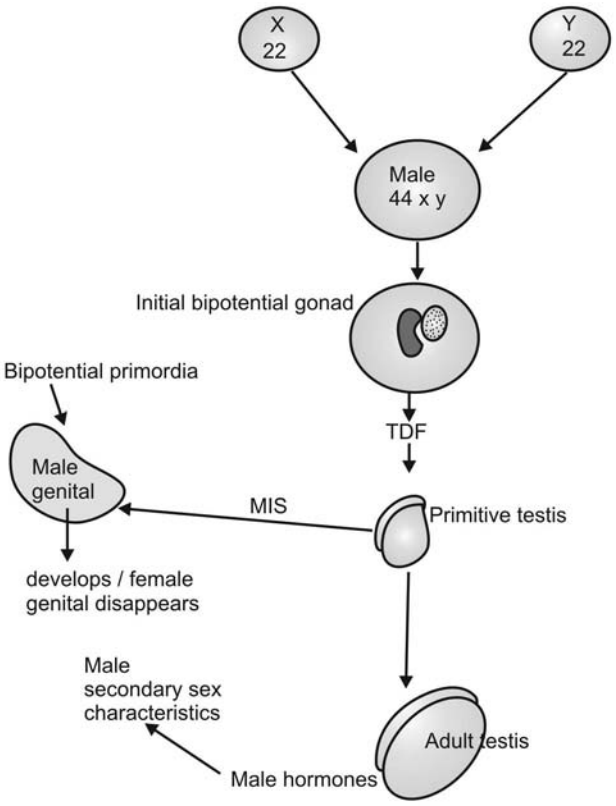


Fig. 2.5a: TDF = Testis Determining Factor, MIS = Mullerian Inhibitory Substance, T = Testosterone

Leydig cell then starts secreting the androgen, which further consolidates the development of foetal testis (Fig.2.5a).[1,3,6,7]

The male reproductive system develops from three embryological sources. The primitive gonad forms the testes, while the urogenital duct (Wolffian duct in male) and the urogenital sinus (primitive cloaca) contribute to other two components. Both the Wolffian and the Mullerian ducts coexist in the early embryonic life in both sexes. Mullerian ducts disappear in male, but some of its remnants can be traced to the prostatic utricle. The epididymis, vas and the seminal vesicle owe its origin to the Wolffian duct. The prostate and the prostatic urethra develop from the urogenital sinus. The urogenital swellings become the scrotum and the urethral folds fuse to form the shaft of the penis and the rest of the male urethra. (Table 2.1)

Testosterone along with dihydrotestosterone (DHT) from the foetal testes stimulates the development of male genital organs like the male urethra, prostate, penis and the scrotum in the IUL. Foetal testicular secretion attains its peak level around 8th to 10th week and the formation of the male phenotype is mostly completed by the end of the first trimester of gestation. Later, in the IUL further development of testes and the external genitalia, and the descent of the testes complete the full process of embryonic development.[6,7]

Table 2.1: Developmental sources of male reproductive system

Source	*Organ*
Primitive gonad	Testes
Mesonephros and *Wolffian duct*	Epididymis, vas and seminal vesicles
Urogential sinus	Prostate and the prostatic urethra
Urogenital swellings	Scrotum
Urethral folds	Shaft of the penis and rest of the urethra
Genital tubercle	Glans penis

Developmental Abnormalities

Cryptorchidism (undescended testis)

It is a common developmental defect. A bilateral undescended testis usually leads to absence of sperms in the semen. Even a unilateral case may sometimes show low sperm count, if there is some structural

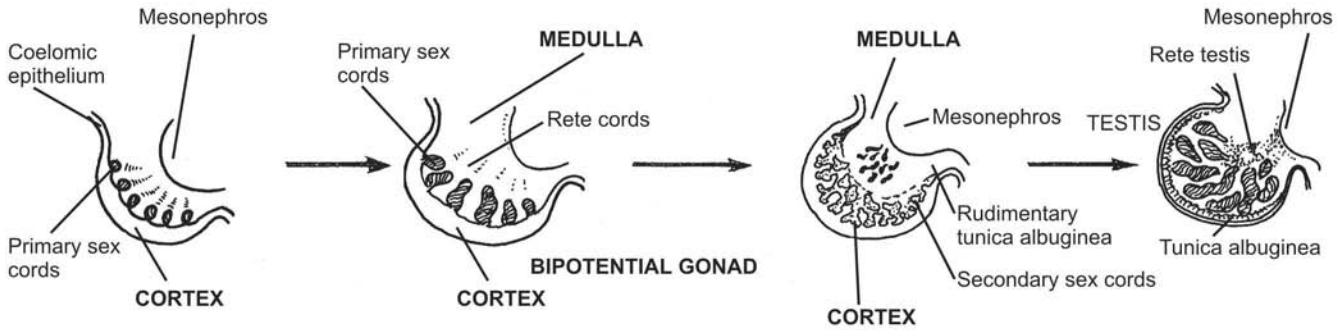


Fig. 2.5b: Diagrammatic representation of the development of testis from the medulla of a bipotential primordial human gonad.

dysgenesis in the other side (details in Chapter 10). Increased temperature within the abdomen has a probable inhibitory effect on the enzymes and proteins that are responsible for the normal sperm production. It has also been theorised that estrogenic influence might be responsible for increased incidence of the testicular cancer in these subjects.[3,8]

Bilateral Anorchia

Bilateral anorchia or anorchidism, or vanishing testes syndrome is an extremely rare disorder affecting about 1 in 20,000 males. These patients present at birth with nonpalpable testes; and later in life, show sexual immaturity due to the absence of testicular androgens. Unilateral cases may escape detection, as the other side is normal (details in Chapter 10). [1,3,4, 8]

Mixed Gonadal Dysgenesis

It is an inherited disorder with a distinctive genetic signature like 45, XO or 46, XY (details in Chapter 10).[3,8]

Vascular Supply of Testis

Arterial Supply

The arterial supplies to the testis and epididymis come from the internal spermatic or testicular artery arising out of the abdominal aorta. Vasal artery maintains a dual supply to the vas and the epididymis through its anastomosis with the testicular artery. This additional supply of the epididymis ensures higher concentration of androgen in the epididymis, perhaps to facilitate maturation of sperms.

Venous Drainage

Venous drainage is provided by the spermatic veins and has been described in details in chapter 9 on varicocele The spermatic vein passes along the vas in a very tortuous course as the pampiniform *(like a vine*) venous plexus, which wraps round and surrounds the spermatic artery in a convoluted manner. This anatomical feature facilitates the countercurrent heat and androgen exchanges between the arterial and venous systems. The testes are suspended outside the body in the scrotal sac. Contraction and relaxation of the cremasteric muscle alter the distance of the testis and the body (which has a higher temperature) depending on the environmental temperature, thus maintaining the gradient of approximately of 2°C between the body and the testis for optimal spermatogenesis (see Chapter 9 on varicocele for details). Dilatation of the component veins of the pampiniform venous plexus leads to varicocele, which often causes impaired spermatogenesis. Injury to the testicular blood supply can occur in a hernia operation or even during vasectomy.

MICROSCOPIC ANATOMY OF TESTIS

Testicular histology and cytology are of paramount importance in the evaluation of infertility, especially where there is a hormonal component in its pathogenesis. For routine purpose, conventional light microscope is considered enough to study the testicular microanatomy in spermatogenesis, but use of an electron microscope is essential for its full evaluation.[9,10] One needs to handle the testicular tissue with care before it is placed under microscope. Bouin's or Stieve's or Zenker's solution should be used instead of the universally used formalin as the latter causes significant shrinkage or distortion of testicular tissue. Either fine-needle aspiration cytology using 0.6 mm needle or open surgery is used, and the tissue needs to be fixed with 96% ethanol.

Basically, the testicular histology shows seminiferous tubules and interstitial tissues. Germ cells (also known as stem cell or primitive spermatogonia) and Sertoli cells constitute the seminiferous epithelium. The interstitial tissue occupies approximately one-fourth to one-third of the total testicular volume and contains the Leydig cells, blood vessels, lymphatics and nerves. In addition, there are collagen fibres, myoid and elastic tissues and a large number of macrophages. Blood supply to the testis passes through the interstitial tissues. As stated earlier, the epididymis has a dual supply from both the testicular and the vasal arteries.

Sertoli Cells

Sertoli cells provide the physical support for the germ cells and are considered to be primary regulator of spermatogenesis. After puberty, the Sertoli cells are a fixed-population of non-dividing cells with its base attached to the basement membrane of the tubule and the apex extending towards the lumen. They surround all germ cells except the stem cells (*spermatogonia*) and its immediate successor cells or *primary spermatocytes* (Fig. 2.6).

The cytoplasmic membranes of the adjacent Sertoli cells are tightly adherent to prevent penetration from the capillaries in the interstitial tissues

of the tubules. The adherent cytoplasmic membranes of the adjacent Sertoli cells coupled with close approximation of the myoid cells of the peritubular contractile cell layers serve to form the tight junction that constitutes the *blood-testis barrier*. This barrier divides the germinal epithelium into basal and adluminal compartments producing a unique functional compartmentalisation. The basal compartment is adjacent to basement membrane and contains the spermatogonia and the early spematocytes.[4,5,10]

The blood-testis barrier provides a unique microenvironment that facilitates spermatogenesis and maintains these germ cells in an immunologically privileged location. This isolation is important because spermatozoa are first produced during puberty, long after the period of self-recognition by the immune system. If these developing spermatozoa were not immunologically protected, they would be recognised as foreign substance and liable to be confronted by the body's immune system. All substances from the capillaries of the interstitial tissues must thus be transported through the Sertoli cell cytoplasm to reach the germ cells of the adluminal compartment. The breakdown of the blood-testis barrier may lead to autoimmune responses.[4,5,11]

Seminiferous tubules contain all the germ cells at various stages of maturation and their supporting Sertoli cells. The germinal or the spermatogenic cells are arranged in an orderly manner from the basement membrane up to the lumen. Spermatogonia lie close to the basement membrane, and next in order progressing up to the lumen, are found the primary spermatocytes, secondary spermatocytes and the spermatids. These cells continually proliferate to replenish themselves and portions of them differentiate through definite stages of development to form sperms. It is estimated that there are thirteen (13) different germ cells representing different stages in the developmental process.[1,4,9,10]

Sertoli cells appear to be involved with the nourishment of developing germ cells and phagocytosis of the damaged cells. The germ cells are surrounded by and in close contact with the Sertoli cells, and are bathed in the tubular fluid secreted by the latter. Sertoli cells exhibit distinct endocrine and other secretory functions, and produce various substances such as ABP (androgen-binding protein), inhibin, activin, MIF (mullerian-inhibiting factor or substance) and the enzyme aromatase, which converts the testosterone to estrogen.[12] The circulating follicle-stimulating hormone (FSH) and intratesticular androgens stimulate functions of Sertoli cells for optimal spermatogenesis (see Chapter 3).

Sertoli-cell-only syndrome or germinal cell aplasia may be caused by the congenital absence of the germ cells, genetic defects, or androgen resistance, and is diagnosed in a testicular biopsy by the complete absence of the germinal elements. Clinically, there would be azoospermia in association with normal virilisation, testes of normal consistency but slightly smaller in size, and no gynaecomastia. Testosterone

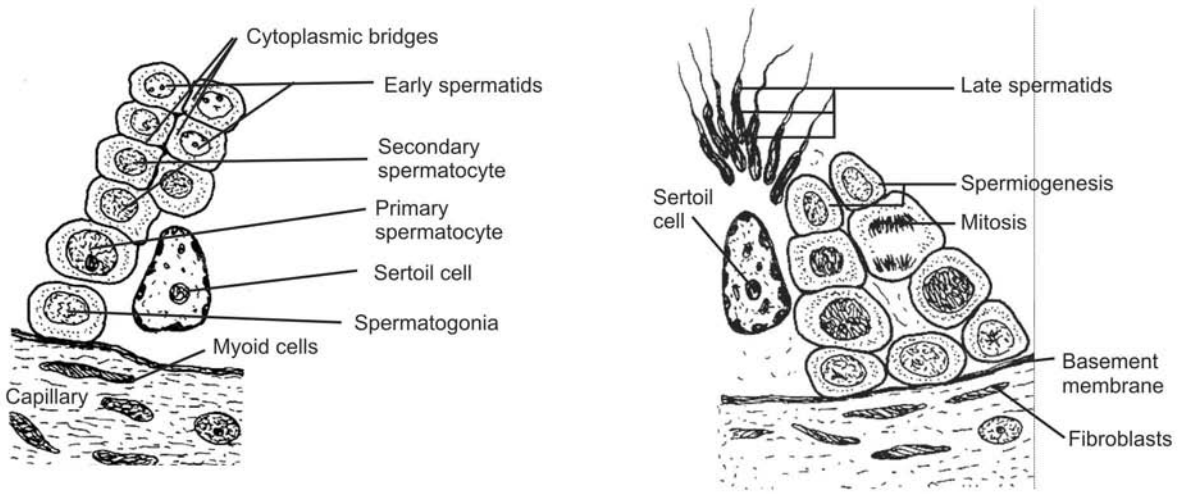


Fig. 2.6: Seminiferous epithelium. Maturing germ cells remain connected by cytoplasmic bridges through the early spermatid stage and these cells are closely surrounded by Sertoli cell cytoplasm as they move from the basal lamina to the lumen.

and luteinising hormone (LH) levels are normal, but FSH levels are usually elevated. Sometimes in patients, who had other testicular disorders such as mumps, cryptorchidism or radiation/toxin-related damage, the seminiferous tubules may also contain only Sertoli cells; but in these men, the testes are small and the histological pattern is not so uniform. These patients are more likely to have severe sclerosis and hyalinisation as prominent features.[12]

Leydig Cells

Leydig cells have a biphasic pattern of development, and are of foetal and adult types. The foetal type cells proliferate between the 8th and 18th week of the IUL. Later, they start regressing slowly and undergo complete attrition in the first few weeks of neonates. The adult type starts replacing the foetal type at about the third week of neonatal life; and usually by the 8th week, a definitive level is reached. Adult Leydig cells most probably have origin from a mesenchymal fibroblast like cells, macrophages, and peritubular myoid cells. After puberty, the numbers of Leydig and Sertoli cells do not increase any further. Consequently, the turn over of these cells in contrast with the germ cells is very low.[9,10,12]

SPERMATOGENESIS

Spermatogenesis is a complex process, whereby primitive stem cells or spermatogonia either divide to renew and replenish the stem cell, or produce daughter cells that will later become spermatozoa**.** At birth, the Sertoli cells are numerous with ill-defined cytoplasmic boundaries. With the advent of puberty, the positions of the Sertoli cells, which are present normally in two or three layers, change from the earlier position along the outer border of the tubular epithelium towards the developing lumen of the seminiferous tubule near the basement membrane. This is achieved by the extension of the cytoplasmic process of the Sertoli cell. This pre-pubertal movement of the Sertoli cell to its adult position is very important in achieving the *blood-testis barrier*. The seminiferous tubules also start their development at puberty.[11-14]

Spermatogonia located along the basal membrane are of three basic types – dark Type A with dense chromatin, pale Type A with pale chromatin and Type B, which has clumps of chromatin. Through three phases– mitotis, meiosis and spermiogenesis, the spermatogonium attains its full development into spermatozoon.[4,5,9,12] (Fig. 2.7)

1. *Type A*—that is thought to be precursor, divides four (4) times (A1 to A4) and then through another intermediate phase (IN) by *mitosis* to produce sixteen (16) Type B spermatogonia.[1]
2. *Type B* migrates towards the Sertoli cells and then divides to produce primary spermatocytes through the *first meiotic division.* In the initial stage of this division, 46 chromosomes are replicated. In this process, each of these 46 chromosomes acquires two *chromatids* that remain bound together at the centromeres having duplicate genes of the particular chromosome. It then goes through another division to produce two secondary spermatocytes, but each pair of chromosomes now separates into two halves, so that 23 chromosomes each containing two chromatids go to one secondary spermatocyte; while other 23 chromosomes go to the other secondary spermatocyte. Secondary spermatocytes then go through the *second meiotic division* within 2 to 3 days to develop into spermatids with haploid number of 23 chromosomes (half of the original number of 46 chromosomes). So each primary spermatocyte with forty-six chromosomes produces four spermatids (immature sperms) each containing

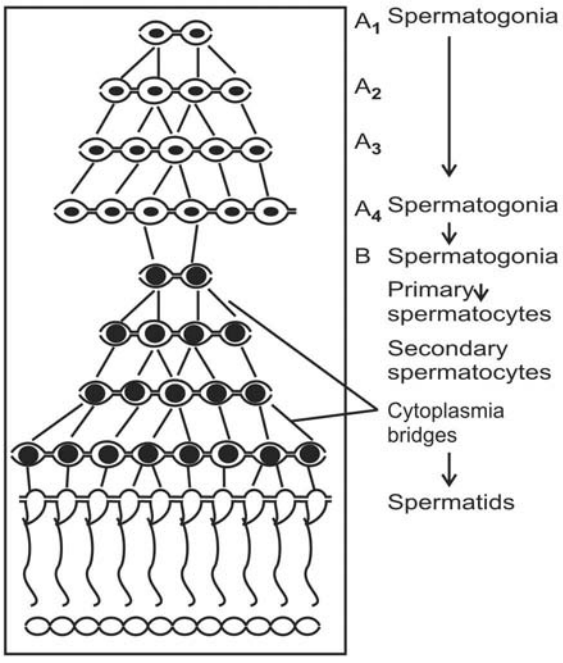


Fig. 2.7: Stages of spermatogenesis showing progression of spermatogonia to spermatozoa

twenty-three chromosomes, but having only half the genes (haploid number) of the original spermatogonia.

3. The third phase (*spermiogenesis)* is the development of spermatids to spermatozoa. During this process of development, the shape of the nucleus of spermatid changes from round to oval and the light granulated chromatin goes through a process of condensation. Accordingly, the spermatids are classified into four successive types **Sa, Sb, Sc** and **Sd.** The last group of Sd spermatids undergoes a transformation into spermatozoa. It thus appears that from one germ cell, 512 spermatozoa develop.

In the process of its transformation into spermatozoa, the spermatid undergoes nuclear condensation, acrosome formation and loss of most of its cytoplasm. It also develops a tail and the mitochondria get arranged in the middle piece of the sperm. Due to incomplete cytokinesis, all cells derived from a single spermatogonia are connec-ted through cytoplasmic bridges and this is replicated till a spermatid is developed.[4,5,9-16]

Spermatogenesis occurs in all the seminiferous tubules during active sexual life, beginning at an average age of 12 years as a result of stimulation by the pituitary gonadotrophin hormones. It continues throughout the remainder of life. Interestingly, the successors of spermatogonia do penetrate the blood-testis barrier; otherwise, they cannot come to the lumen and become totally enveloped within the enfolding cytoplasmic processes of the Sertoli cells. This close relation with the Sertoli cells continues throughout the life.

Groups of germ cells tend to develop and pass through spermatogenesis together. This sequence of developing germ cells is called a generation. Each generation of germ cells is basically in the same stage of development. There are six stages of its development, and progression from stage one through stage six constitutes one cycle. In humans, the duration of each cycle is approximately 16 days and 4.6 cycles are required for a mature sperm to develop from an early spermatogonia. Thus, the duration of the entire human spermatogenic cycle is calculated as 74 days (4.6 cycle of 16 days each equals 74 days).[1,9,17,18]

STRUCTURE OF A SPERM

The structure of a matured sperm consists of a head and tail joined by the middle piece. A narrow portion or neck lies between the latter and the head. Essentially, the head is the condensed nucleus with a very thin cell membrane covering its surface. But its anterior two thirds has a cap known as the *acrosome*. It contains the hyaluronidase capable of digesting proteoglycan filaments of tissues, and powerful proteolytic enzymes (see later "role of acrosome"). The tail or the flagellum has a central skeleton with 11 microtubules called axoneme (very much similar to cilia), a very thin cell membrane and collection of mitochondria surrounding the axoneme in the proximal portion or the body of the tail. (Fig. 2.8)

The motility of the sperm is achieved through energy supplied through ATP (adenosine triphosphate synthesised by the mitochondria) and takes the form of rhythmic longitudinal sliding motion between the anterior and posterior tubules. The fertile sperms exhibit flagellated forward movement in a straight line and not circuitous, at the rate of 1-4 mm/min. The activity is enhanced in neutral and slightly alkaline media, and acidity

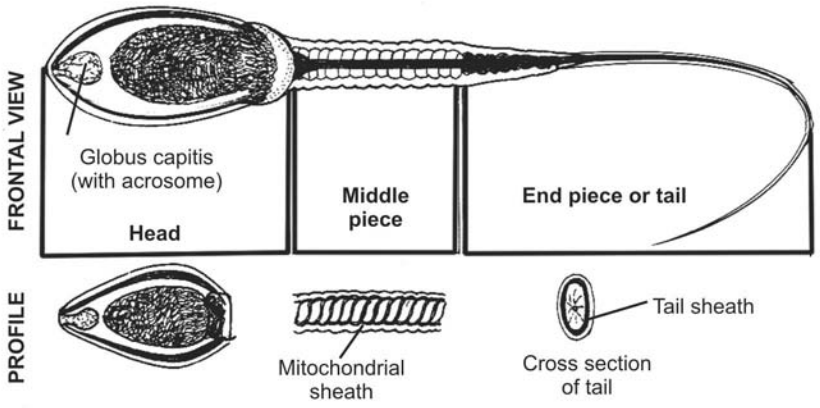


Fig. 2.8: Structure of a sperm

depresses the movement of the sperms. While increased temperature enhances the activity of a sperm; at the same time, it increases the rate of metabolism curtailing its lifespan.[1,4,5,17]

The sperm centriole is located in the neck or the connecting piece. The midpiece contains the mitochondria carrying the paternal mDNA and this portion progressively degenerates soon after fertilisation, as its presence would harm the developing embryo. The point of entry of sperm into the oocyte could determine the polarity of the developing embryo. The entry of sperm into the oocyte cytoplasm produces a new axis, once the sperm aster is developed in the cytoplasm of the oocyte around the centrosome. The sperm centrosome is inherited, replicated and perpetuated in human embryos[19-21] (see later "fertilisation").

SEMEN

Semen is a combination of sperms and fluids from the seminal vesicles, prostate and the bulbourethral glands. It provides a watery environment in which the sperms can swim and supplies nutrients for the sperm cells. A recognised function of the seminal fluid or plasma is its buffering effect on the acidic vaginal environment to protect the sperms. The major volume of the seminal fluid comes from the seminal vesicles (65 percent on an average) and secondarily from the prostate and the bulbourethral glands of Cowper (30%). The sperms constitute approximately 5% of the semen volume.[1,5,6] Semen is usually creamy white in colour. Often, the sperms are not very well mixed making the semen appear to have patches of cloudy and clear areas. It has about the same consistency of a liquid dishwashing detergent.

The seminal vesicular element also provides nutrient fructose, fibrinogen and the prostaglandin. Importantly, the prostaglandin makes sperms receptive to the cervical mucus and aids the peristaltic movement of the uterus and tubes to propagate sperms towards the ovum.

The prostatic portion provides calcium and fibrinolysin and also adds zinc, phospholipids, seminin and phosphatase to the seminal fluid. Prostate-specific antigen (PSA) is a protein made by prostate tissue. The exact function of PSA is not clear, but it helps to keep semen in a liquid state.

The calcium content of the semen helps in movements of flagella. The semen forms a coagulum, as it is expelled into the vagina during ejaculation. The coagulum formation is aided by the fibrinogen from the seminal vesicles. It binds the semen close to the upper part of the vagina near the cervix after its ejection through intercourse. The fibrinolysin and seminin help to dissolve the coagulum of the voided semen. Once released from the coagulum, the sperms are able to start their onward passage to the cervix upwards.

According to various research workers, the volume of the semen has a wide variation ranging from 1.5 to 6.6 ml. After a few days of abstinence, the average volume may range around 3 to 3.5 ml in healthy young adults, while 13 ml has been recorded after prolonged abstinence.[1,3,5,6,16] The semen volume must be judged in relationship to the frequency of ejaculation. Undoubtedly, there is a normal genetic variation and ageing tends to reduce the volume. Alkalinity of semen at an average pH of 7.5 is maintained by the mucus element provided by the seminal vesicle and other mucus glands. Latter also gives the semen its mucoid appearance. Alkalinity of seminal fluid mainly neutralises the acid pH of the vagina that is detrimental to the sperms. The secretion of the prostate gland imparts its odour and whiteness.

The sperms are transported to the epididymis by testicular fluid pressure, ciliary action and contraction of the efferent ductules. The sperms leave the epididymis, when called upon during intercourse or masturbation, and travel through the muscular vas deferens that propels them forward by its peristaltic contractions into the ejaculatory duct. During emission of the semen, secretions from the seminal vesicles and prostate simultaneously are deposited into the posterior urethra.

Prior to ejaculation, peristalsis of the vas deferens and bladder neck occur under sympathetic nervous control. During ejaculation, the bladder neck tightens and the external sphincter relaxes, and the semen is propelled through the urethra by rhythmic contractions of the perineal and bulbourethral muscles. The first portion of the ejaculate contains a small volume of fluid from the vas deferens, which is rich in sperm. Subsequent second portion has a greater volume and is composed primarily of seminal vesicular and the prostatic secretions, but fewer sperms. The coagulum formed by the ejaculated semen liquefies within 20 to 30 minutes as a result of prostatic proteolytic enzymes.

STORAGE AND MOVEMENT OF SPERMS

There is a broad agreement that DSP (daily sperm production) is about 200 to 300 millions. As mentioned earlier, it takes on an average 74 days for spermatozoa to mature and to come to the lumen of the tubules for its transport to epididymis. The process of ripening or the last phase of maturation takes place in the epididymis for a further period of 2 to 3 weeks. The sperms, when they enter the epididymis, are immature, immobile, and infertile, and really attain their full fertilising potential in the epididymis. The storage unit is provided mainly by the vas and its ampulla with a similar amount remaining in the epididymis. It is estimated that the extragonadal sperm reservoir is 440 million and more than 50% of these are located in the tail of the epididymis.[1]

The sperms that are stored in the tail of the epididymis are the first to enter the vas deferens. The sperms released from the epididymis are still nonmotile and incapable of fertilising the ovum. The inhibitory substances produced in the male genital tract cause its inactivity. The reported transit time of sperms in the human epididymis vary considerably. Using thimidine-labeled sperms, Rowley et al[19] estimated it to be 3 to 21 days. During its transit the sperms acquire their maturity. While the sperms stored in the male genital tract can remain viable, but in an inactive state for a period of 4 to 6 weeks, they die within 48 hours of ejaculation into the female genital tract. However, if stored at a lower temperature or frozen below –100°C, it can survive for many months to years.[1] The next process of capacitation occurs in the female genital tract, which confers the full fertility potential of any sperm (Fig. 2.9).

SPERM TRANSPORT AND FERTILISATION

Semen is ejaculated mostly in the form of a coagulum. Normally, within 20 minutes liquefaction of coagulum aided by the prostatic enzymes takes place. If the cervical mucus plug is friendly, the sperms can penetrate through within minutes of their deposition. Sperm transport in the female genital tract is aided not only by the tail movement of the sperms, but also by the contractility of the uterus and the tube, and the ciliary movements in the endometrium and endotubal epithelium.[4-6,9]

Motile sperms even with abnormal heads may at times be able to penetrate the cervical mucus barrier, but are unable to pass through it. The protease enzyme systems in the sperms mainly help this process of entry. The energy needed for motility is produced in the mitochondria situated in the midpiece of the sperm. Approximately 90% of the energy needed for motility is produced as ATP and transported to the flagellum. In the flagellum, the ATPase hydroxylates ATP into adenosine diphosphate (ADP). Comhaire et al[2] and Romac et al[20] reported significant correlation between ATP per ml of ejaculate and the parameters such as density, number of motile sperms, capacity of migration of sperms against gravity and *in vitro* potential of the sperms to penetrate zona free hamster ova. They found that the ATP concentration was significantly lower in the semen of infertile men even with normal sperm concentration and motility.[2, 20]

Cervical factor owes to the rheological properties of the cervical mucus that determine the cross-linking micelles (electrically charged particle built up from polymeric molecules or ions in the foam of the cervical mucus) tend to hinder not only the passage of morphologically abnormal sperms (with abnormal heads, etc), but also even the normal ones during the luteal and early follicular phases of ovulation.[9] During the midcycle, the estradiol-17-β stimulation causes increased fluidity of the cervical mucus with the resultant change in the character of the micelle, and thus helps the passage of the sperms during the most productive period of the fertilisation (see "female factor" in Chapter 6).

Normally, only 500 to 1000 sperms reach the site of ovum even in the midcycle out of many millions present in the semen.[9] Major blocking points appear to be cervix, uterotubal junction and lastly, the

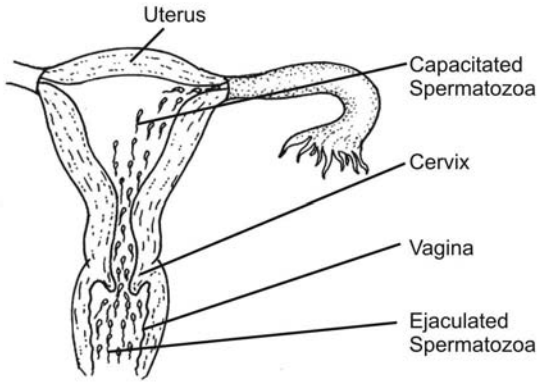


Fig. 2.9

isthmus-ampullary junction of the tube. Not more than 1% of total sperms pass through the cervical barrier.But beyond that point, the transport is fairly rapid and can occur within 15 to 20 minutes of deposition of the sperms at the cervix. Average time taken by the sperms to reach the tube is 4 to 6 hours. If the sperm penetration into the ovum is not completed within 15 to 18 hours of ovulation, the ovum mostly degenerates.[1] (Table 2.2)

Contrary to the rule, first come first served, the sperms reaching in the first lot mostly are incapable of penetrating the ovum, as they probably do not have time to go through the complete process of capacitation. Sperms that reach 2 to 4 hours later at the isthmus-ampullary junction of the tube are better equipped to fertilise the ovum.[5,9,21]

It is still controversial whether the process of hyperactivation, whereby there is a change of sperm movements from linear trajectory to complex nonlinear form play any role in the ultimate process of fertilisation. Some believe this process actually helps penetration after the acrosome reaction has occurred.

Table 2.2: Path of a sperm to ovum

Origin from the germ cell of the testis
Three phases- ↓ *Mitosis, Meiosis and Spermiogenesis*
Spermatid
↓ *Spermatogenesis*
Spermatozoa
↓
Maturation in epididymis
↓
Ejaculation
↓
Capacitation in female genital tract
↓
Acrosome reaction
↓
Sperm binding to Zona
↓
Sperm penetration into Zona
↓
Entry to perivitelline space
↓
Entry into the ovum and Fertilisation

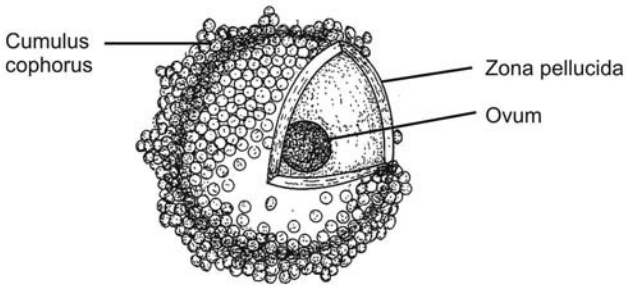


Fig. 2.10: Structure of ovum and its coverings

An oocyte is surrounded by three layers – *cumulus oophorus* and *corona radiata* consisting of follicular cells, and the *zona pellucida*, rich in glyco-proteins. The perivitelline space is located between the zona pellucida and the oocyte membrane. (Fig. 2.10)

Sperms seem to utilise two mechanisms to penetrate oocytes – firstly, through its lytic enzymes in the anterior head portion, especially within the acrosome; and secondly, through its movement of the tail.[9] Creatinine phosphokinase present in the midpiece of sperm allows the phosphorylation of creatinine and its subsequent transfer to the contractile element of the tail for its motion. Thus, all three segments of the sperms play important roles for their movement and subsequent penetration of the ovum.[19,21-23]

Role of Epididymis

Animal studies have shown that the sperm maturation and the storage are major functions of epididymis. Compared to other species, the storage capacity of the human epididymis is limited and this is reflected by the low sperm content of the epididymis.[9] The sperm contents of the ejaculate depend on the number of sperms in the epididymal tail and the proximal portion of the vas at the time of ejaculation. It also depends on the daily sperm production and the frequency of ejaculations. When emptied by multiple ejaculations, healthy human epididymis can replenish the stock over a two-week period.

During this period, the sperms acquire properties to progress forward to undergo capacitation, to attach and to penetrate the zona pellucida of the ovum. Various specific proteins from the secretions from epididymal epithelium, which bind the sperm and remain in the ejaculate, help to induct the acrosome reactions. It thus facilitates to penetrate zona during the fertilisation (Figs 2.11 and 2.12). Lower molecular weight components such as *carnitine*,

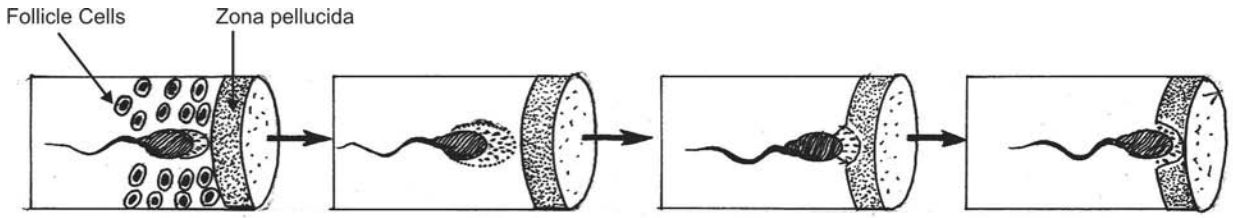


Fig. 2.11: Penetration of the oocyte by a capacitated spermatozoa. After passing through the follicle cell layers, the sperm binds by its head to the zona pellucida, and undergoes the acrosome reaction. During the reaction most of the proacrosin is converted to acrosin and released. Acrosin digests an opening into the zona pellucida and the sperm enters by its forward motility. Some proacrosin remains bound to the inner acrosomal membrane after the acrosome reaction. Proacrosin is converted to acrosin after the sperm enters the zona pellucida, further aiding sperm passage.

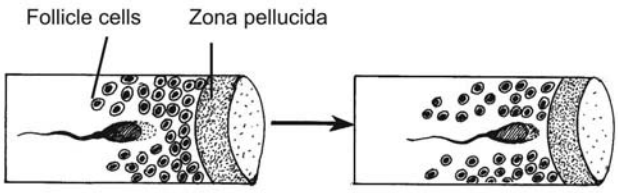


Fig. 2.12: Penetration of the oocyte by a noncapacitated sperm. Penetration through the follicle cell layers (cumulus oophorus and corona radiata) may or may not occur, but penetration through the zona pellucida will definitely not take place.

enter the sperm cells to facilitate energy production for motility.

Capacitation of Sperm

Mature sperms, even when they are coming out of the male genital tract are incapable of fertilising the ovum unless the further changes or *capacitation* takes place for a variable period of 1 to 10 hours in the female genital tract. The fluids from vagina, uterus and tube of a female first wash away multiple inhibitory factors present in the male genital tract. The floating vesicles from the seminiferous tubules containing cholesterol continually bathe the sperms, and it toughens the covering of the sperm acrosome preventing the release of enzymes. The excess cholesterol is gradually washed away as it is bathed in the fluid from vagina upwards and the membrane at the head of a sperm is made weaker.

The membrane of the sperm thus becomes progressively permeable to calcium ion that enters in abundance to initiate the powerful whiplash forward movement of the flagellum or tail instead of its previous undulating motion. Calcium has a further role to bring about further changes in the acrosome intracellular membranes for helping to release its enzyme very rapidly in the female genital tract.[1,3-5]

Changes in the sperm membranes associated with capacitation and the arcrosome reactions most probably are initiated as sperms pass through the epididymis. This observation led to the conclusion that an end-to-end anastomosis is a better option than a side-to-side anastomosis between the vas and the epididymis in cases of vasal obstruction, as it ensures passage of sperms through epididymis for a longer period. Sperms from the distal regions of epididymis are potentially fertilising, since they constitute the sperm reserve that normally enter the ejaculate (see Chapter 12).

Role of the Acrosome in Fertilisation

Histology of the sperm reveals that there is an inner acrosomal membrane (IAM) close to the nucleus and an outer acrosomal membrane (OAM) close to the plasma membrane with the acrosome proper located between the two. For the sperm to enter the inside of the ovum, it must go through the granulosa cells (corona radiata) before reaching the thick covering of the zona pellucida[9] (Figs 2.10 to 2.14).

The lytic enzymes involved in the sperm penetration are mostly located in the anterior sperm head, whereas others such as acrosin are primarily contained within the acrosome. The anterior surface of the sperm head needs to be removed allowing liberation of acrosin before the sperm can penetrate zona pellucida. Removal of this anterior surface of the head is the process called *acrosome reaction*.

Most of the acrosin initially is in an inactive form called *proacrosin*; and during acrosome reaction, the proacrosin gets activated or converted to acrosin. For a successful fertilisation, it is essential that the activation of the proacrosin and release of acrosin take place at the correct time and place, after the sperm gets bound to the zona. Premature occurrence

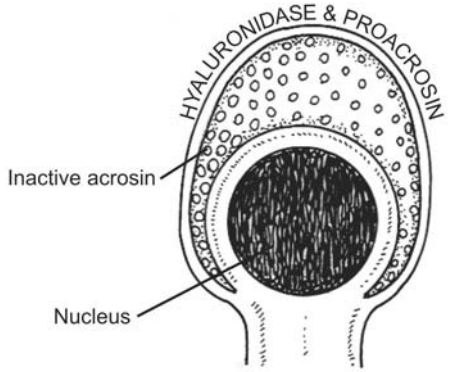


Fig. 2.13: Localisation of sperm hyaluronidase and proacrosin, the inactive (zymogen) form of acrosin.

or lack of it could hinder the process of fertilisation[9-11,24] (Figs 2.13 and 2.14).

The outermost plasma membrane of the sperm and acrosome are involved in the acrosome reaction. The sperm-oocyte union actually occurs between the midsegment of sperm and the oocyte membrane. The sperm is then directly incorporated into the oocyte cytoplasm. Incidentally, the sperm motility is not a factor for this fusion. The oocyte maturation occurs till the metaphase II after ovulation, and progresses no further till it is fertilised.[15,23]

In human beings, acrosome reaction can be induced by the presence of calcium ions. There are, however, inhibitory agents in the form of two high molecular weight glycoproteins and a low molecular weight protein. Removal of these inhibitory agents is mandatory, as they act as membrane stabiliser or receptor blockers. The process of capacitation encompasses the complete process of removal of these agents, destabilisation of the outer sperm head membranes by removal of cholesterol or phospholipid, and aggregation of the membrane protein. The sperm capacitation in the female genital tract initiates the release of small amount of acrosome enzymes.

As stated earlier, the acrosome of the sperm importantly stores hyaluronidase and other proteolytic enzymes. Hyaluronidase from the plasma membrane and the outer OAM helps in the penetration of cumulus oophorus by opening of pathways between the granulosa cells to facilitate entry of the sperm. It depolymerises the hyaluronic acid polymers present in the intercellular cement that holds the granulosa cells of the ovum together. An esterase enzyme is also involved in corona penetration. Acrosin (trypsin like protease) associated with IAM and acrosomal matrix) also helps to penetrate the zona. After penetrating the cumulus oophorus and corona radiata, the sperms reach the zona pellucida. The anterior membrane of the sperm on reaching the zona gets bound specifically to the receptor protein of zona and this binding is species specific.[9,17,18]

At first, the head of the sperm enters the perivitelline space lying immediately beneath the zona, but outside the oocyte membrane. Once firmly bound to the zona, acrosin is released via the acrosome reaction. That enables the sperm to digest the structural elements of the tissues and to make an opening in the zona. The sperm by its forward movement then enters through this opening into the perivitelline space.

After its entry into the perivitelline space, the sperm gets tied to the vitelline membrane. At this critical time, the oocyte undergoes the second meiosis extruding the polar body; and thus, the two polar bodies are located in the perivitelline space during fertilisation. Only acrosome containing sperms having gone through capacitation appear to be able to penetrate the vitelline membrane.[9] Once the sperm enters the oocyte cytoplasm, the ultimate fertilisation with union of male and female pronuclei takes place to form the zygote– the precursor of the new life. The process of this penetration to fertilisation approximately takes 30 minutes.

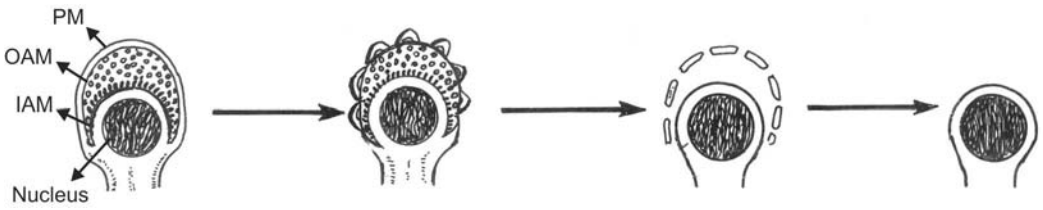


Fig. 2.14: The acrosome reaction. The plasma membrane (PM) and outer acrosomal membrane (OAM) fuse at various sites, forming vesicles. The vesicles and acrosomal contents are released. The inner acrosomal membrane (IAM), surrounding the sperm nucleus (N), remains after completion of the acrosome reaction.

As soon as the sperm penetrates the zona, there is a zona reaction causing hardening of the zona pellicida to prevent penetration by another sperm or the phenomenon of *polyspermy*. Dispersal of zona removes this species-specific property from the oocyte – a phenomenon used for the zona-free hamster oocyte penetration assay.[9]

Why does Only One Sperm enter the Oocyte

It is reasonable to assume that an individual sperm is completely equipped to penetrate the layers of the oocyte by itself, even though fertilisation does not occur until many sperms reach the site. When the fertilisation phenomenon is studied, it is noticed that although one sperm penetrates the zona pellucida, several are found around the same area. With each sperm getting attached to the oocyte, the enzymes from the head of sperm digest the oocyte cells. With the tail movement, it then pushes itself through the passage thus made.

Only a few sperms actually reach the zona pellucida of the ovum and they do not reach at the same time, and there may be time-gap between each reaching the area of zona pellucida. Although one sperm penetrates the zona pellucida, several of them do not complete the process. But they certainly provide the helping hand for the passage of the successful sperm by their liberated enzymes by softening and weakening of the zona of the pellucida.

The evidence of multiple slits in the wall noticed under microscope proves this theory. Within a few minutes of the first sperm penetrating the zona pellucida, calcium ions diffuse through the oocyte membrane causing release of multiple cortical granules from the oocyte to the perivitelline space by exocytosis.[9,10,15] These granules contain substance that permeates into all portions of the zona pellucida to prevent binding of any additional sperms. They would even cause the sperms that are already loosely bound to fall off. Moreover, the oocyte membrane after its fusion with the sperm is believed to cause electrical depolarisation that fends attachment of sperms reaching subsequently. Thus, almost invariably, only one sperm enters the oocyte during the process of fertilisation.

Postfertilisation Event

Fertilisation ensures formation of a new individual by a combination of two haploid sex cells or gametes from separate parents with their respective original genetic information. Each sperm provides the one half of the genetic material to complete the fertilisation, while the other half comes from the oocytes of female.

At fertilisation, when the sperm and the ovum meet, there is a random sorting of chromosomes from both sets. The process of meiosis converts the original diploid cell to a haploid gamete with a single set of chromosomes and causes a change in the genetic information to increase diversity in the offspring.

The depolarization caused by the sperm penetration results in one last round of division in the oocyte nucleus, forming a pronucleus containing only one set of genetic information. The pronucleus from the oocyte or egg merges with the pronucleus from the sperm. Once the two pronuclei merge, the cell division begins immediately. The fertilized egg is now called a zygote with combination of two sets of chromosomes restoring their number to 46 or diploid status.

The dividing zygote gets pushed along the fallopian tube. By approximately four days after the fertilisation, the zygote has about 100 cells and is called a *blastocyst*. When the blastocyst reaches the uterine lining, it floats for about two days, finally, gets implanted in the uterine wall by the sixth day after fertilisation. Once implanted, the blastocyst secretes human chorionic gonadotrophin (hCG), which rescues the corpus luteum and signals that a successful pregnancy has begun.

Sometimes, two dominant follicles develop eggs and ovulate. If both are fertilised and subsequently implanted in the uterus, two embryos develop into twins. They are called *fraternal twins* as they have developed from separate eggs that were fertilised by different sperms. In contrast, the two daughter cells, after a fertilised egg undergoes its first division, may separate and divide independently of each other. In this situation, they remain loosely connected in the fallopian tube, and later two blastocysts implant together in the uterine wall. It subsequently develops into two separate embryos. These are called *identical twins*, as they originate from the same fertilised egg and consequently, have identical genetic material.

The implanted blastocyst continues developing in the uterus for about nine months. As the foetus grows, there is uterine growth to accommodate its increasing size.[1,5,6,25]

Sex Determination of Embryo

(see details in Chapter 10)

As stated earlier, the embryo contains a different and random mix of maternal and paternal genes. Each cell of the body contains a set of chromosomes from the maternal ovum and the paternal sperm. Consequently, two brothers in the same family can look and act totally different from one another, even though they come from the same parents. It all depends on which genes (chromosomes) were randomly chosen, when the division of the sex cells of the mother and father took place.

If the embryo is a male (XY chromosomes), then testosterone will stimulate the wolffian duct to develop male sex organs, and the mullerian duct will get degraded. If the embryo is female (XX), no testosterone is made. The wolffian duct will get degraded, and the mullerian duct will develop into female sex organs. The female clitoris is the remnant of the wolffian duct. If the embryo is a male (XY), but there is a defect with no testosterone made, then the wolffian duct will get degraded, and the mullerian duct will develop into nonfunctional female sex organs.[1] Sex-organ development is determined by the third month of development (see Chapter 10 on Chromosomes). It is worth noting that the sex is always determined at the time of the fertilisation, but really it is declared at birth or not earlier than the third month of the foetal life, if an ultrasonography is used.

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CHAPTER 3

Endocrinal Aspects of Male Reproductive System

INTRODUCTION

Male reproduction is a complex synergy of several factors with successive functioning of the central nervous system (CNS), endocrine glands and the male sex gland. So, to understand the basis of endocrinal aspect of the reproduction in male, roles of the hypothalamus, anterior pituitary and the testis need to be assessed. The testis in real terms is not a pure endocrine gland, as it has an endocrine function provided by the Leydig cells and a nonendocrine or spermatogenetic function carried out by the Sertoli cells.

The endocrine system, in concert with the nervous system, coordinates function of various components of the reproductive system. The CNS draws upon external (e.g. sexual cues, temperature) and internal inputs (e.g. checks and balances between endocrine and tissue functions, metabolic status, etc.), and then acts on the reproductive axis. The four hormones—luteinising hormone (LH), follicle-stimulating hormone (FSH), testosterone, and prolactin are of prime interest in this respect.[1,2]

For the completion of a successful male reproductive process, the hypothalamic-pituitary neurohormonal factors regulate the production of male hormones in the testis, which is also the seat of production and development of the sperms.[1] A major share of the control of sexual functions in both male and female begins with the secretion of gonadotrophin-releasing hormone (GnRH) by the hypothalamus. This hormone, in turn stimulates the anterior pituitary gland to secrete two gonadotrophic hormones named as LH and FSH . LH is the primary stimulus for the secretion of testosterone by the testes and FSH helps to stimulate spermatogenesis.

Cohesive functioning of the testicular and the extratesticular hormonal factors in males without doubt is an essential prerequisite for successful reproduction. It is thus imperative that complex functioning at various levels of the endocrinal activity – CNS-hypothalamic, hypothalamic-pituitary, pituitary-testis axes along with roles of various other factors, such as the growth hormone of the pituitary, adrenal cortical hormone, and thyroid hormone, are coordinated. There are some additional roles of the testicular growth substances and cytokines, and a possible obscure role of pineal gland. This is summarised in Table 3.1

Table 3.1: Hormonal factors in reproduction

Testicular component
a. Adequate androgen or male hormones from Leydig cells to coordinate intratesticular control mechanism and cell interactions.
b. Effective spermatogenesis (production and development of sperms) from the seminiferous epithelium (consisting of Sertoli and germ cells)–which depends on FSH and high intratesticular testosterone.
Extratesticular component
a. CNS-hypothalamic axis.
b. Pituitary gonadotrophins – LH and FSH with additional role of growth hormone of pituitary origin.
c. Adrenal cortex, thyroid and perhaps obscure role of the pineal gland.

Interplay of different stimulating and inhibitory factors involving the endocrine aspect of male reproductive process actually determine the optimum hormonal milieu. Whenever any hormonal secretion reaches a critical level, automatic negative feedback effect operating at various levels would reduce the secretion of the particular hormone. Conversely, when the hormonal level gets reduced to a critical level, the inhibitory factors are thrown out of action by the stimulating factors of the particular hormone (Table 3.2 and Fig. 3.1 Plate 1).

Table 3.2: Stimulatory and inhibitory factors in males

1. CNS-hypothalamic axis
2. Hypothalamus-pituitary axis
3. Pituitary-testis axis
4. Testicular component and role of Sertoli cell growth factors
5. Additional roles of pituitary growth hormone and prolactin, adrenal cortical and thyroid hormones
6. Obscure role of pineal gland.

CNS-HYPOTHALAMUS-PITUITARY COMPONENTS

At the onset of puberty, the resultant sexual changes are initiated by changes in the CNS leading to hypothalamic stimulation. Premature activities would lead to precocious puberty as seen in tumours and certain cases of hydrocephalus. Underlying mechanism that leads to such activities is still not known.

GnRH NEURONAL SYSTEM

The ultimate drive to the male gonads originates in yet to be clearly defined neuronal mechanism located in the hypothalamus. Pituitary gonadotrophin activity is modulated by a variety of direct inputs from the CNS, expressed either indirectly at the hypothalamic or directly at the hypophyseal level. Precursor molecule of GnRH- the Pro-GnRH contains an aminopeptide and a GnRH associated peptide (GAP) stored in the secreting nerve terminals and is encoded on chromosome-8. [3-9]

Indubitably, the prime mover in the chain of endocrinal activities is the CNS. In this context, physiological role of an opiodergic (opiate-like substance) regulation is now known. Endogenous blockade with an opiate receptor antagonist accelerates LH pulse frequency.[10,11]

The nerve impulse from the CNS passes on to the arcuate region of the mediobasal portion of the hypothalamus. It then sends a decapeptide, i.e. GnRH (also known as LHRH) through CNS-mediated periodic discharge of GnRH pulsator into the hypophyseal portal circulation.[3] This initiates gonadotroph cells of the pituitary in their turn to produce the gonadotrophins – LH and FSH.

LH and FSH act on the testes to produce:

a. Testosterone—which is converted partly into an active ingredient called –
b. Dihydrotestosterone (DHT),
c. Estrogen, a part of which originates from testosterone through aromatisation.
d. Inhibin—a nonsteroid product, which has a negative feedback effect on the pituitary gonadotrophins.
e. Activin.

The GnRH-secreting neurons originate from the olfactory placode outside the developing brain. They subsequently migrate through the nasal septum towards the olfactory bulb and olfactory tract, and eventually end up principally at the median eminence of the hypothalamus.[1] The rhythmic activities of GnRH cells (GnRH pulse generator) release the gonadotrophin releasing hormone from hypothalamus. The precise underlying mode of action of the neural mechanism of these terminals in hypothalamus is not fully explained. However, there is a dynamic equilibrium between the stimulating and inhibiting factors of gonadotrophic hormones that ultimately determine their rate and quantity of synthesis.

Episodic and pulsatile nature of release of GnRH is best exemplified by similar variations in the LH level (which commonly is highest at night in pubertal male). Similar variation of FSH is less clear as there is no clear-cut evidence of a FSHRH (unlike LHRH). Moreover, longer plasma half-life of FSH (nearly 4 times that of LH) does not help easy detection of variation of the FSH level to prove pulsatile nature of its discharge.[4-6,9]

GnRH pulses occur about every two hours (90-150 minutes) as a result of episodic GnRH secretion from the hypothalamus into the pituitary portal circulation.[1,9] Intermittent pulsatile discharge of GnRH is an essential prerequisite for the normal functioning of the GnRH-pituitary axis, as is proved by experimental evidence of continuous GnRH administration in GnRH deficiency resulting in profound pituitary desensitisation. Amplitude of LH pulse is dependent on complex interplay of at least three principal factors—intrinsic responses from gonadotrophs, GnRH pulse frequency and size of the GnRH bolus. There is also a fourth unknown factor that determines the LH distribution and elimination

of LH from the circulation.[4-6] For the GnRH therapy to be effective. It must be given in a pulsatile manner (see chapter 11 under medical management).

PITUITARY COMPONENT
(Gonadotrophic hormones)

The LH and FSH are dimeric molecules composed of two dissimilar noncovalently linked glycosylated polypeptides – α and β subunits, encoded by different genes.[7-9,12] The α-subunits are species specific and its structure is common to the LH, FSH, human chorionic gonadotrophin (hCG) and thyroid-stimulating hormone (TSH). The specific hormonal activity of these dimeric glycoproteins is determined by the β-subunits, which are hormone specific and develop later in the evolution. As the structures of LH and hCG are encoded by the same gene, it lends credence to their identical biological activities. Endogenous opiates exert a tonic restraining control of LH release in eugonadal men.[10, 11]

Microsomal enzymes cause glycosylation (addition of sialic acid chains) of structurally and functionally related gonadotrophins– LH and FSH. Removal of these terminal sialic acid chains from the carbohydrate side chains reduces the half-life period of these hormones. The LH contains only one or two sialic acid residues compared to four in FSH and nearly twenty in hCG. This partly explains differences in the plasma half-life periods of these hormones, the LH having least with ½ hour, FSH with 4 hours and hCG longest with 6 hours.[9, 12]

Protein component of gonadotrophins is responsible for binding of hormone to the target cells (Leydig or Sertoli), while carbohydrate component determines the response of these target cells. FSH binding to the cell receptors of Sertoli cell cytoplasmic membrane activates adenyl-cylase activity, which initiates a specific gene to cause spermatogenesis. The LH activity similarly causes androgen production. Spermatogenesis essentially depends not only on adequate FSH, but also on high intratesticular testosterone indicating roles of both gonadotrophins. While secretions of gonadotrophins –LH and FSH are subject to the levels of androgens and estrogens, *inhibin*, a non-steroid substance, has a specific role only in the regulation of FSH.[13,14] Administrations of testosterone and estrogen inhibit secretions of LH and FSH, testosterone acting at the hypothalamic and estrogen acting at the pituitary levels.

Growth hormone of pituitary is necessary for controlling the background metabolic functions of the testes.[1,2] It also promotes spermatogenesis. In pituitary dwarfs, absence or reduction of the level of growth hormone causes spermatogenesis to be severely deficient or entirely absent.

TESTICULAR COMPONENT
(Leydig and Sertoli Cells)

Endocrine Functions of the Leydig Cell

Androgen (main sex hormone)

Both in the testes and in the adrenals, the androgens can be synthesised either from cholesterol or directly from acetyl coenzyme A[1]. The biosynthesis of testicular steroid hormones (androgens) from cholesterol takes place in several tissues with the help of specific cytochrome P-450 enzymes of the mixed-function oxidase type. These enzymes are responsible for mediation of many of the reactions involved in steroid biosynthesis. All steroidogenic tissues, namely the adrenals, testes, ovaries, and placenta, contain these enzymes necessary for cleaving the cholesterol side chains to remove the 6-carbon isocaproic acid, and thus converting the cholesterol to pregnenolone. However, some important target tissues do not have appropriate alpha reductase enzymes in their cells to convert the testosterone into DHT. In these tissues, actions of testosterone works with only half its potency to induce the formation of cell proteins.

Androgens are formed by the interstitial cells of Leydig, which lie in the interstices between the seminiferous tubules and constitute about 20 per cent of the mass of the adult testis. Stimulation of Leydig cells by the LH results in a cascade of intracellular events, which eventually lead to the formation of androgens. It has been suggested that prolactin of anterior pituitary along with the LH exhibits a synergistic effect at the level of Leydig cells, but the exact role of prolactin in human reproduction is still not fully understood.[15]

The LH can generate free cholesterol inside the cell through different mechanisms.[1] Cellular cholesterol is stored in the form of cholesterol esters in the lipid granules. The LH through stimulation of the cholesterase enzyme helps to form free cholesterol, which is transported to the mitochondria for its conversion to pregnenolone. The C_{27} steroid cholesterol, which is the substrate for androgen biosynthesis, may be synthesised by the Leydig cells from acetate or from the lipid in the lipoproteins [(mainly the low-density lipoprotein (LDL) component)] in the circulation. The enzyme involved in

cholesterol biosynthesis is 3-hydroxy-3-methyl-glutaryl coenzyme-A-reductase (HMG-CoA-reductase) (Fig 3.2).

a. If the enzymes involved in the steps 1 to 4 are deficient, it would produce congenital adrenal hyperplasia with male pseudohermaphroditism.
b. If the enzyme in step 5 is deficient, it would produce only male pseudohermaphroditism.

The mitochondrial side chain cleavage cytochrome (P-450) enzyme complexes, consisting of three protein components, catalyse the process of conversion of cholesterol to pregnenolone. The LH stimulates the synthesis of cytochrome and this enzymatic conversion is irreversible and rate limiting.[1] These enzymes catalyse the hydroxylation and oxidation between the C_{20} and C_{22} positions of the steroid backbone to reduce the C_{27} to a C_{21} steroid. Clinically, useful drugs such as *aminoglutethimide* inhibit the side chain cleavage reaction. After the release of pregnenolone from the mitochondria, the predominant steroidogenic pathway in human for testosterone is shown in Figure 3.3.[1,2]

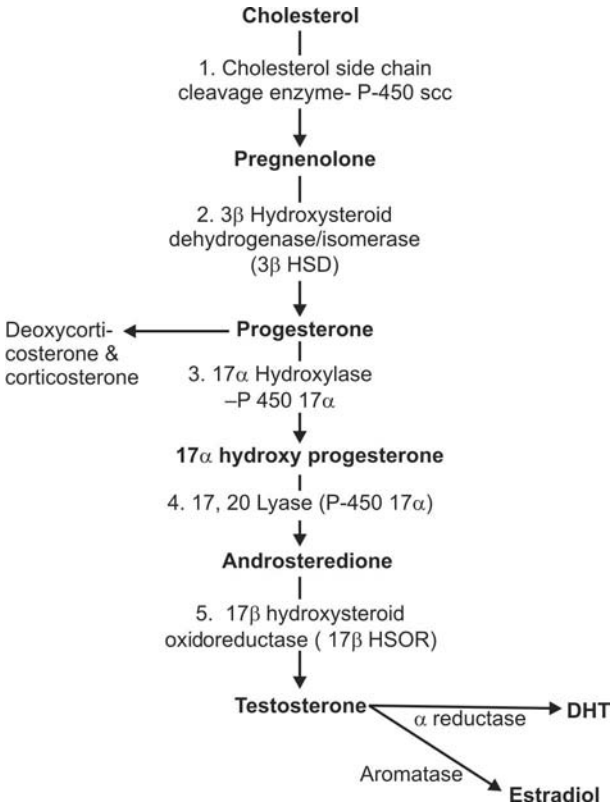

Fig 3.2: Cholesterol to testosterone conversion (with enzymes involved-1-5)

Formation of C_{19} steroid testosterone from the C_{21} pregnenolone requires the activity of the cytochrome enzyme system present in the endo-plasmic reticulum. The LH-dependent P-450 enzyme also belongs to the cytochrome P-450 protein family. It catalyses conversion of pregnenolone to form the C_{19} steroid dehydroepiandrosterone (DHEA) through two separate reactions referred to as 17-α-hydroxylase, and 17,20 lyase activities (cleavage between C_{17} and C_{20}). Both these enzyme activities can be demonstrated in non-steroidogenic cells transfected with a cDNA clone for the P-450 group enzyme. The enzyme also requires the participation of a flavoprotein, NADPH cytochrome P-450 reductase.[1]

Two enzymes not related to the cytochrome P-450 protein family mediate the final conversion of DHEA to testosterone. The 3α-hydroxysteroid dehydrogenase (3α-HSD) enzyme converts DHEA to androstenedione, which is further converted by a reversible enzyme 17β-hydroxysteroid dehydrogenase (17α-HSD)) to testosterone (Fig. 3.3).[1, 2]

Alternatively, DHEA can be altered to androstenediol by the 3β-HSD enzyme followed by transformation of testosterone mediated by 3β-HSD. In addition to this pathway, a parallel reaction (through the formation of progesterone, 17α-hydroxyprogesterone and androstenedione as intermediate products) is predominantly operative in steroidogenic tissue of other species to convert C_{21} to C_{19} steroids. Testosterone secreted by Leydig cells either goes into the general circulation or into the seminiforms tubular lumen.

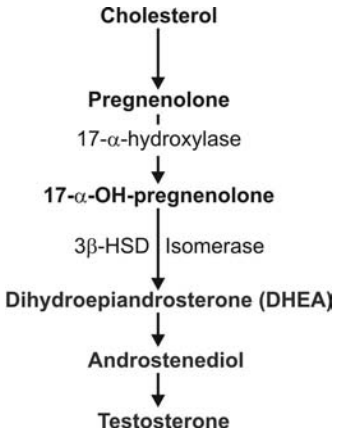

Fig. 3.3: Alternate pathway for cholesterol to testosterone

Deficiencies of the enzymes involved in synthesis of testosterone may manifest in the adrenal gland as well as in the testis.[1] Although most enzymatic disorders are rare, individuals with defective product of each separate enzyme have been described. Patients with defective 17β-HSD enzyme system will have elevated androstenedione and low testosterone levels resulting in partial virilisation at puberty. Rare heredity disorders due to enzymatic defects can result in defective testosterone synthesis and are associated with an inadequate virilisation that is evident at birth with ambiguous genitalia.

Several forms of androgen resistance result in undermasculinisation and infertility in males with otherwise normally developed external genitalia. Characteristically, there is an elevation of testosterone and LH levels. There is no effective treatment for the condition. Diagnosis is made by the finding of abnormal androgen receptors in a tissue culture of genital skin. However, it is not cost effective and only possible through a dedicated research laboratory.

Negative feedback action of androgens on GnRH pulse generator is the most important factor in the maintenance of hormone milieu. Major component of this regulation of the GnRH pulse generator is exerted by the testosterone. In contrast to pre-eminence of the testosterone-mediated feedback at the hypothalamic level, the estrogen acts at the pituitary level by direct inhibitory action on gonadotrophes and by shortening the half-life period of FSH. Estrogen also inhibits some enzymes in the testosterone synthetic pathway and therefore, directly reduces testosterone production (Fig. 3.1). Some amount of estrogen is formed from aromatisation of testosterone in Sertoli cells.

As estrogen has a predominant role, at the pituitary level, estrogen administration to normal men reduces the amplitude of endogenous LH pulses and treatment with antiestrogen causes the LH pulses of larger amplitude.[16]

Regulation of the androgen production is essential for the spermatogenesis. It requires a complex network involving the intratesticular mechanism and cell interactions. In foetal life, maternal hCG from the placenta exerts exactly the same action on the sexual organs as the LH. Testosterone is secreted episodically from the Leydig cells in response to LH pulses and has a diurnal pattern, with the peak level in the early morning and the trough level in the late afternoon or early evening. The LH in prepubertal boys has a large sleep-related nocturnal amplitude. In intact testis, the LH receptors decrease or get downregulated after exogenous LH administration. Large doses of GnRH or its analogues can inhibit LH secretion. This has been applied clinically to cause medical castration in men with prostate cancer. Although testicular secretion also shows a pulsatile pattern, unlike the pituitary response to GnRH, the pulsatile Leydig cell functioning is not an essential prerequisite. It is proved by the evidence of uninterrupted stimulation of Leydig cells following administration of hCG in hypogonadism.[9]

There also appears to be an intratesticular ultrashort loop feedback, so that the exogenous testosterone will override the effect of LH and inhibit testosterone production in the testes.[1]

In normal males, only 2% of testosterone is free or unbound, 44% is bound to testosterone-estradiol-binding globulin or TeBG (also called sex hormone-binding globulin) and 54% of testosterone is bound to albumin and other proteins. These steroid-binding proteins modulate the androgen action. TeBG has a higher affinity for testosterone than for estradiol, and changes in TeBG alter or amplify the hormonal milieu. TeBG level is increased by estrogens, administration of thyroid hormone, and in cirrhosis of the liver, and may be decreased by androgens, growth hormone and obesity. The biological actions of androgens are exerted on the target organs that contain specific androgen receptor proteins. As testosterone leaves the circulation and enters the target cells, it is converted into more potent androgen DHT by an enzyme 5-alpha-reductase. The major functions of androgens in target tissues are shown in Table 3.3.[2,15,17]

Table 3.3: Major functions of androgens

1. Regulation of gonadotrophin secretion by the hypothalamic-pituitary axis.
2. Initiation and maintenance of spermatogenesis.
3. Foetal internal and external male genital system's development.
4. Promotion of sexual maturation and further development starting at puberty.

At the macro level the testosterone has three important functions (See Table 3.4).[18]

Table 3.4: Macrolevel actions of testosterone

1. Enhances sexual interest.
2. Increases frequency of sexual acts.
3. Increases frequency of nocturnal erections.

An intimate structural and functional relationship exists between the two separate compartments of the testis, the seminiferous tubule and the interstitial tissues between the tubules. While the LH effects spermatogenesis indirectly by stimulating androgen or testosterone production, FSH targets Sertoli cells. Thus, androgen, mainly the testosterone, and FSH are the hormones, which are directed at the seminiferous tubular epithelium. Androgen-binding protein, which is a Sertoli cell product, carries testosterone intracellularly and may serve as a testosterone reservoir within the seminiferous tubules in addition to transporting testosterone from the testis into the epididymal tubule. The physical proximity of the Leydig cells to the seminiferous tubules and the elaboration by the Sertoli cells of androgen-binding protein, cause a high level of testosterone to be maintained in the microenvironment of the developing spermatozoa.

The hormonal requirements for the initiation of spermatogenesis appear to be independent of the maintenance of spermatogenesis. This is substantiated by the fact that after a pituitary ablation, only testosterone is required for the maintenance of the spermatogenesis. However, if spermatogenesis is to be re-initiated after the germinal epithelium has been allowed to regress completely, both FSH and testosterone are required.[17]

Other Male Sex Hormones

The testes secrete several male sex hormones, which are collectively called androgens, including testosterone, DHT, DHEA and androstenedione. However, testosterone is so much more abundant than the others that one can consider it to be the significant testicular hormone, although much, if not most, of the testosterone is converted into the more active hormone DHT in the target tissues.

Strictly the term "androgen" means any steroid hormone that has masculinising effects, including of course, testosterone itself. It also includes male sex hormones produced elsewhere in the body besides the testes. The adrenal glands secrete at least five different androgens (principally DHEA and androstenedione). Total masculanising effects of these are normally so slight, that they do not cause any significant masculine characteristics even in women, except causing growth of their pubic and axillary hairs.[1,2] Five to 10 percent of androgen comes from the adrenal glands.

When an adrenal tumour of the androgen-producing cells occurs, the quantity of androgenic hormones become large enough to cause all the usual male secondary sexual characteristics to develop in a greater degree. These effects are seen in the *adrenogenital syndrome*. Rarely, embryonic rest cells in the ovary can develop into a tumour that produces excessive quantities of androgens in women; one such tumour is the *arrhenoblastoma*.[2] The normal ovary also produces minute quantities of androgens, but these are not significant.[1]

In general, the testosterone is singularly responsible for the distinguishing characteristics of the masculine body. Even during foetal life, the testes are stimulated by chorionic gonadotrophin from the placenta to produce moderate quantities of testosterone throughout the entire period of foetal development, and up to 10 or more weeks after birth. Thereafter, essentially, no testosterone is produced during childhood until the onset of puberty (approximately at the age of 10 to 13 years), when its production increases rapidly under the stimulus of anterior pituitary gonadotrophic hormones. It then lasts throughout most of the remainder of life, although there is a slow drop after the age of 30 years (see PADAM later).

Leydig cells are almost nonexistent in the testes during childhood with almost no secretion of testosterone, but they are numerous in the newborn male infant, and also in the adult male any time after puberty (see Chapter 2) with the testes secreting large quantities of testosterone. Furthermore, when tumours develop from the interstitial cells of Leydig, great quantities of testosterone are secreted. When the germinal epithelium of the testes is destroyed by X-ray treatment or by excessive heat, the Leydig cells, which are relatively difficult to be destroyed, continue to produce testosterone.

Metabolic Aspects of Androgens

All androgens are steroid compounds. Most of the testosterone after its secretion by the testes, becomes loosely bound with plasma albumin or more tightly with a betaglobulin called gonadal steroid-binding globulin. It circulates in the blood for about 30 minutes to an hour or so. By that time, it either becomes fixed to the tissues or degraded into inactive products that are subsequently excreted. Much of the testosterone that becomes fixed to the tissues is

converted within the cells to DHT, by 5-α-reductase enzyme and lesser quantities into 5-α-androstanediol, especially in certain target organs like the prostate gland in the adult and in the external genitalia of the foetal male. Androgen receptors bind DHT with much higher affinity resulting in higher target cell response. Some actions of testosterone are dependent on this conversion, whereas others are not.

The testosterone that does not become fixed to the tissues, rapidly gets converted mainly by the liver into androsterone and DHEA, and simultaneously conjugated either as glucuronides or sulfates (glucuronides, particularly). These are excreted either into the gut in the bile or into the urine.[1]

Functions of Androgen (Testosterone)

In Foetus

Testosterone begins to be elaborated by the male testes at about the seventh week of embryonic life. Indeed, one of the major functional differences between the female and male sex chromosomes is that the male chromosome causes the newly developing genital ridge to secrete testosterone, whereas the female chromosome causes this ridge to secrete estrogens. Injection of large quantities of male sex hormone into pregnant animals causes development of the male sexual organs even though the foetus is female, while removal of the testes in a male foetus causes development of the female sexual organs. Therefore, testosterone secreted first by the genital ridges and later by the foetal testes is responsible for the development of the male body characteristics, including the formation of a penis and a scrotum. It also causes formation of the prostate gland, seminal vesicles, and the male genital ducts; while at the same time, suppressing the formation of female genital organs.

Table 3.5: Functions of androgen (testosterone)

1. In foetus before the testicular descent
2. In foetus during the testicular descent, then continuing in boys till prepubertal stage
3. At puberty and after, and during adulthood
4. In elderly and the old age groups.

During Descent of the Testes

Last part of the descent of the testes, when they come out of the inguinal canal to slip into the scrotum, occurs during the last two months of gestation. This necessitates secretion of reasonable quantities of testosterone by the foetal testes. The direct effect of testosterone is essential for the development of male genitals including the epididymis, the vas deferens, and the seminal vesicles. Sometimes clinicians administer gonadotrophic hormones in a child with undescended but otherwise normal testes, to stimulate the Leydig cells to secrete adequate testosterone. It is a common clinical experience that the testis can descend in this situation only, if the inguinal canal is large enough to allow it to pass through.

At Puberty

Male hormone would impart male characteristics in an individual, which naturally occurs with the onset of puberty, when substantial changes in hormone milieu takes place. At the onset of puberty, male hormones cause further development of the sexual organs initiating spermatogenesis. There are also changes in the hair distribution, larynx and general metabolism.[1,2]

Androgen level rises as the adrenal glands and testicles mature at or soon after puberty. The increased testosterone secretion causes the penis, the scrotum, and the testes all to enlarge up to approximately about six to eightfold, and the secondary male sexual characteristics develop at the same time. These secondary sexual characteristics, in addition to the sexual organs themselves, distinguish the male from the female. Testosterone causes change in the growth of body hairs over the pubis upward along the linea-alba; sometimes, to the umbilicus and above, on the face, usually on the chest; and less often, on other regions of the body such as the back. It also causes the hair on most other portions of the body to become more prolific (Fig. 3.4).

Testosterone has a natural effect to cause decrease in the growth of hair on the top of the head, but a man without functional testes does not always become bald. Scalp hair loss can begin in teens and progress at varying rates through adulthood. In fact, baldness is the result of factors like a genetic background for the development of baldness or the presence of large quantities of androgenic hormones often superimposed on this genetic background. Incidentally, patchy loss of hair in the scalp is almost always due to some skin conditions needing attention of a dermatologist. A woman with appropriate genetic background becomes bald in the same manner, as does a man.[19]

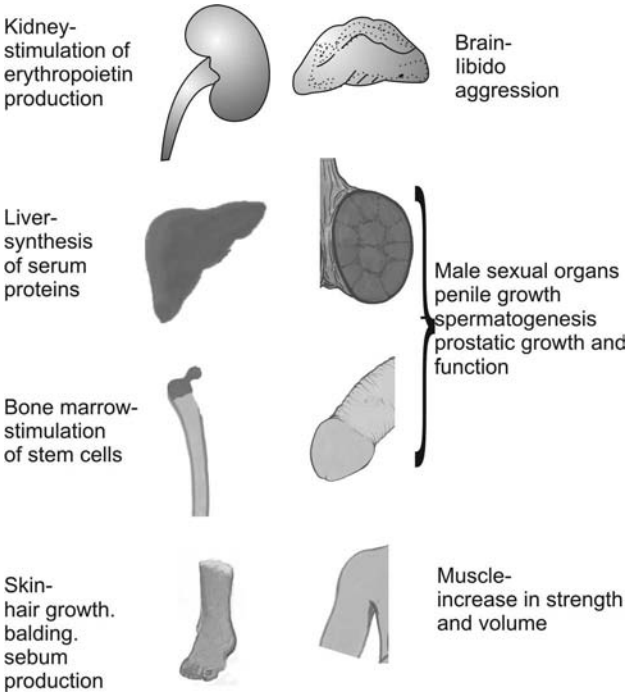

Fig. 3.4: The systemic functions of testosterone

At the onset of puberty, the testosterone also causes hypertrophy of the laryngeal mucosa and enlargement of the larynx. This results in at first a relatively discordant cracking voice, but this gradually changes into the typical adult masculine bass voice.[1]

In Elderly and the Old Age Groups

Androgen production declines with age; but unlike in females, the decline occurs gradually. In general, older men have lower testosterone levels than younger men. While the menopause in a woman initiates faster drop in the level of estrogen in a relatively short period signalling the end of child-bearing for women, men do not have similar reproductive event. Logically, there should be a distinction between two different situations as men can reproduce well into their sixties and at times even later.[19] Todd Nippoldt, an endocrinologist at Mayo Clinic, says, "While every woman goes through menopause, not every man ends up with low testosterone levels." He prefers the term *andropause* that is caused as the name suggests, by the decrease in the testicular androgen secretion. Other terms for this condition include *male climacteric*, *viropause* and *low testosterone syndrome*. A more appropriate designation is androgen decline or deficiency in the ageing male (ADAM) or partial androgen deficiency or decline in the ageing male (PADAM). In practice, perhaps, PADAM is the more appropriate name. The pertinent question is not the choice of the name, but whether gradually declining testosterone level is natural and protective, or a condition to be considered for treatment.[20-29]

PADAM is neither life-threatening nor trivial. In men, bioavailable and free testosterone (T) levels decline by about 1.0 and 1.2% per year respectively, after the age of 40. From the age of 30, the free testosterone (FT) levels decrease continuously with age. The mean total T level at age of 70 is approximately 66% of mean level at age 25, whereas mean FT level at that age is only 40% of the mean level in young adults**.** The decrease in FT levels is, however, only one of the many factors responsible for the signs and symptoms of the ageing male. The diagnostic criteria have been more or less arbitrarily defined as levels below the lowest 1% of levels in young healthy males.[21-24] The diagnosis of androgen deficiency in elderly men should be based on both the clinical symptomatology and the FT levels.

PADAM or ADAM is a clinical entity characterised biochemically by a decrease not only of the serum androgen, but also of other hormones, such as growth hormone, melatonin and DHT. Ageing in males is accompanied by a series of signs and symptoms akin to androgen deficiency in young adults with decrease in muscle mass and strength, increase in abdominal mainly visceral fat, with insulin resistance and atherogenic lipid profile, decrease in libido and sexual hair, osteopenia, decrease in cognitive performances, insomnia, excessive sweating and decrease in general well-being. The character of PADAM or ADAM is distinct from profound hypogonadism in its relation to age, the degree of contributing symptoms and the marginal reduction in testosterone.

The onset of PADAM is unpredictable and its manifestations are subtle and variable, which has led to a paucity of interest in its diagnosis and treatment. Androgen deficiency of this nature in the ageing male affects an estimated 1 in 200 men. Urological practice commonly includes a large proportion of men older than 50 years. Therefore, it is important for urologists to recognise the manifestations and be familiar with evaluations necessary to document PADAM as well as its treatment and monitoring. By the time the ageing men get to an andrologist, they have sweated the decision for months as most men are loathe to discuss their libido problems with others including

doctors. Once other risk factors, such as smoking, stress, and alcohol or drug use, are ruled out, older men with low testosterone require testosterone replacement therapy (TRT).

Careful long-term studies are needed to assess the risk-to-reward ratios of androgen or other hormone replacement therapy (HRT).[23-29] However, it is still a matter of conjecture the magnitude and longevity of the beneficial effects of testosterone supplementation in the older men, and whether only certain subgroups of men would truly benefit from therapy. More importantly, the long-term risks of androgen therapy in this age group really are not known.

Studies examining TRT and normal ageing have not shown consistent benefits. In ageing men, testosterone treatment has been recorded to stimulate noncancerous (benign) growth of the prostate. Other effects include unmasking prostate cancer (testosterone is contraindicated in prostate cancer), aggravation of sleep apnoea and polycythaemia.[21, 29] In the USA, some 4 million men have low testosterone levels, but only an estimated 200,000 receive treatment. Over the last 10 years, there has been an increase in its incidence, and having a low, sperm count in these men very often compounds the problem. Incidentally, a lot of foods have excessive estrogens that can cause low sperm count and problems with virility, libido and the general feeling of wanting to be involved sexually. About 10 to15 per cent of these group of men also suffer from psychological problems that can lower sperm count and cause problem with erection. (See Appendix-4)

Physiological Effects of Androgens

All forms of androgens secreted by different sources are known to increase the rate of metabolism by 5 to 10 per cent with the onset of adolescence and during early adult life. This increased rate of metabolism is possibly attributed to an indirect effect of testosterone on the protein anabolism causing increased quantity of proteins and enzymes, thus enhancing the activities of all cells. Average man has about 700,000 more red blood cells (RBCs) per cubic millimetre than the average woman. This difference may also be due partly to the increased metabolic rate caused by testosterone in general, thus increasing RBC production. When normal quantities of testosterone are injected into a castrated adult, the number of RBCs increases by 15 to 20 per cent. Testosterone also has a minor degree of adrenal mineralocorticoid-like effects on the sodium metabolism increasing reabsorption of sodium from the renal distal tubules. This also to some extent explains postpubertal increase of the blood and extracellular fluid volumes of the male in relation to his weight increase.

Basic intracellular mechanism of action of testosterone causes increased rate of protein formation in the target cells. The study of sequence of these actions of testosterone on the prostate gland, (one of the organs that is most affected by testosterone), has given insight to the knowledge of the intratesticular actions of testosterone. Within a few minutes of testosterone entering the glandular cells, it is converted to DHT and gets bound with a cytoplasmic "receptor protein". This combination then migrates to the nucleus, where it binds with a nuclear protein, and induces the DNA-RNA transcription process. Within 30 minutes, RNA polymerase gets activated and the concentration of RNA begins to increase in the cells. This is followed by progressive increase in cellular protein. After several days, the quantity of DNA in the gland also increases, and there is simultaneous increase in the number of prostatic cells. Therefore, it is assumed that testosterone greatly stimulates production of proteins in general, though increasing more specifically those proteins in "target" organs or tissues responsible for the development of secondary sexual characteristics.[1,2]

Production of Estrogen in Male

In addition to testosterone, small amounts of estrogens are formed in the male (about one-fifth the amount in the nonpregnant female), and a reasonable quantity of these can be recovered from a man's urine. The exact source of the estrogens in the male is also still doubtful, but following facts are known:

1. Sertoli cell forms the estrogen through aromatisation from the testosterone following its stimulation by the FSH. Androgen-binding proteins secreted by the Sertoli cells bind both testosterone and estrogen, and carry these products into the fluid in the tubules making them available to sperms.
2. Concentration of estrogens in the fluid of the seminiferous tubules is thus quite high, and probably plays an important role in spermatogenesis. Estrogen production in the testis is minimal compared to other sources, but it has a definite role in the intratesticular regulatory mechanism involved in the spermatogenesis.[1]

3. Estrogen is also derived from androstenediol in other tissues of the body, especially the liver accounting for as much as 80 per cent of male estrogen production.

Aromatase is the terminal enzyme responsible for estrogen biosynthesis. The aromatase deficiency is associated for instance with severe bone maturation problems and sterility in mouse and man. Conversely, it is well known that estrogens in excess are responsible for the impairment of spermatogenesis. Therefore, the female hormone (or the androgens/estrogens ratio) plays a physiological role in the development and maintenance of male gonadal functions and seem to control especially the spermatid production (both qualitative and quantitative aspects) and epididymal sperm maturation.[30]

ENDOCRINE FUNCTIONS OF SERTOLI CELLS

Steroidogenetic functions of the Sertoli cells convert the testosterone to estradiol with participation of P-450 aromatase enzyme and NADPH cytochrome P-450 reductase. The Sertoli cell estrogen has mainly an intratesticular role and actually gets bound to the specific receptors in the Leydig cells to inhibit androgen production.[1]

Perhaps the other important hormone-like substance – *inhibin* secreted by Sertoli cells has a potent inhibitory feedback action on the anterior pituitary gonadotrophin FSH. There is an interrelated role of testosterone and inhibin in the physiological negative feedback regulation of FSH by direct action on the pituitary. The role of inhibin in spermatogenesis operates simultaneously with and in parallel to the negative feedback mechanism on anterior pituitary for control of testosterone secretion.[12-15] *Activin* feeds back positively on FSH secretion by the pituitary (Fig. 3.1).

Many other proteins produced in Sertoli cells are androgen-binding protein (ABP), transferin, ceruplasmin and plasminogen activators.[9,12] ABP binds the testosterone and maintains the high intratesticular concentration of androgen in the tubular lumen. *Transferin* and *ceruplasmin* are involved in the transport of iron and copper ions to the tubular lumen respectively and *plasminogen* activators mediate the proteolytic process in the migration of germ cell and its successor to the tubular lumen.

FACTORS MODULATING ANDROGEN EFFECTS

Certain factors such as growth factors, cytokines [9,12] and other hormones in the system modulate the metabolic and physiological effects of androgen in males.

Growth Factors and Cytokines

The growth factors and the cytokines are distinct from the classical endocrine hormones. They act only at the site of production, yet appear to be omnipresent. These factors are only involved in the intratesticular control of hormones that is very important to the spermatogenesis.[8, 9, 12,16]

The Sertoli cells produce local factors, growth factors and cytokines, which are also involved in the control of immune system activity, blood flow and energy metabolism of the testis. There also appears to be an intratesticular ultrashort loop feedback, so that the exogenous testosterone will override the effect of LH and inhibit testosterone production. Growth factors and cytokines are involved together with endocrine factor in testicular growth, development and differentiation. All these are probably under the control of different interrelated factors at different stages of life (foetus to adulthood).

The cellular growth, differentiation and death of testicular cells are interrelated and controlled by the growth factors and cytokines, in addition to the role of the endocrines.

Growth factors and cytokines are distinct from the classical endocrine hormones, as their synthesis is ubiquitous and their actions exclusively local at the site of action. Although these substances act locally with different mode of actions, they are potentially influenced by the target cell environment. In the autocrine mode, the factor is secreted and then binds to cell surface receptors on the secreting cells. In the paracrine mode, the factor is secreted by one cell type and acts on the neighbouring cell.[13, 15]

Inhibin and Activin: FSH appears to be one of the major regulators of inhibin production by Sertoli cells. Combined actions of activin and inhibin regulate activities of the Leydig cells. Activin inhibits, while inhibin stimulates LH-induced testosterone production. The inhibin thus reverses inhibitory action of the activin. Activin also affects the Sertoli cell activity by inhibiting FSH-stimulated aromatase activity (for estrogen production), and on the androgen receptors, while stimulating transferin secretion.[12, 13]

Epidermal growth factor (EGF): It has been implicated in the regulation of spermatogenesis through Sertoli cell regulatory factors like inhibin, activin or

insulin-like growth factor (IGF). It is also involved in pro-viding lactate to the germ cells. It is suggested that EGF may have a role in oligospermia in a diabetic patient.[12]

Mullerian inhibiting factor or *substance* (also known as MIF or MIS): It is a glycoprotein secreted by Sertoli cells that inhibits the Mullerian system in a male foetus. Persistent Mullerian duct syndrome with undescended testis and pseudohermaphroditism may be caused by mutation of its gene. Activin, EGF, transforming growth factors (TGFα and TGFβ) have immune-suppressive functions. However, in case of arrest of spermatogenesis, it is difficult to quantify their roles with present state of knowledge. Among the growth factors IGF, TGFβ and related peptides such as inhibin, activin, MIS, epidermal (EGF or TGFα), fibroblast (FGF), or nerve growth factors (NGF) are also identified.

TGFβ may have an important role to prevent activation of the specific functions of Leydig and Sertoli cells before puberty. It reduces hCG or LH-stimulated steroid hormone biosynthesis in Leydig cells and possibly exerts inhibitory actions on other endocrine or even local factors, such as IGF, that stimulate the Leydig cell function. Withdrawal of its inhibitory role may herald the pubertal spurt in Leydig cell activity.

Cytokines, such as tumour necrosis factor (TNF) and interleukin, inhibit spermatogenesis and reduce sperm motility. Inhibin and TGFβ reduce the proliferation of the spermatogonia, while IGF, EGF, TGFα stimulate development of pre-and postmeiotic germ cells in the adult.

Data from different laboratory experiments indicate that growth factors and cytokines are present in the male gonad from early foetal to adult life. In early foetal development of the male gonad, the c-kit/SCF (*stem cell factor*) system is probably involved in the germ cell migration from the yolk sac to the gonadal ridge as well as proliferation and survival of the cells involving the development of gonads.[9]

In the adult testis, two types of interactions between the local and the systemic factors probably take place. Gonadotrophins modulate the production and/or the action (via modulation of cell surface receptors) of the growth factors and cytokines. As these local growth factors generally control several testicular cell types, the gonadotrophins most probably extend their actions beyond their classical specific target cells (Leydig and Sertoli cells). These local factors may synergise or antagonise cellular response of gonadotrophins according to the local requirements.

Presence of large numbers of both enhancers and inhibitors of gonadotrophin action indicates superabundance of local modulators of FSH and LH. These factors are intermediate effectors acting between the reproductive hormones and the different testicular cell types. More specifically, because of *blood-testis barrier*, the local factors involved in the control of spermatogenesis are probably produced in Sertoli cells.

HORMONES

Prolactin

Prolactin is secreted by the lactotrophes of the anterior pituitary. It is controlled by dopamine sometimes referred to as prolactin inhibiting factor, and the thyrotrophin-releasing hormone acts as the stimulator of the prolactin.[1] However, the precise role of prolactin in males is not fully understood, but the secretion of adequate amount of prolactin seems to be necessary for normal testosterone production, whereas elevated prolactin is associated with depressed its production.

The negative effect of prolactin on testosterone production may be due to indirect inhibition of testicular functions secondary to changes at the hypothalamic-pituitary axis or through direct inhibitory action of prolactin on Leydig cells through prolactin receptors. The LH and prolactin exhibit synergistic effects at the level of Leydig cells.

Prolactin has thus a complex inter-relationship with the gonadotrophins, LH and FSH. In males with hyperprolactinaemia, the prolactin tends to inhibit the production of GnRH.[17,31] Besides inhibiting LH secretion and testosterone production, elevated prolactin levels may have a direct effect on the CNS. In individuals with elevated prolactin levels, who are given testosterone, libido and sexual function do not return to normal as long as the prolactin levels are elevated.[17] Mild prolactin elevation produces no symptoms, but greater elevation can reduce sperm production, impair sex drive, and cause impotence.

A few reports concerning its influence on spermatogenesis are contradictory. When hyperprolactinaemia was induced by giving 10 mg metoclopramide three times daily for 12 weeks, a five-fold increase of serum prolactin levels was observed. The semen volume and abnormal sperm

forms decreased, while spermatozoa velocity increased. On the contrary, no influence was noted on the number of spermatozoa per millilitre, the total number of spermatozoa, the percentage of motile spermatozoa, or the index of motility. According to these authors, hyperprolactinaemia seems to improve spermatozoal velocity and morphology, although direct effect of metoclopramide on these parameters cannot be excluded.[32,33]

Thyroid Hormones

The action of the thyroid hormone on the gonadal axis cannot be pinpointed; but, probably results from a combination of direct metabolic effects on the gonads, and excitatory and inhibitory effects operating through anterior pituitary hormones that control the sexual functions.

Hypothyroidism can cause poor semen quality, poor testicular function, and/or disturbances in sex drive. Incidence of male infertility due to a hypothyroid state is not more than 1%. Additional symptoms such as lethargy, intolerance to cold, and overweight, should be looked for before a clinical diagnosis is made, and confirmed by estimation of thyroid hormone profile. In hypothyroidism, initially the pituitary through its TSH would try to stimulate the thyroid gland to produce more T3 and T4. Eventually, thyroid would be unresponsive causing elevation of the pituitary-produced TSH level, with subsequent lowering of T3 and T4 levels. Elevated prolactin levels, frequently found with this disorder, may have its deleterious effect on the testicular functions; and occasionally, it may even lead to impotence.

Hyperthyroidism affects both pituitary and testicular functions with alterations in the secretion of releasing hormones and increased conversion of androgens to estrogens.[17,33-36] Some researchers believe that hyperthyroidism causes marked alterations of the gonadotrophin and prolactin (PRL) axis, and dramatically affects spermatic function. BioT (Bio-available testosterone) measurement is useful to identify hypoandrogenism in these patients in spite of the high concentration of total testosterone.[1,2] Dynamics of androgen and estrogen metabolisms and productions in patients with hyperthyroidism, change with increased conversion of testosterone to estradiol in hyperthyroid state.

Possible explanations are:

1. Anomalies of testosterone binding affinity in the serum of thyrotoxic subjects.
2. An increased serum level of sex hormone binding globulin binds testosterone and DHT, but not androstenedione. Consequently, the metabolic clearance rates of testosterone and estradiol decrease, but those of androstenedione and estrone remain within the normal range.
3. Metabolic clearance rate of estradiol-17 beta (MCRE2) is reduced, but the production rate of the estradiol-17 beta steroid increases above normal.
4. Conversion of estradiol-17 beta (CRE2E1) from testosterone increases in both hyperthyroid men and women. MCRE2 also decreases in women with hyperthyroidism, but the plasma concentration of estradiol and its production rate are unchanged from normal.[37]

Growth Hormone of Pituitary

The growth hormone of the anterior pituitary has an important role in the function of the male reproductive system. Relative growth hormone insufficiency, which may be caused by reduced reactivity to growth hormone-releasing hormone in pituitary growth hormone secretory cells, is strongly related to spermatogenic dysfunction.[38]

Adrenal Cortical Hormones

Several moderately active male sex hormones collectively called adrenal androgens (most important being DHEA) are continually secreted by the adrenal cortex, especially during foetal life. In addition, it secretes estrogen and progesterone. Some of the adrenal androgens are converted to testosterone. In normal physiology adrenal androgens have insignificant effects. However, its role in the early development of the male sex organs is accepted. Only a small fraction of androgen is provided by the adrenal cortex, but overactivity of the adrenal cortex can produce increased levels of adrenal androgens suppressing the pituitary.

In a *congenital adrenal hyperplasia* (CAH-discussed later) semen analysis shows a several abnormalities, hypertension and oedema. Cortisone replacement therapy (CRT) will lower the androgens and allow the pituitary to function normally, and thus corrects the spermatogenic abnormalities.[39-41]

Pineal Gland

Pineal gland is a small, cone-shaped projection from the top of the midbrain of most vertebrates. It

develops embryologically as an outgrowth of the brain. In humans, the structure develops till the seventh year, when it is slightly larger than a pea; thereafter, throughout life, small mineral particles, particularly calcium, may be deposited in the pineal body and is sometimes seen on the skull X-ray as calcified spots (Table 3.6).[1,2]

Table 3.6: Hormonal factors involved in the spermatogenesis *(Summary)*

1. LH stimulates the Leydig cells to produce testosterone. Testosterone acts directly and is essential for growth and cell division of germinal cells.
2. FSH stimulates Sertoli cells to organise full maturation from spermatids to spermatozoa.
3. Estrogen formed from testosterone by the Sertoli cells following their stimulation by FSH is also essential for spermatogenesis.
4. Sertoli cells also secrete ABP that binds both testosterone and estrogen, and carries these into the seminiferous tubular fluid making them available to sperms. Inhibin and activin from Sertoli cells regulate Leydig cell activity with FSH-regulating inhibin production.
5. Growth hormone is necessary for controlling background metabolic function of the testis. Growth hormone promotes early phases of spermatogenesis and in its absence as in pituitary dwarfs, the spermatogenesis is severely deficient or entirely absent.
6. Growth factors and cytokines are involved together with endocrine factors in testicular growth, development and differentiation. There is also an intratesticular short feedback loop. Exogenous testosterone overrides the effect of LH and inhibits testosterone production.
7. It is possible that gonadotrophins modulate the production and/or the actions (via cell surface receptor response) of all these local growth factors, while latter modulate the cellular response according to the local requirements.
8. There are additional roles from adrenal cortex and thyroid gland, but the role of pineal gland is still not fully understood.

Experiments have demonstrated that changes in the level of *melatonin* secreted from the pineal gland in seasonally breeding animals affect their reproductive cycles. Decrease in melatonin brought about by artificial lighting can prolong the breeding activity. Further studies in animals show that the melatonin is secreted almost entirely at night, and its secretion mostly ceases during the day. Light signals from the eyes pass to the hypothalamus, thence to pineal gland for its activation.

Role of pineal body in reproduction or sexual drive in human is still full of conjecture. However, both hyper-or hypofunctions of the testis seen in pineal body tumours secreting excess of pineal hormones notably melatonin, or in its atrophy caused by outside pressure effects by tumours of the surrounding structure, lend credence to its role in the human context. Melatonin affects the functions of other endocrine organs such as the thyroid, adrenals, and gonads. After passing either through the circulatory path or through the fluid in the third ventricle, melatonin reaches anterior pituitary for the control of the gonadotrophin secretion.[1,2]

ENDOCRINE CAUSES OF MALE REPRODUCTIVE DISORDERS

Endocrine defects account for 10% of male reproductive disorders.[17] They are mainly pretesticular causes in terms of anatomical origin. Obviously, they result from developmental or chromosomal defects and are summarised in Table 3.7. The reproductive endocrine status of the male is best established by measuring the hormones in the circulation or in the urine.

Table 3.7: Endocrine disorders of male reproductive system

1. Hypothalamic disease-Isolated gonadotrophin deficiency (Kallmann's syndrome)
2. Pituitary disease -
   a. Isolated LH deficiency ("Fertile eunuch")
   b. Isolated FSH deficiency
   c. Congenital hypogonadrotrophic syndromes
   b. Pituitary insufficiency (tumours, infiltrative processes, operation, radiation)
   e. Hyperprolactinaemia
3. Gonadal deficiency
4. Miscellaneous –
   a. Exogenous hormones (estrogen-androgen excess, glucocorticoid excess, hyper-and hypothyroidism)
   b. Haemochromatosis

HYPOTHALAMIC DISEASE

Kallmann's Syndrome

Kallmann's syndrome is a congenital hypothalamic dysfunction with isolated gonadotrophin (LH and FSH) deficiency. It occurs in both sporadic and familial forms, and is uncommon with an incidence of 1 in 10,000 men. It is second only to Klinefelter's syndrome as a cause of hypogonadism.[17,19] The syndrome, where men have varying degrees of sexual infantilism (prepubertal), underdeveloped testicles and no sperm production, is often associated with anosmia (inability to smell), congenital deafness, hair lip, cleft palate, craniofacial asymmetry, renal abnormalities and colour blindness. The underlying cause of this condition is absence of the hypothalamic

hormone GnRH. Since the hypothalamus fails to stimulate the pituitary adequately, FSH, LH, and testosterone levels are low; but if exogenous GnRH is administered, both LH and FSH are released from the pituitary, as anterior pituitary function is intact. The syndrome appears to be inherited either as an autosomal recessive or an autosomal dominant trait with incomplete penetrance. Distinguishing features of Kallman's syndrome are testes less than 2 cm in diameter and positive family history with the presence of anosmia. One should distinguish Kallman's syndrome from "fertile eunuch", where there is only an isolated LH deficiency causing low serum LH and low testosterone concentration. In an isolated FSH deficiency, which is rare, patients are normally virilised with normal testicular size and baseline levels of LH and testosterone. Kallmann's syndrome, is treated similarly to hypogonadotrophic hypopituitarism. There are reports that men afflicted with Kallmann's syndrome can achieve normal puberty and eventually become fertile, although the prognosis of this condition initially seems hopeless.

GnRH must be given in a pulsatile manner as continuous administration downregulates the pituitary. The initial dosage is 25-50 ng/kg every two hours through a small infusion pump. Although GnRH achieves a more physiologic pattern of gonadotrophin stimulation, its superiority has yet to be proved. Pituitary disease is not amenable to GnRH therapy alone, and combined treatment with hCG and HMG is mostly necessary. Individuals with the fertile eunuch syndrome (partial LH deficiency) may respond to hCG therapy alone.[17]

There are other congenital hypogonadotrophic syndromes causing secondary hypogonadism and a multitude of other somatic findings.

Prader-Willi Syndrome

This is an inherited secondary hypogonadism disorder. Affected male infants may show reduced muscle tone at birth. Some of the distinguishing features of Prader-Willi syndrome include small testes, diminished mental capacity and obesity. It is believed that the disorder is caused by a defective mechanism of GnRH secretion by the hypothalamus.[17,29]

Infertile men with Prader-Willi syndrome may benefit from hormone therapy. Specifically, blood testosterone levels may increase following hCG administration, and LH and FSH levels may increase in response to GnRH therapy. (See also Genetic and Chromosomal Disorders in Chapter 10).

Lawrence-Moon-Biedl Syndrome

This is also an inherited disorder. Like Prader-Willi syndrome, the hypogonadism in Lawrence-Moon-Biedl syndrome is caused by a hypothalamic deficiency of GnRH. This disorder is associated with a number of additional abnormalities, such as mental retardation, polydactyly, and retinitis pigmentosa, causing progressive loss of sight. [17,19, 29]

PITUITARY DISEASE

Pituitary insufficiency may result from tumours, infarctions, iatrogenic causes such as surgery and radiation, or one of the several infiltrative processes. If pituitary insufficiency occurs prior to puberty, growth retardation associated with adrenal and thyroid deficiency is the major clinical presentation. Pituitary tumour can also cause hypogonadism in a sexually mature male. Decreasing libido, impotence and infertility may occur years before symptoms of an expanding tumour such as headaches, visual abnormalities, or thyroid/adrenal hormone deficiency. Once an individual has passed through normal puberty, it takes a long time for secondary sexual characteristics to disappear unless adrenal insufficiency is present. The testes will eventually become small and soft. Low serum testosterone levels with low or low-normal plasma gonadotrophin concentrations clinch the diagnosis. Depending on the degree of panhypopituitarism, levels of corticosteroids, TSH and growth hormones will also be reduced.

Hypogonadotrophic Hypopituitarism

Hypogonadotrophic hypopituitarism is a spectrum of diseases with a complicated name that signifies low pituitary gland output of LH and FSH. Other stages of this disease are called isolated gonadotrophin defect and panhypopituitarism, in which the entire (*pan*) pituitary gland is affected. These diseases arrest sperm development and cause progressive loss of germ cells from the testes. In addition, functions of the seminiferous tubules and Leydig cells also deteriorate. If the condition persists for a long time, there will be cessation of sperm production. When

the disease is associated with a pituitary tumour, occasionally elevated prolactin from the tumour may contribute to occurrence of impotence.

Panhypopituitarism

Complete pituitary gland failure (panhypopituitarism) lowers the levels of growth hormone, adrenocorticotrophic hormone (ACTH) ,TSH, LH and FSH levels. Depending on the degree of panhypopituitarism, plasma corticosteroids as well as plasma TSH and growth hormone levels are reduced.[17] In this rare disease, there are multiple symptoms that include impotence, decreased sex drive, loss of secondary sex characteristics, and a normal or undersized testicle. Resultant hypothyroidism will cause weight-gain, intolerance of cold and lethargy. If the disorder began early enough, there may be dwarfism.

In prepubertal pituitary insufficiency, growth retardation, adrenal and thyroid deficiencies are major clinical presentations. Hypogonadism that occurs in a sexually mature male usually has its origin in a pituitary tumour. In addition, pituitary insufficiency can result from other less common factors such as pituitary damage from surgery or radiation. In these postpubertal individuals it takes a long time for the secondary sexual characteristics to disappear without adrenal insufficiency. However, erectile dysfunction (ED) and infertility may occur much before other symptoms such as thyroid or adrenal hormone deficiency, and pressure effects like headaches, visual abnormalities, etc. The testes will eventually become small and soft. Low serum testosterone levels with low or low-normal plasma gonadotrophin concentrations are the parameters used for establishing the diagnosis.

Replacement or supplementing of missing pituitary hormones, as well as thyroid and other hormones are needed to restore general health and vigour. At times, additional hCG administration is required to stimulate the testes to produce testosterone and to start spermatogenesis.

Delayed Puberty

Individuals with isolated pituitary growth hormone deficiency do not sexually mature until their mid to late twenties. Hormone supplements can make them look virile; but until they go through puberty, they would not be fertile. Pergonal and/or hCG injections can bring about the puberty; although if left alone, sexual maturity is sometimes achieved later.

Fertile Eunuch

In a fertile eunuch, virilisation (acquisition of adult sex characteristics) will be incomplete or moderately advanced. Occasionally, there may be gynaecomastia and normal spermatogenesis. Basic pathology is isolated deficiency of LH. Virilisation is inadequate because of insufficient androgen exposure. In some cases, there may be incomplete sexual maturation and testicular growth. Testicular biopsy would find evidence of sperm production and the potential for fertility. Plasma FSH levels are normal. The cause appears to be a partial gonadotrophin deficiency in which there is enough LH to stimulate testosterone production with resultant spermatogenesis, but there is insufficient testosterone to promote virilisation. Since the arrest of sperm production and low testosterone levels are caused by LH deficiency, administering hCG will raise levels of these hormones and stimulate sperm production. Only a few cases of isolated FSH deficiency (i.e. opposite of this syndrome) have been reported, where there will be adequate LH, but insufficient FSH leading to the arrest of spermatogenesis (Table 3.8).[2,17]

Table 3.8: Endocrine status in some of the disorders

	FSH	*LH*	*Testosterone*
1. Hypogonadotrophic hypogonadism	Low	Raised	Low
2. Primary gonad failure	Low	Raised	Low
3. Androgen receptor deficiency	Normal/	Normal/ Raised	Raised
4. Kallmann's syndrome	Low	Low	Low
5. Panhypopituitarism	Low	Low	Low

Hyperprolactinaemia

Hyperprolactinaemia can cause both reproductive and sexual dysfunctions. Men with severe hyperprolactinaemia frequently show mild hypogonadism and altered spermatogenesis, and many complain of loss of libido and erectile dysfunction.[1,33] Sometimes there may be galactorrhoea and gynaecomastia. A microadenomatous (less than 10 mm) or a macroadenomatous prolactin-secreting tumour of the pituitary gland is incriminated to cause excess secretion of prolactin. Patients with a macroadenoma usually present with visual field abnormalities and headaches. These patients have low serum testosterone levels, but basal serum levels of LH and

FSH are either low or low-normal. They reflect an inadequate pituitary response to depressed testosterone. These patients may produce particularly small amounts of ejaculate, due to abnormal function of the Leydig cells within the testes. In addition, pituitary insufficiency can result from other less common factors such as pituitary damage from surgery or radiation. The testis biopsy will show a lack of mature Leydig cells. In addition, men with postpubertal affection may have below-normal blood levels of corticosteroids, TSH, and growth hormone.

Hypoactive thyroid gland and certain medications such as metoclopramide (Maxolon), or tranquillisers like chlorpromazine (Largactil) or antidepressants such as amitriptyline and fluoxetine (Prozac) cause high prolactin level. These factors need to be excluded before a diagnosis of prolactinoma should be considered.[17, 19, 31, 39, 40]

Men with suspected tumours should undergo scanning by CT or MRI, and they should undergo laboratory testing of the anterior pituitary, thyroid and kidney functions. Since prolactin release is governed by the catecholamine dopamine, the medication of bromocriptine and other long-acting drugs will reduce prolactin levels and restore normal gonadal function in men with prolactin-secreting tumours. (See Chapters 10 and 11 for further details).

GONADAL CAUSES

Two important causes of gonadal deficiency are either due to chromosomal defects such as Klinefelter's syndrome, or gonadal toxicity from various external factors. These have been described in the Chapter 10 (Chromosomal abnormalities) and in Chapter 6 (Environmental factors in infertility).

MISCELLANEOUS

Other Hormonal Disorders

High blood levels of estrogen may be caused by a number of factors, including obesity, tumours of the adrenal cortex and testes (Sertoli cell tumours), and cirrhosis of the liver. Estrogen excess may lead to testicular failure because of inhibited pituitary gonadotrophin secretion. Similarly, androgen excess-caused by adrenal cortical or testicular tumours, CAH, or misuse of anabolic steroids may lead to secondary testicular failure and infertility (see also Congenital Adrenal Hyperplasia and Anabolic Steroids).

Exogenous Hormone Excess

Adrenocortical, Sertoli cell or interstitial cell tumours of the testes may all at times be estrogen producing. Hepatic cirrhosis is associated with increased endogenous estrogens. Estrogens act primarily by suppressing pituitary gonadotrophin secretion resulting in secondary testicular failure. Androgen excess can also suppress pituitary gonadotrophin secretion leading to secondary testicular failure. The current use of anabolic steroids by certain athletes may result in temporary impaired fertility. An androgen-producing adrenocortical or testicular tumour may cause endogenous androgen excess, but it is more evident in a CAH.

Sometimes exogenous glucocorticoid excess (caused by prolonged use of prednisolone) is noticed in the therapy of ulcerative colitis, asthma, or rheumatoid arthritis resulting in decreased spermatogenesis. The elevated plasma cortisone level depresses the LH secretion leading to secondary testicular dysfunction. Correction of the glucocorticoid excess results in improvement in spermatogenesis. Both hyper-and hypothyroidism can also alter spermatogenesis. Hyperthyroidism affects both pituitary and testicular functions with alterations in the secretion of releasing hormones and increased conversion of androgens to estrogens (discussed earlier).

One also needs to look into the stress and excessive exercise factors that may cause hormonal imbalance. When a woman is under a great deal of stress, use of serophene regulates the GnRH pulses from her hypothalamus and restore ovulation. It seems logical to assume that serophene may help a man in the same situation although the therapeutic effects are not certain.

Congenital Adrenal Hyperplasia (CAH)

CAH is an uncommon inherited disorder that may be associated with a lack of adrenal cortical enzyme (most commonly 21-hydroxylase). Hyperplasia of the adrenal cortex causes excessive production of adrenal testosterone, which in turn, inhibits the release of pituitary gonadotrophin. CAH can occur due to any of the enzymes involved in the steps of synthesis of testosterone from cholesterol, and is rare with an incidence of 1 in 20,000 to 64000 male births. The karyotype is 46 XY with the mutant gene X-linked.[41]

Early puberty and short stature (height) are hallmarks of CAH. However, CAH is difficult to

diagnose, since affected men often appear normal and sexually mature, without excessive masculinisation. Men with CAH often will show low/normal blood levels of adrenal steroids such as cortisol. In addition, they may have low or normal urinary levels of 17-hydroxycorticoid and high urinary levels of 17-ketosteroids and pregnanetriol (a byproduct of progesterone). The testicular tumours sometimes are detected in men with CAH.

In a CAH, a semen analysis shows a low sperm count, an increased number of immature sperm cells, sperms with long tapered heads, and low motility along with hypertension and oedema. Early onset of the disease may result in ambiguous genitalia at birth or reaching puberty at an early age. Adult onset may be characterised by infertility, high blood pressure, and/or water retention. Cortisone replacement therapy will lower the androgens and allow the pituitary to function normally and correct the spermatogenic abnormalities.

As a consequence of this disease, the production of androgenic steroids by the adrenal cortex is increased, resulting in premature development of secondary sexual characteristics and abnormal phallic (penile) enlargement. The testes fail to mature because of gonadotrophin inhibition and are characteristically small. In the absence of precocious puberty, the diagnosis is extremely difficult since excessive virilisation is difficult to detect in an otherwise normally sexually mature man. Careful laboratory evaluation is essential. Infertility caused by CAH is treatable with corticosteroids. Dexamethasone may be used to suppress adrenal secretion in men with CAH. In addition, glucocorticoid therapy may provide fertility benefits in men with CAH by increasing sperm output. Physicians have used corticosteroids in individuals with idiopathic infertility, but unless these abnormalities can be documented, steroid therapy has no place.

Haemochromatosis

Approximately 80% of men with haemochromatosis have testicular dysfunction. Hypogonadism may be secondary to iron deposition in the liver causing changes in the estradiol-testosterone conversion, or may be primarily testicular as a result of iron deposition in the testes. Iron deposits have also been found in the pituitary, implicating this gland as the major site of abnormality.

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APPENDIX 1

Testing of Endocrine Status in Male Reproductive Dysfunction

After taking a careful history and physical examination, if the physician suspects that the cause of the infertility has an endocrinal component with some specific signs of hormone imbalance, it is important to have measurement of the levels of reproductive hormones by radio-immunoassay (RIA).

Serum testosterone level usually is low in men with hormone-related hypogonadism (delayed sexual maturity) and with abnormal Leydig cell function in the testes. These men often have a history of reduced libido and impotence. Yet total testosterone levels can be misleading. For example, men with testicular failure (as in alcohol-related cirrhosis or Klinefelter's syndrome) may have testosterone levels within the normal range because of an increase in estrogen-induced, testosterone-binding globulin (TeBG). In such individuals, testicular failure must be confirmed by increased blood levels of FSH and LH, as well as by checking testosterone levels. The reproductive endocrine status of the male is best established by measuring the hormones in the circulation or in the urine. The hormones of interest are luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, estradiol and prolactin.

APPENDIX 2

Male Hypogonadism

Male hypogonadism, where there is absence of testosterone production by the testes, is of two types

- **Primary.** This type of hypogonadism — also known as primary testicular failure, originates from an abnormality in the testicles.
- **Secondary.** The testicles are normal, but function improperly due to lack of stimulation by the pituitary hormones. This type of hypogonadism indicates a defect in the pituitary gland failing to send the chemical messages to the testes causing impaired testicular function.

Common causes of primary hypogonadism:

1. **Klinefelter's syndrome.**
2. **Bilateral undescended testes (cryptorchidism) or Anorchia.**
3. **Bilateral Orchitis caused by Mumps.** If a mumps infection involving the testicles in addition to the salivary glands (mumps orchitis) occurs during adolescence or adulthood, long-term testicular damage may occur. This may affect normal testicular function.
4. **Cancer treatment.** Chemotherapy or radiation therapy for the treatment of cancer can interfere with testosterone and sperm production.
5. **Injury** either direct to parenchyma of the testes or to their vascular supply causing atrophy as in inguinal surgery**.**
6. **Normal ageing**
7. **Haemochromatosis.** Too much iron in the blood can cause testicular failure or pituitary gland dysfunction.

Common causes of secondary hypogonadism

1. **Kallmann's syndrome.** Defective development of the hypothalamus that controls the secretion of pituitary hormones.
2. **Pituitary disorders.** A pituitary tumour or other types of brain tumours located near the pituitary gland may cause testosterone or other hormone deficiencies.
3. **Inflammatory disease.** Certain inflammatory diseases such as sarcoidosis involving the pituitary gland can affect testosterone production and cause hypogonadism.
4. **Medications.** The use of certain drugs can affect testosterone production. Some psychiatric drugs and medications taken for heartburn or gastroesophageal reflux disease (GERD) may cause hypogonadism.

APPENDIX 3

Clinico-pharmacological use

Drugs Interfering with Estradiol Synthesis:

a. Inhibition of synthesis- by Aromatase inhibitor (Testolactone).
b. Blocking its effect on target cells by anti-estrogens.
   i. Clomiphene citrate- a mixture of two isomers.
   ii. Tamoxifen-which is devoid of any intrinsic estrogenic activity.

Effect is usually perceived after 4- 6 months of treatment. Patients, who do not present a normal increase in serm testosterone (ST) with anti-estrogen receptor antagonists may possibly benefit from gonadotrophin treatment. It is hypothesised that the hypothalamus–pituitary functions of these patients are impaired. Pure FSH (Metrodin) can be used in these cases. FSH is not likely to suppress pulsatile release of LH. Other available alternatives are products like Metrodin. It is the same kind of drug, but can now be injected under the skin. Another alternative is Pergonal, which needs deep intramuscular injection.

Androgen and its Derivatives

The safest testosterone delivery methods for adult men are intramuscular injections and absorption through the skin using patches and gels.[19] In USA oral testosterone is not very much in use as it is likely to worsen blood cholesterol levels and increase risk of blood clots, heart disease and liver disease. However, this is still in great demand in the subcontinent. As pure androgen is not absorbed orally, the structure of a synthetic androgen derivative is modified to facilitate its oral absorption. They cause decrease in aromatisation of estrogen and increase the concentration of 5αDHT.

1. Oral

1. Mesterolone (75 mg/day).
2. Testosterone undecanoate or Andriol or Nuvir (120 mg/day) – is preferred as it has minimal suppressive effect on the hypothalamus-pituitary function.

2. ***Injections***

   Intramuscular testosterone injections (testosterone cypionate and testosterone enanthate) are effective, safe and inexpensive. With the recommended biweekly administration, the level of testosterone varies between the doses with consequent fluctuations in symptomatic relief.

3. ***Patches***

   A. *Scrotal patch* (Testoderm). Thin scrotal skin is much more absorbent than other skin sites. The scrotal patch is applied in the morning and removed during bathing and intercourse. In a small percentage of people, itching and skin irritation can occur, but these are usually very mild and diminish with continued use.
   B. *Nonscrotal patch* (Androderm). The nonscrotal patch is applied to areas like back, abdomen, arm or thigh. Skin reaction is experienced in some patients.

4. ***Gels*** *(AndroGel)*

   The most recent advance is testosterone gel. The gel is rubbed on the upper arm, shoulder or lower abdomen. However, unlike the patches, there is always a possibility of transfer of testosterone to the partner, as there is no barrier. This can be conveniently taken care of by covering the area and avoiding skin-to-skin contact for approximately 5 hours after application.[19]

5. ***Striant*** *(buccal cavity)*

   Striant, a small putty-like substance, delivers testosterone through the natural depression above the top teeth, where the gum meets upper lip (buccal cavity). This product rapidly adheres to the gumline. As it is exposed to saliva, it softens into a gel-like form allowing testosterone to be absorbed directly into the bloodstream.

(See also Medical management of infertility –*Chapter 12*)

APPENDIX 4

Signs and Symptoms of Low Testosterone

1. Decreased beard growth.
2. Increased fat
3. Decreased muscle and bone mass
4. Irritability, mood swings, difficulty in concentrating.
5. Depression or fatigue
6. Breast enlargement or tenderness.
7. Erectile dysfunction

APPENDIX 5

Future Drugs for Male Infertile Conditions[42]

1. **Pure LH**- through genetic engineering a pure form of LH has been manufactured and it can be used for sperm production.
2. **Activin**-It is a potent activator of FSH secretion. In males, it is able to stimulate body to produce increased amount of FSH to help spermatogenesis. It stimulates ovulatory function in female.
3. **Relaxin**- It is a recently identified hormonal component of semen that enhances sperm motility.

CHAPTER

4 Erection, Orgasm and Ejaculation

INTRODUCTION

Creativity of nature is best exemplified by its most elaborate efforts in the human system to ensure continuation of the species. Continuance of progeny is a complex path of events that involves in males, arousal leading to erection, orgasm and eventually ejaculation. However, erection, orgasm, and ejaculation are three distinct processes controlled both by conscious mind and by involuntary neural responses. For the sperms to be deposited in the female reproductive system, all three actions must work in unison. Successive phenomenon of erection, orgasm and ejaculation are complex and cannot be brought about as simplistic as one can put a light on by pressing an electric switch. Erectile dysfunction (ED) or impotence encompasses abnormalities that stem out of the deviation of the normal run of events. It should be recognised that desire, orgasmic capability, and ejaculatory capacity might be intact even in the presence of ED. However, when these factors are deficient, they possibly contribute to inadequate sexual function.

The term *impotence*, has traditionally been used to signify the inability of the male to attain and maintain erection of the penis sufficient to permit satisfactory sexual intercourse over a period. However, use of this term has often created confusion and misinterpretation of results in clinical practice. Lack of a simple definition, failure to delineate precisely the problem being assessed, and the absence of guidelines and parameters to determine assessment and treatment outcome and long-term results, have contributed to this state of affairs by producing misunderstanding, confusion, and ongoing concern. The National Institute of Health Consensus Development Conference on Impotence convened in December 1992 has aptly recommended the use of the more precise term *erectile dysfunction*.[1,2] Erectile dysfunction (ED) is the repeated inability to get or keep an erection firm enough for sexual intercourse for some period. In actual fact, ED often is a part of the multifaceted process of inadequate male sexual function.

RELEVANT ANATOMY OF PENIS

The penis has a fixed or anchored, as well as a free or pendulous segments. Anatomically, there is an angle between the fixed and the free portions. It is comprised primarily of three cylinders of sponge-like loose trabecular meshwork of vascular and smooth muscular tissues (sinusoids) that get filled with blood to create an erection. Dorsal portion is composed of two corpora cavernosa and the ventral comprises of the corpus spongiosum, which enlarges into a knobby distal head called the glans.

The corpora cavernosa are cigar-shaped tubes of strong connective tissue filled with spongy muscle-lined cavities. Relaxation of vascular muscle causes inflow of blood and expansion of the corpora's inner sponge during erection. As a result, its wall or the tunica albuginea, is stretched tightly enough to produce rigidity. In addition to defining the shape of the erect penis, the tunica also has a key role in limiting blood outflow during erection. The paired

corpora are joined within the penis, but separate proximally at the base, where they diverge into right and left crura to get attached to the corresponding pubic rami. These two bony anchor points along with a midline suspensory ligament form a sturdy three-point anchor for the penis. The urethra runs dorsally under the corpora cavernosa, while most of the penile blood vessels and nerves run ventrally along the topside (Figs 4.1, Plate 2 to 4.4).

Unpaired spongiosum lies in the ventral groove of the paired cavernosa and contains the urethra for the passage of both urine and the ejaculate. The glans has a distal raised groove called corona. Two cavernosa actually indent the proximal portion of the glans with the distal ends extending beyond the corona of the glans (Fig. 4.5). Cavernosa share a common septum in the free portion with many perforations to allow free flow of blood.

A dense fibrous tissue called tunica albuginea surrounds all three spongy tissues. The tunica albuginea surrounding the corpora cavernosus is bilaminar (two layered). In the inner layer, the fibroelastic fibres are set circularly; and from it, originates the intercavernsoal pillars to fortify the support for the cavernous tissues. In the outer layer, the fibres are oriented longitudinally extending from the glans to the proximal crura and fibres are attached proximally to the inferior pubic rami, except at the 5 o′ clock and 7 o′ clock positions causing its vulnerability at these points. Corpus spongiosum does not have the outer layer or the intercavenosal pillars or struts. The outer layer of the tunical albuginea plays an important part in compression of the emissary veins lying between its two layers. The structures of corpora are almost identical except that the

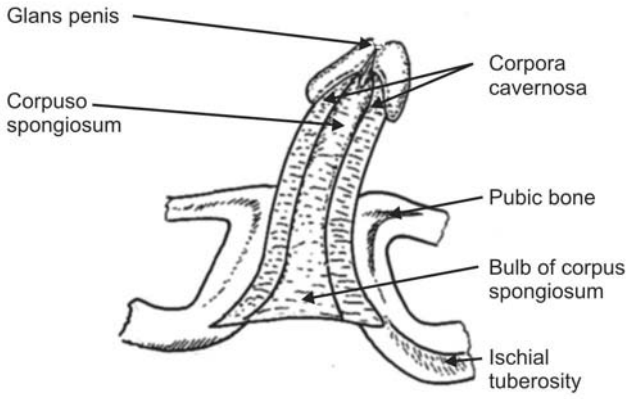


Fig. 4.2: Crura with bony attachments

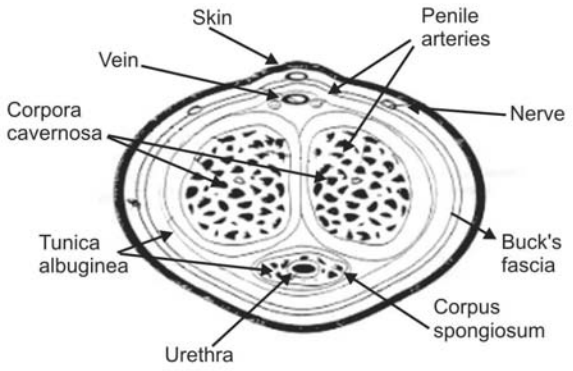


Fig. 4.3: Penile anatomy in cross-section

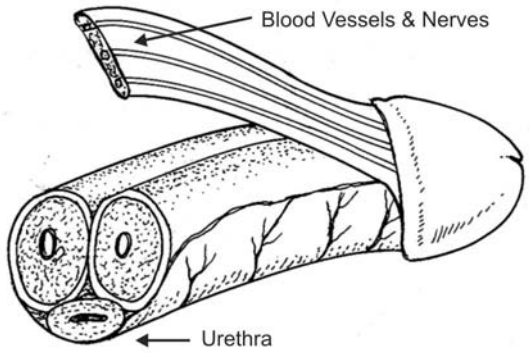


Fig. 4.4: Position of vessels and nerves

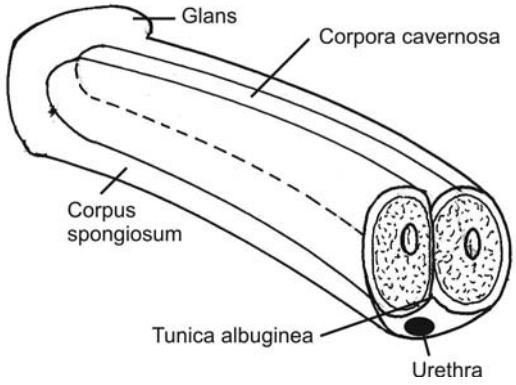


Fig. 4.5: Position of corpus spongiosum and cavernosum

spongiosum has larger sinusoids, and thinner and monolaminar or single-layered tunica albugenia. The tunica albuginea and the midline septum dividing the cavernosa can be identified in sonography by its high echogenicity against the cavernosa appearing as homogeneous low-level echoic areas.

These two major internal divisions of the penis, corpus cavernosum and corpus spongiosum have different functions. The corpora cavernosa, which can be likened to hydraulic cylinders, create structural

rigidity. The corpus spongiosum contains the urethra for the excretory channels for the urine and the semen with the glans providing the instrument for easy penetration of vagina.

All three cylinders are surrounded by another dense fascial sheath called *Buck's fascia,* which extends proximally to fuse with another condensed fibrous tissue or suspensory ligament of the penis (Fig 4.3). This is attached to the symphysis pubis above to provide the anchorage of the penis to the bony pelvis. The proximal portions of cavernosa diverge from each other and are attached to two ischiopubic rami. The proximal portion of spongiusum is attached to the urogenital diaphragm (Fig. 4.1, Plate 2) Ischiocavernosus muscles cover the proximal portions of corpora cavernosa and similarly bulbocavernosus muscle, which has an important role in expressing the last portion of the semen, covers the base of the corpus spongiosum. Superficial to all these structure lies the *Colles' fascia*. Functions of penile components are shown in Table 4.1.

Arterial Supply

Internal pudendal artery with minor contributions from the scrotal and epigastric arteries provides the main source for the arterial supply of the penis.[3] After coming out of the pudendal or *Alcock's canal* and at the level of urogenital diaphragm, it divides into four branches with the main trunk continuing as cavernosal or deep penile artery. Bulbar branch supplies the bulb of the penis or the proximal portion of corpus spongiosum. Urethral or spongiosal artery penetrates the corpus spongiosum and runs longitudinally within the spongiosal tissues supplying the urethra, corpus spongiosum and finally the glans penis. Spongiosal or dorsal penile artery runs beneath the Buck's fascia but outside the tunica albuginea. Between the two dorsal arteries lies the deep dorsal vein. Dorsal arteries terminate in short helical branches in glans penis, and on its ways send circumflex branches, which pass around and penetrate the corpus cavernosum, and ultimately ending in corpus spongiosum. Deep or cavernosal arteries are most important in erectile physiology. They run longitudinally in a relatively medial position in the corpora cavernosa. Terminal branches of the cavernosal arteries are tortuous and known as helicine arteries.[4,5] Each of the helical arteries divides and arborises into several subdivisions and eventually become an end artery. These end arteries pass directly into the cavernosus tissue to end in arteriovenous (AV) shunts that pass through the cavernous venous system without any capillary bed. However, Wagner[6] has demonstrated that some of these helical arteries may have communications with the arteries of corpus spongiosum and that of glans.

Venous Outflow

Venous drainage of penis can be divided into three sets of channels–superficial, intermediate and deep. Superficial dorsal veins drain the skin and the subcutaneous tissues superficial to Buck's fascia. Intermediate set runs deep to Buck's fascia, but superficial to tunic albuginea and comprises the unpaired deep dorsal vein draining the glans, part of the corpus spongiosum and corporus cavernosum. Emissary veins from both spongiosal and cavernosal tissues anastomose with the deep dorsal vein. There is also anastomosis between the superficial and the deep dorsal veins (Figs 4.6, Plate 2 and 4.7).

Deep set of veins provides the main drainage of blood from the corpora cavenosa. Proximal portions of corpus spongiosum drain through bulbar veins into

Table 4.1: Various penile structural components

1.	Tunic albuginea	a. Provides rigidity of the corpus cavernosum b. Participates in veno-occlusive mechanism
2.	Corpora cavernosa	a. Provide the pressurised vascular chambers in erection process. b. Support the corpus spongiosum
3.	Corpus spongiosum	a. Provides the pressurised narrow vascular chamber b. Provides space for the urethra to facilitate passage for the semen
4.	Glans	a. Provides for the sensory input to enhance erection process b. Its cone shape allows easy intromission into the vagina
5.	Smooth muscle in the corpora	Regulates the blood flow in and out of the cavernosal sinusoids
6.	Ishiocavernosus muscle	Pumps blood distally to facilitate and speeds up erection
7.	Bulbocavernosus muscle	Compresses bulb of the urethra to expel semen

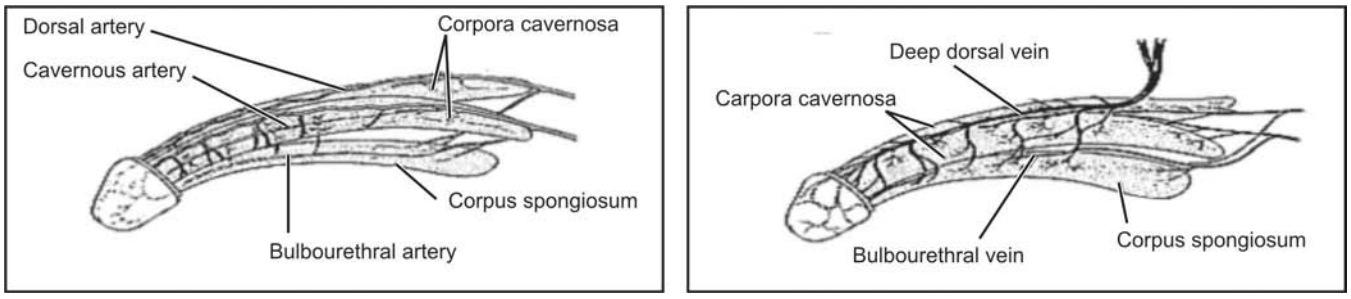


Fig. 4.7: Penile vascular supply

the deep veins of penis, while distal portions of these structures drain into the deep dorsal vein.

Broadly, cavernosal venous drainage originates in the tiny venules from the peripheral parts of the sinusoids lying just beneath the tunica albuginea. It then forms the subtunical venular plexus, which leads to the emissary veins lying between the two layers of the tunica. These emissary veins pierce the tunica albuginea to reach the circumflex veins that end in the deep dorsal vein. The deep dorsal vein provides the drainage for the majority of corpora cavernosa, while the crural veins drain the penis and proximal portion of the corpora cavernosa. The deep dorsal vein and the crural veins ultimately drain into the periprostatic venous plexus, and thence to the internal iliac venous system. The superficial dorsal veins drain distal corpora, skin and supporting tissues and end in the external iliac venous system.

Arteries and veins (Fig. 4.7) penetrate the long, filled cavities running the length of the penis-the corpora cavernosa and the corpus spongiosum. Erection occurs, when relaxed muscles allow the, corpora cavernosa to fill with excess blood fed by the arteries, while drainage of blood through the veins is blocked.

Penile Nerve Supply and Central Control

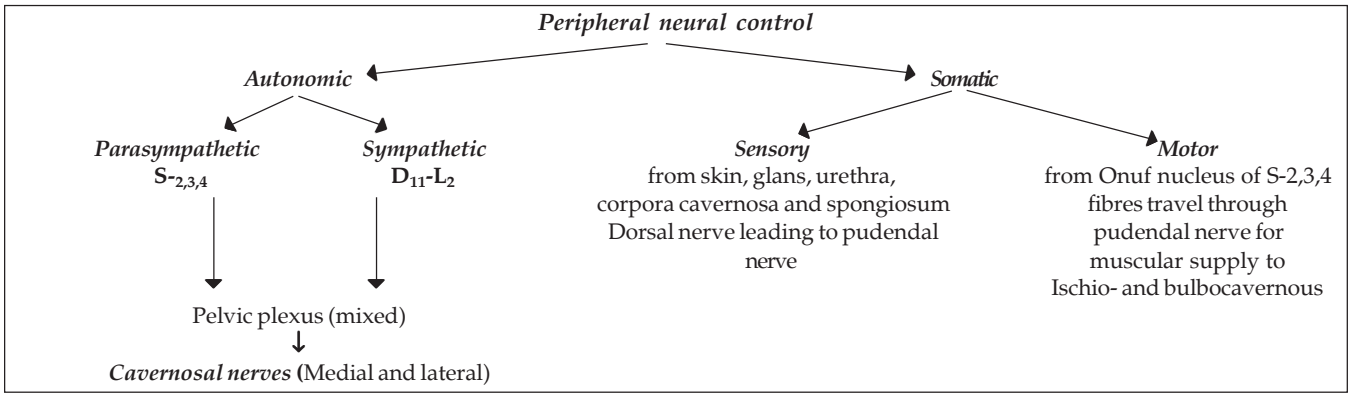
Penis has both autonomic (sympathetic and parasympathetic) and somatic nerve supplies besides a central control. In its most common form, erection is initiated by a central nervous system (CNS) event that integrates psychogenic stimuli (perception, desire, etc.) and controls the sympathetic and parasympathetic innervation of the penis. The medial preoptic area (MPOA), the paraventricular nucleus and the hippocampus act as the important coordinating centres for any sexual response.[7] Neural centres of the brain for sexual function and the peripheral nerve supply of the penis are shown in Tables 4.2 and 4.3.

Sensory stimuli from the penis are important in continuing this process and in initiating a reflex arc that causes erection under proper circumstances. This also helps to maintain erection during sexual activity. Needless to say, the neural input from the brain is extremely important. While the primary neural pathway to penis is parasympathetic from the sacral second, third and fourth segments ($S-_{2, 3, 4}$) of the spinal cord, the erection is not a cholinergic phenomenon. Although the sacral parasympathetic chain is the most important component in the reflexogenic

Table 4.2: Neural centres of the brain for sexual function (*Adapted from Campbell's Urology-7th edn*)[8]

A.	***Cerebral cortex***		***Function***
	1. Medial amygdala and stria terminalis	—	Sexual motivation
	2. Pyriform cortex	—	Inhibition of sexual drive
	3. Hippocampus	—	Penile erection
	4. Right insula and inferior frontal cortex, and	—	Visual arousal of sexual drive
	5. Left anterior cingulate cortex		
B.	***Hypothalamus***		
	1. Medial preoptic area (MPOA)	—	Integration of hormonal and sensory cues as well as recognition of signals from the opposite sex
	2. Paraventricular nucleus	—	Penile erection augmentor
C.	***Brain stem*** –Nucleus paragigantocellularis	—	Penile erection inhibitor
D.	***Midbrain*** - Para-aqueductal grey area	—	Relay centres for the relevant sexual stimuli

Table 4.3: Peripheral neural control of sexual function[8]



erection, the thoracolumbar pathway through synaptic communications[9] can compensate for its derangement caused by spinal cord injury.

PHYSIOLOGY OF ERECTION

The male erectile response is a vascular event initiated by neuronal action and maintained by a complex interplay between vascular and neurological, and perhaps humoral phenomenons resulting in a cascade of events. Erection of penis in simple terms consists of trapping pressurised blood within the confines of a limited space provided by the spongy corpora cavernosa. These blood-filled spaces relax and open up, allowing free inflow of blood leading to expansion of the chambers pulling the tunica albuginea tight. The tensed tunica albuginea makes the corpora hard (resistant to indentation) and rigid (resistant to flexion). Secondarily, it pinches off the veins (that normally let blood leave the chambers) trapping blood inside and contributing to the state of engorgement (see later).

The valves (actually flaps, according to some experts) that control the flow of blood, however, are opened and closed by nerves that run through the spinal cord to the brain. Activation by the nervous system causes a rapid increase in the blood flow into the penis. During erection, as blood flows into the penis, the holes in the spongy tissue in the penis get filled in with it. At the same time, flaps in the veins leading out of the penis enlarge, cutting off the outflow. Thus, more blood flows in than out, and the penis enlarges and becomes harder. Finally, veins in the penis are compressed from the increased pressure from the erection itself. In addition, the heart rate and blood pressure increase, the pressure of blood into the penis increases to maintain its hardness.

In simple terms, physiology of the erection and ejaculation is comparable to combination of *electrical* and *plumbing events*. The electrical system consists of the areas of the brain and spinal cord regulating male sexual response, and the peripheral nervous system. This electrical system provides the stimulus for the plumbing system that consists of a series of arteries and veins controlling the inflow and outflow of blood to and from the penis, and also to several accessory glands. Functioning of the plumbing and electrical systems of male reproductive organs are obviously complex and especially designed to produce synchronisation of the male sexual cycle. According to American Psychiatric Association, there are four phases of male sexual cycle (Table 4.4).

Table 4.4: Phases of the male sexual cycle

1. **Desire** (libido): Fantasies, thoughts, feelings
2. **Excitement**: Pleasure, erection
3. **Orgasm**: Emission and ejaculation
4. **Resolution**: Relaxation, refractory period

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th edn, 1994.[10]

Male sexual cycle begins with desire or arousal leading to a state of excitement or erection of penis. In a normal course of event, the orgasm and the ejaculation (see later) naturally follow, and the cycle ends with a refractory period or period of relaxation. There is a general tendency to consider arousal, erection and ejaculation as one event; but in reality, they are separate but interrelated and synergistic events. Any of these events can occur in the absence of the others.

Firstly, fantasies, thoughts, or feelings excite a man (*arousal*), and messages are transmitted to the portion of the brain dedicated to sexual response. This sexual arousal is often described in slang as: "*I'm turned on.*"

There is an erroneous perception that sexual arousal is always accompanied by an erection, but this is not necessarily the case at this stage. The experience of arousal sends signals to two tiny Cowper's glands or bulbo-urethral glands lying along the sides of the urethra (directly above a point behind the scrotum or the perineum) to go into action. This is outside the conscious control and an automatic result of sexually exciting stimuli.

These glands are primarily responsible for the production of glistening drops of a clear, slippery fluid sometimes called *pre-ejaculate* or *precum*, but known also in slang as "*ooze*". For most men, the first physical indication of sexual arousal is the appearance of a drop or two of this fluid at the tip of the penis even before an erection occurs. There is often a perception amongst many men that they are already starting to leak ejaculate as soon as the fluid or secretion from these glands appears at the tip of the penis; but more often than not, this is not the case. Main function of this fluid is to act as a lubricant for the tip of the penis to facilitate the penetration of vagina. High or alkaline pH of these secretions have an additional function to neutralise or to minimise the acidity of the vaginal secretion, thereby facilitating the passage of sperms in their path to fertilise the egg (see Chapter 2).

In the early stages of sexual arousal, the brain is flooded with natural chemicals called *endorphins*, with actions akin to drugs like cocaine. These chemicals are responsible for the man suddenly getting into a psychological feeling of well-being. His mental state gives a feeling that the sexual arousal is very enjoyable, and that its continuity is desirable.

To perpetuate nature's ultimate goal of continuing the species, it is important that sexually attractive mates elicit a response that maximises the chance of two individuals of opposite sex eventually to engage in sexual intercourse. The arousal mechanism works identically for gay males, but the object of the sexual attraction there, is of course another male. For most men, whenever there is a sexual excitement be it from reading a sexually explicit story, watching a sexually explicit movie or video, or watching nude female, it would elicit a pleasant feeling and production of pre-ejaculate. But, this does not necessarily mean that this state of mind would invariably lead to erection or orgasm on every occasion. Pre-ejaculate may, on occasion, contain some sperms, and a woman can become pregnant, even if actual orgasm and ejaculation do not take place. Although rare, such pregnancies are on record.

Excitement Phase

A penile erection is the culmination of first physiologic response to effective sexual stimulation (Fig. 4.8). Once full penile erection is attained, the excitment phase may vary according to the intensity of successful sexual stimulation. Some men can maintain penile erection for extended periods by carefully varying the intensity of stimulation. Erection may even be partially lost and subsequently be rapidly regained many times during an intentionally prolonged excitement phase[11] (Fig. 4.8).

Psychosensory diversions caused by a sexual stimuli like a sudden loud noise or an extraneous subject, or an obvious change in lighting, temperature, or attendant personnel may impair penile partial or full erection despite maintaining the somatogenic penile stimulation. After the penis apparently has achieved full erection during excitement phase, it undergoes a minor involuntary vasocongestive increase in diameter as the orgasmic (*ejaculatory*) phase approaches. A mottled reddish-purple colour due to venous stasis may occur in this pre-ejaculatory phase (*Plateau phase*) (Fig. 4.9).

Orgasmic Phase

The orgasmic phase ends with ejaculation or expulsion of semen into the vagina. The penile ejaculatory reaction (Fig. 4.10) is manifested by regularly recurring involuntary but coordinated contractions of the muscle groups-sphincter urethra, bulbospongiosus, ischiocavernosus, and transverse superficial and deep perineal muscles. Expulsive penile contractions start at intervals of 0.8 second. However, after the first three or four major expulsive efforts, the penile contractions get rapidly reduced in frequency and its expulsive force naturally decreases.

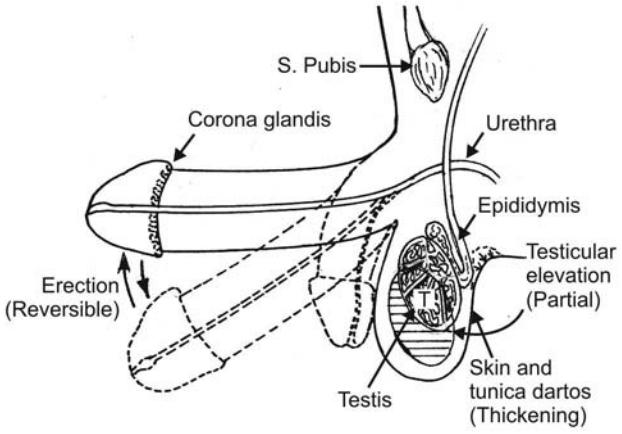


Fig. 4.8: Excitement phase

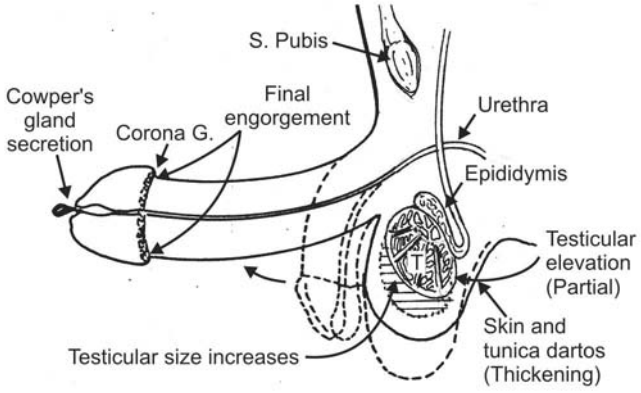


Fig. 4.9: Plateau phase

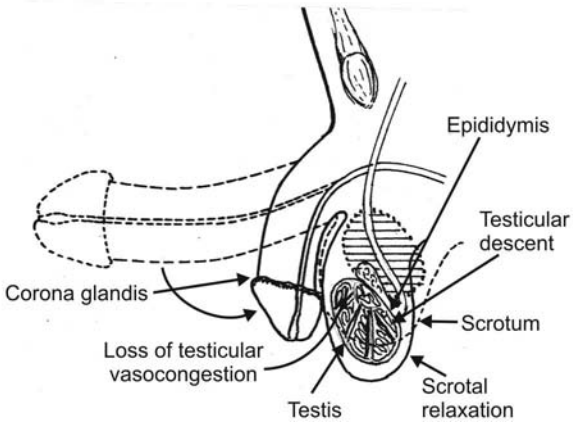


Fig. 4.11: Resolution phase (penile detumescence and involution)

Minor contractions of the penile urethra continue for several seconds in an irregularly recurrent manner with little expulsive force, ejecting only a minimal amount of seminal fluid. In the end the intervals the contractions extend to several seconds till it stops.

Resolution Phase

In the resolution-phase penile tumescence gradually decreases in two distinct stages (Fig. 4.11). The primary stage of penile detumescence occurs early in the refractory period of the resolution phase and reduces the size of the penis at its full erection state to approximately 50 percent of its size in the unstimulated flaccid state. This primary or initial stage of penile detumescence usually occurs fast. The secondary stage of the penile involution ultimately restores the penis to its normal unstimulated size. However, it may be an extended lasting well past the refractory period of the resolution phase.

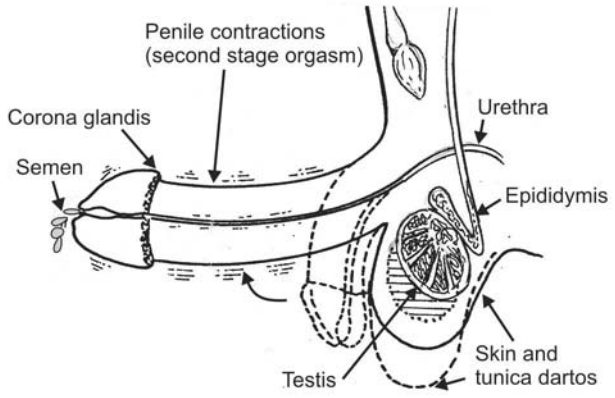


Fig. 4.10: Orgasmic phase

The primary stage of penile involution usually is prolonged, when the excitement or plateau phases of the particular sexual cycle have been extended by direct intent. Many males learn to restrain or delay their ejaculatory reaction until their sexual partner is satisfied. If the penis is taken out of the vagina immediately following an ejaculation, full detumescence is accomplished much more rapidly than if the penis in the postejaculatory phase remains inside the vagina.[11]

Chemical Transmitters for Erection

There are numerous chemical transmitters (Table 4.5) involved in the penile erection phenomenon. This includes acetylcholine (Ach), prostaglandins, epinephrine, norepinephrine and nitric oxide (NO), but the precise role and influence of the hormonal milieu on the erection is still not known.[12] Real explanation eludes the scientific world and it remains controversial, whether erection is a neurologically driven vasoconstriction or a passive anatomical obstruction to the penile venous outflow that determines the events involving shunt mechanism of filling of corpora cavernosa with blood and control of venous effluent (Fig. 4.12).

Table 4.5: Various chemical transmitters for erection

1. Acetylcholine
2. Prostaglandin
3. Serotonin
4. Endothelin
5. Norepinephrine
6. Nitric oxide (NO)
7. Dopamine
8. Opioids
9. Oxytocin

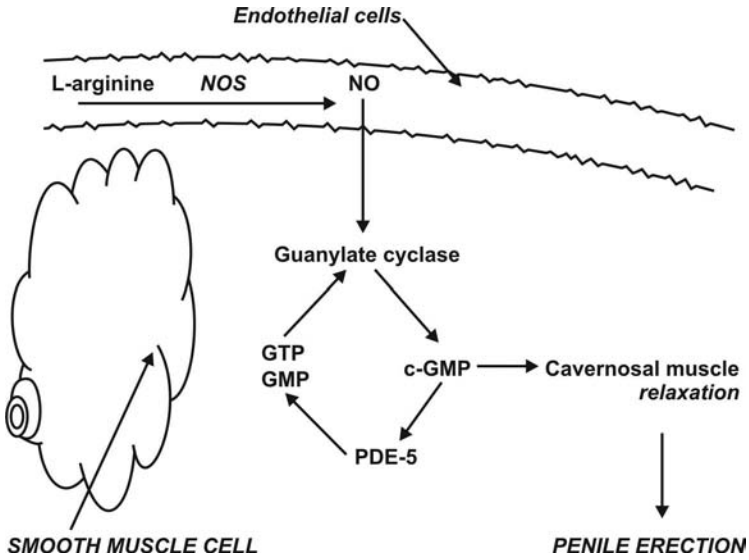


Fig. 4.12: Nitric oxide-cGMP mechanism of action in corpus cavernosum, smooth muscle relaxation and penile erection.
(Some actions take place in the endothelial cells and others in the smooth muscles)

Acetylcholine released by the parasympathetic nerves is thought to act primarily on the endothelial cells to release a second nonadrenergic noncholinergic signal that relaxes the trabecular smooth muscles. It appears that the vasodilator response mediated by the release of Ach or polypeptide mediators cause relaxation of arteriolar resistance and sinusoids. Acetylcholine in the human erectile tissues cause penile erection by parasympathetic inhibition of the adrenergic neurons and help to release the NO from the endothelial cells. Alpha-adrenergic fibres in the corpora, endothelium of the smooth muscles and prostaglandin F_2 help in flaccidity.[13, 14]

Cholinergic effect on the endothelial cells probably results in production of EDRF (endothelium-derived relaxing factor), which in turn, is believed to cause relaxation of the smooth muscle lining of the sinusoidal spaces through NO mechanism. When the psychoerotic stimulus subsides or lessens, the smooth muscle relaxation and dilatation of the penile blood vessels diminish. Sinusoids then shrink resulting in less compression of the emissary veins, thus allowing venous outflow to occur unimpeded, with resultant flaccidity.

Acetylcholine is thought to decrease sympathetic tone. When the trabecular smooth muscle relaxes and helicine arteries dilate in response to parasympathetic stimulation and decreased sympathetic tone, increased blood flow fills the cavernous spaces, increasing the pressure within these spaces, so that the penis becomes erect. As the venules are compressed against the tunica albuginea, penile pressure approaches the arterial pressure, causing rigidity. Once this state is achieved, subsequent arterial inflow is reduced to a level that matches venous outflow, sustaining erection.

There are many dopaminergic systems in the cell bodies in the hypothalamus, midbrain, etc. Dopinergic neurons initiate erection through its oxytocinergic neuronal effect. Oxytocin is a neural hormone secreted by the neurons in circulation. Oxytocin activity induces penile erection with calcium probably acting as the second messenger. Serotonin (5-HT) is an inhibitor for sexual drive. Norepinephrine with its central action has a positive effect on the sexual function. Opioids in small amounts enhance, while in large amounts exert inhibitory effect on sexual activities.[8]

Nitric oxide released by the endothelial cells, and possibly from its neural origin, is currently thought to be this nonadrenergic noncholinergic transmitter; but this has not yet been conclusively demonstrated to the exclusion of other potentially important substances (e.g. vasoactive intestinal polypeptide). The relaxing effect of NO on the trabecular smooth muscle may be mediated through its stimulation of *guanylate cyclase* and the production of *cyclic guanosine monophosphate* (cGMP), which would then function as a second messenger in this system (Fig. 4.12).[15,16]

Role of Nitric Oxide in Erection

The NO-cGMP pathways play an essential role in initiating cavernosal smooth muscle relaxation and

erection.[16] Immunohistopathological studies have demonstrated presence and localisation of NOS (*nitric oxide synthase*) throughout the male genitourinary tract. [17] It is generally accepted that NO is the principal agent responsible for relaxation of penile smooth muscle. The NO is derived from two principal sources: directly from nonadrenergic noncholinergic parasympathetic nerves and indirectly from the endothelium lining cavernosal sinusoids and blood vessels in response to cholinergic stimulation.[18]

The NOS catalyses the production of NO from its substrate L-arginine. There are two types of NOS. The constrictive NOS is dependent on the calcium ions, and is commonly seen in the endothelial cells and neurons, while the inductible NOS, which is independent of calcium ions, is primarily found in macrophages, but is expressed after cytokine induction. With ageing, NOS in corpus cavernosum and in the nerve fibres, and the penile NOS proteins decrease in quantity.[19-21]

The penile NOS activity can be achieved possibly by three methods–increasing the NOS substrate concentration, stimulation of NOS activity through related pathways or blocking of the endogenous NOS inhibitors. Long-term oral administration of high doses of L-arginine have been found to have considerably improved the erectile dysfunction of ageing rats by elevating intracavernosal NOS substrate. Evidence supporting the second method is based on the discovery that the main stimulating pathway for NOS, the NMDA (N-Methyl D-Aspartate) receptor is present in the penis. However, NMDA receptor antagonists *in vitro* behave unpredictably possibly because of existence of an unknown NO-independent pathway. A protein inhibitor of NOS (PIN) that is expressed in the corpora cavernosa has been detected. Penile NOS content may be pharmacologically increased by induction of NOS expression, or by gene therapy with NOS cDNA as evidenced by improved erectile response on the gene transfer of PiNOS into the rat corpora cavernosa.[22]

When a man is sexually aroused, the nerve ending and penile cells release NO, which activates certain enzymes that cause an increase in cGMP levels. The cGMP allows tissues around the penile arteries to relax. When the tissues relax the arteries dilate causing more blood to flow. Phosphodiesterase (PDE)-5 is an enzyme that binds to cGMP and makes it ineffective. An erection weakens if cGMP is made ineffective too fast. Its effect on smooth muscles of the penis is then reversed.

Chronic ischaemia is, therefore, associated with fibrosis but also with NO-cGMP. The sensitivity of the alpha-adrenoceptors on the smooth muscle cells increases with ageing. The atherosclerosis of the penile vessels that occurs with ageing causes a decrease in penile oxygen tension.[23]

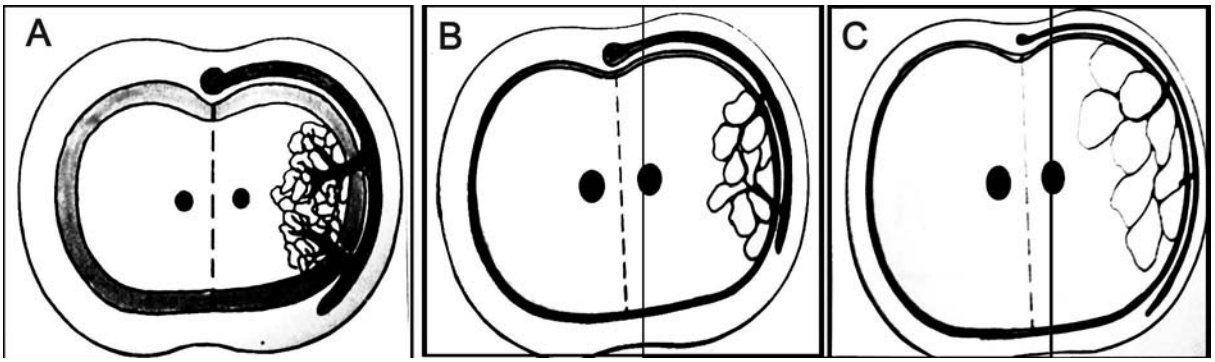
Erection is primarily initiated from an erotic thought (mental stimulation) or when the penis is touched (sensory stimulation) or both. Erection has three types of origin–psychological, reflexogenic and nocturnal.

Psychogenic erection has its origin in the brain from where the impulse passes on to spinal centre at the thoracolumbar and sacral segments of the spinal cord to set up the erectile process.

Reflexogenic is caused by the tactile stimulus from the glans reaching the spinal centre to initiate the reflex activity. Nocturnal is initiated during rapid eye movement (REM) sleep phase in the pontine reticular formation, where the cholinergic neurons are activated, while the adrenergic neurons remain silent. This impulse then passes on the spinal centres to cause erection.[81]

Impulses from the brain and local nerves cause the muscles of the corpora cavernosa to relax, allowing blood to flow in and fill in sinusoidal spaces. The blood creates pressure in the corpora cavernosa, making the penis expand. Consequent rigidity completes the process of erection.

The tunica albuginea helps to trap the blood in the corpora cavernosa, thereby sustaining erection. As the sinusoidal distension progresses, the efferent venous channels are compressed against the resilience of tunica albuginea. The obstruction to the venous outflow allows intercavernosal pressure to rise above the arterial pressure to produce penile rigidity. The increase in the volume of blood in the cavernous tissues is determined by the length and circumference of the tunica albuginea that can be stretched. It is singularly important that for the initiation and sustenance of the erection amount of blood entering the cavernous tissues must exceed the outflow to ensure increased blood volume in the cavernous tissues. Concomitantly, to sustain the erection, this excess blood needs to be prevented from leaving the penis by cutting the outflow off. From a normal flaccidity to rigidity, three stages are recognised as depicted in Figures 4.13A to C and 4.14A and B, Plate 2. It is evident from studies of several workers mentioned above that an active mechanism for this



Figs 4.13A to C: Three stages of penile erection. (A) Normal flaccid position–penile arterial tree and trabecular smooth muscles are contracted. Emissary veins are open allowing free outflow of blood. (B) Stage of tumescence–relaxation of arteries and intracavernosal smooth muscle allowing sinusoidal dilatation. (C) Stage of rigidity–expanded sinusoid compresses against subtunical venous plexus and compression of emissary veins due to stretching of tunica. (Courtesy: Dr S Thulkar)

reorientation must exist and appear to be under neurological control.

Friction from manual and vaginal stimulations of penis send continuous and repetitive signals to the brain to maintain the erectile state of the penis. Erection also stimulates orgasm and ejaculation. While psychological factors interfere with erection; once orgasm is reached, automatic reflex actions run their course. When muscles in the penis contract to stop the inflow of blood and open outflow channels, the erection is reversed. In the absence of an erection, the inflow of blood and the outflow is maintained in balance and the penis remains flaccid.

Erection can be elicited by the so-called reflex erection as seen in patients with spinal cord injury, or can be caused by psychogenic stimulation. Numerous sexual stimuli are processed by the brain and transmitted to the penis via the nervous system. Normally, arousal is followed by erection.

There is a general unanimity amongst most physiologists that decrease in corporal vascular resistance and concomitant increase in the arterial inflow into the corpora is mainly responsible for the phenomenon of penile erection. The pathophysiology of structurally based corporeal veno-occlusive dysfunction is related to elevated corporeal connective tissue content.[24]

There is experimental evidence that the increased flow through the internal pudendal artery does not produce erection. This led to the conclusion that without an active neurological mechanism, flow of blood into corporal spaces erection cannot be achieved. On the other hand, erection achieved in cadavers by direct perfusion of cavernous tissues would indicate the absence of constriction of veins in the erection process. However, passive venous occlusion may well contribute to the vascular mechanism of erection as proved by Xenon washout technique before and after visual sexual stimulation. Wagner [61] interpreted definite reduction of venous outflow during tumescence as an evidence of some sort of venous regulatory mechanism. Incidentally, arterial inflow substantially increases during erection. While the normal blood flow in the internal pudendal artery is 10 ml/min, there is a 5 to 6-fold increase during erection.[25] Marked reduction of penile vascular resistance could provide a plausible explanation of the absence of change in the aortic pressure during erection.

At present, there are two major views regarding the pathophysiology of ED.[26] In the first hypothesis, the oxygen tension-dependent changes in the penis during erection are proposed to impact corpus cavernosum structure by inducing various cytokines, vasoactive factors and growth factors at the two different oxygen tensions (flaccidity and erection), which, in turn, alter smooth muscle metabolism and connective tissue synthesis. Decreases in the corpus cavernosum smooth muscle/connective tissue ratio have been correlated with an increased likelihood of diffuse venous leak and a failure of the veno-occlusive mechanism in prospective patient studies. Evidence for such a hypothesis incorporates nocturnal penile tumescence and circadian changes in oxygenation as important in maintaining erectile health.[27-31]

The alternate hypothesis proposes that ED is the result of a metabolic imbalance between relaxatory and contractile processes within the trabecular smooth muscle, where the contractile processes predominate. Consequently, this hypothesis postulates that the therapy can be accomplished by drugs that shift this balance towards vasodilatation, or, by

gene therapy to supplement the deficient components favouring smooth muscle relaxation.[19-22]

Regulator and Modulating Factors

Many independent factors converge on the modulation of corporal smooth muscle tone. Neuronal and local neurotransmitter produces effects via gap junction, potassium channels, and calcium channel. The NO/cGMP mechanism as well as cyclic aminomonophosphate (cAMP) have an important role in mediating the corporal smooth muscle relaxation necessary for erectile function.[32] Moreover, it is affected by hypercholesterolaemia, atherosclerotic vascular occlusive disease, smoking, veno-occlusive dysfunction and cavernosal fibrosis.[33]

Animal studies and clinical experiments have shown that the androgens are essential in the maintenance of NO oxide-mediated erectile activity. The results indicate that in men with erectile dysfunction low free testosterone may correlate independently of age with the impaired relaxation of cavernous endothelial and corporeal smooth muscle cells to a vasoactive challenge.[34-36]

The structure of the corpus cavernosum is altered by the defect in the connective tissue synthesis induced by the transforming growth factor or TGF-beta-1. This has been evidenced by the dose-dependent decreases in percentage of corporal smooth muscle after injection of recombinant human (rh)-TGF-beta-1 impregnated alginate microspheres into the corpus cavernosum.[37]

The modulators of erectile function in the penile tissue such as NO and endothelin-1 (ET-1) and growth factors such as TGF-beta-1 and vascular endothelial growth factor (VEGF) undergo age-related changes. This is in evidence in the immunohistochemical studies in the old population of rats showing a decreased expression of endothelial nitric oxide synthase (eNOS) protein and an increased staining for ET-1. Quantitative analysis also revealed decreased levels of TGF-beta-1 and VEGF.[38-40]

Nature of regulatory mechanism that directs the blood flow either into efferent venous system during flaccidity or into the trabecular spaces of corpora cavernosa during erection, establish the presence of some sort of intraluminal control of penile arterioles. Microscopic studies of penile vasculature structure by Conti (1952)[41] revealed presence of intraluminal intrusions or projections described as penile pads, cushions or pollsters. These muscular pollsters known as *Ebson's pad* in both the efferent and afferent vasculature of corpora probably act as the control for the inflow and distribution of blood flow.

Conti postulated a shunting of arterial blood to the venous return during flaccidity. During active contractions of cushions or *polsters*, AV shunts would close and blood would be trapped in the cavernous spaces.[14] However, some investigators doubt the role of these pollsters, as they are also present elsewhere in the body, yet only function in the penile arterioles. Pollsters are also absent in a newborn and in patients receiving estrogen.

Deysach[42] contradicted their existence and opined that erection is produced by a series of sluice valves in corpora cavernosa. One can thus draw a conclusion on the existence of some active mechanism played by the pollsters or a sluice mechanism under probable neurological control.

Parasympathetic input allows erection by relaxation of trabecular smooth muscle and dilatation of the helicine arteries of the penis. This leads to expansion of the lacunar spaces and trapping of blood by compressing venules against the tunica albuginea, a process referred to as the corporal veno-occlusive mechanism. The tunica albuginea must have sufficient stiffness to compress the venules penetrating it so that venous outflow is blocked and ensure sufficient tumescence and rigidity. Constriction of the trabecular smooth muscle and helicine arteries induced by sympathetic innervation makes the penis flaccid, when the arterial blood pressure in the cavernosal sinuses of the penis nears the venous pressure.[19] There is also a role of the intercellular communication channels called gap junctions for the synchronised and coordinated erectile response. Any malfunction of these structures alters the smooth muscle activities.[43,44]

Erectile Failure

The underlying pathology of erectile failure or impotence in simple terms is a result of the failure of the plumbing mechanism. Either the blocked blood vessels do not allow rush of blood to get into the penis, or there is a leakage of blood from the penis into the veins around the penis.

The valves or the flaps controlling the veins that must be shut off at the time of erection may leak and cease to prevent the complete outflow of blood as a man ages. The blood flow into the penis may also be restricted, as with age the main penile artery may be filled with sludge reducing blood inflow.

Smoking may also be a contributory factor, as it has a role in the buildup of sludge in other portions of the circulatory system. Nicotine not only decreases arterial blood flow, but it also blocks cavernosal smooth muscle relaxation.[45] Use of alcohol may decrease the ability of the nervous system to close off the necessary valves and thus inebriated men often fail to achieve or to maintain erection.

Patients with spinal cord injuries are frequently unable to attain erection, because the nerves that control the valves in the veins and arteries have been severed. Consequently, these valves cannot be opened and closed, making an erection impossible. Normally, the cerebral impulses pass through sympathetic (inhibiting release of norepinephrine), parasympathetic (releasing NO and acetylcholine) and somatic (releasing acetylcholine) pathways to produce erectile response. In a sacral cord lesion, the cerebral pathways no doubt inhibit the norepinephrine release, but the NO and acetylcholine can still be released through synapses and postganglionic parasympathetic and somatic neurons.[70] Bicycle and other injuries to the perineum can be dangerous as the main artery controlling blood flow to the penis may be damaged, making a firm erection difficult or even impossible.[46,47]

Autonomic dysfunction of the penile vascular innervation or degeneration of penile smooth muscles is the most likely cause of the venous leakage in diabetic patients, where more often than not the spontaneous cavernosal activity is absent.[48]

Erectile dysfunction has been classified as psychogenic or organic. Until the early 1970s, this was believed to be primarily a psychological disorder. The treatment usually consisted of empiric testosterone administration and psychotherapy that were often ineffective.[3] An organic ED is different from a psychological one, and is classified based on the penile components that are involved in erection (neurogenic, arteriogenic, endocrinologic or cavernosal) (See Tables 4.6 and 4.7).[8,49]

Table 4.6

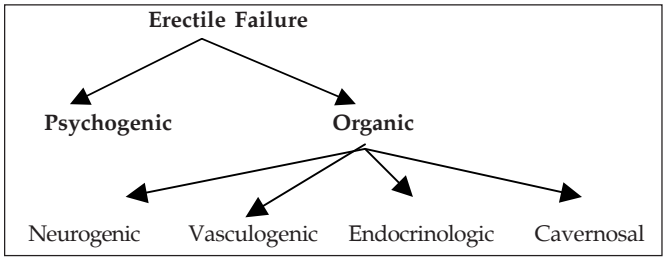


Table 4.7: Organic erectile failure

1. ***Neurogenic*** : Lesions in the brain, spinal cord, cavernous and pudendal nerves, and smooth muscle receptors caused by deficiencies in the sensory, motor, autonomic neural pathways or in the neurotransmitters.
2. ***Vasculogenic*** :
   a. Arteriogenic : Hyperlipidaemia, hypertension, pelvic and perineal trauma, pelvic irradiation, smoking, diabetes.
   b. Venogenic or cavernosal: Fibrotic lesions causing insufficient smooth muscle relaxation, degenerative changes like peyronie's disease, veno-occlusive disorders.
3. ***Endocrinologic***: Hypogonadism from various causes, hyperprolactinaemia, hyper- and hypo- thyroidism, diabetes.
4. ***Drug induced***: Antipsychotic and antidepressant drugs (interfering with 5-HT, noradrenergic or dopanergic pathways), alcoholism, cimetidine, etc.
5. ***Miscellaneous***: Ageing, systemic diseases.

Bancroft (2000)[50] postulated that normally there is a balance between the excitatory and inhibitory impulses within the CNS. Psychogenic erectile failure is probably a result of either direct inhibition of the spinal erection centre by the brain or due to excessive sympathetic tone preventing smooth muscle relaxation that is responsible for erection phenomenon.

Among the organic types, the neurogenic ED may be the most common, and quite a few cases are due to probable deficiency of neurotransmitters seen in many diseases and conditions.[51] Testing the nocturnal erections perceived during sleep can be used for monitoring an organic dysfunction, where the numbers of such erections are few. The nocturnal penile tumescence can differentiate organic and psychogenic erectile dysfunction more precisely with an exception that the recording parameters cannot distinguish subgroups with a vascular cause of ED.[52]

However, strict differentiation of two varieties of ED is made difficult, as often they are coexistent. Men with organic dysfunction may have a psychological overlay stemming from their earlier inability to perform sexually. This phenomenon of psychological factor is often referred to as *performance anxiety*. Performance anxiety is a state, where subconscious memory of previous failure to perform becomes singularly the most important incriminating factor in subsequent failure to achieve adequate erection to complete the sexual act.

Erectile dysfunction, when present from the outset of man's life, is described as primary. In contradistinction, when it develops later in life, it is termed secondary.

Erectile dysfunction is estimated to have an impact more than 150 million men every year worldwide.[26]

For some men, erectile function may not be the best or the most important index of sexual satisfaction, but for many, it creates mental stress that affects their interactions with families and associates. In spite of many advances in both diagnosis and treatment of ED, its various aspects remain poorly understood by the general population and by most health care professionals. Erectile dysfunction still remains a taboo in the subcontinent, where public has often been shielded from the advanced knowledge on the subject. Even in the Western countries, lack of communication with the public has effectively compounded the problem further.

Erectile dysfunction is often assumed to be a natural sequel to the ageing process, to be tolerated along with its other associated conditions. This assumption oversimplifies the actual state of affair as for some elderly, ED may occur as a consequence of specific illnesses or medical treatment for certain illness, resulting in fear, loss of image, self-confidence and depression.[53]

Many men with diabetes mellitus may develop ED during their young and middle adult years.[54] Although the overall incidence of ED in the general population between the ages of 40 and 70 years is 52%, men with diabetes mellitus have impotence at an earlier age and with a significantly higher prevalence, ranging as high as 75%. Physicians, diabetes educators, patients and their families are sometimes unaware of this potential complication.[55] Underlying causes of ED in diabetes are shown in Table 4.8.

Table 4.8: Causes of erectile dysfunction in diabetes (see chapter 5 as well)

1. Diabetic autonomic neuropathy in the afferent pudendal pathway causing altered perception of the glans due to loss of vibratory sensation.
2. Vascular changes in the penile arteries causing either deficient blood flow or degeneration of penile smooth muscles to allow venous leakage or both.
3. Gonadal dysfunction.
4. Accompanying depletion of NOS and NO c′ -GMP in corpus cavernosum.
5. Accompanying dyslipidaemia negatively affects blood circulation in the penis.
   P.S. *In addition ejaculatory dysfunction contributes to the erectile failure in diabetes.*

Whatever the incriminating factors, reticence of patients and inadequate knowledge of most health care providers, especially in the subcontinent, in discussing sexual issues, act as the stumbling block in the efficient management of the condition. Hyperprolactinaemia also interferes with erectile process not only by inhibitory dopaminergic activities in the MPOA, but also by decreasing testosterone level. Prolactin may also have a direct effect on the penile smooth muscle contractility.[56]

Erectile dysfunction can be effectively treated with a variety of methods (see Chapter 5). Many patients and health care providers are unaware of these treatments, and the dysfunction thus often remains untreated compounding its psychological impact. Concurrent with the increase in the availability of effective treatment methods, there has been increased availability of new diagnostic procedures that may help in the selection of an effective and cause specific treatment.

Major advances in science and medicine have led to improved understanding of the haemodynamics, functional anatomy, neurophysiology, and neuropharmacology of penile erection and dysfunction at the cellular and molecular levels. Consequently, there is at present a much better understanding of the physiology and pathophysiology of ED. The change of smooth muscle tone has emerged as a key factor in erection and detumescence.[32,57]

Penile Size

Normal variations in the size of male external genitalia are of considerable interest to scientific studies. The penile dimensions are highly correlated with anthropometric parameters such as height and weight, which display a considerable normal variability in general population across the world. There is also expected variation of the penile dimensions in flaccid and erected state in various races. We do not have any authentic published data from the subcontinent available.

The median values of penile dimensions recorded in an Italian study are flaccid length 9.0 cm, and stretched length 12.5 cm.[58] Studies show that the typical erection is roughly 5 inches in length. The amount of body fat dictates the length of the penis as well. A general rule of thumb is that for every 30 pounds over ideal body weight, one can generally expect to lose an inch of penis size. The penis does not actually shrink, but more of it is concealed under a layer of fat. As more fat surrounds the base of the penis, the less it becomes apparent. While it is unusual to see a very large penis in an obese men, it is also

true that a short penis can look quite long in an extremely thin man.[50]

According to the book, "Man's Body"(1995), the average flaccid penis is about 3¾ inches long with most falling between 3¼ and 4¼ inches, though a few fall outside this range. The average erect penis is 6¼ inches, with most between 5 and 7 inches, though a few are smaller or larger. An article in Men's Health Magazine (June 1996) indicates that these data are overly optimistic, and that the average erect penis length now widely accepted by doctors is 5.1 inches. This seems a bit short, at least for an average number; but if these data become widely known, most men may be happy to find out that they are "above average." Part of the problem is that scientific data on this is difficult to collect by other parties. Men, if they measure themselves in private, are perhaps sometimes prone to brag a bit.

Artificial Penile Enlargement

Artificial penile enlargement can be achieved by surgery. An appearance of a longer penis can be done without actually increasing its length by cutting the suspensory ligament. Unfortunately, it also makes the penis unstable during intercourse; and in due course, makes it more susceptible to injury. The other method of enlargement is by liposuction of tissue from one part of the body and injecting it around the penis to create a fatter penis. This gives the appearance of a fat, wide penis but not longer. These procedures are not recommended and have very high complication rates.

The penis being singularly the most significant flag bearer of manhood, a desire for a long penis is common in the male population. Consequently, penile size has often caught the imagination of the young and the adolescents. It is likely that sexual enlightenment from books and magazines, and increasing access to the erotic video, play a significant role in increasing the number of young men seeking consultations for the problem of 'short penis' to andrologists and plastic surgeons. However, most men seeking penile lengthening surgery are ignorant of the normal penile length and tend to overestimate its size. In one Italian study[58,60] forty-four (65.7%) patients complained of a short penis while flaccid, 22 patients (32.8%) while both flaccid and erect, and only one patient (1.5%) was worried by the erect length of the penis. Fifteen patients (22.4%) also complained about their penile circumference. Fifty-seven (85%) patients thought a 'normal' penile length should range from 10 to 17 cm (median value of 12 cm). Ten patients (15%) were not able to estimate 'normal' penile size. No patient was found to have a penile length under the 2.5 percentile according to the data concerning measures of penile length and circumference recorded in both the flaccid and fully stretched states and compared to the normal reference range described in the nomogram.[52] Documentation of such a demonstration should be made for any man seeking an opinion on penile lengthening surgery.

Furthermore, it is important to note that the length of an erect penis varies with the degree of erection. The degree of the erection may be relatively unrelated to the size of the nonerect penis. Typically smaller penises tend to enlarge to a greater degree when erect, so the differences in the size of the erect penis may not be that important.

In the age group past puberty, in the teens, and perhaps during the twenties, it is possible to get a full erection without any manual stimulation at all. As men age beyond the 20s, this occurs less frequently and increasingly some manual manipulation of the penis is needed. As the penis becomes more and more erect, the nerve endings located there gradually become more and more sensitive to touch. In general, the harder the penis become, more pleasant are the sensations from the touch. As the erection grows, the heart and breathing rates increase. During the initial stages of arousal, before the erection occurs, the testes and scrotum feel quite large and soft, and are very sensitive to touch. Gentle pressure on the testicles with the fingertips produces particularly pleasant sensations. As the erection proceeds, the testicles change as well, increasing in size as they also get filled with blood. They become harder and are drawn up to the body as the point of ejaculation becomes imminent. (Figs 4.8 to 4.10)

ORGASM AND EJACULATION

Orgasm is a state of physiological and sensory thrill that accompanies ejaculation. The first stage of orgasm, called *ejaculatory inevitability*, occurs two to four seconds before actual ejaculation takes place. In this state, one senses the imminence of ejaculation and unless the brain executes a great volitional effect (as in *coitus interruptus*), progress of the ongoing process cannot be stopped or controlled. Prostate gland and seminal vesicles also get into a pulsatile state.

The second stage of orgasm begins, when involuntarily semen is expelled in several convulsive waves. The psychological and physical pressure to ejaculate is released in a series of muscular contractions, usually about 6 to 8 major contractions spaced a second or so apart, followed perhaps by several smaller ones that can last 45 seconds or so. In essence, a pump has swung into action. This is accompanied by rhythmic contractions of perineal muscles and bulbocavernosus to help expressing the semen. This process, which propels the semen through the urethra, is known as ejaculation (See earlier also).

Technically, an orgasm is similar to a sneeze and it involves a series of involuntary muscle contractions in response to an "irritation," though, of course, it is usually a good deal more fun. Once the orgasm is complete, the valves, which maintained the erection, are opened, and the penis is drained of blood; so that within a space of a few minutes, it has returned to its flaccid state. (Fig. 4.11)

Normally, one ejaculates about one to two teaspoons of semen. The first squirt of the semen fraction generally contains the largest number of sperms. After orgasm, most men and many women experience a recovery (refractory) period, when they cannot have another orgasm. This period may last many minutes and sometimes even hours. One may also experience an erection refractory period.

Premature ejaculation is a condition, where one cannot control the ejaculatory responses sufficiently for a period to ensure semen to be deposited into the vagina. Normally, this period is at least twenty to thirty seconds after penetration of vagina. Some researchers although in minority, broaden the clinical definition of premature ejaculation to "inability to control ejaculatory responses for a sufficient length of time to satisfy his partner at least half of the time" (See later for details).

Many men think that an erection must ordinarily proceed to an orgasm and ejaculation, but this is not necessarily so. By repeatedly massaging and then stopping the manual stimulation of the penis, a man can go through many erection cycles that do not necessarily need to lead directly to orgasm.

Research indicates that stimulation of a portion of the brain results in the feeling of an orgasm, but this stimulation produces neither an erection nor ejaculation. These findings support the theory that ejaculation and orgasm, though often linked together, are, indeed, separate events. Interestingly, this research finding also lends credence to the theories of those authors, who advocate the view that men can learn to have multiple, closely- spaced, orgasms.

Learning how to achieve an erection just below the level, which will lead to ejaculation, is an important part of sexual enjoyment. It is important for the man to learn how to read his body's signals that orgasm and ejaculation are near. Psychologists call the point, where the men can no longer delay orgasm the "*point of inevitability*". There are several physical indications. First, the hole in the tip of the penis will become more slit-like. Precum production will stop. Generally, if the fluid at the tip of the penis becomes milky, the point of inevitability is already past.

With practice, a man can learn a degree of control over the point when he proceeds to orgasm and ejaculation. Some men learn techniques for having multiple orgasms without ejaculating. Many of these techniques involve squeezing of the urethra so that the semen is not allowed to escape. This sounds potentially painful and perhaps even dangerous.

PHYSIOLOGY OF EJACULATION

Ejaculation naturally follows the erection and orgasm. It is the final act in the scenario; and without a successful ejaculation, sperms cannot be delivered to the female genital tract to complete the process of fertilisation or union of sperm and ovum. Ejaculation is a complex and coordinated neuromuscular mechanism to deliver the sperm to the female reproductive system.The ejaculatory reflex is initiated and coordinated by cerebral integration of visual, auditory, tactile and olfactory stimuli and modulated by psychosocial cognitive processing (see ejaculatory pathways in Fig. 4.15).[61]

Ejaculation comprises of three synergistic phenomena–seminal emission (delivery of semen into the posterior part of the urethra), ejaculation (propulsion of semen from posterior urethra to outside), and bladder neck closure ensuring no passage of semen towards the bladder. These events are reflex actions and require coordination of autonomic and somatic nervous systems. The afferent reflex arc involves sensory pathways through the dorsal nerve of penis from the sensory branch of the pudendal nerve. Sensory impulse then passes through this nerve to reach the sensory cortex of brain. Efferent limb passes from the brain through anterolateral column of the spinal cord to the thoracolumbar sympathetic

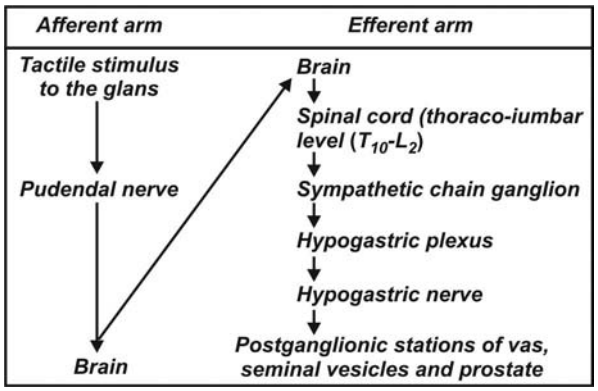


Fig 4.15: Ejaculatory pathways (afferent and efferent paths)

outflow. Stimulation of thoracolumbar segment causes smooth muscle contractions of prostate seminal vesicles, epididymis and the bladder neck, which gets closed. The ability to propel the semen through urethra is provided by the somatic pudendal efferent nerve in the striated muscles of pelvic floor. Interaction between the somatic and autonomic nervous systems produces rhythmic expulsion of semen through external urethra.

A concurrent sympathetic stimulation causes closure of bladder neck, and contractions of prostate, ampullae of vas and seminal vesicle to propel semen to the prostatic urethra. Opening of urogenital diaphragm and rhythmic contractions of bulbocavernosus, ishiocavernosus and pelvic floor muscles under somatic control of pudendal nerve ($S_{2,3,4}$), finally expels the semen from the prostatic to the anterior urethra. As discussed earlier, the role of nerve transmitter NO in the regulation of smooth muscle contractions of vas, seminal vesicles and prostate involved in ejaculation is also very important.

Ejaculatory Distance

Generally, more frequently a man has an ejaculation, less will be its force resulting in a shorter shooting distance. However, it appears to be both genetic and age-related. Some men are able to shoot longer distance than others, and younger men tend to have greater force of ejaculation than older men. Thus, one may coin a word some shoot, while some dribble. The book "Man's Body" indicates that after prolonged abstinence – more than three days – a man may be able to shoot 3 feet or more, but the average is 7 to 10 inches with more frequent ejaculation. If one is able to ejaculate two to three hours after his previous ejaculation, the semen after just dribbles out. The ability to shoot long distances not only declines generally with age, but probably also varies somewhat according to the hardness of the erection.

There is a wide variation in semen production with volume ranging from 1.5 ml to 6.6 ml (see Chapter 2 and 7). The book "Man's Body" notes that 3.5 ml (about a teaspoonful) is average after a few days without ejaculation, while 13 ml has been recorded after prolonged abstinence. Volume must be judged in relationship to the frequency of ejaculation, but there is undoubtedly normal genetic variation.

Frequency of Ejaculation

According to a number of studies, many post pubescent young men report daily ejaculation, if not more frequently than that. This frequency gradually declines for most males to 2-3 times per week, which is typical of men in their forties onwards. But there is still a considerable variation among adult men of a given age.

A "Newsweek" article cited the following data on "average" frequency of orgasm per year by age. These data appear 'conservative' to me, but perhaps that is good, if nearly all men, like the children in the mythical Minnesota town, are "above average." Here is the data (Table 4.9):

Table 4.9: Average frequency of orgasm

Age	*Frequency*
20	104 orgasms per year
30	121 orgasms per year
40	80 orgasms per year
50	52 orgasms per year
60	35 orgasms per year
70	22 orgasms per year

[From Newsweek article (September 16, 1996, p. 73)][62]

[I wonder how many 20-year-old men are content to "survive" on an average of fewer than 3 orgasms per week! Older men should feel pretty good about their frequency of orgasm, based on these data!]

EJACULATORY DYSFUNCTION

Failure to achieve erection indubitably includes failure of ejaculation, but there are some exclusive conditions of isolated ejaculatory dysfunctions like premature ejaculation (PE), deficient ejaculation (DE), anejaculation (AE), retrograde ejaculation (RE) and ejaculatory incompetence (see Chapter 10 as well). Diagnosis of these disorders can often been made, if a careful history of the ability to ejaculate including

nocturnal emission and the ability to experience orgasm is taken. Initial investigations mainly include the demonstration of sperm in the postmasturbation urine, analysis of the ejaculate and transrectal ultrasonographic assessment of the ejaculatory ducts (see Chapter 8).

Premature Ejaculation

According to "Masters and Johnson"[63] premature ejaculation (PE) is inability to control ejaculation for sufficient length of time during intravaginal containment to satisfy the female partner in at least 50% of coital events. Approximately 25 to 40% of US males have difficulties with PE at some time in their lives. Some surveys have reported that as many as 60% of men have intermittent concerns about rapid ejaculation. Although relatively few men seek professional treatment for the disorder, it remains among the most prevalent and troublesome of male dysfunctions.[64]

At least two types of premature ejaculation are seen – *primary* or lifelong and *secondary* or situational. Patients with secondary PE generally present with a higher incidence of ED and associated other sexual performance difficulties. It has also been proposed that men with primary PE have increased penile sensitivity or a decreased threshold for the bulbocavernosus reflex.[64]

Learning how to lengthen the arousal and erection period, while delaying orgasm is an important part of maximising enjoyment from sex. As the erection proceeds, the physical sensations become more and more exciting, and the psychological pressure to ejaculate becomes increasingly intense. The trick is to learn to keep the stimulation just below the level required for ejaculation, while learning to deal with the increasing psychological pressure to ejaculate. Like driving a racing car closer and closer to a wall at high speeds, the psychological pleasure becomes more and more intense. Longer can the arousal be maintained without ejaculation, greater is the enjoyment for the man. Furthermore, the longer this stage can be maintained, the more powerful and enjoyable the orgasm will be for the man. Thus, developing skills for doing this and dealing with the psychological desire to ejaculate for as late as possible are essential for the full enjoyment of sex for the partner and self, and this is what requires practice. Women usually require a somewhat longer period of time to become fully aroused, so being able to delay orgasm potentially increases the enjoyment of sex for both partners.

Metz et al[65] have suggested that PE may result from altered perception of the genital area such as glans due to a lesion in the afferent pudendal pathway. Consequently, they have downgraded the importance of the psychological factor hitherto recognised as the primary cause of PE. They have recognised two a etiological factors of PE, biogenic or physiological and psychological types. The physiological type is predisposed by (a) neurologic constitution, (b) acute physical illness, (c) physical injury, and (d) pharmacologic side effect. The psychological type has a background of (a) psychological constitution, (b) acute psychological distress, (c) relationship distress, and (d) psychosexual skill deficit.[64]

Premature ejaculation becomes a fertility problem, when ejaculation takes place prior to inserting penis fully into the partner's vagina. Artificial insemination with sperm is one of the methods to achieve pregnancy; but given a choice, most couples prefer using natural coital technique. Some are even averse using of behaviour modification procedure.

Treatment of Premature Ejaculation

Behavioural Therapy

Behavioural conditioning approach (e.g. squeeze, stop-start) has been used for many years with mixed results. For compliant patients, these techniques have moderate short-term efficacy. The treatment requires a high level of motivation and active involvement of the sexual partner. However, partners frequently voice dislike or aversion to the procedures. Long-term follow-up indicates a high degree of relapse, unless treatment is continued at periodic intervals.

The squeeze technique helps to desensitise penis so that one can participate in sex without experiencing premature ejaculation. When using this procedure, female partner places her thumb on the frenulum on the underside of the penis (Fig 4.16). She places her first and second fingers on either side of the coronal ridge on the top of the penis. Squeezing her fingers together for three to four seconds in this manner will make male partner lose his urge to ejaculate and at times some degree of erection. After fifteen to thirty seconds, female partner can stimulate the penis again and just before ejaculation, and repeat the squeeze technique. If female partner is concerned about how much pressure to use, male partner can place his fingers over hers and press with her. This demonstration of ejaculatory control improves male

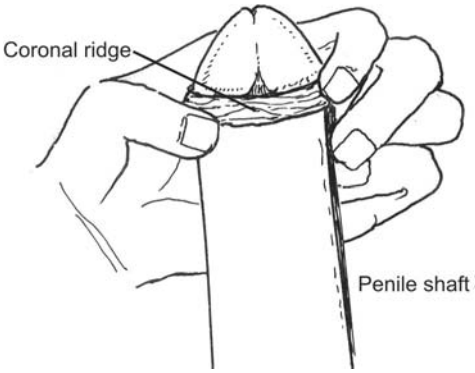


Fig 4.16: Squeeze technique

self-confidence and will be a major step toward re-establishing communication and improving marital relations.

Once this technique is practised, one can try it with the female-superior coital position (female on top). After using the squeeze technique several times, the female can insert penis into her vagina without thrusting her pelvis for male stimulation. With counselling, many couples are able to establish normal coital patterns.

As all portions of the erect penis are not equally sensitive, the man can perhaps delay orgasm by varying locations being stimulated. Stimulation of the base of the penis near the body may elicit pleasant sensation; but normally, it will not be sufficient to achieve orgasm. The underside of the tip of the penis, called the *frenulum*, is very sensitive to manual stimulation. Its stimulation in an erect penis may evoke an orgasm and even ejaculation. It is thus desirable that stimulation of this area is delayed until late in the sex play.

Medicinal Therapy

Pharmacological treatment approaches are used increasingly in the treatment of PE. Serotonergic antidepressants (e.g. paroxetine, clomipramine) are especially effective. Although the mechanism of action has not been examined, these drugs appear to improve ejaculatory control by decreasing alpha-adrenergic tone or increasing serotonin levels in the blood.

Among the serotonergic agents currently used for treatment of PE, clomipramine (Anafranil) has been shown to be highly effective in several double-blind studies.[65, 66] The drug is usually administered in low doses (25-50 mg), and can be taken 4-6 hours prior to intercourse. Clomipramine is effective in approximately 70 to 80% of cases, although patients report a high rate of adverse side effects (e.g. dry mouth, sedation, dizziness). Most patients experience a return of symptoms following discontinuation of the drug.[67]

Other serotonin reuptake inhibitors or SSRIs (fluoxetine, sertraline, paroxetine) have been shown to be effective in some cases. Few double-blind studies have been conducted, although sertraline and paroxetine have been found to be well tolerated and effective in two recent studies. In a European study, patients taking paroxetine (40 mg/d) showed a marked improvement in control of ejaculation compared to placebo.[68,69] Independent partner ratings confirmed the significance of the findings.

Although serotonergic drugs offer potential for simple and cost-effective treatment of premature ejaculation, several limitations and risks should be considered:

1. Treatment is likely to be effective only as long as drug administration is continued. Most patients are reluctant to use psychiatric medications on a long-term basis.
2. Chronic use of SSRIs can cause diminished libido or erectile difficulties for some patients. These drugs are strongly contraindicated for patients with PE as a long-term measures, especially with concomitant desire or arousal difficulties.
3. Other side effects in particular, sedation or sleep difficulties are reported in a significant number of cases.
4. Use of SSR may mask the contribution of relationship factors or inadequate sexual technique on the part of the male or his partner.

Paroxetine hydrochloride appears to be a useful agent in the pharmacological treatment of premature ejaculation when administered initially or later as an '*on-demand*' basis in chronic cases.[70]

In India, several medicinal and ayurvedic products (like Speman forte, Tentex forte, Bangsheel, Fortege, etc.) claim varying success in treatment of PE and ED. It is very difficult to assess their efficacy in the absence of proper well-documented trials as per scientific norms, and without knowing the pharmacological basis of the ingredients in these medicines.

Recently, after an appraisal of the psychological background of PE, some therapists like putting more stress on the systematic and in-depth investigations to arrive at a definitive diagnosis before formulating the treatment of PE. They have shown doubts in the

long-term efficacy of behavioural therapy in many men treated for the condition. Apart from using the medicinal treatment, importance of pelvic floor muscle exercise has now come to light. Pelvic muscle exercise should now be a routine measure for the treatment of this condition.[71] (See Appendix 1, Chapter 5 – *Kegel' exercise*). Effective psychosexual treatment combines multiple strategies such as physiological relaxation, pubococcygeal muscle training, cognitive and behavioral pacing strategies, and the involvement of the partner in the therapy.

Anejaculation

Anejaculation (AE) is defined (see Chapters 10 and 11) as complete absence of antegrade ejaculate. It is not a very common disorder and may occur as a result of spinal cord injury to D-10 segment from injury, retroperitoneal node dissection, diabetic neuropathy, multiple sclerosis or transverse myelitis. Patients may have decreased or even normal orgasm, but no ejaculation takes place. At times, psychogenic factors also come into play. If the efferent or afferent nerve pathways are damaged, electroejaculation can be employed to ensure semen collection. If the reflex arc is intact, vibratory stimulation may be useful. Use of hCG injections has been reported to increase the incidence of nocturnal emissions, which can then be collected for artificial insemination.[72, 73]

Retrograde Ejaculation

Retrograde ejaculation (RE) is a condition in which semen is ejaculated into the bladder rather than out through the urethra, because the bladder sphincter does not close at the moment of ejaculation (see Chapters 10 and 11 as well). It is found in 1.5 per cent of infertile men, and is the most common cause of absent ejaculate. In this disorder, one may notice that the ejaculate volume is small (below one millilitre). Urine after intercourse looks turbid or cloudy. The diagnosis can be confirmed by examining a urine specimen taken soon after intercourse. Postejaculation urine showing 15 sperms per high power field is put as the benchmark for the diagnosis (Table 4.10). Retrograde ejaculation becomes a fertility problem if large quantities of sperms are found in urine.[74]

Table 4.10: Diagnostic criteria of RE

1. Ejaculate volume is small (below one millilitre).
2. Postintercourse urine looks turbid or cloudy.
3. Postejaculation urine showing 15 sperms or more per high power field.

Retrograde ejaculation often occurs in diabetics, paraplegics, and men taking blood pressure medication (antihypertensives). The disorder may also occur in men with urethral stricture or men who have undergone operation for bladder neck, prostate, or other abdominal structures. Many times, medications such as decongestants, which contract the bladder sphincter, will control retrograde ejaculation. In certain circumstances, surgical reconstruction of the bladder neck can restore normal ejaculation.

Some patients with RE have success, when they have intercourse with full bladder. Those, who do not have any bladder neck pathology or neurological disease, may benefit from alpha-adrenergic sympathomimetic agents to ensure closure of internal urinary sphincter.

Glander[73] has enunciated three principles of management–conversion of retrograde into antegrade ejaculation by drug therapy, harvesting of sperms from the postejaculate urine and surgical treatment (Table 4.11).

Table 4.11: Principles of management of RE

1. Conversion of retrograde into antegrade ejaculation by drug therapy.
2. Harvesting of sperms from postejaculate urine.
3. Surgical treatment.

Drugs like with α agonist action (ephedrine, pseudoephedrine, imipramine, phenyl propalamine, etc) are first used to treat RE. The success rate of the drug treatment being suspect, sperm-harvesting is mostly used followed by some form of assisted reproductive methods. Surgical treatment is rarely feasible.

Denil et al[75] have also successfully tried electroejaculation for anejaculation and retrograde ejaculation. The most accepted method nowadays is to collect the sperms from the post-ejaculate urine after ensuring optimum environment, so that the acidity in the urine does not come in the way of their survival. For the ART like IUI, the collection of sperms from urine needs to be modified from the routine. The male partner is advised to take one teaspoon of bicarbonate of soda in a glass of water four times a day for two days prior to female partner's most fertile time of the month. About twenty minutes prior to collecting the semen, male partner empties his bladder. A catheter into the bladder now drains any excess urine and a small amount of sperm nutrient media is introduced into the bladder. The man is

asked to ejaculate into a specimen cup. The collected semen is washed, and the motile sperm fraction isolated before the artificial insemination (AI) of the partner. A major obstacle to success is the severe asthenospermia and the poor functional quality of the obtained sperm samples.

In another method, the patient is first asked to empty the bladder and the residual urine is then taken out through a catheter followed by instillation of alkalinising medium with sodium bicarbonate solution. Lim et al[75] successfully tried a silicone balloon catheter to tamponade the bladder neck to prevent the retrograde passage of sperms to the bladder. The success rates are quite good, provided the couple can stick to the regimen.

Ejaculatory Incompetence

This is very similar to anejaculation (AE) and is often referred to retarded or disturbed ejaculation. Men with ejaculatory incompetence rarely have difficulty in achieving or maintaining erection; however, they cannot ejaculate during sex. Often, even the female is unaware of her husband's condition, as the male partner has orgasm. Diagnosis is established by comparing the normal semen analysis with the postcoital test. The semen test is normal, but there are no sperms in the postcoital cervical mucus, as the ejaculation did not occur. This rare psychological condition sometimes responds well to behaviour therapy. Combining masturbation and manual stimulation with eventual insertion into the vagina may stimulate ejaculation. If the condition persists, one may have to resort to artificial insemination by husband (AIH) with a masturbated ejaculate.

Ejaculatory Duct Obstruction

This is discussed in details in Chapters 10 and 12.

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CHAPTER

5 Management of Erectile Dysfunction

INTRODUCTION

Erectile dysfunction (ED) or impotence is the repeated inability to get or keep an erection firm enough for sexual intercourse. The word impotence is often used to describe other problems that interfere with sexual intercourse and reproduction such as lack of sexual desire, and problems with ejaculation or orgasm.

Erectile dysfunction or male impotence seems to be a common problem that is faced by the world male population irrespective of race or country. However, precise and exact information of the prevalence of ED is not known (even in a country like the United States). Moreover, how this prevalence varies according to individual characteristics is also not documented in the available literatures. A recent estimate of ED is shown to affect over 30 million US men to some degree, which probably includes individuals with partial ED.[1-10] The majority of these individuals probably would be older than 65 years. According to Dr F Eid: "Ten percent of the male population and 35 percent of men over 60 years of age suffer from ED in the US alone." [11] Comparative authentic figure of such incidence in the subcontinent is not available.

Treatment of ED includes psychotherapy, drug therapy, vacuum devices, and surgery.[12] Drug treatments help many of them, but do not work for all to regain their sexual lives. In other cases, invasive methods such as injections, pumps or implants, have been tried with no guaranteed or permanent success. Difficulty in scientific management of ED is compounded by the multiplicity of causative etiological factors. Notwithstanding these facts, ED usually has a physical cause and most cases are treatable at all ages.

ETIOLOGICAL FACTORS

Natural history of the ED such as information on the age of onset, incidence rates stratified by age, progression of the condition, and frequency of spontaneous recovery, is often vague. Moreover, the data on associated morbidity and functional impairment are mostly inadequate.

Prevalence of Erectile Dysfunction

Estimates of prevalence of ED vary depending on the given definition of ED or impotence. There are ample evidence of racial, ethnic, and other cultural diversity in perceptions and expectation levels for satisfactory sexual functioning, but very little is known about the roles of these variable factors. Authentic data are predominantly available from the Western world. Majority of these data constitute studies in whites with other racial and ethnic population representing only in small numbers. Consequently, these data cannot be used for comprehensive appraisal or critical analysis as a function of race or ethnicity. Naturally, these differences are reflected in some research groups' reaction and perception of ED.

Ageing and ED

The prevalence of ED has been found to be associated with age. According to research cited in a study on sexual dysfunction in the US (referenced below), sexual dysfunctions are highly prevalent in both sexes, ranging from 10 to 52% of men and 25 to 63% of women. However, data published by different investigators vary to a great extent. Feldman [13] (*New England Research Institute, Watertown, Mass, USA)* reported, "Long-term ageing studies have estimated that 10 per cent of men between 40 and 70 are completely impotent, and ageing is still the biggest single cause of impotence." ED is highly prevalent in men over 40 and this condition showed a clear relationship to ageing, as demonstrated in many studies published. Other researchers state that impotence or ED affects about 10 per cent of men in their sixties, 25 per cent of men in their seventies, 40 per cent of men in their eighties, and more than half of those in their nineties. In young couples, the incidence is about 7 per cent.[14]

When the individual and his partner as a natural consequence of the ageing process find changes in sexual function, they often modify their behaviour to maintain sexual satisfaction. But men at times do not perceive ED as a normal part of ageing and seek to identify means by which they may return to their previous level and range of sexual activities. Such levels of expectation and desire for sexual interactions are important aspects in the evaluation of patients presenting with the chief complaint of ED. [15, 16]

We do not have any relevant and reliable statistics from South-East Asia including India, where a strong taboo exists amongst the population on any free discussion on sex. Most Indian men would brood and suffer in silence rather than discussing this with competent health care personnel. Mostly, this discussion would be limited to their close associates, who are equally ignorant, or so called quacks with no scientific knowledge of the subject. It is a common experience in India that these quacks often advertise in local magazines or even put pamphlets in the prominent public places to attract these hapless patients.

In the past, any discussion on the subject of sexual problems were fraught with fear complex of getting exposed, as any form of sexual problem of an individual was likely to be equated with having venereal disease! Fortunately, in the urban context, there is a change in the attitude in recent years and this is an important development in India. Unconfirmed report from a recent community survey concluded that the erectile disorder was the leading complaint of males attending sex therapy clinics. Erectile dysfunction may be associated with depression, loss of self-esteem, poor self-image, increased anxiety or tension with one's sexual partner (see Chapter 14).

Male-female Perceptions

The impact of ED depends very much on the dynamics of the relationship of the individual and his sexual partner, and their expectation of performance.

In men of all ages, erectile failure may diminish willingness to initiate sexual relationship because of fear of previous inadequate sexual performance or rejection. In the subcontinent, older males are particularly sensitive to the lack of social support of intimate relationships, but consequent withdrawal from these relationships may have negative effects on their overall health. Female perception in the subcontinent has traditionally been overtly negative with ageing. The situation is also compounded by the lack of privacy for elderly couple in an overcrowded urban housing situation with children growing up and subsequent arrival of grand children.

In the Western world, many women believe that men are happier, if they can have more frequent intercourse. This is not really accurate. Men truly enjoy being aroused, when their partners are also aroused, and both remain in an aroused state for a long period of time delaying orgasm for as long as is comfortable and possible. A continuing theme of the story lines in erotic literature is a situation in which the woman arouses the man and keeps him for a long time at a level just below what is needed to achieve an orgasm. It is important in achieving marital happiness for women to learn how to do this for their husbands.

During intercourse, when the man's penis is fully inside the woman's vagina, the tip of the penis may touch the cervix, only if the man is deep inside the woman and the penile size is long enough. If the orgasm takes place almost precisely when the tip of the penis comes in contact with the cervix, the sperms will have the shortest distance to travel to reach the waiting ovum. This situation ensures that the sperms travel a shorter distance to maximise the chance of pregnancy. In addition, a man feels a pleasant sensation, when the tip of the penis touches the cervix. In sex play, the man can reproduce this pleasant sensation outside of intercourse by touching the tip

of the penis particularly its underside or the frenulum. But in this situation, there is always a possibility of orgasm and ejaculation following almost immediately. The frenulum is often referred to as the *male G spot* because of its sensitivity and pleasant sensation it can elicit.

A significant portion of the penis, perhaps one-third to one-half of it, lies inside man's body. This portion of the penis like the part outside also responds to manual stimulation. It can be felt and externally massaged at a spot in the perineum directly behind the base of the scrotum. This is a little recognised, but highly sensitive spot on the body of a male. It is sometimes referred to as the *second male G spot*. Many men are not even aware of the possibility of externally massaging this interior portion of the penis and that it is a sexually sensitive area. The main artery providing blood for erections runs through this area and a bundle of nerves also terminate here.

CAUSES OF ERECTILE DYSFUNCTION

Close to 20% of all infertile men [17] have some form of erectile problems in the USA. In India, the incidence could be higher as the actual statistics is difficult to get in the subcontinental set up. If one adds the incidence of temporary failure in any couple's conjugal life that normally goes unreported to the medical personnel, the incidence of erectile failure would certainly be higher (Fig. 5.1, Plate 3).

As adequate vascular supply is critical for erection, any disorder that impairs blood flow may be implicated in the etiology of erectile failure.[18] Most of the medical disorders associated with ED appear to affect the arterial system. Some disorders may interfere with the corporal veno-occlusive mechanism and result in failure to trap blood within the penis, or produce leakage, so that erection cannot be maintained or is easily lost.

Lesions of the somatic nervous pathways may impair reflexogenic erections and interrupt tactile sensation needed to maintain psychogenic erections. Spinal cord lesions may produce varying degrees of erectile failure depending on the location and completeness of the lesions. Not only do traumatic lesions affect erectile ability, but also the disorders leading to peripheral neuropathy that may impair functions of the neuronal innervation of the penis or the sensory afferents. The endocrine system, particularly the production of androgens appears to play a role in regulating sexual interest and the erectile function.[17]

Etiological factors for erectile disorders (mentioned in Chapter 4) are commonly categorised as o*rganic* or *psychogenic*, but these two factors mostly appear to act in concert [19] (see Chapter 4). In the past, ED was thought to be largely the result of psychological or lifestyle problems. [20] Stress, depression and trouble in a relationship are often blamed for erection problems. Smoking, heavy drinking and use of recreational drug may also adversely affect a man's ability to have an erection. Erectile dysfunction is not hereditary, but some specific conditions that may cause ED tend to run in families. After taking into consideration all risk factors, such as age, lifestyle and medical conditions, the effect of occupation remaines significant. Men, who worked in blue-collar occupations were one and a half times more likely to develop ED compared to men in other occupations.[21]

Men, who experience a sudden loss of erectile capability, often have a psychological origin to their condition. Just as an erection can result from thinking about sex, negative thoughts can prevent an erection from occurring. Psychological processes, such as stress, depression, anxiety, and relationship problems, can impair erectile functioning by reducing erotic focus or otherwise lessen awareness of sensory experience (Table 5.1). This may lead to inability to initiate or to maintain an erection.

Table 5.1: Psychological causes of ED

• Stress and anxiety from work or home
• Worry about poor sexual performance (performance anxiety)
• Marital discord
• Unresolved sexual orientation
• Depression

Impotence quad hoc is one disorder, where one can be normal with one partner, yet fails to achieve erection with another. "Fetish" is one disorder worth mentioning in this respect.[22] In a fetish some inanimate object can initiate or stimulate sexual response. It is an exclusive male disorder, where sexual arousal and orgasm can only be achieved by some objects like even sight of women's underwear, shoes, or any such objects, which in real terms act as a crutch to aid their sexual expression. A lot of these people are really quite shy and find it difficult to maintain normal sexual relationship in a healthy way. They also have a hard time maintaining intimacy and erection. So fantasies of these men get down to particular objects for

initiation of their sexual pleasure. In reality, while the majority of patients with ED are thought to demonstrate an organic component, psychological aspects, such as lack of self-confidence, anxiety, lack of communication, and partner conflict, are important contributing factors.

Research has shown that there are quite a few medical conditions responsible for ED (listed in the Table 5.2). Medications prescribed to treat a variety of medical conditions, such as hypertension and depression, may also affect a man's ability to have an erection. Any disease that affects nerves, arteries or veins may cause ED. Hormone imbalances can also decrease sex drive and cause impotence. Conditions affecting normal hormone status of thyroid or adrenal glands may also cause ED.

Diabetes is one of the most common causes of sexual dysfunction seen in men. It has been estimated that up to 50-60% of diabetic men have ED. [23,24] One of the complications of diabetes is diabetic neuropathy causing loss of vibratory sensations of the lower part of the body including the glans. *Vascular changes* in the arteries, *autonomal neuropathy* and *gonadal dysfunction* all contribute in diabetic patients, where onset of ED occurs perhaps 10-15 years earlier than the corresponding age groups in general population. Men in their 30s and younger with diabetes may also experience sexual dysfunction.[25] Incidentally, both ageing and diabetes cause depletion of nitric oxide synthase (NOS) and nitric oxide-cyclic guanasine monophosphate NO-cGMP in corpus cavernosum and thus contribute to dysfunction of the erectile process (see Chapter 4).

Table 5.2: Common medical conditions that may cause erectile dysfunction

1. Diseases that affect the arteries, such as hardening of the arteries, which causes decreased blood flow.
2. Diabetes mellitus, which may affect both nerves and blood vessels.
3. Diseases that affect hormone production, including pituitary tumours and thyroid dysfunction.
4. Chronic medical conditions, such as kidney failure, liver failure and AIDS, chromic alcoholism and drug addiction with cocaine or marijuana.
5. Diseases that affect nerve function including stroke, multiple sclerosis, Alzheimer's disease, Parkinson's disease and epilepsy.
6. Trauma to the pelvis, such as a pelvic fracture or even long-distance bicycle riding, which may affect nerves and arteries.
7. Pelvic surgery including radical prostatectomy and surgery/ and pelvic irradiation for rectal and anal carcinomas.
8. Peryonie's disease, causing the penis to bend to one side.

It is estimated that fifty per cent or more of the men with multiple sclerosis have ED as an extension of the bladder dysfunction caused by the neurological deficit. Parkinson's disease and temporal lobe abnormalities (where the sexual impulse most probably originate) are common risk factors. Many other chronic illnesses such as cancer, rheumatoid arthritis, herpes zoster, anaemia, stroke, chronic renal failure, chronic alcoholism and drug addiction with marijuana, very often cause erectile problems, and are considered high risk factors. The diseases associated with peripheral neuropathies (apart from diabetes) and hereditary neuropathies are also known risk factors for ED. Peyronie's disease interferes with normal erection due to changes in the corpus cavernosum (this has been described in details in Chapter 10).

Preventive Aspect

Erectile dysfunction is clearly a symptom of many conditions, of which a few have been identified, some of which may be amenable to prevention strategies. Although ED increases progressively with age, it is not an inevitable consequence of ageing.[13] Knowledge of the causative factors can guide prevention strategies. It is important that physicians and other health care providers treating patients for chronic conditions periodically inquire into the sexual functioning of their patients and be prepared to offer counsel for those, who experience erectile difficulties.

Lack of sexual knowledge and anxiety about sexual performance are common contributing factors to ED. Education and reassurance may be helpful in preventing the cascade of events leading to serious erectile failure in individuals, who initially experience minor erectile difficulty due to medications or common changes in erectile functioning associated with chronic illnesses or with ageing. Changes in the lifestyle are one of the recommendations to improve erectile problem. In the subcontinent, the enlightened and educated men almost never take recourse to this preventive measure for societal reasons.

A lot of food having excessive estrogens can cause low sperm count and problems with virility, libido and the general feeling of wanting to be involved sexually (see Chapter 6). About 10 to15 per cent of men suffer from psychological problems that can lower sperm count and reduce the size of erections.

Medications

In individual patients, the physician can modify the regimen of specific antihypertensive, antidepressant, and antipsychotic drugs in an effort to resolve the erectile problem. Body-building supplements containing creatine and androdione sometimes suppress testosterone production. As some men indulging in these supplements develop ED, it is imperative that physicians warn these men about the consequences and advice accordingly (Table 5.3).

Table 5.3: Drugs interfering with erectile mechanism

1. Estrogen, antiandrogens (flutamide and Lupron) used in prostate cancer.
2. Beta blockers, calcium channel blockers, methyldopa and diuretics used for heart disease and hypertension, and older drugs like Digoxin used for heart failure
3. Tranquilizers and decongestants
4. Medicines used for prevention of seizure or epilepsy
5. Drugs to lower cholesterol
6. Cimetidine
7. Anticancer drugs

It is important to stop habits, such as smoking, heavy alcohol drinking, and recreational drug use, as these can adversely affect erectile function. Persistently, high cholesterol levels may cause hardening of the arteries to limit blood flow in any organ including the penis. Specific measures such as restricting cholesterol in diet; and if necessary, use of cholesterol reducing medicines should be advised. Exercise is important in maintaining cardiovascular health and lowering the cholesterol level.

Some studies suggest that the long-distance bicycle riding on a standard bicycle seat may cause erectile problems or numbness in the genitals. The blood vessels or nerves that supply the penis are positioned in the perineum. According to an article published recently in *Urology Times*, constant pressure on these structures in frequent long bicycle rides can lead to ED (see Chapter 10). Even perineal injuries can also damage these vessels and nerves culminating in erectile problem. [26, 27] (Table 5.4)

Table 5.4: Common forms of treatment for ED

1. Changing habits and medications
2. Professional counselling
3. Hormone medications
4. Vacuum devices
5. Injection therapy
6. Oral therapy
7. Surgical treatment (like penile prostheses)

EVALUATION AND ASSESSMENT OF PATIENTS WITH ERECTILE DYSFUNCTION

The evaluation of ED follows the same pattern practised for any diseased state. Since ED is often a result of other medical conditions, appropriate investigation and treatment of these conditions are mandatory. The physician needs to look into, if ED is adversely affecting patient's quality of life, and whether there are additional or superadded relationship problems necessitating counselling. The doctor also needs to identify any change in lifestyle that can help to prevent the problem from getting worse. This may include changes in diet, giving up smoking and cutting back on alcohol consumption. A review of daily activity or cardiovascular status is important. There is always a potential risk of enhancing ED in patients with sedentary lifestyle. One should also identify patients, who may be at risk for an adverse cardiac event, when sexual activity is potentially increased.

The appropriate evaluation of all men with ED should thus include a medical and detailed sexual histories (including practice and technique), a physical examination, a psychosocial evaluation, and basic laboratory studies. When available, a multidisciplinary approach to this evaluation may be desirable. In selected patients, further physiologic or invasive studies may be indicated.

Elicitation of History

For a clear communication, it may be necessary to offer the patient several alternative terminologies that describe the same phenomenon in different ways. It is prudent not to accept the patient's own diagnosis and label a disorder without first ascertaining a clear picture of the complaint. It is common for less educated patients to misuse medical or technical terminology as is very commonly encountered in this subcontinent, where general perception level about sex, even in so-called educated class is suspect. In India, one should be careful while one inquires into the sexual need of the patients. The cultural and religious differences in sexual perception amongst general population of India should be kept in mind. To facilitate communication, use of clear and simple terminology that is also respectful of the patient's religious and cultural feelings, is very important to build up the patient-doctor rapport.

The general medical history is important in identifying specific causes that may account for or

contribute to the patient's ED. Any work up of these patients should include vascular factors such as hypertension, diabetes, smoking, coronary artery disease, peripheral vascular disorders and blood lipid abnormalities. Decreased sexual desire or history suggesting a hypogonadal state could indicate a primary endocrine disorder. Neurological causes may include a history of diabetes mellitus or alcoholism with associated peripheral neuropathy. Neurological disorders, such as multiple sclerosis, spinal injury, or cerebrovascular accidents, are often obvious or well defined.

It is essential to obtain a detailed history of medication since an estimated 25 per cent of cases of ED may be attributable to medications for other conditions. Past medical history can reveal important causes of ED, including radical pelvic surgery, radiation therapy, Peyronie's disease, penile or pelvic trauma, prostatitis, priapism, or voiding dysfunction.

The medical history should also include review and screening for the past and present psychiatric illnesses such as depression or neuroses. To elicit more honest response in a case of depression, phrasing of the sentence in the form of "do you develop lack of interest in the surrounding" rather than "are you depressed" is often preferred. Anxiety state is often responsible for ED. It is also very critically important to look for stress in a relationship and substance abuse of alcohol, cigarettes and narcotics, especially marijuana. Tobacco use should document its detailed amount and the length of time that the patient has been smoking.

A well-organized brief sex history can be an effective diagnostic tool. Information regarding prior evaluation or treatment for impotence should be obtained. A sensitive sexual history, including expectations and motivations, should be obtained from the patient. The sexual partner's own expectations and perceptions should also be sought since they may have important bearing on diagnosis and treatment recommendations. A written patient questionnaire may be helpful, but is not a substitute for the interview. The sexual history is needed to accurately define the patient's specific complaint and changes in sexual desire, and orgasmic or ejaculatory disturbances.

Specific situational circumstances, performance anxiety, the nature of sexual relationships, details of current sexual techniques, expectations, motivation for treatment, and the presence of specific discord in the patient's relationship with his sexual partner are often found to have significant contribution in the causation of ED. Attention should be paid to changes in mental status.

History of nocturnal erections, whether a patient wakes up in the morning with an erection, how are the erections when not having intercourse, how are the erections during oral sex or masturbation, and how do these compare with the normal heterosexual functioning need a comprehensive assessment. Inquiries into the specific sexual dysfunction must encompass a discussion of important personal problems such as stressful job situation, impending divorce, separation, and sex with other partners. Whether the partner provides the patient enough stimulation to allow adequate sexual relationship is singularly important as a man could easily be impotent in relation to one woman yet have most fulfiling relation with another as in an *impotence quad hoc*.

Physical Examination

The physical examination should be comprehensive. It should be focussed on presence of obesity, evidence of insufficient secondary sexual characteristics including hair distribution, breast swelling (gynaecomastia), and inadequate penile and testicular developments. These are important parameters for the diagnosis of hypogonadism. Evaluation of blood pressure and peripheral pulses may show vascular disease. Symptoms of vascular diseases, such as intermittent claudication and specifically, diseases of arterial insufficiency such as "Lerich syndrome" (claudication of buttocks and loss of erection), should be elucidated. A neurological examination including pelvic sensory function, perianal sensation, anal sphincter tone, and bulbocavernosus reflex are needed to confirm both sympathetic and parasympathetic functions. More extensive neurological tests including dorsal nerve conduction latencies, evoked potential measurements, and corpora cavernosal electromyography lack control data, and thus they appear at present to be of limited clinical value. A digital examination of the prostate is a must to exclude any diseased condition of the prostate. A detailed examination of the penis will reveal any anatomic defects. Presence of possible tender plaques located in the tunica albuginea would identify Peyronie's disease. The anatomy of the meatus, urethra and testicles (size, locations, presence or absence of

masses) and the presence or absence of hernia need to be looked into.

The International Index of Erectile Function (IIEF) is a widely used, multidimensional self-report instrument for the evaluation of male sexual function. It has been recommended as a primary endpoint for clinical trials of ED. The simplified IIEF-5, as a diagnostic tool, showed to be an easy method, which can be used to evaluate this condition in studies with large number of men. The possible scores for the IIEF-5 range from 5 to 25, and ED is classified into five categories based on the scores: severe (5-7), moderate (8-11), mild to moderate (12-16), mild (17-21), and no ED (22-25). It has been linguistically validated in 32 languages and used as the primary endpoint in more than 50 clinical trials.[28- 32]

Investigations

Investigations are important as they often reveal many aspects of ED that cannot be foretold by clinicians. But in developing countries, such as India, where the costeffectiveness is a factor, they should preferably be chosen according to the clinical findings. As there are quite a few disorders that may lead to ED, a plethora of investigations is often a necessity in most cases for critical evaluation (Table 5.5).

Table 5.5: Blood and urine tests

1. Urinalysis
2. Complete blood count (CBC)
3. Blood sugar, liver and kidney functions
4. Lipid profile
5. Hormone profile (FSH, LH, testosterone and prolactin)
6. Thyroid function test

Complete blood count (CBC) for any potential haematological disorder is one of the preliminary tests to be done. A lowered level of red cells limits the body's utilisation of oxygen, and can lead to fatigue initiating erectile problem. Nearly 60% of men with diabetes experience impotence some time in their lifespan. Checking of blood sugar level, (if necessary glucose tolerance test) for diabetes, thus is very important. Urine analysis could demonstrate renal disease or infection, which can then be confirmed by detailed renal function tests. Liver and kidney diseases can create hormonal imbalances, if these organs are not adequately removing metabolic wastes from the body. Liver is particularly important regarding steroid hormone metabolism. Lipid profile is done to rule out hyperlipidaemia. The level of blood lipids such as cholesterol and triglycerides, may signify the presence of arteriosclerosis, which negatively affects blood circulation in the penis.

Prostate specific antigen (PSA) and serum acid phosphatase and transrectal ultrasonography (TRUS— see Chapter 8) are important for men over age 50, to evaluate size of the prostate as well as for the presence of any cancer. PSA estimation especially assumes significance, if hormonal treatment is a possibility.

Evaluation of thyroid function is required, when thyroid is suspected to be an incriminating factor as the thyroid hormone regulates metabolism and production of sex hormones. The status of thyroid hormone levels may be a factor, where low sex hormone levels are contributing to impotence. Low blood levels of testosterone can diminish libido (sexual desire), and excess prolactin can lower testosterone levels (see Chapter 3). Endocrine evaluation with clinical and hormone profile is generally indicated. If they are persistently low, an endocrinologist may have to be consulted. The value of routine testing for endocrine disorders is costly in India, and one should back clinician's judgement in an individual case. If there is any evidence of hypogonadism, full hormone evaluation assumes a high priority. Breast enlargement in men (gynaecomastia) may be an indication of testosterone deficiency or estrogen excess, either of which can diminish erectile function. In our series, testosterone, estradiol, luteinising hormone (LH), follicle-stimulating hormone (FSH), and prolactin levels are routinely estimated for all infertile male patients (see Chapters 3 and 11).

Table 5.6: Special tests

1. Penile circulation studies
2. Penile nerve function test
3. Nocturnal penile tumescence (NPT)
4. Biothesiometry
5. Vasoactive injection (PIPE).
6. Specific nerve testing.
7. CDDU and other imaging tests
8. Angiography

Special Tests

These tests (Table 5.6) focus directly on the erectile function through examination of the blood vessels, nerves, muscles and other tissues of the penis and surrounding areas. The advanced diagnostic evaluation for ED includes tests such as nocturnal penile tumescence (NPT) studies, papavarine-induced penile erection (PIPE), vascular evaluation with Color duplex

Doppler ultrasonography (CDDU), biothesiometry, cavernosometry, cavernosogram and if necessary, selective internal pudendal angiography.

The urologist or andrologist is supposed to do these tests with help from a specialist radiologist. Cavernosometry, Duplex ultrasonography, and angiography performed either alone or in conjunction with intracavernosal pharmacological injection of vasodilator agents rely on complete arterial and cavernosal smooth muscle relaxation to evaluate arterial and veno-occlusive functions. All tests are basically aimed at distinguishing psychogenic and vasculogenic ED. CDDU would detect the plaques in Peyronie's disease and cavernosal fibrosis as hyperechoic areas.

Penile Circulation Tests

Penile circulation is evaluated by assessment of the penile blood flow by measuring penile blood pressure with a special cuff, or with color Duplex Doppler ultrasonography in both flaccid and erect states of penis (CDDU and PIPE tests— see later).

Penile Nerve Function Tests

These tests are conducted to find out if there is sufficient sensation in the penis and surrounding area. One such test is the bulbocavernosus reflex. The physician squeezes the glans penis, which causes the anus to contract, if the nerve function is normal.

Nocturnal Penile Tumescence (NPT)

It is normal for a man to have five to six erections during sleep, especially during rapid eye movement (REM), which occurs during the dream segments of sleep. These erections occur about every 90 minutes and last for about 30 minutes. Failure to have these erections may indicate a problem with nerve function or blood supply in the penis. Although not indicated for routine use, NPT testing may be useful in patients, who report a complete absence of erections (exclusive of nocturnal "sleep" erections), or when a primary psychogenic etiology is suspected. This test requires expertise and knowledge in interpretation. Various methods and devices are available for the evaluation of NPT, but limitations of diagnostic accuracy and availability of normative data restrict their clinical usefulness. Further study regarding standardisation of NPT testing and its general applicability is indicated. Nocturnal erections can be measured at home by three main methods:

Snap gauge test: A band with three plastic strips is wrapped around the penis. These strips will snap, when they are stretched. Each of the strips has a different strength so that a rough measure of penile rigidity and the change in its circumference during nocturnal erection can be assessed.

Strain gauge test: A circular device is placed at the base and tip of the penis. It stretches during erection, providing a measurement of change in penile circumference.

Stamp test: The third method that can be used at home by the patient is the stamp test (*erection self-test).* Men with organic impotence have few, if any, erections during their sleep. One can confirm an organic impotence by monitoring the nocturnal erections for several nights even at home using this procedure. A strip of four to six stamps (any kind) is used for each test (Fig. 5.2).

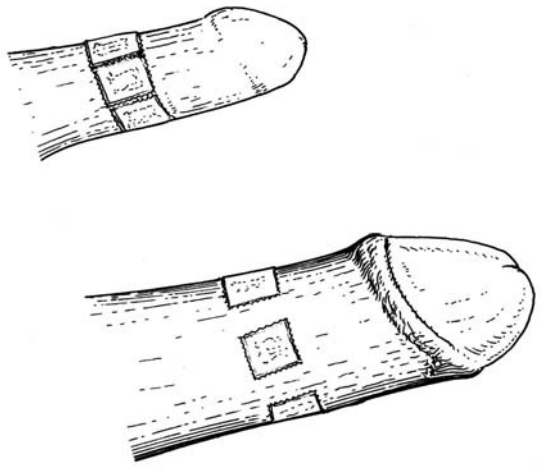

Fig. 5.2: Stamp test

The test needs to be done for three nights. The man needs to wear brief-type underwear that has a fly. The penis is brought through the fly leaving most of the pubic hair against the body. Just before going to bed, the strip of stamps is wrapped and stuck snugly around the centre of the shaft of the penis like a ring. The stamps need to overlap so that the overlapped stamp can be moistened to seal the ring. When the stamp has dried, the penis is carefully replaced inside the shorts. One should particularly try to protect the stamps from falling off. The stamps are often felt as an unusual sensation. In the morning,

checking is done, whether the stamp-ring has been broken along the perforations. With nocturnal erection, tearing of the stamps occurs and it may even awaken the man during the night. One also needs to assess how rigid the erection is. For at least 2 days before the test, alcohol or sleep-inducing medication or sedative should be stopped. (Fig. 5.3)

Men may have erection without being aware of it during certain period of sleep, and the test confirms if the man is unable to maintain an erection at all. The test may be helpful in differentiating psychological or organic causes. During at least one of the three nights, if the ring of stamps is not broken between any two stamps, it may indicate that the erection problem is related to physical conditions or medications. On the other hand, the stamps may be broken on any of the three nights indicating nocturnal erection that is suggestive of erection problem from psychological causes. The test, however, is not foolproof and does not indicate many important factors, such as firmness of the erection or the duration or number of erections during the night. The procedure is repeated for several nights in a row before any conclusion is drawn.

Rigiscan

Rigiscan monitor is used to monitor penile size and rigidity during any study. It is strapped around the thigh. One loop of the monitor is then placed around the participant's penis about ¼ inch from its base. Another loop is placed around the end of the penis about ¼ inch from the glans penis. The patient sits in a comfortable armchair with a towel placed over his lap. Videos with neutral content followed by one with erotic content, and then another with neutral content, are successively presented. The erotic video includes explicit scenes of heterosexual activities such as vaginal intercourse, oral sex and manual stimulation. Erectile function is recorded during the videos, and blood samples are drawn at the end of each one. Heart rate and blood pressure are recorded at one-minute interval, while the subject watches.[33-36]

Biothesiometry

It is a test that uses vibration to measure the perception of sensation. A decreased perception of vibration may indicate nerve damage in the pelvic area that can lead to impotence.

Vasoactive Injection Test

When injected into the penis, certain medicines can cause erection by dilating its blood vessels and erectile tissues. Normally, these injections will produce an erection lasting about 20 minutes. During this procedure, the penile pressure is measured and X-rays may be taken of the penile blood vessels using a special contrast agent. Papaverine-induced penile erection (PIPE test)[37] is performed after injecting 60 mg of papaverine intracavernosally and erection is assessed after 10 minutes (see later).

Broderick et al[38] has graded the erectile response to distinguish the psychogenic and organic ED. Grades 0-3 denotes organic, while Grades 4-5 are suggestive of psychogenic ED (Table 5.7). Using a combination of papaverine, phentolamine and prostaglandin E-1 (Caverjet) is certainly better, as the incidence of priapism is 1 to 2% as against as high as 10%, when papaverine alone is used.

Table 5.7

Grade	***Erectile response** (visual grading)*
0	No response
1	Elongation of shaft
2	Moderate tumescence, no rigidity
3	Full tumescence, no rigidity, bendable
4	Full erection, partial rigidity
5	Full rigidity for 20 min.

Normal spectral waveform after papaverine injection has been described in five phases (Table 5.8).

Table 5.8

***Phase** I*:	Increase in both systolic and diastolic velocities.
***Phase** II*:	Progressive decrease in end-diastolic velocity and appearance of dicrotic notch.
(*Patients with severe venous leakage do not proceed beyond phase II.*)	
***Phase** III*:	Diastolic flow becomes almost zero.
***Phase** IV*:	Reversal of diastolic flow.
***Phase** V*:	Loss of both systolic and diastolic signals.

One of the complications of PIPE test is persistent erection or priapism. If tumescence persists, withdrawing 20 ml of blood from the cavernosa normally relieves the condition. Injection of 2 ml of 1 in 2,00,00 adrenaline almost invariably relieves any persistent tumescence. Pharmacologically induced priapism should subside within an hour and its persistence between 1 and 3 hours can cause cavernosal fibrosis with its attendant unfavourable effect on erection. If the aspiration of blood fails, 200 µg of phenylephrine hydrochloride diluted in 1 ml of normal saline can

be injected intracavernosally to induce vasoconstriction. Men with neurogenic impotence, sickle cell trait and on heparin therapy are particularly prone to develop priapism (see later—in penile intracavernosal injection).[39]

Specific Nerve Testing

These tests are indicated for patients with potential neurological causes such as diabetes with suspected nerve damage, a history of nerve disease, or where the physical examination uncovers an abnormality of the nervous system.

IMAGING STUDIES OF PENIS

Imaging studies include various invasive and noninvasive radiological examinations. Selective angiography of internal iliac and internal pudendal arteries is considered gold standard in the evaluation of vasulogenic erectile dysfunction. However, its invasive nature precludes its use as screening examination. Thus, color Duplex Doppler ultrasonography (CDDU) has become the most important investigation in the assessment of ED because of its noninvasive nature. Earlier, penile brachial index (PBI) was a very popular screening test to identify patients with arteriogenic impotence. PBI was calculated by dividing the mean systolic pressure in the penile arteries by the mean systolic pressure in brachial artery and values less than 0.7 suggest arteriogenic aetiology. However, it lacks the specificity as results show a considerable overlap between normal and abnormal patients.

Color Duplex Doppler Study

Color Duplex Doppler ultrasonography (CDDU) is commonly used noninvasive screening procedure. It is superior to continuous wave sonography as it allows clear visualisation of the deep cavernosal arterial tree. It is usually combined with intracavernosal injection of vasodilators, and measurements of blood velocity are done before and after the injections of vasodilators. In flaccid state, the cavernosal artery can follow a tortuous course; but in erect state, it becomes straighter. The transducer is placed on the dorsal aspect of the penis (Fig. 5.3).

The vasodilator drugs can be injected into any of the cavernous tissue at its distal third, as there is communication between two sides. There are various

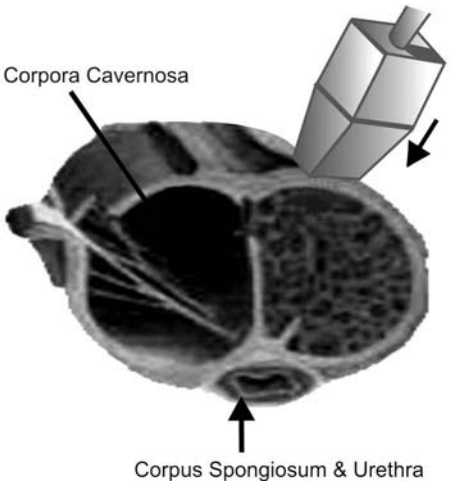

Fig. 5.3: Position of the transducer

options (see *intracavernosal penile injection therapy* later in the chapter) that are used:

a. Papavarine 60 mg in 1 ml solution.
b. Papaverine 40 mg with phenolamine 2.5 mg in 1 ml solution.
c. Papaverine 4.4 mg, phentolamine 0.15 mg with prostaglandin E-1 1.5 µg in 0.5 ml.
d. Sildenafil citrate - 50 mg (Table 5.9)

Table 5.9: Sildenafil citrate in CDDU

Advantages
– Non-invasive, oral
– No complications
– Accurate (Sensitivity 90%, specificity 100%)
Disadvantages
– Requires sexual stimulus
– Slow and delayed action

Interpretation

1. Adequate blood flow through one cavernosal artery is all that is needed for a satisfactory erection. A blood flow of above 30 cm/sec is considered a normal flow, but that of less than 25 cm/sec after the injection of vasodilator is suggestive of arterial disease. A flow between 25 and 30 cm/sec is considered a borderline value.
2. Apart from the velocity, calibre of the penile artery should also be assessed. If the calibre of the penile artery does not significantly increase after vasodilators, diffuse small vessel disease due to diabetes or diffuse vasospasm from nicotine or drug abuse is suspected.
3. Reversal of blood flow during systole may be seen in corporal fibrosis, trauma or arteriosclerosis.

This occurs due to proximal penile artery occlusion with collateral flow in retrograde direction.

4. Venogenic impotence occurs due to excessive venous leakage from the corpora. It can occur secondary to stretching of tunica, which prevents the compression of emissary veins draining the sinusoids during the erection process.[39] Traditionally, venogenic impotence is evaluated with cavernosometry and cavernosogram.

Normal values are mentioned in Table 5.10 and interpretation of results shown in Table 5.11.[40]

Table 5.10: Normal values

PSV (Peak systolic velocity) — 30 cm/sec or more
EDV (End-diastolic velocity) — 5 cm/sec or less
AT (Acceleration time) — 0.11 sec or less
RI (Resistive index) — 0.85 or more

Table 5.11: Interpretation of results

- **Arteriogenic ED-**
  1. PSV < 30 cm/sec
  2. AT > 0.11 sec
- **Veno-occlusive ED**
  1. EDV > 5 cm/sec
  2. RI < 0.85

NB-Veno-occlusive ED cannot be diagnosed in presence of arterial insufficiency

Cavernosometry and Cavernosogram
(see Chapter 8 for details)

Cavernosometry is done after induction of pharmacological relaxation of sinusoids using papaverine injection.[39,40] Two scalp vein cannula are put into each cavernosa near their bases, keeping them away from the urethra. Heparinised saline is then adminis-tered from a height using the gravity for the inflow. Intracavernosal pressure (ICP) is then measured. Cavernosmetry is usually combined with cavernosogram by injecting 40 ml of Urograffin and taking films in both oblique and anteroposterior positions of the penis. Cavernosogram is mainly used for confirmation of diagnosis of venogenic impotence. Venous leakage into deep dorsal vein can easily be identified through cavernosogram.

Assessment of Invasive Studies

Clinical effectiveness of these invasive studies is severely limited by several factors, including the lack of normative data, operator dependence, variable interpretation of results, and poor predictability of therapeutic outcomes of arterial and venous surgery. At present, these studies might best be done in referral centres with specific expertise and interest in investigation of the vascular aspects of ED.

After the history, physical examination, and laboratory tests, a clinical impression can be formed of a primarily psychogenic, organic, or mixed etiology for ED. Patients with primary or associated psychogenic factors may be offered further psychological evaluation, and patients with endocrine abnormalities may be referred to an endocrinologist to evaluate the possibility of a pituitary lesion or hypogonadism.

Unless previously diagnosed, suspicion of neurological deficit may be further assessed by complete neurological evaluation. No further diagnostic tests are necessary for patients, who favour noninvasive treatment (e.g., vacuum constrictive devices, or pharmacological injection therapy). Patients, who do not respond satisfactorily to these noninvasive treatments may be candidates for penile implant surgery or further diagnostic testing for possible additional invasive therapies.

A rigid or nearly rigid erectile response to intracavernous injection of pharmacological test doses of a vasodilating agent indicates adequate arterial and veno-occlusive functions. This suggests that the patient may be a suitable candidate for a trial of penile injection therapy. Genital stimulation may be of use in increasing the erectile response in this setting. This diagnostic technique may also be used to differentiate a vascular from a primarily neuropathic or psychogenic etiology.

Patients, who have an inadequate response to intracavernous pharmacological injection may need further vascular testing. It should be recognised, however, that failure to respond adequately may not always indicate vascular insufficiency, but can be caused by patient's anxiety or discomfort. The number of patients benefiting from more extensive vascular testing is few. They include young men with a history of significant perineal or pelvic trauma with anatomic arterial blockage (either alone or with neurological deficit) to account for ED.

Further clinical research is necessary to standardise methodology and interpretation, to obtain control data on normal (stratified according to age), and to define what constitutes normality in order to assess the value of these tests in their diagnostic accuracy, and in their ability to predict treatment outcome in men with ED.

MANAGEMENT OF ERECTILE DYSFUNCTION

Lack of unequivocal and clear definitions of clinical entity of ED causes a great deal of diversity in therapeutic interventions with resultant variety of entry criteria for patients in therapeutic trials. However, there is consensus that the least invasive procedures should be tried first and invasive therapy should not be the primary treatment of choice.

All patients must be evaluated and investigated thoroughly by taking detailed medical histories followed by investigations before any form of treatment is advised. The patient and his partner should be well informed about all therapeutic options including their effectiveness, possible complications, and costs.

Preventive aspects should always be looked into in details, wherever it is possible. Hormone replacement is certainly an indication, if the impotence is due to a hormone imbalance and/or systemic disease. Withdrawal of offending agents, whether it is drug or alcohol, is an important consideration.

Table 5.12: Principles of management

1. Evaluation
2. Prevention
3. Psychotherapy
4. Pelvic exercises
5. Medication
6. Other invasive measures

Principles of Management

In most cases, simple etiological evaluation of ED, whether the problem is organic or psychogenic, can be done by night-time sleep studies. Normal men should have three to seven erections per night.

Psychotherapy and/or behavioural therapy may be useful for some patients with ED without obvious organic cause, and for their partners as well. The therapy may also be used as an adjunct to other therapies directed at the treatment of organic ED. Data from such therapy, however, have not been well documented or quantified in the subcontinental context. In future comprehensive studies are indicated. Efficacy of therapy may be best achieved by inclusion of both partners in treatment plans.

Importance of pelvic muscle exercise has come to light in a study conducted by the researchers at the University of West of England, Bristol. They concluded that the pelvic floor muscle exercises showed same overall improvement in men with ED as seen in a large trial of Viagra.[41] This study included 55 men (with average age of 59), who had experienced ED for six months or longer. The men were given five weekly sessions of pelvic floor exercises and did daily home exercises. They were assessed at three and six months. The study found that 40 per cent of the men regained normal erectile function, 35 per cent had improved function and 25 per cent failed to show improvement. The pelvic floor exercises (*See Appendix 5)* also resulted in dramatic improvement in the 65.5 percent of the men with ED, who suffered dribbling after urination.[42]

Oral medications (discussed in details later) have a distinct edge over other forms of treatment. One can overcome irreversible organic impotence with a surgical penile implant or by the use of an injection to stimulate and maintain an erection. In many Western countries, thousands of men use penile implants, and many of their wives report that during intercourse, they cannot sense any difference from a normal erection. In the subcontinent, any operation is fraught with apprehension and with cost being a factor, a penile implant is within reach of only few urban rich.

Treatment should be individualised to the patient's desire and expectation. Even though there are several effective treatments currently available, long-term efficacy in general, is relatively low. Moreover, there is a high rate of voluntary cessation or drop-outs for all currently popular forms of therapy for ED. In India, the majority lives outside the urban areas and has no access to treatment by qualified doctors. Even today, treatment of ED is sadly confined to prescribing hormones. In urban India, mostly men go to the sex clinics run by non-doctors, who prescribe aphrodisiacs or hormone supplements with impunity because of lack of suitable legislation. Consequently, failure and drop-out rates are high. Better understanding of the reasons for each of these phenomena is needed. An andrologist should consider always counselling as part of the treatment regime.

CURRENT TREATMENT FOR ERECTILE DYSFUNCTION

Various methods that are employed in the treatment of ED currently are shown in Table 5.13.

Table 5.13: Various methods of treatment of ED

1. Psychotherapy or counselling, if appropriate.
2. Oral medication: Viagra, Yohimbine, Endocrinal therapy, etc.
3. Urethral suppository and local application of cream.
4. Penile rings and injection
5. External vacuum devices.
6. Internal pump devices and penile implants
7. Vascular surgery

PSYCHOTHERAPY AND BEHAVIOURAL THERAPY

Psychosocial factors are important in all forms of erectile dysfunction. Careful attention to these issues and attempts to relieve sexual anxieties should be a part of the therapeutic intervention for all patients with ED. Changing lifestyle is also one of the recommendations. The therapy has been reported to relieve depression and anxiety as well as to improve sexual function. Counselling may also help patients, who refuse medical and surgical interventions.

Psychotherapy should be directed to the coexistent problems such as issues related to the loss of a partner, dysfunctional relationships, psychotic disorders, or alcohol and drug abuse. Psychological treatment focusses on decreasing performance anxiety and distractions, and on increasing a couple's intimacy and ability to communicate about sex. Education concerning the factors that create normal sexual response and ED, can help a couple to cope with sexual difficulties. Working with the sexual partner is useful in improving the outcome of therapy. However, data of this form of therapy have not been quantified and evaluation of the success of specific techniques used in these treatments is poorly documented. Studies to validate their efficacy are, therefore, strongly indicated.

Along with psychotherapy, some andrologists have also experimented with some medicines like apomorphine with variable success.[43] Similarly, Moclobemide (MAO-A inhibitor) has a beneficial effect on ED in patients suffering from psychogenic ED.[44]

PELVIC MUSCLE EXERCISE

See appendix 1 at the end of the chapter.

MEDICINAL THERAPY

Before formulating any medicinal therapy, it is imperative to exclude potentially reversible medical problems contributing to ED (Table 5.14). Accordingly, in patients with medication-induced ED, reduction or substitution of the offending of medications that can do away with erectile problem or lessen its chance, should be considered first, before any other definitive therapy is started.

Table 5.14: Potentially reversible causes of erectile dysfunction

1. Patients on medications for high blood pressure
2. Patients on medicines for depression
3. Patients, who have endocrine problems, like thyroid or pituitary problems
4. Patients, who have partner conflict
5. Patients, who smoke cigarettes
6. Patients who use recreational drugs; alcohol, marijuana, cocaine, heroin
7. Patients with anatomical abnormality of penis
8. Men less than 45 years of age may have a correctable cause of vascular impotence

Hormone Medication

For some patients with an established diagnosis of testicular failure (hypogonadism), androgen replacement therapy may sometimes be effective in improving erectile function. A trial of androgen replacement may be worthwhile in men with low serum testosterone levels, if there are no other contraindications. In contrast, for men who have normal testosterone levels, androgen therapy is illogical and may carry significant health risks, especially in those with unrecognised prostate cancer. If androgen therapy is indicated, it should preferably be given in the form of intramuscular injections of testosterone. Currently available oral androgens are mostly not equally effective. For men with hyperprolactinaemia, bromocriptine therapy often is effective in normalising the prolactin level and improving sexual function. [45, 46]

Testosterone

At present, there are various options that are available for treatment with testicular hormones (Table 5.15). A severe deficiency of testosterone can in some cases cause impotence, where the effective treatment is to give either oral testosterone or an intramuscular injection to raise the hormone to acceptable levels. Only about 4% of the male population, however, benefits from this form of treatment.[47,48] (See appendix of chapter 3).

Table 5.15: Methods of testicular hormone treatment

1.	**Injection therapy:**
	a. Free testosterone preparation (Aquaviron)
	b. Various combinations of testosterone propionate, phenyl propionate, isocaproate, enanthate and undecanoate (Sustanon, Testoviron depot, Testanon)
2.	**Oral therapy:**
	a. Testosterone undecanoate (Nuvir, Andriol)
	b. Mesterolone (Provironum, Restore)
3.	**Skin application:** Androstenalone or dihydrotestosterone (Androlactin) — scrotal patch
4.	**Other methods:** Insertion of pellets, gels, etc.

Side effects of testosterone replacement therapy can be serious, and patients with a medical history that includes liver, heart or kidney problems, or prostate cancer, should avoid testosterone supplement. Testosterone therapy is also known as HRT (hormone replacement therapy) or TRT. It can cause side effects like retention of fluids, enlargement of the prostate, and damage to the liver. As pure androgen is not absorbed orally, in a synthetic androgen derivative the structure is modified to facilitate its oral absorption. They cause decrease in aromatisation of estrogen (see Appendix in Chapter 3) and increase the concentration of 5α dihydrotestosterone (DHT). Testosterone undecanoate has minimal suppressive effect on the hypothalamic-pituitary function. Androgen administration to eugonadal men with ED may activate their sexual behaviour without enhancing erectile capacity and without effects on mood and psychological symptoms.[49]

ORAL MEDICATIONS

Viagra *(Sildenafil citrate)*

Viagra is a new medication by Pfizer for the treatment of ED. The drug was approved Food and Drug Administration (FDA), USA in March 1998 for distribution in the USA. FDA reports that Viagra has helped two out of three impotent men to improve their sexual function. This prescription in pill form became the first nonsurgical treatment for ED that does not require either an injection or insertion directly into the penis. In India, several pharmaceutical companies have launched the product in different names like Penagra, Androz, etc. in three strengths of 25, 50 and 100 mg.

Sildenafil is effective for most types of organic, psychogenic, and mixed ED. It requires sexual stimulation to enhance the erectile mechanism and does not provide a spontaneous erection. It is an erection enhancer, but does not affect the libido or desire in normal men. Moreover, it is not an aphrodisiac. If a patient is unable to initiate an erection, Viagra may not work. Libido is not affected, although if a man feels that an erection is more likely to occur, libido may be increased as a secondary effect of using the medication.

Mode of Action

Viagra works by storing penile blood flow in an already-stimulated penis. To be effective, one must be able to initiate a partial erection in response to sexual stimulation. In the absence of this partial erection, or without sexual or tactile stimulation, it does not work. It enhances the normal erectile process by regulating vasodilatation in the penis by inhibiting the enzyme phosphodiesterase-5 (PDE-5) that helps to break cGMP, the chemical substance produced during sexual stimulation (Figs 5.4 and 5.5). By inhibiting the PDE-5 enzyme, Viagra allows continuation of the action of the cGMP. Success with its treatment, thus inherently depends on the presence of cGMP in the body (Fig. 5.4)

According to reports released in May, 1996, the success rate of Viagra was 65 to 88% compared to

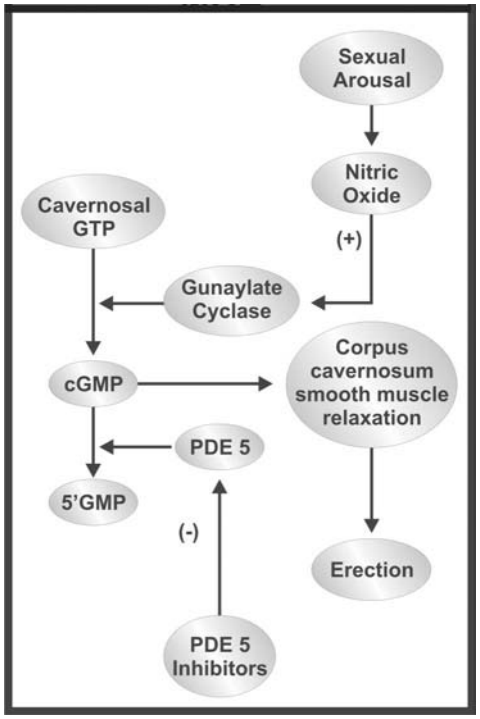

Fig. 5.4: Oral medication (Mode of action)

39% for those subjects receiving a placebo. According to an Associated Press article (27.10.97),[50] Viagra achieved a success rate of 80%, (no placebo results were published). Later reports showed that the dose response curve to Sildenafil is statistically better than that of placebo, especially over a period. However, it is less effective in diabetic and postprostatectomy patients, and those with complete organic ED.

Administration

Viagra should be taken in empty stomach to enhance its absorption as delayed gastric emptying following a meal decreases its efficacy. Diet rich in fat or excessive protein such as meat that delays gastric emptying should be avoided. For its optimum action, one should give about an hour to an hour-and-a-half before engaging in sexual activity. The bottom line is sex with Viagra needs planning. Excess of alcohol may at times diminish a man's already diminished erectile function. So it should better be avoided; otherwise, it may make it a little bit counter-productive.[51-57]

Initial recommended dose is a single tablet (50 mg to start with) taken approximately one hour before initiating sexual activity. Lower doses of 25 mg may be used in patients, who are elderly, sedentary, have renal or hepatic insufficiency, already taking cytochrome P_{450} inhibitor, have spinal cord injury (who are more susceptible to all types of erectogenic medications), or moderate to severe cardiovascular insufficiency. No more than one dose is recommended daily and doses greater than 100 mg is not recommended. The timing of taking the medicine may need to be individualised because of its half-life of 1 to 4 hours and variability of physiological response in different men.

Side Effects

The most common side effects are headache (25.4%), flushing of the face (30.8%), stuffy nose 18.7%) and heartburn (10.5%).[58] Some patients complain of hypersensitivity or a bluish tinge to light. The side effects for most men are very mild, transient, and they are very well tolerated. Modest changes in blood pressure can occur, but these changes are generally not significant and do not cause symptoms or increased morbidity in most men. Normally, the erection starts after a period of 30 to 60 minutes, but one of the complications is persistence of erection — a disturbing side effect noted in some cases.

One of the important side effects that have been occasionally reported is defect in colour perception particularly blue, especially in diabetic patients or those having vascular disorders. The visual disturbance is probably due to some weak inhibition of PDE located in the retina. Some men report a slightly blurred vision or a blue-tinge to their vision. No long-term problems have been reported and these visual effects are totally reversible. Most men, who reported this phenomenon, still continued to take Sildenafil in the trials because of its positive effects.

However, Dr Howard Pomeranz from the University of Maryland, in November 2000 reported five men with permanent visual damage following taking of Viagra. According to him, "Viagra regulates chemicals in the body to constrict the arteries and the drug may cut off the supply to people with low cup-to-disk ratio. Cup-to-disk ratio is measured by noting the small circular indentation, where the optic nerve connects the eyeball." About three per cent of patients develop visual disturbances at 100 mg dose.

Most talked about side effects which at times could be life-threatening, is when Viagra is used along with nitrate containing medication. In these patients, Sildenafil can cause excessive vasodilatation and hypotension. Concomitant use of nitrates is the most important contraindication to Viagra. According to a statement released by the American College of Cardiology (ACC) and the American Heart Association (AHA), combination of Viagra (Sildenafil citrate) and nitrate-containing medicines may be lethal (*Viagra combined with nitrates may be lethal* —statement of ACC and AHA on 8.11.98**).** The statement provides recommendations for the prescription of Viagra for cardiac patients with diverse medical profiles as well as treatment procedures for patients on Viagra, who experience a cardiac event. "Viagra should not be prescribed to patients taking nitrates. We want physicians and the public to know that the combination can be deadly," said Dr Adolph Hutter Jr of Massachusetts General Hospital, co-chairman of the ACC expert panel that issued the statement. [59]

The ACC and the AHA urged physicians to take precautions in prescribing Viagra to patients with certain other cardiovascular profiles, even if they are not taking nitrates. Markers for concern include active coronary ischaemia, congestive heart failure combined with borderline low blood pressure, or a multidrug regimen for high blood pressure. Patients taking certain drugs, such as erythromycin or cimetidine,

and patients who have severe liver or renal disease, may prolong the action of the drug. When treating conditions like cardiovascular diseases including heart attacks and unstable angina, physicians must establish the time of the patient's last dose of Viagra, which may remain in the body for up to 24 hours or more. In addition, the combination of Viagra and inhaled nitrates, such as amyl nitrates or poppers (an illicit recreational drug) could prove to be fatal and should be avoided.

The ACC also recommended caution when using Sildenafil in patients having strong recent history of angina or myocardial infarction, or who have severe left ventricular dysfunction. However, there is a scenario, when a man uses Sildenafil for a brief period and then, with confidence restored by improved erections, manages to continue satisfactory sexual activity without it.

Recently, the WHO has come out with a protocol for prescribing Sildenafil. This includes proper screening of patients with physical examination, blood sugar estimation, ECG and penile Doppler study to avoid indiscriminate use. WHO also recommends bi- or triweekly administration only once a day and strict monitoring by competent specialists to obviate any untoward effects. Alpha-blockers taken with drugs like sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis), may interact and cause low blood pressure. There is, however, no evidence of cardiovascular problems associated with the use of Viagra in men in contact with inorganic nitrates, such as those commonly found in food products or from environmental sources such as automobile exhaust and cigarette smoke.

Yohimbine

Yohimbine, which is made from the bark of the yohimbe tree, has been available for many years. Doctors have been prescribing Yohimbine as a natural aphrodisiac for men with intermittent ED. Its efficacy in improving ED has never been clearly proven, especially when the strong placebo effect of any oral medication for this problem is considered. Results have been slightly better in men with primary psychogenic etiology. The drug is used to stimulate desire and improve the quality of the erection.

Yohimbine is an effective therapy to treat organic ED in some men as evidenced by improved and increased rigidity on RigiScan testing.[60] There is substantial evidence available suggesting that noradrenergic and opiate transmitter systems are involved in regulation of sexual function. Yohimbine is supposed to be working on the adrenergic system. Guay[60] also evaluated the effects and safety of a potential novel treatment combination for ED consisting of both, the alpha-adrenoreceptor antagonist yohimbine, and the opiate antagonist naltrexone.

Dispensed in tablet form in doses of 10 to 20 mg, yohimbine is taken three times a day for 4 to 6 weeks to test its effect. Success is probably achieved in 15 to 20% patients, and in most cases stopping the tablets often reverts the patient to his former state of impotence. Side effects may include headache, sweaty palms, dizziness and nausea. Men with ulcers or hypertension probably should not take this drug. Adverse effects of hypertension and increased heart rate, and its proscription with most antidepressants and in patients with cardiovascular disease make Yohimbine significantly less useful than Sildenafil.

In a study published in the New England Journal of Medicine, the herb "yohimbehe" was found to increase libido and frequency of ejaculation. Yohimbehe is thought not only to be effective for some types of impotence, especially of vascular, diabetic or psychogenic origins, but can in some cases also improve the quality and staying power of erections, usually without increasing sexual excitement. But dizziness, nervousness and anxiety are some of the side effects. The American Urological Association (AUA) does not recommend Yohimbine hydrochloride for ED. However, in one European study shows, on-demand oral administration of 6g L-arginine glutamate and 6 mg yohimbine combination is effective in improving erectile function in patients with mild to moderate ED. It appears to be a promising addition to first-line therapy for ED.[61]

TAP

'TAP Holdings' apomorphine acts by antagonising dopamine in the central nervous system (CNS).[43] In a carefully selected group of impotent patients with no documented organic causes of ED, but with proven erectile potential, significant durable erections have occurred with oral or sublingual dose of 3 or 4 mg of apomorphine. Its injectable forms are used in veterinary medicine to induce vomiting. Oral administration of 6 mg results in lower blood levels of dopamine that can trigger nerve impulses in the brain to cause an erection in more than 70% of

patients. Predictable side effects include nausea (14%), vomiting (3%) and yawning. Its different mode of action (central versus peripheral) could prove useful in Viagra failures.

Herbs

Herbs or other botanical medicines such as s*aw palmetto*, which were originally used for enlarged prostate, possibly have some effect on testosterone levels in the body. *Saw palmetto* helps to maintain the proper hormone balance of prostate, which is needed for sexual function, especially in older men. An optimally functioning prostate gland not only produces and delivers semen, but is also a part of the biochemical and mechanical process involved in achieving an erection. Selenium is one of the important ingredients that is very often lacking in older men and can be found in *horsetail*, which has been used with success in ED following prostatic enlargement. Helpful herbs include *ginger, prickly ash bark, chickweed, sarsaparilla, garlic, turmeric and pumpkin seeds.*

For men with low testosterone levels, "*Korean* and *Siberian ginseng*" may be able to enhance virility and is thought to be a remedy for infertility. Korean red ginseng can be used as an effective alternative for treating male ED.[62]

Not withstanding the evidence that certain herbs could be helpful in some cases of ED, there is unanimity amongst the medical experts that these herbal remedies are not as effective for medical problems like infertility or erectile dysfunction like the conventional drug therapies. Documented scientific research on the herbal products is often never published to prove their authenticity. Some herbs, in fact, may cause infertility or damage sperms genetically. A study at Loma Linda University suggests that the side effects of such herbs as *St. John's wort*, *Echinacea* and *ginkgo biloba* might include blocking conception and the possibility of genetic damage to sperms.

NEWER ORAL MEDICINES

Vardenafil and Levitra

Two pharmaceutical giants Bayer and Glaxo-Smith-Kline jointly have developed Vardenafil — a potent and selective PDE-5 inhibitor. It is active at lower doses, and targets the same erection-inhibitory chemical a little more specifically than Viagra.[63, 64] In Britain, the companies launched the verdenafil as Levitra claiming it to be more potent than Pfizer's Viagra or Eli Lilly's Cialis. Like Viagra, men taking these drugs still require sexual stimulation and arousal before getting an erection. Participants achieved peak levels of erection within 30 to 36 minutes of taking vardenafil and reported good results regardless of the cause of ED. Its efficacy is 90% for most men taking the drug.[33] The results of phase III clinical trials of the drug were presented at the annual meeting of the AUA in May 2002. In one North American trial over 26 weeks, 85 per cent of men taking the drug reported improved erections, compared with 28 per cent taking a placebo. It is not associated with the visual disturbances occasionally reported with Viagra.

Table 5.16: Newer oral medicines

1.	Vardenafil and Levitra
2.	Cialis (Tadalafil)
3.	Uprima
4.	Vasomax
5.	Trazodone
6.	Melanotan

Pharmacodynamic studies showed that the maximum plasma concentration after oral administration of 20 to 40 mg of vardenafil occurred in 0.7 to 0.9 hour, the half-life was 4 to 5 hours, and negligible amounts remained in the circulation after 24 hours.[65] The findings of many workers confirm that vardenafil dispensed in 20 mg and 40 mg tablets were able to generate stronger erections of longer duration than placebo under conditions of visual sexual stimulation in patients with ED. In trials with 1020 patients, who took the drug for one year, the average success rate in maintaining erections improved to 82% from the initial 14% with a dose of 10 mg, and to 86% from 16% with a 20 mg dose.

The FDA approved Levitra (vardenafil), the second impotence drug to be marketed in the United States and the first to rival Viagra. There have been no head-to-head comparisons of Levitra and Viagra, but that data are suggestive. Levitra seems to work faster than Viagra, with a "window of opportunity" of 16 minutes to five hours vs the average one to four hours that are indicated on Viagra's package insert. There are also no food or alcohol restrictions on Levitra. The general recommendation for Viagra is to take it after a low-fat meal or on an empty stomach because fatty foods inhibit absorption of the drug.[66]

Men using alpha-blockers for high blood pressure or prostate problems, also should not take Levitra as such combinations could cause blood pressure to drop to an unsafe level. Moreover, in patients taking certain medications such as ritonavir, indinavir, ketoconazole, itraconazole, and erythromycin, lower doses of Levitra are recommended, and time between doses may need to be extended. In clinical trials, reported side effects were headache, flushing, and stuffy or runny nose. Levitra is available in 5 mg, 10 mg, and 20 mg tablets. The starting dose of Levitra is 10 mg taken no more than once per day.[67-73]

However, Cialis (see below) can work all day unlike Levitra with a half-life of 4 to 5 hours. But the merits of each drug in terms of potency, speed and duration of action are hotly disputed by respective companies. Bayer and GSK said that Levitra had been shown *in vitro* to be a more potent inhibitor of PDE-5 than its rivals. The companies (Bayer and GSK) also argue that the drug Levitra is not circulating in the body for any longer than that is necessary and allows a patient to be confident of its use. Moreover, they claim that the adverse effects were not severe and tended to decrease with time.

Cialis

Cialis (Tadalafil), which is made and marketed by Lilly ICOS, a US-based joint venture of Eli Lilly & Co. and ICOS Corporation, is yet to receive final clearance from the FDA in America. But it has received marketing authorisation from the European Union (EU) in December 2002. Cilais is a second generation selective PDE-5 inhibitor. The selectivity ratio vs PDE-5 is more than 10, 000 for PDE-1 through PDE-4 and PDE-7 through PDE-10, and 780 for PDE-6.[74] Cialis has been claimed to have 93% efficacy with a 50 mg dose in European trial. It is reported to work faster than vardenafil. It is absorbed slightly faster than Viagra and can be taken with food, making sex more spontaneous. It lasts longer in the bloodstream for about 34 hours, thus one needs to take it once almost for the whole weekend. Drug-related adverse effects, such as headache were observed in 23% of patients (placebo, up to 17%), dyspepsia in 11% (placebo, up to 7%), back-pain in 4.7% (placebo, 0%), and myalgia in 4.1% (placebo, up to 2.4%). The effects were mostly mild to moderate.[74] Neither drug-related serious cardiovascular adverse events nor colour vision disturbances were encountered.

According to Dr Ann Taylor, Director of Impotence Association in UK, " It is always an irritating problem that couples using Viagra have to make love within a short time frame." With Cialis, this difficulty can be obviated with an opportunity even several hours later. In one study by Dr Gerald Brock, associate professor of urology at the University of Ontario, 88 per cent of the 212 participants with ED were able to have sex within 16 minutes of taking the drug. Many were still able to have sex the next day, before the drug wore off. A few experienced side effects such as headaches, mild muscular aches and facial flushing, much the same as in the case of Viagra. According to urologists, the biggest demand is expected to come from men above 70, who are not prepared to accept that their sex lives are over. Cialis should not be used with nitrate-based drugs (nitroglycerin) or certain alpha-blocker medications used to treat enlarged prostate and high blood pressure. This combination could lower blood pressure enough to cause fainting and even death.[75] Comparative merits and demerits of different PDE-5 inhibitors are shown in the following Table 5.17.

Table 5.17: PDE-5 inhibitors

	Sidenafil	*Verdenafil*	*Tadalafil*
1. Brand names	Viagra	Levitra	Cialis
2. Availabiltiy in mg	25/50/100	5/1/20	10/20/50
3. Onset of action in minutes	30	25	30
4. Duration of action in hours	4 to 5	4 to 5	Up to 36
5. Reaction with food	Yes	Yes	No
6. Side effects			
1. Headaches	Yes	Yes	Yes
2. Flushing	Yes	Yes	Yes
3. Dyspepsia	Yes	Yes	Yes
4. Nasal congestion	Yes	Yes	Yes
5. Visual disturbances	Yes	No	No
6. Back pain and myalgia	No	No	No
7. Contra-indications	–	–	
Organic nitrates or nitric oxide donors and Alpha-blocker:	Yes	Yes	Yes

Uprima

Extensive experimental data on the proerectile effect of apomorphine support its exclusive central site of action on the brain as well as on the spinal cord. The treatment of ED in human being with apomorphine is validated by the mechanism of action seen in the

animal models. It also emphasises the key role of the dopaminergic system in the control of erection. Clinical effects of apomorphine do not rule out the occurrence of undesirable side effects due to its actions on the CNS.

In clinical practice, however, approved doses of apomorphine SL (Uprima) are well tolerated. It is noteworthy that no modification of sexual desire was observed with apomorphine. Its action within the CNS may more likely to interact with sexual desire than peripherally acting drugs, and care should be taken to assess this point in future research. Although our knowledge of the control of penile erection by the CNS is restricted, there are many potential sites for CNS-acting ED drugs. New centrally acting therapy for ED should concentrate on receptor targets more specific to erectile command. Clinical efficacy of the new centrally acting compound will assess the well-founded purpose of this rationalisation. Uprima is to be taken sublingually about an hour before intercourse. One of the areas of concern is the rare event of syncope induced by apomorphine, and further reasearch should be conducted before it is allowed universal use.[76,77]

Vasomax

Vasomax is said to contain the formulation of phentolamine mesylate, a generic antihypertensive drug that has been on the market since 1952. In clinical studies, a 40 mg dose of rapidly oral dissolving tablets has produced erections in 30 to 40% of patients compared to 15 to 20% with placebo. It acts as an alpha-adrenergic receptor antagonist. However, as Vasomax has been found to cause liver abnormalities and cancer in animal studies, it is not likely to be made available clinically in the present form.

The major advantage of Vasomax over Viagra is a faster onset of action of 15 minutes against almost one hour for Viagra. Overall, sildenafil was more effective and appeared to be better tolerated than phentolamine for the treatment of ED.[78] The drug produces an alpha-adrenergic block that dilates vessels in the smooth muscle throughout the body, and therefore, is contraindicated in people with history of myocardial infarction, angina or coronary insufficiency. Side effects include tachycardia, hypotension, dizziness and rhinitis. It is believed that this drug's modest efficacy limits its use.

Trazodone

The antidepressant drug Trazodone, taken one hour before sexual activity, has been found to prolong erections in men, who are able to obtain, but not maintain an erection during intercourse. Trazodone, however, is much less effective than Viagra.

Melanotan

Preliminary trials with a synthetic version of a hormone melatonin produced in the brain have enabled men with ED to maintain an erection. In the most recent study by Dr Hunter Wessells,[79,80] 10 men with ED caused by such condition as diabetes, heart disease, injury or high blood pressure, were given subcutaneous injections with Melanotan II at a dose of 0.025 mg/kg body weight. Accordingly, 60% achieved erection and men experienced about 2.5 erections over a period of six hours, when the effects of the drug wear off.

Melanotan acts on a region in the hypothalamus and caused men to get an erection without sexual stimulation. A remarkable feature is its effect causing increased sexual desire for men. The drug takes about 1.5 hours to take effect and about 20 per cent of men experienced nausea as side effects. The researchers are continuing their evaluation and at this early stage they are primarily concerned with drug safety, side effects and figuring how exactly it acts on the brain region responsible for stimulating the penis. Wessells[79] pointed out in the interview, "Future work will assess just how low a dose has an erection-causing effect. Lower doses or altering the molecule may reduce side effects." The erectogenic properties of Melanotan II are not limited to cases of psychogenic ED, and men with a variety of organic risk factors also had penile erections.

URETHRAL SUPPOSITORY AND TOPICAL CREAMS

Urethral suppositorly (MUSE or Medicated Urethral System for Erection) is another form of drug treatment for impotence. It is based on the finding that the urethra can absorb certain medications into the surrounding tissues, creating an erection. Muse urethral suppositories use prostaglandin (PG) E1 (alprostadil), the same medication used in a self-injection therapy. An erection usually begins within 5 to 10 minutes after administering a prefilled MUSE dosage. The erection lasts approximately 30 to 60 minutes, though this can vary from person to person.

The most common side effects associated with MUSE are aching in the penis, testicles, legs and in the area between the scrotum and the rectum, a warmth or burning sensation in the urethra, redness of the penis due to increased blood flow, and minor

urethral bleeding or spotting due to improper administration. Psychologically, it is perhaps more pleasing than an injection, but its effectiveness and side effects preclude its use.[80,81]

The pellet acts by widening the penile blood vessels, increasing blood flow to the penis. Since its FDA approval in 1996, patients response to MUSE has been very disappointing. This method is less effective and more painful than penile injection therapy. MUSE has become much less popular with the advent of various PDE-5 inhibitors like Viagra.

Procedure: After urination, a small applicator tip (1/8"wide) is inserted just over approximately 1½ inch inside the penile urethra. By gently pushing the button on top of the applicator, a dose of alprostadil in the form of a suppository is delivered. Absorption occurs through the urethra wall (urethral mucosa). This relaxes the smooth muscle in the penis resulting in rapid arterial inflow and penile rigidity. MUSE was shown to begin the erectile process in 5 to10 minutes after application. Urethral insert had an advantage that it avoided the injection and is applied approximately 1½" inside the penis using an applicator. Pressing the plunger puts a little pellet of medication into the urethra. Unfortunately, it does not give a very firm erection. It has more side effects than the injection and is more painful[81,82] (Fig. 5.5).

Topiglan (Urethral Gel)

MacroChem Corporation's Topiglan, in the form of topically applied urethral gel achieved 80 to 90 per cent response rate for ED. Alprostadil is a naturally occurring substance that is present in male semen. Sepa®, a patented drug delivery technology permits the drug to be absorbed through the surface of the skin. The data presented by Irwin Goldstein, Professor of urology at the Boston University School of Medicine, at the 93rd annual meeting of the AUA held in San Diego, confirms the safety and efficacy of Topiglan. Topiglan differs from other ED treatments in that *it is a topical gel* that is applied directly to the glans, thereby minimising the potential for systemic side effects. Transurethral alprostadil and alprostadil-prazosin combinations produced erections in men with complete organic ED. This combination therapy may be an option for patients, who do not respond to transurethral alprostadil alone.[82] However, Topiglans may cause enlargement of the glans penis. However, this method also causes some penile ache and does not produce a very rigid erection.[81-84]

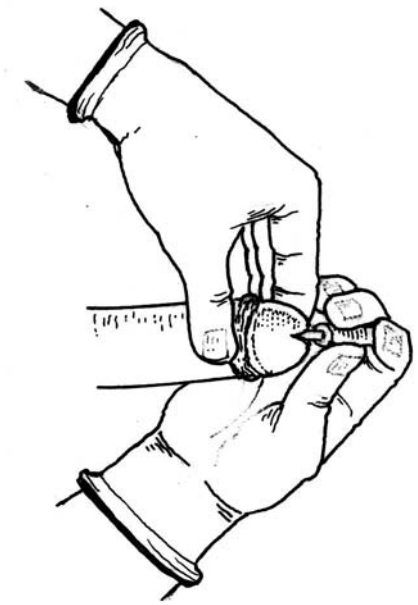

Fig. 5.5: Intraurethral application

CONSTRICTION RINGS

Rings are useful in men, who can obtain good erections initially, but cannot maintain them for completion of intercourse. Rings may also be used in conjunction with penile injection therapy or intraurethral treatment.

INTRACAVERNOSAL PENILE INJECTION THERAPY

History of 'intracavernosal penile injection therapy' dates back to early 1980s with the discovery that some medications injected directly into the corpora cavernosa would produce an erection within a few minutes. Injection of vasodilator substances into the corpora of the penis has been found to be effective for a variety of causes of ED. Urologists can generally teach a person the technique of self-injection at home in one or two visits.

Drugs Used

Several medications have been used for this purpose. Papaverine, which acts as a vasodilator, was the first drug used. Phentolamine, an alpha-blocker, was used next, (initially as an additive to papaverine) and lately PG-E-l. Phentolamine an α-adrenergic agent potentiates the effect of papaverine, and PG-E-1 minimises the priapism, which can occur in 2 to 8% of cases.

Papaverine and prostaglandin both act on smooth muscle tissue in the corpora cavernosa, while phentolamine has effect on the tiny penile arteries to prolong the erection. Most doctors use combination of all three drugs together (see CDDU in Chapter 8).

The drugs typically used for injection therapy include prostaglandin (PGE-1 or Prostin or Alprostadil or Caverjet), papaverine hydrochloride and phentolamine (Regitine). The most effective and well-studied agents are papaverine, phentolamine, and PG E-1.

Papaverine and phentolamine have not yet been approved for treating impotence by FDA, USA even though they were the first ones used for injection therapy. It has approved all these drugs for other medical uses, with prostaglandin approved specifically for treating impotence. Nevertheless, urologists have gained considerable experience over the past decade with all three drugs, which are now considered safe for injection therapy. PG E-1 has been the drug used for injections since 1995. Alprostadil is a naturally occurring substance in the penile tissue. In addition to PG-E-1, use of a combination medication called Trimix (which contains papaverine, phentolamine, and alprostadil) is very successful and safe, and is currently the preferred penile injection method.

Method

Diabetic needles (27 or 28 gauge, a half-inch long) are used for these injections. The patient must learn to inject the base of the penis using less than 1 ml to either corpus cavernosum safeguarding the urethra (Fig. 5.6). Hand pressure is applied afterward for 2 to 3 minutes to prevent bleeding. The injections are relatively painless and create an erection that begins about 5 to 15 minutes after the injection. Ideally, the erections should last for 30 to 60 minutes and become more rigid with stimulation. Not all patients respond to this type of treatment and 70% of men achieve satisfactory erections with injections. Failure of the therapy in 30% cases is often due to poor blood flow or venous leak.

Self-injection therapy can be used any time and only involves a short preparation (Fig 5.7). It creates an erection that is very similar to the body's own spontaneous erection, and generally lasts long enough for successful and pleasing intercourse. It also does not interfere with orgasm or ejaculation. Nevertheless all men are not good candidates for self-injection therapy and the cost can still be prohibitive for some people in the subcontinent and other developing countries. Patients must return for follow-up visits, particularly at the beginning of the treatment process, as there may be necessity for adjustment of doses or changing of drugs.

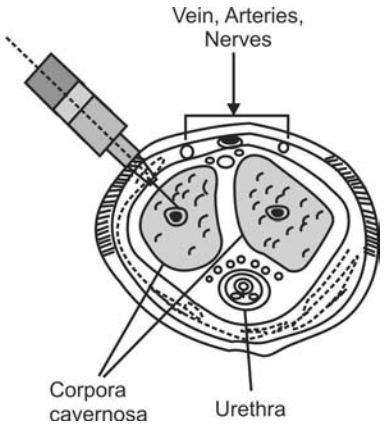

Fig. 5.6: Site of penile injection

Side Effects

There are areas of concerns with injections. The pain is primarily at the site from the needle puncture. A dull penile ache is experienced by 40 per cent of patients using PG E-1, but this is transient and well tolerated in the majority of patients.

Priapism (prolonged or inappropriately persistent erection) is an unwanted side effect. Overdose of the drug may cause an erection, which lasts much longer than intended. Priapism is treated with adrenergic agents (see PIPE test), which can cause life-threatening hypertension in patients receiving monoamine oxidase inhibitors. If the erection lasts for more than four hours, medical help for reversal of the erection is warranted (see priapism, later).

Use of the penile vasodilators also can be problematic, even risky in patients, who do not tolerate transient hypotension and those with severe psychiatric disease, poor manual dexterity to be able to inject

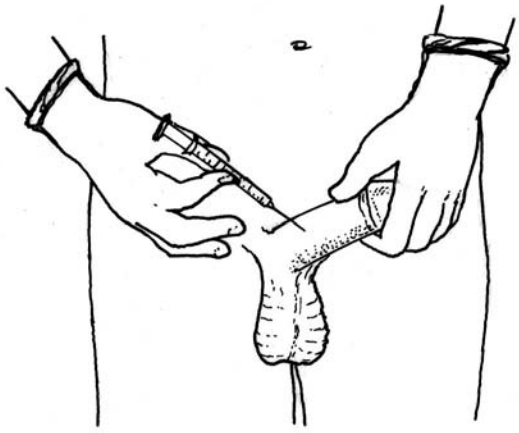

Fig. 5.7: Self-injection therapy: Injection is chosen for the specific area of the penis

the medication at the precise point, poor vision, and those receiving anticoagulant therapy. Some men complain of dizziness, palpitations and/or a flushed feeling when using these medications. There is a minor chance of infection and the possibility of bleeding or bruising during injection.

Another complication is the possible development of permanent scarring within the penis. Scarring from PG E-1 injections is minimal (occurring in only 5 per cent of cases) and the satisfaction rate is high. Scarring is generally seen in patients, who use the drugs too often. Consequently, self-injection therapy should be limited to once every four to seven days, depending on the medication is used and response to the initial treatment. Scarring can also interfere with erections. If the scarring is severe, later placement of a penile prosthesis can be difficult.

Although the penile injections are very effective, instance of long-term use is poor. Nearly 80% of patients in the first year, and over 50% of the patients in the first two months of therapy drop out of injection therapy. So certain amount of motivation is required for it to be effective in the long term. Quite a few men, however, are frightened to think of injecting the penis with a needle. This apprehension may partly account for the high dropout rate for men on injections. A 1990 study (University of Chicago) showed that 51% of the group dropped out after receiving only a test injection.[85]

Patients treated with these agents should give full informed consent as all medications have potential risks and side effects. Patient education and follow-up support might improve compliance and lessen the dropout rate (Tables 5.18 and 5.19).

EXTERNAL VACUUM THERAPY (Vacuum Constrictive Devices)

External vacuum therapy is probably the most widely recognised treatment since it works for most forms of impotence with minimal side effects. ErecAid® System is the original external vacuum device. Vacuum devices are simple mechanical tools that allow a man with ED to have sexual intercourse by initiating and maintaining erections. It works best in men, who are able to achieve partial erections on their own. Geddings Osbon created the external vacuum device in the early 1960s to solve his own impotence problem. He created the ErecAid® System, based on negative pressure, and tension rings to produce and maintain a naturally engorged erection every time one was needed. Its works on the principle of bringing more blood into the penis at first, and then trapping it. Of the two models, ErecAid Esteem System (battery and manual models) and ErecAid Classic System are available without a prescription.

Table 5.18: Mode of actions of common erection enhancing drugs

1. **Yohimbine**	α-adrenergic antagonist — Blocks presynaptic autoreceptors and enhances adrenergic receptors activities, which also alters serotonin and dompamine transmission.
2. **Trazodone**	α-adrenergic blocker and central inhibitor of serotonin reuptake.
3. **Apomorphine**	Dopaminergic agonist with its main action on the paraventricular nucleus.
4. **Phentolamine / Moxisylyte**	α-adrenergic antagonist (competitive blocker of α_1-adrenoreceptors).
5. **Alprostadil**	Stimulates adenyl cyclase to increase intracellular level of cGmp lowers intracellular concentrations of calcium — thus relaxes the arterioles and smooth muscles.
6. **Papaverine**	Inhibitory effect on the PDE causing increased calcium influx and calcium accelerated potassium and chloride channels.
7. **Sildenafil citrate and similar products**	Phosphodiesterase inhibitor (PDE).

Table 5.19: Summary of various drugs used for erectile dysfunction[17]

	Route	*Mechanism*	*Effectiveness*
1. **Yohimbine** (Yocon)	Oral	Alpha receptor agonist	Poor
2. **Testosterone**	Injection, patch, oral		Good (only with low testosterone levels
3. **PG E1** (Caverject)	Penile injection	Dilates penile arteries	Very good
4. **Papaverine/regitine/ prostaglandin**	Penile injection	Dilates penile arteries	Very good
5. **PG E 1**(MUSE)	Intraurethral pellet	Dilates penile arteries	Moderate
6. **Sildenafil** (Viagra) **other similar/drugs**	Oral	Increases penile blood flow	Research data Very good response
7. **Phentolamine**	Oral	Increases penile blood flow	Research stage

Mechanical vacuum devices cause erection by creating a partial vacuum, which draws blood into the penis making it engorged and expanded. It works very much like a breast pump.

The devices have three components: a *plastic cylinder*, into which the penis is placed; a *pump*, which draws air out of the cylinder; and an *elastic band*, which is placed around the base of the penis to maintain the erection after the cylinder is removed and during intercourse by preventing blood from flowing back into the body. The user stretches the tension ring around the open end of the cylinder, and then inserts his penis into that end. Holding the device firmly against his body to form an air seal, he uses the pump to remove air from inside the cylinder. This creates a partial vacuum around the penis, causing the extra blood to enter the corpora cavernosa. This engorges the penis in a way similar to a natural erection. They have to use a lot of lubrication over the penis and shave the base of the penis.

To maintain the erection, it is necessary to reduce the outflow of blood from the penis. Therefore, while the penis is still under vacuum pressure, the tension ring is pushed from the cylinder on to the base of the penis. This breaks the seal of the vacuum, allowing the cylinder and pump to be removed and laid aside. Using a vacuum device involves a mechanical process that takes generally 5 to 10 minutes to set up. So it can interfere with foreplay. The user can maintain an erection for up to 30 minutes, wearing only the tension ring. The body shapes of some men can make it difficult to apply these devices. Once the O-ring is applied, there is no erection between the rubber band and the body, making the penis somewhat floppy. In some men the O-ring inhibits the normal flow or ejaculation after orgasm. This is not harmful, and semen does pass once this rubber band is removed. The erection stops, when the tension ring is removed. FDA recommends its use no longer than 30 minutes.

The ErecAid® System has been effective for over 90% of men including men after prostatectomy and also after removal of penile implants. It has also been used successfully with blockage of vessels, in psychological and in diabetic patients. Of 200,000 subjects after using the system for 90 days, 80% said they were having sexual intercourse at least twice a month. Initially, it takes practice to use the System. About 42% of patients learn to use it eventually and 90% master it in two weeks. Almost 69% can create a usable erection within two minutes.[86,87]

Advantages and Disadvantages

The most significant advantage of external vacuum device is that it is safe, noninvasive and works without medications, injections or surgery. It is used on the body (not in the body), and can stay in a dresser drawer or on a shelf, when not in use. The second advantage of ErecAid is that the erections are of high quality lasting longer than natural ones and do not usually disappear after an orgasm. Therefore, the system is also effective for treating premature ejaculation. Once the erection technique has been learned, the patient can achieve reliable and consistent erections each time. The vacuum device can be used any time at a patient's convenience. Another major advantage is safety. These devices work for almost any type of erectile problem, and cost less than surgery or ongoing self-injection therapy (Figs 5.8 and 5.9).

An unexpected statistics that emerged from the survey is occasional restoration of natural erection

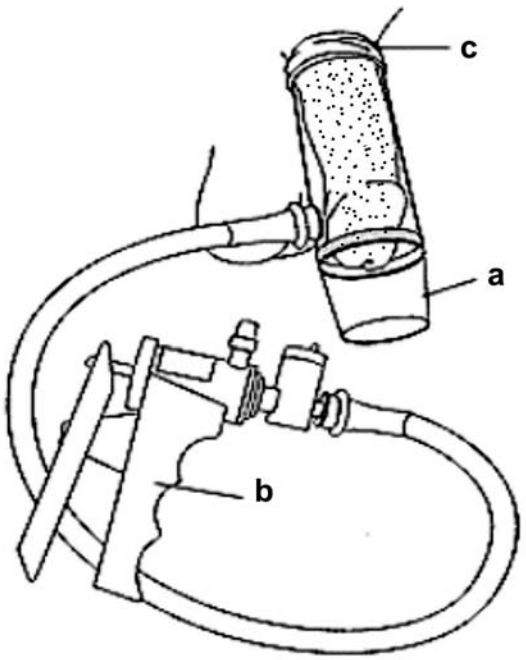

Fig. 5.8: A vacuum-constrictor device causes an erection by creating a partial vacuum around the penis, which draws blood into the corpora cavernosa. Pictured here are the necessary components: (a) a plastic cylinder, which covers the penis; (b) a pump, which draws air out of the cylinder; and (c) an elastic ring, which, when fitted over the base of the penis, traps the blood and sustains the erection after the cylinder is removed (www.noah-health.org).
http://kidney.niddk.nih.gov/kudiseases/pubs/impotence/index.htm. · [10]

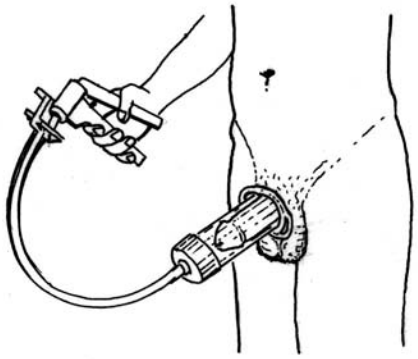

Fig. 5.9: Vacuum device

after its use for a while. About one in four (26%) reported that after using the system for a number of months, they were sometimes able to have intercourse without using the device. This means that the use of a vacuum device to force blood into the penis may have the effect of bringing back some sexual power in some cases. This was also noted in a case by the Western Reserve University Medical School study (Cleveland, Ohio) in 1989-1990.

With some men, minor side effects can occur such as petechiae and ecchymosis. Placing the penis under negative pressure too rapidly causes petechiae or reddish pinpoint-size dots on the surface of the penis. The penis may need to be reconditioned slowly after a prolonged period of inactivity. The penis held under vacuum pressure for too long also causes ecchymosis or a bruise. This condition, however, is neither painful nor serious, and passes off without any active treatment. Another side effect is penile temperature drop of 1 to 2 (degree) caused by the tension ring. In addition, some men complain of coldness and/or numbness of the penis after the O-ring is placed. The O-ring should be removed after 25 to 30 minutes. Otherwise, it can restrict blood flow. No major injuries have ever been reported concerning the ErecAid® Systems.

While the initial success rate is high with vacuum devices, less than one-third of the men ends up using the vacuum pump long term. However, often patients complain that the whole process is painful and that the erection is not natural. Its proper use requires some manual dexterity and average hand strength.

Use of tension rings and the loss of spontaneity in lovemaking are two main criticisms of these devices. This is definitely not the preferred treatment option for couples that enjoy spontaneous, normal, and frequent sexual activity.[87-89] This device may not be an appropriate treatment for men with sickle cell anaemia, leukaemia, or blood clotting problems or who take aspirin regularly.

With overall low incidence of side effects, many couples, especially the men, believe that they are far better sexual partners with the device. There is still a significant rate of patient dropout.

SURGICAL TREATMENT

Surgery usually has one of the three goals:[12]

1. To implant a device that can cause the penis to become erect.
2. To reconstruct arteries to increase flow of blood to the penis.
3. To block off veins that allow blood to leak from the penile tissues.

Common surgical procedures performed for erectile problems are insertion of penile prosthesis and corrective vascular surgery.

Penile Prosthesis

Implanted devices, known as penile prostheses, can restore erection in many men with ED. This treatment involves the surgical placement of a stationary or movable device into the two sides of the penis, allowing erections as often as desired. This treatment is not recommended until other methods have been considered or tried first.

The main indication for penile prosthesis is for patients refractory to or having contraindications to any medicinal therapy. Ideal prosthesis should allow acceptable penile erection to achieve satisfactory vaginal penetration. Education of the patients and their partners is very important to have its satisfactory use. These implants come mainly in two forms: an *inflatable device* and *semirigid rods*. The semirigid prosthesis can be malleable or mechanical types[90] (Table 5.20). Malleable implants usually consist of paired rods, which are inserted surgically into the corpora cavernosa. The user manually adjusts the position of the penis and therefore the rods. Adjustment does not affect the width or length of the penis (Fig. 5.10A).

Table 5.20: Types of common penile prosthesis

1. ***Semirigid***	
a. Malleable	Mentor malleable Accuform
b. Mechanical	Dura II
2. ***Inflatable***	
a. Two piece	Ambicor
b. Three piece	Alpha 1 (normal, narrow base or with backout valve

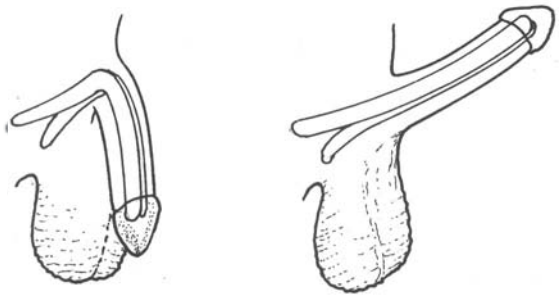

Fig. 5.10A: Semi-rigid penile implant. Penis is always erect with semi-rigid implant. To hide erection the rod has to be bent.

Inflatable implants consist of paired cylinders, which are surgically inserted inside the penis and can be inflated using pressurised fluid. Tubes connect the cylinders to a fluid reservoir and a pump, which are also surgically implanted. The patient inflates the cylinders by pressing on the small pump, located under the skin in the scrotum. Inflatable implants can somewhat expand the length and width of the penis. They also leave the penis in a more natural state when not inflated (Fig. 5.10B and C).

Internal Penile Pump

Inflatable prostheses include**:**

1. A multicomponent prosthesis consisting of a fluid pump located in the scrotum, a reservoir in the lower abdomen, and two inflatable cylinders, and

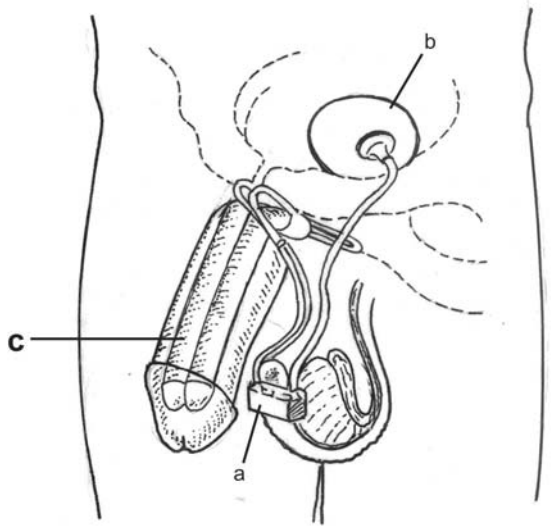

Fig. 5.10B: In an inflatable implant, erection is produced by squeezing a small pump (a) implanted in a scrotum. The pump causes fluid to flow from a reservoir (b) to two cylinders (c) residing in the penis. The cylinders expand to create the erection.[88]

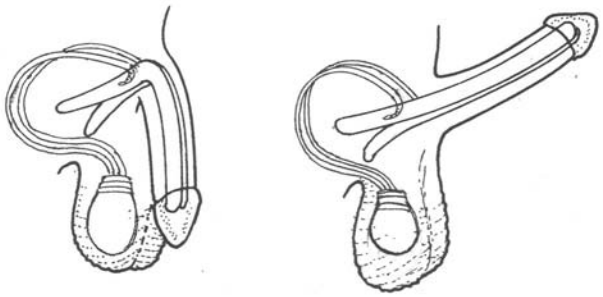

Fig. 5.10C: Inflatable penile implant. Inflatable implant uses a tiny pump and a reservoir. Pump pushes the fluid from the reservoir into the inflatable tiny cylinders placed in the penis to cause erection.

2. A self-contained inflatable prosthesis is composed of two-sealed cylinders, each containing fluid, a pumping mechanism, and a release valve.

In an inflatable implant, works with discrete squeeze of the pump that produces an instant erection that is maintained till such time as the release valve is similarly squeezed.[10,89,90]

According to J François Eid, New York Presbyterian Hospital-Weill Cornell Medical College,- "Inflatable penile prosthesis (also known, as the internal penile pump or IPP) is a remarkable device that can restore the possibility of intercourse to men suffering from severe sexual dysfunction."[90]

In the USA, 250,000 men are currently the secret keepers of the IPP. The device is surgically implanted in the penis and scrotal sac. The IPP is a soft saline-filled device that can expand and contract without losing its elasticity. It consists of three small components: very thin tubes, a pump, and a reservoir. The reservoir contains the saline, which is transferred into the penis by a gentle squeeze of the scrotal sac, where the pump is housed, causing the tubes in the penis to fill and become rigid.

The IPP has some excellent advantages over oral and injectible medications, and its surgical predecessors. There are no drug side effects and the entire IPP can be placed through a 2 to 3 cm opening in the scrotal skin in less than an hour unlike other methods that require much larger openings with far more painful recoveries. The IPP is entirely invisible in both the flaccid and hard penis, and does not interfere with normal sensation or ejaculation. A man is able to have a full erection at any time without having to plan ahead that is necessary with other treatments.

The operation is done through a small one-inch opening in the scrotum. As there is no cuts in the penis, the sensitive penile skin is preserved. The device is put through underneath from the scrotal sac, where the opening is made. Two little sleeves are put through a tiny opening about a quarter of an inch in the penile shaft. The sleeves are extended inside the penis all the way down at the base of the penis underneath the scrotal sac. Two inflatable cylinders are put in the little sleeves. The pump is placed between the two testes in a way that is accessible, yet looks cosmetically appealing so that a patient can go to a locker room without being embarrassed in front of other men. The penis feels totally normal and the patient does not feel its presence inside. Squeezing the pump to transfer the fluid from the reservoir into the cylinders results in erection. Feeling of erection is entirely normal, so is the ejaculation and orgasm. The IPP, thus restores patient's anatomy and at the same time his ability to have a spontaneous erection.

The IPP is an excellent alternative for men who do not respond to Viagra or similar drugs. It can be used for elderly men, who are sexually motivated and active even after treatment for prostate, bladder, or colon cancer treatment, and who have penile deformity and/or atrophy. It is still one of the safe and successful cures for organic impotence. While a penile implant or injectible medication can improve sex life, it does not guarantee fertility.

The effectiveness, complications, and acceptability vary among the three types of prostheses, with the main problems being mechanical failure, infection, and erosions. The mechanical problems have diminished in recent years with technological advances. In some silicon particle shedding has been reported, with migration to regional lymph nodes. However, no clinically identifiable problem has been reported as a result of the silicon particles.

Although the inflatable prostheses may yield a more physiologically natural appearance, they had a higher rate of failure requiring re-operation. Men with diabetes mellitus, spinal cord injuries, or urinary tract infections have an increased risk of prosthesis-associated infection. This form of treatment may not be appropriate in patients with penile corporal fibrosis, or severe medical illness. Circumcision may be required for patients with phimosis and balanitis. Common complications of prosthesis applications[91] are summarised in Table 5.21.

Table 5.21: Common complications of prosthesis

1.	Crural perforation during operation
2.	Postoperative infection
3.	Postoperative pain
4.	Cosmetic problem related to position
5.	Pressure erosion and encapsulation of the prosthesis
6.	Mechanical problem.

Vascular Surgery

Vascular surgery aims to restore blood flow in patients with arterial block or to stop the venous leak during erection. Penile revascularisation and venous ligation are microsurgical procedures similar in technical complexity of a heart bypass operation, although they clearly do not carry anywhere near the same risk. In principle, the surgery to repair arteries relieves the obstruction to the flow of blood to the penis. The best candidates for surgery are young men with discrete blockage of an artery from an injury to the crotch or fracture of the pelvis. The procedure is contraindicated in older men especially with widespread blockage.

Surgery of the penile venous system involves an intentional blockage by venous ligation on patients, who have been demonstrated to have venous leakage. Blocking off veins (ligation) can reduce the leakage of blood that diminishes the rigidity of the penis during erection. Experts have raised questions about the long-term effectiveness of this procedure. It is rarely done, as the selection of patients for a predictably good outcome is difficult. Moreover, decreased effectiveness of this approach has been reported as longer-term follow-ups have been obtained. This has tempered enthusiasm for these procedures, which are therefore best done in specialised medical centres by experienced surgeons.

Arterial revascularisation procedures have a very limited role (e.g., in congenital or traumatic vascular abnormality) and probably should be restricted to to medical centres with experienced personnel. All patients who are considered for vascular surgical therapy need to have appropriate preoperative evaluation. This may include dynamic infusion pharmacocavernosometry and cavernosography, Duplex ultrasonography, and possibly arteriography. The indications and interpretations of these diagnostic procedures are incompletely standardised, and there are difficulties in using these techniques to predict the success of surgical therapy.

Overall result of surgical treatment can be summarised as excellent for implants especially the penile in flatable devices, moderate to good for discrees arterial block and rather poor in venouslization.

CHOICE OF TREATMENT

After a long period of frustration in the management of ED, advancement of medicine has now made it reversible in many patients. Research for an effective oral medicine with rapid action was on for a number of years, but no effective medicine was discovered till fourth quarter of the last century. In patients in whom psychogenic ED is suspected, sexual counselling should be offered first. Taking an oral drug such as 'Yohimbine' did help a few men, but undesirable side effects occurred, and results were perceived often weeks later. A few did also benefit from taking hormone medications in cases of severe hormone deficiency. Urethral gel and Topiglan often get patients' preference due to their easy noninvasive application. Urethral suppository represents a different delivery of the drug 'Alprostadil', but its success rate is less than that of injections. Even if other noninvasive treatments such as 'Yohimbine' or counselling are tried first, the vacuum treatment with a success rate of nearly 90% can be used at the same time to obtain immediate results.

Penile injections have been used for over a decade with nearly 70% success rate, but many men express disdain for this treatment, when they learn that it involves a needle stuck into the penis. However, there are many impotence clinics, which continue to use this therapy.

Advent of 'Sildenafil' or 'Viagra' indubitably has changed the whole perception of the treatment of ED, and no doubt has added a new dimension to the treatment of impotence or ED. Notwithstanding its side effects and contraindications for its use in heart patients on nitrite medication, it has caught the imagination of general public. However, long-term results are awaited. There are promising new drugs such as Cialis or Levitra on the horizon that may work even better. Viagra still has some limitations such as being not so effective in diabetic and postprostatectomy subjects.

In the subcontinent, quite a few indigenous medicines (some of herbal origin) were claimants for effective oral treatment. A wide variety of other substances taken either orally or topically have been suggested to be effective in treating ED. But these claims are unsubstantiated, and no authentic scientific data were published. Most of these medicines have not been subjected to rigorous clinical studies. Their use should, therefore, be discouraged until further evidence in support of their efficacy and safety is available. If a universally acceptable and safer oral medication eventually is discovered, it will surely require healthy corpora cavernosa for it to work. An implant unfortunately destroys this part of the penis from being useful again.

Implanted devices, of course, involve surgery. Experts now believe that this treatment, once considered as the "gold standard" therapy, should only be done as a last resort, when the lesser invasive treatments have failed. Penile prostheses should be considered only after patients have been carefully screened and informed. Only an expert with extensive clinical experience should undertake vascular surgery after thorough clinical investigations. With any form of therapy for ED, long-term follow-up by health professionals is required to assist the patient and his partner.

Psychotherapy and behavioural treatments, and sexual counselling alone or in conjunction with other treatments may need to be used in all patients with ED. The motivation and expectations of the patient and his partner, and education of both, are critical in determining which therapy is chosen and in optimising its outcome. If single therapy is ineffective, combining two or more forms of therapy may be useful.

Follow-up is an essential part of management of ED. Reviewing the success of treatment or its lack, any adverse effects, and considering alteration of dose treatment is more likely to achieve the patient's goal. Follow-up should include continued patient education and exercising the option for a change, if earlier therapies are unsuccessful. Careful assessment of reasons for cessation of a particular form of therapy by the patients should also be a part of any follow-up regime.

It is the sole responsibility of the physicians to have a free and sympathetic dialogue with the patient as well as his partner. Often, a clinician fails to appreciate that ED can result from problems in the patient's partner and/or difficulties in their relationship.[92] He should then put forward his opinion about the suitability of a particular form of treatment after he has explained the full spectrum of available

alternatives. If initial treatment modalities fail, more invasive alternatives or combination therapy should be offered to cure the patient's ED.

IMPROVING PUBLIC AND PROFESSIONAL KNOWLEDGE

Plethora of scientific information about ED is now available, yet it has not percolated down to great majority of health care professionals let alone public at large. In fact, most of the doctors and obviously the patients remain relatively uninformed or even misinformed. In India, many doctors continue to consider testosterone supplement as the panacea for any erectile problem. Patients in the subcontinent are far too inhibited to initiate any detailed discussions themselves. Lack of information together with reluctance of physicians to deal candidly with sexual matters has resulted in patients being denied the benefits of modern treatment for ED. Improving both public and professional knowledge about ED is a must to usher in more open communication and more effective treatment of this condition. The primary care clinicians with good communication skills, who are knowledgeable about the first-line management for sexual problems, can plan initial work-up and treatment. Consultation with specialists may be done at appropriate intervals after the initial work-up of men with ED.

Education and reassurance would be helpful in preventing escalation of minor into serious erectile failure in individuals with only minor erectile difficulty caused by medications or chronic illness. Elderly men are often conditioned to accept ED as an accompaniment of progressive ageing, but their problems are often compounded by illness and medicines, they take for other unrelated ailments.

In India, inaccurate public information regarding sexual function and dysfunction often appears in the form of advertisements in which enticing promises are made. Patients become even more demoralised, when these promised benefits fail to materialise. Herein lies the importance of seeking the benefit of only expert professional help. At the same time, doctors must be aware of these men nurturing the feeling of embarrassment to come out with their erectile problems.

Strategies

To reach a sizable audience, communication strategies should include informative and accurate newspaper and magazine articles, and special educational programmes in radio and television. To summarise, one needs to have following strategies:

1. To define specific information about the etiology and management of ED suitable for the knowledge of medical personnel and general public.
2. To introduce courses in human sexuality in the curricula of medical schools.
3. To include sessions on diagnosis and management of ED in continuing medical education courses.
4. To put emphasis on the importance of obtaining a detailed sexual history as part of every medical history as sexual well-being is an integral part of general health.
5. To put emphasis on an integrated multidisciplinary approach in the diagnosis and treatment of ED.
6. To encourage presentations on ED at scientific meetings of speciality medical associations or medical societies.
7. To distribute scientific information from lectures, and panel discussions on ED to the news media (print, radio, and television) and to provide them with active supports to disseminate accurate information on this subject.
8. To counteract misleading news reports and false advertising claims.

FUTURE THERAPY

The ever-increasing interest in the field of erectile function and dysfunction has stimulated ongoing research in the subject for crystallising the choice and direction for therapies. Improving the horizon of current therapies with further developments in the second and third generation of medications such as active PDE inhibitors and new dopamine receptor agonists are on the anvil. Newer medications are on trial and may soon be available to supplement treatment with sildenafil. Oral phentolamine, apomorphine, newer PDE type-5 inhibitors, and topical agents are currently in phase III trials.[93]

Combinations of existing therapeutic principles such as PDE-5 antagonists (Viagra, Vardenofil and Cialis) and an alpha (1)-adrenoceptor (AR) antagonists (apomorphine-Uprima) appear to be an attractive alternative for resistant cases with one set of drugs. Nitrosylated alpha (1)-AR antagonists, combining NO donation and alpha (1)- or alpha (2)-AR antagonism are currently being evaluated.[94]

Drugs targeting CNS such as Melanocortin-receptor agonists have shown promise not only in animal models, but also in preliminary studies in humans. Other possible targets such as growth hormone-releasing peptide receptors are being explored.

New peripheral targets such as Rho-kinase antagonism and non-NO-mediated stimulation of soluble guanylyl cyclase have been suggested as possible new principles for drug development. Progress has been made in intracavernosal somatic gene therapy.

Gene Therapy

The past two decades have seen revolutionary changes in the treatment of ED from inventions of penile prosthesis and the oral agents. However, despite their efficacy, all forms of treatments have significant drawbacks and side effects. On the other hand, gene therapy has the potential to be the best and most physiologic treatment for ED.

Findings in future population-based studies may disclose the presence of a particular mutation of a gene or gene variants that may predispose to the development of ED. Altered expression or activities of some smooth muscle regulatory components of the ischaemic, diabetic, or ageing penis have been reported.[95]

Gene therapy is an exciting method of restoring erectile function, but regulatory requirements may not allow its use for many years to come.[96] Gene therapy involves the transfer of selected genes into a host with the hope of ameliorating or curing a diseased condition. Initially, gene therapy was envisioned for the treatment of genetic disorders, but is currently being studied in a number of acquired diseases. One important consideration for a gene therapy strategy includes: a sufficient understanding of the pathogenesis of the targeted disorder, potential side effects of treatment, and understanding of the target cells to receive the gene therapy.

The most elementary step in gene therapy is that the relevant gene must be identified and cloned. Upon completion of the Human Genome Project, gene availability is expected to be unlimited. Once the gene has been identified and cloned, the next consideration must be expression. Questions pertaining to the efficiency of gene transfer and gene expression remain at the forefront of gene therapy research. Currently, much debate in the field of gene therapy revolves around the transfer of desired genes to appropriate cells, and then obtaining sufficient levels of expression for treatment of the disease.

Penis is an ideal organ for gene delivery because of its external location for delivery of specific genes. In penis, only a small number of cells need to be transfected as its syncytial tissues having interconnected gap junction, allow a collective response.[97] Since smooth muscle relaxation is the final step for penile erection, many molecules and enzymes in the signal transduction pathway for smooth muscle relaxation can be potential targets for the treatment of ED, e.g. NOS and potassium channel (maxi-K channel, hSlo). In addition, many growth factors have been shown to enhance angiogenesis and regeneration of nerves and thus can also be used to treat vasculogenic or neurogenic impotence.

The most commonly used vehicles for gene transfer are:

1. Plasmid DNA vector (naked DNA) or liposome-DNA complexes, and
2. Viral vectors (retrovirus, adenovirus and adeno-associated virus). Theoretically, the penis is an ideal organ for gene therapy because of its external location and slow circulation.

Several groups of researchers have successfully performed gene therapy in animal experiments to date. Garban and associates[98] transfected NO gene into old rats' penis and were able to improve erection in those rats. Christ et al performed gene therapy with potassium channel opener and showed significantly improved erection in diabetic rats. Huard et al[99] showed that myoblast-mediated gene therapy was more successful in delivering iNOS into the rat penis than direct virus or plasmid transfection methods. Wessells and Williams[100] also showed successful endothelial cell transplantation into the corpus cavernosum and suggest that it may be a more efficient method of gene transfer.

Two experiments were performed: one with VEGF-165, the other with adeno-associated virus-VEGF. Both were injected into the corpus cavernosum several minutes after the internal iliac arteries were ligated. In the VEGF treated group, the erectile function was restored at six weeks. In the AAV-VEGF group, the function did not return until ninth week, because of the delayed production of VEGF by the transfected VEGF gene. The control groups only showed minimal restoration of erectile function. Although gene therapy is an exciting method of restoring erectile function, regulatory requirements

may not allow its use for many years to come[101-104] (See chapter 4-VEGF).

Growth Hormone

Cavernous nerve injury resulting in ED is common after radical pelvic surgery patients. Studies have been conducted on the feasibility of using growth hormone to enhance the cavernous nerve recovery in the rats. The result was quite exciting since many of these rats recovered erectile function much faster and more completely than those did not receive growth hormone. In another animal experiment, erectile function was also successfully restored in rats, which had undergone ligation of both internal iliac arteries.

Many of the growth factors known to enhance nerve growth and angiogenesis are available for clinical use today and may become the best preventive and therapeutic option in the next decade. Growth hor-mone (GH) may be of major importance in the maintenance of male erectile capability, probably through a stimulating effect on cGMP generation in human cavernous smooth muscle, and that a decline in GH release may contribute to the manifestation of ED.[105-106]

The final direction is prevention strategies. Strategies to prevent cavernosal degeneration and/or to restore cavernosal function will be one of the most exciting challenges for future research. Long-term prospects of all these new exciting therapies are awaited. But they have to prove their efficacies against advantages over conventional pharmacological therapies already established.

CONCLUSION

Great advancement in the diagnostic, investigative and therapeutic aspects of ED in the last decade of the twentieth century has ensured that in many patients the condition is now reversible. However, advances in dynamic investigations of ED using various inducing agents such as papaverine may still lead to an incomplete erectile response. This may potentially lead to patients being incorrectly labeled.[107]

Psychotherapy and behavioural treatments, and sexual counselling alone or in conjunction with other treatments may need to be used in all patients with ED. The motivation and expectations of the patient and his partner are critical in determining which therapy is chosen and in optimising its outcome. If single therapy is ineffective, combining two or more forms of therapy may be useful.

There is a sea change in the individual interest and societal awareness of the problem of ED in recent times. There has been virtual explosion of knowledge in this subject, but there are still lacunae in our knowledge. Moreover, controversies have also crept in as regards definition of what constitutes ED, standardisation and development of precise protocol for management.

There is unanimity of opinion that good communication is the foundation for an enduring relationship. When couples encounter sexual difficulties, communication can be strained or break down entirely even in the best of relationships. Erectile dysfunction may divide and distance couples often causing conflict and emotional pain. Couples may intentionally or unintentionally ignore or deny the condition, thus delaying treatment. Fortunately, many sexual difficulties can be improved or resolved through open communication and a mutual commitment to learn about the condition and treatment options.

In the subcontinent, there is now increasing awareness amongst men and women. Lifestyle and social values have changed and old joint family concept is slowly giving way to nuclear family system with its attendant stress. Consequently, the incidence of ED is certainly on the rise. In the USA, 80% men with ED are not treated, because they do not seek medical attention or their physicians do not initiate a dialogue about sexual problems during their visits.[108] The incidence is much more in India given the reticence of patients and the doctors to open a dialogue.

Cause-specific assessment and treatment of male sexual dysfunction will require recognition by the public and the medical community that ED is a part of overall male sexual dysfunction. Erectile dysfunction changes the societal and individual perceptions and expectations.[108] Public and the medical community need to understand these consequences. The multifactorial nature of ED comprising of both organic and psychological aspects, often requires a multidisciplinary approach. Dedicated research teams with experts representing urology (with a special interest in male reproductive functions and ED), geriatrics, medicine, endocrinology, psychiatry, psychology, epidemiology, bio-statistics and basic sciences should form a team. Different types of ED often have overlapping pathophysiologies, but may also have common pathways contributing to ED. Such pathways may be potential treatment targets.

A coordinated, holistic and focussed approach encompassing an in-depth study of demographics, aetiology, pathophysiology, diagnostic assessment and treatment (both generic and cause-specific) is thus absolutely necessary to assess the risk factors of ED. Only after this exercise, a comprehensive formulation of ED would really be possible.

Table 5.22: Treatment of priapism

Low-flow venous type	*High-flow arterial type*
1. Immediate aspiration of blood.	1. Ice-packing to cause vaso-spasm and spontaneous thrombosis.
2. ICI with α-adrenergic agonist. (250-500 mcg of phenylephrine diluted in normal saline/ 5 mins till detumescence is achieved.	2. Angiographic embolisation of internal pudendal artery. 3. Rarely surgical ligation

*NB. For **chronic and acute recurrent types**—first the ICI with phenylephrine is tried for sexually active patients. For others antiandrogens or GnRH agonist can be used.*

PRIAPISM

Priapism is a form of erectile disorder that is caused by stagnation of accumulated blood in the cavernous tissue after erection. Almost exclusively the corpora cavernosa are affected, and the glans and the corpus spongiosum escape the pathological process except in rare cases of tricorporeal priapism. Mainly, two varieties are recognised. Acute low-flow (veno-occlusive) priapism is painless to start with; but if it lasts for several hours, pain follows from resultant tissue ischaemia. High flow priapism is caused by the arterial factor following perineal or direct penile injury.

In low-flow types, the corpora cavernosam are always in a fully rigid state. In the high-flow type, it could be either partial or at times fully rigid. However, all cases of priapism are always due to initial high flow of blood. At times, the priapism could be chronic or acutely intermittent, which are always difficult to diagnose. Intercavernous injection (ICI) of pharmacological agents and sickle cell disorders are common etiological conditions. Patients with neurogenic and psychogenic EDs commonly predispose to priapism after ICI.

Blood gas estimation of the aspirated corporal blood is the most important diagnostic measure. In low-flow type, the blood gas would reveal a venous type of oxygenation value, while the high-flow type shows an arterial type value. CDDU is the most convenient diagnostic tool used for establishing diagnosis. But in an advanced andrological set up, Tc^{99} scan can confirm the diagnosis. Minimal blood flow with distended corpora would be revealed in a low-flow type; but in a high-flow type, there is unregulated blood flow from the traumatised arterial source.

Diagnosis of priapism is usually based on history and physical examination. The treatment is aimed to relieve firstly, the pain and secondly, the erection to prevent permanent damage to corpora, which may lead to impotence. Methods of treatment are summarised in Table 5.22[109] (See PIPE Test earlier).

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APPENDIX 1

Pelvic Floor Exercises (Kegel's exercise)

Strengthening of the pelvic floor muscles has been shown to improve control of the bladder and the bowel. These exercises are used with a bladder-training program, in people with urge incontinence as well as for ED.

How to Contract the Pelvic Floor Muscles

1. The first thing to do is to correctly identify the muscles that need to be exercised.
2. Sit or lie comfortably with the muscles of your thighs, bottom and stomach relaxed.
3. Tighten the ring of muscle around the back passage as if you are trying to control or wind and then relax it.
4. Practice this movement several times, until you are sure you are exercising the right muscles. Try not to squeeze your bottom.
5. When you are passing urine, try to stop the flow mid-stream, and then restart it. Do this only to learn which muscles are the correct ones to use, and then do it no more than once a week to check your progress. If you do it more often than this, it may interfere with normal bladder emptying.
6. If you do not feel a definite squeeze and lift action of your pelvic floor muscles, or are not able to even slow the stream of urine as described in point 3, talk to a health worker, a continence adviser, a doctor, or physiotherapist. They can help to get your pelvic floor muscles working correctly. Even men with very weak pelvic floor can be taught these exercises and will benefit.

While doing Pelvic Floor Exercises

You can feel the muscles working and exercise them by:

1. Tightening and drawing in strongly the muscles around the anus and the urethra all at once. Lift them up and inside. Try and hold this as you count to five then release and relax. You should have a definite feeling of 'letting go'.
2. Repeat ('squeeze and lift') and relax. It is important to rest for about 10 seconds between each contraction (tightening of the muscles). If you find it easy to hold for a count of five, try to hold for longer - up to ten seconds.
3. Repeat this as many times as you are able to up to a maximum of 8-10 squeezes.
4. Now do five to ten short, fast, but strong contractions.
5. Do this whole exercise routine at least 4-5 times every day.

What You Should Not Do

1. DO NOT hold your breath.
2. DO NOT push down instead of squeezing and lifting up.
3. TRY NOT to tighten your tummy, bottom or thighs.

Do your exercises well - the quality is important. Fewer good exercises are better than lots of half hearted ones.

Make the Exercises Part of Your Daily Routine

Once you have learnt how to do these exercises, they should be done regularly, giving each set your full attention. It might be helpful to have at least five regular times during the day for doing the exercises. For example, exercise after going to the toilet, when having a drink and when lying in bed. Other things you can do to help your pelvic floor muscles are

1. Share lifting of heavy loads
2. Avoid constipation and prevent any straining during a bowel movement
3. Seek medical advice for hay-fever, asthma and bronchitis to ease sneezing
4. Keep weight within the right range for the height and age.

APPENDIX 2

Sources of Indian Herbal Medicine

1. **Carom seed** (*ajwain*), which is rich in thymol should be crushed and fried in ghee, butter or olive oil together with an equal quantity crushed kernels of tamarind seeds. A teaspoon taken with honey or milk before going to bed improves virility and cure premature ejaculation.
2. **Asafetida** (*hing*) is also known as devil's dung or ferula. It is available either as a light brown resin or as a powder. It is aphrodisiacal, when used liberally in cooking.
3. **Powdered cardamom** (*ealaichi*) seed boiled with a spoonful of honey is used as a remedy for impotence and premature ejaculation.

4. **Ginger** *(adrak)*-Indian literature recommends a mixture of ginger juice with honey and half boiled egg taken at night for a month as remedy of impotence.
5. **Garlic** (*lasan)* has been used not only by Indians, but also by ancient Egyptians, Greeks, Romans, Chinese and Japanese. It generally contributes to general feeling of well being, which is transformed into aphrodisiacal effect. It has also been used as a paste in crushed form mixed with lard for local application to genitals for fortifying erection.
6. **Clove** (*lang)* is the dried flower bud of *Jambosa Caryophyllus*. It has been recognised by Chinese as an aphrodisiac since third century BC. Swedish herbalist Anders Mansson Rydaholm wrote in 1642 – "drinking of five grams of cloves with milk fortify a man and makes him desire his wife" indicating its virtue to activate the sexual act.
7. **Nutmeg** (*Jaiphal*) is the ripe seed of *Myrtistica fragrans*. Some claim that a mixture of nutmeg honey and a half boiled egg prolongs the duration of the sexual act, if ingested hour before intercourse.
8. **Pepper** (*Kaalimirch*). Ancient Egyptians, Greeks, Romans and Arabs have used pepper or kaalimirch in various forms to act as an aphrodisiac. A few pepper cons can be chewed before intercourse to prolong erection. Others recommend daily consumption of a glass of milk with six crushed black pepper cons and four crushed almonds for the same purpose.

CHAPTER

6 Basic Information on Male Infertility and Working-up of Patients

BASIC INFORMATION ON MALE INFERTILITY

The word "infertile" in Oxford dictionary has been described as "barren or some one incapable of giving offspring". Going by this description, infertility apparently denotes a sense of finality so far as the reproductive function is concerned. However, quite a few of these patients branded as infertile, do father children negating the meaning of this expression in strict sense of the term. So the "infertility" simply means that begetting offspring is a challenge rather than impossibility, while the "sterility" denotes a problem in the male or the female that precludes the ability to conceive. Fertility, in real terms, is a matter of degree and could vary from high, normal or optimal, to non-existent.

The relative fertility of each couple depends on several factors. Medical treatment of many of these factors aiming to increase the probability of successful conception can bring about favourable changes in the relative fertility. In fact, most couples seeking medical advice should actually be described as subfertile rather than infertile or sterile. It is certainly within the realms of probability for them to attain conception independent of any treatment. Needless to say as the probability of such occurrence of pregnancy is never zero, it may possibly influence their decision to undergo any treatment at all for their infertility. Some of them even with normal fertility potential may have delay in having pregnancy without any scientific explanation. This could even be attributed to bad luck or lack of optimality. According to the data published by various study series,[1-4] 13 to 18% of couples (approximately 15%) meet with failure to get their pregnancy.

Growing evidence from the clinical and epidemiological studies suggests an increasing incidence of male reproductive problems. Conception normally is achieved within twelve months in 80 to 85% of couples, who use no contraceptive measures.[4] Indubitably, each couple cannot hope to have pregnancy with each cycle, even if they are highly fertile, and thus one wonders at the vagaries of nature. The positive gain from this phenomenon probably has limited the world population in the past, when the birth control or voluntary limitation of pregnancy was alien to couples.

Without any treatment, probability of conception certainly decreases in a subfertile couple. According to the World Health Organisation (WHO) definition of infertility, a couple should be considered infertile, if they do not achieve pregnancy after 12 months of regular unprotected intercourse. Long period of infertility worsens their chance for an effective cure.

In demography, "fecundity" is defined as the physiological ability to reproduce or biological capability of individual to produce live birth, [5] while "fertility" is the capacity to conceive or induce conception. Obviously, conception is only possible once in each menstrual cycle of a female and fertility of each couple can thus be expressed as probability of conception per menstrual cycle of exposure or as fecundability or fecundity per cycle (P/C). For a better

understanding of the concept, a simple model using the term fecundability or fecundity was developed, and it was found to vary from "0" to "30" depending on whether the couple is sterile or highly fertile.[6] This was substantiated by statistical analysis of data about conception after extrapolating the results of two hypothetical model populations with P/C 15% and 0%, respectively. Influence of duration of infertility was also analysed and the mean probability of conception for the entire population was calculated at different duration of the infertility.

In model "A" in a population of 100 couples with identical degree of fertility of 15% probability of conception per cycle were chosen as this value was close to one reported for the normal population. If all these couples try to achieve pregnancy starting at time of 0, 15 of 100 will be successful at the end of the first cycle and 15% of the remaining 85, i.e. 13 would be successful at the end of the second cycle and so forth. At the end of 12 months, it would be found that 86 would achieve conception, leaving only 14 to be classified as infertile as per the WHO norm. After analysing the results of various statistical models including the multicentric WHO studies on 8350 couples, [7] three working formulae were arrived at (Tables 6.1 to 6.3). However, it must be emphasised that these calculations were based on the models from population group that may lack universal application and may not have any relevance in the subcontinent.

Similarly, a formula for calculating the probability of conception per cycle (P/C) in percentage related to the duration of infertility in months (n) in couples consulting for infertility of 12 to 48 months duration was arrived at. Based on the reports of data provided by Collins et al[8], a correction factor for primary and secondary infertility was calculated. In Collins' series conception rate per cycle for the entire population was 0.020, that with primary infertility was 0.018 and with secondary infertility it was 0.027. Thus, P/C rate for the entire population needs to be multiplied by 0.018 /0.020 or 0.9 to estimate the value for couples with primary and by 1.35 (0.027/0.020) for secondary infertility.

Table 6.2: Formula 2[6]

Probability of conception per cycle (P/C) in percentage related to the duration of infertility in months (n) can be obtained from the following formula using the correction factor for the duration between 12 and 48 months: $P/C = 4 \times 0.97^{n}$, and beyond 48 months using a formula P/C-1.3-0.1, × being the number of years.

Table 6.3: Formula 3[6]

Probability of conception per cycle (P/C) in percentage related to duration of infertility, n = in number of months, type of infertility—primary or secondary (a), and severity of male (bm) or female factor (bf) can be obtained from the formula: $P/C = 4 \times 0.97^{n} \times (a) \times (bm) \times (bf)$

Here (a) could be either 0.9 or 1.35 depending on the type of infertility whether primary or secondary (see Table 6.3), and correction factors for male and female factors have also been deduced from statistical analysis of data from different series. Data from various studies have shown that the conception rate naturally depends on male factors such as severity of sperm defects and sperm concentrations; and on female factors such as age, ovulation disturbance, cervical factor, endometriosis and block in the passage. Chance of pregnancy after treatment is certainly brighter, if demonstrable male causes of infertility such as varicocele or accessory gland infection are found. Expected conception success rate is the most important criteria for evaluating different treatment modalities.[8] Most untreated infertile couples have average pregnancy rates of less than 5% per month, whereas pregnancy rates in the general community average 20% per month.[9]

A *primary male infertility* in a man is defined as his failure to father a child after 12 months of unprotected intercourse with his partner. A *secondary male infertility* is a condition, when the man has achieved pregnancy with his partner at least once. Duration of pregnancy is calculated from the day of fertilisation irrespective of completion of pregnancy. However, as fertility is a quotient of male and female factors,

Table 6.1: Formula 1[6]

Incidence of infertility	$= (1 - P/C)^{n} \times 100$
Incidence of fertility would be	$= [1- (1- P/C)^{n}] \times 100$
	Using formula 1:
In model "A" :	Incidence of infertility = $(1 - 0.15)^{12} \times 100 = 14\%$ after 12 cycles.
In model "B" :	Where the P/C was chosen as 5% using the same formula
	Incidence of infertility would be $(1 - 0.5)^{12} \times 100 = 54\%$ after 12 cycles, i.e. 1 year.
	Incidence of infertility would be $(1 - 0.5)^{24} \times 100 = 29\%$ after 24 cycles (2 years).

deficiency of fertility status of one partner adversely affects the overall chance of pregnancy. Data available over the past twenty years reveal that in approximately 30% of cases pathology is found in the man alone, and in another 20% both the man and woman are abnormal. Therefore, the male factor is at least partly responsible in about 50% of infertile couples. The WHO statistics in 7273 couples is shown in Figure 6.1.

Important issues related to the evaluation of the male factors in infertility ideally should include the most efficient format for comprehensive clinical and other investigations. This would enable the physician or andrologist to arrive at its causation, and to formulate a rational and an effective medical and surgical regimes in the treatment of these disorders.

It is extremely important in the management of infertility to consider the couple as one unit in an evaluation and to proceed with parallel investigative procedures of both partners until an appropriate diagnosis is arrived at. Many couples experience significant apprehension and anxiety after only a few months of failure to conceive. In countries like India, societal pressure from the elders of patriarchal family singularly aiming for the continuation of progeny, often drives the couple within a very short period of marriage to the doctor's clinic. It goes without saying that consequent mental pressure is certainly not a desired optimal situation for a pregnancy. The other spectrum of unduly prolonged unprotected intercourse should not be advocated before a workup of the couple is instituted. Initial screening of the man should always be considered, whenever the couple presents with infertility. This initial evaluation should be relatively quick, noninvasive and cost effective. However, it is worth noting that the pregnancy rates of up to 50% have been reported, when only the woman has been investigated and treated, even when the man was found to have moderately severe abnormalities of semen quality.

It is difficult to know the incidence of proportion of male factor in infertility in India, as no reliable data have been published, but it is commonly believed that a third of the couple (30-40%) is made infertile from the male factors. However, often the females are investigated first, even when the male factor may be equally if not solely responsible. This phenomenon of female first is not a subcontinental phenomenon, as when it comes to research—male infertility comes poor second to its female counterpart. Female role being more dominant and women more forthcoming to their roles in the reproductive process, they easily come forward, when the couple has the problem of infertility. Historically, men are reluctant to reveal their inability to father a child, even their incapacity in the consummation of a marriage. In the subcontinent, the taboo that men are superior creatures pushes the wives to the doctor's chambers much before their husbands.

FACTORS INFLUENCING MALE INFERTILITY

Why does a male become infertile? In today's marketing jargon, either there is a manufacturing defect or a marketing failure. Some of the manufacturing defect or spermatogenic abnormality can be corrected by medical intervention, so are some of the marketing incompetence or the sperm-transporting defect.

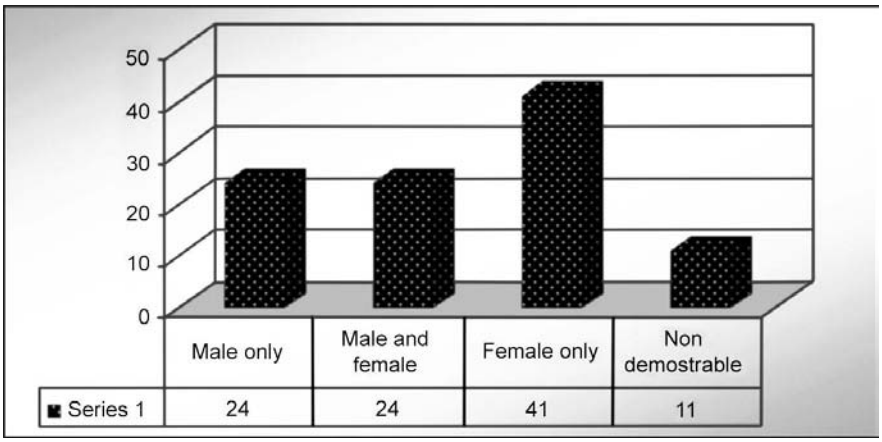


Fig. 6.1: Proportion of male and female factors in 7273 infertile couple in percentage (WHO)

Often, the erectile dysfunctioned or impotence is excluded notwithstanding that it should also be in the second category.

Any scientific research should aim at a systematic approach to find out the most effective therapy for any diseased condition. Consequently, the first step to achieve this goal would be to classify the etiological factors, so that the process of diagnosis and treatment can be streamlined. Many investigators and researchers have put forward their own classifications, but most of their methodology centres round the semen analysis—admittedly the singularly important investigation in male infertility. Others have preferred to enumerate them with a simple classification as pretesticular, testicular and post-testicular causes (Tables 6.4 to 6.6).

Table 6.4: Pre-testicular causes of infertility

1. Hypothalamic disease—isolated gonadotrophin deficiency (Kallmann's syndrome).
2. Isolated LH deficiency ("Fertile eunuch").
3. Isolated FSH deficiency.
4. Congenital hypogonadrotrophic syndromes.
5. Pituitary disease-Pituitary insufficiency (tumours, infiltrative processes, operation, radiation), hyperprolactinaemia,
6. Haemochromatosis.
7. Exogenous hormones (estrogen-androgen excess, glucocorticoid excess, hyper- and hypothyroidism.

Table 6.5: Testicular causes of infertility

1. Chromosomal abnormalities (Klinefelter's syndrome, XX disorder (sex reversal syndrome), XYY syndrome) and sperm maturation defects.
2. Noonan's syndrome (male Turner's syndrome)
3. Myotonic dystrophy
4. Bilateral anorchia (vanishing testes syndrome) and cryptorchidism
5. Sertoli-cell-only syndrome (germinal cell aplasia)
6. Gonadotoxins (drugs, radiation).
7. Orchitis (bilateral)
8. Trauma /Torsion (bilateral)
9. Systemic disease (renal failure, hepatic disease, sickle cell disease)
10. Defective androgen synthesis or action
11. Varicocele
12. Neoplasm in single testis

Table 6.6: Post-testicular causes of infertility

1. Disorders of sperm transport or sperm motility.
2. Congenital disorders
3. Acquired disorders
4. Functional disorders
5. Immunological disorders
6. Infection.
7. Sexual dysfunction

I prefer to put the etiological factors in a chronological order following the onward passage of the sperms in the process of fertilisation from the testis to the ovum. So the causative factors of the male infertility as the sperm, erectile, female and the environmental factors are put in this order, and add the societal and stress factors for its contributory role in certain situations. Infertility may often be caused by combination of one or more of these factors (Table 6.7).

Table 6.7: Causative factors in male infertility

1. **Sperm factors**
   a. Arrest of production of sperm
      i. Primary testicular failure
      ii. Secondary testicular failure.
   b. Apparent normal production, but subsequent deterioration:
      i. Rise in intratesticular temperature as in varicocele and other similar conditions
      ii. Inflammatory and infective factors
      iii. Immunological changes.
   c. Partial or complete obstruction in the normal passage.
2. **Erectile factors**: Failure to deliver the sperms to the female genital
3. **Female factors**
4. **Environmental factors**:
   a. Gonadotoxic agents.
   b. Occupational hazards.
   c. Environmental pollutions.
5. **Societal factors**—Mainly individual.
6. **Stress factor**

SPERM FACTOR

To achieve fertilisation not only must the sperm be morphologically normal, but also they must have energy to penetrate the zona pellucida. This penetrative power is partially provided by the motility of sperms. In such a complex process, it is obvious that any reduction in the quality, motility and morphology will drastically reduce the chance of fertilisation *in vivo.* Pregnancy naturally depends on the quality of sperm, and its probability factor works in favour, if the causative factor is treatable. The primary testicular defects or failure is often caused by bilateral anorchia or cryptorchidism, and by chromosomal defects such as Klinefelter's syndrome (Chapters 2, 3 and 10). Similar effect is possible in endocrinal defects caused by the pituitary and other endocrine diseases (Chapter 3). The secondary testicular defects often result from the testicular atrophy subsequent to trauma or torsion, pituitary or adrenal diseases. As the sperm quality

often is dependent on the intra-testicular temperature, varicocele has been incriminated as an important cause of infertility (discussed later in details in Chapter 9). A wide range of chemical substances including certain medications can affect sperm quality and quantity. The medications listed below all have been associated with male infertility and prolonged use of these drugs has been found to cause spermatogenetic abnormalities (Table 6.8).

Table 6.8: Common drugs causing male reproductive dysfunction

Allopurinol, anabolic steroids, antihypertensives (α blockers), Chemotherapy, Cimetidine, Colchicines, Cyclosporine, Dilantin Macrolides (like Erythromycin), Gentamicin, Nitrofurantoin, Tetracycline.

Even in an *in vitro* fertilisation programme, sperm counts are important. When the husband is moderately (5.1 to 11.9 million motile sperm per millilitre) or severely (less than or equal to 5 million motile sperm per millilitre) oligospermic, fertilisation rates were 56 and 30%, respectively. This is in comparison to a fertilisation rate of 72% in normospermic couples.[10,11] The fertilisation rates achieved using the husband's spermatozoa (9/31; 23%) are significantly lower than with donor (26/35; 74%), despite similar insemination densities, suggesting a functional defect in the spermatozoa of oligospermic husbands. Nonetheless, the pregnancy rates following embryo transfer were similar for all groups, suggesting that once fertilisation is achieved, all embryos have the same potential to result in a successful pregnancy.

Other drugs or substances associated with infertility include alcohol, tobacco, excessive caffeine, marijuana, heroin and methadone. Recreational drugs such as marijuana, heroin, and methadone, are associated with lower serum testosterone levels without a concomitant elevation in LH levels, by their effects both at the testicular level as well as on the central nervous system (see- Chapter 3 *Opidergic Effect*).

TEMPERATURE FACTOR

Thermoregulatory Mechanism of Scrotum

The scrotal temperature at the scrotum is a result not of its intrinsic activity, but of a passive thermodynamic network consisting of heat from the arterial inflow, venous outflow, the countercurrent heat exchanger, and scrotal dissipation of heat. Pampiniform plexus acts as a countercurrent heat exchanger, which serves to precool the incoming hotter arterial blood by the outflowing cooler venous blood. The scrotum is incapable of regulating outflow of heat to the surroundings and allows dissipation of heat. This has been dealt with in details in chapter on varicocele (Chapter 9).

Elevated temperature brings disruption of the normal absorptive and secretory functions of the cauda epididymis. Rise in temperature diminishes the storage capacity of the cauda epididymis. It also reduces sperm numbers in the ejaculate. Zorgniotti et al, MacLeod and Mirone et al[12-15] all postulated that the intrascrotal temperature has to be steadily kept lower than the corporeal temperature by 2 to 2.5°C in order to let the testes perform its normal spermatogenetic function.

Some job environment (see environmental factor later) may overheat the scrotum such as the foundry workers or the sedentary long-distance truck drivers. Oligospermia in the wheelchair-bound paraplegic may also be due to excessive scrotal heat. In some situations, changing from jockey shorts to boxer shorts may offer a solution. Removal of the heat exposure will usually resolve this type of fertility problem. It is postulated that a varicocele may also damage testicular tissue, because of the excessive heat caused by the pooled blood. Measuring the temperature difference between the right and left sides of the scrotum has been used even to diagnose varicocele. However, this method is not standardised and could be unreliable at times.

Commonly, the varicose condition of the pampiniform plexus of veins or varicocele is blamed for the spermatogenetic abnormalities. Frequent sauna bath, overexercise, and wearing tight under-wear for a long period are incriminated similarly in sperm abnormalities, as these conditions are likely to interfere with one of the important factors in the thermoregulation of scrotum—the scrotal dissipation of heat. Increased scrotal skin thickness seen the conditions such as elephantiasis of scrotum—a subcontinental phenomenon following filarial affliction, could easily explain spermatogenetic abnormalities. I encountered two such cases out of 2061 cases of male infertility in eleven years. One of these patients was lost to follow-up, but the other successfully fathered a child three years after the operative correction by scrotectomy.

INFLAMMATORY AND INFECTIVE FACTORS

Recurrent urogenital tract infections in the male are associated with impairment of sperm quality (oligospermia or oligoasthenospermia). At times, there may not be evidence of any active infection, but effects of past infection can lead to abnormalities of the sperms. WHO conducted a study in 25 countries and concluded that the effects of infection vary from country to country with highest incidence in Africa and South America (12%). Asian regions had relatively low incidence at 3.3%. The WHO study and studies by several workers have supported the ill effects of the male accessory gland infection (MAGI) on the reproductive system of male[16] (Chapter 10).

Role of Infection on Infertility *(see Chapter 10)*

Chronic infections of the male genital tract may be an unsuspected factor to impair fertility in male. It may hinder the sperm motility and cause death of immature sperm cells. Chlamydia is a common cause of epididymitis, while tuberculosis can cause an epididymo-orchitis (See also Chapter 10). The main influence of Chlamydia trachomatis on male fertility is due to sexual transmission and negative influence on the tubal function of female partners. It may also reduce sperm functional capacity.[17]

The resultant granuloma and scarring may not only cause abnormality of semen-analysis, but also some immunological changes in the sperms. Gonorrhoea, tuberculosis and the common gram-negative bacteria of the urinary tract may cause inflammatory changes of the ductal system, and produce blockages within the epididymis or vas deferens besides producing immunological changes from the chronic inflammatory state. Gonorrhoea is still prevalent in many countries and remains a potent cause of infertility. Its acute phase often produces inflammatory changes in the seminiferous tubules followed by fibrosis and thickening of their basement membranes. Since the process is bilateral, spermatogenetic abnormalities can readily occur. Even with successful treatment, the abnormalities in sperms can continue up to 2 years in 60% of patients.

Under normal circumstances, there is always a balance between proinflammatory cytokines and natural oxidants. Chronic infection or inflammation of genitourinary tract causes increase in leucocytes in the semen (*leucocytospermia*) and excessive release of biologically active chemokines or cytokines. This leads to decrease in antioxidant potential affecting the sperm motility, viability and functions. According to recent publications, the presence of peroxidase-positive white blood cells (WBCs) in chronic infection is associated with biochemical alterations in the seminal plasma and raised concentration of cytokines causing deleterious effects on the sperm membrane.

Chronic urogenital tract infection is associated with generation of reactive oxygen species (ROS) detrimental to sperm activities and functions. The ROS are intimately involved in physiological control of capacitation, acrosome reaction and zona penetration. Recent evidence also suggests that the spermatozoa and oocyte possess an inherent but limited capacity to generate ROS to aid the fertilisation process.[18]

At the same time, the ROS inhibit sperm movement and have been implicated in male infertility. The results from the works of some researchers indicate that H_2O_2 is the toxic ROS produced by the activated leucocytes causing inhibition of both sperm movement and adenosine triphosphate (ATP) production.[19] It inhibits sperm movement subsequent to ATP depletion, and causes lipid peroxidation (LPO) of the sperm membrane phospholipids to impair sperm function.[20-22] These changes in the lipid composition of the cell membrane are also dependent on the duration of the exposure of the sperms to the ROS.

The LPO has an inhibitory effect on human spermatic ATP causing adverse changes in the motility, velocity and linearity of the spermatozoa.[23,24] A variety of defence mechanisms encompassing antioxidant enzymes (SOD, catalase, and GSH peroxidase and reductase), vitamins (E, C, and carotenoids), and biomolecules (GSH and ubiquinol) are available in the human system. A balance between the benefits and risks both from ROS, and antioxidants appears to be necessary for the survival and functioning of spermatozoa.[18]

Role of Leucocytes

It is worth mentioning some facts that are known about the role of leucocytes in male genital system

1. Leucocytes play an essential role in male fertility, as they are responsible for immune surveillance and phagocytosis of degenerated sperms.
2. Leucocytes seen in all semen specimens are not necessarily an indicator of sperm dysfunction. A monoclonal antibody, now available for identification of leucocytes, can accurately distinguish them from degenerated sperm cells.

3. Increase in the number of leucocytes is an important feature of acute infections, but their numbers mostly do not show any increase in asymptomatic infections.

IMMUNOLOGICAL FACTORS

Rumke and Wilson in 1954[25,26] demonstrated occurrence of the anti-sperm antibody (ASA). Since then, the immunological aspect of the infertility has been a subject of intense discussion. Antibody coated spermatozoa reduce the male fertility potential and do not allow even the normal sperm to impregnate an ovum.[27] The immunological response occurs both at cellular and humoral levels. Presence of ASA on the surface of the sperms interferes with sperm's fertilising ability independent of the other sperm characteristics (see Chapter 10). Immunofluorescence studies located the main sites of the antibodies in the acrosome of the sperm and in the spermatids. The immunological factors may play a role in the pathogenesis of 10 to 20% cases of unexplained infertility.[4] Researchers have detected antibodies in nearly 10% of infertile couples.[28]

Genesis of immunological response is traced to two facts. Firstly, the spermatozoa come into existence only at about puberty, and have different chromosomal structure from the rest of the somatic cells. With the immune system of the body developing much earlier, the sperm specific antigen is viewed as foreign intrusion to the system, and it creates a situation to activate the protective antisperm antigen reaction.

Secondly, nature protects the sperms from this possibility under normal conditions by organising barriers at the testicular level through blood-testis barrier (see chapter 2), and similarly at the epithelial level of the genital tract of the male. The latter is achieved either through a local cellular immunosuppressive barrier of the epithelial lining or through activation of the suppressor "T" lymphocytes inhibiting the activation of the antisperm immune response.

The origin of ASA is traced to breaking down of these "blood-testis" and the epithelial barriers as a result of injurious effects on the testis, which release excessive sperm antigens overriding the natural immune mechanism.[6] Commonly, this occurs in any form of testicular damage that releases the testicular cells outside. Trauma, torsion, destructive lesions (e.g. mumps) and tumour conditions of the testis, recurrent and chronic infections of the genital tract, congenial and acquired obstructive lesions like postvasectomy state, absence of vas or ejaculatory ducts, etc., deposition of semen in the nongenital epithelium, and sometimes even a long-standing varicocele are common such conditions. However, in certain cases causative factor remains undefined. Diseased conditions inducing immunological response are mentioned in Table 6.9.

Table 6.9: Immunological factors

Congenital: Absence of vas, ejaculatory duct obstruction, etc.
Acquired
1. Infective lesions:
a. Chronic and recurrent granulomatous lesions like accessory gland infections, tuberculosis, STD, chlamydia, etc.
b. Mumps and syphilis
2. Destruction of testis by trauma, torsion or tumour.
3. Obstruction to the vas: Congenital and acquired (post-vasectomy, injuries, etc).
4. Deposition of sperm to the nongenital epithelium.
5. Varicocele, if long-standing.
6. Idiopathic.

FEMALE FACTOR

The roles of the female and male in fertilisation are complementary very much like the roles of the two wheels to run a chariot. Fertilisation is the end product of supposedly normal female and male factors. But biological behaviour of a female is different as the menopause determines the end of her reproductive life much earlier and in her forties unlike the male counterpart, who can go on till much later. Moreover, the fertility potential of females decreases with the advancing age as the eggs remaining in their ovaries also age. Consequently, these women become less capable of completing the fertilisation by the sperm. Data from the European Fecundity study show that "the percentage women failing to conceive within a year was 8% in 19 to 26 year olds, 13-14% in 27 to 34 year olds and 18% in 35 to 39 year olds." .

From the age of 28 years, the cumulative pregnancy rates at 12 months and 24 months decreased gradually, reaching 75 and 80% respectively.[29] In today's society, age-related infertility is becoming more common. In the USA, approximately 20% of women wait until after the age 35 to begin their families; and in the subcontinent urban working women are similarly delaying their pregnancy adding

to the infertility problem. The American Society for Reproductive Medicine has cited several contributory factors (Table 6.10).

Table 6.10: Reasons for delayed pregnancy

1. Ready availability of contraception, which are being used by young women going to work and otherwise;
2. Women are marrying at an older age and the divorce rate is creeping up all over the world with higher education rate amongst women;
3. Married couples are delaying pregnancy until they are more financially secure.

The fertility in women begins to decline in their late 20s or early 30s. The age affects their natural ability to get pregnant particularly after the age of 35, thus the success rates of infertility treatments decline as the women age (Table 6.11).[30] A healthy 30-year-old woman has a little above 20% chance per month to get pregnant and by the age 40, the chance dwindles to 5% per month. These percentages are true for natural conception as well as conception using ART. Most untreated infertile couples have average pregnancy rates of less than 5% per month, whereas pregnancy rates in the general community average 20% per month.[31]

Table 6.11: Infertility increases with age

Age group (years)	*% infertile*	*% chance of remaining childless**
20 - 24	7	6
25 - 29	9	9
30 - 34	15	15
35 - 39	22	30
40 - 44	29	64

**Note*: Historical data based on the age at which a woman marries.

Fertility of women declines due to normal age-related changes that occur in ovaries. The biological clock in female begins to tick even in the foetal life. At 20 weeks of foetal life, a female foetus has about seven million eggs, but it drops to about one to two million at birth. Over the next 35 to 40 years or during women's entire reproductive years, women ovulate 300 to 400 eggs. The rest gradually perishes on their own by menopause, which takes place around age 45-50. This degenerative process or atresia occurs regardless of occurrence of normal menstrual cycles, use of contraception or pregnancy. In life, women are born with all the eggs or oocytes in their ovaries. There is no new or fresh production of eggs throughout their life unlike men, who continue to produce sperms all their entire life. Even with nearly over a million eggs in one's ovaries at birth, women are left with only about 300,000 eggs at puberty.

Women aged 32 years or less had a cumulative pregnancy rate (CPR) of 65% and those over 32 years had a CPR of 31%. Couples with previous pregnancy had a CPR of 88%, while those with primary infertility had a CPR of 45%.[32] The process of smoking appears to accelerate the degenerative changes and is linked to earlier menopause.

Although age is the most powerful predictor of fertility, other factors also play significant role in depleting ovarian reserve. According to Dr Fady Sharara, Medical director for the Virginia Center for Reproductive Medicine and an associate clinical professor at George Washington University[33] in Washington, DC- "there are some factors that can contribute to this, such as genetic background, smoking, ovarian surgery and exposure to radiation."

Ovarian Reserve

The more eggs a woman has in her ovaries, and the higher the quality of those eggs, the better is her chance of conceiving. In order to assess a woman's ovarian reserve, there are a number of blood tests that are routinely used in women seeking treatment for infertility. These include- the *'day 3 FSH'* test, the clomiphene citrate challenge test (CCCT) and the inhibin-B test. These tests may help determine the appropriate dose of fertility medications, or how well a woman will respond to fertility treatments such as in vitro fertilization (IVF).

1. *FSH test* - The FSH test, which is generally considered the standard test, measures the follicle-stimulating hormone. If a woman's FSH level is elevated on the third day of her menstrual cycle, it suggests that her ovarian reserve is reduced signifying that the pituitary is forced to release an excess amount of the hormone in its effort to stimulate the ovaries. However, the FSH test results are not a definitive assessment of fertility. "The FSH test is really reliable only when it's abnormal, so a reassuring *'day 3 FSH'* test does not assure fertility," says Dr Janis Fox,[33] Director of egg donation at the Brigham and Women's Hospital in Boston. She adds that this could in part be due to the fact that the eggs that are responding to FSH might not be of good quality. At the same time, an abnormal or elevated test

result does not mean that a woman could not conceive. The younger the woman is, less predictive the FSH levels are. So one can have an elevation in FSH, yet still be fertile.

2. *CCCT test*- The CCCT test may provide additional information in women over the age of 35 having normal FSH test results, yet with unexplained infertility. This test measures FSH and estrogen levels at day three of a woman's cycle and again at day 10, after she is given clomiphene citrate. If the FSH levels are still high, that may indicate a diminished ovarian reserve.
3. *Inhibin-B test*- It is not widely used and measures hormone *inhibin-B*. Its low levels are associated with low pregnancy rates.

Ovarian Size

Human reproduction study suggests that the ovarian volume may reflect the number of follicles remaining in the ovaries. This may help determine the ovarian reserve and therefore the reproductive capacity. The ovarian volume can be determined using a transvaginal ultrasound by measuring the size of the ovaries in three dimensions. The researchers after analysing large studies, found that smaller ovaries and a low number of follicles per ovary correlate with a low ovarian reserve, and the vice versa.

Dr Sharara says that these findings suggest that if the ovarian sizes are good and healthy, the chance of fertility is more. On the contrary, if the ovaries are not of good size and healthy, the woman should set her priorities to get pregnant early as the time is running out for her.

Dr Fox, however, does not think that there is sufficient evidence to justify using the test in general population, and notes that the quality of the eggs cannot be adequately assessed with the ultrasound. After all, in addition to the difficulty in accurately assessing ovarian reserve, there are factors other than ovarian reserve that can affect fertility.[33]

In addition, fertilisation of these ageing ova is associated with a higher risk of genetic disorders. Fall in the pregnancy rates in women over forty is largely ascribed to the increased number of ova with chromosomal problems. Disorders involving the chromosomes, such as Down syndrome, are more common in children born to older women. When eggs with chromosomal problems are fertilised, they are less likely to survive and grow. Consequently, women over 40 have increased risk to abort even after successful fertilisation. Risk of chromosomal abnormality in newborns in a 20-year old is 1/1,667 and that of Down syndrome is 1/526, while that increases to 1/106 and 1/66 respectively in a 40-year old.[34] Similarly, risk of miscarriage in mothers in the age groups 15 to 19 years is 9.9%, while that in the age groups 30 to 34 years is 11.7% and that in 35-39 years show it to be 17.7%.[35]

There is also a positive correlation between the age of women with the incidence and severity of ovulatory dysfunction, tubal occlusive disease, pelvic adhesions, endocrine conditions and endometriosis.[36] When the ova from women in their 20s and 30s are used for in vitro fertilisation, and the fertilised ova transplanted to the uteri of women over 40, the probability of successful pregnancy increases considerably, and is much higher than they possibly could have achieved with their own eggs. It is thus obvious that the age of women could be an important deterrent to achieve pregnancy due to pelvic organ diseases and chromosomal problems of the ova.

However, inadequacy of the male factor in a couple can be compensated, if the wife is very fertile. With a higher fertility, the wife could become pregnant despite her husband's oligozoospermia. Consequently, the male partner would never see a physician for his relative subfertility.[37] Variable female factor itself thus assumes an important role, notwithstanding the possibility that fertility of a couple could sometimes be woman dependent, as is seen often in the experiments in animal husbandry. However, it would be socially incomprehensible to test the biologic potency of normal men using multiple female recipients as is practiced in animal husbandry.

In a female, ovulation takes place around the middle of each menstrual cycle, when the ovum or oocyte burst out from the ovaries to enter the fallopian tube, where the fertilisation actually takes place. Later, the fertilised ovum travels to the uterus to get implanted to consolidate the process of pregnancy. This period lasting for 3 to 5 days in the midcycle, thus provides in each menstrual cycle, the window of opportunity for a sperm to fertilise the ovum, unlike the relatively safe or unproductive days of the cycle. These fertile days are calculated by several methods such as serial US ovulation studies, recording changes in the basal temperature and noting the mucus cycle. The beginning of the mucus cycle begins with a sticky (a quarter of an inch), cloudy or tacky vaginal discharge until the last day,

when the mucus becomes clear and stretchy or lubricative. The fertile period is defined as the 1st day of mucous discharge through three full days past the peak day. There is always a higher probability of conception when the couple uses this peak fertile time.[38]

With ovulation occurring once in four weeks in most women, a couple gets only twelve or thirteen opportunities per year for a pregnancy. Research indicates that even fertile couples achieve pregnancy only 20 to 30% of the time with properly timed intercourse in each menstrual cycle.[39,40] So infrequent sex especially during this critical midcycle period seriously hampers odds for success. Moreover, long period of abstinence can also lead to decreased sperm motility and abnormal sperm morphology *(see* Chapter 7-*Period of abstinence).* Epidemiological studies statistics reveal and an addition of approximately 7% newly formed couples per year needing treatment for infertility.

When the male partner was under 40 years, only 3% of 19 to 26-year old, 6% of 27 to 34-year old and 9% of 35 to 39-year old females failed to conceive in the second year. Starting in the late 30s of women, the male age becomes also important. The percentage of failures for women aged 35 to 39 years after one-year rose from 18 to 28%, if the male partner was over 40. After the second year, the failure figure was 9% with male partners under 40 and 16% with those over 40.[41,42]

ENVIRONMENTAL FACTORS

With ever-increasing environmental pollutions and occupational hazards from various industries, the sperm environment is getting vitiated with consequent fall in the sperm quality. The male reproductive system is known to be highly sensitive to some physical and chemical agents. Unlike the small laboratory animals, human spermatogenesis is more vulnerable to the toxic actions of some chemicals.[43-45] Thus, environmental pollution has been often and justifiably incriminated for the deterioration of sperm functions. Most researchers incriminate decreasing sperm counts to the modernity of lifestyles with smoking (both tobacco and marijuana), alcohol, leaded petrol fumes, excessive use of antibiotics for trivial reasons, estrogenic factor (discussed later), some drugs (see list) and general stress, in addition to the environmental factors. We have overwhelming evidence of chronic exposures to some physical agents such as heat, radiation, etc., various chemicals such as lead, cadmium, mercury, etc., and in industries dealing with solvents or pesticides, causing damage to the gonads.[46] Temporary deterioration in the quality of sperms may also be caused by long periods of staying still, as in long air travels, or after taking frequent long hot showers or sauna bath. Moreover, global warming may also partly be responsible for lower sperm count and motility.

In the subcontinent as well as in the warm climates all over the world, the birth rates show a seasonal variation.[47] In India greater number of pregnancies, however, takes place, when the temperature is relatively down resulting in higher rate of deliveries in the summer. This is related to deterioration of the semen quality during the summer, and the drop is reflected in the fall in the birth rates in the following winter and spring. Sperm specimens in both summer and winter reveal significant variations in sperm concentration, total sperm count per ejaculate, and concentration of motile sperms. Moreover, the lower is a subject's average sperm count and motile-sperm concentration, the greater is the reduction. Substantial summer deterioration in semen quality is more likely to occur only among men whose work places are probably not air-conditioned. These findings suggest that the deleterious effects of summer heat on spermatogenic cells or on epididymal spermatozoa may reduce male fertility and account, at least partially, for the lowering of spring births in warm climates throughout the world.[48]

However, according to a study from Israel by Rojansky, these changes also correlate with the absolute number of light hours and its increment over time, and not solely with the temperature, humidity or other environmental changes, possibly because of the light/dark variations.[49] The findings of Levine et al also do not support the hypothesis that the heat of the summer is detrimental to male reproductive capacity. The available evidence suggests instead, a possible role of photo-period in causing the seasonal changes in semen quality.[50]

Women show a birth distribution that was best represented with a bimodal curve with zenith in January and July (P = 0.06). The study by Smits et al[51] provides evidence for the existence of differences in fecundity by the month of birth. The cause of this relationship is unclear, but may again lie in a mela-tonin-dependent circannual variability of the quality of the oocyte. Seasonal variability in fertilisation and embryo quality rates in women undergoing *in vitro* fertilisation (IVF) has also been reported. Seasonality seems to have a significant

influence on the fertilisation process and on the quality of the human embryos that are obtained *in vitro.* [47,49]

Estrogen Factor

There has been spate of reported incidence of excessive estrogen in the diet and atmosphere affecting the male reproductive system. The term environmental estrogen refers to chemical substances that exhibit some degree of estrogen-like activity. Many researchers believe that increasing volume of estrogen products are seeping into the environment through use of chemicals in the plastics and non-domestic cleaners. Moreover, synthetic estrogens are used in the dairy products, contraceptive pills, shampoos, and skin cream and even in some talcum powders. London[52] aptly described the situation that we now live in an environment that can be viewed as "a virtual sea of estrogens".

The relationship between the estrogen and male sexual development is best viewed by examining the effects of synthetic estrogen diethylstilbesterol (DES). Between 1945 and 1971, several million women were treated with DES. By 1970, the side effects of DES became adequately known. DES and other synthetic estrogens were not only used in humans, but also for 20 to 30 years in the livestock industry to fatten the animals and to help them grow faster. Although many of these synthetic estrogens like DES are now banned, many livestock such as dairy cows and poultry are still hormonally manipulated. Cow's milk contains substantial amounts of estrogen due to modern farming techniques. The rise in dairy consumption since the 1940s inversely parallels the drop in sperm counts.

Avoidance of hormone-fed animal products and milk products are important for male sexual vitality, especially in men with low sperm counts or testosterone levels. According to some researchers, there are reports that estrogens have been detected in drinking water. Presumably, the estrogens are recycled in the water treatment plants from the excreted synthetic estrogens having a potent source from women taking birth control pills. These estrogens may be harmful to male sexual vitality. They are more potent as they do not bind to sex hormone binding-globulin (SHBG). Purified or bottled water may be a suitable option to prevent exposure. There are other sources of estrogen from the environment (food, water, and air) that can weaken male sexual vitality. Many of the chemicals contaminating our environment in the past 50 years are weakly estrogenic.[53-56]

These estrogenic factors are thought to have their greatest impact during foetal development. Dr Richard Sharpe of Edinburgh has performed tests with pregnant rats, which led him to believe that if a male foetus is exposed to even tiny amount estrogen in the womb, it can have dramatic effect on their fertility in later life. He also postulated that one could get biological effects from estrogen at levels so low that it escapes measurement by any analytical method.[57,58] Inhibitory effects of these products on the Sertoli cells have been in evidence in animal studies. Sertoli cell multiplication (discussed in Chapter 2) occurs primarily during the foetal life and before puberty. Each Sertoli cell can only support a fixed number of germ cells that develop into sperms under control of the follicle-stimulating hormone (FSH).

Animal studies on mice as well as in some control trials in humans have lent credence to deleterious effects of excessive estrogen administration.[59] When estrogen is administered early in life, it inhibits FSH secretion resulting in reduced number of Sertoli cells, and in adult life, this would cause reduced sperm counts. This theory is further proved by the reduced sperm counts in male offsprings of women exposed to DES during pregnancy in the US study. Typically low-fibre, high-fat diet of most American mothers, even if they did not take DES, may be an important factor as the diet of this nature often leads to higher levels of estrogens, as without the fibre excreted estrogens are reabsorbed.[54-56]

Another biological significance of these changes is emphasised by a concomitant increase in the incidence of genitourinary abnormalities such as testicular cancer and cryptorchidism.[13,59,60] Exposure to DES induces persistent structural and functional alterations in the developing reproductive tract in males. This fact is now recognised to have led to substantial increase in the number of men suffering from developmental problems of the reproductive tract as well as decreased semen volume and sperm counts. It is also possible that xenoestrogen other than DES may account for the increasing incidence of developmental disorders of the reproductive tract in men and wild animals.[61]

It thus appears that there are several irrefutable evidences that the environmental estrogens can negatively influence mammalian sperm capacitation,

acrosome reactions and fertilising ability in male.[62-64] However, a few researchers have cited contrary evidence. [65-70] They argue that no population-based evidence regarding the effect of prenatal estrogen exposure on the male fertility has been generated and strict criteria were not adhered to before the conclusion was drawn on the falling sperm counts. They showed that massive maternal exposure to oral estrogen has negligible effects on male fertility and sperm output.[64]

However, several observations suggest that the male reproductive health has been declining since the World War II in many countries and the downward trend of the semen quality over the past 50 years provides its ample evidence. Conclusion drawn from the analysis of data based on both animal and human records, show that exposure to exogenous estrogenic compounds reduce sperm production in adult men.[53-56] New pathways through which such changes could be induced, have been identified. These include suppression of testosterone production by the foetal testis, suppression of androgen-receptor expression and suppression of insulin-like factor.[71]

In the nineties, Danes began their extensive research into the both quality and quantity of sperms in human beings and came out with astounding information. Skakkebaek of Denmark reviewed the sperm count surveys of 15000 men with no history of infertility since 1938, and found that the average sperm counts had come down to 66 million in 1992 from the level of 113 million in 1940.

Carlsen et al[72] in 1992 prompted the debate claiming falling human sperm output. Sperm count or density is one of the factors that determine fertility in male. There has been a genuine decline in semen quality over the past 50 years. The overall reduction in male fertility thus can be correlated with the declining sperm counts.

Olsen et al[68] expressed contrary views on the inference of 50% reduction in mean sperm counts in the last 50 years published through linear regression model. They contended that the potential selection errors might have occurred in the 61 assembled studies. So, they are not true representative of the studied populations. There was variability in collection methods, in particular the lack of adherence to a minimum prescribed abstinence period, as had been stated for the largest study, and paucity of data in the first 30 years of the 50-year trend analysis. The data of the last 20 years, which contains 78.7% of all studies and 88.1% of the total number of subjects, revealed no decrease in sperm counts. Use of other mathematical models that perform statistically better than the linear model, suggest constant or slightly increasing sperm counts.[68,69]

We do not have comparative figures in India. But from my personal experience during the last twenty-five years in Delhi, I find that the number of persons with higher than 100 millions count have come down drastically and more often than not, we find a count of 60 to 80 millions in normal individuals. Scandinavian reports revealed that the sperm counts had nearly halved from 56.4% in 1981 to 26.9% ten years later. Reports from other countries also register diminishing trend in sperm counts. In Pakistan, it is now 79.5, Germany—78, Nigeria—64 and Hong Kong—62. In one Scottish study, it was found that the average sperm counts for men born before 1959 were 98 million and those after 1970, it had come down to 78 million.[72-79] Notwithstanding the contrary views, which questioned the methodology of collection of data rather than the conclusion, it is a reality that the sperm counts are showing a downward trend over the years.

Gonadotoxic Agents

Gonadotoxic agents that are present in places of occupation and work, and in day-to-day environment can basically affect one or multiple sites of the male reproductive system. When these injurious effects target the early stages of spermatogenesis, it could last long or even cause permanent damage. There are four different effects[6] as shown in Table 6.12.

Table 6.12: Injurious effects of toxic environmental factors on gonads

1.	Disruption of spermatogenetic function resulting in reduction in the number of sperms and increase in the number of abnormal sperms due to the damage to sperm DNA.
2.	Impairment of sperm motility by accumulating in the accessory sex glands secretions (prostate, seminal vesicles or seminal fluid).
3.	Disruption of the neuroendocrinal control by acting at the testicular level on the Leydig or Sertoli cells or at the CNS level to interfere with GnRh or gonadotrophin secretions.
4.	Disruption of the vascular supply essential for the supply of nutrient at the blood-testis barrier.

Genetic damage is difficult to detect in human sperms because most methods require an induced replication, which cannot occur in haploid cells. Genetically damaged sperm may alter fertilisation, lead to developmental anomalies, or spontaneous

abortions. Epidemiological studies of large populations have demonstrated increased frequency of spontaneous abortions in women, whose husbands were working as motor vehicle mechanics[79] or had lead exposure.[80] Kurinczuk and Clarke[81] from the UK suggest an unfavourable effect at relatively high levels of exposure to lead. There was some evidence that the leatherwork is a risk factor for teratozoospermia. Workers with aromatic solvents were at an increased risk of presenting with infertility, although this was not mediated through effects on standard of semen quality.

A toxicant or its metabolite may act directly on accessory sex glands to alter the quality or quantity of their secretions. Alternatively, the toxicant may enter the seminal plasma,[82] and thereby, affect the sperms, be carried to the site of fertilisation on the sperm membranes, and eventually may cause damage to the ova or *conceptus*, or be absorbed into the body of the female partner after intercourse.

Integrity and fluidity of the cell membranes are extremely important for proper functioning of sperms making them sensitive to free radicals. If the integrity of the membrane is not maintained, enzymes are activated leading to impaired motility, abnormal structure, loss of viability, and ultimately death of the sperm. The major determinant of membrane fluidity is the concentration of polyunsaturated fatty acids, particularly omega-fatty acids such as decosahexanoic acid, which are very susceptible to free radical damage. The sperms have relative lack of superoxide dismutase and catalase, which can prevent or repair oxidative damage. Adding to this more susceptible state is the fact that sperms generate high quantities of free radicals to help breaking through the barriers to fertilisation.

Biochemical analysis of seminal plasma provides insight into the function of the accessory sex glands. Chemicals that are secreted primarily by each of the glands of this system are typically selected to serve as a marker for each respective gland. The epididymis is represented by glycerylphosphorylcholine (GPC), the seminal vesicles by fructose, and the prostate gland by zinc. Measuring of semen pH and osmolarity provides additional general information on the nature of seminal plasma.

Seminal plasma should be analysed for the presence of a toxicant or its metabolites. Heavy metals have been detected in seminal plasma using atomic absorption spectrophotometry,[83] while halogenated hydrocarbons have been measured in seminal fluid by gas chromatography after extraction or protein-limiting filtration.[84] There are a few reports of effects of some toxicants on the accessory sex glands in humans. Ethylene dibromide (EDB) is one such toxicant that exerts post-testicular effects. Short-term exposure to this toxicant causes reduced sperm velocity and semen volume.[85] Chronic exposure leads to decreased sperm motility and viability, decreased seminal fructose levels, and increased semen pH.[79] An EDB metabolite was present within the semen of some exposed workers.[82] Other potential toxicants that have been detected in semen include: lead, cadmium, hexachlorobenzene, hexachlorocyclohexane, dieldrin, and polychlorinated biphenyls.[83-85]

Physical Agents

Heat Exposure

Exposure to heat is a well-established suppressor of spermatogenetic function. Sperm counts in outdoor workers were substantially reduced in the summer compared to winter, and fertility in the USA is closely correlated with the summer temperature.[47,48] Transient drop in sperm count up to 50% has been observed with the rise in the body temperature of 0.93°C following sauna bath lasting 15 minutes. Naturally, workers in iron foundries, bakeries and steel-works are particularly vulnerable to heat exposure with consequent deleterious effects on the sperm counts and motility.

Ionising Radiation

Testis is one of the most radiosensitive organs in the body. A radiation dose as low as 0.15 GY is capable of causing reduction of sperm counts, while 0.3 GY and 2 GY doses can cause a temporary and permanent azoospermic condition respectively. The sperm-forming germ cells of the testis are very sensitive to radiation probably, due to their high rate of cell division (see also 'normal process of sperm development' in Chapter 2).

Radiation-associated injury and reparability of germ cell tissue is dose-dependent. Germ cell damage is reversible at single exposures below 600 rads. However, above 600 rads, permanent infertility is likely. After exposure to 200 to 300 rads (radiotherapy protocol for Hodgkin's disease patients), it may take up to 3 years for sperm production to recover fully. At 400 to 600 rads, the recovery time is

roughly 5 years. Thus, to preserve fertility, every effort should be made to guard the testes from radiation, when radiotherapy is applied to the pelvis and other abdominal sites. The problem of "radiation scatter" is particularly important, if there is less than 30 cm distance between the testes and the edge of the radiation field. In such cases, use of a testicular shield can minimise radiation exposure by three to ten-fold.[86]

High Frequency Electromagnetic Radiation (HHF) and Short Waves (500 kHz-300 mHz)

Studies on the effects of high HHF used in the plastic sealing and glue hardening, and in the manufacture of radar and telecommunications equipment on the sperm functions have shown disturbing trends. Earlier, American survey reported a severe reduction in the sperm concentration of military radar equipment operators with high HHF exposure. Recent study suggests that the French military population, who worked as submariners in a nuclear-powered submarine in very hot conditions, is also considered to be susceptible to the risk factors for infertility.[87] There have been reports of continuous exposure to microwave (100 mHz-300 mHz) causing oligo- and astheno- and terato-spermia. However, in these cases heat exposure component may have a contributory role.

The Pennsylvania State University, USA, demonstrated that artillerymen with potential microwave exposures had lower sperm counts and sperm per ejaculate than the comparison group; but variables used to assess endocrine, accessory sex gland, and sperm cell function were not different from the comparison group.[88]

Chemical Agents

There have been several attempts to develop a list of male reproductive toxicants. Documentation of such a list is complicated by the quality of reported studies, whether to include animal studies, and pharmaceuticals. To determine the quality of a study, it requires an intense evaluation by qualified individuals. Excluding a potential toxicant from this list following a qualitatively poor study may lead to a chemical given "clean bill of health." Results from animal studies often complicate matters. These include the extrapolation of species difference, dose, and often the route of exposure. Pharmaceuticals that are designed and produced specifically for their effects on the reproductive system (e.g. androgens, estrogens) are frequently not included in the lists of reproductive toxicants. However, there are potential occupational exposures in the manufacturing and dispensing of such drugs.

The registry of Toxic Effects of Chemical Substances (RTECS)[6,7] using a computer search programme called CCINFO prepared a list of 1191 chemicals with effects on male reproduction. However, RTECS included all substances (including drugs) that have tested positive in either human or animal studies.

Smoking

Deleterious effect of smoking on fertility has been a subject of discussion for several years. Multiple factors are likely to operate for smoking to cause decrease in fertility. Effects of smoking on erectile process have already been dealt in Chapter 4. There is a close association between smoking and low sperm count, poor sperm motility, and abnormal sperm. A higher incidence of cigarette smoking was observed in subfertile than in fertile males.[89] Cigarette smoking continued to show a significant decrease in sperm penetration assay score.[90] Smokers are estimated to take 3.4 times longer than nonsmokers to conceive, and are reported to be less fertile. Spermatozoa are both abnormal morphologically and genetically compromised in proportion to the dose.[91]

Current cigarette smokers, marijuana smoking and heavy alcohol users showed greater numbers of leucocytes in the seminal fluid than did non-users of these agents.[90] The data from a Chinese study also revealed that cigarette smokers, who had worked in a petrochemical plant, had significantly poorer quality semen (sperm density, total sperm count, and forward progression rate) compared with the control (Tables 6.12 and 6.13).[92]

Mechanism of the toxicity of cotinine (active ingredient of nicotine) on sperm morphology is probably due to additional compounds in cigarette-smoke other than nicotine.[89] Smoking a large quantity of cigarettes per day severely affects the ultrastructure of the flagellum and, more specifically, it affected the axoneme of the human sperm as evidenced by the electron microscope studies of smokers and nonsmokers .[93]

The nicotine in cigarettes and cigars also constricts a smoker's blood vessels, which reduces blood flow throughout the body including that to the penis. The

Table 6.13: List of chemical agents and substances known to cause injurious effects on sperms[6,7]

- Alcohol
- Aluminum
- Anaesthetic gasses (e.g., nitrous oxide, enflurane, halothane)
- Arsenic
- Benzene/benzene hexachlorides (lindane)
- Boron
- Cadmium
- Chlorinated hydrocarbons (e.g., PCBs, TCDD)
- Cobalt
- Cotinine of nicotine (smoking)
- Dibromochloropropane (DBCP)
- Fungicides (e.g., captan)
- Herbicides (e.g., "Agent Orange" (dioxin), paraquat)
- Hydrazines
- Insecticides/Pesticides (e.g., carbamates, DDT, dieldrin)
- Marijuana and related substances
- Metals like lead, manganese, mercury, methyl mercury, molybdenum, nickel, etc.
- Various paints, silver solvents (e.g., benzene, xylene, hexane, toluene), vinyl chloride
- Pesticides and rodenticides (e.g., fluoroacetamide) polycyclic aromatic hydrocarbons (PAHs)
- Radiation (e.g., X-ray exposure) and exposure to radioactive materials (e.g., uranium)

increase in blood flow required for erection is comparable to that required by the heart for vigorous exercise. A cigar-smoker is worse off with the rate of impotence at 30%, than a cigarette-smoker with a 24% impotence rate.

A recent study by the New England Research Institute, Massachusetts, USA, Henry Feldman[94] followed 513 men for 10 years, and after adjusting for age and lifestyle factors, he found moderate or complete impotence in 26% of the nonsmokers, who were exposed to second-hand smoke both at home and in the workplace as against 14% of men with no exposure to second-hand smoking. "The group that showed an elevated rate was the group exposed to second-hand smoke both at home and at work," Feldman says. A double dose of passive smoke approximately doubled the risk of ED. Feldman's study though not conclusive, also indicates that some of the hundreds of chemicals in cigarettes may interfere with fertility by elevating the number of abnormal sperm forms.

Alcohol

A brief overview of recent literature concerned with ethanol sensitivity of various components of the reproductive tract reveals a lack of a critical survey of ethanol-induced functional and physical effects on the male reproductive tract. Anderson et al[95,96] conclude that ethanol is a male reproductive tract toxin, but some of the effects of ethanol-induced manifestations of male infertility may be incriminated to concomitant secondary factors such as hepatic dysfunction and nutritional deficiency induced by alcohol. Future clinical studies of alcoholics afflicted with testicular dysfunction but having normal liver histology, will be of great value in efforts to identify the mechanisms by which chronic ethanol ingestion cause reproductive impairment.[95,96]

Consumed in a large enough quantity over a long enough period of time, alcohol can cause infertility. Alcohol consumption beyond a certain limit can lead to premature ejaculation (PE) even an-ejaculation (AE), and is associated with an increased number of defective sperms. However, critical dose of alcohol could be person specific—some showing higher threshold than others. Fortunately, findings from animal research suggest that the damage alcohol causes to the sperm population is partially reversible, if subjects avoid drinking alcohol over a period.[97]

Occupational Exposure

Occupational exposure in work environment (occupational hazards) to many chemical agents or substances may damage the sperm-producing testicular germ cells causing infertility. Potential toxicants that have been detected in semen include: lead, cadmium, dieldrin, hexachlorobenzene, hexachlorocyclohexane, dibromochloropropane (DBCP), some pesticides, organic solvents, and polychlorinated biphenyls.[83,84] Chemical agents causing injurious effects on sperms are shown in Table 6.13.

An Italian study finds that the traffic pollution slows sperm movement and vitality in toll workers.[98] "Environmental levels of occupational pollutants, except carbon dioxide, at the tollgates exceeded the maximum legal levels and the workers were exposed to significantly higher levels of nitrogen oxides, sulphur oxides, carbon monoxide and lead than the controls," says study author Dr Michele De Rosa. Researchers from the University of Naples [98] opined that their study should be an alert to health authorities on the insidious health hazards of pollution. Their findings should prompt research on workers in other jobs, who are exposed to similar levels of pollution.

Lead

Lead has been found to interfere with the hypothalamic-pituitary-gonadal axis—the hormonal "feedback mechanism" of the reproductive system (see also Endocrine Disorders in Chapter 3). Excessive lead exposure can cause suppression of testosterone level in blood. Men with lead poisoning (e.g., battery plant workers) also may exhibit direct testicular damage in the form of fibrosis around the tubules, cavity formation, and decreased sperm production. High exposure of lead exceeding 70 microgram (µgm) per decilitre without any clinical signs of intoxication is common in battery workers. Relatively low exposures (30-50 microgram per decilitre) are seen in metal painting industries. Recommended biological exposure (currently 50 µgm in Denmark[98] needs to be reviewed in the light of these findings. There is a strong evidence that high level exposure to lead with a blood lead level (PbB) > 70 microgram/dl, is associated with male infertility. Some effect even at a lower PbB (i. e. < 50 microgram/dl) was reported in the study from the UK.[81] But some contributory factors attributable to personal and social conditions of the workers in this study cannot be ruled out.

According to Benoff, lead could be responsible for 12% of infertility problems among men. The good news is that men can reduce the amount of lead in their bodies by eliminating exposure to it. Zinc supplements may also help reduce lead's presence in the body. Lead exposure and ageing damage sperm and their swimming acumen.[100,101]

Mercury

In experiments with mice, it has been established that both mercury and methyl mercury interfere with early stages of spermatogenesis.[102,103] Koos and Longo[104] studied environmental exposure to mercury in Minamata Bay, Japan, to pregnant women, foetuses and newborn infants. They observed that human foetuses were at risk due to chemical exposures to mercury. Exposure to mercury may lead to its deposition in the pituitary gland and the testicular tissues causing impairment of testicular function, especially spermatogenesis.[105]

Other Metals

Cadmium affects testicular capillaries causing increased permeability. Manganese (present in sperm mitochondria) affects the sperm motility. Chromium has effect on epididymal sperm count and motility. Thus, all these metals have inhibitory effects on spermatogenesis at different levels. Change in the semen characteristics in welders involving steel (not stainless steel) showed hexavalent chromium and nickel being offending agents. In welders radiant heat may be an additional factor.[106]

Pesticides

Dibromochloropropane (DBCP): DBCP, an agricultural soil fumigant, is associated with severe testicular toxicity. Men, who have been exposed to DBCP frequently, will suffer from small, soft testes, elevated blood gonadotrophin levels, low testosterone levels, and few or no sperm in their semen. The reduction in sperm production is dependent upon the length and degree of DBCP exposure. Slutsky et al[107] reported that DBCP-exposed workers had adverse reproductive health effects. In some cases, sperm production and fertility can be restored, if the patient later remains free from any contact with DBCP. Agriculture workers using this chemical have been conclusively proved to have low sperm counts.[108-110]

Organic Solvents

Organic solvents, especially carbon disulfide, produce effects on semen characteristics probably by acting on the pituitary-hypothalamus axis. Solvents with low volatility such as ethylene glycol, ethyl or methyl alcohol and ethers are being used increasingly in paints, adhesives, thinners, printing ink and anti-icing fluid. The fact that they cause low sperm counts, is of great concern.[111-112] An association between aromatic solvents and reduced semen quality was

demonstrated irrespective of the exposure assessment method used.[113]

Plastic Monomers

Chlorprene is a monomer used in the production of synthetic rubber. Workers in these industries have been shown to have decrease in sperm motility and their wives have three-fold more chances of having abortions.[113-114]

Most of these chemicals such as PCBs, dioxin, and DDT, consequent to their resistance to biodegradation get recycled in our environment to find a safe haven in our system. DDT has been banned for nearly 20 years, yet it is still often found in the soil and root vegetables, such as carrots and potatoes. These toxic chemicals are known not only to interfere with spermatogenesis, but more importantly may have their effects on sexual development.

The present knowledge about occupational and environmental hazards from different industries could be a tip of the iceberg, considering the fast pace of the industrialisation that is taking place especially in the third world countries, where the checking of norms of health of these workers are suspect, if not absent. The matter is compounded by the fact that the economy of these countries forces the authorities to use migrant labourers often from a distant area, and at times from another poorer country. This leads to difficulty in studying and sampling the affected persons for a scientific data. International cooperation with strict implementation of the World Trade Organisation (WTO) norms seems to be an urgent need for the health of our next generation. One bit of caution should be borne in mind that researchers usually had to rely on the testimonies of the workers regarding their sexual health. This testimony, in the absence of scientific control trials, may often be confounded by the bias of the individual worker to guard his ego or masculine image. They often attribute a pre-existing libido problem to exposures at work. However, some effects attributable to personal and social conditions of the workers should not be ruled out.

ERECTILE FACTOR

Inability to deliver the sperms to the female with resultant infertility can result from either due to an erectile dysfunction or ejaculatory failure. Physiology and pathology of ED have already been dealt with in Chapters 4 and 5. It is reported that while 2% of couples have some sexual or ejaculatory problems, only 1% of men with adequate erectile function may have ejaculatory problem compared to 20% with inadequate erection. There is significant increase in erectile inadequacy with increasing age and 5% of male above 40 years have erectile problems. Masturbatory and early morning erections are common features in 59.2% healthy males. Absence of these features is not diagnostic of any ejaculatory and EDs, but it certainly indicates a strong probability factor.[115]

EJACULATORY FACTOR

Ejaculatory failure can be grouped under four headings: anejaculation (AE). retrograde ejaculation (RE), premature ejaculation (PE) and ejaculatory obstruction (see also Chapter 12).

Anejaculation is a phenomenon, where there is no ejaculation of semen—antegrade or retrograde (dry intercourse). Diagnosis of this condition can only be clinched, if a postcoital urine specimen shows absence of sperms as well as that of fructose. In a retrograde ejaculation the semen is ejaculated, but it finds its way into the bladder to be voided with urine. Premature ejaculation and erectile failure have been discussed in Chapters 4 and 5. Ejaculatory duct obstruction, which does not allow free flow of semen during ejaculation, could be partial or complete.

Etiological Factors of Ejaculatory Failure

Possible etiological factors of ejaculatory failure are as follows (Table 6.14).

1. *Congenital:* Absence of vas or ejaculatory ducts, or a mullerian duct cyst usually causes ejaculatory obstruction, while epispadius or extrophy of bladder and posterior urethral valve can cause RE.

Table 6.14: Various causes of ejaculatory dysfunction

1. Congenital.
2. Some systemic diseases.
3. Iatrogenic from some operations.
4. Pharmacological or drug induced.
5. Inflammatory cicatrisation.
6. Post-traumatic.
7. Idiopathic

2. *Systemic diseases:* Diseases such as diabetes or multiple sclerosis and bone marrow transplantation can cause either AE or at times PE. Diabetes

is the most common case of autonomic neuropathy and ED is seen in nearly 50% patients either in the form of AE or RE.

3. *Iatrogenic factors:* These include sympathectomy, retroperitoneal lymph node dissection, or repair of abdominal aneurysm, prostatectomy (almost always in open, but less often in TUR), Y-V plasty of bladder neck or posterior urethroplasty. They could be responsible for either AE or RE. Injuries to vas during repair of hernia or vasectomy are the common causes of obstruction in the passage.
4. *Pharmacological or drug-induced ejaculatory dysfunction:* These are seen after administration of various medicines, especially antihypertensive and psychotrophic agents. Alcohol, Amitryptine, Chlordiazepoxide, Chlorpromazine, Haloperidol, Hexamethonium, Imipramine hydrochloride, Methadone, Naproxen, Prazosin, Thiazides and Trifluoroperazine hydrochloride are common agents that can cause impairment of ejaculation. Alcohol consumption beyond a certain limit can lead to AE and even PE. However, critical dose of these agents could be person specific—some showing higher threshold than others. Any medicine that disrupts the normal sympathetic activities can affect both seminal emission and ejaculation, and with some psychotrophic agents there could be in addition, changes in the volume and quality of semen.
5. *Inflammatory:* Recurrent attacks of prostatitis, tuberculosis and gonorrhoeal affection of urethra and prolonged catheterisation may cause ejaculatory obstruction.
6. *Traumatic*: Injuries of the spinal cord and posterior urethra can cause either AE or RE.
7. *Idiopathic:* In many cases, the cause of AE or PE remains undefined, notwithstanding the important role of a psychological factor in the etiology.

SOCIETAL FACTOR

In the subcontinent like India, the sex education remains a taboo. The timing of the sexual acts of many couples may sometimes have a contributory role in infertility as they often have little or no understanding about the ideal timing of intercourse. Consequently, the sexual habits of these couples are not often conducive to timely pregnancy. I have noticed while interviewing quite a few couples that they use nonmedicinal lubricants (oil or jelly) to facilitate intercourse. Many of these lubricants are often toxic to sperms and could in fact impede the sperm motility. The situation is not frequently compounded by the lack of privacy for young couple from overcrowding in many urban lower middle class households of Indian metropolis, thus depriving them of the most fruitful time to achieve pregnancy.

It is a medically acknowledged fact that it is essential to have the presence of live sperms during the 12 to 24-hour period in which the egg is available to be fertilised. Most propitious time to achieve pregnancy for the female partner is the period midway through the menstrual cycle. At this time, the most desirable and effective frequency of intercourse is every 24-48 hours for five days leading up to and including the day of ovulation, which normally coincides with the middle of the menstrual cycle. Infertility of a couple is a possibility without an average frequency of vaginal intercourse of at least twice during this fertile period of the female.

Present-day business commitments may even find the wife and the husband spending themselves in different cities. Executives travelling frequently may not be able to have sex during their wives' fertile period of the month. The demographic factors, particularly a high level of education, a high professional level and a high level of family income, were associated with the desire to have the first child at a relatively late age.[29] I often describe these couples as "corporate couple". With the limited chance of achieving pregnancy in each menstrual cycle (see female factor in infertility), infrequent sex by these couples is not conducive to successful conception. Obviously, they need to exercise some control over the travel schedule to improve their chances by having sex during wife's most fertile days. One should also note that infrequent sex can also lead to decreased sperm motility and abnormal sperm morphology. Today, science has made it possible to have sperm frozen, so that an insemination can be performed in husband's absence. It is debatable, whether such option of bypassing the natural method, should ever be taken recourse to.

Young couples often defer conception by choice to achieve economic betterment or to prolong their unencumbered life, thus pushing women to the wrong side of the thirties for the first pregnancy. This age unfortunately coincides with the period of reduced fertility in women (see 'Female factor'). This societal factor operates naturally to the detriment of fertility.

STRESS FACTOR

Stress is another factor that determines the fertility, and could play a significant contributory role, especially in the urban context.[116] It is well known that stress and excessive exercise can interrupt the normal flow of hormones from the hypothalamus and pituitary. Resultant abnormal hormone levels can interfere with women's menstrual cycles and her fertility. Some believe that endorphins (natural narcotics) released by the brain to minimise pain and stress may block the normal release of GnRH,[116,117] that is essential for maintaining balance between the male and the female reproductive hormones. Abnormal (infrequent or absent) menstrual cycles often seen in women, who run fifteen to twenty miles a week, can thus be explained. There are many similarities in the hypothalamic-pituitary hormone systems of men and women. The response to stress and excessive exercise could possibly have similar effects in males too. Seasonal variation of occurrence of pregnancies as stated earlier could possibly have an underlying stress factor with higher environmental temperature causing increased physical stress and consequent reduced fertility (see 'Environmental factors' and also Chapter 14).

WORKING-UP OF A CASE OF MALE INFERTILITY

History and Physical Examination

In the light of plethora of factors incriminated for the abnormalities in the male reproductive system, history and physical examination assume a very important role in arriving at the diagnostic, therapeutic and prognostic assessment of male infertility. It is also critically important that the couple should have an interview with the andrologist, as the fertility is dependent on both male and female factors. I make it a point to see the female partner after I have finished examination of the male. Often she throws light to some important aspect, which her male counterpart has conveniently omitted. I have seen umpteenth time that a male perception is altogether different from that of a female, yet this has a bearing on the fertility of the couple. Often, she comes out with facts like PE, STD infection or alcohol abuse of husband. However, her complaints about the sexual satisfaction may be overlooked, as it may not have the direct bearing on the fertility.

A specialist andrologist is not supposed to miss a diagnosis. However, it would be practical to look for common causes first, before going into the rare ones. On the other hand, one needs to have pragmatism, as some conditions such as genital tuberculosis are certainly more common in the subcontinent than in Europe or Australia. A comprehensive history from the male should be the first step, and herein lies the importance of having a ready proforma lest a busy clinician misses details. WHO proforma is comprehensive for the purpose. In our infertility clinic, a simpler form of record keeping is used (Appendices 2 and 3).

Importance of Taking History

A detailed history taking readily distinguishes a case of primary from a secondary infertility. A patient may fail to impregnate his present partner after been successful with the same or another one in the past. This information would establish the approximate duration of his secondary infertility. It would be injudicious to conclude about or to prognosticate his chance to father a child in a case of secondary infertility, although there may be some truth in assumption that once a man has been able to get a pregnancy with any partner, his probability of subsequent success is somewhat bright. The crux of the matter is to assess his present state of infertility, and whether it is redeemable or not.

Environmental and occupational factors must be obtained in the history. As discussed earlier, many occupational hazards such as workers in lead, cadmium and mercury industries and those in various other factories, are prone to have gonadal damage. The study by Thonneau et al concluded that the occupational heat exposure was a significant risk factor for male infertility, affecting sperm morphology and resulting in delayed conception.[118]

A special note about the recreational substances like smoking, alcohol, marijuana, cocaine, etc. should be included while eliciting the history. As stated earlier, excessive smoking can cause damage to the sperms and interfere with erection. Marijuana has deleterious effects on the fertility potential. The active ingredients in marijuana negatively affect the sperm qualities of men, who regularly smoke the drug, leaving them vulnerable to infertility. Frequent pot smokers have significantly less seminal fluid, and lower sperm count, and their sperm functions

abnormal.[119] *In vitro* study shows impairment of testosterone synthesis after taking recreational substances like cocaine.[120]

History of alcohol excess is also very pertinent. In normal men with good nutrition, there is an episodic release of testosterone. After just 5 days of consuming a sizable amount of alcohol (220 ml/day), the testosterone level may show a downward trend. After 4 weeks, there could be further fall in the testosterone secretion. Chronic alcohol use can lead to impotence, poor sperm quality, and further complications from liver damage due to altered androgen metabolism.[94,95,121] History of taking medications should be noted as they often have some bearing on the presenting symptoms.

Loss of libido associated with headache, visual abnormalities and galactorrhoea may suggest a pituitary tumour. Anosmia may be associated with oversecretion of the pituitary hormone prolactin (caused by prolactin-secreting micro- or macro-adenomas), or with Kallmann's syndrome (see also in Chapter 3). Impaired visual fields and galactorrhoea (spontaneous milk production by the breasts) may also be symptomatic of a prolactin-secreting tumour.

History of any respiratory infection or generalised illness is important. Temporary depression of sperm functions is also seen after administration of general anaesthesia and persistent fever exceeding 40°C. Macleod in 1966[13] had clearly demonstrated that viral and bacterial infections causing temperature of high degree could damage the testicular function and recovery may take months. Viral fever can cause impaired testicular function affecting sperm development for 1 to 3 months, even after the symptoms have cleared. Chromic respiratory infections are sometimes associated with disorders of sperm cilia resulting in asthenospermia or secretory disturbances in epididymis causing an obstructive azoospermia (*Kartagener syndrome*). Teratospermia or oligospermia may be caused by previous treatment with some gonadotoxic agents such as chemotherapy and irradiation for cancer, with medicines such as sulphasalazine for ulcerative colitis, spiralactone for renal and cardiac disorders. Prolonged use of nitrofurantoin, cimetidine and colchicines may at times lead to abnormal semen parameters.

History of urethral surgery or TURP may explain the cause of ejaculatory disturbance, so are sympathectomy and spinal injury involving L1 and L2 segments. Inadvertent injury to the vas can occur during repair of hernia in children. *(Incidentally, I have encountered a man, who had bilateral hernias repaired as a child in a hospital in Mumbai with azoospermia due to vasal injury).*

History of diabetes assumes importance as diabetic neuropathy may lead to either RE or impotence (see also Chapter 4). History of dysuria, urethral discharge, haematuria or pyuria may be associated with male accessory gland infections leading to abnormalities in the sperms.

It is important to elicit the past history of chronic infections of the male genital tract, as they are potent factor to impair fertility in male.[122] Chlamydial infection of the male gential tract has been observed in a large number of cases in recent years (see Chapter 10).[17]

While taking history, an andrologist should also be aware that the patients or even the primary care physicians might fail to distinguish between an epididymitis and epididymo-orchitis. History of an acute generalised scrotal pain is more likely to be due to epididymo-orchitis; while in a chronic epididymitis, there is recurrent localised pain in the scrotum.

It is of paramount importance to inquire into the past history of any testicular swelling and trauma as they have an important bearing on the fertility. Orchitis due to mumps, syphilis or small pox (now almost eradicated) may cause a testicular swelling and later infertility. Severe interstitial oedema, increased numbers of mononuclear leucocytes, and possible irreversible damage of the seminiferous tubules characterise affliction with mumps orchitis. Prepubertal mumps normally does not cause testicular damage to produce impaired fertility, but in a postpubertal affection the sperm abnormalities result from overt or covert testicular involvement.[123] About 15 to 25% of adult men who contract mumps, can develop orchitis, which is more commonly unilateral. Bilateral involvement occurs in about 10% of affected men. Testicular atrophy can develop within 1 to 6 months or may take years. One-fourth to less than one-third of men with bilateral orchitis recovers normal semen parameters.[122,123]

The exposed position of the testes makes them susceptible to trauma and subsequent atrophy. Testicular trauma, blunt or penetrating can destroy the testicular tissues to varying degrees and cause deterioration of testicular function, even if the loss

of actual volume of tissues is minimal, and the lesion is unilateral. Injury to one testis thus, may also result in male infertility, the diagnostic criteria being trauma followed by a reduction in the size of the injured testicle and/or the detection of antisperm antibody in the semen. Such infertility results not from the wasting of testicular tissue, but rather from an immune reaction that occurs due to penetration of the "blood-testis barrier" in the testes (see Chapter 2).

In a testicular torsion, the testis twists on the spermatic cord. The cord, which acts as a narrow pedicle from some congenital abnormality of the testicular suspension mechanism, allows the testis to twist on it. Consequent anoxaemic changes produce damage at the cellular level similar to what occurs with repeated infections. Patients with unilateral torsion also carry a high risk of subsequent torsion on the contralateral side. Bilateral testicular torsion is rare, but not unknown and would naturally damage both sides. Approximately 30 to 40% of men with a history of testicular torsion have abnormal semen analysis that is most likely to be due to some inherent defects in its sperm-producing potential, as shown by the findings of impaired spermatogenesis in tissue samples from the opposite testis. Duration and degree of testicular torsion have a direct proportional effect on the fertility. The fact that a unilateral affection often produces deterioration of the sperm quality disproportionate to the actual volume of tissue damage is explained by the phenomenon of *sympathetic orchidopathy* involving the contralateral or opposite testis. Either one or combination of the following reasons (Table 6.15) is cited for its occurrence.[124 -126]

1. Immune reaction caused by the anoxaemic destruction of the testis releasing 'autogenic' testicular tissue following breakdown of the "blood-testis barrier of Sertoli Cells" set the autoimmune reaction thus resulting in the damages to the contralateral testis.[127]
2. The congenital abnormality of the testicular suspension mechanism may be coexistent with an abnormality of testicular parenchyma. A torsion-prone testis may have inherent defects in its sperm-producing potential. It is shown by the findings of impaired spermatogenesis in the tissue samples from the opposite testis.

Table 6.15: Possible causes of sympathetic orchidopathy

1. Immune reaction.
2. Congenital abnormality of the testicular suspension mechanism.
3. Subclinical testicular torsions.

3. Possibility of subclinical testicular torsions occurring bilaterally may precede actual acute episode, and these repeated subclinical torsions might induce damage to the testes thus explaining the abnormal semen analysis.

Besides the trauma and the torsion, a neoplastic lesion is another important cause of testicular swelling. Hodgkin's disease produces spermatogenetic abnormality by the diseased process itself, and following its treatment by chemotherapy and radiation, both detrimental to sperm function. Other testicular malignant tumours have similar negative effects on the sperm functions.

Recent studies have established that the CIS (*carcinoma in situ*) factor[128] acts in foetal life on the primordial gonad cells to transform them to CIS cells, and thus predisposes their development into full-fledged carcinoma at a later date. Importantly, there is evidence that men with abnormal sperms at an early age have propensity to develop malignant lesions of testis later in life. It is postulated that the abnormal stimulus that ushers in transformation of some primordial gonad cells to CIS, could also be operative to bring about qualitative changes in some of the other gonad cells to behave abnormally from early life to produce excess of abnormal sperms.

Importance of Physical Examination of the Patient

Weight, Build, Height and Facial Characteristics

Excessive body weight reduces the testicular volume with resultant relative testicular deficiency. Incidentally, morbid obesity in women (BMI more than 39) is also an important negative factor and prolongs the TTP (time taken for pregnancy). Limb length is often disproportionate in Klinefelter's syndrome. During physical examination, particular attention should be paid to the features suggesting hypogonadism. Characteristic features of hypogonadism are typical poorly developed secondary sexual characteristics (abnormally sparse axillary, pubic, facial, and body hairs in conjunction with lack of temporal hair recession). There may be eunuchoid skeletal proportions such as arm span two inches greater than

height, ratio of upper body segment (crown to pubis) to lower body segment (pubis to floor) less than 1 and the lack of normal male hair distribution. In these patients one should also be on the lookout for infantile genitalia such as small penis, testes, underdeveloped scrotum and diminished development of muscle mass.

In Down syndrome, mongoloid facial appearance with receding hair in the nape of the neck are often seen. On the other hand, thyroid deficiency can be diagnosed by noting the coarseness of skin with nonpitting oedema, excessive body weight with changes in facial expression. Adreno-cortical disease like congenital adrenal hyperplasia is suspected, when there are premature development of secondary sexual characteristics and abnormal penile enlargement from the increased production of androgenic steroids by the adrenal cortex. If pituitary insufficiency occurs prior to puberty, growth retardation associated with adrenal and thyroid deficiencies is the major clinical presentation (See Chapter 3).

Importance of noting down the blood pressure cannot be ignored as in many endocrinal conditions such as adrenal hyperplasia or hyperactivity of thyroid, there may be changes in the blood pressure.

Gynaecomastia is a consistent feature of a feminising state and Klinefelter's syndrome. Men with congenital hypogonadism may have associated defects such as anosmia, colour blindness, cerebellar ataxia, hair lip and cleft palate. Hepatomegaly may be associated with problems of hormonal metabolism.

Proper examination of the neck can easily rule out a thyromegaly. A bruit or nodularity is often associated with a hyperactive thyroid gland. Neurological examination should include testing the visual fields to exclude pituitary tumours and the reflexes to rule out any spinal cord injuries.

The scrotal contents should be carefully palpated with the patient in both supine and standing positions. The testis, epididymis and the vas deferens should be individually palpated. It is important to make the patient relax while palpating, and to ensure that the temperature of the examination room is above 25°C. This ensures that the cremasteric reflex does not come into play to draw the testes up in a stripped patient. Palpation should start gently from the root or upper portion of the scrotum and gradually working its way down to feel the testis and the epididymis separately. Feeling for the testicular sensation should not be tried, as the resultant sickening pain felt by the patient may be enough for him to loose confidence in the examining doctor.

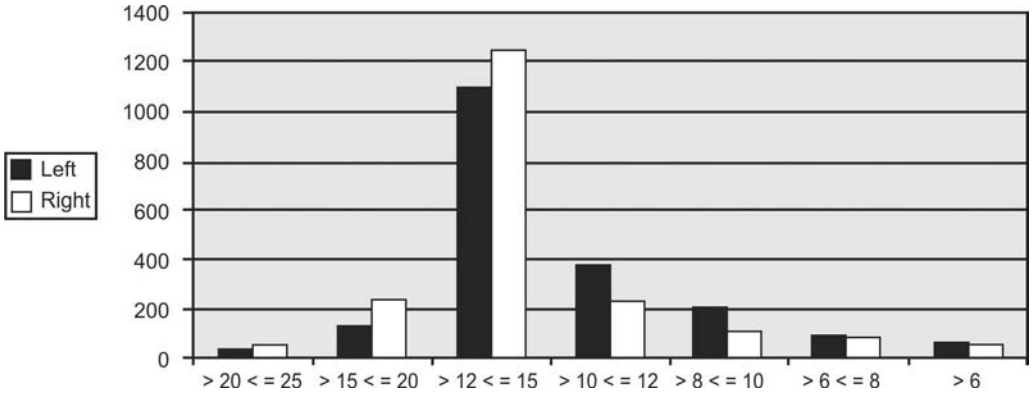
A careful examination of the testes is an essential part of the examination. A caliper or orchidometer may be used to measure testicular size.[4] If the seminiferous tubules are damaged before puberty, the testes are small and firm. With postpubertal damage, they are usually small and soft.[4] The length and the volume of the testis should be noted. The volume can be easily estimated by the use of an orchidometer available in a series of graded sizes of plastic model of testis (see also Fig. 2.1). Normal adult testes are on an average about 4.5 cm long and 2.5 cm wide with a mean volume of about 15 to 20 ml or cubic centimetres (cc). The testicular volume has a positive correlation with the sperm quality.

I have been using Prader's orchidometer (see also Fig. 2.1) for the last fourteen years. With the patient in a recumbent position, the scrotal skin is stretched to outline the testis and then it is compared with corresponding model of the orchidometer. A size of 20 cc or ml in volume is supposed to be the standard lower limit for Caucasians, but in India I often find testicular sizes between 12 to 15 adequate in most men. Comparison of testicular dimension (length and width) and volume of Caucasian testis is shown in Table 6.16. [129]

However, there is often a discrepancy in the sizes of testis measured by the orchidometer and the computerised ultrasonography (US). US measurement often shows a lower testicular volume than what is revealed through the orchidometer. This discrepancy can be explained by the facts that minimal amount of fluid in the tunica vaginalis and slight thickness of the scrotal wall would escape detection by an orchidometer to show a larger sized testes. Testicular sizes measured in the subcontinent recorded in my series and comparisons with Caucasian sizes are shown in Table 6.16 and Figure 6.2.[127,129]

Comhaire found that there is a significant relationship between the total testicular volume and the frequency of inadequate coital erection. With a total testicular volume (sum total of the sizes of two testes) 10 or less, the incidence is 4% approximately, while it is 2% with a total testicular volume of 20 and less than 1%, when it is 25 or above.[115]

Varicocele should be looked for not only in supine, but also with the patient standing using Valsalva manoeuvres (see chapter 9 on Varicocele). Many



	> 20 < = 25	> 15 < = 20	> 12 < = 15	> 10 < = 12	> 8 < = 10	> 6 < = 8	< 6
Left	39	133	1098	378	207	92	58
Right	54	242	1247	234	106	79	43

Fig. 6.2: Testicular size in 2061 male infertility cases (January 1990-June 2001)
(Both right and left included separately; 11(eleven) undescended and 45 (forty five) maldescended testes excluded)

varicoceles are not visible and may only be discernible, when the patient stands or performs the Valsalva manoeuvre. Varicoceles can often cause reduction of the testicular size. A marked discrepancy in the sizes of two testes should arouse suspicion. Both vas deferentia should be palpated, as 1 to 2% of infertile men have congenital absence of the vas and seminal vesicles. Thickening, nodularity or tenderness of the vas indicates presence of any inflammatory lesions (e.g. beaded vas in tuberculosis).

Table 6.16: Testicular size

Clinical status	*Volume in ml*	*Length (in cm)*	*Width (in cm)*
1. Prepubertal	8	3.1	2
	10	3.4	2.1
	12	3.7	2.3
	15	4.0	2.3
2. Adult	20	4.5	2.7
	25	5.0	3.0
	30	5.5	3.2

Adapted and modified from Sherins RJ and Howards SS. In Campbell's Urology, 5th edn, 1986

The actual position of the testis should be looked into in details. True undescended testes are positioned along the normal route of descent, but cannot be manually lowered into the scrotum. Retractile testes usually occur between the ages of 3 and 6 years due to hyperactivity of the cremasteric muscles. Ectopic testes are positioned outside the normal route of descent in areas such as the upper groin, floor of the pelvis, penile shaft or thigh. An ectopic testis unlike the undescended often attains normal or near normal size.

One needs to be very gentle while palpating epididymis. The epididymis is situated behind the testis at a posterior position and in healthy individual, it is a very soft structure unlike the testis. Irregularities in the epididymis may be caused by previous or current infective process and obstruction.

Examination of the prostate may reveal a small size in androgen deficiency or slight tenderness (bogginess) in men with prostatic infection. Any penile abnormalities, such as hypospadias, abnormal curvature and phimosis, should be looked for. Multiple scrotal cysts and elephantiasis of the scrotum may cause greatly thickened skin that may have a bearing in raising the intrascrotal temperature. Seminal vesicles are not normally palpable by a digital examination; but if it is tender and palpable, it signifies an infective process conclusion.

Conclusion

It should be evident from the above discussion that a clear understanding of the etiological, physiological and pathological aspects help a great deal to give an insight of male reproductive dysfunction. Some unnecessary investigative procedures can easily be

eliminated, if the clinician or andrologist is able to narrow down the probabilities in terms of diagnosis. A thorough taking of history and clinical examination of the patient thus assume a very important role, before the doctor uses the investigative tools to arrive at a final diagnosis. Obviously, some of these investigations would be essential, but criteria of choosing others may need to be prioritised on the basis of cost-effectiveness, especially in many developing countries like this subcontinent. Particularity and not universality may thus be the guiding factor in formulating the investigative procedures on a case-to-case basis.

The ultimate goal of management of male infertility or male reproductive dysfunction is to achieve pregnancy of the female partner and success of all methods of treatment are assessed in terms of attaining this end point. However, occurrence of pregnancy really is an indirect proof of male fertility. In this context, the results of treatment should ideally be expressed as overall conception rate per month of follow-up. But this may give also incorrect results, if cases are lost to follow-up (drop-out) as commonly seen in an Indian context.

In theory the *cumulative pregnancy or conception rate* (CPR or CCR) also is an expression of the probability of conception. The *effective CCR* should really be called the *take home baby rate*.[6] This can be calculated from the observed number of pregnancies and the total number of cases including those lost to follow-up or drop-out, or still under treatment. This presupposes the hypothesis that the probability of these cases achieving pregnancy is the same as that of the observed cases. This approach may be used to compare the different modes of treatment among cases with identical selection criteria. However, it is hazardous to extrapolate the results of this approach to specific cohorts of cases, since there may in fact be differences between the drop-out and lost to follow-up on the one hand, and those continuing treatment or still under observation on the other. Particularly, the group that terminates treatment early may consist of couples with poorer prognosis, or lower probability of conception. They decide to give up treatment, when no improvement has occurred in the initial phases of treatment and follow-up. Hence, the need to calculate effective cumulative pregnancy rate, which is limited to the cohort of cases under well controlled follow-up.

If P/C is the probability of conception per menstrual cycle, then 1 – P/C is the probability of not conceiving per menstrual cycle. Thus, probability of not conceiving after the second menstrual cycle is $(1 – P/C)^2$, and the probability of not conceiving after the third menstrual cycle is $(1 – P/C)^6$. In this manner, the formula can be extended to the nth menstrual cycle, as probability of not conceiving after the nth menstrual cycle = $(1 – P/C)^n$, where n = number of months. Therefore, the percentage of infertile couples = $(1 – P/C)^n \times 100$, and the percentage of fertile couples = $[1 – (1 – P/C)^n] \times 100$. This percentage in real term would be CPR, which can be represented by the following formula:

$$CPR = [1 – (1 – P/C)^n] \times 100$$

From the statistical data collection, CPR can be found and by applying the above formula, the value of P/C can be calculated for the population under evaluation.[6]

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APPENDIX 1

Glossary of Terms

Aspermia	– No semen ejaculated
Haematospermia:	– Blood present in semen
Leucocytospermia:	– White blood cells present in semen
Azoospermia:	– No spermatozoa found in semen
Normozoospermia (Normospermia):	– Normal semen concentration
Oligozoospermia (Oligospermia):	– Low sperm concentration
Asthenozoospermia (Asthenospermia):	– Poor motility and/or forward progression
Teratozoospermia (Teratospermia):	– Reduced percentage of morphologically normal sperm
Necrozoospermia (Necrospermia):	– No live sperm in semen
Globozoospermia (Globospermia):	– Round headed acrosome-less sperm

APPENDIX 2

CASE PROFILE USED IN OUR SERIES

Name _____

Date _____ Computer code _____

Age _____ address _____

_____ Phone _____

Referral by Doctor / Self/ Friends/ others

Marital Status- 0/ 1/ 2/3 Married for _____ yr.

Spouse age _____ Yrs. Contraception use-Yes/No.

Period without contraception _____ months/years.

Previous conception - Yes / No– Period since _____ months/years

Past Illness/operation (Inguinal and Scrotal operations)

Past history of TB, STD, Mumps, and Testicular swellings.

Medications, Smoking, Alcohol _____

GENERAL EXAMINATION

Height _____ cm. Weight. _____ Kg. Build _____.

Anaemia _____ Lymphadenopathy _____

Congenital Anomalies _____

Secondary Sex Characters _____

SYSTEMIC EXAMINATION

Abdomen/ Chest/ Limbs. _____

Endocrine status _____

Neck / Nervous system _____

Scrotum and Genitals

1. **Skin –** WNL/ Thick / Cysts _____
2. **Penis –** WNL / Abnormalities _____
3. **Testes-**

Right	**Left**
– Size- _____	– Size- _____
Consistency - _____	Consistency - _____
Epididymis- _____	Epididymis- _____
Hydrocele _____	Hydrocele _____

4. **Spermatic cord**- WNL / Thick /absent

5. **Varicocele**- Right – Nil / Early / 1^0 / 2^0 / 3^0.
   Site of varicosity – Uniform/ Lower / Upper
   Left – Nil / Early / 1^0 / 2^0 / 3^0.
   Site of varicosity – Uniform/ Lower / Upper
6. **Temperature gradient** (measured with Feverscan)- Not significant/ Significant. — °C.

7. **Semen Analysis**
   1. Volume
   2. Liquefaction time.
   3. Count—millions/ml.
   4. Total-millions/ml
   5. Motility Grade- % of Grade 3 and 4.
   6. Morphology- Normal — %. Abnormal — %.
   7. Acrosome — %
   8. Agglutination
   9. Sperm serum antibody titre—— mIU/ml.
   10. Cells._____
   11. Fructose-contents —-

8. **Hormone studies**

Serum Testosterone-	ng/ml.
Serum FSH-	M IU/ml.
Serum LH	MIU/ml.
Serum Prolactin	ng/ml.
Serum Estradiol	pg/ml.

9. ***General Remarks on Fertility***

APPENDIX 3

GENERAL PHYSICAL EXAMINATION

Height (cm)	☐☐☐	
Weight (kg)	☐☐☐	
Blood pressure (mmHg)	☐☐☐☐☐☐	
General physical examination	☐ normal	☐ abnormal
Signs of virilization	☐ normal	☐ hypoandrogenism*
Gynecomastia	☐ normal	☐ tanner stage

URINO-GENITAL EXAMINATION

Penis	☐ normal	☐ scars ☐ plaques	☐ hypospadias ☐ other*
			side: left-right
Testes	☐ both palpable	non-palpable	☐ ☐
Site	☐ both normal	abnormal*	☐ ☐
Volume (ml)	☐☐ left ☐☐ right		
Epididymis	☐ both normal	thickened	☐ ☐
		tender	☐ ☐
		cystic	☐ ☐
		non-palpable	☐ ☐
Vasa deferentia	☐ both normal	thickened	☐ ☐
		non-palpable	☐ ☐
Scrotal swelling	☐ none	hydrocele	☐ ☐
		hernia	☐ ☐
Varicocele	☐ none	grade III	☐ ☐
		grade II	☐ ☐
		grade I	☐ ☐
		subclinical	☐ ☐
Inguinal examination	☐ normal	lymphadenopathy	☐ ☐
		infectious scars	☐ ☐
		surgical scars	☐ ☐
		hernia	☐ ☐
Rectal examination	☐ normal		
– prostate		☐ soft swelling	tender
		☐ hard swelling	other
–Seminal vesicles		☐ palpable	
Contact thermography	☐ normal	☐ abnormal*	

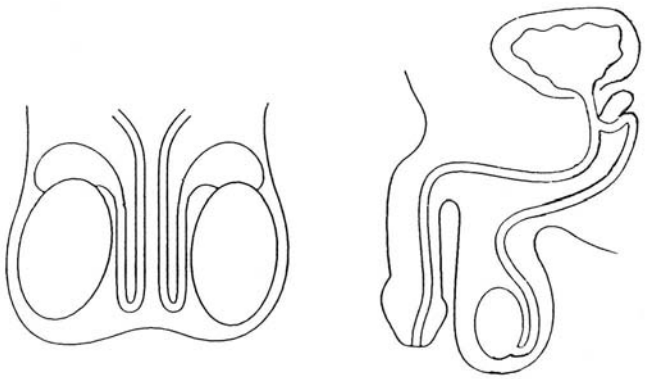

Fig. 6.3: Checklist for diagnostic investigation of the male partner (WHO)

MALE PARTNER

Date of history taking | Day | Month | Year | | | | Date of birth | Day | Month | Year | | | |

History of (in) fertility

Infertility
- ☐ primary
- ☐ secondary

Months since last fertilization
Duration of infertility
Previous investigation(s) and/or treatment(s) for infertility
- ☐ no
- ☐ yes

Pathology of treatment(s) with possible influence on fertility

History of medical treatment
- ☐ no
- ☐ diabetes
- ☐ chronic respiratory tract disease
- ☐ neurologic disease
- ☐ tuberculosis
- ☐ fibrocystic disease of the pancreas
- ☐ other*

History of medical treatment
- ☐ no
- ☐ yes*

High fever in past 6 months
- ☐ no
- ☐ yes*

History of surgery
- ☐ no
- ☐ urethral strictures
- ☐ prostatectomy
- ☐ vesectomy
- ☐ inguinal hernia
- ☐ sympathectomy
- ☐ hypospadias
- ☐ bladder neck operation
- ☐ hydrocelectomy
- ☐ other*

History of urinary infection
- ☐ no
- ☐ yes*

History of sexually transmitted disease
- ☐ no
- ☐ syphilis
- ☐ chlamydia
- ☐ gonorrhea
- ☐ other*

side: left-right

History of epididymitis
- ☐ no
- ☐ yes*

History of pathology possibly causing testicular damage
- ☐ no
- ☐ orchitis: mumps
- ☐ orchitis: other*
- ☐ injury*
- ☐ torsion*

History of varicocele treatment
- ☐ no
- ☐ yes*

History of testicular maldescent
- ☐ no
- ☐ yes

Treatment for testicular maldescent
- ☐ none
- ☐ surgical
- ☐ medical
- ☐☐ age at treatment

Other factors with possible influence on fertility

Environmental and/or occupational factor
- ☐ no
- ☐ heal
- ☐ toxic fractors
- ☐ other*

Excess consumption of alcohol
- ☐ no
- ☐ yes*

Drug abuse
- ☐ no
- ☐ yes*

Sexual and ejaculatory function

Average frequency of vaginal intercourse per month
- ☐ normal
- ☐ inadequate

Erection
- ☐ normal
- ☐ inadequate*

Ejaculation
- ☐ normal
- ☐ inadequate*

*Give details

CHAPTER

7 Semen Analysis

INTRODUCTION

Semen analysis still remains singularly the most informative investigation in the management of male infertility, notwithstanding the plethora of investigative tools now available to us. Its role in the etiological diagnosis of male infertility, however, is limited to azoospermia. Perhaps no other aspects of infertility are more controversial than the evaluation of male factor and in particular—the evaluation of semen. Often, the erroneous results stem from failure to ensure rigid criteria for transport and age of the specimen, and to set the standard technique for its evaluation under the microscope.

Any evaluation of semen must be based on overall picture that relates the volume, count, morphology, acrosome reaction and other cellular contents. No single factor can be considered in isolation, but motility is perhaps the most important criteria[1] for fertilisation. The "minimally adequate parameters" of semen are not the same as the "average parameters" of fertile men because of the variable female factor.[1]

Besides containing sperms, normal semen contains a number of other substances. These substances include water, simple sugars such as fructose for the nourishment for the sperms and alkaline chemicals that buffer the sperm against the acidic environment of the urethra and vagina. In addition, it contains the prostaglandins, which are fatty acid compounds that spur contractions in the muscles of the uterus and fallopian tubes aiding sperm's journey through the uterus, vitamin C, zinc, cholesterol and a few additional compounds. The semen can carry the bacteria or viruses of sexually transmitted diseases (STDs) including the acquired immunodeficiency syndrome (AIDS) virus, but normal healthy semen does not contain any harmful substance.

In most Indian laboratories, semen analysis is a relatively inexpensive test. But its relative inexpensiveness leads many patients and doctors too to believe that it is also a very easy test to perform, and they often choose to have it done from the neighbourhood laboratory for convenience. However, the apparent simplicity of performing semen analysis can be very misleading; as in reality, it requires lot of skill and expertise. It is a very easy to do this test badly, as is wont when performed by poorly trained technicians in most Indian laboratories. Consequently, the report causes confusion for both the patient and the doctor. Thus, it is crucial to go to a reliable andrology laboratory preferably the one that specialises in semen analysis, since the reporting is very subjective and depends entirely upon the skills of the doctor and the technician.

A normal sperm report is always reassuring to patients and usually does not need to be repeated provided a specialised laboratory having necessary expertise does it. However, one caution needs to be exercised. If the semen analysis is normal, a certain amount of complacency may creep in laboratory doctors resulting in skipping the detailed examination

of the patient. But the sperm count and the motility in normal range do not necessarily mean that the person is fertile. Even a normal motility pattern does not guarantee capability of the sperms to fertilise the ovum as the fertility of a particular couple depends on various factors (see Chapter 6). In real term, the only foolproof way of proving adequate sperm functions is having a pregnancy naturally or artificially by doing *in vitro* fertilisation (IVF).

Poor sperm tests can result from a host of factors such as incorrect semen collection technique, inadequate expertise, history of recent illness, long period of abstinence, etc. Several factors such as the sample not collected properly, dirty container, long delay between providing the sample and its testing in the laboratory, would change the semen parameters, so would too short an interval since the previous ejaculation or recent systemic illness in the last three months. Even flue or a high fever can temporarily depress sperm counts (see Chapter 6 and later in this chapter). Consequently, one should refrain from arriving at a conclusion on the basis of just one report, as the sperm parameters tend to vary because of various intrinsic and extrinsic factors.

Before making any judgement about semen quality, it is customary for specialists to obtain over a period of 3 to 6 months at least two preferably three, samples in which the semen characteristics are within the same 20% range[3, 4] (see later). As it takes a minimum of ten weeks for the testes to produce a new batch of sperms (see Chapter 2), there is a rationale of repeating the test after a period. In practical field, it also makes sense to repeat it from another competent laboratory to ensure validity of the report, especially in a patient with male factor infertility. When the sperm count is persistently poor, other investigations, such as specialised sperm tests, are mandatory to arrive at the core problem. For the man with a poor semen sample, additional tests such as hormone and other blood tests, ultrasonogram and testis biopsy, should be done. These are described in details in the next chapter.

PARAMETERS OF SEMEN ANALYSIS

Detailed information regarding various para-meters of the semen analysis and WHO prescribed norms are appended at the end of the chapter. The parameters are shown in the Table 7.1.

Table 7.1: Various parameters of semen analysis

Collection	Morphology
Transport	Cellularity
Period of abstinence	Biochemical
Volume and liquefaction time	Immunological
Count	Acrosome reaction
Motility	

Collection

The semen specimen should be collected in a small, clean wide mouthed jar of 10 to 20 ml (using larger jar may cause drying of some portion, when it is transported to the laboratory). The container must be spotlessly clean. I have seen umpteenth time a laboratory providing narrow-mouthed bottles for semen collection, which is not very conducive for collection of semen. Masturbation (self-stimulation) is the most preferred method. *Coitus interruptus* (withdrawal of penis just prior to ejaculation during sexual intercourse) may be used, but there is always a possibility of loss of the sperm rich initial portion.[4] If the patient objects to both masturbation and withdrawal because of religious or other beliefs, special condoms can be used during sexual intercourse.

The container must be ideally warmed to the body temperature, as sperms are especially susceptible to cold. This holds good if the container is transported; and moreover, while examining, the slide must be warmed, as otherwise motility studies may show erroneous result.[5] Providing a semen sample by masturbation can be very stressful for some men—especially when they know their counts are low, or if they have had problems with masturbation on demand for semen analysis in the past.

The condition of the toilet room, where the patient has to go for procuring the specimen, is often related to their not providing the proper specimen. My experience with most of the laboratories in this country is rather disappointing, as overwhelmingly great majority of them have dirty collection rooms not ideal for the purpose. Men failing to provide a specimen could be advised either to have female partners beside or to see sexually arousing pictures for helping them to provide sample. They can also use a mechanical vibrator to get an erection.

In some cases, additional assistance by using liquid paraffin helps in masturbation. If the problem still persists, it is possible to collect the ejaculate in a special silicon condom (which is nontoxic to the sperms and not ordinarily available in India) during

sexual intercourse, and then send this to the laboratory for testing. Ordinary condoms should not be used for semen collection, since they may contain spermicides or substances toxic to sperms.

Ideally, the semen sample should be collected in the laboratory or andrologist's office or clinic, although an acceptable sample may be obtained at home as long as it is kept warm at body temperature during transit, and is analysed within the hour of its actual collection. The semen sample must be kept at room temperature.[5] If the sample spills or leaks out, the test is invalid, and needs to be repeated. Except for liquid paraffin, no other lubricant should be used during masturbation for semen analysis as other lubricants can kill the sperms. The infertility centres should have a special private room to allow the patients for the masturbation on demand.

Most andrologists recommend that the semen samples be collected after a sexual abstinence 3 to 5 days, or at least 72 hours after the last ejaculation (no sex or masturbation). It is very important to keep with the chosen abstinence schedule, because variations in the time period between ejaculations interfere with the accuracy of test results. For up to one week, semen characteristics, such as volume and sperm concentration, may increase with each day of abstinence; but after that period, the sperm motility is usually impaired, (Table 7.2).

Table 7.2: Collection and delivery of a semen sample[6]

1. The sample should be collected after sexual abstinence of at least 72 hours but no longer than five days in duration. The name of the person, the period of abstinence, and the date and time of collection should be recorded.
2. Two semen samples should be collected for initial evaluation. The interval between the collections will depend on local circumstances, but they should not be less than seven days or more than three months apart. If the results of these assessments are remarkably different, additional semen samples should be tested, because marked variations in sperm output may occur in one individual. If the result of the first analysis is completely normal, there is no need for a second sample.
3. The sample should be collected in the privacy of a room near the laboratory. When collected elsewhere, it should be delivered to the laboratory within one hour of collection. If the tests of sperm functions, e.g. zona-binding test or zona-free hamster oocyte assay, are to be performed, it is critical that the spermatozoa are separated from the seminal plasma within one hour of production of the ejaculate.
4. The sample should be obtained by masturbation and ejaculated into a clean, wide-mouthed, glass or plastic container. If plastic is used, it should be checked for lack of toxic effects on spermatozoa. The container should be warm to minimise the risk of cold shock. If a bacterial analysis is to be done, the patient should pass urine, and then wash and rinse his hands and penis before the sample is collected into a sterile container.
5. Lubricants except perhaps the liquid paraffin should not be used to facilitate semen collection as they may interfere with the viability of the spermatozoa.
6. If ordinary method of masturbation is not possible for some reasons, specific plastic condoms are available for semen collection.[6] *Coitus interruptus* is not acceptable as a means of collection because it is possible that the first portion of the ejaculate, which usually contains the highest concentration of spermatozoa, will be lost. Moreover, there will be cellular and bacteriological contaminations of the sample and the acid pH of the vaginal fluid will adversely affect sperm motility.
7. Incomplete samples should not be analysed, particularly if the first portion of the ejaculate is lost. The sample should be protected from extremes of temperature (not less than 20°C and not more than 40°C) during transport to the laboratory.

Transport

It is always ideal to have the specimen collected in the laboratory. Transport from patient's house to the doctor's clinic is quite acceptable, provided it can be done within half an hour, as no examination is possible unless the semen liquefies (see Table 7.2).

Period of Abstinence

For initial evaluation, there should be 3 days abstinence and not more than 5 days is advised. The subject is dealt with later.

Volume and Liquefaction Time

A lot of men feel that their semen volume is too little or not enough, but abnormalities of volume are not very common. Abnormalities of volume usually reflect a problem with the seminal vesicles and prostate. Normal volume is about 2 to 6 ml. A low semen volume may suggest a block (see also Chapter 1), retrograde ejaculation (RE) into the bladder, infection or lack of androgen (Table 7.3). Men with congenital absence of the vas deferens or the seminal vesicles may have low semen volumes. Dubin and Amelar (1971)[7] in their studies with 1294 infertile patients have recorded less than 1 ml in 1.8% and above 4.5 ml in 10%. In our study, volume of 1.5 ml has been seen in many cases. The largest volume in the study has been 6.8 ml (Table 7.4).

Table 7.3: If the volume is less than 1 ml—one should consider

1. Collection error.
2. Stressful collection—a very common occurrence.
3. Retrograde ejaculation—when postejaculatory urinary examination is required.
4. Hypogonadism.

Table 7.4: Distribution of volume in ml
(Analysis of 2061patients—Jan. 1990 to June 2001)

		<1	*1*	*1.5*	*2*	*2.5*	*3*	*3.5*	*4*	*>4*
Azoospermia	(124)	7	27	23	36	9	6	9	7	0
Oligospermia	(1381)	0	12	67	345	594	227	69	39	28
Severe oligospermia	(502)	7	10	234	154	72	25	0	0	0
Normospermic	(54)	0	0	5	23	11	8	5	0	2

An abnormal semen volume may affect the fertility, only when it is less than 1.0 ml or greater than 5.0 ml. The bulk of the volume is contributed by the seminal vesicles and accessory sex glands. Prostatic fraction contributes normally between 0.5 and 1 ml. A low semen volume (less than 1.0 ml) is unlikely to provide enough fluid to bring the sperms to come in contact with the female partner's cervix or to neutralise vagina's natural acidic environment. The vaginal acidity may keep the bacteria under control, but it is detrimental to sperms. On the other hand, a high semen volume (greater than 6.0 ml) may actually dilute the sperm density to impede fertility. In these cases, artificial *in vitro* methods can be used to concentrate the semen to increase the sperm density. Later, this can be reintroduced through intrauterine insemination into the uterus (see Chapter 13-Artificial Insemination).

The total volume of the ejaculate should be measured using either a graduated cylinder or by aspirating the whole sample into a graduated syringe or pipette. Strict sterility is mandatory in case of any bioassay or semen culture.

The viscosity or consistency of the liquefied semen sample is estimated by pushing gently the semen through an injection needle (21G with an approximate internal diameter of 0.03 inch or 0.8 mm) and the length of the thread is carefully observed. A normal sample leaves the needle as small discrete drops. Each drop will form a thread of more than 2 cm, if the consistency is abnormal. Another method of estimating consistency is by introducing a glass rod into the sample and observing the length of the thread that forms.

Normal semen forms a coagulum after ejaculation. The enzymatic activity of the proteolytic enzymes such as seminin, later liquefies the coagulum to ensure its easy flow.[8] Liquefaction can occur in some patients as early as 5 minutes; but normally, it takes about 25 to 30 minutes. The laboratories normally start the test after waiting for about 30 minutes after ejaculation to allow the semen to liquefy. The concentration of the seminin cannot be the sole index of determining the eventual viscosity of the semen.[2] However, it is logical to assume that semen of higher viscosity would not release the sperms and may contribute adversely to human fertility. Incidentally, semen of some species such as rat never liquefies.[9]

During ejaculation, the semen spurts out as a liquid, which coagulates or gels promptly. In normal men, ejaculated semen liquefies within 20 to 30 minutes. If the liquefaction is delayed more than 60 minutes, the sperms may become trapped in this jelly-like mass. Since the prostate gland produces the substance needed for liquefaction, nonliquefying semen or if the semen is still thick after some liquefaction, it may signal a disorder such as prostatic infection or at times that of the seminal vesicles (see Chapter 2).

pH

The semen is alkaline with a normal pH between 7.05 to 7.8.[10] No positive correlation has been found in the fertilising capacity with any change of pH. An alkaline pH protects the sperms by counteracting the acidity of the vaginal fluid. An acidic pH suggests problems with seminal vesicular function such as absence of the seminal vesicles or an ejaculatory duct obstruction.

Count

Most Indian laboratories for the past few decades had set the lower limit of normal sperm count at 60 millions/ml, while clinicians were content with a minimum count of 40 millions/ml. It is well documented that pregnancies often occur even with a count of 20 millions per ml or less. On the other hand, many infertile men have a higher total count, but their inability to establish conception may be related to lower motility or higher proportion of abnormal morphology of sperms. In addition, the variable female factor still remains a subject unexplored in

clinical medicine unlike the animal husbandry. What is thus the minimum adequate count still remains controversial.

The total count alone without any reference to other parameters is an unreliable index of fertility.[11] Moreover, there is a large variation in the counts of an individual day-to-day, often compounded by stress, illness and taking of medicines such as nitrofurantoin, cimetidine, prolonged use of aspirin and colchicine, etc. So, as mentioned earlier, at least 2 or 3 counts should be done before any critical assessment is made.

Normally, a human ejaculate can be made into four fractions.[12] The pre-ejaculatory fraction or precum contains mainly mucous secretions from urethral (Littre's) and bulbouretheral (Cowper's) glands. The second fraction is opalescent fluid of low viscosity mainly derived from the prostate; and normally, it does not contain any sperms. Next comes the most important or the principal fraction contributed by the prostatic fluid and the major portion of the sperms from the vas deference and the distal epididymis. The terminal fraction contains the gel-like secretions from the seminal vesicles and few sperms. Thus, the first portion of the ejaculate characteristically contains most of the spermatozoa and the prostatic secretions, while the second portion is composed primarily of seminal vesicular secretions and fewer spermatozoa. It is thus important to ensure that the first portion of the ejaculated semen is never missed in the semen specimen sent to the laboratory for analysis. The daily sperm production (DSP) is approximately 300 millions in healthy males (till about 30 years), yet it is not explained why the sperm count in an ejaculate is much less.[13] In real terms, the sperm production is related to the state of the germinal epithelium.[14]

One of the pioneer researchers in the seventies Macleod had set the minimum count at 40 millions/ml.[11] At present, the World Health Organisation (WHO) and American Society for Reproductive Medicine have set the standard of the lower limit of normalcy at 20 millions per ml (Tables 7.5 to 7.7). The total sperm count is a more reliable index of sperm output by the testicular germinal epithelium than the sperm concentration as it is unaffected by the semen volume. Automated method is not reliable if the count is less than 10 millions per ml.[2]

Hyperspermia or polyzoospermia is a condition when sperm concentration in excess of 200 millions/ml is seen. It is rather rare and according to most observers, the condition also interferes with the fertility. However, huge normal values of sperm density are seen in most mammals; and keeping this background in mind, the significance of hyperspermia in infertility cannot be fully understood unless faults in the germinal epithelium, sperm structures or enzymatic activity are proved.[15] Hyperspermia with sperm counts above 200 millions /ml was recorded in 1.7% patients in their series by Singer et al[16] They found that this phenomenon was common in low volume semen samples and in relatively older individuals. Their explanation that this may be due to impaired response of testes to endocrinal and possibly other influences is contradictory to normal hypospermatogenesis seen in the older individuals. Often, these patients have low fructose value.[17, 18]

Table 7.5A: Normal semen parameters (WHO manual 1992—3rd edn)

1. Volume = > 2 ml.
2. pH = 7.2 – 8.0.
3. Viability = 75% or more within hour of collection.
4. Sperm concentration = 20 millions per ml or more.
5. Total sperm count = 40 millions per ml or more.
6. Morphology = 30% or more with normal morphology.
7. Motility = 30% or more with (A) or 60% or more with A + B within 1 hour of collection.

Table 7.5B: The normal semen parameters established in 1998 by the WHO

1. Volume: 1.5-5.0 ml.
2. Sperm density: >20 million sperm/ml.
3. Motility: >60%
4. Morphology: >60% normal forms.
5. Forward progression (scale 1-4): 2+
6. Viscosity: No hyperviscosity
7. White blood cells: 0-5 per high power field

Table 7.6: Normal values advocated by the American Society for Reproductive Medicine[19]

1. *Liquefaction* (conversion into a liquid): complete within 60 minutes.
2. *Appearance:* Homogeneous, gray-opalescent ejaculate.
3. *Volume* (amount): 2 millilitre or more.
4. *Consistency:* Not viscous (not thick).
5. *Morphology* (structure): 30% has normal shape* (WHO) > 15% have normal shape**(Kruger)
6. *Concentration*: 20 million per millilitre.
7. *Total count*: 40 million sperm per ejaculate.
8. *Motility* (movement): 50% at one hour.
9. *pH* (acidity): 7.2.
10. *White blood* cells: < 1 million per millilitre.

* Normal morphology according to WHO.
**Normal morphology values according to Kruger.

Table 7.7: Semen analysis and semen quality

Semen analysis		*Semen quality*		
	WHO	Normal Standard	Borderline	Abnormal
Semen volume (ml)	> 1.5 ml	2-6	1-2	< 1
Sperm count [million [10^6]/ml]	20 million/ml	20-250	10-20	< 10
Sperm motility (movement) %	> 60%	> 50	40-50	< 40
Curvilinear velocity (VCL)	x	> 40	30-40	< 30
Agglutination (clumping)	None	Minimal	Minimal	Significant
pH	7.2-8.0	7-8	<7	> 8

Adapted from: J. Raifer: Common Problems in Infertility and Impotence. (Chicago: Year Book Medical Publishers, Inc., 1990),

In our infertility clinic, we have followed the norms presented by Macleod[11] and set 40 millions as the lower limit of normalcy as shown in Table 7.8. In our series of 2061 infertile patients, we had 54 patients with normal count, i.e. above 40 millions, but there was deficiency in other parameters to account for infertility (Table 7.9).

Table 7.8: Norms followed in the series

1. Up to 40 millions/ml or above: **Normospermic**
2. 20-40 millions/ml: **Oligospermic**
3. Less than 20 millions/ml: **Severely oligospermic**.

Motility

The motility and the morphology (discussed later) are criteria highly vulnerable to individual observer's personal impression. Herein lies the importance of setting a standard. Unfortunately, this is not possible in most Indian laboratories unless we have a dedicated pathologist or a technician, who has been specially trained. If the motility is poor, this suggests that the testes are producing poor quality sperms and not functioning properly. This situation leads to apparently motile sperms not being able to fertilise the ovum or the egg. The percentage of motility is determined by judging the number of active vs inactive forms in 4 or 5 estimations and a mean is taken. At least 100 successive sperms should be studied for a proper and critical evaluation.

The sperm motility is the most important feature of a semen quality. The motility is usually estimated by direct microscopic examination of the semen to determine the percent of the sperms that are swimming. The quality of the sperm movement is often more significant than the count. The sperms are mainly classified into two types—those which swim, and those which do not. The sperms, which move forward fast, are only able to swim up to the ovum to fertilise it. Others are of little use.

Overwhelming numbers of laboratories of this subcontinent have no access to sophisticated machineries[2] such as laser Doppler technique, videomicrography or photon-related spectroscopy. New technologies now incorporate computer-assisted semen analysis (CASA) with video systems (see later). In our series, we have followed criteria for motility advocated by Hotchkiss in 1970, corresponding to different WHO grading. The motility is graded from A to D, according to the WHO manual criteria (Tables 7.10 and 7.11).

Table 7.9: Distribution of sperm counts in millions/ml in 2061 patients

Sperm counts in million/ml	*Azoospermic (124)*	*Oligospermic (1381)*	*Severe oligospermic (502)*
Less <10	—	0	79
Bet. >10<15	—	0	169
Bet >15<20	—	0	254
Bet >20<25	—	484	—
Bet >25<30	—	614	—
Bet >30<35	—	209	—
Bet >35<40	—	74	—

***NB**: 54 Patients had counts above 40 millions/ml, but had other abnormalities to account for infertility (Excluded from the list)*
(Report presented in the annual conference of the International College of surgeons, Indian section in 1998)

Table 7.10: WHO grading

a.	**Grade A** (*fast progressive*) sperms swim forward and fast in a straight line—like guided missiles.
b.	**Grade B** (*slow progressive*) sperms swim forward, but either in a curved or crooked line, or slowly *(slow linear or nonlinear motility*).
c.	**Grade C** (*nonprogressive*) sperms move their tails, but do not move forward (local motility only).
d.	**Grade D** (*immotile*) sperms do not move at all. Sperms of grades C and D are considered poor.

Table 7.11: Hotchkiss and WHO grading

	Hotchkiss [20]		*WHO*
Grade	**0** = Nonmotile	=	WHO grade D
	1 = Sluggish	=	WHO grade C
	2 = Poor to fair motion	=	WHO grade C
	3 = Good forward movement	=	WHO grade B
	4 = Highly active progression	=	WHO grade A

The doctor should make the detailed studies after half an hour, as physiologically the sperms should penetrate the cervical mucus plug by ½ to ¾ hour. The motility should also preferably be recorded at successive period of every half hour for a period of three hours. We have followed MacLeod's lead to set 60% of grades 3 and 4 as the lower limit of the normalcy, and below this limit was described as *asthenospermia*.[20] Hudson and Baker and American Society of Reproductive Medicine[19] had set the normal level at 50%.

One of the best discrimination factors is the proportion and concentration of sperm with rapid linear progressive motility, based on the cut-off value of linear velocity greater than or equal to 22 microns/sec. This parameter has been found to be 90% accurate in discriminating semen of infertile men from that of subfertile patients.[21] A morphologically normal sperm can move or swim faster and straighter than an abnormal one[22] *Necrospermia* is a condition, where no movement is recorded in the sperms. In strict sense this expression is a misnomer as even these immotile sperms are alive. Most likely physiological cause of this condition is due to lack of dynein arm of the microtubules in the tail.[23]

Computer-assisted Semen Analysis (CASA)

The new technologies such as CASA incorporate the video systems to measure the types and the speed of sperm motility. CASA systems couple video technology and sophisticated microcomputers for automatic image digitalisation and processing. This technology was developed for more objective measurements of seminal parameters over the subjective measures of standard semen analysis. Structurally, normal sperms swim faster and straighter than the abnormal ones. The average speed of a human sperm is roughly 48 to 96 mm per second. CASA permits the measurement of additional motility parameters such as curvalinear velocity (VCL), straight-line velocity (VSL), linearity, and flagellar beat frequency.[4]

CASA measures the parameters such as VCL, VSL and amplitude of lateral head (ALH). The VCL is measured as the average distance per unit time between successive sperm positions. The VSL is measured as the distance between first and last sperm positions per total elapsed time. The linearity is taken as the ratio of VSL and VCL. The ALH is measured by the average perpendicular distance of lateral positions of the sperm head in relation to the average path of swimming. While CASA may appear to be more reliable as the test is done objectively by a computer, there are still many controversies about its real value, since many of the technical details have not been standardised, and vary from lab to lab. The lower limit for VSL is 25 mm/sec, the lower limit for VCL is 40 mm/sec, and the lower limit for linearity is 5 mm/sec. The quality of sperm movement is based on a classification system of 0 to 4, wherein 0 represents no movement and 4 represents excellent forward progression; for example, a semen sample with 60% motility would be characterised as 3 + to 4.[2]

Quality Assessment of CASA

Three levels of quality assessment are generally accepted: structure, process and results. Quality of structure mainly concerns the quality of laboratory assessment, in particular the skill of the staff and the equipment used.[24] From reports of the literature it may be stated that:

a. In the dimensions of structure and process quality, CASA is superior to other methods of measuring sperm motility;
b. The evaluation of results and quality of results, however, is highly problematic;
c. CASA systems do not appear to be superior to the visual estimation of sperm motility with respect to the fertilising capacity of spermatozoa;
d. The guidelines of the WHO task force form a basis for sufficient process quality;
e. Further efforts should actually focus not on the improvement of investigation technology, but on

the improvement in the qualification of investigators; and

f. CASA systems are also superior to other methods regarding the documentation of laboratory values.

Measurements of sperm motility and velocity should be conducted using a microscope stage warmed to 37°C. An attempt to record 200 motile sperms per sample is desirable, if one is interested in the distribution of velocity measurements.[25] However, record of 50 motile sperms will suffice, if means are to be compared. If the videotapes are being used to calculate the percent motility, one should avoid hunting for motile sperms. All fields examined or searched should be included in the calculations. Therefore, recording a certain number of arbitrary fields is advised. Some researchers have found it useful to record a given number of fields to determine sperm concentration and percent motility, and then additional fields for velocity estimates. If a CASA system is being used for motility and velocity estimates, the number of sperms per field needs to be reduced to minimise cell collisions. Using a 10 to 20 m deep chamber, the sperm concentration should be less than 40 millions/ml. Diluents (including seminal plasma), however, alter sperm velocity up to a dilution of about 1:1. The current recommendation is to dilute all samples 1-part semen in 1-part iso-osmotic buffer.[25-27] If this dilution does not reduce the sperm concentration below 40 millions/ml, then an additional dilution in the same buffer should be performed on those concentrated samples.

The collected data from the sixty-four laboratories from Connecticut, Massachusetts, and California indicate that there is a wide range of normal values for each parameter considered in a semen analysis.[28] Similar observations were made in a study conducted in Edinburgh, UK.[25] There were no unanimity in giving simples and precise instructions for collection of the specimen. In many instances, there were no records of the collection or the arrival time of the specimen at the laboratory. Most laboratories did not report motility or forward progression at time intervals, and abnormal morphology was not broken down according to the types of abnormalities involved. These data indicate the absolute need for adequate standardisation of semen analysis, as these informations form the basis on which the clinician makes the important decision on the male factor infertility problem. It is absolutely essential that there should be an agreement on the designated values for threshold velocities for grades A and B sperms in the assessment of sperm motility grades. Without any unequivocal standardisation of such values, CASA analysis is not expected to provide the expected percentages of grades A, B, and C forms for quality control samples recorded and distributed on videotapes.[29-32] Thus, presently under certain circumstances, CASA has been found to be less accurate than the standard semen analysis, and the biological and clinical relevance of some of these new parameters has yet to be validated.[4] These observations based on findings of various laboratories amply elucidate the problem of standardisation. This also illustrates the enormity of the task to standardise semen findings from different Indian laboratories.

Morphology

Morphological analysis of sperms or seminal cytology is the most time consuming and requires special expertise of pathologist. Any variation in the shape or size of sperms relates to the germinal epithelial activity and thus it acts as its sensitive index.[33] Normal sperm has regular oval head, uniform has size and a single long tailpiece separated by a constriction called the neck from the connecting midpiece (Chapter 2). If too many sperms are abnormally shaped (round heads; pin heads; very large heads; double heads; absent tails), man may find it difficult or not be able to fertilise the ovum. A study of detailed morphology thus assumes very important role in assessment of semen. However, various forms of deviations in small proportion are often found even in fertile men. Depending upon the pathologist's expertise, normal semen should contain 60% or more normal and 2 to 3% immature sperms. Some labs use Kruger strict criteria (developed in South Africa) for judging sperm normality (Fig. 7.1). In Kruger's criteria only the sperms, which are "perfect", are considered to be normal. A normal sample should have at least 15% normal forms, which means even up to 85% abnormal forms is considered to be acceptable!. There is a positive correlation of the percentage of oval forms and the sperm concentration with fertility. About 75% of normal men have 60% oval forms of sperms.[34, 35] High percentages of abnormal sperms such as tapering forms and often spermatids, are found in varicocele or related disorders such as elephantiasis of scrotum. I have encountered 2 such patients of elephantiasis with abnormal semen parameters. Some specific forms of

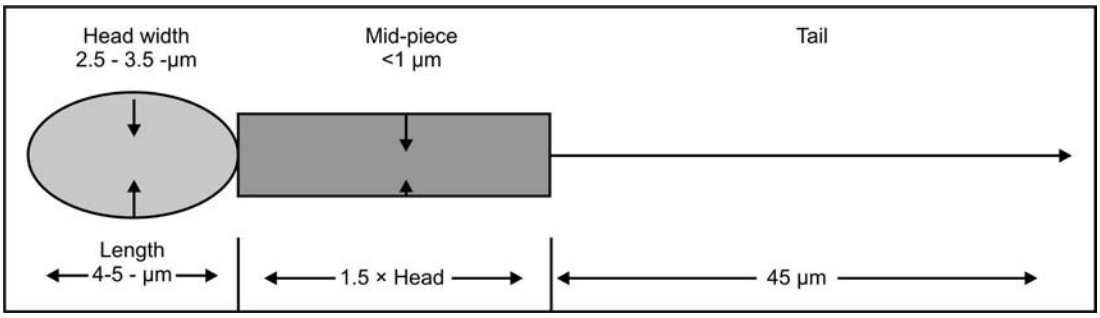

Fig. 7.1: Kruger's specification

sperm abnormalities include small or enlarged heads, coiled tails, duplicate heads, immature sperm shape, and sperms with absent or multiple nuclei (see Chapter 5 Gonadotoxic agents) (see details of morphology in Appendix 10).

Normal sperm has a smooth oval head, an acrosome that is 40 to 70% of the volume of the head, no abnormalities of the neck, midpiece or the tail and no cytoplasmic droplets of more than half the size of the head. Length of the head should be 4.0 to 5.0 microns and the width 2.5 to 3.5 microns with length to width ratio 1.50 to 1.75. The midpiece should be slender and not more than 1 µm and 1.5 times the length of the head and attached axially to the head. The tail should be approximately 4.5 microns (µm) long, straight and uniform, and thinner than the midpiece (Fig. 7.1).

Normal morphology, as evaluated by the strict criteria, was also highly predictive of the outcome of certain functional tests, such as the hemizona assay and the acrosin activity.[39] The sperm morphology (or shape) is determined by an average scoring of at least 100 sperms. It is considered within the normal range, if more than 50% of the sperms have normal sized oval head, with a customary midpiece and tail.[11, 40]

Sperm morphology should be estimated on air dried, stained semen smears. During the past 30 years, several schemes have been presented for the assessment of normal and abnormal sperm morphologies. Variations in sperm size and shape are not often distinct. This provides a challenge within and especially among laboratories to establish a repeatable system for morphological classification.[28-32] With recent advances of computerised image analysis, several methods of sperm morphometry have been introduced.[41-46] These morphometric analysis systems provide objective assessments of individual sperm head size and shape. Comparisons of measurements between different analysis systems should be avoided. Sperm morphometry is now routinely used as part of the assessment of reproductive hazards to the male industrial workers.[42]

In the newer system advocated by Kruger and others set the criteria for morphology very strict and more stringent and 15% is considered as the lower limit of normalcy (see Appendix 6) . When examined directly under microscope, a fraction of nonmotile sperms is not necessarily dead cells. A certain number may still be in a nonmotile live (NML) state. Many investigators still believe that nonmotile sperms though still alive do not take part in the fertilisation. Commonly, the supravital staining using 1% eosin is used to differentiate the live and dead sperms.[47] A wet preparation of the slide is preferred as the dry smear may cause death to many living sperms while the preparation is made. Normally, the ratio of NML to dead is 2:1. However, the supravital staining procedure has not been found to be foolproof in the human context.[2]

The study of seminal cytology by the conventional method suffers from an inherent difficulty in distinguishing the leucocytes from the immature spermatogenetic cells. Thus, one has to depend almost entirely on the subjective assessment of the pathologist (see discussion on cellularity later in this chapter). The problem in the assessment of the sperm morphological characteristics is their pleomorphism.[48] The number of sperm in the ejaculate is directly correlated with the number of germ cells per gram of testis.[14] One tends to assume easily that those looking morphologically normal are the ones that are capable of fertilising. On the other hand, a very small change (which may only be detected by the electron microscopy and is passed as normal under ordinary microscope) could particularly be important. Examination of spermatozoa with the light microscope can provide only limited information on their internal

structure but electron microscopy often reveals major ultrastructural abnormalities.[47] Thus, one of the grey areas in morphological interpretation is, whether a pathologist should classify spermatozoa as normal or abnormal on the basis of anatomical variations or in the physiological sense in terms of its fertility potential. Without having the luxury of electron microscope, most pathologists of the subcontinent would tend to go by the norm that those sperms, which looked obviously normal, are capable of fertilising. They obviously gloss over the fact that even minor variations in the shape and size may be the real deciding factor in fertilisation. Patients with severe sperm head abnormalities have a lower chance of establishing successful pregnancies, even though fertilisation may be achieved.

Sperm Clumping or Agglutination

While looking for the motility during microscopic evaluation, the doctor or the trained technician of the laboratory should also note any sign of sperm agglutination or the clumping of sperm. Under the microscope, agglutination or clumping is seen as the sperms sticking together to one another in bunches. Such clumping can keep the sperms from swimming properly through the cervical mucus and can prevent them from attaching to the ovum. Increased agglutination may suggest an inflammatory condition (e.g., bacterial infection) or an immunological abnormality. The sperms may clump head-to-head, tail-to-tail, or head-to-tail. In particular, tail-to-tail agglutination of motile sperm is noteworthy and usually is followed up with tests for antisperm antibodies (see also other tests of 'Sperm Function').

Taking three most important parameters (count, motility and morphology) all together, one looks in a semen sample for the total number of good sperms as the products of the total count, the progressively motile sperm and the normal shaped sperm. A crude index of the fertility potential of the sperms is the count of progressively motile normal sperms.

The motile sperm concentration is calculated by multiplying sperm concentration by percentages of the motility and the normal morphology. If a man has a total count of 40 million sperm per ml of which 40% are progressively motile and 60% are normally shaped; then his progressively motile normal sperm concentration is:- 40 × 0.40 × 0.60 = 9.6 million sperm per ml. If the volume of the ejaculate is 3 ml, then the total motile sperm count in the entire sample is 9.6 × 3 = 28.8 million sperms. Other indices of assessing semen are shown in Tables 7.12 and 7.13.

Table 7.12: Other indices of assessing fertility

1. Motile sperm concentration	Normal > 10 millions/ml.
2. Total functional sperm	0-3 = poor 3-13 = average >13 = good
3. Sperm motility index	0-80 = poor 80-160 = average >160=good

Table 7.13: Gross guidelines for interpretation

Parameter/Grade	*Good*	*Medium*	*Poor*
Sperm motility index-SMI	> 160	80-160	< 80
Motile sperm conc. - MSC	> 26	10-26	0-10
Functional sperm conc.-FSC	> 13	03-13	0-03
Total sperm count	> 60	20-60	0-20
Morphology-Normal	> 30	20-30	0-20
Motility	> 50	30-50	0-30

Very often, a low percentage of motility is found along with high sperm concentration and vice versa. The product of sperm concentration and percentage of motile sperms provide the percentage of the motile sperms in the specimen. It is still debatable, whether a specimen with 30×10^6 per ml and motility of 60% should be equated as equal in quality with another specimen with 120×10^6 per ml with a motility of 15%, since both specimens have the same concentration of motile sperms. Nevertheless, it is reasonable to assume that high sperm concentration should somehow compensate for low percentage of motility in the same manner as the high rate of motility may compensate the low sperm density. However, Macleod[11] had found that the total count alone should not be considered alone without taking into account other parameters, such as morphology, and motility is an unreliable index of fertility and the motility is the most singular factor in determination of fertility.

Period of Abstinence

With each day of abstinence (up to one week), the semen volume increases by 0.4 ml, sperm concentration by 10 to 15 million per ml, and the total sperm count by 50 to 90 millions. Sperm motility and morphology appear to be unaffected by 5 to 7 days of abstinence, but longer period beyond 7 days leads to impaired motility.[4, 49, 50]

In a study of 378 men (between September 1995* and November 1997), semen was analysed to ascertain the effects of period of abstinence on the motility and count (Table 7.14). Same individuals

Table 7.14: Average motility and count after different periods of abstinence in 378 patients

Two specimens in 378 patients* examined-first with abstinence a period of -3 days and later after 10 days	*Average motility*		*Average count*	
	Decreased	No change	Increased	No change
	277	101	216	172

*Note: *In continuation of the preliminary report on 108 cases presented at the annual conference of the International College of Surgeons, Indian section in Mount Abu, 1996.*[51]

were asked to submit semen specimens on two successive weeks after an abstinence of 3 and 10 days.

On the basis of these observations, we tend to conclude that abstinence of 7 days or more to save up sperms may be counterproductive as the gain is offset by the lower motility. In the same series, there was increase in the volume of semen in 63% (238) patients.

Cellularity

There are various forms of cellular elements in the semen such as pus cells, immature spermatogenic or testicular cells, RBC, bacteria, or protozoa epithelial cells, etc. The presence of these cells may indicate specific disorders that can affect potency. However, testicular cells, and leucocytes at times could be difficult to differentiate under the conventional microscope unless the pathologist is really experienced.

Numerous germinal cells, along with debris from dead or immotile (nonmoving) sperm, may suggest recent testicular stress due to fever-related illness or infection (e.g., a severe episode of the flue) or effects of various toxic agents. In such cases, after a few months of recovery, the sperm characteristics usually return to normal. Excess of pus cells may be due to overt genitourinary infections. In the subcontinent, tuberculosis and *Chlamydia* must be thought of to cause pus cells in the semen. Number of leucocytes greater than 5 millions/ml may imply fertility-hampering infections such as STD, gonorrhoea, chlamydia, ureaplasma, etc, in the male patient and/or his female partners. In males, prostate, infections of bacterial origin often tend to linger on or take some time to resolve completely[52-56] (see also "Medical Management of Infertility" in Chapter 11).

Even for an experienced doctor or technician, it is often very difficult to evaluate the morphological differences between 'round cells' of spermatogenic and nonspermatogenic origin in semen. The latter group includes inflammatory cells such as neutrophils, lymphocytes, macrophages and epithelial cells. Amongst the nonspermatogenic cells in semen samples, only the inflammatory cells such as the neutrophil leucocytes (both eosinophilic and basophilic) show a positive reaction, when exposed to the peroxidase stain. The lymphocytes, macrophages and other 'round cells' such as epithelial cells and the spermatogenic cells, all show negative reactions. So, the presence of specifically stained neutrophils in semen is considered to signify an infection and/or an inflammatory reaction in the male genital tract.[57, 58]

It is also difficult to differentiate between the white blood cells (WBCs) and the immature spermatozoa on routine analysis as they both may appear as round cells in the semen. The peroxidase stain and more recently the monoclonal antibodies have been utilised to aid in this differentiation. Even excessive white cells (>1 million/ml) may contribute to subfertility (see 'ROS factor' in Chapter 6). WBC concentration has been negatively correlated with progressive motility, percentage of morphologically normal sperm, and hypo-osmotic swelling (HOS) test (see later) in infertile men.

Conventional Semen Examination

Semen analysis should be conducted in an andrological laboratory in two phases. The initial evaluation of the sample should be conducted, when the sample arrives at the laboratory. This evaluation consists of recording the temperature, turbidity, colour, liquefaction time, volume, osmolarity, and pH of the semen. The sperm counts, viability estimates, video recordings for motility assessments, preparation of slides, and preservation of seminal plasma should also be conducted at this time.

Morphologic and morphometric analysis of sperm on slides and motility and velocity analysis of the sperm recorded on video tapes may be conducted at a later time. The motility analysis may be performed manually or using a computer assisted sperm analysis system (CASA).

As stated earlier, most andrologists before establishing a baseline for semen quality recommend analysis of at least two or three specimens and the seminal parameters of these samples should be within 20% of each other. The semen specimen is best obtained by masturbation after a three to four days of abstinence. Examination and assessment of the specimen should start within 45 to 60 minutes of collection. The samples obtained by *coitus interruptus* or from the condoms are not a desirable option. Ideally, the collection should be done at the site of analysis.

Besides laboratory errors, as stated earlier, there are variations in sperm density, motility and morphology among multiple samples from the same person at different times. These variations must take into consideration in interpretation of a sample of semen. The minimum number of specimens to define good or poor quality of semen is three samples over a 6 to 8 week interval with a consistent period of abstinence of 3 to 4 days. In a longitudinal analysis of the semen from both fertile and infertile men, it was found that 97% of men with initial good sperm concentration would continue to show good density after as many as 3 to 6 specimens.[4, 34] Those rated poor at first, mostly remained poor in future visits. For those rated equivocal, first visit was of little value and at least two or three visits were needed for critical assessment of the semen. It has also been found that the sperm count may go up because of the phenomenon of regression toward the mean as revealed in careful longitudinal analysis.[59, 60]

Failure of produce semen specimen either through masturbation or intercourse is sometimes encountered in the laboratory. The absence of semen may have many causes.

1. Retrograde ejaculation, where the semen may be entering the bladder instead of emerging through the penis This may result from bladder, urethral or prostate surgery, diabetes and certain medications for psychiatric and neurologic disorders, and for high blood pressure.
2. Organ failure— Failure of organs that normally make semen volume, such as the prostate and seminal vesicles. This may result from surgical removal of the prostate gland (prostatectomy), surgical removal of part or whole of the bladder (cystectomy) or pelvic radiation therapy that damages the pelvic nerves.

In our study, the lower limit of normal semen analysis has been set as follows with certain modifications from McLeod (Table 7.15).

Table 7.15: Lower limit of normal semen analysis

Volume	:	1.5 ml
Sperm concentration	:	40 millions/ml
Total sperm count	:	60 millions/ml
Sperm motility	:	60% grade 3 and 4
Sperm morphology	:	60% oval forms

Semen analysis can be conducted in phases. The initial evaluation of the sample should be conducted by recording the temperature, turbidity, colour, liquefaction time, volume, osmolarity, and pH of the semen. The sperm counts, viability estimates, motility assessments, preparation of slides, and preservation of seminal plasma should follow. Morphologic and morphometric analysis of sperm on slides and motility and velocity analysis of the sperm recorded on videotapes may be conducted at a later time. The motility analysis may be performed manually or using a CASA system.

Abnormal Colour Change

1. *Haemospermia* or *haematospermia* is a condition that is characterised by blood in ejaculated semen. It is an uncommon but rarely a serious condition. In most cases, the cause is not determined, and the problem resolves without treatment. Known causes of haemospermia include inflammation, infection, blockage or injury to the prostate gland or seminal vesicles. Experienced andrologist can diagnose this condition using physical examination, urine and blood tests. Treatment is directed to the underlying cause when possible.
2. *Gold and clumpy sperms*. Certain chemicals in semen may cause it to liquefy several minutes after ejaculation. Changes in the appearance of semen due to gold and clumpy sperms are usually temporary and do not indicate a serious underlying condition. However, an unusual colour or consistency may be due to infection or bleeding into the prostate gland, deficiency of male hormones reducing the volume of semen.[61]

Semen Culture Test

In a semen culture test, the semen sample is tested for the presence of bacteria. Testing the bacterial sensitivity to antibiotics is mandatory if there is any presence of bacteria. However, it is important for the laboratory physician to interpret accurately the difference, whether the bacteria present in the specimen are that are usually seen in normal semen or those of a bacterial disease. Without the evidence

of inflammation or infection, there is no indication for routine culture or antibiotic treatment in infertile men. If urinalysis is abnormal or bacterial prostatitis is suspected from the history or the physical examination, semen culture is certainly indicated. The common sexually transmitted organisms[52-54] such as C*hlamydia trachomatis, Mycoplasma hominus* and *Ureaplasma urealyticulum* have been implicated in reproductive failure in animals and humans. Some physicians prescribe antibiotic therapy even without any overt evidence of these infections in the hope of improving fertility. However, most andrologists have contrary views and doubt the role of asymptomatic infection caused by above organisms in male infertility.

Biochemical Tests

Biochemical analysis of seminal plasma mostly provides insights into the function of the accessory sex glands. The fructose content of semen (normal value—250-400 mgm or 4-28 mmol/litre) should be routinely tested. Low fructose content (less than 120 mgm) is often due to seminal vesiculitis, androgen deficiency or partial ejaculatory duct obstruction.[2]

Its absence indicates complete obstruction either due to a congenital block at the level of ejaculatory duct or proximal to it like agenesis of the vas and the seminal vesicle, or following acquired post-infective cicatrisation. Almost invariably, these conditions are associated with azoospermia or severe oligospermia. Chromosomal studies should form a part of the work up of these cases. The fructose is produced by the seminal vesicles and provides energy for the sperm motility. Agenesis of seminal vesicle is often associated with partial or complete agenesis of vas. It may also cause failure to form coagulum and low volume. Estimations of acid phosphatase and zinc are not routinely done, as these substances are indices of prostatic function.

Comhaire et al[62] and Mahmoud et al[63] reported significant correlation between adenosine triphosphate (ATP) per ml of ejaculate and the density, number of motile sperms, capacity of migration of sperms against gravity and *in vitro* potential to penetrate zona free hamster ova. They found ATP concentration was significantly lower in the semen of infertile men even with normal concentration and motility.

Chemicals that are secreted primarily by each of the glands of this system are typically selected to serve as a marker for each respective accessory gland. The epididymis is represented by glycerylphosphorylcholine (GPC), the seminal vesicles by fructose, and the prostate gland by zinc. However, this type of analysis provides only gross information on glandular function and little or no information on the other secretory constituents. Measuring semen pH and osmolality provide additional general information on the nature of seminal plasma. Seminal plasma may need to be analysed for the presence of a toxicant or its metabolite. Heavy metals have been detected in seminal plasma using atomic absorption spectrophotometry. Halogenated hydrocarbons have been measured in seminal fluid by gas chromatography after extraction or by protein-limiting filtration.[41-46]

Immunological Tests

The antibodies are immune system molecules that interact with the specific antigens (foreign substances such as proteins, toxins, or bacteria) and caused them to be manufactured by the host body. The antisperm antibodies are supposed to lessen the fertilising potential of sperms[1] by interfering with the physical changes that the sperms must undergo to swim successfully through the cervical mucus and eventually to penetrate the ovum for fertilisation.

Some men (or their wives) possess antibodies against the sperms to immobilise or kill them preventing their movement up towards the ovum. The presence of these antibodies can be tested in the blood of both partners, in the cervical mucus, and in the seminal fluid.

The role of antisperm antibodies in male infertility is controversial as there is little correlation between the circulating antibodies in the blood and the sperm-bound antibodies in the semen. There are various methods to detect the antisperm antibodies. These tests are difficult to standardise resulting in a lot of variations between the reports from different laboratories.

In all patients with azoospermia or severe oligospermia tests for the antisperm antibody should always be done. Specialised andrological laboratories do this test routinely. In the enzyme-linked immunosorbent assay (ELISA) test, the antisperm antibodies measuring up to 20 units/ml in 32 or 64 dilutions is considered normal. Some laboratories express the antisperm antibody titre up to 1 in 20 dilution as normal.

Perhaps, the best method available today is one using immunobeads, which allows determination of the location of the antibodies on the sperm surface. If the antibodies are present on the sperm heads, they can interfere with the sperms' ability to penetrate the ovum; and when present on the tail, they can retard sperm motility. Presence of fewer than 50% beads bound motile sperms is considered as the normal parameter in a semen specimen. It is always reassuring both to the patient and the treating doctor if the test is negative. But real significance of a positive test is still a vexatious issue as this is not an absolute criterion to judge fertility. Pregnancies have been reported in some series in spite of positive antisperm antibody titre. The postcoital test (PCT) may be more important parameter in this context.

The immunobead test, which is conducted *in vitro* in a test tube, uses tiny polyacrylamide beads that are coated with specific antibodies. These antibodies, in turn, bind to antisperm antibodies and identify any immunoglobulins (an immune system protein with antibody activity). Common immunoglobulins are IgG or IgA.[2]

There are two types of immunobead tests. The direct method measures the binding of beads to the target sperm surface. The indirect (passive) method includes an additional procedure in which antibody is transferred to the donor sperm from the body fluid such as cervical mucus, follicular fluid, blood or semen. Although direct assessment of antibodies on the sperm surface is preferred, serum IgG alone can be used as a sensitive screening assay for antisperm antibodies in men. However, Hjort et al[64] in their observations during the last few years have indicated that antisperm antibodies of the IgA class primarily cause this immune disease, and recent results from with vasovasostomy men lend further support to this theory. Antibodies of the IgG class appear to be of little significance for fertility and do not inhibit sperm penetration.[64-66]

Antisperm antibodies have been found in some men after vasovasostomy or who have experienced any form of testicular disease such as testicular trauma, testicular torsion, repeated STD, recurrent orchitis and long-term pyospermia (see Chapters 6 and 13).

Current approaches to the treatment of antisperm antibodies include methods of sperm processing to remove surface antibodies through rapid washing, freeze thawing and enzyme cleavage. All these methods have modest success rates. In vitro fertilisation (IVF) with or without sperm processing may provide a better alternative for couples with positive immunobead tests (see also *in vitro* Fertilisation, Chapter 13).

Acrosome Reaction

The acrosome reaction is absolutely necessary for fertilisation. Normally, at least 15% of all sperms must show acrosome reaction. Evaluating the ability of sperm to undergo the acrosome reaction may provide an additional assessment of sperm function. Electron microscopy, staining, immunofluorescent technique and monoclonal antibodies can all determine the acrosomal status of a sperm. It is also possible to induce an acrosome reaction with ionophores and human zona pellucida. Most of these techniques are labour-intensive and the ability of the acrosomal status to predict fertility must be assessed in terms of the cost effectiveness.[4] Another type of test used is *Acrosome Intactness test*, where the normal range is intactness of acrosome at 60% or above.

The acrosome status can also be detected either by TST (*triple-stain technique*) or by phase-contrast microscopy. Garde et al[67] modified the triple-stain technique (TST) developed for human sperms. This staining procedure was used to study the time course of the true acrosome reaction of deer sperm in vitro. They used 0.4% trypan blue to identify the percentage of live sperms in culture and subsequent staining of fixed sperms to differentiate the acrosome. This staining procedure enables differentiation of spermatozoa that have undergone a true acrosome reaction (TAR) from those that have undergone a false acrosome reaction (FAR).

A modified technique "Blutstan" has utilised trypan blue to identify the percentage of live sperms. Blutstan, a prestained glass slide coated with dyes, is able to identify activated sperms quickly and easily. Blustan method is perhaps the quickest method and is often utilised in IVF.[68]

Another novel method to evaluate the acrosomal status of mammalian spermatozoa is based on the ability of the lectin Pisum sativum agglutinin (PSA) to bind specifically to glycoproteins of the acrosomal matrix released during the acrosome reaction.[69] The amount of released acrosomal contents is proportional to the fraction of spermatozoa that underwent acrosome reaction. The released glycoproteins present in the supernatant portion and separated from the cells were detected via an ELISA-like assay. The

authors suggest that one of these glycoproteins might be the acrosin as identified by antiacrosin antibodies, using Western blot analysis. The new method (demonstrated with ram and bull spermatozoa) correlates well with the results obtained by conventional methods. Its advantages are simplicity, objectiveness, rapidity, and low cost. In addition, many samples can be processed in parallel. The method can be used in experimental as well as clinical applications.[69] However, immunofluorescence method is the most recent and perhaps the most accurate method.

Sperm Function Tests

The semen analysis yields critical information regarding the etiology and prognosis of many types of reduced male fertility. The basic semen analysis does not directly measure the fertilising capacity of sperms or many of the biochemical events both prior to and subsequent to fertilisation. The sperm function tests assess the sperm's ability to fertilise the ovum and allow accurate diagnosis of many infertile patients not diagnosed by the routine semenanalysis. Naturally, it helps to improve treatment modalities. However, there is a drawback that these tests are often not standardised adequately. So interpretation of their results from different laboratories remains a genuine problem. In the last two decades, various assays of sperm function have been developed[70] and they are summarised in Table 7.16.

Table 7.16: Sperm function assays

1.	Assays of postpenetration events like sperm-cervical mucus interaction tests.
2.	Assays of zona binding and oocyte penetration like Hemizona or Hampter assay.
3.	Assays of general biochemistry and ultrastructure—like electron microscopy and sperm chromatin structural assay (SCSA).

Sperm Viability or Sperm Survival Test

This is a simple test, which provides crude, but useful information on the functional potential of the sperm. The sperm viability may be determined by two methods—*Eosin Y stain exclusion*[47] and hypo-osmotic swelling or *HOS assay*.[71] The former method uses the principle that the dead sperms take the eosin stain. Nigrosin is sometimes added to accentuate the contrast between the sperms and the semen plasma.

These techniques test for the structural and functional integrity of the cell membrane, respectively.[2] The sperm concentration and motility characteristics should be measured in a chamber at least 10 micron deep for the sperm to move freely in all dimensions. The sperms are washed using the same method, which is used for IVF (either a Percoll spin or sperm swim up), and the washed sperms are then kept in a culture medium in the laboratory incubator for 24 hours. After 24 hours, the sperms are checked under the microscope. If the sperms are still swimming actively, they have the ability to survive *in vitro* for this period, and are considered adequately viable for fertilisation. If none of the sperms are alive after 24 hours, they may be functionally incompetent. The vital staining determines the numbers of living and dead sperms.

Hypo-osmotic Swelling Test

This test is based on the observation that 33 to 80% tails of viable sperms swell, when exposed to a solution of fructose and sodium citrate. The normal cell membrane is able to maintain an osmotic gradient, while the nonviable sperms with nonfunctioning membranes do not exhibit this phenomenon.[4] It has also been statistically observed that the semen of a particular person shows fertilising potential if 60% swelling is recorded, while less than that is mostly seen in infertile semen. This test, however, is mostly a research tool, as it has not been widely accepted by the clinicians.

Tests for Sperm-cervical Mucus Interaction

Postcoital Test

The postcoital test (PCT), otherwise known as the *Sims-Huhner* or sperm-mucus interaction test, examines whether the sperms are able to complete their passage through the female reproductive tract. The test is a bioassay performed *in vivo* and assesses the ability of sperms to penetrate and to progress through the cervical mucus. It provides information concerning sexual function, motility of the sperm and the sperm-mucus interaction. For the fertilisation to take place *in vivo*, the sperms must be able to get past the cervical mucus. This test is conducted during the midcycle of the menstruation period, corresponding to time of ovulation. At this time, the cervical mucus, which normally acts as a barrier to seal the uterine

cavity from outside, is thin and watery to enable the sperms to swim easily through the cervix and fertilise the awaiting ovum in the fallopian tube[9,72] (Table 7.17).

Table 7.17: Interpretation of Post-coital test

1. Normal test	Adequate sperm function and compatible cervical mucus
2. Poor result with normal semen parameters	Cervical abnormality or presence of sperm antibodies
3. No sperms in the cervical mucus, but present in the vagina	Hostile vaginal or sperm factor.
4. Many "shaking," motionless, clumped or dead sperms in the cervical mucus	Incompatibility between sperms and mucus.

NB: *Internal factors like an allergic response of female to the sperms or the production of antisperm antibodies by the man or external factors like the use of vaginal lubricants may cause such reactions.*

Prior to postcoital testing, the ovulation is tested using ultrasonography. There is also a test kit available to do it at home to determine the exact day of ovulation (a few drops of the woman's urine are placed on a test stick; a colour change in the stick will indicate that ovulation should occur within the next 24 hours). Intercourse is recommended late evening the day before or early morning the same day. The reproductive physician or the gynaecologist having ensured that enough semen was delivered to the cervix, examines the cervical mucus 2 to 8 hours after intercourse at the expected time of ovulation. Presence of greater than 10 to 20 motile sperms per high power field is generally accepted as normal. The sperms should be healthy, do not show large numbers of clumped, motionless or dead cells, and swim energetically through the cervical mucus.

A normal test implies adequate sperm function and compatible cervical mucus. A poor result with apparently normal semen parameters implies either a cervical abnormality or presence of sperm antibodies. If no sperms are found in the cervical mucus, but they are present in the vagina, hostile vaginal or sperm factor is suspected. If many "shaking," motionless, clumped or dead sperms are found in the cervical mucus, obvious interpretation is that the sperms and mucus are incompatible. Internal factors such as an allergic response of female to the sperms or the production of antisperm antibodies by the man, or external factors such as the use of vaginal lubricants, may cause such reactions.

If other important parameters of the semen analysis are normal, the woman may be inseminated with washed sperms to overcome the sperm factors and to help the sperms to pass through the cervix. An abnormal test needs to be repeated. If the problem is persistent, one needs to determine whether the defect lies in the sperms or in the mucus, by cross-testing with the husband's sperm, donor's sperm, wife's mucus and donor's mucus (from another woman).

Bovine Cervical Mucus Test

The sperm-mucus interaction may also be assessed *in vitro*. The bovine cervical mucus test is another form of testing for the ability of the sperms to penetrate and swim through cervical mucus. The difference is that in this test the mucus used is that of a cow. This is commercially available abroad in a test kit. The sperms are placed in a column of human or bovine ovulatory cervical mucus in a capillary tube. Sperm penetration into the mucus is measured over a fixed period of time under the microscope. These *in vitro* techniques enable one to compare patient's specimen with fertile sperm and to control some of the variables associated with standard postcoital testing.

Sperm Penetration Assays

Penetration of an oocyte requires sperm capacitation, acrosome reaction, fusion and incorporation into the oocyte (see Chapter 1). Yanigimachi[73] in 1972 was one of the first few researchers to conduct these tests to assess the fertilising potential of sperms.[70] Barros et al[74] and Rogers et al[75] found excellent correlation of fertility with the sperm penetration assays (SPA).

Cross-species fertilisation is normally prevented by the zona pellucida. But the human sperm can penetrate the hamster eggs stripped of the zona pellucida. This *in vitro* functional test measures the ability of penetration of the sperms. The end point of this assay is penetration of the ovum and decondensation of sperm heads. The percentage of oocytes penetrated and the number of sperms penetrating each oocyte are measured. The sperms that are capable of multiple penetrations per oocyte appear to have greater fertilising potential than the sperms that do not penetrate.

The results of the SPA have primarily been used to predict the results of assisted reproductive techniques (ART) in particular *in vitro* fertilisation.

Men with sperm of low SPA score are less likely to achieve a spontaneous pregnancy than those with high SPA score. Although variations still exist between laboratories, there appears to be general agreement that less than 10% penetration is an evidence of sperm dysfunction leading to decrease in the fertility potential in males. The indications for SPA include unexplained infertility, and its use is also recommended prior to performing ART. Although SPA is a reliable indicator of the fertilising capacity of human spermatozoa, neither does it predict the ability of sperms to get bound to and penetrate the zona pellucida, nor the sperm's motility and its progression in the female reproductive tract.

Hamster Assay

This SPA test is based on a normal sperm's ability to penetrate a denuded (zona-free) hamster egg. It uses hamster eggs as a substitute for human eggs in order to measure the ability of a patient's sperm to undergo capacitation, penetration into an egg and its ability to fertilise human eggs. The swelling of the sperm head, which occurs after penetration, identifies penetrating sperm.

A zona-free hamster egg is obtained from hamsters and the covering (the zona) removed by using special chemicals. The egg is then incubated with sperms in an incubator in the laboratory. After 24 hours, the eggs are checked to ascertain how many sperms have been able to penetrate them. The result gives a penetration score, which gives an index of the sperm's fertilising potential. In addition, incubating the sperm/egg complex until the formation of the male pronucleus may be useful in sperm chromosome karyotyping.[76-78]

A penetration rate of greater than 10% is good evidence of the fertility potential of sperm, whereas a penetration rate of less than 10% may indicate less-than-adequate fertility. Men with low sperm counts and normal follicle-stimulating hormone (FSH) levels make up the largest subset of the infertile male population. For these men, the SPA tests can help reproductive physicians to determine the fertilising potential of the sperm, and thus to decide upon appropriate medical therapy (see also Medical Management of Infertility, Chapter 11). This is a very delicate technique and is perhaps available in a few advanced research centres of the subcontinent. However, the research scientists are not overly impressed with poor correlation between the IVF results and the SPA (the ability to penetrate zona-free hamster eggs).

Hemizona Assay

The SPA with zona-free hamster eggs can demonstrate completion of the human sperm acrosome reaction and sperm oocyte plasma membrane fusion. However, only tests with human zona pellucida can assess the capability of human sperms to bind to the human oocyte. The hemizona assay uses zona pellucida from nonliving human oocytes that have been microsurgically bisected into two halves. The sperms are allowed to interact and bind with the hemizona.[79, 80] The binding powers of the patient's and the fertile donor sperms are compared utilising the identical halves of hemizona. The results are expressed as the hemizona index, i.e. bound sperm by the subfertile man divided by bound sperm from the fertile donor multiplied by 100. This assay requires significant expertise in micromanipulation and is not used in the routine evaluation of the subfertile man.

Electron Microscopy

Electron microscopy, which requires a high degree of expertise, is the most sophisticated and ultimate test for assessing the sperm characteristics and its ultrastructures. It provides without doubt the best method for detailing the motility and the detailed morphology. Examination of spermatozoa with the light microscope can provide only limited information of their internal structure. More detailed examination of the sperm structure using electron microscopy can reveal major often unsuspected ultrastructural abnormalities.[46] Human sperm undergoing slight and severe chromatin decondensation following incubation in medium containing heparin sulphate can be observed in an electron microscopy. Chromatin decondensation is indicative of diminished semen quality and fertilising ability. Unfortunately, even in developed countries, not to speak of the subcontinent, its accessibility is limited to advanced research centres.

Sperm Chromatin Structural Assay (SCSA)

New researches have unearthed that the sperm with certain levels of deoxyribonucleic acid (DNA) fragmentation serve as a strong predictor of reduced male

fertility. The sperm that appears to be normal by traditional semenanalysis parameters (motile and morphologically normal sperm) may even have extensive DNA fragmentation.[81-83] However, the SCSA is not a replacement for the semen analysis as the test analyses different parameters in the sample and both should be performed. Georgia Reproductive Specialists[4] offer the SCSA, to measure the level of DNA fragmentation in the sperm and to help the diagnosis and treatment for male infertility.

The sperm with high levels of DNA fragmentation have a lower probability of producing a successful pregnancy. The patients with a DNA fragmentation level of greater than 30% are likely to have significantly reduced fertility potential as well as a greater risk of miscarriage. Another study confirmed that greater than 30% DFI (% sperm with damaged DNA) has a significant lack of fertility potential, 15 to 30% DFI reasonable potential, and less than 15% DFI has high fertility potential.

In an effort to achieve the most effective measurement of male fertility potential, the SCSA reports the percentage of the following major populations of sperms present in a semen sample (Table 7.18).

Table 7.18: Different sperm population in SCSA reports

1. Sperms with a low level DNA fragmentation,
2. Sperms with increased susceptibility to DNA denaturation classified into a moderate and a high level of defragmentation,
3. Sperms with immature chromatin due to less chromatin condensation, allowing for a higher degree of DNA stainability.

The SCSA is performed using a flow-cytometer in which cells that have been stained with a fluorescent dye, are sent through a glass channel in liquid suspension. The cells pass through a laser beam and the light from the beam causes the dye to emit fluorescent light of a certain colour.

Several factors are responsible for certain individual to have high DNA fragmentation in the sperm, resulting in low fertility potential. The length of sexual abstinence, age (significant increase in DFI after age 46), smoking history and exposure to high levels of air pollution all cause significant variations in results. Sperm chromatin structure is also compromised in patients with leucocytospermia, febrile illness and testicular cancer. Significant exposure to prolonged heat in the testicles can also contribute to high levels of fragmentation; for example, excessive hot tubbing, truck driving and avid cycling are all key factors in poor SCSA results. Drug use, exposure to chemicals or radiation and testicular trauma are other potential causes of abnormal results. The SCSA is also helpful in identifying men, who may have varicocele. More importantly, it also helps to identify the best sperms for *in vitro* fertilisation in which intracytoplasmic sperm injection (ICSI) is performed.

Other tests that are performed in a specialised laboratory are Nuclear Chromatin Decondensation test (normal range above 75%) and Sperm Mitochondrial Activity index (normal range above 50%).

CONCLUSIONS

Semen analysis is singularly the most important investigation for the subfertile or infertile men, but it is also the potentially the most fallible and vulnerable to many external and temporary factors. Normal sperm characteristics vary according to age, seasonal or environmental factors. A study of semen, however, does not give information regarding etiological diagnosis. Many of the highly sophisticated tests are not easily available to the infertility centres of the subcontinent except in a few research centres. The semen analysis is fundamental to the clinical work up of the infertile couple; but unlike most other laboratory investigations, it is not a standardised test so that interpreting their results can be quite confusing.

No definite conclusion on the results of semen analysis is advisable without strictly adhering to the norms such as repeating the tests after an interval with a consistent abstinence period and setting a standard for the laboratory. Statistics with fertile men show that 97% of men with initial good sperm concentration would continue to show good density, and those rated poor at first, also remained poor in future visits. For those rated equivocal, first visit was of little value and at least two or three visits are needed to obtain stability.

Certain semen analysis parameters are positively correlated with a high degree of statistical probability with the time required for the occurrence of conception. The quantitative impact of the male fertility potential on conception rates was shown to correlate not solely with the sperm concentration (SC) or percent motility (MOT) values, but even more so with their derivatives like motile sperm concentration (MSC) and total motile sperm count concentration (TMSC) (Table 7.13). Therefore, in an *in-vivo* environment, it is not only the number of sperms

and their motility, but also their derivatives that provide a quantitative insight into the male fertility potential. The Texas Institute for Reproductive Medicine and Endocrinology conducted a study on 1,055 infertile couples and came to a conclusion that further studies were necessary to clarify the effect of the other semen analysis parameters (i.e., morphology, velocity, linearity, and MSC) on conception rates, relative risk ratio for conception, and time until conception, in a large population of infertile couples.[84]

Although semen analysis is routinely used to evaluate the male partner in infertile couples, sperm measurements that discriminate between fertile and infertile men are not well defined. Although each of the sperm measurements helped to distinguish between fertile and infertile men, none was a powerful discriminator. The percentage of sperm with normal morphologic features had the greatest discriminatory power.[85] The sperm motility index provides a reliable and objective reflection of semen motility parameters and quality.[86] However, apart from azoospermia, there is no absolute criterion beyond which semen can be considered to be infertile.[87]

CASA needs further standardisation. When the parameter settings of the CASA system and the handling of the sample are defined, the reproducibility of the CASA values is clearly better than that of the visual estimation of motility. CASA scores over other methods regarding the documentation of laboratory values.

Electron microscopy of sperms without doubt provides the best method for detailing the motility, morphology and count, but its limited accessibility and dependence on expertise restrict its use solely to research centres.

The best and perhaps the ultimate sperm function test is IVF or ICSI, since this directly assesses whether or not the male partners' sperms can fertilise the female ova. However, even this test is not infallible, since it has been shown that about 5% of sperm samples, which fail to fertilise an egg in the first IVF attempt, can do so in a second or subsequent attempts.

In the ultimate analysis, evaluation of any semen must be based on overall picture that relates to volume, count, motility, morphology and other cellular contents. No single factor can be considered in isolation, but the mortility is perhaps the most important criteria.[1,11] No semen test can fully predict the fertilising ability of sperm because of the variability in other factors like the fertility of the female partner. Therefore, a complete evaluation of the female partner is also necessary.[88,89]

Silber[90] assessed the extent to which the standard semen parameters influence male fertility. Having reviewed many studies relating pregnancy rate to sperm counts in 'fertile' and 'infertile' couples, he concluded that the standard semen parameters, though much maligned at time, were still useful in evaluating the degree of 'male factor' in an infertile couple.

The average semen parameters of fertile men are arrived at with an optimal female factor. However, the complimentary role of the partners, if one of them is subfertile, could not be overlooked altogether. If the wife is very fertile, pregnancy could occur despite her husband's low sperm count. The fact that conception can be achieved with very low sperm counts in various artificial reproductive procedures like ICSI, IVF or GIFT procedures is a weighty argument in favour of the compensatory role of the female factor in male infertility.[90] Consequently, the minimally adequate fertile parameter of semen would always remains an enigma because of the variable female factor. However, in human context, it would be socially incomprehensible to test the biologic potency of sperms amongst multiple female recipients as is practised in animal husbandry.[91]

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APPENDIX 1

COMPREHENSIVE SEMEN ANALYSIS (Report required for an analysis)

Name		Age	
Period of abstinence	days.	Examination done after	mins.
Referred by-			

PHYSICAL PROPERTIES

Volume ml. pH Liquefaction time mins.

MICROSCOPIC FEATURES

Sperm Count and **density**- Total and per ml.

Motility pattern- *Normal= at least 60% of Grade 3 and 4.*
Grade -0 = Non motile = %
Grade -1 = Sluggish = %
Grade-2 = Poor to fair motion = %
Grade-3 = Good motion = %
Grade-4 = Highly active forward progression = %

Gross morphology –(Normal— at least 60% of total should have oval heads, uniform in size and long tapering tail.)

Normal = % *Abnormal* = %

Cellular Elements

Epithelial cells
Pus cells
Testicular cells
Red blood cells
Macrophage cells
Bacteria/ Monilia/ Trichomonus/ Chlamydia

Precursor Cells-

Spermatogonia Spermatocytes Spermatids

Biochemical - Fructose *content*- mg/litre or m.mol.
Acid phosphatase

Acrosome Study- Normal - at least 15% of sperms must have normal acrosome content.

Miscellaneous-

Agglutination
Granular debris
Crystals
Gram stains for bacteria

OTHER INFORMATION (See also Appendix 6)

Detailed morphology (*Menkveld-1990*)

Normal-	%		Abnormalities	%
1. Abnormalities of head:				
Amorphous	=	%		
Megalo	=	%		
Elongated	=	%		
Micro	=	%		
Duplication	=	%		
Loose heads	=	%		
Normal head with duplicated or short tail	=	%		
2. Other abnormalities:				
Tail- Long, short or duplicated	=	%		
Absent neck	=	%		
Absent tail	=	%		
Spermatids or other precursors	=	%		
Cytoplasmic droplets	=	%		

Remarks regarding fertility of the specimen

APPENDIX 2

MACLEOD

Grade IV	=	Excellent swift progressive motility	}	Equivalent to WHO- A =	Rapid Linear Progressive motility
Grade III	=	Moderate progressive motility			
Grade II	=	Struggling progressive motility	}	Equivalent to WHO- B =	Sluggish Linear Progressive motility.
Grade I	=	Poor sluggish Non-Progressive	}	Equivalent to WHO- C =	Poor sluggish motility

APPENDIX 3

WHO-1998

Normal semen values as outlined by the World Health Organization 1998

Parameters	*Minimum value*
Volume (ml)	2.0
Sperm Concentration (million/ml)	20
Motility (%)	50
Forward Progression (3-4)	60
Normal Morphology (%) (WHO)	60
Normal Morphology (%) (Kruger)	15
Total Sperm Count (million)	40
Total Motile Sperm (million)	20
Total Functional Sperm (million)	6

APPENDIX 4

FURTHER INFORMATION—Motility of spermatozoa (at 37^0 C)

Nature of Motility (WHO)		*Macleod Grade*	*After ½ hours*	*After 2 hours*
A.	Rapid linear Progressive	IV and III	%	%
B.	Sluggish linear Progressive	II	%	%
A+ B	Total forward Progressive			
C.	Non Progressive	I	%	%
D.	Non Motile	0	%	%

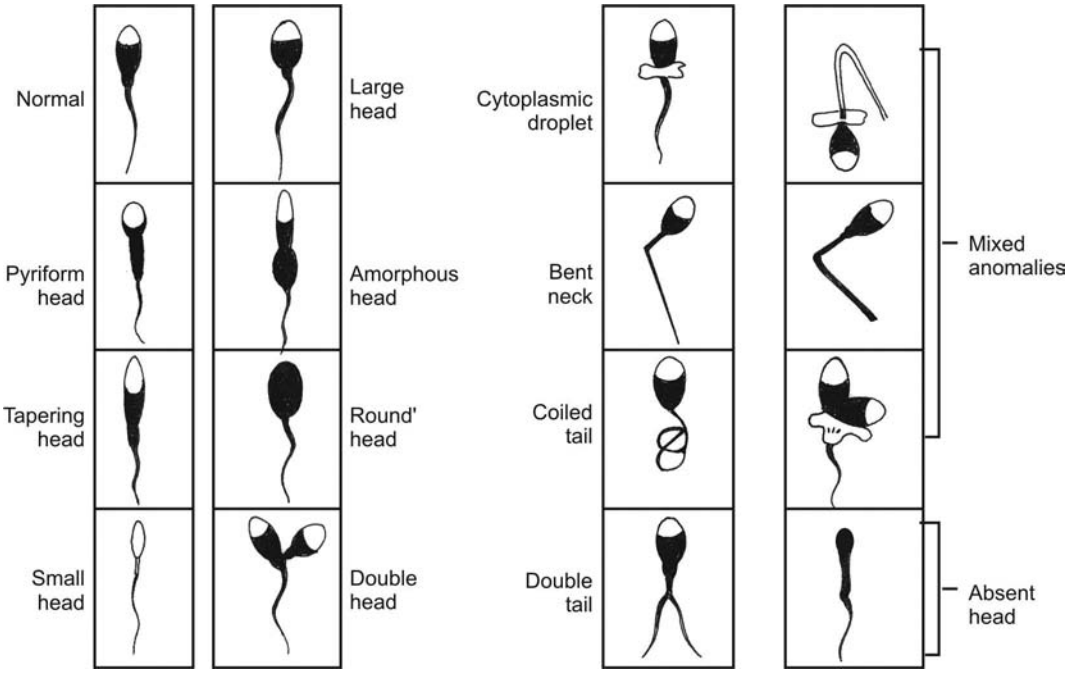
APPENDIX 5

Abbreviations used normally

1.	Sperm concentration	=	SC
2.	Total sperm count	=	TSC
3.	Percent motility	=	MOT
4.	Motile sperm concentration	=	MSC
5.	Total motile sperm count	=	TMSC

APPENDIX 6

Kruger's criteria (at least 15% of sperms should be normal morphology)



CHAPTER

8 Other Investigations in Male Infertility

INTRODUCTION

The semen analysis is always the first choice investigation done in males for infertility. Its ready accessibility and relative simplicity fulfils the criteria of a screening test for male infertility. But limitations of semen analysis mentioned in the previous chapter (Chapter 7) necessitate dependence on other investigations for a complete work up of a patient. Advances in the surgical treatment of male infertility are dependent on honest evaluation and reporting of results, innovation and fine tuning of technologies, and a well-coordinated multidisciplinary approach. Without doubt, advancement of other diagnostic tools has in recent years transformed the assessment of the overall state of infertile male patients (Table 8.1).

Table 8.1: Investigations for male infertility (other than semen analysis)

1. Blood tests and hormone estimation
2. Microbiology—Urine and prostatic smear
3. Radiological—
   a. Ultrasound and TRUS.
   b. Color Doppler—scrotum and penis
   c. Vasography
   d. Venography and angiography
   e. CT scan of head, abdomen and pelvis
   f. MRI of pelvis and abdomen
   g. Radionuclide scanning or scintigraphy
4. Testicular biopsy
5. Chromosome study
6. Scans for thyroid disorders and adrenal pathology

BLOOD TESTS

Complete blood count (CBC), tests for sexually transmitted disease (STD), enzyme-linked immunosorbent assay (ELISA) test for tuberculosis and the kidney function tests such as estimation of blood urea nitrogen (BUN) and serum creatinine are indicated only in special circumstances, when a relevant pathological condition is suspected.

The antisperm antibodies are directed against sperm surface antigens and play a significant role in the etiology of immunological infertility. About 10 to 20% cases of unexplained infertility can be traced to immunological causes. Sperm-reactive antibodies are found attached to the sperm and can be detected in both serum and semen. Level of serum antisperm antibody is considered normal up to 20-mIU/ ml.

Immunobead tests have been dealt with in the chapter 7. Evaluation of the fertilising potential of sperm is an important procedure in *in-vitro* fertilisation and embryo transfer (IVF-ET) clinic in order to avoid performing invalid conventional IVF-ET or unnecessary intra-cytoplasmic sperm injection (ICSI). However, none of the standard semen parameters are reliable indices for predicting IVF outcome. The sperm-zona interaction test is the best amongst present methods, though the short supply of the human zona component needs to be solved. IVF-ET treatment is useful for pregnancy in women having sperm immobilising antibodies, as it avoids exposing sperms to antibodies that block fertilisation (see Chapter 13).[1]

Data support pathophysiological role of sperm antibodies in some cases by their interference with sperm motility and/or sperm-egg interaction. These antibodies are formed following disruption of either the cellular barrier separating sperm antigens from the immune system (e.g. testis trauma or obstruction to sperm outflow), or to dysregulation of normal immunosuppressive activities within the male reproductive tract. The epididymis is likely to be the key site of antibody generation, especially in cases of obstruction.[2] In males, antisperm antibodies have also been detected after vasectomy, and following epididymitis, orchitis or seminal vesiculitis.

HORMONE ESTIMATION

Only fewer than 10% of cases of male infertility are caused by primary endocrine defects.[3] Several hormones are involved in the causation of reproductive dysfunctions in males (see Chapter 3 on the "Endocrine aspects of infertility"). But mainly the testosterone (or androgen), luteinising hormone (LH), follicle-stimulating hormone (FSH), and the prolactin (PRL) are involved in the intricate mechanism of normal reproductive function. The secretion of the LH from the pituitary gland is controlled by gonadotrophin-releasing hormone (GnRH) produced by the hypothalamus. The LH directly regulates the testosterone production from the Leydig cells of the testes. The PRL, another pituitary hormone, affects the GnRH release from the hypothalamus. For initial screening, the pituitary hormones like FSH, LH and prolactin, and the testosterone are estimated in most andrological laboratories dealing with male infertility.

Estimations of these hormones are done by the radioimmunoassay or ELISA test, and should be performed by a quality laboratory with specialised equipment. In the subcontinental perspective with resource crunch, ordinary laboratories outsourcing these tests to specialised centres for the authenticity of the results should be an acceptable alternative. At least, this would ensure quality results for the benefit of both patients and the treating doctors.

Serum Testosterone

The level of the serum testosterone provides information, whether or not the testes are producing adequate amounts of male hormone. Most infertile men have normal serum testosterone levels, because the physiological component for testosterone production is separate from the site of production of sperms, and is often intact in infertile men. The serum testosterone level usually is low in men with hormone-related hypogonadism (with delayed sexual maturity) and in men with abnormal Leydig cell function in the testes. Yet, total testosterone level, can be misleading. Men with testicular failure (as in alcohol-related cirrhosis or Klinefelter's syndrome) may have testosterone levels within the normal range, because of an excess estrogen-induced testosterone-binding globulin (TeBG). In these individuals, increased blood levels of FSH and LH and accompanying testicular atrophy would confirm testicular failure. However, both serum testosterone and serum dihydro-testosterone (DHT) significantly decrease in azoospermia caused by the testicular atrophy. A low testosterone level may cause decreased libido especially in androgen deficiency of ageing male (ADAM- see chapter 3).[4,5]

Some researchers estimated the seminal plasma levels of FSH, LH and testosterone. Micic and Dotlic[5] found that the seminal plasma FSH and LH levels were significantly higher in oligospermic men, and in males with reduced motility than in fertile men. The seminal testosterone level was significantly decreased in many patients with severe oligozoospermia.[6,7] The levels of seminal plasma DHT and testosterone have been found to be persistently low in all azoospermic patients, and there is a positive correlation between the seminal androgen and concentration of motile sperms.[8]

Serum FSH and LH

The serum FSH level test is very useful for assessing the testicular function. If the testicular failure is the underlying cause of the azoospermia or severe oligospermia, it is reflected in a raised FSH level. In these patients, the testis also fails to produce the hormone inhibin, which normally suppresses FSH level to its normal range (see Chapter 3).

Basically there are only two reasons for the absence of sperms in patients with azoospermia. Either the sperms are not produced due to testicular failure, or the outflow passage of the sperms is blocked due to ductal obstruction. A high FSH level is usually diagnostic of testicular failure, but a normal FSH level provides no useful information. So, in azoospermic or severely oligozoospermic patients with a normal FSH level, a testicular biopsy is indicated. Evidence of normal spermatogenesis in a testicular biopsy

would confirm the diagnosis of an obstructive pathology in the ductal system. A low FSH level is found in patients with hypogonadotrophic hypogonadism. In fertile eunuch syndrome, there is a low LH level.

The antioestrogen receptor Clomiphene, which is often used for male infertility, can also raise the FSH level in some men. To avoid this fallacy, the FSH estimation should be done before any such medication is started in cases male infertility.

Serum Prolactin

The level of serum prolactin (PRL) should always be estimated in infertile men, who complain especially of sexual dysfunction or show signs of any pituitary disease. Significance of high PRL level has been discussed in Chapter 3. There are three likely causes of a high PRL level in a blood sample. The first is certain medications, such as metoclopramide (Maxolon), chlorpromazine (Largactil) and antidepressant drugs such as amitriptyline and fluoxetine (Prozac). The second possibility is an underactive thyroid gland, which can be diagnosed by estimation of thyroid hormones. Once these two causes are excluded one needs to consider the third possibility of a prolactinoma.[9] Seminal PRL levels are also significantly elevated in all infertile males[7] with pituitary macroadenoma (Table 8.2).

Table 8.2: Causes of high PRL

1. Drug related-Metoclopromide, chlorpromazine, antidepressants, etc.
2. Hypothyroid state
3. Prolactinoma-macro or micro

Serum Estradiol

Estradiol is a form of estrogen and is not routinely estimated except in men with gynaecomastia. But in our series serum estrogen is added in the list of routine hormone estimation for the reasons outlined in Chapter 6.

Other Hormones

Routine estimation of the thyroid hormone is unnecessary, unless the patient has a history or evidence of thyroid disease. Both hypo- and hyperthyroid states can affect the reproductive hormonal milieu in males. Likewise, routine measurement of adrenal steroids is unnecessary unless the patient shows signs of adrenogenital syndrome (see Chapter 3).

MICROBIOLOGICAL TESTS

Microbiological tests assume importance as chronic infection or inflammation of the genitourinary tract is often incriminated as a causative factor in infertility (see Chapters 6 and 10). Not only does the urogenital infections cause excess leucocytes in the semen (*leucocytospermia*), but they may also lead to appearance of pus cells in the prostatic smear. Consequently, bacteriological examinations of semen, prostatic smear and urine after a prostatic massage and culture are included in the investigation proforma.

IMAGING STUDIES (US, TRUS WITH CDU)

In specialised andrology clinics, high-resolution gray scale ultrasonography (US) along with the use of transrectal (TR) probe and Color Doppler Ultrasound (CDU) imaging are now used routinely to evaluate the testis and the ductal systems in infertile men. Use of transrectal probe has mostly replaced vasography. Previously, before US became an important diagnostic method, vasography was the only imaging modality available for evaluation of the distal ductal system. TRUS with CDU is particularly useful in evaluating selected patients with obstructive azoospermia, when a block at the level of the seminal vesicles is suspected. TRUS enables an accurate diagnosis of certain congenital and acquired anomalies of the lower urogenital tract (vas deferens, seminal vesicles and ejaculatory ducts) associated with male infertility. Furthermore, it helps to formulate appropriate clinical and surgical management.[10] CDU is singularly the most important diagnostic tool in the detection of subclinical varicocele.[11] In an infertile male, combinations of US, TRUS and CDU should all be used for arriving at a definitive diagnosis.

Testis

The sonographic examination of the testis is important in the evaluation of infertile male, specifically for measurement of the testicular size for atrophy and for comparison with the contralateral side. In a scrotal US, the normal adult testis appears as a homogeneous structure with low-to-medium echogenicity and measures 3 to 5 cm in length, 2 to 3 cm in width and 2 to 3 cm in anteroposterior thickness. The testicular volume can be calculated using the formula: *length* × *breadth* × *thickness* × *0.53*, which gives the testicular volume in cubic centimetre or ml.[12] The normal volume is between 15 to 20 ml or cc with usual variation related to race or ethnicity (for further

details-see Chapter 9). However, the overall assessment of the testicular size should always be based on the findings of clinical examination and measurement with an orchidometer and US.

Spectral Doppler of testicular arterial flow demonstrates relatively low resistance in contrast to the cremasteric and deferential arteries, which have a relatively high-flow resistance. The normal testicular arterial resistance indices range from 0.46 to 0.78 (mean value = 0.64) and those of intratesticular arteries in postpubertal patients range from 0.48 to 0.75 (mean value = 0.62). Supra-testicular arteries (of vas and cremaster muscle) have higher impedance with low diastolic flow and resistive indices ranging from 0.63 to 1.0 (mean value = 0.84).[13-15]

Color Doppler study has 91% sensitivity and 100% specificity in the diagnosis of scrotal inflammation.[16] It can demonstrate the hyperaemic[16] response to scrotal inflammatory disease, and in a proper clinical setting, it can supplement the grey scale findings to enhance the diagnostic accuracy.

Color Doppler is almost always abnormal in early stages of torsion. If arterial flow cannot be detected in the symptomatic testis; but can be in the contralateral testis, the diagnosis of torsion can be effectively suspected. Color Doppler sonography is often not of much benefit in the evaluation and characterisation of adult testicular neoplastic masses.[13,15]

Epididymis

Under normal circumstances, the epididymis is usually isoechoic or slightly heperechoic in comparison to testes.[13] Scarring of epididymis from a chronic inflammation or infection results in epididymal obstruction. It is detectable in US as hyperechoic areas with foci of calcifications. Acute cases often show reactionery hydrocele (Fig. 8.1).

Vas Deferens

The vas deferens or the vas can be seen in a TRUS in axial plane as round or tubular structure just cranial to the prostate, but medial to the seminal vesicles. In sagittal plane, the vas appears behind the prostate projecting medially towards the seminal vesicles.

Seminal Vesicles

Seminal vesicles appear as saccular elongated normally symmetrical hypoechoic areas above the prostate like a *bow-tie* in an axial plane (Fig. 8.2). Bilateral agenesis of seminal vesicles thus can be detected easily by absence of this sign. Almost invariably, this condition is accompanied by bilateral vasal agenesis resulting in azoospermia (Figs 8.3 to 8.4A and 8.4B).

Ejaculatory Ducts

Normally, these ducts are difficult to visualise in TRUS. Occasionally, they can be seen as tubular structures obliquely traversing through the prostate into the verumontanum. Presence of motile sperms in the seminal vesicles provides indirect evidence of ejaculatory duct obstruction, as motile sperms are absent in normal seminal vesicles. Ejacutatory duct cyst can be seen in a TRUS (Fig. 8.5).

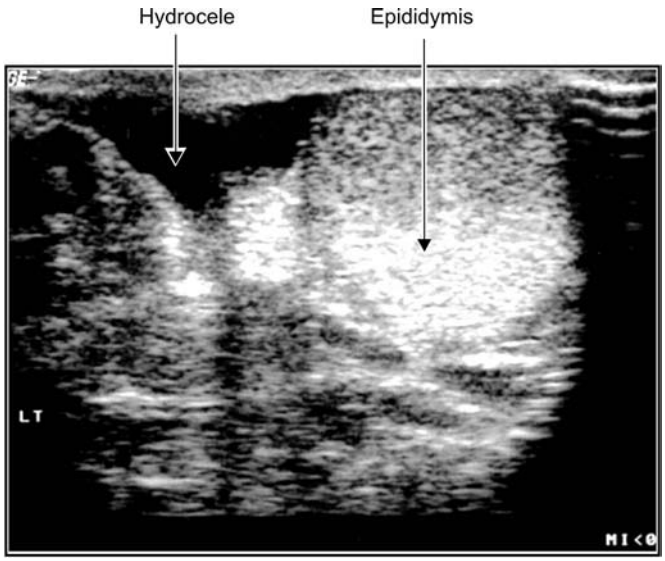


Fig. 8.1: Epididymitis with hydrocele

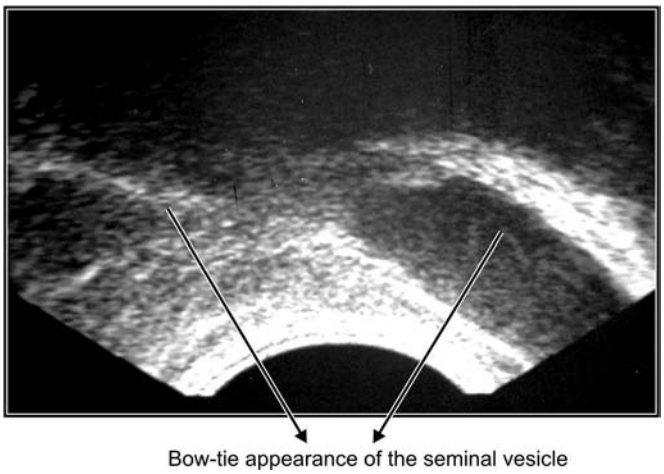


Fig. 8.2: Bow-tie appearance of the seminal vesicle

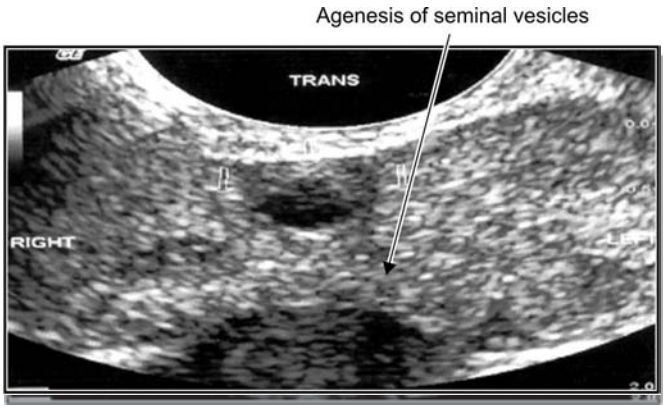


Fig. 8.3: Agenesis of seminal vesicle

The new method of TRUS guided needle aspiration can be extended to perform a *seminal vesiculography* by injecting a nonionic contrast into the seminal vesicles to delineate the inguinal and pelvic portions of the vas as well. However, MRI with endorectal coil is considered superior in detecting otherwise the inaccessible portions of the vas deferens.[17-19]

Varicocele

For detection of varicocele, Color Duplex flow imaging has further enhanced the sensitivity of US (see Chapter 9). The Doppler pencil-probe stethoscope was one of the earliest instruments utilised in an office setting. Now, all andrology clinics routinely advocate the scrotal US, as it is a non-invasive and simple method for the diagnosis of a varicocele. However, CDU is decidedly superior because of its objective

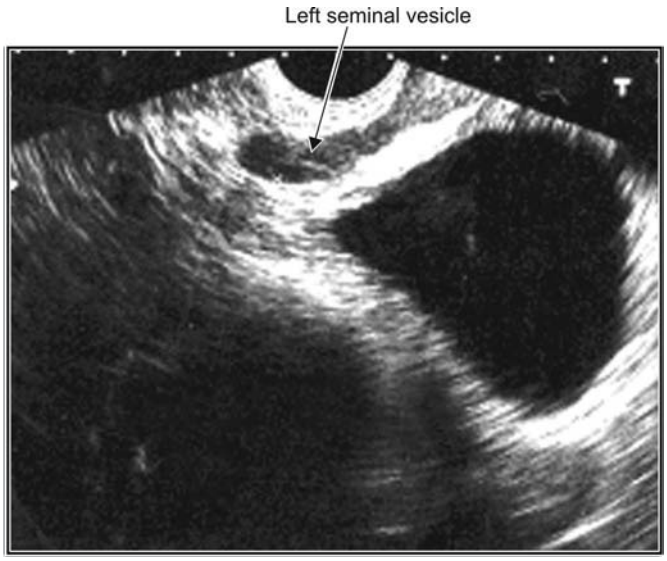


Fig. 8.4A: Left seminal vesicle

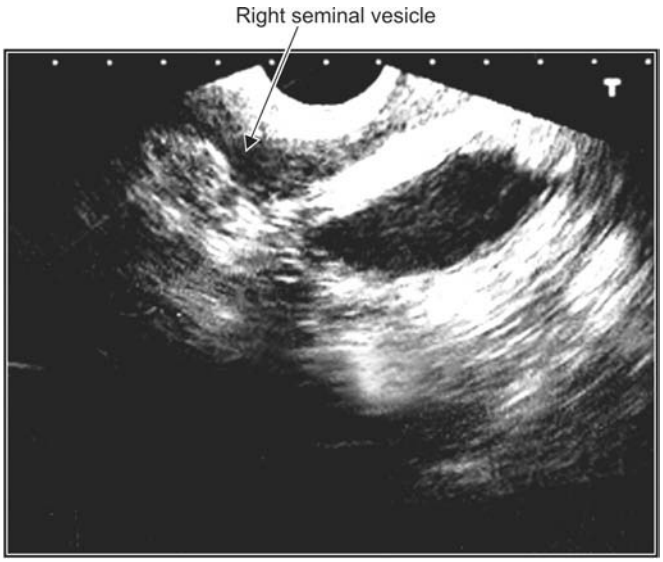


Fig. 8.4B: Right seminal vesicle

characterisation of the pampiniform veins, especially when there is an element of doubt of the presence of varicocele on physical examination.

CDU measures the diameters of the spermatic cord veins by imaging these vessels, at rest and during Valsalva manoeuvres. Moreover, it quantifies and qualifies the flow of blood through these veins. The diameters of the veins surrounding the testes should be less than 0.8 mm; but in advanced grade III varicoceles with multiple serpeginous and tortuous veins, diameters of 12 to 14 mm may be seen. CDU has been shown to be 85% sensitive in the detection of subclinical varicoceles, when venography is used as the "gold standard".[18]

CDU being the most important diagnostic investigations, certain details need to be highlighted. Most

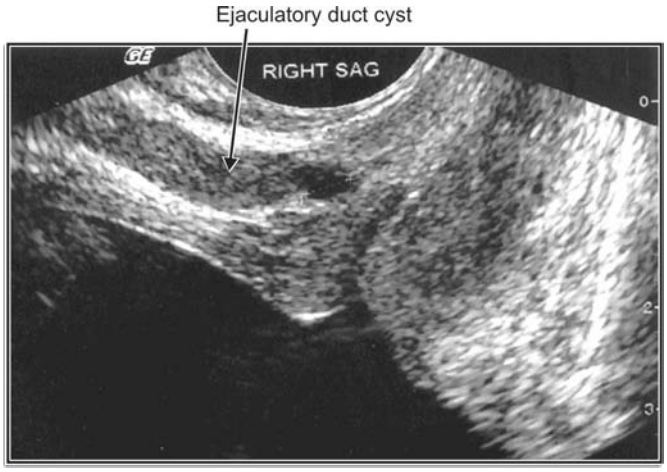


Fig. 8.5: Ejacutatory duct cyst

importantly, the room should have an optimum temperature and certainly not too cold. When exposed to cold, the contraction of the cremaster and dartos muscles would lift the testes up to make the examination difficult.

While CDU is performed, the patient holds the penis up against the lower abdomen for a proper exposure of the scrotum. The ultrasonologist starts the examination preferably sitting on a low stool with the patient standing. It is, however, mandatory that the patients should always be examined in both supine and standing positions during quiet breathing, deep breathing and with Valsalva manoeuvres to demonstrate the reflux (Figs 8.6A and B, 9.14 in Plate 3). Use of linear transducer of 7.5 mHz (or more) is considered ideal, and this has replaced the past improvisation of using a water bag with a 3.5 mHz transducer.

The transducer should first be placed at the root of the scrotum and then worked down the spermatic cord alongside the testis ending at the lower end of the scrotum. This drill ensures that the varicosities at the lower and the posterior ends of the testis are not missed. The vessels are scanned on each side from the scrotal neck to the lower pole of testis. Visualisation of three or more dilated veins with at least one of them having a diameter of 2 mm or more during Valsalva manoeuvre or increase in flow or reflux as seen on Color Doppler study during Valsalva manoeuvre is taken as a positive sign for varicocele. If the reflux lasts for more than one second, varicocele is more likely to have associated infertility.[17]

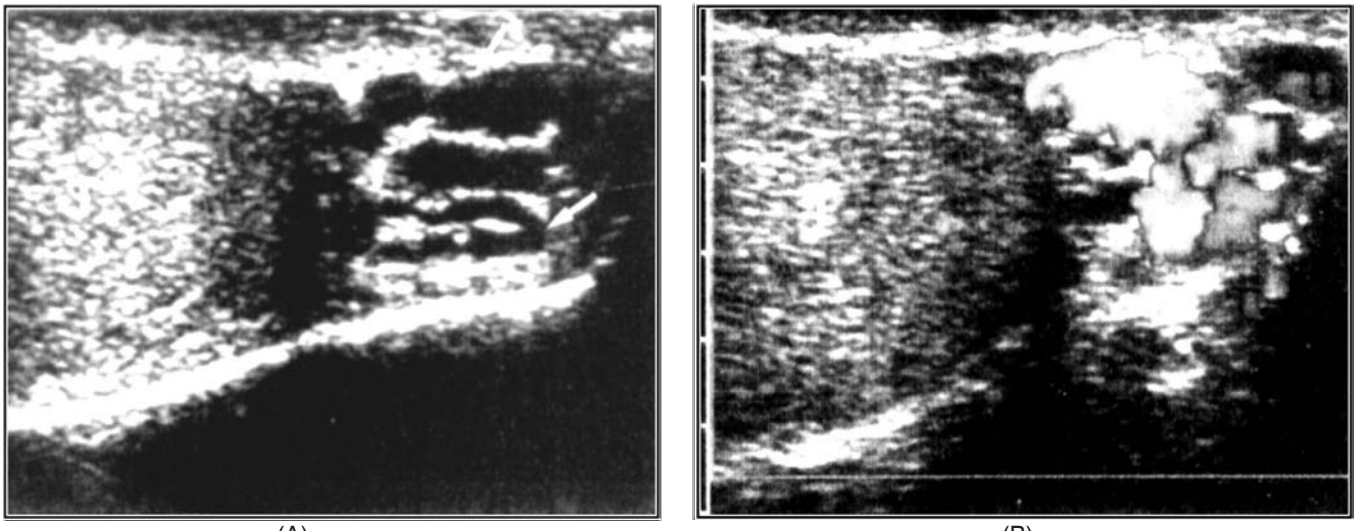
As the flow in pampiniform plexus is very slow, Doppler parameters must be adjusted to detect very slow flow during Color Doppler examination. No doubt, a Doppler ultrasound would diagnose even a subclincal varicocele, but there should not be overdependence as the presence of a varicocele *per se* is usually of little clinical significance in its overall management (see Chapter 9 on varicocele), as it does not correlate with degree of impairment of spermatogenesis. The radiological assessment of the size of pampiniform veins is not directly proportional to the degree of impairment of spermatogenesis.[20] At times, the flow is so slow, that no blood flow is demonstrated; but on reflux, some colour flow is evident. One should bear in mind that clinically felt small cysts at the upper pole of testis could prove to be varicoceles in CDU.

CDU-PIPE TEST (See also Chapter 4)

CDU-PIPE test is used for the assessment of erectile dysfunction (ED). While performing this test, self-genital or visual erotic stimulation and application of tourniquet can augment the erectile response.

Normally, following procedure is adopted:

1. Rubber band is placed at the base of the penis.
2. US image of the flaccid state in transverse plane is taken to measure the diameter of cavernosal artery.
3. Using 27G needle 'Caverjet' with 60 mg of Papavarine is injected into any one cavernosum avoiding a urethral puncture.
4. Visual grading (see Table 8.3)[21]
5. Scanning is done from the base at the penoscrotal junction to the distal end both in the transverse end longitudinal planes.



(A) (B)

Fig. 8.6A and B: CDU showing normal (A) and dilated veins in varicocele with (B) reflux after Valsalva manoeuvre

6. Doppler scanning in sagittal plane is important in identifying penile deformities in Peyronie's disease.
7. Post-injection diameters of cavernosal arteries are measured.
8. Angle correction velocities are measured in cavernosal arteries. Initial flow velocities are taken 5 minutes after the injection, and especially at the height of rigidity.

Table 8.3: CDU-PIPE test

Grade	*Erectile response (visual grading)*
0	No response
1	Elongation of shaft
2	Moderate tumescence, no rigidity
3	Full tumescence, no rigidity, bendable
4	Full erection, partial rigidity
5	Full rigidity for 20 mins.

While performing the test PSV or peak systolic velocity (30 cm/sec or more), EDV or end-systolic velocity (0 05 cm/sec or less), AT or acceleration time (0.11 sec. or less) and RT or restive index (0.85 or more) are all noted.[22] Postpapaverine injection spectrum has five phases.[23]

I. Increase in both systolic and diastolic velocities.
II. Progressive decrease in end-diastolic velocity with dichrotic notch.
III. Diastolic flow approximates zero.
IV. Diastolic flow gets reversed.
V. Eventual loss of both systolic and diastolic signals.

The results are interpreted in the form of five categories from A to E.

A. If there is venous leakage, Phases III, IV and V are not seen.
B. If there is arterial deficiency, PSV and AT would be low.
C. If there is venous leakage, EDV >5 cm/sec and RI < 0.05 (RI more important).
D. If there is increase in cavernosal artery diameter by less than three fourths of the baseline diameter.
E. Veno-occlusive disease cannot be assessed, if there is concomitant arterial disease.

CAVERNOSOMETRY

Cavernosometry is done by injecting 40 ml of iodinated contrast with infusion line keeping intracavernosal pressure just above mean arterial pressure. In research centres, cavernosometry is often combined with cavernosogram where the pressure is also measured.[17,24]

TESTICULAR BIOPSY

The testicular biopsy is performed mainly to differentiate a primary testicular failure from the obstructive ductal lesions in azoospermic patients. Jarrow[24] reported an incidence of azoospermia in 10 to 20 % of the men assessed in an infertility clinic. In our series spread over 11 years we have found an incidence of 6% (see Chapter 7, Table 7.4).

The best criterion to predict ductal obstruction preoperatively is serum FSH level of less than double the normal value and the clinical absence of bilateral testicular atrophy (100% sensitivity and 71% specificity).[25] Azoospermia in a patient with soft, small testes and a borderline FSH level is very likely to be caused by testicular failure, but for confirmation biopsy is absolutely essential.

If the semen has adequate fructose content, andrologists naturally assume that there is no major obstruction in the passage of the sperms to the urethra. As the source of fructose in semen is the seminal vesicles, men born without the vas deferens or seminal vesicles and with bilateral ejaculatory duct obstruction have no fructose in their semen. Yet, fructose-positive semen does not necessarily ensure a totally obstruction-free path. So, in addition to the testis biopsy, vasography, TRUS and MRI are sometimes needed to rule out completely any obstruction (see also Vasography, TRUS-CDU and MRI).

Testicular biopsy remains the definitive test to differentiate these two disorders. This is still the *gold standard* for judging the testicular function, since the testicular tissue is being examined directly and later under microscope for histological assessment by expert pathologists. Sometimes, azoospermia may occur in a man with apparently normal testes and vas deferens, and normal levels of reproductive hormones such as testosterone and FSH. Some andrologists, such as Marc Goldstein, advocate the testis biopsies in all patients preoperatively to assess the state of spermatogenesis.[26] However, in a subcontinental set up, it is cost-effective to eliminate the testicular biopsy in men with an elevated serum FSH level and bilateral small soft testes. Zukerman et al demonstrated a significant correlation between sperm density and germ cell counts in a testicular biopsy. They found that in men with sperm counts

below 5 million/ml, the number of germ cells in the biopsy was lower than in men with higher sperm counts.[27]

If there are indications of ductal obstruction or testicular failure, both testes should have the biopsy, as state of each testis more often than not has different degrees of dysfunction. It is thus important to know the side, that in future, is likely to give a better functional result after correction of the block.

There is an argument of doing a unilateral biopsy to keep the other side completely untouched to obviate adhesions or fibrosis in the subsequent reconstructive surgery. But lack of uniformity of testicular damage in two sides would make this argument untenable. In a routinely followed procedure, the biopsy is performed from one area of the testis at its anterior aspect. But this may not be representative of the state of the rest or the entire testis, as sperm production is not uniformly distributed throughout the testis, especially in men with testicular failure. Ideally, four-quadrant areas of the testis should be taken out as biopsy material for an accurate assessment. But in the practical field this is rarely done.

When a specialist does not perform the testicular biopsy, this relatively minor and easy procedure is often done badly. This makes future reconstructive surgery on the epididymis more difficult due to adhesions and fibrosis. However, the commonest problem with the biopsy is that the pathologist does not report the result accurately either due to his inexperience or an adequate representative tissue has not been supplied to him.

Since testis biopsy is a surgical procedure, most patients in the subcontinent are often reluctant and apprehensive as it involves cutting into the testis. I often use this little anecdote to convince them.

When someone opens any tap, water should run immediately. If it does not, one looks for two situations—firstly, the tap is dry because the tank is empty as there is no water supply, or else the pipe from the tank to the tap is blocked for some reason. It is a common knowledge that in that event all we do is to open the lid of the tank to see whether the tank is really empty or not? If one substitutes the tank for the testis and the pipe for the vas, it is very easy to explain away the rationale of doing a testicular biopsy (Figs 8.7 and 8.8).

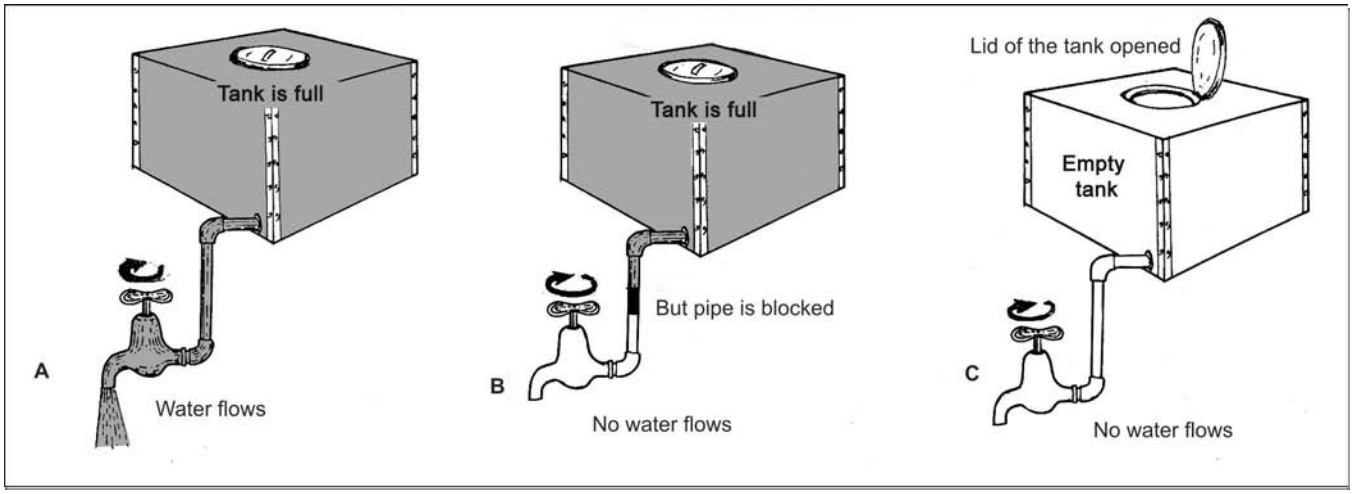


Fig. 8.7: A. Tank is full and opening the tap drains the water. B. Tank is full, but no water drains because of block in the outlet tube. C. Tank is empty, so no water flows down the outlet. Tank is inspected by opening the top lid

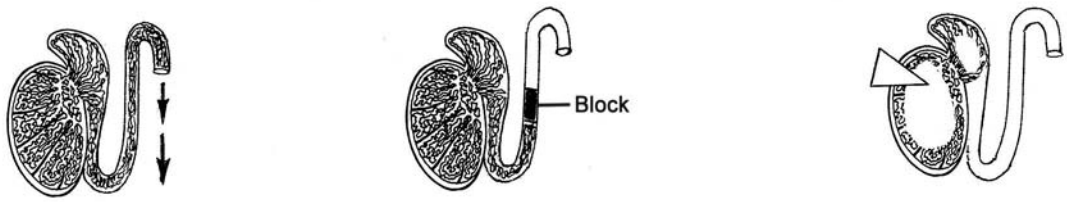


Fig. 8.8: A. Normal testis with patent outlet tube—semen with sperms flow normally. B. Normal testis, but with outlet obstruction. Sperms do not show in the semen (azoospermia.) C. Testis is devoid of function. So there is no sperm production and sperms do not show in the semen (azoospermia). Testicular biopsy is required to diagnosis the state of testicular function for B and C.

Procedure

Open Testis Biopsy

It allows visualisation of the exposed structures and it is the preferred method in most centres. This procedure generally is performed in a hospital on an outpatient basis, or in a well-equipped doctor's clinic. The simple surgical procedure of testicular biopsy is almost exclusively done under a local anaesthesia. Approximately, the procedure takes about 10 minutes for each side to perform. A short general anaesthesia is rarely required, unless the patient is very apprehensive.

A window technique is used, when a simple biopsy is planned, and no inspection of the epididymis is done. The frontal skin of the scrotum over the testis is stretched. With the testis lifted and fixed between fingers a small incision is made through the skin, dartos sheath and tunica vaginalis till a small hole is made in the tunica albuginea. Gentle pressure is applied to squeeze out a small amount of testicular tissue using a no-touch technique, and then the testicular tissue is placed in an appropriate preservative solution. The removed bit of tissue is placed in a special preservative fluid such as Bouin's or Stieve's or Zenker's solution. Universally used formalin causes significant shrinkage or distortion of testicular tissue. Customary tissue preparation techniques, such as fixation in formalin, embedding in paraffin and staining by haematoxylin and eosin, are not recommended for the testis biopsy sample. Instead, newer methods, such as fixation with glutaraldehyde, embedding in plastic and the use of high resolution microscopy are recommended by andrologists.

The pathologists look for the evidence of sperm production in the seminiferous tubules or its arrest at a particular stage (*maturation arrest*). If the sperm production in the testes is completely normal, and yet there are no sperms in the ejaculated semen, it implies a block in the male reproductive tract.

Fine-needle Aspiration Cytology (FNAC)

It is another procedure that may be used to obtain a tissue sample from the testis. It is performed under local anaesthesia with a special cutting device. This method is a blind technique that does not permit the surgeon to see within the testis itself, thus exposing a risk of unintentional injury to either the epididymis or the underlying vessels. In addition, some specialists criticise the quality of percutaneous biopsy samples and do not recommend the procedure for a testicular sampling. Methods such as flow cytometry (cell-counting device) are used to analyse the sample after the FNAC. Whether the percutaneous testis biopsy (FNAC using 0.6 mm needle) or an open surgery is used, the obtained tissue needs to be fixed with 96% ethanol.

Interpretation

Interpretation of a testicular biopsy requires special expertise. Perfunctory and incomplete reports could altogether change the course of management in hapless couples, who would be left with no hope of having their biological children. These couples can be driven to despair with the prohibitive cost for availing of different forms of assisted reproductive technologies (ARTs) in this subcontinent. It is thus imperative either to repeat the biopsy with its attendant sequela of producing more adhesions and fibrosis, or to send the slide and block for an expert second opinion. Often, a testicular biopsy needs to be repeated simply because the results could not be accurately interpreted, as the first biopsy has been done badly not following the protocols. It is, therefore, advisable for an andrologist to instruct the pathological laboratory to keep the tissue (blocks) carefully so that it can be checked by an experienced pathologist later. Better option would be for the surgeon to retrieve, retain and preserve one's own slides for further assessment by an expert.

Histological Features

The following terms often are used to describe testis biopsy results.[3,28,29]

1. *Hypospermatogenesis* or *germ cell hypoplasia*: (Slow rate of sperm production). This may be due to reduced activity and/or loss of germ cells that eventually mature to become sperms. Gonadotoxins, drugs and varicoceles are often incriminated for hypospermatogenesis.
2. *Maturation arrest*: Arrest of sperm development is a common biopsy result. The germ cells are found to divide and produce early forms of cells. However, at some stage of sperm development, maturation stops throughout the testicular tubules. Maturation arrest may be complete, as in azoospermia or partial, as in oligospermia. Common causes of maturation arrest are similar to hypospermatogenesis such as toxins, drugs and varicocele (see Causes of Infertility in Chapter 6).

The sperm production can at times be restored in a patient with maturation arrest with a low level of FSH. Unfortunately, maturation arrest in a patient with a high FSH level usually signals severe untreatable testicular damage. In a germ cell aplasia or Sertoli cell-only syndrome, only Sertoli cells line the seminiferous tubules. The germ cells are not developed in affected patients, so the sperms cannot be produced. Common causes of germ-cell aplasia include exposure to toxins, chemotherapy or radiotherapy. Most cases are caused by unknown factors.

3. *Tubular/peritubular sclerosis:* (Hardening of the interiors of the seminiferous tubules and surrounding tissues). In tubular sclerosis, there are no cells lining the hardened seminiferous tubules.The testosterone-producing Leydig cells that lie around and between the seminiferous tubules may be missing. Affected men may have small testes and high levels of LH and FSH. In some instances, the tubular sclerosis may suggest Klinefelter's syndrome.

The finding of motile and nonmotile sperms on a wet preparation has positive predictive values of 100% and 81% for the presence of obstruction, and 94% and 86% for complete spermatogenesis, respectively. When any complete sperm with tail is found in a testis biopsy wet preparation with Ringer's lactate, obstruction is likely. When motile sperms are present, obstruction is almost certain, and immediate exploration and reconstructive surgery is justified.[29]

A testicular biopsy is extremely useful in the evaluation of the azoospermic male to determine, if there is a block to sperm transport. As the needle core biopsy and needle aspiration biopsy are now more widely used, they may replace in future the open testicular biopsy. According to Nagler[30] and Schoor,[31] the isolated diagnostic testicular biopsy is rarely, if ever indicated. Men with FSH 7.6 mIU/ml or greater, or testicular long axis 4.6 cm or less may be considered to have nonobstructive azoospermia and counseled accordingly. These men are best treated with therapeutic testicular biopsy and sperm extraction followed by IVF or ICSI.[31]

VASOGRAPHY

Vasography is an invasive technique and requires surgical exploration of the vas in the scrotum.[9,28-30] Its main indication is for men with azoospermia, where the testicular biopsy has shown normal spermatogenesis. The procedure itself may cause iatrogenic scarring and obstruction of the vas. CDU has now replaced vasography as the primary imaging modality for the distal ductal system. It is now widely used to diagnose congenital and other obstructive lesions that produce low volume ejaculate and azoospermia.

In vasography, the contrast is injected into the vas deferens to determine its patency. It is possible to find out the exact site, if there is a block in its course.

This test requires a very delicate surgery and advanced X-ray equipment. However, possibility of the test itself damaging the vas precludes its frequent use at present. The procedure usually is conducted under local or at times under general anaesthesia. A small vertical cut is made over the testis, which is then pulled forward. If the patient has a history of repair of inguinal hernia, the cut may be made directly over the previous surgical scar. Sometimes the obstructed site of the vas is clearly found at this site, and vasography is not even necessary. The vas deferens is identified, and using an operating microscope and microsurgical tools, the lumen of the vas is inspected for the presence of sperm-containing fluid. If no fluid is present, a fine flexible catheter is passed through the vas to the epididymis, which is milked for fluid. If there is still no fluid, the seminal vesicle end of the vas is filled with a salt water and/or contrast solution to confirm that this region is free from any obstruction.

If a significant amount of sperm-containing fluid is noticed on opening the lumen of the vas deferens, there is probably a block in the seminal vesicle end of the vas. A catheter is passed up through the vas and is filled with water-soluble contrast media. The procedure is then repeated with the vas on the other side. If a block is found at the ejaculatory ducts, surgical correction is performed preferably at the same sitting. If the vas deferens ends blindly, far away from the ejaculatory ducts, no further surgery is performed. If a block is found in the inguinal region, the surgeon could perform an inguinal vasovasostomy to surgically by pass the obstructed portions of the vas deferens (see also Vasovasostomy in Chapter 12).

If there are no sperms in the fluid from the vasography site, and there is no block at the seminal vesicle end of the vas, the vas may be cut and readied for microsurgical vasoepididymostomy between the vas deferens and the epididymis.

After vasography, microsurgical methods should be used to close the operative site. Vasography should always be performed only at the time of planned definitive correction of obstructive lesions of the genital duct system. Emphasis is placed on deferring vasography until the time a definitive surgery is planned to correct the patient's ductal obstruction, to avoid two operations for the same pathological condition. A vasography, therefore, should not be performed as an isolated outpatient procedure.

CHROMOSOME STUDIES

The chromosomal studies should be considered in men with severe oligospermia or azoospermia to look for autosomal and sex chromosomal abnormalities. Several somatic chromosomal abnormalities are associated with the male infertility. About 13% of cases of nonobstructive azoospermia are caused by deletion of the azoospermia factor (AZF), a gene or gene complex normally located on the long arm of the Y chromosome. Oligozoospermia is far more common than azoospermia, but little is known about its genetic causes.[32] Deletion of any of three regions of the human Y chromosome results in spermatogenic failure and infertility[33] (see also Chapter 10, chromosomal abnormalities).

In a study of 1,263 barren couples, it was found that the overall incidence of male chromosome abnormalities was 6.2%.[3] For some men with testicular failure, a study of the chromosomes (karyotype) is useful, because it allows one to determine, if a chromosomal problem (e.g., Klinefelter's syndrome, 47 XXY, with an extra X chromosome) is responsible for the azoospermia. Some research clinics in USA and Japan offer testing for microdeletions on the Y chromosome—a newly discovered cause for testicular failure in about 15% of infertile men.[32,34-36] While there is no treatment for this disorder, at least the test provides an answer to the question of why the testes have failed, which unfortunately medicine today still cannot answer in the majority of patients.

Scrapings of buccal mucosa were used in the initial chromosomal study, but all modern genetic scientists use peripheral blood. This requires use of special media and microculture method followed by harvesting. Chromosomes are then prepared and fixed, slides are treated by *giemsa-trypsin* for banding metaphase chromosomes.[37] In each case, at least 30 to 50 well-spread metaphases are scored for detecting chromosomal abnormalities. In case of mosaicism or mixed samples, at least 50 to 70 metaphases are scored. Subsequently, *quinacrine* fluorescent staining[38] is done to find out the presence of terminal fluorescent heterochromatic region on the long arm of Y chromosome.[39]

MAGNETIC RESONANCE IMAGING (MRI)

The MRI is the most important complimentary modality in evaluation of male infertility. A scrotal MRI is an important adjunct to a scrotal US in the diagnosis of abnormal pathology and anatomy of the testes. For the diagnosis of cryptorchidism, it certainly acts as the gold standard. Using endorectal coil, MRI provides additional usefulness to provide intricate detailed information of the distal ductal system of the male genital tract, especially the anatomy of seminal vesicles, ejaculatory ducts, pelvic and the inguinal parts of the vas, hitherto inaccessible to conventional US.[17]

The characteristic high signal intensity of testis on T2 weighted sequences aid in easy recognition of undescended testis. MRI scores over CT and US in localisation of an atrophic undescended testis. However, routine use of MRI is neither cost effective nor accessible in the medical set up of the subcontinent. Even in the developed countries, MRI is used infrequently and only as a complimentary to US, TRUS and CDU.

CT SCAN

CT scans of different regions such as pelvis or abdomen, are used to locate the anatomical lesions. Pelvis may be scanned for testicular cryptorchidism or tumours pressing on the veins to cause secondary varicocele.[17] Similarly, scan of head or abdomen may be necessary for the diagnosis of pituitary or adrenal lesions causing male reproductive dysfunction.

RADIONUCLIDE SCANNING

Prior to availability of Color Doppler sonography, scintigraphy was the standard diagnostic modality for evaluating testicular arterial flow and perfusion. This is done by injecting ^{99m}Tc pertechnetate, with a gamma camera so placed that the symphysis pubis is at the centre of the crystal. Color Doppler sonography and scintigraphy show similar sensitivity in detecting testicular blood flow. Scintigraphy lacks the sonographic advantage to provide anatomic information as well as arterial perfusion status. Moreover, it exposes the patients to radiation. Presently, the scintigraphy is reserved for situations, when the Color Doppler studies for the patients with low

velocity and low volume testicular arterial flow show unsatisfactory sensitivity.[13,15,40] Radionuclide scanning is also useful in the diagnosis of chronic epididymitis or acute torsion of testis. It can also detect pooling of blood in the pempimiform plexus to substantiate a diagnois of varicocele.[17]

ANGIOGRAPHY

Spermatic Venography

The spermatic venography is considered the gold standard for the diagnosis of a varicocele. It also has a therapeutic application for embolisation treatment for varicocele. In this technique, an angiographic catheter is placed into the testicular vein via transfemoral venous route. About 5 to 10 ml of contrast is injected during Valsalva manoeuvre before radiographs of abdomen and pelvis are taken. Venous embolisation can be performed using balloons, coils or sclerosants like 70% glucose. Spermatic venography is especially suitable for the detection of postsurgical recurrent varicocele.

Internal Pudendal Angiography

Internal pudental angiography is indicated in young patients suffering from ED with suspected isolated arterial disease, especially of post-traumatic origin, where a surgical repair is expected to cure the condition. It is contraindicated in older patients with arteriosclerosis.[17,18] It can only be done, if there is patency of the proximal arterial trees of common iliac, internal iliac and internal pudendal arteries. Papaverine is injected into the anterior division of the internal pudendal artery to visualise the penile arteries.

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APPENDIX 1

Diagnostic Testing for Male Factor Infertility

(*American Society of Reproductive Medicine*)

1. ***Semen Analysis***: At least two semen samples collected by masturbation on separate days are recommended. Each sample should be collected after abstaining from ejaculation for at least 48 hours, but not longer than five days. The complete ejaculate should be collected in a sterile container provided by the clinic or laboratory, and should be examined within one hour of collection. Agglutination (clumping or sticking together) of sperm should also be noted.
2. ***Endocrine (Hormone) Evaluation***: Normal sperm production and sexual function are dependent on a normal hormonal environment. An endocrine evaluation should be performed if:
   1. A low sperm concentration is detected,
   2. There is impaired sexual function,
   3. There are other signs of endocrine disease.

   Endocrine evaluation includes measurement of follicle stimulating hormone (FSH) and testosterone. Luteinising hormone (LH) and prolactin are also commonly measured.
3. ***Additional Semen Tests***: These optional tests may provide more information about the semen or sperm. They can help define specific abnormalities or diseases of the male reproductive system. These tests include:
   1. *Vital staining* – determines numbers of living and dead sperms.
   2. *Antisperm antibodies* – tests for antibodies that bind to sperm and may reduce fertility.
   3. *Semen fructose* – the absence of fructose means either the vas deferens is obstructed or the seminal vesicles are absent.
   4. *Peroxidase staining* – differentiates white blood cells from immature sperm cells to assess for possible infection.
   5. *Semen culture* – checks for bacteria that may cause genital infection.
   6. *Biochemical analysis of semen* – measures various chemicals in semen such as fructose.
4. ***Specialised Tests***: These tests may be useful in a small number of patients for identifying a potential male factor in a couple with otherwise unexplained infertility. The value of these tests in selecting therapy is controversial.

1. *Hypo-osmotic swelling test* – assesses the sperm membrane for structural integrity.
2. *Sperm penetration assay* (Hamster egg penetration test) – measures sperm-egg membrane fusion, using hamster eggs and the man's sperm to test the capability of the sperm to penetrate the egg during in vitro fertilization (IVF).
3. *Human zona pellucida binding test* – measures the ability of sperm to bind to the zona pellucida of the egg. This test is also called the hemizona assay.
4. *Computer-assisted semen analysis* (CASA) – measures precise characteristics of sperm motion.

5. ***Genetic evaluation***: The importance of genetic evaluation in infertile males with severe oligospermia (sperm counts of less than 5 to 10 million per ejaculate) or nonobstructive azoo-spermia (absence of sperm in semen, not due to blockage) has recently been established.

APPENDIX 2

Changed Hormone Levels in Males

	Increased	*Decreased*
Serum FSH	a. Primary hypogonadism b. Primary testicular failure c. Gonadotrophin secreting pituitary tumours	a. Hypothalamic GnRH deficiency (Kallmann's syndrome) b. Pituitary FSH deficiency c. Ectopic steroid hormone production d. Isolated FSH deficiency
Serum LH	a. Primary hypogonadism b. Primary testicular failure c. Gonadotrophin secreting pituitary tumours d. 5-alpha-reductase deficiency	a. Hypothalamic GnRH deficiency (Kallmann's syndrome) b. Pituitary LH deficiency c. Ectopic steroid hormone production d. Fertile eunuch or isolated LH deficiency
Serum testosterone	a. Precocious Puberty b. Androgen resistance (Androgen receptor deficiency)	a. Delayed puberty. b. GnRH deficiency c. Congenital adrenal hyperplasia d. Gonadotrophin deficiency e. Primary and secondary testicular failure, PADAM f. Some systemic diseases
Serum Prolactin	a. Pituitary Prolactinoma b. Hypothalamic lesions c. Hypothyroidism d. Treatment with anti-depressants e. Stress	a. Dopamine agonist treatment

CHAPTER

9 Varicocele and Male Infertility

INTRODUCTION

A varicocele is described as a varicose condition of the venous plexus in the spermatic cord leading to dilated and tortuous state of the veins. Essentially, it is caused by retrograde or inversions of blood flow into the internal spermatic venous system. Even in the first century, Greek medical history accurately described the varicocele as "the veins are swollen and twisted over the testicle, which becomes smaller than its fellow".[1] The resultant bulging veins cannot support the column of blood returning to the circulatory system, so the blood pools in the swollen vessels surrounding the testicles. In early cases, these dilated veins are at times difficult to feel. Exerting a downward pressure by bending over and as though forcing a bowel movement or a forcible cough, makes the veins to bulge out and be easily palpable. There are documentary evidences of British surgeon Barwell describing varicocele in 1885 and Bennett in 1889 changes in semen characteristics associated with it.

In an unscreened male population, various workers estimated the incidence of varicocele to be 4.4 to 22.6%, which works out approximately at 15 percent in general population. Amongst infertile men, its incidence is between 21 and 40 percent, while Kursh reported it to be 8 to 23% for clinically evident varicoceles.[2-7] In our series till August 2002, out of 2178 infertility patients, 936 (42.9%) were found to have varicoceles (see later) while Steckel et al[8] put it at approximately 35% (21-41%). The current report indicates that bilateral varicoceles are much more common than previously suspected.[9] Using high resolution US color Doppler and careful clinical examination in a warm room with relaxed scrotum to obviate any cremasteric and dartos contraction, it may be high as 70% or higher. This is also borne out in our series with an incidence of 76.5% (see later) =(716 out of 936). Anatomical, physiological and clinico-pathological aspects of the varicocele need a detailed discussion to understand their significance on the altered spermatogenesis.

ANATOMICAL ASPECT

The venous drainage of the testis and the epididymis is provided by three sets of venous channels running parallel to the testicular (internal spermatic), vasal or deferential and cremasteric venous systems. All three have free communications at the scrotal and the suprapubic levels. However, the internal spermatic or testicular veins provide the major drainage. While the left testicular vein normally ends at the left renal vein and the right at the inferior vena cava, cremasteric group of veins drain into the infraumbilical superficial veins ending in the iliac veins. But the deferential veins end at the plexus around the seminal vesicle and prostate (presumably to supply the accessory sex organs with testicular steroids), where there is a free communications between the right and the left venous systems. From the prostatic venous plexus, outward flow goes towards the hypogastric veins, and ultimately to the inferior vena cava. There is another bypass, which

connects adrenal veins with the internal spermatic veins, commonly known as adrenogonadal bypass.

Anatomy of Spermatic Cord

Spermatic cord is a fasciomuscular tube with three layers that are extensions of the anterior abdominal wall musculature. These layers comprise the external and internal spermatic fasciae, and between them lie the cremasteric muscle and fascia. These fibromuscular fasciae consist mainly of elastic fibres impregnated with collagen and arranged in a crisscross pattern to provide them with an intricate textile design.[10] The histological structure of the fasciomuscular tube lends credence to the observation that it not only provides the fascial covering for the cord, but also acts as an autoelastic stocking type support for the cord veins. The crisscross disposition of fibres helps in pumping the blood up in the cord and contraction of the cremasteric muscle greatly potentiates this pumping action. The collagen fibre component of the cord helps to limit excessive relaxation of the spermatic tube to prevent venous stagnation in the cord (Figs 9.1 and 9.2).

The fibromuscular tube also plays an important role in testicular thermoregulation[10] (see later). When the atmospheric temperature falls, contractions of the cremaster and the dartos muscles draw the testes up towards the relatively warm abdominal surface area to maintain the normal testicular temperature. The

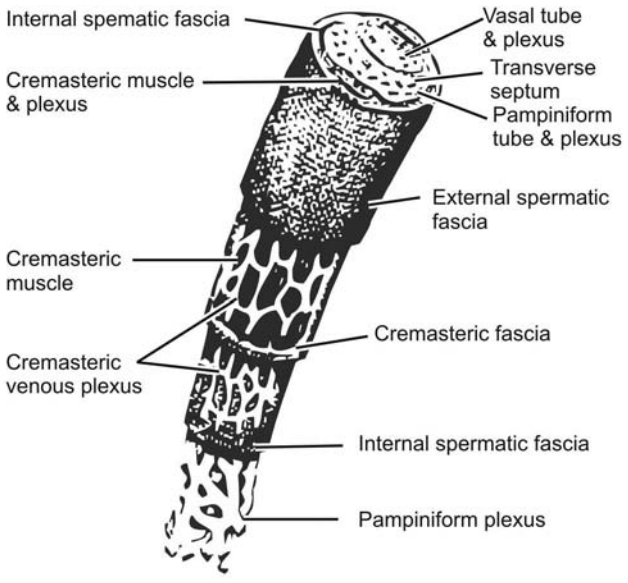

Fig. 9.1: Fibromuscular spermatic cord
Reproduced with permission of Prof. Shafik, Cairo, Egypt

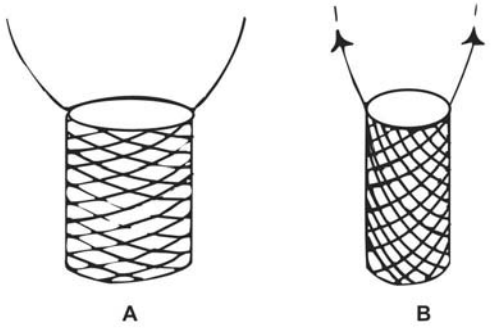

Fig. 9.2: Sphincteric action of the fibromuscular tube
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fasciomuscular tube by its contraction mainly of its cremasteric component, compresses the cord veins, thereby decreases the blood volume in the cord with resultant shrinking of the exposed cord surface to prevent heat loss. When the atmospheric temperature rises, the tubal muscles relax with resultant dilatation of the cord veins to increase blood volume. Greater portion of the surface of the spermatic cord becomes consequently more exposed due to a lowered position of the testes (Fig. 9.3).

This up and down movements of the testes with the change of atmospheric temperature is more pronounced in certain animals, where the testes can

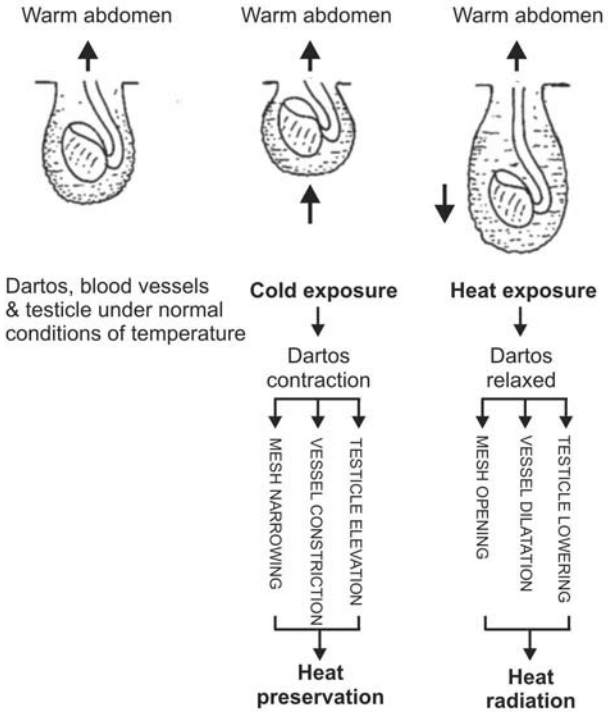


Fig. 9.3: Thermoregulation by the fibromuscular tube
(Modified after Prof. Shafik)

retract to the abdomen or take a position in the scrotum with the change in the outside temperature. The dartos muscle bundles are also arranged in a crisscross plywood pattern and act as potential sphincters around the blood vessels between their decussations to help in the testicular thermoregulation.

Ergun et al[11] analysed the vascular organisation of human spermatic cord by means of injection and by casting preparation of the testicular artery and veins of the pampiniform plexus after orchidectomy. They found that the testicular veins are organised in two main groups and form two venous plexuses after their exit from the testis. One of these vein groups forms a tight plexus around the testicular artery. Computer-aided 3-D reconstructions based on the paraffin serial sections of the human spermatic cord demonstrated the relation between the blood vessels and the other tissue structures of the spermatic cord.

On its basis, the human spermatic cord can be divided into three compartments (Fig. 9.4). The dorsomedial compartment contains the vas deferens and its blood vessels and the middle compartment contains the testicular artery and the veins forming a tight plexus around this artery. The ventrolateral compartment also contains a vein group, which shows no topographic relation to the testicular artery. This vein group is embedded in a large, macroscopically visible volume of fatty tissue and should be targeted during antegrade sclerotherapy, so that the testicular artery is spared.[11]

At the root of the scrotum, all three venous systems coalesce to form the pampiniform venous plexus. The venous hypertension in the pampiniform venous plexuses is the incriminating factor in the causation of varicocele. As the spermatic veins drain against gravity, defective valvular systems obviously play a predominant part in the causation of varicocele. If the valves at their upper end or outlet become anatomically or functionally incompetent, venous pressure in the renal vein causes a retrograde flow into the internal spermatic vein. A varicocele develops because of these defective valves that normally allow for blood to flow away from the testicle towards the abdomen.

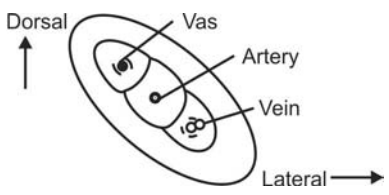


Fig. 9.4: Compartments of spermatic cord (Ergun)

Testicular damage occurs due to abnormal back flow of blood from the abdomen into the scrotum and this creates a hostile environment for sperm development. Rooney et al[12] found that although in most cases, incompetence of the internal spermatic vein was the main cause of varicocele; in 18 of 165 patients, they demonstrated through venography competent internal spermatic vein indicating that the varicocele may at times be due to incompetence of some other anastomotic connections to the vein.

Initially, the varicose condition may be limited to one system; but in later stages, all three venous systems (spermatic, cremasteric and vasal) may get into the varicose state. However, the varicocele primarily almost never occurs due to the venous reflux in the other two systems.

Most varicoceles are described as *primary*, where there are no secondary pressure effects. A *secondary* varicocele can occur due to external compression of the internal spermatic venous system by a pelvic mass or due to an intrinsic block caused by a tumour embolus or thrombosis.

One of the explanations for such venous hypertension and subsequent causation of the varicocele is the popular *"nut-cracker phenomenon"* (proximal type) causing compression of the renal vein of the left side between the aorta and the superior mesenteric artery (Fig. 9.5), especially in the upright position.[13] Morphometric analysis of the pre- and post-segment portions of the left renal vein in relation to the superior mesentereic artery shows variation in its diameter with post-segment narrowing. This also corroborates existence of possible ontogenic etiology or embryogenic disturbances in the development of the secondary venous system like the left renal vein. Furthermore, an association is found between the decrease of superior mesenteric artery angle and the increase in testicular vein diameter. Coolsaet et al[14] and Takihara et al[15] have described a distal type of "nut cracker" effect (Figs 9.5 and 9.6), where common iliac artery especially the right, compresses the left common iliac vein. Collateral venous anastomosis is an additional factor in its causation. (See later – anatomical variables). All these factors predispose to the development of idiopathic left varicocele (Table 9.1).

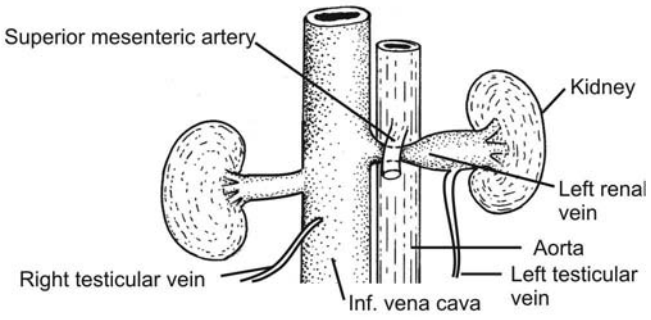


Fig. 9.5: Proximal nut-cracker phenomenon

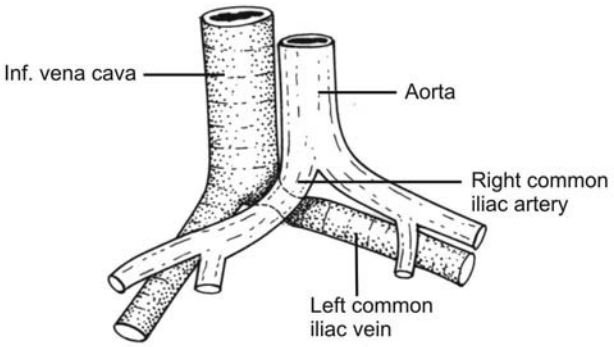


Figure 9.6: Distal nut-cracker phenomenon

Presumably, the left-sided varicocele is more frequently encountered due to the longer vertical course of the internal spermatic vein on the left side in contradistinction to the oblique course of the right internal spermatic vein opening into the vena cava (Table 9.1). In addition, the left renal vein enters the inferior vena cava 8 to 10 cm more cranial than its counterpart from ontogenic causes and consequent extra pressure in the left spermatic system. Thus, any backflow of blood secondary to incompetent or absent valves in the system would have an easier back flow into the left side compared to the right.

Table 9.1: Possible anatomical causes of left preponderance of varicocele

a.	Incompetent or absence of valves in the internal spermatic venous system.
b.	Drainage of left spermatic system into the left renal vein at a right angle and 8 to 10 cm cranial to its right counterpart.
c.	Nut-cracker phenomenon—proximal and distal.
d.	Collateral venous anastomosis.
e.	Possible ontogenic etiology due to embryogenic disturbances in the development of the secondary venous system like left renal vein originating from composite sources.

A unilateral right-sided varicocele of sudden onset normally suggests a venous thrombosis/tumour. Obviously, rare congenital anomalies, such as *situs inversus*, would make right-sided varicocele a common possibility. The right-sided varicocele results commonly from shunting of blood from the advanced left-sided varicocele and infrequently from the similar valvular defects of the right testicular vein. In our series, out of 936 patients with varicocele, four (4) patients had exclusive right-sided varicocele proved by clinical and color Doppler examinations.

A varicocele often casts its shadow in male infertility and is often a common causative finding in infertile men with impaired semen quality. Paradoxically, many men with varicocele are still fertile.

Anatomical Changes in a Varicocele

For practical purpose, the cord veins can be grouped into two systems. The superficial consists of the cremasteric plexus and the deep one have pampiniform and the vasal plexuses. Numerous small valveless communicating veins connect the three venous plexuses and these veins traverse the cremasteric and spermtic fasciae and transmit blood in both directions.

The anatomical changes in a varicocele consequent upon raised venous hypertension occur in three stages.[10]

Stage I: Cremasteric Varicosity

The veins of the cremasteric plexus lie between the cremasteric muscle bundles with a few scattered in the cremasteric and external spermatic fasciae. With the occurrence of the venous hypertension, the cremasteric plexus of veins reacts first, as they are the least supported, valveless and poorly musculariseed. Increased venous pressure in the spermatic veins gets readily transmitted to the cremasteric venous plexus through the numerous communications between the cremasteric and other venous plexuses.

Stage II: Pampiniform Varicosity

Pampiniform group is the largest among three venous plexuses. At the lower part of the cord, these veins are small with poor musculature and there are two or three valves in the central veins. Higher up the cord, near the external ring, the veins are relatively larger, more muscular but valveless. In the inguinal canal or near the deep ring, the veins coalesce to form two or three larger veins that are well muscularised but still valveless.

Subsequent to sustained cremasteric venous hypertension, the pampiniform plexus gradually starts to dilate and becomes congested. Initially, this

plexus escape from the effects of venous congestion due to relative abundance of the muscles in their venous walls and their internal support within the spermatic cord. It is important to note that the cremasteric varicosity is mainly positioned anteriorly relative to the pampiniform varicosity.

Stage III: Vasal Varicosity

The vasal plexus is positioned further posteriorly and consists of small poorly muscularised and valveless veins around the ductus deferens. The vasal varicosity occurs very late in the pathogenesis of varicocele as the vas is best supported within the cord. Consequently, the vasal venous plexus can withstand venous hypertension for a very long time before becoming varicose. However, the vasal varicosity can readily occur, if the testicular vein is obstructed primarily as seen in tumours.

All varicose conditions, however, go through three distinct phases.[10, 16]

1. *Compensated or hypertrophy phase:* The veins are thickened, but the stasis is absent as the venous return is kept normal by the compensatory medial coat hypertrophy.
2. *Concealed varicocele phase:* Continuous rise of venous pressure causes eventual failure of the muscle coat to provide support and leads to the venous stasis. The resultant testicular congestion leads to disruption of the thermoregulatory mechanism of testis and eventual decreased spermatogenesis.
3. *Manifest varicocele phase:* Sustained venous hypertension eventually leads to the testicular changes such as oedema, infarction and atrophy.

Anatomical Variables

As is wont, the vagaries of nature are evident in the anatomy of spermatic venous systems. While a unilateral varicocele is generally considered to be a lesion solely due to ipsilateral involvement of the pathological process, communications between the venous systems of the either side could lead later to bilateral involvement. Salemo et al (1999)[17] found many variations of the anatomic pattern of the internal spermatic vein. In fact, many vascular variants are reported in surgical and radiological studies demonstrating the existence of visceral-spermatic-lumbar and perirenal anastomoses. Uncommon spermatic veins variants were found in 34 (11.1%) patients; in 11 patients more than one vascular variants were present. The presence of a single or more commonly double colonic venous trunk was found in 24 (7.8%) and the presence of cross-communication in small vessels in 10 cases (3.2%). More rarely, lumbar (2.2%), inferior mesenteric (1.3%) and perirenal or ureteral connections were detected.

Free communications between the external and internal spermatic venous systems are more frequent in advanced varicoceles and were reported in about 20% of the cases of idiopathic varicocele in phlebographic studies.[18, 19] Niedzielski et al[20] in their series identified left-to-right cross-communicating vessels as well as communications between the internal and external spermatic veins through venography done during operative procedure in 12% patients. Comhaire[21] offered similar explanation for the distension of the right pampiniform plexus due to shunting of blood through the left-to-right venous connections in bilateral varicoceles. The high percentage of bilateral varicoceles detected by the sonography is mostly likely to be due these cross-communications between two sides. Progression of an initially considered unilateral anatomical abnormality to bilateral affection can thus be anatomically explained.[22]

Hirokawa et al[23] revealed the possibility of overlooking the small internal spermatic venous branches communicating with the external spermatic venous channels during routine ligation of internal spermatic veins for varicocele. Goluboff [24] noted the presence of external spermatic veins on the floor of the inguinal canal travelling posterolateral to the spermatic cord and subsequently exiting the spermatic cord before passing through the internal inguinal ring. Dudai et al[25] suggested that the ligation of the internal spermatic vein alone was not adequate, and cited a comparative study yielding better surgical results with transinguinal ligations of both the internal and external spermatic (cremasteric) veins.

In course of operating on varicocele patients, I came across the presence of accessory venous channels mostly on the floor of the inguinal canal (Fig 9.7). This observation prompted me to modify the steps of suprainguinal routine high ligation of varices. This modification enabled me to have a look into the floor of the inguinal canal; and consequently, I managed to find the accessory venous channels, which were duly ligated. I continued to practice this exercise and found the occurrence of such accessory venous channels in 7-8% cases (see Table 9.2). These

Table 9.2: Incidence of accessory venous channel

	Period	*Number of* cases operated	*Acc. venous channels looked* for in inguinal exploration *since 1992 September*	*Accessory venous* channels found
a.	Jan. 1990 - Jun 1994*	109	60	5 (8.3%)*
b.	Jan. 1990 - Jun 2000	499	344	26 (7.5%)
c.	Jan. 1990 - August 2002	556	401	29 (7.2%)

a. Total no. of cases operated = 109 (1.1.1990- 30.6.1994); Accessory venous channel looked for in inguinal exploration = 60 (since 1992 September); Accessory venous channels identified = 5 (8.3%)* *(These findings were presented in the World Congress of International College of Surgeons in the paper "Varicocele and male infertility" in November 1994 at London, U.K)*

b. Total no. of cases operated = 499 (1.1.1990 to 30.6.2000): Accessory venous channels looked for in inguinal exploration = 344: Accessory venous channels found = 26 (7.5%).

c. Total no. of cases operated = 556 (1.1.1990 to 31.8.2002): Accessory venous channels looked for in inguinal exploration = 401: Accessory venous channels found = 29 (7.23%).

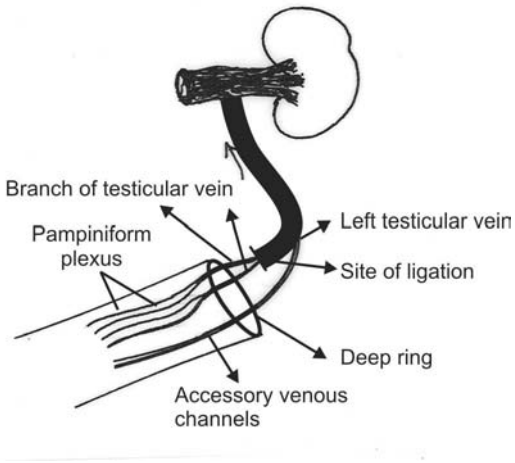


Fig. 9.7: Accessory venous channel

accessory venous channels[26] on the floor of the inguinal canal are likely to be missed, if routine high ligation or laparoscopic technique is adopted. Leaving these channels would not have ensured complete cure of the condition. The resultant possibility of residual varicosity would defeat the very purpose of the operation.

PHYSIOLOGICAL ASPECTS

Association of varicocele with abnormal semen parameters is well documented. Elevated testicular temperature without varicocele may be present in patients with idiopathic infertility.[27] Over the years, various researchers have cited several incriminating factors in varicoceles that contribute towards the subfertile semen. But these effects are variable and capricious, and often determined by hitherto unknown factors not always been explained by scientific reasoning. Several theories have been put forward to explain causation of abnormal spermatogenesis in varicocele. Apart from the intrascrotal hyperthermia, other factors such as reflux of renal and adrenal metabolites from the renal vein, hypoxia, intratesticular hormonal changes and hyperperfusion-induced testicular damage, have all been incriminated for the testicular dysfunction (see Table 9.3). As knowledge of male reproductive physiology expands more, insight into these theories could unfold in future.

Table 9.3: Probable factors causing testicular dysfunction leading to abnormal semen in varicocele

1. Changes in the temperature gradient.
2. Blood stagnation in the testis and epididymis causing hypoxic changes in the tissue.
3. Retrograde flow of toxic metabolites from the adrenal or kidney.
4. Alterations in the testicular hormonal status.
5. Intratesticular hyperperfusion-induced testicular injury.

Temperature Gradient

Various research workers consider the elevation of testicular temperature causing alteration in the temperature gradient between the body and the scrotum very important in the pathophysiology of the varicocele. Experimental evidence has demonstrated that increase in both testicular blood flow and temperature cause altered spermatogenesis. Even elevated testicular temperature without a varicocele may be present in as high as 54% of all patients with idiopathic infertility.[27] Preliminary results using the microwave testicular thermography showed altered testicular thermoregulation in a group of patients with impaired spermatogenesis with and without varicocele.[28]

It is also important to note that the temperature gradient would affect not only the seat of sperm production in the testes, but the epididymis as well. The varicocele is bound to influence epididymal circulation and temperature. In severe cases, the epididymis may be surrounded by the vascular structures causing severe epididymal tubular damage.[29] The epididymis plays a stellar role in the sperm maturation, capacitation and acrosome reaction (see Chapter 2).

An elevated temperature brings disruption of the normal absorptive, secretory and storage capacity of the cauda epididymis reducing the sperm numbers in the ejaculate. Based on animal experiments, Bedford suggested that chronic hyperthermia would affect the normal functions of the testes and the epididymis.[30] Effects of elevated temperature on the epididymis eliminates the special ability of the cauda to store, causes significantly faster sperm transport through it, and consequent reduction in the time required for capacitation of spermatozoa. Notwithstanding a normal sperm production by the testis, a much smaller number of spermatozoa in the first ejaculate were seen. Some of these temperature-related phenomena described in animals could have a bearing on the functions of the human male reproductive tract.

A temperature gradient of 2°C between the body and the scrotal temperature is the accepted norm by most research workers. With a lesser gradient, the spermatogenesis is inhibited. Several researchers[27,31-33] postulated that the intrascrotal temperature had to be steadily kept lower than the corporeal or body temperature by 2°C in order to let the testes perform its normal spermatogenetic function.

Using sensitive needle thermistors, Kurz et al[34] and Goldstein et al[35] found that the intratesticular temperatures were elevated significantly in men with varicocele. The scrotal skin surface temperature being related to intratesticular temperature was also raised. Furthermore, they demonstrated that a unilateral varicocele was associated with bilateral elevation of temperature. Lund et al[36] and Hjollund et al[37] confirmed these observations showing a relationship between testicular temperature in varicocele and impaired sperm quality.

The scrotal temperature shows a wide variation from time to time in course of a day and it is important that the body has a mechanism to keep the temperature gradient at an acceptable level to prevent prolonged variation of the gradient. The temperatures during sleep (TS) were generally higher than daytime values (TD), probably as a consequence of thermal insulation in bed.[38] Nocturnal scrotal temperatures were significantly higher, while sleeping on the side compared with periods lying on the back. The scrotal temperature was very significantly correlated to room temperature and its variations.[39] It is also strongly correlated with sedentary work position with dose-effect association. The work position is an important determinant of testicular temperature.[40]

Basic Mechanisms

It is postulated that the testes keep the euthermic condition through three basic mechanisms:

1. *Countercurrent thermoregulatory system*—consisting of the testicular artery and pampiniform venous plexus. The pampiniform plexus wrapping round the artery acts as a countercurrent heat exchanger, which serves to precool the warm arterial blood by the cooler venous blood (Fig 9.8).
2. *Dartos and cremasteric muscles*—depending on the corporeal and scrotal temperatures (often determined by the room temperature) gradient contractions of these muscles vary and adjust the distance between the testicle and the body. (Fig 9.3)
3. *Thinness of the scrotal skin*—that allows a fast and easy thermal dissipation. Scrotum acts as a fixed passive dissipater that is incapable of regulating outflow of heat to the surroundings.

Maloney et al [41] in an experiment with ram found that during cold exposure the tunica dartos muscles contract and the scrotal temperature drops, but the body temperature increases due to the general

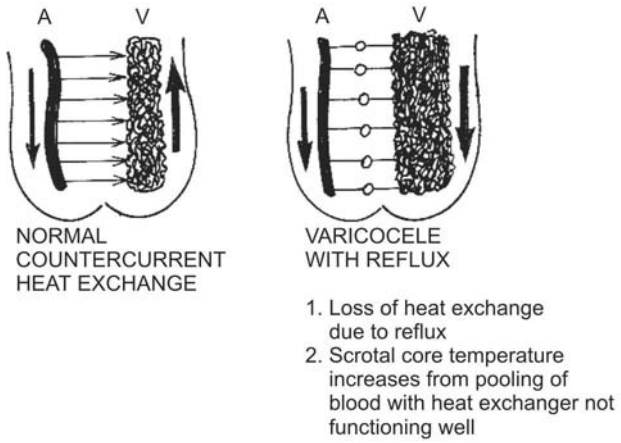


Fig. 9.8: Countercurrent heat exchange

thermoregulatory mechanism of the body. During heat exposure, the tunica dartos muscles relax and the scrotal sweat glands are activated. They concluded that the scrotal temperature is regulated independently of the body temperature via a feedback circuit involving scrotal thermoreceptors and effectors in the tunica of the dartos muscle and scrotal sweat gland activities. This local circuit is not affected by the adjustments to the general thermoregulatory control system. The effector mechanisms were insufficient to maintain the scrotal temperature during the extremes of heat and cold exposure.

The temperature at the scrotum is thus not a result of its intrinsic activity, but of a passive thermodynamic network consisting of heat from the arterial inflow and venous outflow, the countercurrent heat exchanger and scrotal dissipation. At the local level, the thermoregulatory responses of the scrotum would benefit the testis through the heat exchange in the pampiniform plexus, which acts to isolate thermally the scrotum and testes from the body.

Significant increase in the sperm concentration and total sperm output were achieved by Jung et al[42] after nocturnal scrotal cooling continuously for 12 weeks to effect moderate decrease in the factors leading to genital heat stress. Noticably, significant improvements in the sperm motility and morphology were also observed, but the changes in sperm concentration was markedly less pronounced. The study underlines the importance of genital heat stress as a cofactor in fertility impairment in men and indicates nocturnal scrotal cooling as a therapeutic option. Aggar also found correlation between lowered scrotal temperature and improved semen parameters after successful treatment of varicocele.[43]

Tight fitting thermally insulating clothing, prolonged physical activity or obesity affects the heat loss through the scrotal skin, and thus has unfavourable effect on the temperature gradient.[44] High fever also results in substantial warming of the testicles. Combination of these unfavourable factors could affect spermatogenesis leading to infertility.

However, in some cases, these effects are temporary and reversible. Likewise, electromagnetic waves may impair spermatogenesis by heat induction in the testicles, but only when exposure is excessive. Poor semens of paraplegics confined to wheelchair may also be reflection of the temperature effects on the epididymis.[44] Fujisawa et al[45] also incriminated higher than 34°C intratesticular temperature detrimental to optimal functioning of germ cell enzymes involving DNA and polymerase activities to produce negative changes in the semen parameters. The most probable explanation of this decreased spermatogenesis due to the temperature gradient also explains another important observation that the conventional varicocele of the pampiniform plexus is not the only cause of male infertility, but similar conditions in the adjacent epididymal plexus or the tunica vaginalis may also cause the temperature gradient to drop contributing to the inhibition of spermatogenesis.[27]

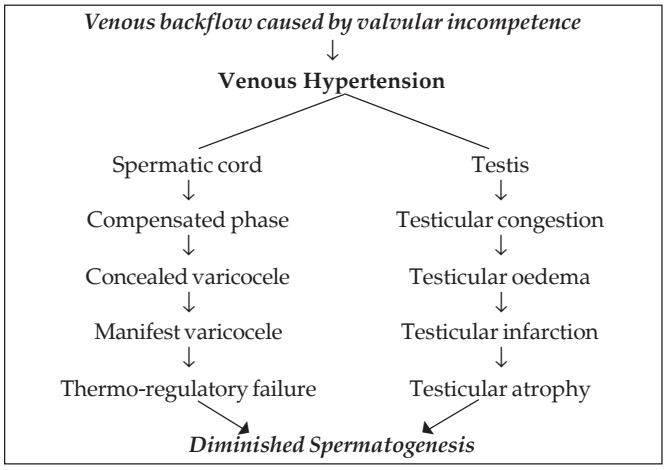
Incidentally, extensive thickening of the scrotal skin caused by long-standing dermatitis, multiple sebaceous cysts and elephantiasis of scrotum of filarial origin seen in some parts of the world, may at times be a contributory factor to cause drop in the temperature gradient and induce inhibition of spermatogenesis. Eight (8) such cases of scrotal thickening were encountered in our series—2 (dermatitis), 2 (elephantiasis), 2 (multiple cysts) and 2 (very large bilateral hydrocele).

Hypoxia Consequent upon Venous Congestion

Saypol et al created a varicocele model in animals to study alterations in testicular physiology. They surgically induced unilateral varicocele in rats and dogs and found that it resulted in bilateral increased testicular blood flow and temperature.[46] The secondary dilatation of the left internal spermatic vein was achieved by partial ligation of the left renal vein in one group of dogs and rats and by surgical destruction of the valve of the left testicular vein in the second group.

Venous dilatation secondary to partial vein ligation or testicular vein valve obliteration was followed by large bilateral increase in the testicular blood flow in these two species. As a consequence of this, increased flow was observed with elevation in the bilateral testicular temperatures leading to impaired spermatogenesis in some of these animals. This lends support to the view that the varicocele may impair spermatogenesis by a similar physiologic mechanism in men. Increase in the blood flow occurs first, followed by the sequence of elevated temperature, decreased spermatogenesis and seminiferous tubular atrophy (Table 9.4). Obviously, increased blood flow causes venous congestion, and subsequent hypoxia is due to decreased perfusion of oxygen from stagnated blood. However, Donohoe et al[47] and Netto et al[48] failed to find adequate evidence to substantiate testicular hypoxia thus caused to effect spermatogenic abnormalities.

Table 9.4: Probable mechanism of decreased spermatogenesis in varicocele



Reflux of Venous Blood with Chemical Compounds (adrenal steroids, catecholamines, NO)

Comhaire and Vermeulin[49] put forward a theory that increased quantity of norepinephrine reaches the spermatic venous system from the backflow through the andrenogonadal bypass in varicocele patients. A part of this norepinephrine then finds its passage into the testicular arterial system at the level of pampiniform plexus due to the countercurrent exchange phenomenon or due to increased scrotal venous pressure from the nut-cracker effect.[50, 51] This causes contraction of testicular arterioles with resultant hypoxia of the testes and epididymis. The concentration of norepinephrine has been found to be higher in spermatic venous system in patients with varicoceles. Catecholamine concentration in the internal spermatic veins was also found higher in patients with varicoceles than in controls. Ito et al also found evidence of prostaglandin (PG-E and PG-F) formed in kidneys causing impaired spermatogenesis.[52] These compounds can reach the affected testis through backflow into the spermatic vein from the renal vein or via the venous crossover channels. Normally, alterations of semen are more apparent in patients suffering from moderate and advanced varicoceles. MacLeod[53] had suggested influx of adrenal steroids into the spermatic venous system through the adrenogonadal bypass in the pathogenesis, but this was not borne out by later workers.[54,55] Ozbek et al[56] have also found evidence of increased level of NO (nitric oxide) in the internal spermatic vein as possible additional cause for spermatogenetic dysfunction.

Hormonal Changes

Leydig cell dysfunction[57] has been encountered in some research studies in patients with varicocele in spite of having an apparently normal histological appearance and normal FSH, LH and serum testosterone levels. Concentration of intratesticular level of testosterone and not the serum testosterone is more critically important for functioning of the Leydig cells. The latter may still be normal, yet the Leydig cell dysfunction may exist.[58] Pirke et al[59] found decreased testosterone in some patients with varicocele and reported improvement of the hormonal and spermatogenic functions after varicocelectomy. Increased FSH and LH levels after GnRH stimulation may indicate irreversible parenchymal damge to Leydig and germ cells.[60] Exaggerated gonadotrophin response to GnRH stimulation correlated with abnormal semen parameters.[61]

Intratesticular Hyperperfusion

Independent experiments in laboratory conditions of varicocele in animals by Green and Turner[62] revealed histological changes in the testes of these animals. Enhanced blood flow in varicocele not only produces higher temperature, but also increased perfusion in the intratesticular microvascular circulation. Consequent upon these changes, there is increased metabolism and phosphorylase activity in the testis leading to depletion of intratesticular glycogen and eventual parenchymal damage to the testis[3,63] (Table 9.5).

Table 9.5: Probable toxic effects in the testis from varicocele.[3,63]

1. Arrest of testicular growth (more perceived in adolescents).
2. Deranged semen parameters.
3. Leydig cell dysfunction.
4. Histological changes like tubular thickening, interstitial fibrosis and maturation arrest of sperms.

Significant advances have been made in the understanding of varicoceles, yet a clear pathophysiologic mechanism remains elusive. Most likely, a varicocele is the result of a multifactorial process and the theory of alteration of the temperature gradient seems to be most acceptable. However, concomitant presence of certain other factors such as smoking and environmental factors, may accelerate the deleterious effects on the sperms in patients with varicocele.

CLINICOPATHOLOGICAL ASPECTS

As stated earlier in the chapter, there is an incidence of varicocele in unselected male population, while the incidence is much higher amongst infertile men. However, there is a variation in the incidence from different countries and we do not have an authentic data in the subcontinent.[7,8,64,65] Incidence of varicocele at 42.9% in our series (Table 9.11) was recorded amongst the more enlightened urban popution, who come to the doctor more often than their rural counterparts. In large majority of patients, varicoceles are clinically palpable in standing position.

A subclinical varicocele is not clinically detectable even with the patient standing and with Valsalva manoeuvre. Its detection is only possible with technical aids such as color Doppler sonography and scrotal thermography. The presence of a varicocele can also be confirmed by using venography to delineate the anatomy of venous system of the scrotum using Seldinger's technique. But its clinical application is limited except for the transcatheteric embolisation treatment of varicocele or for the detection of residual varicocele after operation.

Clinically, the varicocele is classified into three degrees by palpation of the thickness of the varicosity in the cord with patient standing and using Valsalva procedure. It is considered *first degree,* when the thickening is up to one cm, *second degree,* when it is between one and two cm and *third degree*, when it is beyond two cm. The clinical classification of varicoceles into these three degrees roughly corresponds to the anatomical changes in the pampiniform plexus described in three grades (Tables 9.6 and 9.7).[66] Commonly, the degree and the grade are used synonimously.

Table 9.6: Clinical classification

First Degree	=	Thickening of cord up to 1 cm = Grade I anatomical changes
Second Degree	=	Thickening of cord between 1-2 cm = Grade II anatomical changes
Third Degree	=	Thickening of cord beyond 2 cm = Grade III anatomical changes

Table 9.7: Corresponding anatomical changes described in three grades

Grade I	:	Dilatation of the pampiniform plexus is neither visible nor palpable, but becomes palpable with Valsalva manoeuvre.
Grade II	:	Dilatation of the pampiniform plexus is not visible, but is easily palpable in standing position.
Grade III	:	Very large dilatation of the pampiniform plexus causing visible and bulging of the scrotal skin is seen.

The ambiguity regarding the different stages, phases (mentioned earlier), degrees and grades may appear confusing. The stages and the phases of the varicocele are based on the pathological anatomy, while the degrees and the grades are essentially a clinicopathological connotation. In practice, clinical grading is considered as the most important criterion for describing a varicocele.

Clinical Assessment

Most men with varicocele have no symptoms. Asymptomatic cases are often diagnosed during routine physical examinations. Clinical symptomatology may take the form of ache or feeling of heaviness in the testis. An observant patient may notice shrinking of the affected testis (more often the left) or papable veins over the scrotum. Recurrent or constant discomfort or pain in the scrotal region should be reported to an urologist, andrologist or primary care physician to determine the cause. However, all patients reporting with infertility should have a thorough examination of the scrotum and testes.

The clinical assessment of a patient with varicocele needs to focus on the degree of varicocele, the size of testis and detailed analysis of semen for count, motility, morphology and other parameters. After establishing the diagnosis, the modalities of treatment (nonsurgical or surgical) and the surgical approach are formulated. Ultimate aim of treatment in any infertile couple is to achieve pregnancy. Consequently, an appraisal of success and failure in terms of pregnancy is singularly important to formulate the modalities of treatment by any clinician or andrologist.

It has been my experience that the assessment of varicocele by clinicians is not always perfect. They often fail to adhere to the protocol. I have often found that a varicocele is missed, if the protocol is not strictly adhered to. Palpation must be done from just above the testis and going down rather than from below upwards. Consequently, many physicians tend to err on the negative side and the varicocele is missed. For a critical assessment of varicocele, the clinicians need to be thorough spending some time with the patient. The patients must be examined in both standing and lying postures in a warm room to prevent contractions of dartos and cremaster muscles. It is also mandatory to use Valsalva manoeuvre (bending over and exerting a downward pressure as

though forcing a bowel movement) with the patient coughing or straining for one minute. A cursory examination may often fail to reveal a varicocele, and only with the patient standing and straining for a minute or so, would actually reveal that the varicocele is indeed present.

Subclinical Varicocele

Some varicoceles could be missed, if the andrologist depends solely on his clinical findings. Yet these missed cases do not escape detection by an ultrasonography (US). In a subclinical varicocele, the clinical examination is negative, but the ultrasonographic finding is positive. Statistical analysis of the palpatory findings in scrotal physical examinations for infertile men reveals a sensitivity of 73.90 percent and a specificity of 90.60 percent in the detection of clinical varicocele.[67] So the diagnosis of any varicocele must be confirmed on the basis of the ultrasonographic findings. Some andrologists advocate measurement of the temperature gradient as well. In one series, Preutthipan et al[68] reported that in patients whom initial scrotal physical examinations failed to detect varicocele, 17.14% were found to have its presence by the scrotal ultrasound. Gonda et al[69] opined that the subclinical varicocele seems to be an important causal factor in infertility and found that the ultrasonographic diagnosis was positive for subclinical varicocele in 95% of patients, while nuclear scanning was considered positive in only 55%. Yarborough et al[70] used venography for the diagnosis of subclinical varicocele in 22 out of 40 infertile men declared normal in physical examination. A major difference between the venograms of clinical and subclinical varicoceles is the degree of reflux, which is expectedly more pronounced in the former group.[71]

Some authors such as Jarrow[72] state that the results of subclinical varicocelectomy are of questionable benefit. But most other authors express contrary views. Pierik et al[73] had found significant improvement in sperm motility after treatment of subclinical varicoceles. Dhabuwala et al[74] showed in their statistical analysis similar improvement in fertility potentials in the subclinical groups following improvement in sperm density and morphology after surgical treatment. Postoperatively, all patients showed improvement in their semen and 40% became fertile. Gonda et al[69] and Bsat et al[75] both agreed that the Doppler studies solely are able to diagnose patients for subclinical varicocele. The subclinical varicocele is now a definite entity; and with the advent of ultrasound, the diagnosis has become less controversial. Moreover, several workers have found its treatment beneficial for patients.

Prepubertal and Adolescent Varicoceles

With the advent of ultrasound and other diagnostic facilities, a varicocele is no longer a disease confined to adults and is being detected in children and adolescents as well. Varicoceles can be found in boys as young as 5 years and can cause testicular growth arrest as early as at 9 years. Works of Paduch and Neidzielscki[76] documented statistically significant deteriorations in sperm parameters of boys with varicocele, when compared with control without varicocele. Consequently, these boys must be followed with serial testicular measurements before and after puberty until late adolescence, as repeated semen analysis is not practicable.[77] Long-term risks for impaired fertility that these patients could face should also be made known to their family members.

Our present state of knowledge is unable to guarantee any immunity from the future risk of infertility in prepubertal and adolescent varicoceles. It would not be prudent on the basis of the existing data, simply to ignore the potential for future infertility in these patients.[77] If infertility becomes an issue in the adulthood of these patients, the chance for its reversibility may be lost without an early treatment. However, discussion with the family members on the merits and demerits of surgical interference would be important and they should also be made aware of the fact that a certain number of patients even with varicoceles would be fertile. It is well known that varicoceles, especially the subclinical type, are extremely common findings even in a group of fertile men.[7, 78, 79]

It is not possible to predict accurately the rate of progression in individual cases of prepubertal and adolescent varicoceles. Current recommendations for repair of the adolescent and prepubertal varicoceles are based on the findings of impaired testicular growth and/or spermatogenesis.[80] Vasavada et al[81] conclude that all prepubertal boys should be screened in the standing position for the presence of varicoceles. They recommended that these children do well with early surgical repair with improvement of testicular growth.

Significant prognostic features (see later) in adolescents with varicocele include the testicular atrophy or arrested testicular growth, high-grade varicocele (grade II or III), bilateral lesions, pathologic GnRH stimulation test, and histologic picture of Leydig cell hyperplasia. The presence of these features either alone or in combinations is considered as indications for treatment.[80]

Unlike an adult varicocele, it is not practical to follow children and adolescents with semen analysis. Consequently, the clinical assessment using orchidometer and ultrasonographic assessments are the avenues left for the follow-up studies.

At present, there are several options like open or microsurgical operations, but some workers prefer the embolisation treatment at puberty or during adolescence.[77,82,83] Riccabona et al[84] recommended modified Palomo method as the optimal surgical technique for varicocele treatment in males of this young age. Ultimate aim for any treatment is to prevent deterioration of testicular function. Data from series of Greenfield et al[77] and Lund et al[85] show that there is a testicular catch-up growth after varicocelectomy. Greenfield recorded a testicular catch-up growth in more than 80% of patients after microsurgical correction of these varicoceles.

Laterality of Varicocele

In infertile men, clinically varicoceles are mostly bilateral[6, 86] and the left varicocele is usually larger in men with bilateral varicoceles.[87] Sophisticated thermographic measures in both animal and human studies reveal that a unilateral varicocele is associated with bilateral elevation of intratesticular and scrotal surface temperatures.[35] McClure and Hricak[22] in a small series of 50 patients recorded 70 percent bilateral varicoceles as detected by sonography, and 24 percent subclinical varicocele on the left side.

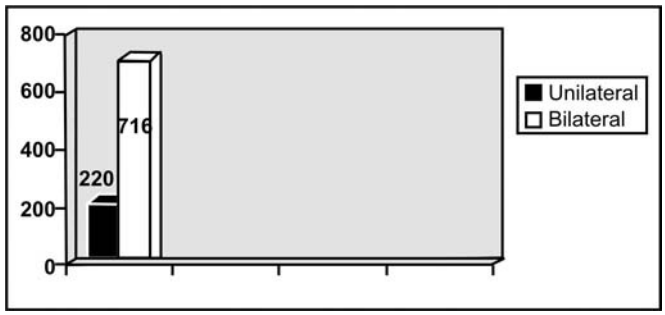


Fig. 9.9: Laterality of varicocele
936 varicocele out of 2178: Unilateral = 220 (left = 216, right = 4); Bilateral = 716

In a series of 100 fertile patients reporting for vasectomy, Kursh[7] estimated the incidence of clinical left varicocele at 17% and subclinical varicocele at 44%. Out of the subclinical group, overwhelming 43 out of 44 men had at least left-sided lesion; while in 8 men, the lesions were bilateral.

Anatomical reasons for the preponderance of the left-sided lesion have been dealt with earlier. However, the laterality of varicocele assumes a real significance, when it is right-sided for the reasons discussed earlier. In my series, a great majority of patients had bilateral affection as evidenced by color Doppler (CDU) studies. The high percentage of bilateral varicoceles detected by sonography may substantiate the pathophysiological mechanism involved in the progression of an initially considered unilateral anatomical abnormality to bilateral affection. Numerous anatomical communications between the right and the left venous systems[22] are cited as the explanation for this progression in due course of an initial unilateral varicocele to the other side.[17] Four (4) cases of strictly unilateral right-sided varicocele confirmed by US were found in our series (Fig. 9.9).

Progression of Varicocele

Varicocele rarely is clinically evident before adolescence, but its regression probably does not occur.[63] An individual with a varicocele, even with a previously documented normal semen parameters or fertility, is still at a risk for subsequent loss of testicular function leading to infertility.[87] Many of these patients would need treatment because there is convincing evidence that a varicocele may have a progressive toxic effect on the testes that may ultimately result in irreversible infertility. It is still beyond our current clinical capabilities to identify these individuals with varicoceles, who ultimately will have impaired fertility.[88] However, other unfavourable factors such as environmental effects, (see Chapter 6) pre-existing genetic or medical risk factors.[89] would continue to operate for all patients contributing to the severity of infertility.

Compensatory Varicocele

Any atrophy of testis would naturally lead to loss of testicular volume in the scrotum. This could open up a relative empty space in the scrotum above the testis and may cause the venous plexus occupying therein

to lose some of its supports. Consequent to this loss of support, varicose condition of these venous plexus could easily result. It, therefore, appears that the development of these varices somewhat compensate for the loss of volume of tissue inside the scrotum causing creation of the empty space. This type of varicocele in association with testicular atrophy due to some reason has been termed as *"Compensatory varicocele"* (Fig. 9.10). Till 2002 August in a 12-year study of 2178 patients with infertility, we have encountered a total of 81 such cases of compensatory varicoceles. In these patients, variable degrees of testicular atrophy were present for reasons known or undetected, and the testicular sizes were 8 cc or below. Other noteworthy observations were that these varicoceles were mostly of degree I, occasionally of II, and none in degree III or more advanced (Fig. 9.11). Some loss of testicular volume is common in varicoceles. But these varicoceles mentioned may not have been the primary cause of testicular atrophy as most of these men were also azoospermic (63 out of 81) caused by some other conditions.[26]

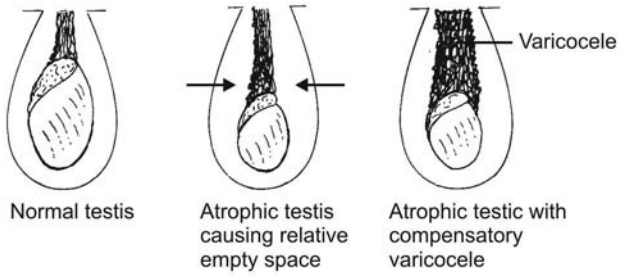


Fig. 9.10: Compensatory varicocele

Secondary Infertility and Varicocele

As stated before, varicocele is a progressive and not a static lesion. Over a period, it could lead to the loss of previously established fertility with progressive reduction of sizes of the testes and subsequent deterioration of sperm functions.[90] The term secondary infertility is referred to a situation, where an individual is able to impregnate his partner at least once, but later fails to do so. Majority of these cases are due to varicocele.

The incidence of varicocele is much higher in male factor secondary infertility compared with primary infertility. Gorelick et al [3] recorded 81% incidence of secondary infertility (79 out of 98) in their series. Witt et al[91] recorded an overall incidence of 8.5% in men

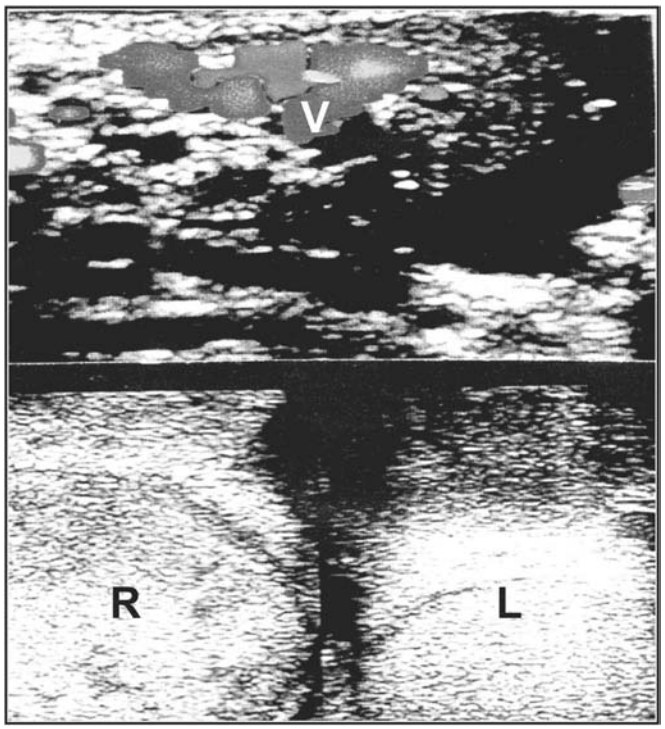


Fig. 9.11: (R) Testis size 10.8 (no varicocele); Smaller (L) Testis size 6.8 with 2° varicocele (V) (Compensatory varicocele)

with secondary infertility in a series of 2,989 patients evaluated for infertility. They identified varicocele as the cause of the patient's infertility in 177 out of 285 (69%) men with secondary infertility.[91] As these men have already fathered children, they belonged to slightly older age groups. Expectedly, these men are likely to have an age-related lower mean sperm concentration and more abnormally shaped sperms compared with men with primary infertility with varicocele.

Interestingly, I can cite quite a few couples, who got one or two children born within a short period of their marriage although they did not take any contraceptive measures, but failed to get any pregnancy later. I have encountered 117 such patients out of a total of 2178 cases of infertility seen in the clinic in the last twelve years (January 1990 till August 2002). Out of these, 81 (74%) were found to have varicocele as the cause of secondary male factor infertility.

Testicular Size

The clinical assessment of varicocele must include measurement of the testicular size. Two methods for measuring testicular size have been developed using

calipers for measurement and comparison with plastic testicular models or orchidometer.[92] Varicoceles have a negative impact on the testicular volume and size on the affected side due to ipsilateral testicular atrophy caused by the effects of various factors outlined earlier. The effect may be more in the growing testes of adolescents and children. With the incriminating factor continuing to operate, progression is a natural sequel of a varicocele. The spermatogenesis is not affected initially. However, follow-up of these patients over a period of time would eventually reveal reduced sperm counts. Witt et al[91] concluded after following 2,989 infertile patients that the varicocele in most men is a progressive and not a static lesion resulting in loss of previously established fertility. Findings of Ku et al[93] suggest that men with higher grades of unilateral varicocele may have hypertrophied testis on the contralateral side. Men with large varicoceles had significantly decreased testicular volumes than those with small ones. So, the arrest of testicular growth is more significant in patients with larger varicoceles.

Zin et al [94] suggested that the left testicular volume is usually less than the right in most varicocele patients. According to them, both clinical and subclinical left varicoceles have a negative impact on the left testicular volume. In adults and adolescents, the left and the right testes should almost be of equal size and volume. The difference between the two if at all, should be within 2 ml (cc) or 20% of the other.[95]

Lyon et al [96] noted that the left testis was smaller in 77 percent of the patients in their series and indicated a preponderance of the left-sided varicocele. Thomas et al [97] measured the testicular volumes with calipers and computed the figure in cubic centimeter or cc (formula-length × width × breadth × 0.521). They set the norm of a testicular growth arrest, when the left testis is at least 15% smaller than the right testis and found that the testicular growth arrest occurred irrespective of the size of the varicocele.

Several reports have also demonstrated disorders of both testes by testicular biopsy with no histological differentiation in both sides. Brown, Dubin and Hochkiss[98] have observed right-left difference in only 10% of cases. Takihara[14] and Kamada[99] used FCM DNA analysis and flow cytometry for evaluation of testicular function. The results confirmed impairment of both testicular functions even in a unilateral affection with greater changes in the affected side.

There is one moot point, whether the testicular volume increases after correction of the varicocele. Cayan et al[100] concluded that the testicular consistency achieved normal firmness after corrective operations in all boys with preoperative soft testes. They are of the opinion that there is a catch-up growth in comparison to the contralateral testis. At ages older than 14 years, the testicular consistency often improves, but the testicular volumes do not increase significantly after operations on varicocele. Lund et al[85] reported testicular catch-up growth in 27 adolescents after a follow-up for 48 months after varicocelectomy. Culha et al[101] found that both the right and left testicular volumes increase significantly after operation in patients with grades II and III varicoceles. Curiously, the right testicular volume improved more than the left in most of their patients. Paduch et al[102] also reported testicular catch-up growth within 12 months of surgery.

The testicular sizes were measured in all cases using Prader's orchidometer in our series. We did not use any caliper to record the testicular length, breadth and the thickness, and depended on the calibration of the orchidometer in recording the testicular size. The size of the testes was found to be smaller than normal in most patients with varicoceles. There was preponderance of the left-sided varicocele; and as expected, the left testicular sizes were smaller than the right in most cases. But the size of the testis, when reviewed through US invariably showed a smaller volume than what was revealed by the orchidometer. This could, however, be explained by the fact that the testicular size measured through orchidometer does not exclude the added volumes of the testicular coverings and even minimal collection of fluid in the tunica vaginalis. Analysis of testicular size recorded in our series is shown in Figures 9.12.

Semen Parameters

MacLeod in 1965[53] first described the triad of decreased count, decreased motility and increase in

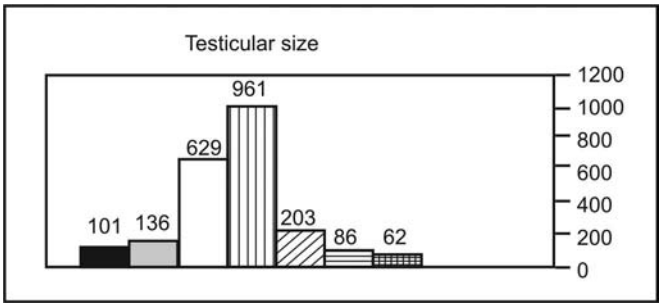


Fig. 9.12: Testis size (average of both sides) Total = 2178 (> 18 = 101; > 15 < 18 = 136; >12 < 15 = 629; > 10 < 12 = 961; > 8 < 10 = 203; >7< 8= 86' < 6= 62)

immature and tapered forms as main characteristics of semen of infertile men with varicocele. However, the effects of varicocele on various semen parameters still remain a matter for discussion. In spite of having varicoceles, some patients continue to have some normal parameters in the semen analysis. Unless all three important parameters of the semen analysis—count or density, motility and morphology are carefully analysed, one should not incriminate varicocele as the sole male factor in infertility. Out of all these parameters, the study of morphology is most controversial, and also needs the help of sophisticated microscope as well as great degree of expertise. In terms of morphology, overwhelming majority (91%) of patients with varicocele showed moderate to marked stress pathology with large numbers of sperms with tapered heads and many immature spermatids, amorphous cells and exfoliation of immature cells of the germinal line in the ejaculate. In a later study in 1969, MacLeod[103] further reiterated that the sperm motility was the most important factor in semen quality, and that pregnancy was possible with very low count provided the motility of the sperms present was good.

Cockett et al [86] found that two thirds of the infertile men, who had varicocele, also had reduced sperm motility and abnormal sperm morphology with more than 25 to 35% immature and tapered sperms. The motility of the sperms was usually low. Parikh et al[104] found that the sperm count, motility parameters such as curvilinear or straight-line velocity, lateral head displacement and normal morphology were significantly lower in men with varicocele.

Postoperatively, there was significant improvement in count, motility, and normal morphology, with a decrease in proportion of acrosome-deficient heads and tapering forms. After operation, they found that 46.2% of the men had normal semen parameters. Similar opinion was voiced by Naftulin et al,[105] who found that the varicocele patients had significantly more tapered sperms (36% +/– 3% versus 15% +/– 2%) and significantly fewer oval (41% +/– 3% versus 47% +/– 2%) sperms. Tinga et al[106] concluded that the changes in some semen characteristics after operation suggested a relationship between the varicocele size and the semen improvements. There was also an inverse relationship between preoperative value of semen and its improvements, and a similar inverse relationship seemed to exist between varicocele grades and deterioration of semen values.

In the present series, 386 patients had sperm count below 20 millions, while 454 had it between 20 and 40 millions. Interestingly, 96 had normal or near normal sperm counts in spite of having confirmed varicoceles (Fig. 9.13). Overwhelming numbers of patients (498 out of 936) showed grade III and IV motility less than 20%, while 293 had the motility in the 20 to 40% range. Thus, most sperms showed lowered motility or asthenospermia. Thirty-three patients had practically no motile sperms (Fig.9.14).

DIAGNOSTIC TOOLS

Once the clinical diagnosis is established and the semen characteristics studied, other diagnostic tools are used to confirm the diagnosis. However, the diagnosis of varicocele in prepubertal patients, especially in children below 12 years, poses additional problem due to impracticablity of the seminal examination in these patients. Consequently, the

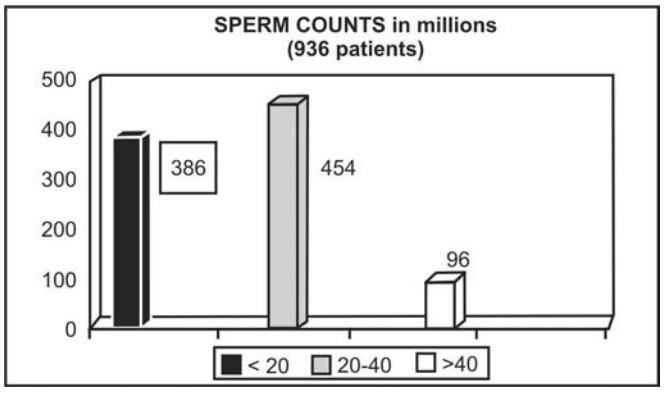


Fig. 9.13: Sperm counts in millions (936 patients)

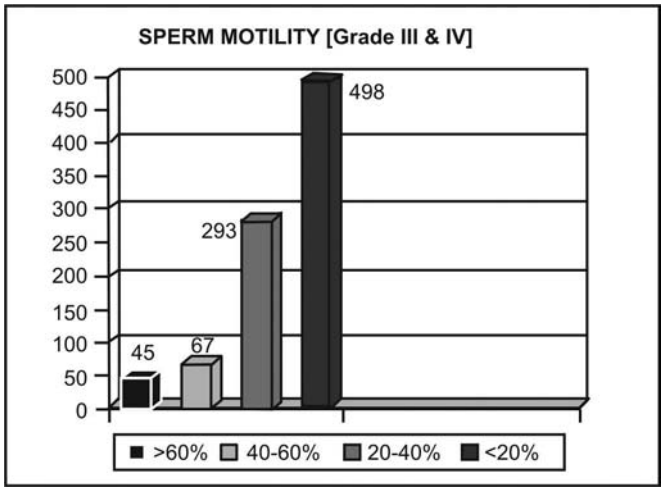


Fig. 9.14: Sperm motility (Grades III and IV)
A. = >60% = 45; B = 40-60% = 67; C = 20-40% = 293;
D= <20% =498 *[33 patients had no motile sperms]

clinical diagnosis of varicocele in these patients can only be substantiated with use of an orchidometer.

For andrologists, a varicocele is always investigated keeping the prime consideration in mind that it is one of the important causes of infertility. The microbiological examinations of the semen, prostatic fluid and urine, hormone studies, Transrectal ultrasonography (TRUS) of lower genital tract including the prostrate and testicular biopsy may not have special diagnostic value in varicoceles. However, without TRUS, an accurate diagnosis of certain congenital and acquired anomalies of the urogenital tract often associated with male infertility is not possible. Furthermore, it helps to formulate appropriate clinical and surgical management.[107] Three important and essential diagnostic tools that are used for varicocele are Color Doppler ultrasonography (CDU), measurement of scrotal temperature and venography.

Ultrasonography (US) and Color Doppler US (CDU)

The US of the scrotum has been one of the most important additions in the armamentarium of andrologists. The Doppler pencil-probe stethoscope was one of the earliest techniques and was easily utilised in an office setting. Presently, scrotal Doppler ultrasonography is a readily available in all recognised andrology clinics. In the hands of suitably trained doctors, this provides the most rapid, simplest, least costly and most reliable noninvasive method for the diagnosis of varicoceles.

Presently, use of linear transducer of 7.5 mHz or more is considered ideal, and this has replaced the past improvisation of using a water bag with a 3.5 mHz transducer. With this high-resolution apparatus emitting at 7.5 mHz, the sensitivity of US for the detection of varicocele has been further qualitatively improved by the Color Duplex flow imaging, which complements the haemodynamic data in the ultrasonographic images obtained. The pampiniform plexus has an alveolar appearance with increase in the diameters of the draining vein. Ideally, the data obtained should be compared with those obtained by venography.[108] The color flow Doppler Duplex US (CDDU or CDU) is decidedly superior, because of its objective characterisation of the pampiniform veins, especially when there is a doubt of varicocele on physical examination. There is an added advantage of accurate estimation of the testicular size, which is negatively affected by the varicocele. However, the accuracy of US is operator-dependent and one must be aware of the limitations of this diagnostic modality.[109]

CDU measures the diameters of the spermatic cord veins by imaging these vessels, at rest and during a Valsalva manoeuvre. It also quantifies and qualifies the flow of blood through these veins.

The diameters of the veins surrounding the testes should be less than 0.8 mm at rest; but in advanced grade III varicoceles with multiple serpeginous and tortuous veins, diameters of 12 to 14 mm may be seen. The patient is examined in supine and erect positions during quiet breathing, deep breathing and Valsalva manoeuvres. The vessels are scanned on each side from the scrotal neck to the lower pole of testis. Visualisation of three or more dilated veins with at least one of them having a diameter of 3 mm or more during Valsalva manoeuvre or increase in flow or reflux as seen on Color Doppler study during Valsalva manoeuvre is taken as a positive sign for varicocele. If the reflux lasts for more than one second, associated infertility is more likely.[110] However, other researchers have set the outer limit of the diameter at a lower level of approximately 2 mm[111] (Table 9.8, Fig. 9.15, Plate 3 and Fig. 9.16, Plate 4).

Table 9.8: Ultrasonography of varicocele

1.	**Color Doppler—**Increased flow on Valsalva manoeuvre.
2.	**Pulsed Doppler—**Reflux for 2 sec/more
3.	**2D US—**3 or more dilated venous channels (>2 mm)

The diameter appeared larger with increasing clinical grade (*subclinical*: 3.40 +/– 1.64 mm; *grade 1*: 2.74 +/– 0.84 mm; *grade 2*: 3.70 +/– 1.09 mm; *grade 3*: 4.38 +/– 1.30 mm).[112] However, not only the diameters but also the reflux, especially during quiet respiration, is as important in diagnosing all clinical and subclinical varicoceles.[113, 114] CDU has been shown to have 93% sensitivity and 80% specificity in evaluation of varicocele. With venography as the "gold standard", it is 85% sensitive in detection of subclinical varicoceles.[69]

As the flow in pampiniform plexus is very slow, Doppler parameters must be adjusted to detect very slow flow during any Color Doppler examination. No doubt a Doppler ultrasound would diagnose even a subclincal varicocele, but there should not be overdependence as the presence of a varicocele *per se* is usually of little clinical significance in its overall management, as it does not correlate with degree of impairment of spermatogenesis. The radiological

assessment of the size of pampiniform scrotal veins is not directly proportional to the degree of impairment of spermatogenesis.[115]

CDU being the most important diagnostic investigations, certain details need to be highlighted. Most importantly, the room should have an optimum temperature and certainly not too cold. When exposed to cold, contractions of the cremaster and dartos muscles would lift the testes up to make examination difficult. While CDU is performed with the patient standing, he holds the penis up against the lower abdomen for proper exposure of the scrotum, and the ultrasonologist conveniently sits on a low stool. It is, however, mandatory that patients should always be examined in both sitting and standing positions as well as with or without Valsalva manoeuvre to demonstrate the reflux.

The transducer should first be placed at the root of the scrotum and then worked down the spermatic cord alongside the testis ending at the lower end of the scrotum. This drill ensures that the varicosities at the lower and the posterior ends of the testis are not missed. At times, the flow is so slow, that no blood flow is demonstrated; but on reflux, some color flow is evident. One fallacy needs attention. Clinically felt small cysts at the upper pole of testis could prove to be varicoceles in CDU and vice versa.

Tasci et al[116] combined CDU with venous flow spectral analysis to study the characteristics of blood flow in the internal spermatic vein. They analysed with CDU and venous flow spectral analysis of 100 infertile men with clinical left varicocele and 50 fertile men without clinical varicocele serving as control. The results showed three types of pattern in the spectral analysis of venous flow (Table 9.9).

Table 9.9: Venous flow spectral analysis

Type I:	If the venous flow was directed to the heart and did not change direction with an intra-abdominal pressure increase.
Type II:	Venous flow is directed to the heart, but changing direction with an intra-abdominal pressure increase.
Type III:	Blood flow is directed to the testicles and augmenting with an intra-abdominal pressure increase.

Tasci et al concluded that the spectral analysis of Doppler waves should be used in combination with CDU for the diagnosis of varicocele. Varicocele should not be diagnosed with a type II flow pattern that occurs only during Valsalva manoeuvre. For the diagnosis of varicocele, the main criterion must be a type III pattern flow, as well as a type II pattern during normal breathing.

Measurement of Scrotal Temperature

As discussed in the physiological aspect of varicocele, an elevated testicular temperature is a potential risk factor for impaired spermatogenesis. The temperature gradient between the body and the scrotum supposedly plays an important role in the pathophysiology of varicocele.[27,31-36] Thus measurement of the scrotal temperature assumes an added importance in investigation of a varicocele, especially in children, where it can be used suitably to substantiate the diagnosis.

Methods of Measuring Scrotal Temperature

There are various methods of measuring scrotal temperature.

1. *Computer-assisted infrared thermography:* The change in scrotal temperature in patients with varicoceles and controls with different positions are measured with an infrared thermometer.
2. *Microwave technology:* It is a new method reported by Gazvani et al[28] for the reliable assessment of testicular core temperature. The testicular temperature profile obtained by microwave thermography may be of value in the assessment of infertile men with or without a varicocele. Furthermore, there was a temperature gradient between the scrotal neck and the testicular core in all groups; testicular core temperatures were lower than scrotal neck temperatures.
3. The scrotal skin surface temperature is elevated in men with varicocele and linear regression analysis revealed a strong correlation between intratesticular and scrotal skin temperatures.[34-36,117] This principle was used to device a method to measure the scrotal temperature using Feverscan strip. While it is very simple and inexpensive, it may not be very accurate. But for day-to-day clinical practice, it is certainly worth its use.

Method Used in this Series

A simple method of measuring the skin temperature was adopted in our study using Feverscan. It was started later in our series, and till August 2001 out of 738 patients, 585 showed temperature changes, while 153 did not. The procedure involves keeping the patient stripped for a period of 5 to 7 minutes to

expose the scrotum to the room temperature. The scrotum is shaved of hairs beforehand in these patients; and with the patient in standing position the Feverscan strip (Fig. 2.1, Plate 1) is wrapped on the scrotum over the varicocele area. Another strip is simultaneously put on the forehead to note the body temperature.[26] *(Preliminary report on 106 patients in 1994 showed changes in 85 patients).*

Venography

Venography is still considered as the gold standard and the most specific method of identification of varicoceles. But it suffers from the disadvantage of being invasive in nature and is associated with some morbidity. Percutaneous transvenous retrograde venography was originally used to delineate the venographic anatomy of recurrent varicoceles following conventional inguinal varicocele ligation.[118] In the subcontinental context, it is only possible in specialised centres, where competent radiological services are available. It is also expensive and so should be reserved for use in recurrent varicoceles for postoperative detection of aberrant veins.[119]

TREATMENT

Association of varicocele with male infertility has over the years posed an area of controversy. While most uro-andrologists and specialists are in favour of treating varicocele by surgical or allied methods, some infertility specialists in the world are still sceptical of the precise role of varicocele in male infertility and interventional procedures in the treatment of varicocele associated with male infertility.[120-122]

Evers et al[121, 122] reviewed relevant trials for the period 1966-2002 in the Cochrane Menstrual Disorders and Subfertility Group's specialised register of controlled trials. They noted that the concepts of varicocele causing male subfertility, and varicocelectomy curing male subfertility have been around for almost fifty years. However, the mechanisms by which varicocele would affect fertility have not yet been satisfactorily explained, and neither have the underlying physiological device by which varicocelectomy resolves subfertility. Furthermore, they have even gone to the extent of questioning the causal relation between the varicosity of the pampiniform plexus and impairment of fertility. They contended that the evidences for the treatment of varicocele in men with subfertility improving the chance of partner pregnancy were insufficient. In the article published in 2003, they further observed that the repair of varicocele did not seem to be an effective treatment for male with unexplained subfertility.

In spite of assertions by Evers and Collins, overwhelming majority of infertility specialists subscribe to the opinion that there is little doubt that the varicocele definitely casts its shadow on semen analysis. Most of the data drawn from the published series support a beneficial effect of varicocelectomy on semen quality and rates of pregnancy in the female partner of the patient.[123,124.] However, there is no general consensus in some areas, and partner pregnancy is known to occur without any treatment in certain number of patients.

Medical Management

After an appropriate clinical, laboratory and ultrasonographic assessment of patient's fertility status, the clinician must decide whether specific or empiric treatment is indicated. Specific treatment may take the form of replacement therapy (exogenous gonadotrophins or GnRH) for pituitary or hypothalamic failure, inhibition of prolactin secretion, antimicrobial or immunosuppressive therapy, for demonstrable immunologic infertility. Finally, ejaculatory dysfunction often requires sympathomimetic agents. Alternatively, in the normogonadotrophic oligospermic patient, the major form of empiric therapy relies on the enhancement of physiologic hormone levels that influence spermatogenesis. GnRH analogues, antiestrogens, exogenous gonadotrophins, or androgens may achieve such "stimulation" therapy.

Details of the medical management in male infertility have been discussed in Chapter 11. Specific measures for varicocele such as avoiding tight briefs have a few advocates. Cold compress of scrotum to reduce the temperature has been tried, but its efficacy is doubted by majority of researchers and andrologists. Wearing of scrotal support similarly has no curative value in varicocele, but it may give some relief in reducing the dragging discomfort felt in the scrotal area.

However, when potentiated by other cofactors such as gonadotoxins, the varicocele assumes a key role in causing male infertility. Nicotine and the presence of a varicocele were more gonadotoxic than either the varicocele or nicotine by itself.[125] So, while treating a varicocle patient, he should be advised strictly to stop smoking and be taken off any gonadotoxic agents including medications. Concomitant

genital infection (Chlamydia, U. urealyticum and Tuberculosis in the subcontinent) should always be looked for and appropriately treated. Any hormonal imbalance should also be corrected. One uncommon, but treatable cause of male infertility is gonadotrophin deficiency in which gonadotrophin replacement therapy is highly effective in inducing spermatogenesis and fertility.

Use of antiestrogens such as clomiphene has mostly not found favour with andrologists, but some researchers found encouraging results with clomiphene and tamoxifen. Unal et al found that although clomiphene citrate did not increase the sperm density and motility as effectively as a surgical correction of varicocele, there was no statistically significant difference between surgical and medical therapy methods in terms of seminal improvement and pregnancy rates.[126] Check has advocated that medical therapy with clomiphene citrate may prove not only to be a useful adjunct to varicocelectomy, but may prove to be the first-line therapy for counts less than 10 million/ml.[127] Encouraging results have also been reported using another antiestrogen Tamoxifen.[128]

In our series, we have recorded some improvement in semen analysis and in partner pregnancy rate in patients with first degree varicoceles with the use of clomiphene along with measures such as antioxodants and control of infection (Tables 9.10 and 9.11).

Table 9.10: Analysis of semen report in 392 cases treated with medical treatment for 1° varicocele

	Before treatment	*After treatment*
Average count per millions/ml	21	39
Average percentage of motility (Grade III and IV)	32	48
Average of abnormal morphology	47	33
Incidence of partner pregnancy	Nil	50 (12.7 %)

Table 9.11: Varicocele (medical treatment) January 1990-August 2002

Total patients	=	2178
Varicocele	=	936 = 42.9%
Varicocele treated by medical treatment	=	392.
Overall incidence of pregnancy	=	50 (12.7%).
Incidence of pregnancy in 162 patients, who had operation at a later date	=	80 (49.3%)

After studying various parameters for more than a decade in a male infertility clinic, where we have been able to organise referrals from other practitioners and with certain numbers of patients coming directly, a treatment schedule was formulated for the conservative nonsurgical medical management of varicoceles. We set the criteria depending on the degree of varicocele, quantum of abnormalities in semen analysis and age of the female partner. For all patients with first-degree varicoceles, an initial trial with medical measures for 6 to 12 months was advocated, especially if the female partner was less than 30 years, the semen analysis showed sperm density of 20 millions/ml or more, and the sperm motility was more than 40%.[129]

Interventional Treatment

The guiding principle of all interventional management is based on the interruption of the back flow of blood due to venous reflux into the internal spermatic vein. This is accomplished with a variety of approaches, including modified Palomo or high ligation (Palomo and Bernardi operations), transinguinal or subinguinal with or without magnification, laparoscopic ligation, and transvenous percutaneous occlusion[130] (Figs 9.17 and 9.18).

Several infertility specialists over the years have used the expression varicocelectomy or ablation of the varicocele to describe the surgical ligation of the internal spermatic vein. But strictly speaking, these terms are misnomers, as the varicocele is never excised or removed. The procedure should really be termed internal spermatic vein ligation (Table 9.12 and Fig. 9.19). However, I prefer to use the expression

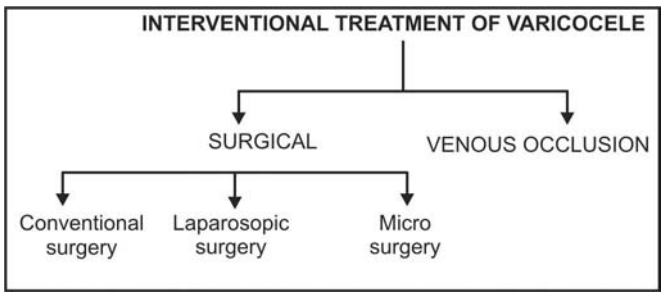


Fig. 9.17: Interventional treatment

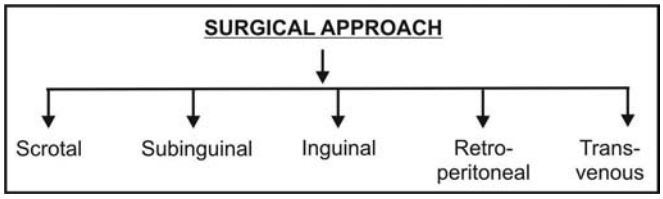


Fig. 9.18: Surgical approach

disconnection of veins, which is actually the exact procedure that is carried out.

To andrologists treating male infertility, the surgical repair of varicocele offers most encouraging results of all the therapeutic approaches for infertile males in terms of restoration of spermatogenetic function of the testes, and more importantly occurrence of partner pregnancy.

SURGICAL TREATMENT

There are controversies in the surgical management of varicocele.[131] The controversy stems from:

1. Whether varicocele should be treated or not?
2. Whether to treat it with medical conservative measures or surgically?
3. When to treat it surgically?
4. Whether there is any role of medical treatment before and after surgery?
5. Which is the choice of surgical approach?

Obviously, there are a few takers for the radical view expressed by Evers et al[121, 122] that varicocele should not be treated, even when there is abnormal semen parameters with incontrovertible evidence of its presence in CDU. Currently, there is little doubt that varicocele is associated with a substantial risk of deteriorating testicular function with time,[7-11] and so its treatment is obviously justified.

The core issue is whether to treat patients with varicocele with medical conservative measures, or surgically. The treatment with fertility drugs is still controversial. A great majority of infertility specialists are of the view that the medical management with the use of estrogen-receptor antagonists does not have any effect on the ultimate improvement in the prognosis of infertile males. Many of them are also not very convinced about the role of nutrients in spermatogenesis (see Medical management in Chapter 11).

But as mentioned earlier, we had encouraging results in some patients and chance of their fathering has improved with the use of combination of estrogen-receptor antagonist and nutrients. We recorded a partner pregnancy rate approximately at 12%.[129] This naturally brings another vexed question to the forefront, whether there is any role of medical treatment before and after surgery? If one accepts that the medical treatment has a role, there is no reason to discontinue the medical treatment with sperm nutrients in the postoperative period as it may continue to further the chance of improving the semen quality.

If the surgical option is decided upon, timing of the operation needs attention. Our criteria is to recommend an early surgery, if the sperm count is less than 20 millions/ml, motility (grades 3 and 4) less than 40% or morphological aberration more than 40%, varicocele is of grade 2° or 3° and the age of female partner is more than 30 years. For all others, initial medical management was recommended.

In many cases, after an adequate trial of not less than six months, surgery was carried out later. The criteria, we set for not advocating surgery for early or 1° varicocele, was based on the observations of many andrologists that infertile men with large varicoceles may have poorer preoperative semen quality, but postoperatively improvement of semen parameters is greater than in smaller varicoceles. Moreover, fertility index increased marginally in men with 2° and significantly in men with 3° varicoceles. Fertility index (motile sperm concentration) is calculated by multiplying sperm concentration by percent motility.[132] Steckel et al [8] reported 128% improvement in third degree (3°) varicocele after operation.

Once the decision for the surgery is taken, one needs to look into the three surgical options—open conventional, laparoscopic and microsurgical methods. Conventional open surgery, which has been practiced for a very long time, can be done through scrotal, inguinal and suprainguinal routes.

Conventional Open Surgery

With several surgical approaches available for treatment of varicocele by conventional open operations, there is a controversy whether to follow the scrotal, transinguinal or suprainguinal route (Fig 9.18). The scrotal approach, where the access is certainly the closest to the site of varicocele, has long been given up, as there is likelihood of encountering numerous small veins, which need to be ligated. These veins being relatively small in calibre, may be missed resulting in residual varicocele, or be injured to cause scrotal haematoma. Moreover, trauma to the testicular blood supply remains a distinct possibility, if the dissection is not meticulous.

Transinguinal route can possibly weaken the inguinal shutter mechanism. Most surgeons now advocate the suprainguinal high or laparoscopic methods, as these methods can achieve most complete ligation of the veins without endangering any other inguinal structures.

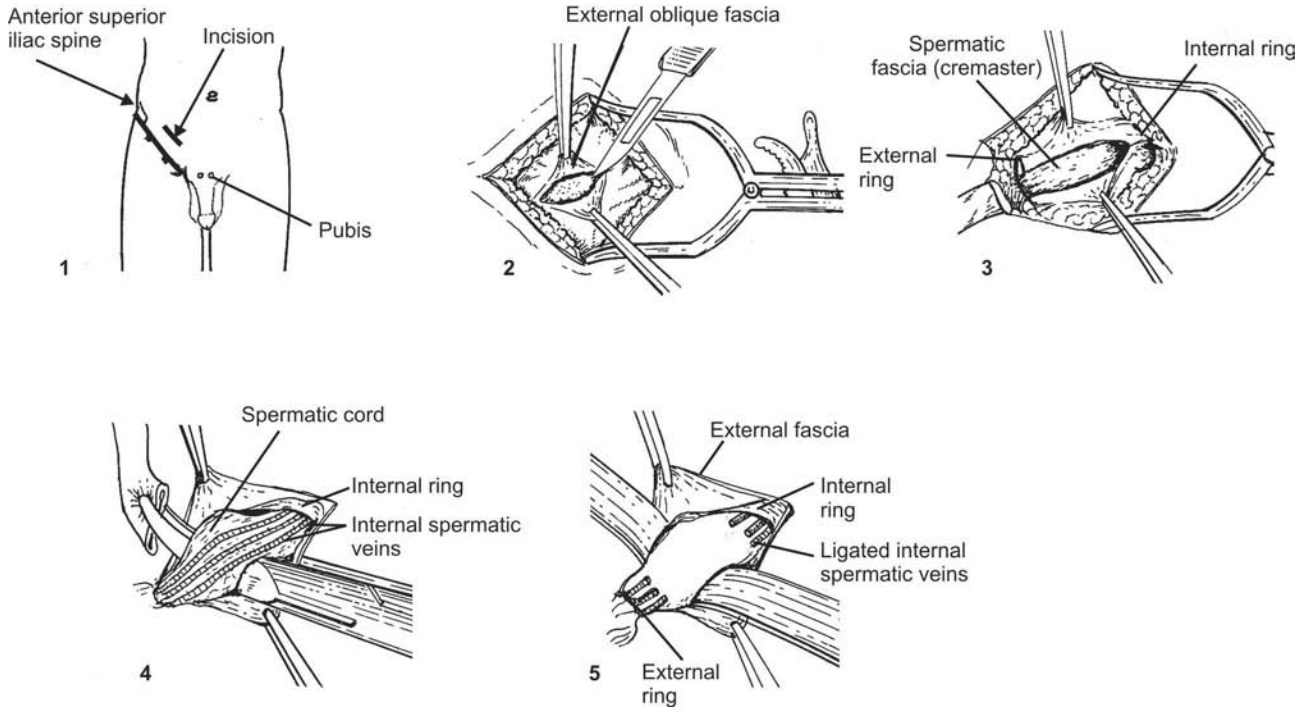


Fig. 9.19: Conventional inguinal approach

Several urologists over the years have used the open surgical approach advocated by Palomo, Ivanissevich or Bernardi.[133-135] Ivanissevich popularised the methods of the inguinal and the suprainguinal or high ligations (essentially a retroperitoneal approach), but Palomo, who published his observation in 1949, pioneered the high ligation technique.[1, 136]

Inguinal Approach

All over the world, the general or regional anaesthesia (spinal or epidural) has been in use for the conventional open surgery. However, Ross and Ruppman[136] advocated local blocks instead of general anaesthesia for the high inguinal ligation. They reported satisfactory results, minimal requirements for postoperative analgesia and rapid return to work as also claimed by the advocates of the laparoscopic method.

Briefly, the procedure involves the identification of the internal or deep inguinal ring at the midpoint between the anterior superior iliac spine and pubic tubercle, and approximately one finger-breadth medial to Poupart's ligament (Fig. 9.19). For local anaesthesia, field block of the area is performed using 0.5% lidocaine. Once adequate anaesthesia has been obtained, a small skin incision is made overlying the internal inguinal ring. The external oblique fascia and external inguinal ring are identified. A small amount lidocaine is injected under the external oblique fascia, which is then incised in the direction of its fibres above down, but the external ring is spared. The ilioinguinal nerve is identified and carefully preserved. The cord is identified and using 25 G needle a small amount of 0.5% lidocaine is injected into its overlying spermatic fascia before opening it. The cord is then gently grasped with special DeBakey forceps and the external spermatic fascia is teased away using a peanut dissector. The portion of the inner spermatic fascia containing the vas deferens and its vessel is identified. A small amount of 0.5% lidocaine is again infiltrated into the internal spermatic fascia away from the vas deferens. This fascia is sharply incised, and using a 'no touch" technique, the vas deferens, its artery and vein are bluntly dissected away from the internal spermatic veins. The peritoneum is seen over the surface of the internal veins. The placement of retractor into the internal inguinal ring aids this dissection. No attempt is made to identify the internal spermatic artery. However, care is taken to preserve the artery and vein of the vas. When the peritoneal reflection is noted on the surface of the veins, a small amount of lidocaine is injected into the spermatic fascia surrounding this group of veins. Veins are

secured with right-angle clamps at the level of reflection. Small portions of the veins are excised and the two clamped ends are ligated with silk. The external oblique fascia is then closed with interrupted sutures followed by the skin closure. The steps for doing it under general anaesthesia are similar, but no infiltration of lidocaine is required.

We have used general anaesthesia in all cases, as with spinal and epidural anaesthesia the patient may have to stay overnight. In the subcontinent, general mental disposition of patients and their natural apprehension and fear about operation may not be congenial for the routine use of local anaesthesia. Most of our patients went home in the evening and certainly next morning. Going back to work was mostly dictated by the mindset of these patients. In our experience, most self-employed men went back to their normal chores within a few days, but those on paid medical leaves, tended to delay their resumption to work. For bilateral cases, perhaps there is less justification to use local anaesthesia, as a large volume of anaesthetic medicine would be required, and the longer operating time would naturally necessitate more intra- and postoperative analgesics. However, I find that one disadvantage of routine high ligation is that accessory veins on the floor of the inguinal canal are likely to be missed (see "anatomical aspect of varicocele" Fig. 9.7).

Retroperitoneal or Extraperitoneal Approach

Retroperitoneal ligation was first described by Palomo in 1949, and has since been advocated by Ivanessivich, Bernardi and others. This is done through a 4.5 cm muscle splitting incision at the level of anterior superior iliac spine and retroperitoneal space is entered into after reflecting the peritoneum medially to identify internal spermatic vessels. Ligation is performed at this level, where usually one or two at the most, internal spermatic veins are encountered. Some prefer to combine with mass ligation of both the artery and the vein, and others the artery sparing method, which is often difficult in adolescents even with assistance from magnification through loupe. Using this approach, Kass and Marcol[137] in 1992 found 11% incidence of persistence of varicocele, but no recurrence if the artery is ligated.

Table 9.12: Types of surgical ligations

1. Internal spermatic veins only.
2. Internal spermatic and external spermatic veins
3. Internal spermatic vein and the artery

Laparoscopic Operation

The laparoscopic surgery uses the same principle of an open operation, i.e. high ligation of the internal spermatic venous system, but uses either the transperitoneal or retroperitoneal (extraperitoneal) route. There is no significant difference between the transperitoneal and extraperitoneal techniques in terms of effectiveness and morbidity. The difficulty in identifying the internal spermatic vein and the additional cost of the balloon dissector for the extraperitoneal technique makes it less favoured method by some operators.[138] Some surgeons have been recommending transinguinal ligation of both the internal and external spermatic (cremasteric) veins. They cite a comparative study showing better surgical results with ligation of both sets of veins instead of ligation of the internal spermatic vein alone.[25]

The testicular artery can either by spared or ligated. No difference in the postoperative improvement of seminal findings was observed between the artery ligating and the artery preserving groups.[139] There was no incidence of testicular atrophy in the study, regardless of whether the testicular artery was ligated or preserved during surgery.[140] Despite the theoretical advantage of artery preservation, Matsuda et al[141] claimed no significant difference between the artery-preserving varicocelectomy and the artery-ligating operation, when improvements in semen quality and postoperative pregnancy rate were evaluated. However, preventing ischaemic injury to testicular vessels is more relevant, when operating on a solitary testis, or in adolescents and young men in whom the operation is performed prophylactically by some andrologists (Table 9.12)

Main advocacy for the laparoscopic method has been less postoperative pain and early return to work. But cost-effectiveness, that is put forward by the surgeons of developed countries, as one of the main arguments in favour of laparoscopic method, may not be tenable in some developing countries like India.

There is also a puzzling debate, whether the ligation of a unilateral varicose spermatic vein should be performed either by open surgery or by laparoscopy. Mandressi et al[142] recorded significant improvement in seminal analysis in both groups, but the laparoscopy costs about 60% more than the open surgery. The cost of equipment and availability of trained surgeons would make this option less favourable in many centres of developing countries. For

the artery-sparing method, Ulker et al found superiority of laparoscopic surgery to other methods.[143]

The majority of laparoscopic cases require general anaesthesia and intubation with its own attendant risks. A Foley catheter and nasal gastric tube must be placed in the patient before the abdominal trochar is pushed into the peritoneal cavity. Notwithstanding these small, but possible risks that are eliminated by an open approach, the optical magnification of a laparoscope certainly enhances ability of the operator to identify and to preserve the testicular artery and lymphatics.

The laparoscopic surgeons claim that repair of bilateral lesions by this method is likely to result in more rapid to return to strenuous activity than even the minimally invasive open techniques. In addition, it offers lower morbidity and allows for microscopic dissection with preservation of the spermatic artery. In addition to its better cosmetic results, it ensures excellent exposure and control of the affected vessels, and a bilateral ligation can be done without a second incision.[144]

However, after weighing the pros and cons, the advocates of open operation[136, 145] contest this claim. They put forward the argument in favour of an open operation, as it is a safe and effective method without the potential for complications associated with laparoscopy. According to them, duration of operation was longer for laparoscopy, when compared with the open method (50 min against 30 min for unilateral cases and 70 min against 55 min for bilateral cases). Moreover, there is not much difference between the two in terms of morbidity, pain, fertility and hospital stay with most of them leaving by the next day, if not the same evening.[136]

Overlooking the usual risks of general anaesthesia and wound problems, a laparoscopic surgery may still encounter common complications such as pneumoscrotum, post-operative hydrocle (5.3%), pain or discomfort associated with insufflation.[146] However, most experienced laparoscopic surgeons acknowledge the risks of inadvertent vascular injury or viscus perforation during insertion and subsequent manoeuvres of the trochar and its with resultant intraabdominal morbidity.

Microsurgery

Microsurgery is the newer method, where under an operating microscope, the surgeon individually ties off the enlarged veins in the spermatic cord. The varicocelectomy is performed by an inguinal (aponeurosis of external oblique opened) or subinguinal (external oblique aponeurosis intact) technique.[147]

The testicular artery and lymphatic ducts can be preserved confidently, because the surgery is done under high magnification. The surgeons, who have perfected the microsurgical treatment of varicocele by subinguinal approach, claimed it to be very safe and reliable procedure, which can be performed even under local anaesthesia. Depending on the preference of the surgeon, the microsurgical operation can be done with or without delivery of testis. Cabone et al[148] favoured ligation of the varicocele without delivery of testis. Goldstein[145] and Du et al[149] preferred delivery of the testis (Table 9.13).

Table 9.13: Microsurgery

1. Transinguinal
2. Subinguinal
a. With testis delivery method
b. Without testis delivery method

Microsurgery is indicated especially for men with prior inguinal surgery or recurrent varicoceles.[136] The protagonists of microsurgery claim to eliminate hydrocele as a complication[123] and low postoperative recurrence rates, as they ligate each vein individually, thus are able to preserve the perivenous and intervenous lymphatics, and the testicular artery. Furthermore, semen parameters and partner pregnancy rates showed improvement with minimal morbidity.[8] The published complication rate for nonmicrosurgical varicocele ligation ranges from 5.4 to 7.2%, with the most common complication being hydrocele formation. In contrast, the overall complication rate was 2.9%, with no patient developing hydrocele in one series.[133] However, like all microsurgical methods, only a few are adept and there is a long learning curve.

Hsieh et al[150] modified the microsurgical method specifically using a ×3.0 loupe for high inguinal varicocelectomy instead of the usual microscope during the spermatic cord dissection at the level of the internal inguinal ring. They found loupe-assisted high inguinal varicocelectomy a safe, simple and effective method. This could put a new dimension to the medical centres of the developing countries without microscopic equipment.

Microsurgery is mostly done by a 2 to 3 cm inguinal incision with delivery of testis as advocated by Goldstein et al.[145] All external spermatic and the gubernacular veins are ligated. The testis is then

returned to the scrotum and the spermatic cord is dissected under operating microscope. The testicular artery, perivenous and intervenous lymphatic channels, and the vas with its arterial supply are precisely identified and preserved. All internal spermatic veins are then identified and ligated with double ligatures or clips.

Although the subinguinal approach in a microsurgical varicocelectomy obviates the need to open the aponeurosis of the external oblique, it excounters with a greater number of internal spermatic veins and arteries compared to the inguinal approach.[147]

Testini et al[151] in their series recorded both immediate and long-term complications such as transient pain (4.7%), ecchymosis (2.0%), transient hydrocele (0.7%), permanent hydrocele (0.7%), palpable recurrence (1.3%), Doppler recurrence (1.3%) and long-term recurrence (0.7%). They did not have any testicular atrophy after operation. Eighty percent (80%) or 120 out of 150 patients showed an improvement of semen analysis. Cyan et al recorded recurrence rate of 2.11% and higher increase in sperm motility.[152]

At present, the subinguinal artery and lymphatic-sparing microsurgical varicocelectomy or subinguinal varicocelectomy by optical magnifying devices represents the gold standard in the varicocele treatment in adults. It minimises relapse, limits postoperative complications, and improves the reproductive potential of sperms. In addition, it is claimed to be cost-effective (certainly in developed countries) and significantly cuts down the operation time.[153, 154]

The subinguinal varicocele repair performed with local anaesthesia is a safe technique offering a quicker recovery period than the laparoscopic approach. The length of time off from work was significantly longer for the laparoscopic patients (6.4 days) as compared with that for the patients undergoing subinguinal varicocele repairs (2.6 days).[155]

There is a general agreement on the desirability treatment for prepubertal varicocele.[156] The best of therapeutic approach for prepubertal or adolescent varicocele, however, is still under discussion, but consensus is in favour of less invasive measures like percutaneous venographic sclerotherapy. Silveri[154] claimed subinguinal microsurgical operation to be very effective and successful in his preliminary experience with paediatric patients. The procedure was successful in all but one patient (2.1%), who showed recurrence of the disease at the time of the first postoperative follow-up. He also used loupe in some cases and the average operative times were 45 and 60 min for loupe and microscopic procedures, respectively. In principle, before recommending any interventional treatment for prepubertal or adolescent varicoceles, the following criteria are to be kept in mind as shown in Table 9.14.

Table 9.14: Indications for intervention in prepubertal and adolescent varicoceles

1.	The results of semen analysis are abnormal,
2.	The volume of the left testis is at least 3 cc less than that of the right,
3.	Above-normal response of either LH or FSH to GnRH stimulation,
4.	Bilaterally palpable varicoceles are detected,
5.	A large symptomatic varicocele is present
6.	Histological picture of Leydig-cell hyperplasia

Percutaneous Venographic Occlusion

Percutaneous transvenous embolisation is an alternative to surgery, and presently it has gained wide acceptance as a primary therapeutic technique in the management of varicocele. However, small veins outside the spermatic cord that are visualised during an open surgical procedure are not easily embolisable with the percutaneous approach. According to some andrologists, this is the method of choice in treating prepubertal or adolescent varicoceles.[156] However, relatively small calibres of the veins in adolescents are more susceptible for potential vascular complications.[157]

Originally, the venographic occlusion sclerotherapy was done through transvenous retrograde route following its earlier use to delineate the venographic anatomy of varicoceles. Percutaneous embolisation should be offered as an alternative to open ligation only in hospitals, where experienced interventional radiologists having expertise in imaging are available. This method is especially indicated for postsurgical recurrent varicoceles (Table 9.15).[158] Retrograde method of embolisation requires a spermatic venography done by placing an angiographic catheter in the testicular vein via transfemoral venous route. Various modifications like use of balloons, coils or sclerosants such as 70 percent glucose are employed for the embolisation.

Table 9.15: Relative merits of different varicocele operations (From Campbells' Urology p-2388)[158]

	Methods	*Hydrocele*	*Recurrence/ failure*
1.	Open (inguinal or subinguinal)	3-9%	15%
2.	Microsurgery (inguinal or subinguinal)	<1%	1-3%
3.	Retroperitoneal (mass ligation)	7.2%	2%
4.	Retroperitoneal (artery-sparing)	<7.2%	11%
5.	Laparoscopic method	3-9%	15%
6.	Embolisation**	Nil	10-25%

**NB. Failure is often due to anatomical and technical factors.

The radiologic balloon occlusion method uses a silicon balloon catheter passed under X-ray guidance into the testicular vein. The balloon is inflated and left in place permanently, thus blocking the engorged veins and repairing the varicocele. However, recurrence may still occur even after embolisation in some patients due to development of high parallel or renal vein collaterals.[159]

As a less invasive means of treatment, the retrograde (interventional) sclerotherapy of varicocele has produced long-term results as favourable as those of open surgical procedures.[160] Nieschlag et al[120] demonstrated that surgical ligation or radiological embolisation of the internal spermatic vein is equally effective in terms of partner pregnancy rates following treatment. Simultaneous microsurgical spermatic vein ligation and sclerotherapy have also been combined for the treatment of recurrent or persistent varicocele.[161] This method combines the advantages of both methods. Precision of the microsurgical technique is combined with quickness of the sclerotherapy.

Tauber[162] introduced antegrade sclerotherapy as an equivalent, but even less invasive treatment modality, which he had used since 1987 to treat patients with varicocele. The antegrade sclerotherapy is competitive to the retrograde embolisation. It has the advantage that the treatment can be performed in all cases, even for a recurrence after an open surgery. The method has proved to be easy to perform, safe, economical and effective, and can be performed with a local anaesthetic through a small scrotal incision. A straight vein merging into the spermatic vein is isolated and cannulated to inject the sclerosant. The whole process in expert hands takes between 12 and 60 min.[163]

Embolisation offers the potential advantage of shorter recovery to full activity as compared to surgical ligation. The sclerotherapy is possible approximately in 80% of the patients. Failures are due to anatomical and technical factors (Table 9.14) .

Possible complications of sclerotherapy are painful induration of the pampiniform plexus and testicular ischaemia. In the subcontinent, the method is yet to achieve its due recognition as the sophisticated imaging facilities and expert radiologists are only available in handful of specialised hospitals.

Postoperative Assessment

The venous reflux is the main parameter for diagnosing varicocele. Consequently, latter's persistence or recurrence is the crux of all postoperative assessment of any interventional treatment. Follow-up should always be done by the clinical examination, semen analysis and Color Doppler ultrasonography (CDU). Immediate and long-term complications after the operations are transient pain, ecchymosis, transient or permanent hydroceles, and persistent or recurrent scrotal varicoceles. Fortunately, this occurs in less than 5 to 10% of patients. There have been reports of unusual complications like epididymitis and injury to the internal spermatic artery.

The hydrocele presumably develops as a result of the lymphatic obstruction due to severance of the lymphatic channels. Ross and Ruppman[136] reported its incidence at 7.3% using a technique that does not attempt to preserve these structures. Some patients develop hydrocele in the postoperative period. Mostly, hydrocele is detected after 6 months and its detection within 6 months of operation is not common. But it is also known to appear even after 3 years. Hydrocele develops more often after bilateral repair regardless of the technique used.[164] Some of these hydroceles may even grow to a size necessitating operative correction. This complication can be obviated to a great extent by the microsurgical technique.

In the subcontinent, occurrence of hydrocele in some parts of India is endemic in any age group. While an urologist should notice a preoperative associated hydrocele, sometimes with such endemic incidence of hydrocele in India, it may make it very difficult to incriminate formation of a delayed hydrocele as a definite sequel to operation on varicocele.

Recurrence of varicocele after operation still remains a distinct possibility. Even in experienced hands, the persistence of varicocele due to residual varicosity can occur, and it may interfere with getting

the desired result. Recurrent varicoceles may appear as late as 76 months after varicocelectomy in patients, while none had been detected at a mean of 27 months after surgery.[165] As discussed earlier, the common cause of recurrence is failure to recognise the anatomical variations or abnormal venous connections. Leaving these channels would not ensure a complete cure of the condition. Possibility of residual varicosity would defeat the very purpose of the operation. Varicoceles detected soon after the operation are most probably the result of missed communications between the internal spermatic and other venous systems or due to left-to-right cross-communicating vessels. Palazzo et al[166] recorded a recurrence rate was 3.1% by postoperative scrotal CDU after 3 months.

Over the years, surgeons advocated various modifications to reduce or to eliminate recurrence or residual varicosity. Tefekli et al[119] used selective internal spermatic venography (SISV) for detecting recurrent venous reflux and radiologically documented much higher recurrence of 22% (32% for surgery and 21% for laparoscopy). It is doubtful whether this rigorous follow-up schedule using invasive procedure like venography can be used in most centres. Certainly, this is beyond the realms of most developing countries. Niedzielski et al[20] used intraoperative venography to reduce the recurrence rate after varicocele repair in adolescence. Overall, the recurrence rate was 2.8% (5 of 177 cases) in the venography group and 11% in controls without venography. Similar effort was made by Palmer et al.[167] Tosato et al[18] advocated simultaneous tying of the external spermatic veins to offset any possible reflux through the external spermatic venous system. Goluboff et al[24] found the presence of accessory veins through loupe magnification of veins on the floor of the inguinal canal travelling posterolateral to the spermatic cord then leaving the spermatic cord before passing through the internal inguinal ring. Similarly, we made modification in the operative procedure in routine high ligation to enable us to have a look into the floor of the inguinal canal to tie the accessory veins, which existed in 7 to 8% patients (Table 9.2 and Fig. 9.7).

Results of Treatment

Two criteria that are normally used to assess the results are improvement of semen qualities and more importantly pregnancy rate (PR) of the female partner. Surgery significantly increases sperm values in most men within 4 months, the highest values being obtained at 8 and 12 months.[153] However, even normal characteristics in semen cannot guarantee pregnancy, which is also dependent on the variable female factor (see Chapter 6). While it is clear that the semen analysis can be improved by the treatment[168] especially if applied in adolescence or early in the man's reproductive life, improvement in the PR remains uncertain. This appears to suggest that the age and fertility status of the female partner might be a crucial element in all studies. So, it is important to observe all treated patients until they complete their families. Improvement of the semen qualities certainly better the probability or chance of pregnancy in the female partner.

Many workers in this field have confirmed improvement of semen parameters and partner PR after correction of varicocele. The percentage improvement in sperm count in subclinical varicoceles is not statistically different from the improvement in clinical varicoceles.[75] The ratio of improvement in semen analysis and the PR of female partner after treatment of varicocele have been reported in the range of 53 to 92% and 20 to 58%, respectively.[8,169,170] Some of the poor results of varicocele treatment reported in some studies can only be explained by inadequate techniques.[21] Treatment of varicocele also appears to improve pregnancy and live birth rates among couples undergoing intrauterine insemination for male factor infertility (Table 9.16).[171]

Steckel et al[8] reported PR two years after surgery at roughly 40% for grade 1, 46% for grade 2 and 37% for grade 3 varicoceles with overall figure of 42%. Steckel et al, Tinga et al and Scott et al[8,106,132] all confirmed that larger the varicocele, more significant is the improvement after operation. In my series PR of 44.8% was recorded, which is comparable to other researchers' results (Tables 9.16 and 9.17). PR after varicocelectomy is reported to be between 20 and 60 percent with most series averaging about 40 percent.

Steckel et al[8] calculated the *fertility index* by multiplying sperm concentration by percent motility and reported that while infertile men with large or grade III varicoceles may have poorer semen characteristics, they show greater improvement after operation. If the increase in the temperature gradient between the testes and the body is presupposed as the prime factor in the pathogenesis, there should be expectedly less damage in grade I or early varicoceles,

Table 9.16: Partner pregnancy rate after operation and venous occlusion

1.	Kibar et al, 2002	— 20%
2.	Perimenis et al, 2001	— 46.6% (Nonoperated—12.9%).
3.	Cavallaro et al, 2001	— 58%
4.	Parikh et al, 1996	— 50%
5.	Mellinger et al, 1995	— 32%
6.	Zuckerman et al, 1994	— 57.6%
7.	Dewire et al, 1994	— 39%
8.	Hirokawa et al, 1993	— 55.2%
9.	Steckel et al, 1993	— 42%
10.	Rageth et al, 1992	— 42%
11.	Gonda et al, 1987	— 40%
12.	Shuman et al, 1986	— 48% (Balloon-embolotherapy)
13.	Zepnick et al, 1986	— 49%
14.	Buch & Cromie, 1985	— 55% (Nonoperated—7%)
15.	Riedl et al, 1985	— 47.7% (Sclerotherapy)
16.	Cocket et al, 1979	— 25% (Nonsurgical group—12%)
17.	Dubin & Amelar, 1977	— 53%
18.	Present series, 2002	— 44.8%

and poorer semen parameters in advanced or grade III varicoceles. So, greater improvement of semen in these patients with grade III compared to early or grade I varicocele after varicocele operation appears paradoxical. One possible explanation is that improvement is more noticeable in grade III patients as the difference from the normal is more in larger varicoceles, while it is less in smaller varicoceles. So improvement appears more dramatic. In formulating our treatment schedule, we have tried nonsurgical treatment initially in these cases with early or grade I varicoceles.

Table 9.17: Varicocele (surgical treatment) (January 1990-August 2002)

Total no of patients	= 2178;
Varicocele incidence	= 936 = 42.9%.
Varicocele operated	= 522 (162 had initial medical treatment).
Incidence of pregnancy	= 234 (44.8%).
Incidence of pregnancy in previously medically treated 162 patients	= 80 (49.3%)

Prognostic Factors in Assessing Results

There has been a lot of discussion on the predictor and prognostic factors for the success of various modalities of treatment for varicocele (Table 9.18). There are some conflicting statements by various research workers and andrologists. According to some authors, preoperative semen characteristics had no prognostic value for the postoperative fertility, as there is no correlation between preoperative sizes of varicocele to postoperative findings[172, 173] in semen analysis, although sperm motility is the most important factor in semen quality.[53, 103]

Yet others have found some correlation in the preoperative semen analysis, grade of the varicocele and the postoperative results. Matkov reported that men with mild to moderate oligoasthenospermia (total motile sperms = TM >5 million) had significantly better seminal improvement following varicocelectomy.[173] Fujisawa et al[174] found that:

1. Small testes, or a grade III varicocele decreased the likelihood of improvement.
2. Patients with a sperm count of 10-20 million were significantly more likely to respond to varicocelectomy than those with sperm counts <5 millions/ml.
3. Patients with elevated FSH were less likely to respond, as were those with a Johnsen score below 6.

Table 9.18: Prognostic factors in assessing results

1.	Preoperative sperm count.
2.	Preoperative testicular size.
3.	Grade of the varicocele.
4.	US findings of internal spermatic vein diameter.
5.	FSH and GnRH levels.
6.	Histological changes

The best ultrasonographic cutoff to predict a positive outcome after subclinical varicocelectomy is venous diameter greater than 2 mm. Patients with larger clinical varicoceles have greater post-operative seminal improvement than those with small or subclinical varicoceles regardless of the baseline sperm count.[72,175]

The diameter of internal spermatic veins measured directly during operation correlated well with clinical grade. It is the diameter of internal spermatic vein and the patient's age at operation, and not the clinical grade, that determine the reversibility of testicular changes following varicocelectomy.[112]

Pontonnier et al[176] found the defects of maturation in the histological examination of testes corresponding to reversible lesions in 36% of cases. They contend that these cases have a very good chance of postoperative improvement with restoration of spermatogenesis. This improvement is more marked, when the lesions of the basement membrane and interstitial region are less severe. The histological lesions predominantly involved the tubules(66%)

followed by the basement membrane (52%) and the interstitium (42%) in their series.

Segenreich et al[177] and Foppiani et al[178] have recommended that a positive preoperative GnRH test (increased gonadotrophin response particularly the FSH to GnRH) is a good predictor of improvement in semen parameters and pregnancy after varicocele surgery. They suggest that the GnRH test can serve as an additional indicator for varicocelectomy.

It is also worth noting that improvement of semen analysis and occurrence of pregnancy start after a lag period of 3 to 6 months. This minimum lag period of three months can be explained by the fact the average period of sperm maturation is 80 days and the effects of interventional treatment is expected to take effect after this period. Maximum incidence of pregnancy mostly occurs between 12 and 24 months. This was also observed in our series till June 1994 (results presented in the World Congress of Surgeons, International College of Surgeons at London, UK). Even after eight years (2002 series), the similar trend was observed in our series (Table 9.19 and Fig. 9.20). Similar results have been seen in the series of Takihera et al[14] in 1991. Cleveland Clinic Foundation[179] and Comhaire et al[180] also had similar observations that the results of varicocele in terms of pregnancy are achieved mostly after six months with progressive rise till 24 months after operation. Cavallini et al[168] observed that the improvement of sperm parameters mostly starts after 4 months and that it is most pronounced between 8 and 12 months after surgery.

Table 9.19: Comparison of number of partner pregnancies after varicocele operation

Months	*6*	*12*	*18*	*24*	*30*	*36*
1994 series	5	12	15	24	3	2
2002 series	11	28	63	81	39	12

There is a still a debate about the timing of treatment for adolescent varicocele. Currently, one should recommend varicocele repair for adolescents, if the criteria shown in Table 9.14 are fulfilled:[80, 95, 156]

CONCLUSION

Varicocele is certainly one of the common accompaniments of male infertility and overwhelming evidence from the works of researchers proves its causal relation. It seems to induce a number of changes in the testicular microenvironment with alterations in temperature, haemodynamics, reactive oxidative species and antioxidant concentrations. However, despite current knowledge of the pathophysiology of varicocele-associated male infertility, the precise mechanism involved in the impairment of fertility by varicoceles still remains elusive.[181]

Most workers consider temperature gradient very important in the pathophysiology of varicocele. It is considered as a progressive lesion, and has deleterious effects on the testicular volume and semen quality. The changes in the seminal parameters are more evident in the secondary infertility caused by varicoceles. While clinical examination and semen analysis are important in initial assessment, the Color Doppler Ultrasonography (CDU) is the most effective

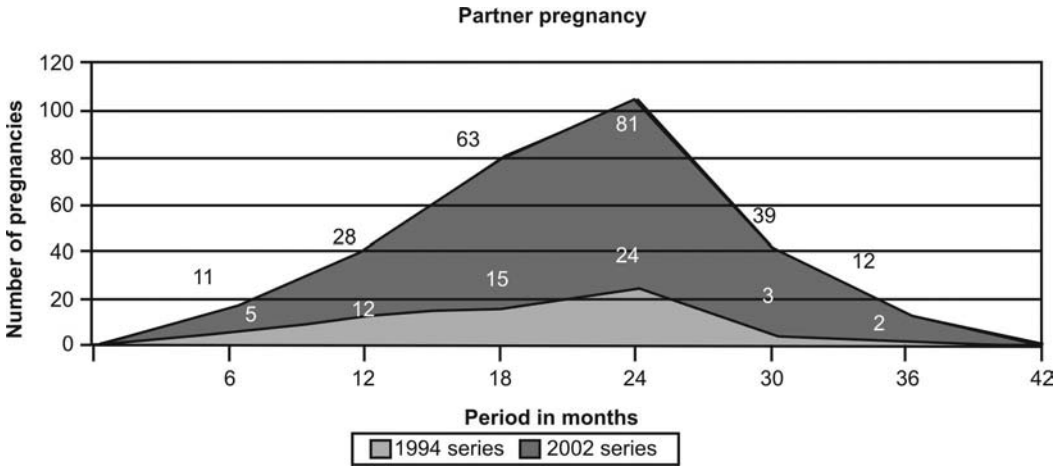


Fig. 9.20: Occurrence of partner pregnancy

diagnostic tool for confirming the diagnosis, postoperative recurrence, surgical complications and associated conditions. However, no method alone is adequate for overall assessment.[182] Current investigative modalities such as analysis of semen, testicular measurement, serum gonadotrophin and testosterone levels, gonadotrophin-releasing hormone (GnRH) stimulation test, CDU and testicular biopsy may all be needed to detect early changes in testicular physiology produced by a varicocele.

Review of current data published by various workers suggest that an individual with varicocele, even with a previously normal semen analysis or documentation of previous fertility, is at risk of subsequent loss of testicular function and fertility.[89] But a section of non-urologist infertility specialists continue to be sceptic of the role of the interventional treatment for varicocele in male infertility. They argue that the results of surgery for varicocele with male infertility remain unpredictable, even with improvement of surgical skill and the availability of sophisticated equipments like laparoscope and operating microscope. Non-surgical treatment with medicines only has few takers, but we still advocate this measure with moderate success.

However, overwhelming numbers of andrologists agree that varicocele should be treated with some sort of interventional measures, and there is also a general agreement that prepubertal or adolescent varicocele should be attended to as its progression have a changing effect on the testes. It is still a matter of individual opinion, whether the correction of the subclinical varicocele is helpful or not.

Consensus still eludes regarding indications or timing of the interventional approach (laparoscopic, microsurgical, open operations or sclerotherapy). For a unilateral varicocele, conventional open surgery is probably preferred to laparoscopic surgery, as it is less costly and significant postoperative improvement in semen analysis is expected.[142]

Anatomical variations contribute to the occurrence of a residual varicocele, which remains a distinct possibility irrespective of the method used. Most surgical recurrences are due to low (inguinal) parallel collaterals, while the majority of post-balloon occlusion recurrences are due to either high retroperitoneal parallel or renal vein collaterals.[183] Formation of hydrocele may occur as sequel to the varicocele operation due to tying of the lymphatics, which can be eliminated or reduced considerably by the use of microsurgery.

Although microsurgery is a great advancement, but lack of trained surgeons and the cost of the equipment, may limit its universal use in the Indian subcontinent and most developing countries. Venous sclerotherapy or embolotherapy, especially the antegrade method, certainly holds great advantage over others. Advent of microsurgery and sclerotherapy may have pushed down the importance of the laparoscopic and open surgeries. If considered in the context of the subcontinent, open surgery is still the method most used, as surgeons can even be trained to do it under local anaesthesia to cut down the cost and the hospital stay.

There is an argument of using laparoscope in bilateral cases, but the cost of equipment and the long training curve of a laparoscopic surgeon could still sway the balance in favour of an open operation in developing countries like India. Moreover, an open operation can easily be extended to ligate the abnormal venous channels in the inguinal canal.

Where experienced interventional radiologists are available, sclerotherapy or embolotherapy can be performed on an outpatient basis. In view of its rapid recovery time, long-term success and complication rates comparable to, and often less than those with other methods, the sclerotherapy should be the treatment modality of choice for all varicoceles in near future. It is especially indicated for the prepubertal and adolescent varicoceles, and for the recurrent or residual cases following conventional surgical treatment.[184]

Improvement of semen parameters and partner pregnancy are two important goals in the treatment of varicoceles. Most workers have been able to achieve improvement of semen parameters. Even if IUI (Intrauterine insemination) is used as an option for achieving pregnancy, prior treatment of varicocele improves partner pregnancy and live birth rates.[171] However, ensuring the anatomical ligation of the internal spermatic vein should not solely be relied upon to get the best result, as the partner pregnancy is also dependent of female factor. Consequently, it may not be achieved in many patients. The positive results of varicocele operation in terms of partner pregnancy are perceived mostly after six months with progressive rise till about two years.

Presently, there is no foolproof method to identify the predictor factors for the success of various modalities of treatment for varicocele in spite of

claims of a few andrologists regarding GnRH test. But most andrologists agree that patients with larger clinical varicoceles have greater chance of post-operative seminal improvement than those with small or subclinical varicoceles regardless of baseline sperm count. Poor results of varicocele treatment reported in some studies can only be explained by imperfect technique, and more importantly inadequate knowledge of the anatomy resulting in incomplete interruption of reflux in the internal spermatic vein.[21]

To summarise the salient features, it can be concluded:

1. Surgery has a definite role for primary and secondary male infertility caused by varicocele, notwithstanding the controversies in its indications and capriciousness of its results.
2. Surgery for varicocele still gives the most encouraging results in terms of restoration of spermatogenic function of the testes, and more importantly, occurrence of partner pregnancy.
3. Microsurgical and venous occlusive methods have gained considerable grounds in recent years, and are the methods for future, but conventional surgical approach could continue to be used for its cost-effectiveness in most centres of the developing countries.

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CHAPTER 10 Other Causes of Male Infertility

INTRODUCTION

The varicocele maintains a high profile amongst the lesions for its relatively satisfying outcome in terms of treatment and its prognosis, but many other diseased conditions need to be focused in a holistic assessment of male infertility. Treatment of these groups of congenital and acquired lesions of male infertility often poses most challenging and at times not a feasible scenario. Causes of these lesions are shown in Tables 10.1 and 10.2.

Table 10.1: Congenital causes

1. Congenital bilateral anorchia (absent testes).
2. Cryptorchidism.
3. Idiopathic non-obstructive azoospermia or oligospermia.
4. Obstructive lesions.
5. Congenital ejaculatory dysfunctions
6. Chromosome abnormalities

Table 10.2: Acquired causes

1. Infections of genital tract.
2. Testicular atrophy—trauma, torsion, tumours, chemotherapy and radiation.
3. Acquired obstructive lesions.
4. Iatrogenic trauma.
5. Acquired ejaculatory dysfunctions.
6. Miscellaneous—Environmental causes, Peyronie's disease and bicycle injuries.

CONGENITAL CAUSES OF INFERTILITY

Congenital abnormalities account for approximately 2 percent of male fertility problems.[1]

Congenital Bilateral Anorchia (Anorchidism or Absent Testes)

Congenital bilateral anorchia (absent testes) is a rare disease having an incidence of 1 in 20,000 male births.[2,3] Since the disease is characterised by male differentiation but with absence of testes, this disease is presumed to be due to atrophy of the testicular tissue after 12 weeks of the foetal life. Patients will present at birth with nonpalpable testes and sexual immaturity later in life, because of absence of the testicular androgens. The karyotype is normal, but LH and FSH levels are elevated and testosterone is extremely low.

The probable cause is complete disappearance of the testis following damage to the testicular blood supply caused by intrauterine infection, trauma or torsion. However, the testicular tissue must have been present in the first trimester of foetal life for the differentiation of the external genitalia to the male lines and development of the male reproductive ducts. These patients have eunuchoid proportions, but no gynaecomastia.

It is differentiated from bilateral cryptorchidism by intrinsic low androgen level, which does not change to stimulation with administration of FSH. Treatment with androgen replacement starting at or near puberty should be continued for life to ensure pubertal growth and to prevent osteoporosis. Unilateral anorchia has been recorded, but it often escapes detection, as it does not affect the fertility potential or androgen production.

Congenital Absence of Vas Deferens

Congenital absence of the vas deferens (CAVD) is a syndrome, whereby a portion or all of the reproductive ducts (including the epididymis, vas, and seminal vesicle) are missing. Consequently, the sperms produced in the testes are trapped or obstructed. CAVD is associated with diseases like cystic fibrosis (CF) and renal malformation. It is imperative that all men with CAVD and/or their wives have genetic screening for CF gene mutations prior to undergoing *in vitro* fertilisation.[4]

Cryptorchidism

Failure of testicular descent (cryptorchidism) is one of the most frequent congenital malformations affecting 1 to 3% of newborn boys.[5] It is conjectured that excessive maternal estrogen during foetal life inhibits FSH production from the foetal pituitary by negative feedback mechanism, thus reducing the Sertoli cell population. MIS factor (an important component in testicular descent) that is secreted from Sertoli cells (see Chapter 2) is therefore reduced.

Cryptorchidism may be part of a broader testicular dysgenesis syndrome, wherein a disturbance in steroid hormone metabolism possibly through a perturbed hypothalamic-pituitary-gonadal axes, could be involved.[6] Orchidopexy may restore the anatomy, but reduced fertility potential still remains a possibility even in a unilateral cryptorchid patient due to structural dysgenesis. There is a 20 percent increased incidence of cancer in the testis (normal risk for testicular cancer is 8 in 100,000 men).

Oligospermia is often associated with unilateral undescended testis, whereas uncorrected bilateral cryptorchidism usually causes azoospermia. Increased temperature within the abdomen has a probable inhibitory effect on the enzymes and proteins that are responsible for the normal sperm production. The clinical consequence of this abnormality is infertility in adulthood and a significantly increased risk of testicular malignancy. High maternal estrogenic factor in foetal life could also be an incriminating factor to predispose to increased incidence of malignant lesions in an undescended testis.

Statistically, it is common in the first-born and 'small for date' babies (pre-term birth and low birth weight), and in maternal toxaemia. In a large series of 10,730 neonates born in the period 1978-1997, Ghirri et al found that the rate of cryptorchidism in the pre-terms is almost ten times higher than that in the full-terms (30.1 and 3.4%, respectively). They recorded late spontaneous descent in 75.7%.[7]

It is also important to undertake the surgical procedure before the age five, if not earlier, to preserve the normal fertility potential and to prevent permanent damage to the seminiferous tubules. Since some testes descend during the first year, making a diagnosis before one year of age is probably not fully justified.

Idiopathic Nonobstructive Azoospermia or Oligospermia

Recent research has identified the genes that control sperm production to the Y chromosome and even small mutations or deletions in any of these genes can cause problems with fertility. Ten to 13% of men with absent sperm count are be found to have a mutation on one of these genes. If a man with a Y chromosome deletion has a male child through intracytoplasmic sperm injection (ICSI), then the child will also have this same type of problem.[4]

A certain number of patients with azoospermia or severe oligospermia present with below normal sized testes (i.e. below 15 cc) and high FSH without any other demonstrable causes like varicocele or other diseases of the gonads. Histology in these cases shows features of 'Sertoli cell only' or varying degrees of 'maturation arrest' (often at the level of spermatocyte). Maternal estrogenic excess in foetal life is similarly blamed for its causation. Diagnosis is only confirmed by an electron microscope showing deletion of AZF (azoospermic factor) of the gene at the long arm of the Y chromosome (see later in the chapter).

Obstructive Lesions

Obstructive lesions almost invariably cause defects in sperm transport and occur at various levels from epididymis to ejaculatory ducts.

1. *Epididymis:* obstruction at the epididymis is mostly acquired due to repeated infective process ending in cicatrisation. It can also be congenital in origin involving any of its segments (caput, body or cauda). The chance of success of treatment improves with reparative bypass surgery or micromanipulation techniques (see-Chapters 12 and 13), if the testicular function is normal.
2. *Vas deferens:* Agenesis of vas is common with men with cystic fibrosis (CF). Characteristic features are normal testes with normal FSH level, but the

ejaculate volume and the fructose level are low. If there is concomitant seminal vesicular agenesis, seminal fructose may be absent. Although the IVF and various micromanipulation techniques are achieving increasing success, the testing for CF mutation gene should always precede IVF to its prevent transmission to the offspring.

3. *Seminal vesicle:* Seminal vesicular agenesis may be bilateral or unilateral. A unilateral case would not affect fertility, thus may not be even diagnosed. But in bilateral cases, low ejaculate volume usually of less than 1 ml and the absence of fructose are diagnostic features. Transrectal ultrasonography (TRUS) and magnetic resonance imaging (MRI) with endorectal coil are now used for the confirmation of diagnosis, and have replaced older method of vesiculography (see Chapter 8).
4. *Immotile cilia syndrome:* This is an autosomal recessive disease occurring in 1 in 20,000 births.[8] This disorder of ciliary dysfunction leads to chronic sinus and pulmonary disease manifested with chronic sinusitis, otitis media, nasal polyposis, and ultimately bronchiectasis. The term *Kartagener's syndrome* applies to this syndrome, when accompanied by infertility and dextrocardia or *situs inversus*. The more common types of ciliary dysmotility syndromes are characterised by structural abnormalities such as missing dynein arms, inner sheath, radial spokes or nexin links in electron microscopic study.[9] Autosomal recessive inheritance has been proved in primary ciliary dyskinesia, but dominant new mutations cannot as yet be excluded in sporadic cases .[10] Treatment of this condition has seen a sea change with the advent of ICSI (see Chapter 13).
5. *Ejaculatory dysfunctions* are discussed later with acquired lesions.

SEX CHROMOSOME ABNORMALITIES

Sex chromosome abnormalities are one of the most important causes of congenital lesions causing male reproductive dysfunction. An outline of abnormalities in chromosome and its genetic aspect is discussed below.

Outline of Chromosome Abnormalities

(See Appendix at the end of the chapter)

Human beings have cells with 46 chromosomes, two sex chromosomes X and Y, and 22 pairs of non-sex chromosomes. In 22 pairs, both members are essentially identical, one deriving from the individual's mother, the other from the father. These pairs are known as *autosomes*. The 23rd pair is different and known as the *sex chromosomes*. In females, this pair has two like chromosomes both called"X". In males, it comprises of two very dissimilar chromosomes, one 'X' and the other 'Y'. It is these chromosomal differences that determine the sex of an individual.

During the production of sperm and eggs (gametes), the paired chromosomes separate, so that each gamete ends up with only one member of each chromosome pair. However, before separation occurs, the paired autosomes swap pieces of their DNA with each other. In women, this exchange process also takes place between the two X chromosomes, but in men, unmatched X and Y-chromosomes do not exchange DNA except at the end parts of the two chromosomes known as the *pseudoautosomal* regions.

Fertilisation restores the chromosomes to their normal paired condition. Thus, a Y sperm fertilising the X egg produces an XY zygote (cell produced by union of two gametes), which develops as male. On the contrary, fertilisation between the X sperm and X egg gives rise to a female XX zygote. Males, thus have karyotype 44,XY and females 44,XX. Since every male must possess a Y chromosome, which can be inherited only from his father, a man's Y chromosome represents a unique record of his paternal inheritance. There is another form of DNA, which follows the female line of inheritance. Outside the nucleus, DNA contained in the energy-producing mitochondria is inherited only through the female line (the sperm's mitochondria is discarded in fertilisation), providing its own unique record of female inheritance.

Each chromosome is comprised of two long DNA molecules in combination with chromosomal proteins. Most genes carry information, which is necessary to synthesise the protein. The pairs of autosomal chromosomes (one from mother and other from father) carry basically the same information or the same genes, but there may be slight variations in the DNA sequence of nucleotide bases in each gene. The information contained in the nucleotide sequence of a gene is transcribed to messenger RNA (mRNA) by enzymes in the cell's nucleus and then translated to a protein in the cytoplasm. These proteins form the structural constituent of a given tissue (Fig. 10.1).

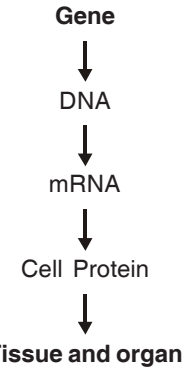


Fig. 10.1: Developmental basis of tissues and organs

The genetic information of a cell determines the sequence of all proteins produced and indirectly that of complex carbohydrates, lipids and polysaccharides, which these proteins enzymatically synthesise in the cell. The sequence of the amino acids of each protein is encoded by a sequence of many thousand nucleotides, which constitute genes. Estimated number of genes in a single cell is thought to be 50,000-100,000 (although the recent research has reduced the number to 30000 to 40000). DNA nucleotide polymer carrying all genetic materials comprises of nucleotides with four bases-*adenine, guanine, cytosine* and *thymine*.

If a gene is abnormal, it may code for an abnormal protein or for an insufficient amount of a normal protein necessary for the tissue. Since the autosomal chromosomes are paired, there are two copies of each gene. In a recessive disease, one of these genes is defective, but the others may code for sufficient protein so that the abnormality is not clinically apparent. If one abnormal gene somehow produces disease, it is called a dominant hereditary disorder. In case of a dominant disorder, if one abnormal gene is inherited from either parent, the offspring will show the disease. In case of a recessive disease, if one abnormal gene is inherited, the child will not show clinical disease, but will pass the abnormal gene to 50% (on average) of the offspring.

A person with one abnormal gene is termed *heterozygous* for that gene. If a child receives an abnormal recessive disease gene from both parents, the disease will show up in the child, who will be *homozygous* for that gene. If two parents are each heterozygous for a particular recessive disease gene, then approximately 25% of their children will be homozygous for that gene with manifestation of the disease. When one parent is homozygous and the other heterozygous, 50% of their children will be homozygous. Almost all diseases have a genetic component with varying importance. The genetic disorders, where genetics play an important role, could have single gene defects, chromosomal disorders or multifactorial causes. Single-gene defects are also called *Mendelian* disorders. In chromosomal disorders, the defect is due not to a single gene, but to an excess or deficiency of the genes contained in a whole chromosome or chromosome segment.[11]

The important role of genetic abnormalities is increasingly being recognised in the causation of human male infertility from impaired spermatogenesis, defective sperm function, and defects in delivery of sperm. The genetics plays a major role in providing a normal hormonal milieu, development of the testis and ductal system, and in control of the stepwise maturation of sperm in the testis. The Y chromosome plays a key role in the testis determination and control of the spermatogenesis.[12]

Several somatic chromosomal abnormalities are associated with male infertility. In a study of 1,263 barren couples, it was found that the overall incidence of male chromosome abnormalities was 6.2%.[2] Over 150 genes have been shown to be associated with infertility in mouse models, although translation of these findings to human male infertility has been slow.[13] It is now incumbent on the andrologist, who evaluates and treats infertile couples, to have a working knowledge of these disorders. Chromosomal studies for autosomal and sex chromosomal abnormalities should be considered in men with severe oligospermia or azoospermia. Moreover, an understanding of the genetic basis of male infertility allows for the appropriate counselling of patients about treatment options and risks to their potential offspring.

The identification of genes linked to disorders in spermatogenesis and male sexual differentiation has increased exponentially in the past decade. The genetic defects leading to male factor infertility can now be explained at the molecular level using the 'Recombinant DNA technology'. Consequently, the etiology of many previously classified idiopathic cases can now be explained to facilitate the treatment of infertile men.[14] Nevertheless, complete knowledge and understanding of this subject still eludes us, especially why in many cases normal development fails partially or completely.

At fertilisation, the genome of the sperm and the egg fuse to start the development of an individual embryo. For the rest of the life, the genetic information will remain unchanged and will be passed on

through cell divisions to form tissues and organs of the particular individual.

The chromosome pairs responsible for the sex determination event in human being during embryonic development have been named arbitrarily as "X" and "Y". They are very different in size and gene content. The X is large and bears thousands of genes, while the Y is small and heterochromatic, and carries only a few genes in addition to the testis-determining factor.

An important difference in the maternal and paternal contribution to the genomes of foetus is the result of spermatozoa carrying either the X or Y-chromosomes. As the female ovum always contributes the X, a male (XY) is born when a sperm carrying Y fertilises the oocyte. A female (XX) is conceived when the X-carrying sperm is involved in the process. It is thus the sperm or the male that decides the gender of the embryo. It is also important to note that the sex of the unborn is determined precisely at the time of fertilisation, although it is really declared at birth.

Our knowledge of sex-determining genes has crystallised in the last one and a half decades. In 1990, the SRY (*sex-determining region Y*) gene was isolated and it is expressed in the genital ridge of the coelome from where the testis originates. In recent years, it has become obvious that some of the crucial genes expressed during male germ cell differentiation exist on the long arm of Y-chromosomes (Yq).

The most frequent pathogenic causes of male infertility are Y-chromosomal microdeletions (8-15%) in the long arm of the Y chromosome, where the loss of specific DNA segments leads to the loss of vital genes for the sperm production.[15] Approximately 7% of infertile men harbour submicroscopic deletions of the Y chromosome that are not detectable on routine karyotype.[16]

The sex-determining region of the human Y chromosome contains a gene ZFY, (*zinc-finger protein*). ZFY may also prove to be the testis-determining factor.[17] There is a closely related gene, ZFX in human X chromosome. Male infertility can be traced either to the loss of a large chunk of the male Y chromosome or to some other large chromosomal abnormality like the presence of a second X chromosome. Impact of a mutation on infertility can be hidden depending on the identity of other genes present in an individual.

Microdeletions of the long arm of the Y chromosome do not always appear to adversely affect the fertilisation and pregnancy can still occur. But the male offsprings from these pregnancies usually inherit similar Y deletion from their fathers. Large microdeletions most certainly occur *de novo*, but such individuals can still produce children in 4% cases, even with severe oligospermia or with azoospermia with sperm retrieval (see Chapter 13). On the contrary, smaller microdeletions are due to mutation and likely to cause sperm count in the region of 2 million per ml.[18]

The chromosomal abnormalities incriminated to the causation of the various reproductive dysfunctions in males, are either *numerical, structural or both.*

NUMERICAL CHROMOSOMAL ABNORMALITY

Klinefelter's Syndrome

Klinefelter's syndrome is perhaps the best known of the genetic disorders that cause infertility in men. The incidence of this syndrome is about 1:500 males live births (0.1% according to others). Patients with this condition have an extra "X" chromosome. This produces the genetic signature "XXY" and represents a total of 47 chromosomes within each cell. In its commonest form it has 47,XXY chromosomal abnormality and is characterised by hypogonadism, gynaecomastia, and azoospermia.[2]

It occurs due to meiotic nondisjunction causing the presence of an extra X chromosome in the male. About 50 to 60% of cases are due to *maternal nondisjunction* with 75% of them due to meiosis-I errors in the elderly maternal age group. The remaining cases are due to *paternal nondisjunction*.

Various karyotypes are known, most common being 47,XXY (about 80-90% of all cases). The extra X chromosome material affects all major areas of development, including expressive and receptive language and coordination. Each supernumerary X chromosome approximately accounts for 15-point reduction of the intelligence quotient (IQ), but conclusions about reduced mental capacity must be drawn cautiously.

Characteristically, these individuals have small, firm testes, delayed sexual maturation, azoospermia and gynaecomastia. In adolescent boys, Klinefelter's syndrome may create distinguishing physical features, such as small firm testes, gynaecomastia and sparse growth of hairs in face, chest and pubic regions.

Incomplete masculine body builds results in most young men with Klinefelter's syndrome not having coordinated muscle action, even if they are tall. Psychological, social and learning problems are common in this group, as is mental retardation. Other associated conditions include glucose intolerance and varicose veins in the legs. They may exhibit incomplete development of the scrotum or penis. As the features of hypogonadism are not evident until puberty, the diagnosis is Klinefelter's syndrome is often made after puberty in the subcontinent, where access to the chromosome studies are still limited to very few urban centres.

Klinefelter's syndrome typically results in infertility due to the decrease in testicular mass from sclerosis and hyalinisation of the seminiferous tubules (see also Chapter 2). Although sexual function may be normal, sperms are not produced leading to azoospermia. The testes characteristically have a length of less than 3 cm and the volume less than 12 cc. The LH and FSH levels are elevated. The testosterone levels can range from normal to low and decrease with age. The serum estradiol levels are often increased. The higher estrogen levels relative to testosterone causes the feminised appearance with gynaecomastia. Mild mental deficiency and restrictive pulmonary disease occur more frequently in these patients than in the general population. Although most adult men with Klinefelter's syndrome may have adequate erection and ejaculation, many have erectile dysfunction (ED) with diminished libido or low sex drive.

Early identification and anticipatory guidance are extremely helpful, although the syndrome rarely is diagnosed in prepubertal males. The treatment addresses three major facets of the disease: hypogonadism, gynaecomastia, and psychosocial problems. Sex hormone therapy may be very beneficial for prepubescent boys, especially if the testosterone levels are low. The hormone therapy is generally recommended to ensure optimal development of secondary sexual characteristics such as growth of pubic and facial hairs, normal size of the penis and scrotum, deepening of the voice, and increased muscular size and strength. The synthetic testosterone in the form of intramuscular injections, oral or buccal preparations, or transdermal (skin) patches can be used. Unfortunately, the infertility is irreversible; and later in life, most of these men will require androgen replacement therapy for optimal virilisation and normal sexual function.

Klinefelter's Variants

About 10% of these patients have chromosomal mosaicism.[2] Other variant karyotypes, including 48,XXYY, 48,XXXY, 49,XXXYY, and 49,XXXXY, are rare. Patients with chromosomal "mosaics" (XXY/XY) have less severe forms of Klinefelter's syndrome and may be fertile, since a normal ("XY") group of sperm-producing seminiferous tubules may exist within the testes. Skeletal abnormalities are more common among men with multiple X chromosomes.

a. *48,XXYY variant:* Patients typically have mild mental retardation, tall stature, eunuchoid body habitus, sparse body hair, gynaecomastia, long thin legs, hypergonadotrophic hypogonadism, and small testes.
b. *48,XXXY variant:* Patients typically have mild-to-moderate mental retardation, speech delay, slow motor development, poor coordination, immature behaviour, normal or tall stature, abnormal face (epicanthal folds, hypertelorism, protruding lips), hypogonadism, gynaecomastia (33-50%), hypoplastic penis, infertility, clinodactyly, and radioulnar synostosis. They may benefit from testosterone therapy.
c. *49,XXXYY variant*: Patients typically have moderate-to-severe mental retardation, passive but occasionally aggressive behaviour and temper tantrums, tall stature, dysmorphic facial features, gynaecomastia, and hypogonadism.
d. *49,XXXXY variant:* The classic triad is mild-to-moderate mental retardation, radio-ulnar synostosis, and hypergonadotrophic hypogonadism. Other clinical features include severely impaired language and behavioural problems, low birth weight, short stature in some individuals, abnormal face (round face in infancy, coarse features in older age, hypertelorism, epicanthal folds, prognathism), short or broad neck, gynaecomastia (rare), congenital heart defects (patent ductus arteriosus is most common), skeletal anomalies (genu valgus, pes cavus, fifth finger clinodactyly), muscular hypotonia, hyperextensible joints, hypoplastic genitalia, and cryptorchidism. Pea-size testes, micropenis, and infantile secondary sex characteristics are characteristic in patients with 49,XXXXY, whereas patients with 48,XXXY exhibit milder hypogonadism similar to that of patients with 47,XXY.

Autosomal Trisomy (Down Syndrome)

Down syndrome (DS) is a major cause of mental retardation and heart disease. It is usually caused by the presence of an extra chromosome 21 due to impaired meiosis. In the adult Down syndrome, often there are oligospermia, mental handicap and several dermatoglyphic changes. High β-hCG in the second trimester of the pregnancy is one of the criteria of suspecting Down syndrome in the unborn child. For screening, this should be combined with estimation of maternal serum α-fetoprotein and unconjugated estradiol.

Noonan Syndrome (Male Turner's syndrome)

Noonan syndrome is the male counterpart of female Turner's syndrome (X0) and it occurs due to mitotic nondysjunction of the Y chromosome. In chromosomal analysis, a sex chromosome abnormality such as X0/XY mosaicism is noticed. Men with Noonan syndrome usually are infertile due to cryptorchidism and insufficient sperm production. They have many distinctive physical features, such as short stature, low-set ears, webbed neck, upper eyelid ptosis, and elbow deformity (cubitus valgus). Cardiovascular abnormalities also may be present. Testicular malfunction in these individuals cause increased serum levels of follicle-stimulating hormone (FSH) and luteinising hormone (LH). Replacement hormone therapy may help to relieve their androgen deficiencies and cryptorchidism, but impaired sperm production and resultant infertility is irreversible.

XYY and XX Male

XYY male or XXX female is caused by nondysjunction in the first or second meiotic division. Strictly speaking, XYY syndrome is a variant of Klinefelter's syndrome. Although affected men have a normal number of chromosomes, the sex chromosome signature is "XX," with a displacement of the Y chromosome somewhere within the other pairs of somatic (bodily) genes.

The signs of XX disorder, otherwise known as *sex reversal syndrome* are comparable to those of Klinefelter's syndrome, yet most individuals are short statured and are less likely to be mentally deficient, and may exhibit hypospadias. No consistent syndrome has yet been defined, and XYY men may suffer from abnormalities such as seminiferous tubular sclerosis, or may have normal testes. Generally, men with XYY syndrome are extremely tall and may suffer from a pustular form of acne. Some individuals express antisocial behaviour. Ejaculate samples from XYY men vary between azoospermia and normal sperm counts. Serum levels of testosterone, LH, and FSH often are normal. Abnormalities in these hormone levels are related to the extent of germ cell damage within the testes.

Mixed Gonadal Dysgenesis

Mixed gonadal dysgenesis is an inherited disorder with a distinctive genetic signature. Sixty percent of cases have 46 XY and in others have 45, XO/46, XY. It is defined by the presence of a testis on one side and a "streak" (primitive) gonad on the other side. The mixed character of this disorder in some patients is exhibited by having male external genitalia that appear female (although ovaries are not present internally), whereas others appear like normal men with one-sided undescended testes.

There is a high probability of malignant (cancerous) transformation in the tissues of the undescended testis and/or streak gonad among adults with this disorder. Non-metastasising gonadoblastomas are the most frequently occurring tumours, but metastasising germinal cell tumours may occur along with them. Thus, most physicians recommend early removal of the gonads, except when the testes are in the scrotum.

5-alpha-reductase Deficiency

The 5-alpha-reductase deficiency is a familial disorder that falls under the banner of "*male pseudohermaphroditism,*" where the gonads are of one sex, but the overall physical characteristics are of the opposite sex. Iin male cases, the individual is a genetic and gonadal male with unfinished masculinisation. The 5-α-reductase is an important enzyme in the pathway of androgen (male sex hormone) activity. The 5-α-reductase deficiency is typified by external female features at birth, presence of well-developed testes, epididymis, vas deferens, seminal vesicles, ejaculatory duct, etc. (see also chapter 2). Individuals with 5-alpha-reductase deficiency often are raised as girls; however, at puberty, they may develop enlargement of penis and experience masculinisation (beard growth, etc.). People with this disorder have a slightly increased blood level of LH and a normal or increased level of testosterone. Since the external genitalia may not be completely developed in affected

individuals, those who are raised as men should undergo surgical repair of any defects such as cryptorchidism.

Androgen-receptor Deficiency

Like 5-α-reductase deficiency, androgen-receptor deficiency is a genetically linked expression of abnormal androgen (male sex hormone) activity. The clinical features of androgen-receptor deficiency, also known as *Reifenstein's syndrome*, may range from infertility alone to pseudohermaphroditism (incomplete masculinisation of the external male genitalia in men with bilateral testes). Cryptorchidism may be present, along with defective vas deferens and incomplete sperm production. Patients often show high serum levels of testosterone with increased levels of LH and estradiol (natural estrogen). The enhanced estradiol output leads to feminisation (development of female sex characteristics), androgen resistance and incomplete degrees of masculinisation. Resultant infertility is irreversible.

The androgens determine the expression of the male phenotype. Their actions are mediated by a single androgen-receptor (AR) and its binding translocates to the nucleus to regulate the expression of androgen-responsive genes. Mutations that disrupt AR function totally, result in the complete feminisation of 46XY individuals and the complete androgen insensitivity syndrome. Studies have revealed that AR mutations that do not lead to complete abrogation of its activity, can cause a wide spectrum of milder androgen insensitivity syndromes from ambiguous genitalia in newborn infants to idiopathic male infertility.[19] A variety of mutations have been attributed to two molecular mechanisms in the human androgen-receptor gene associated with male infertility. These can be due to mutations in the androgen-receptor gene causing alterations in the amino acid sequence and, hence, lead to apparently slight changes in the androgen-receptor effecter mechanisms and mild androgen insensitivity. Secondly, it could be due to variations in the polymorphic polyglutamine segment within the androgen-receptor.[20]

STRUCTURAL CHROMOSOMAL ABNORMALITY

A structural chromosomal abnormality results from chromsome breakage. When the chromosome breaks, its two unstable segments and sticky ends normally rejoin through DNA repair mechanism. If the repair mechansism fails often due to multiple breakage points, a segment of chromosome carrying vital genetic material can be deleted or lost.

The structural anomalies of chromosomes may take the form of X- chromosome translocations and X/Y translocations. Molecular biological studies of the genes on X-and Y-chromosomes have revealed in some 46XX males and 46XY females with wide ranging abnormalities. One of many genetic disorders associated with male infertility is primary hypogonadism characterised by genetic abnormalities resulting in irreversible testicular defects and sexual maturity.

Azoospermia

Azoospermia is one of the common causes of male infertility and is found in up to 10 to 20 percent of the men reporting to an infertility clinic.[21] Normal sperma-togenesis is a complex process that depends on many factors. After excluding the obvious urological reasons and Klinefelter's syndrome, azoospermia may be caused by an abnormality in the crucial gene(s) expressed during male germ cell differentiation. In these cases, there is evidence of existence of genes controlling spermatogenesis-AZF (*azoospermic factor*) and DAZ (*deleted* in *azoospermia*). The AZF is involved in sperm production. In humans, deletion of any one of three Y-chromosomal regions (AZFa, AZFb or AZFc) disrupts the spermatogenesis causing infertility in otherwise healthy men.[22]

In 1995, Reijo and coworkers reported another AZF candidate known as DAZ.[23, 24, 25] The DAZ genes are expressed exclusively in the human germ line[26] and are candidate genes for the expression of the azoospermic factor AZFc. A deletion of the DAZ genes is supposed not to interfere with human sperm maturation, but to cause a graded reduction of the mature sperms. After screening 576 infertile and 96 fertile men, Page[17] was able to sequence the AZFa region of the Y chromosome and identified two functional genes—USP9Y (also known as DFFRY) and DBY. *De novo* (not hereditary) mutation in USP9Y causes spermatogenic failure.[21] In one study, an overall incidence of 6.2% of male chromosomal abnormalities was noted. But the incidence was 11% in the subgroup, where sperm count was less than 10 million and 21% in azoospermic subjects.[27-30]

X-chromosome gene defects can cause *Kennedy disease* (an adult onset motor neuron disease with reduced fertility), *Morris disease* (complete androgen

insensitivity) and *Kallmann's syndrome* (hypogonadotrophic hypogonadism, anosmia, mental deficiency, etc.). As genetic abnormality and infertility often coexist, uses of different forms of intracytoplasmic injections of sperms (ICSI, DICSI, MESA or TESA-See Chapter 13) in *in vitro* fertilisation techniques are often advised. However, it would be appropriate to warn the couple that the foetus may also inherit the genetic defect of father, which has in the first place caused the underlying pathology leading to azoospermia or severe oligozoospermia and subsequent male infertility in offspring (see Chapter 13).

OTHER GENETIC DISORDERS CAUSING INFERTILITY

Cystic Fibrosis

Cystic fibrosis is characterised commonly by low ejaculate volume, and azoospermia among men with an inherited gene for cystic fibrosis. These individuals usually have congenital bilateral absence of the vas deferens and malformations or absence of seminal vesicles (see also Chapter 2).

Sickle Cell Anaemia

Sickle cell anaemia is an inherited blood disorder caused by an abnormal form of haemoglobin. Men with sickle cell anaemia may show evidence of hypogonadism (delayed sexual maturity), as well as slowed skeletal growth, small testes and low sperm density. Hypogonadism usually is related to testicular malfunction as well as hormonal imbalances (e.g. pituitary hormone and hypothalamic hormone irregularities). Testosterone level generally is low in men with sickle cell disease, although LH and FSH levels are variable and may be normal, low or even increased.

Prune Belly or Prader-Willi Syndrome

Prader-Willi syndrome (PWS) first described by Drs. Prader, Labhart, and Willi in 1956, is an uncommon inherited disorder characterised by mental retardation, decreased muscle tone, short stature, labile emotion and insatiable appetite (often leading to life-threatening obesity). PWS is caused by the absence of segment on the long arm of the paternally derived chromosome.[11-13,31] This is a disorder typi-fied by genitourinary abnormalities; in some indivi-duals, early puberty may occur due to increased levels of testosterone, LH and prolactin (PRL).

Fragile X Syndrome

Fragile X syndrome is caused by a defect in the X chromosome and its effects are seen more frequently and with greater severity in males than in females. It has an incidence of occurring 1 in 1500 male births. In normal individuals, the FMR1 gene is transmitted stably from parent to child. However, in fragile X individuals, there is a mutation in one end of the gene. Fragile X syndrome is the most common inherited form of mental retardation.

Myotonic Dystrophy

Mytonic dystrophy is an inherited disorder that is characterised by delayed muscle relaxation after initial contraction. Individuals with the disorder usually have major clinical features such as frontal baldness, lenticular opacities and testicular atrophy. Inheritance is autosomal dominant and the expression is variable, though 80% will develop testicular atrophy. Such atrophy is attributed to abnormalities of the seminiferous tubules. Pubertal development is usually normal and testicular damage occurs later in adult life. Leydig cell function remains normal and there is no gynaecomastia. Levels of FSH are usually increased in proportion to the degree of testicular atrophy. There may be some evidence of spermatogenesis, but testicular biopsy usually shows disorganisation of the sperm maturation process with breakdown of primitive germ cells, Sertoli cells and seminiferous tubules, and eventual tubular sclerosis. Men with myotonic dystrophy normally do not require androgen therapy, as testosterone levels are normal in most individuals. Unfortunately, there is no treatment for infertility in myotonic dystrophy patients due to testicular damage.

Kallmann's Syndrome

Kallmann's syndrome or isolated gonadotrophin deficiency is a genetically inherited disorder that affects the function of the hypothalamus (see Chapter 3). Male infertility occurs due to abnormalities in the migration, proliferation and survival of germ cells. This can be the consequence of mutation in the c-KIT genes. MIS or MIF (*mullerian inhibiting substance or factor*) secreted by Sertoli cells has been mapped to a particular gene factor known as AMH. Overexpression of the AMH can cause persistent Mullerian system in males, cryptorchidism, etc. Similarly, *Kartagener's syndrome* and *primary ciliary dyskinesia*

(PCD) causing dysfunction of sperm motility are inherited in an autosomal recessive fashion.

Exstrophy of Urinary Bladder

Erectile dysfunction (ED) and ejaculation problem occur in *exstrophy of urinary bladder*, (where there is absence of part of the lower abdominal wall and frontal wall of the bladder) or *epispadias*, characterised by the absence of the upper wall of the urethra, which may open anywhere on the dorsum of the penis.

ACQUIRED LESIONS

Genitourinary Infections [including Male Accessory Gland Infection]

Male genitourinary infections approximately account for 15% of cases of male infertility. Infections can affect different sites of the male reproductive tract such as the testis, the epididymis, and male accessory sex glands.[32] Male accessory gland infection (MAGI) is a common occurrence, but its prevalence varies in different countries as revealed in the WHO studies.[33] Increase in white blood cell counts in semen represents a hallmark and important diagnostic criterion for male genitourinary infections. Bacterial infections are more frequently found in semen samples from asymptomatic infertile patients than in those from fertile men.

Various research workers now acknowledge the role of specific bacterial, fungal and viral infections of the genital tract and their possible association with male infertility.[34] Common organisms with spermicidal effects *in vitro* are viridans and haemolytic streptococci, gonorrhoea, Proteus vulgaris, Escherichia coli, Ureaplasma urealyticum, Mycoplasma hominis and chlamydia trachomatis.[35]

Infective processes may lead to deterioration of spermatogenesis, impairment of sperm function and/or obstruction to the seminal tract. Recent studies have identified and evaluated infectious mediators that appear to be responsible for specific molecular processes in infections that particularly affect the motility of spermatozoa.[32, 36] Detection of bacteria in semen does not necessarily signify infection since bacteriospermia may represent contamination, colonisation or infection.[34,37,38]

E. coli probably represents the most frequently isolated microorganism in genitourinary infections including the male accessory gland infections (MAGI). Bacterial growth with *E. coli* (serotype 06) could cause a significant inhibitory effect on the sperm motility. Experiments with the Enterococcus strain and Staphylococcus saprophyticus indicated no significant influence on sperm motility parameters. Tests with Pseudomonas aeruginosa showed a decrease of progressive motility according to time, but not to different bacterial concentrations. A significant inhibitory effect of Candida albicans was only detected in the samples with the initial bacterial concentration of 2×10^7 microorganisms ml.[39] In bacteriological studies of semen and urethral secretion, Gram-Nicolle stain is utilised for the direct examination and enriched blood Columbia agar and Thayer Martin agar are used in cultures for common organisms.[40] Enterobacteria can even be found in up to 90% of semen samples depending on the sensitivity of detection methods used.

Reported prevalence of ureaplasma urealyticum in human semen varies from 10 to 40%. Although the overall prevalence of ureaplasma urealyticum is higher than that of mycoplasma hominis, the prevalence of mycoplasma hominis is significantly higher in the infertile men and women. For mycoplasma identification, both A7 Sheppard solid and liquid media can be used.[40]

The prevalence of chlamydia trachomatis, positive culture also was higher in infertile couples.[41] The researchers found that a couple's chance of achieving a pregnancy was reduced by 33%, if the male partner had antibodies against chlamydia. About 20% of male partners of women, who could not conceive, showed evidence of past infection. Chlamydia trachomatis is the most frequent sexually transmitted bacterial organism in industrialised countries. Possible effect of C. trachomatis is not due to alterations of sperm quality or function, but is due to sexual transmission to the female partner and promotion antisperm antibodies rather than a direct influence on the male reproductive functions.[32, 42-45]

Chlamydial culture is often a difficult proposition and beyond the capability of most laboratories. Several studies including that conducted by Barratt et al[46] arrived at similar conclusions. Chlamydial infection can be determined by serologic methods and by proof of chlamydia-specific DNA.[47] Presence of chlamydia trachomatis in urethral secretion can be detected by direct immunofluorescence.

Men with chlamydia infection may have the symptoms such as painful urination or itching when urinating, discharge from the penis, cloudy urine or

tender scrotum.[48, 49] Chlamydia was found in 9% of 1,300 males between ages 12 and 24, who were tested by the Minnesota Department of Health over a two-year period. This new research, unveiled at the 2004 National STD Prevention Conference, suggests that more efforts should be placed on screening men and possibly on developing new strategies to combat this bacterial infection.[50]

Role of Leucocytes and Reactive Oxygen Species (ROS) (see Chapter 6)

The evaluation for a genital tract infection focuses on urine and semen cultures as well as on the accurate quantification of seminal leucocytes. An elevated seminal leucocyte count and pyospermia can be associated with male infertility, and may reflect an infective or inflammatory disorder. The data show that leucocytospermia occurs frequently in infertile patients and is associated with poor semen quality parameters.[51] In men with varicoceles, the increased number of immature germ cells might play a pivotal role in the pathogenesis of abnormal spermatozoa.[52]

Recent publications have proved convincingly that the presence of peroxide positive leucocytes is associated with biochemical abnormalities of semen. Infections and inflammatory process generate reactive oxygen species (ROS) detrimental to sperm activities and functions.

The presence of 2 million or more peroxidase-positive WBCs per ml of semen is the benchmark for the diagnosis of MAGI. It is associated with important biochemical and biological changes in semen plasma and in the spermatozoa, reducing latter's fertilising potential *in vitro* and *in vivo*.[53] However, Wolff[54] considers a lower value of one million leucocytes per ml for a diagnosis of leucocytospermia.

Among male infertility patients, the frequency of leucocytospermia is between 10% and 20%. By conventional light microscopy or sperm staining techniques, it is not possible to reliably differentiate WBC from immature germ cells in semen. In contrast, the cytochemical peroxidase method reliably identifies granulocytes.

The gold standard for the detection of all WBC populations in semen is immunocytology using monoclonal antibodies. However, it is expensive and time-consuming to measure the granulocyte elastase in semen, which provides information on the number of granulocytes and their inflammatory activation. Commercial granulocyte elastase enzyme immunoassays are expensive and often delay the results for more than one week. Leucocyte esterase dipstick tests lack both sensitivity and specificity for the detection of inflammatory changes in semen. For clinical purpose, the peroxidase method is ideally suited to detect inflammatory changes in semen.[54]

Only neutrophil leucocytes (both eosinophilic and basophilic) show a positive reaction, when exposed to the peroxidase stain. Lymphocytes, macrophages and other round cells such as epithelial cells and spermatogenesis cells remain negative. It could be concluded that the neutrophil polymorphonuclear leucocytes are the only round cells showing a positive reaction in the semen samples.[55] In asymptomatic patients (in terms of genital tract infection), the majority of round cells consist of immature germ cells and < 5% are WBCs. The streptavidin-biotin system and the mABs used in this study proved to be useful to identify patients with elevated rates of leucocytes in semen.[56] Immature germ cells can be differentiated by the Papanicolau stain.[40] However, Sanchez et al[57] suggest that the determination of peroxidase positive cells is not a reliable indicator of leucocytes in the seminal plasma and their absence do not discard a silent genital tract infection.[57]

ROS are a group of oxidising oxygen containing species that are capable of injuring biomolecules such as proteins, lipids, and nucleic acids of cells and tissues.[58] ROS typically are gene-rated and released from the macrophages and the polymorphonuclear granulocytes (PMN) in response to invading micro-organisms. ROS, which are physiologically produced during metabolic reactions in all cells, are mostly confined in the mitochondria.

Chronic urogenital tract infection is associated with generation of ROS detrimental to sperm activities and functions, leading to subfertility in male.[59,60] Recent evidence suggests that the spermatozoa and oocytes also possess an inherent but limited capacity to generate ROS.[61] The markers in the seminal plasma have been utilised in recent years to arrive at explanations regarding how MAGIs might interfere with the molecular functions of spermatozoa. The spermatozoa are protected from the detrimental effects of ROS by the powerful antioxidants in seminal plasma. Individual cells are also inherently protected from the oxidative damage by its internal enzyme systems.

The lipid composition of the sperm membrane has been shown to exert a significant effect upon the

functional quality of spermatozoa[58] and influence to a great extent in impairment of fertilising capacities of sperms. Evidence gathered so far, conclusively proves the damaging effects of excessive number of peroxide positive WBC independent of any infection.

The generation of ROS by PMNs or seminal macrophages results in the alteration of the lipid composition of the sperm membrane phospholipids. This process of lipid peroxidation (LPO) causes decreased membrane fluidity and impaired sperm function.[62,63] The lipid peroxidation also has an inhibitory effect on human spermatic adenosine-triphosphate (ATP) causing detrimental changes in the motility, velocity and linearity of the spermatozoa.[64] This is probably one of the molecular mechanisms that account for changes in the motility and the motility characteristics of sperms after their exposition to seminal leucocytes. These changes in the lipid composition of the cell membrane are also dependent on the duration of the exposure of the sperms to the ROS. Another deleterious effect of ROS on the reproductive capacity of spermatozoa perhaps occurs through their interference of the acrosome reaction in human spermatozoa.[65]

The sperm ATP being responsible for its movement, its reduction runs parallel to the inhibition of sperm energy metabolism and forward progression. The toxic ROS produced by activated leucocytes is H_2O_2, which causes the inhibition of both sperm movement and ATP production. Even low concentrations of H_2O_2 can effect inhibition of sperm movement and ATP levels.[60, 63]

ROS in seminal plasma are increased profoundly, when the MAGI or leucocytospermia is evident. In addition to the effects of ROS on the essential fatty acid composition of the sperm membrane, unfavourable effects can occur through increased concentration of certain cytokines. Increase in WBCs in the semen correlates with the elevation in proinflammatory cytokines having potential unfavourable effect, and with decreased activity of enzymes such as alpha-glucosidase and gamma-glutamyl transferase.

The presence of two million or more peroxidase-positive WBCs per ml of semen is associated with the quantitative and qualitative reductions of the sperms due to a significant biochemical and biological changes in semen plasma.[53,66] In contrast, lower numbers of WBCs exert some beneficial effects on the spermatozoa from increased production of hepatocyte growth factor/scatter factor (a tissue repairing substance) and stimulation of immunocompetent cells by some cytokines (e.g., Interleukin-6). The cytokines in the seminal fluid are secreted by the activated seminal leucocytes. A variety of other defence mechanisms encompassing cellular antioxidant enzymes (SOD, catalase, and GSH peroxidase and reductase), vitamins (E, C, and carotenoids), and biomolecules (GSH and ubiquinol) are also available. A balance between the beneficial factors and risks from ROS through actions of the pro-inflammatory cytokines and the antioxidants appears to be necessary for the survival and functioning of spermatozoa.[59, 60]

Recurrent and chronic infections of male urogenital tract are often associated with oligo- and asthenospermia.[66] More importantly, MAGI may predispose to immunological changes in the sperms (see Chapter 6).

Site of Affection

Infections of the male genitourinary tract may contribute to infertility to a various extent depending on the site of inflammation. Chronic infections of the prostate, epididymis or urethra often contribute to changes in the ejaculate and altered sperm parameters.[67] Emphasis is placed on the need to stick to rigorous bacteriological protocols and to undertake at the same time well-codified clinical, biological, epidemiological and experimental studies. After an extensive review of the literature regarding the role of infection in the etiology of male infertility, conclusions drawn by Purvis et al[68] are shown in Table 10.3.

Diagnosis of acute prostatitis is relatively easy, but chronic prostatitis of both bacterial and non-bacterial etiology poses a challenge. Amongst the nonbacterial group, *Chlamydia* is an important cause. Presence of increased numbers of leucocytes in the expressed prostatic secretion (EPS) without any bacterial presence remains the most important criteria for diagnosis.

Seminal vesicular infection is usually the result of ascending infection travelling from the prostate or bladder. Tender and palpable seminal vesicles can be felt in many cases just above the prostate through digital examination of rectum. *Gonococcus or Chlamydia* mostly causes epididymitis in younger men. Gonorrhoea infection invades the mucous membranes of the genitals and urinary tract, and can facilitate transmission of HIV.

Table 10.3: Male genital tract infections (*Purvis et al*)

1. Temporary inflammatory episodes in the male reproductive tract, which are self-limiting, are probably common.
2. Caution should be exercised in the use of leucocytospermia or bacteriospermia as parameters for glandular infection.
3. There is a need for alternative techniques for detecting nonsymptomatic deep pelvic infections in the male. Transrectal ultrasound has been found to be extremely useful as a large number of men with poor sperm quality often have a nonsymptomatic, chronic prostatovesiculitis that can be detected by its use.
4. Increasing evidence implicates *Chlamydia trachomatis* as being a major cause of chronic nonbacterial prostatitis. However, an important aspect of chlamydial infections in men may be that the male accessory sex glands may function as reservoirs for the organism, increasing the probability of infection in the female.
5. *Ureaplasma urealyticum* may also play an important aetiological role in male infertility, but its significance is confounded by its acknowledged function as a commensal in the reproductive tract.
6. One of the manifestations of male reproductive tract infection is the induction of sperm autoantibodies.
7. There is a need for more systematic controlled studies of the effects of antibiotic treatment on sperm quality with different preparations for extended periods using patient groups in which a glandular infection has been verified by the bacterial culture and the rectal ultrasonography.

Diagnosis of such affection rests on the clinical features of intrinsic scrotal pain with palpable and tender epididymis. In older men, bacterial infection due to coliform bacteria is more common. The sperm transport mechanism may be affected in recurrent and chronic epididymal infections due to inflammation and subsequent scarring.

In the subcontinent, chronic infection with specific bacteria such as *Mycobacterium tuberculosis* are frequently encountered resulting in profound destruction of testicular structures, although the primary location of manifestation usually is the epididymis. Parasitic infections due to filarial affection in the subcontinent are also known to cause epididymitis, funiculitis and later elephantiasis of scrotum.

Analysis of data from various studies has so far failed to resolve the controversy, whether pathogens in semen have any effect on the sperm function. Criteria for precise identification of pathogens and commensals in semen have not been standardised and confusion in this regard still persists. Bacteriological studies of semen can only be informative and significant, when strict measures are taken to prevent contamination during collection. In future, advances in identification of molecular players are expected to facilitate better pathophysiologic understanding of infectious disease.

Diagnostic Criteria of MAGI

According to the WHO criteria laid down in 1993 (Table 10.4), MAGI is diagnosed, if the patient has oligo-, astheno- or teratozoospermia. The study was based taking into consideration the clinical symptoms and signs, biological abnormalities and the classical signs of infection and inflammation.

Table 10.4: Diagnostic criteria of MAGI

A. History and physical signs:
   1. History of urinary infection,
   2. and/or epididymitis,
   3. and/or sexually transmitted disease,
   4. and/or thickened or tender epididymis,
   5. and/or thickened vas deferens,
   6. and/or abnormal rectal examination.

B. Prostatic fluid:
   1. Abnormal prostatic expression of fluid and/or
   2. Abnormal urine after prostatic massage.

C. Ejaculate signs:
   1. More than I million/ml white blood cells,
   2. Culture with significant growth of pathogenic bacteria,
   3. Abnormal appearance and/or viscosity and/ or abnormal pH and/or
   4. Abnormal biochemistry of the seminal plasma.

Any of the following combinations should be present:

1. A history or physical sign with a prostatic sign;
2. A history or physical sign with an ejaculate sign;
3. A prostatic sign with an ejaculate sign;
4. At least two ejaculate signs in each ejaculate,

One of A and one of B, or one of A and one of C or one of B and one of C or two of C in each ejaculate.

Besides the genitourinary infections generalised acute and chronic inflammatory diseases, such as sepsis and rheumatoid arthritis, are associated with disorders in steroidogenesis and spermatogenesis that result in temporary or permanent infertility.

ACQUIRED LESIONS OF TESTIS

As a natural corollary to almost exclusive role of testis in spermatogenesis, lesions of testes disturb the fertility potential of males. Torsion, trauma, tumour, and infective lesions of testis at times produce sperm abnormalities of different degrees.[70] Environmental and occupational factors that expose testes to various toxic substances have been discussed in Chapter 6.

Testicular Infective Lesions

The role of testicular infection in causing spermatogenetic abnormalities has raised a considerable

debate amongst andrologists and reproductive physicians. Two common conditions that affect the testis are gonococcal epididymoorchitis and mumps orchitis. *Ureaplasma ureacalyticum* belonging to *Mycoplasma* species frequently causes urethritis, and ascending infections of the urinary tract, but its effect on the sperm parameters have not been proved conclusively. Testicular biopsies in unilateral epididymoorchitis produced evidence of bilateral testicular tissue damage.[71] It is possible that leucocytes in semen consequent to infective process initiate an autoimmune response as evidenced in animal experiment data.[72] As discussed earlier, these leucocytes produce ROS that damages cell membranes to impair fertilising potential of sperms.

Gonococcal infection has still not been eradicated and it is still common in the developing countries. An acute phase of gonococcal infection is followed by fibrosis of epididymis and also affects germ cells. Sixty percent of patients suffering from this condition remain subfertile after an acute attack.

Mumps orchitis occurs due to direct viral invasion of the testis resulting inactivation of autoimmune mechanism with resultant testicular atrophy. About 15 to 25% of adult men, who contact mumps, can develop orchitis. Mostly, it is unilateral, but statistically bilateral involvement occurs in about 10% of affected men. However, Shulman et al[73] have found the incidence to be much lower. Testicular atrophy can develop within 1 to 6 months or may take years. Less than one-third of men with bilateral orchitis recover normal semen parameters. The presence of antisperm antibodies does not play a role in the etiology of mumps orchitis.

It is possible that preventive treatment with mumps vaccine and antibiotics during acute phase reduces the damage to the testicular tissues and brings down its incidence. Incidentally, treatment with *interferon α-2b* has been successful in preventing testicular atrophy in recent times.

In the subcontinent, emergence of tuberculosis, especially in the form of MDR (multiple drug resistant) type, still has a causative role in some cases of infertility. Involvement of the epididymis, vas and the seminal vesicles and subsequent cicatrisation due to various infections would lead to obstructive lesions later. These are grouped under acquired obstructive lesions (see Table 10.1).

Testicular Torsion (see also Chapter 6)

Testicular torsion is one of the common urological emergencies. It results from abnormality in the testicular suspension mechanism that allows the testis to twist on a narrow pedicle. Clinically, it is commonly unilateral, but concomitant defect in the contralateral side is very frequently found. As the testicular arterial supply is cut off following torsion, the testis undergoes vascular changes leading to varying degrees of ischaemia or infarction.[70, 74, 75]

Even in unilateral atrophy, apparently normal contralateral testis goes through an autoimmune phenomenon called *"sympathetic orchidopathy"* inducing physical changes. Both duration and degree of torsion determine the effect on subsequent fertility. An orchidectomy of the testis with torsion of one testicle will limit potential histopathological alterations in the contralateral testis. An early surgical intervention undoing the torsion and fixation to achieve testicular salvage could prevent subsequent deterioration of semen quality. However, late surgical intervention, even with removal of the nonviable testes, may result in significant impairment of semen quality. Following morphologic alterations causing necrosis of the testis also cause significant increase in plasma prostaglandin (PG) E2 levels.[76-79] So even in a unilateral affection, ipsilateral orchidectomy or an orchidopexy depending on the vascular state of the affected side, should be combined with contralateral orchidopexy to prevent such occurrence.

Testicular Trauma

Testicular injuries can be caused by either blunt or penetrating trauma. The exposed position of the testes makes them susceptible to trauma and subsequent atrophy. Bilateral lesions are uncommon, but closed intrascrotal haematoma after a unilateral injury can eventually cause damage to the other side by pressure effects due to raised tension within the confines of the closed scrotal sac. Penetrating injuries pose not much problem in diagnosis, while a blunt injury can escape early attention, unless the patient is examined thoroughly. Fortunately, with advent of advanced US technique and MRI if necessary, the diagnosis of blunt testicular injuries has been made easy.

Testicular Tumour

Not only do the testicular tumours cause destruction of testicular tissues, but also its treatment with

chemotherapy or radiation may cause additional damage. Chemotherapy and radiation have maximal effects on the rapidly growing germ cells causing severe oligospermia or azoospermia, which may be permanent with doses beyond 0.35 Gy. Leydig cell functions are affected with a dose greater than 15 Gy.[70] Plasma levels of β-hCG levels are raised in choriocarcinomas and in some cases of embryonal carcinomas and teratomas, but rarely in seminomas.

In Western countries, Hodgkin's disease of testis is one of the common testicular neoplasms in young adults and abnormal sperm quality is a common accompaniment. Hodgkin's disease causes dysfunction by direct infiltration of the seminiferous tubules and indirectly by inducing gonadal dysfunction. Other testicular malignant tumours have similar negative effects on the sperm function. Sperm concentration below the normal limit is a common finding in 80% of cases of testicular cancers. This is noticed even before any treatment, implying that subsequent orchidectomy is not entirely responsible for changes in the sperms. This fortifies an argument in favour of cancer having a bilateral effect. While ipsilateral changes can be explained by the direct tumour effect destroying the gonad cells, changes in the contralateral healthy side can only be due to other changes initiated by the tumour.

Recent studies have established that the CIS (*carcinoma in situ*) factor acts in foetal life on the primordial gonad cells to transform them to CIS cells, which develop into full-fledged carcinoma at a later date. Importantly, there is evidence that men with abnormal sperms at an early age have propensity to develop malignant lesions of testis at a later date. It is postulated that the abnormal stimulus that ushers in transformation of some primordial gonad cells to CIS, could also be operative to bring about qualitative changes in some of the other gonad cells to behave abnormally from early life to produce excess abnormal sperms. Increased level of FSH is another manifestation of impaired spermatogenesis in men with testicular cancer. In addition, impairment of Leydig cell function is also observed. Even in a unilateral undescended testes, histological abnormalities in the other testis are commonly observed. Propensity of undescended testis to develop malignancy is well documented. These observations lend credence to a hypothesis of a common prenatal cause of infertility in these patients.[70]

Iatrogenic Trauma

Iatrogenic injures to the vas and testicular arteries during surgery are known to cause acquired obstructive lesions with resultant infertility. Unilateral injuries often escape detection, as the fertility potential is not affected. Bilateral injuries are extremely rare and usually encountered, when the normal anatomy is distorted by adhesions and the surgeon is not careful enough to keep this in mind. Bilateral repair of recurrent hernias, exploration of inguinal region for bilateral recurrent varicocele, scrotal approach for varicocele (now given up), and bilateral herniotomy for infants and children (where the vas is thin) are some of the instances, where bilateral injuries to vas are possible. Vasovasal anastomosis for these conditions has fairly satisfactory outcome (see vasovasal anastomosis in Chapter 12).

MISCELLANEOUS CAUSES

Peyronie's Disease

Peyronie's disease is characterised by a plaque or hard lump on the penis affecting approximately 1% men. The plaque begins as a localised inflammation of the tunica albuginea and can develop into a hardened scar involving the layers containing erectile tissue. Although Peyronie's disease has been noted in print as early as 1687, the French surgeon to Louis XIV of France, François de la Peyronie, first described the disease in 1743.

Peyronie's disease may develop slowly or appear very rapidly in a short period and has an incidence of 0.3 to 0.7% of all urological patients. It ranges from mild to severe in degree and is found to be more common in middle-aged men in their forties or above, but is also noticed in younger men. Infrequently, a few men are born with a curvature of the penis, but they do not have the characteristic and pathognomonic plaques of Peyronie's disease.

Studies have shown that at some point, up to 40% of men with Peyronie's disease have experienced some degree of ED and medical attention is commonly sought for painful erections. Erectile problem often leads to strong psychological impact. Peyronie's disease rarely causes complete loss of erection, though its effect on the erectile mechanism can reduce rigidity or hardness of an erect penis. However, it may change the shape of the erection by way of penile indentation, diameter reduction, or loss of length. A plaque on the top of the shaft would

cause the penis to bend upward and that on the underside to bend downward. If it happens to develop on both top and the bottom, indentation and shortening of the penis may result in addition to curvature. Penile deformities are disabling (when greater than 30 degrees) in 62.5% of cases.[80]

Peyronie's disease may develop slowly or appear very rapidly in a short period. About 30 percent of men with Peyronie's disease develop fibrosis in other elastic tissues of the body. It is known to develop in blood-related men suggesting related genetic factors in its etiology. Other risk factors such as serum lipid abnormalities, diabetes, gout and hypertension seem to have significant impact on the severity of symptoms and outcome.[80]

Peyronie's disease is progressive in 30.2% without treatment. In a small percentage of patients with the milder form of the disease, the process may resolve within a year of onset without causing significant pain or permanent bending. In severe cases, the hardened plaque reduces flexibility causing pain and forcing the penis to bend or arch during erection. In many cases, the pain may decrease over a period, but the bend in the penis persists rendering sexual intercourse difficult.

Pathogenesis of Anatomical Distortion

Peyronie's disease is a disorder of the tunica albuginea. The firm or hard plaques in the tunica albuginea focally interfere with the expansion of this normally pliant tissue. Most researchers believe that these plaques of Peyronie's disease develop following a trauma (hitting or bending) that causes localised bleeding inside the penis. The inner-surface membrane of two corpora cavernosa (which run the length of the penis) is a sheath of elastic fibres. The connecting tissue septum runs along the centres of two corpora and is attached at the top and bottom. The area, where the septum is attached to the elastic fibres of the erectile chamber may stretch beyond a limit in an erect penis, especially if it is bent, and cause injury to its lining rupturing the small blood vessels. Probably, all sexually active men experience some degree of wear and tear particularly over the vulnerable areas of the erection mechanism. Both the structural arrangement of the corpora and the inherent elasticity of its connective tissues counteract the strong mechanical stress imposed by active intercourse. But by the time men reach their mid-forties or fifties, inherent connective tissue elasticity is on the wane rendering the chance of injury relatively more.

Deposition of fibrin, the first step in the wound healing process and the precursor to Peyronie's plaque, usually develops in the area of trauma (Fig. 10.3, Plate 4). The plaques in early phases of the disease show areas of reversible inflammation and permanent scars later on. The damaged area might heal slowly or abnormally either due to repeated trauma or minimal amount of blood flow in the sheath-like fibres. If the healing takes place within a year or so, the plaque may not advance beyond the initial inflammatory phase. However, in case the inflammatory reaction persists, the plaque undergoes fibrosis with formation of tough fibrous tissue and even calcification.

Autopsy studies on many middle-aged and older men have shown microscopic changes of scarring in the corpora as an evidence of Peyronie's disease. Quite a few men probably develop these changes; but only in a very small percentage of cases, it culminates in typical Peyronie's plaques. Clear answer to what triggers off the final changes from the state of normal wear and tear to abnormal activation of the process of wound healing still eludes us. Peyronie's disease is also more common in conditions that can affect connective tissue healing like diabetes or gout.[81]

Some researchers theorize that Peyronie's disease may be an autoimmune disorder or result from vasculitis. It also occurs more frequently in men with family members, who have connective tissue disorders like Dupuytren's contracture or systemic lupus erythematosus. An association between these conditions and the HLA-B27 histocompatibility antigen has been discovered suggesting a genetic or autoimmune link in the causation of Peyronie's disease.[82] Researchers from the Tulane University School of Medicine, New Orleans, have postulated potential roles of the NOS (Nitric oxide synthase) isoforms in the pathophysiology of Peyronie's disease, with particular emphasis on the regulation of endothelial and inducible NOS isoforms.[82]

The side effects of a number of drugs such as the beta-blockers for blood pressure, interferon for multiple sclerosis, phenytoin for epilepsy, etc, are also incriminated in the etiology of Peyronie's disease, but the possibility of developing Peyronie's disease from any of these medicines are very low (Table 10.5).

Table 10.5: Possible etiology of Peyronie's disease

1. ***Trauma***: Often an overt history is absent. Commonest form - Hitting or bending of erect penis during intercourse.
2. ***Vasculitis*** in the area of the tunica albuginea leads to scar tissue formation. Trauma often predisposes to vasculitis.
3. ***Autoimmune disease*** - Like other connective tissue diseases. 30% may develop fibrosis in other parts and 10% Dupuytren's contracture.

Probably, all sexually active men experience some degree of wear and tear, but structural arrangement of the corpora and inherent elasticity of tissues counteract the strong mechanical stress, till inherent connective tissue elasticity is on the wane.

Genesis of Bending of Penis

Complex mechanism of bending of an erect penis somewhat can be explained by the normal functioning of the erection mechanism in the corpora cavernosa. This cigar shaped, paired balloon-like chambers must inflate with blood to create an erection. Their connective tissue wall, or tunica albuginea, produces rigidity only when maximally stretched. It is elastic to a point, but unlike the flimsy wall of a balloon, tunica albuginea is interlaced with strong connective tissue fibres. These fibres control expansion, determine the shape of the erect penis, and translate internal filling into structural rigidity (Table 10.6).

Underlying mechanism of the ED in Peyronie's disease is venous leakage. The blood that should normally be trapped within the taut confines of the tunica albuginea to maintain erection slowly leaks out (Figs 10.4A,B Plate 4).

The plaques by causing local hardening of the tunica may prevent the exit veins from pinching off in the normal fashion. When fully expanded, the rigid corpora cavernosa forms something like an inflatable "I" beam. Mechanical forces on this structure will create a unique area of tissue stress at the top of the "I". Peyronie's plaques most commonly appear along the top of the penis. It is this region between the paired corpora, along the upper edge of the "inflatable I-beam" created by their inflation that is vulnerable to stress-induced delamination (Figs 10.3, Plate 4). Delamination at this point from any buckling stress or injury is common because of the bilaminar structure of the tunica albuginea covering the corpora cavernosa (see Chapter 4, penile anatomy).

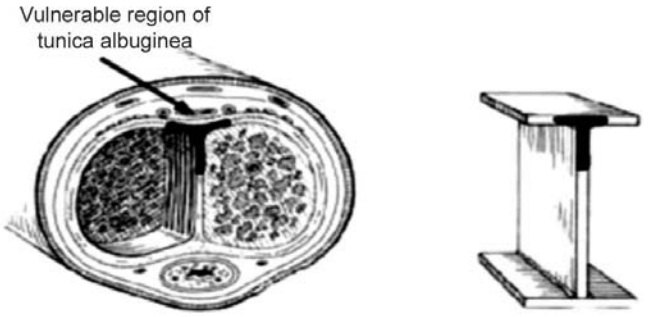
The ventral portion of the tunica is monolaminar and devoid of its longitudinal layer. This also contributes to proneness of the top area to delamination. Relative avascular nature of the tunica albuginea is likely to impede clearance of TGF β factor, which tends to cause lingering of any inflammatory process once that sets in.[83] Histologically, this is the region, where fibrin (Fig. 10.2, Plate 4) involved in activating wound healing, is often found in men with Peyronie's disease.

Major portion of the tunica albuginea gets compressed with stretch during erection, but the top most strip is subject to an opposite delaminating force. The mid-top of the penis is the area most commonly involved by Peyronie's disease (Figs 10.5A and B). If Peyronie's plaque forms in the hoop (circumferential) direction, it causes indentation or segmental loss of penile diameter. These so-called hour-glass areas have a profound effect on overall penile rigidity. The resistance to bending of an inflatable tube is related to its cross-sectional area. But the indented areas or plaques of Peyronie's disease make it easier to bend an erect penis even at high internal fluid pressures[82-92] (Fig. 10.6A, B).

Regardless of their composition, they alter the shape of the distended corpora cavernosa and distort the resulting erection. By looking at the expanding corpora as a series of stacked elements, it is possible

Table 10.6: Why does a penis bend to cause erectile problem?

1. Cigar shaped, paired balloon-like chambers (corpora) must inflate with blood to create an erection.
2. Their connective tissue wall-tunica albuginea, produces rigidity only when maximally stretched.
3. Connective tissue fibres of tunica albuginea are elastic to a point and control expansion, determine the shape of the erect penis, and translate internal filling of blood into structural rigidity.
4. By producing firm areas, or plaques, it focally interferes with the expansion of this normally pliant tunica albuginea material.
5. Like a piece of cellophane tape on the wall of a balloon, they cause uneven inflation and bending.



Figs 10.5A: Vulnerable top portion of penis at the area of 'I' beam.

Figs 10.5B: Top edge of crura showing vulnerability to delamination like an inflatable 'I' beam.

to calculate how much tunica must lose its elasticity to produce a given amount of bending. A plaque about six centimetres long is required to produce a 90-degree bend. In other words, little plaques cannot cause big bends (Fig. 10.6A and B). Microscopic and chemical studies have shown that plaques represent stages of the wound healing process, whether early or late. Peyronie's disease appears to be result of occurrence of inappropriate or dubious modification of this normally healthful process.

Diagnosis

An urologist is expected to confirm Peyronie's disease without taking recourse to any test as it has a characteristic history—thickened area in the penis causing its bending and painful erection. In some cases, pain may be absent. Specific findings on examination are one or more hardened areas, or plaques within the wall of the erection chamber and reduced elasticity of the flaccid penis.

History of any overt trauma, especially to an erect penis, should always be inquired. This can range from painful unexpected angulations during sex, to actual rupture of the corpora cavernosa, that may produce immediate loss of the erection, and subsequent severe swelling. Mostly, men with Peyronie's relate no such occurrences.

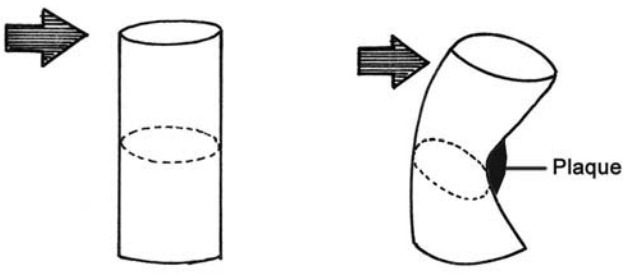
During the initial six months of the disorder, erections can be painful. Eventually, even in the absence of treatment, the pain usually goes away. Unfortunately, bending does not always follow the same pattern. Though it may improve or resolve spontaneously in a minority of men, most untreated men with Peyronie's disease will retain some degree of penile distortion.

Photographs of an erect penis taken by the patient at home, can establish the degree and type of distortion present. This is an important record of the condition helpful in assessing therapeutic response. X-rays or ultrasound pictures are not mandatory, but they can show calcification of the plaque. However, US findings can be used for following the size of the plaque, as there is a potential of reversibility of the condition in some cases and more importantly, when surgery is being considered. Testing the integrity of the erection mechanism (see PIPE test in Chapter 4 and 5) is occasionally recommended prior to surgery. Penile palpation in combination with ultrasound represents the method of choice to diagnose plaque formation in Peyronie's disease. MRI provides better information on plaque formation at the penile base. Calcification can only be proven by ultrasound, not by MRI, which however, may give additional information about local inflammation[93] (Fig. 10.7).

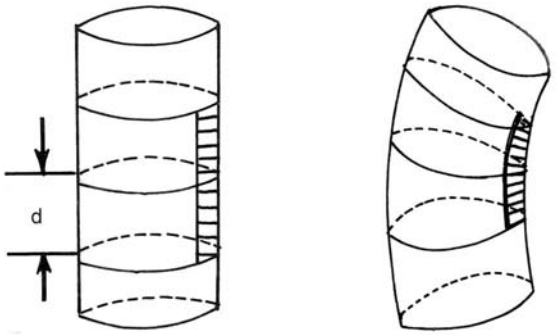
TREATMENT

The ultimate goal of therapy is to keep the Peyronie's patient sexually active. The course of Peyronie's disease is different in each patient, and some patients experience improvement without treatment.

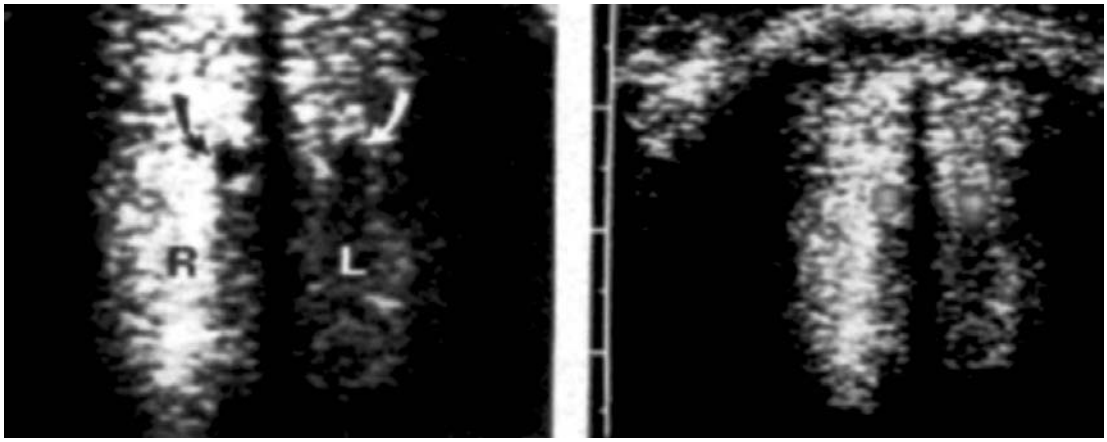
Medical experts suggest waiting for 1 to 2 years before attempting to correct it surgically. During this waiting period, patients are often put on other methods of nonsurgical treatment. In general, changes in tissue elasticity that accompany the inflammation of early Peyronie's disease are reversible, whereas the loss of elasticity associated



Figs 10.6A: Internal fluid pressure causes hourglass like bending if there is a plaque



Figs 10.6B: Longer the plaque greater is the bending



Figs 10.7: Plaque seen in left crus in CDU causing distortion; right crus is normal

with the end-stage scarring characteristic of the later illness is not. Since it is a consequence of local change in elasticity, bending responds best to medical therapy in the early stage, a period that generally lasts for about six months.

A wide variety of nonsurgical treatment options are available to the practicing physician, including oral and topical medications, intralesional injection therapy (directly or by iontophoresis), radiotherapy or direct stimulation of plaque by shock wave therapy.

Peyronie's disease has been treated surgically with some success. Some men choose to receive an implanted device that increases rigidity of the penis. In some cases, an implant alone will straighten the penis adequately. In other cases, implantation is combined with a technique of incision and grafting or plication (pinching or folding the skin), if the implant alone does not straighten the penis.

Most types of surgery produce positive results, notwithstanding the complications of surgical procedure and its failure to correct many of the phenomena associated with Peyronie's disease (for example, shortening of the penis). Consequently, most surgeons prefer to perform surgery on small number of men and only, when the penile curvature prevents sexual intercourse.

Conservative Medicinal Treatment

Over the years, a wide variety of medications have been used to treat Peyronie's disease. Some patients have benefited, while others have found that these medications seemed to make little difference. This variation in treatment outcome and the resulting lack of consensus regarding the drug of choice reflects in part an uncertain strategy, as till now doctors are unsure about the precise cause of the disorder in an individual case. However, there is near unanimity in the understanding of the disease-producing process in all men.

In order to evaluate medical treatment, one needs to take stock of how a case of Peyronie's disease evolves on its own in the absence of therapy. Knowledge of the natural history of the disease provides a basis for comparison, and a means for deciding, when surgical intervention may be appropriate. Most medical treatments have a success rate of about 60% in improving bending. Presence of Dupuytren's contracture, heavy calcification of plaque and severe (greater than 45 degrees) curvature are common factors associated with a tendency for the bending to persist.

Some researchers have given vitamin E orally to men with Peyronie's disease in small-scale studies and have reported improvement. Yet, no controlled studies have established the effectiveness of vitamin E therapy. Similar success attributed to oral para-aminobenzoate, a substance belonging to the family of B-complex molecules, is also doubtful.

Researchers have injected chemical agents such as verapamil, collagenase, steroids, Interferon and calcium channel blockers directly into the plaques. Verapamil has occasionally caused keloid at the injection site. These interventions mostly include a small number of patients and lacked adequate control groups and thus, are still not universally accepted. Steroid or cortisone, have produced unwanted side effects like the atrophy or death of healthy tissues. Another intervention involves iontophoresis, the use of a painless current of electricity to deliver verapamil or some other agents into the plaque.

Nevertheless, nonsurgical treatment of Peyronie's disease clearly has a place in the armamentarium of practicing urologists. Yet, no single nonsurgical treatment stands out as the most effective remedy for all men with this disease.

Contemporary Medications

Some of the contemporary medications in use are shown in Table 10.7.

Table 10.7: Contemporary medications for Peyronie's disease

1. Vitamin E
2. Colchicine
3. Potaba or potassium para-amino benzoate.
4. Verapamil
5. Collagenase
6. Tamoxifen
7. Corticosteroid and combinations
8. Propoleum
9. Interferon-Alpha 2β.

1. *Vitamin E:* This antioxidant has other uses in the treatment of scars, and has been employed in the treatment of Peyronie's disease since 1945. Vitamin E is supposed to promote healing and prevents scarring.
2. *Colchicine:* It is a medication used for many years in the treatment of gout. It acts against inflammation, and interferes with the formation of scar tissue.

   Kadioglu et al and Prieto Castro et al claimed that the use of colchicine plus vitamin E during the early stages of Peyronie's disease (time from onset < 6 months) in patients with penile curvature of < 30 degrees and no ED, is an effective and well-tolerated way to stabilise the disease.[80,95]
3. *Potaba or potassium para-amino benzoate:* This is designated by the Food and Drug Administration (FDA) as "possibly effective". Large doses are often required and can cause intestinal upset.[96]
4. *Verapamil:* It is usually given by direct injection into the plaque and is supposed to interfere with the synthesis of scar tissue precursors. Intraluminal Verapamil is given at biweekly dose through multiple intralesional puncture to ensure proper distribution of the medicine. A total of 10 ml may be required in one session (1 mg/ml).[97,98] Employing external energy sources to drive medicine into the tunica albuginea by iontophoresis have been found to be superior to direct injection by some workers.
5. *Collagenase:* It is an investigational drug that enzymatically digests scar.[99,100]
6. *Tamoxifen:* Tamoxifen treatment may benefit early disease. Inflammation in the subintimal layer activates cytokine network like TGF, which suppresses inflammation and fibrinogensis. Administration of Tamoxifen increases the TGF *in vitro* and *in vivo*.[101, 102]
7. *Corticosteroid and combinations:* Peyronie's diseases were treated by some with ultrasound using hydro-cortisone ointment as the vehicle with some success in 19 out of 30 patients. Lamprakopoulos et al used bethamethasone with hyaluronidase for intra-lesional injection.[103] However, Cipollone et al opined that the data showing usefulness of corticosteroid therapy in some series were due to the mechanical effect of injected volume and not to the drug action itself.[104]
8. *Propoleum:* Lemourt Oliva et al reported some success with Propoleum powder (300 mg) administered for 6 months in patients with Peyronie's disease. Propoleum was accidentally found to be effective in improving penile curvature in a patient with Peyronie's disease, who received propoleum for a giardiasis infection.[105]
9. *Interferon-alpha 2β:* Results of intralesional IFN-alpha-2β injections in improving ED were found to be promising by some researchers, especially in the early noncalcified stage. It had demonstrable impact on the plaque and also alleviated the plaque-associated pain.[106-108] On the contrary, Brake et al[109] found the results of this mode of therapy generally unconvincing for the conservative treatment of Peyronie's disease.

   We have found reasonably good result using local injection of Tricort (Triamcinolone) with Hyalase into the plaque and combined this with other oral medications such as vitamin C and Vitamin E. Follow-up of these cases had been done with ultrasonography to assess the progress of the plaque (incidence 9 out of 2178 cases).
10. *Extracorporeal shock wave therapy* (ESWT) is one of the new methods of treatment for Peyronie's disease. Some workers have recommended ESWT as an effective and safe treatment for this condition.[110, 111] The initial results with ESWT are promising with minimal complications, but the long-term follow-up and results are awaited. At present, it cannot be recommended as a standard procedure for Peyronie's disease as data

pertaining to ESWT are limited and do not appear to be significantly effective for decreasing penile curvature and plaque size, or improving sexual function in the total population of patients with Peyronie's disease despite improvements in some individuals.[112]

11. *Radiation therapy*, in which high-energy rays are aimed at the plaque has also been used. Like some of the chemical treatments, radiation appears to reduce pain, but it has no effect at all on the plaque itself and can cause unwelcome side effects.

Although the variety of agents and methods used points to the lack of a proven treatment, new insights into the wound healing process may one day yield more effective therapies.

Surgical Treatment

The two most common surgical procedures are removal or expansion of the plaque followed by placement of a patch of skin or artificial material, and removal or pinching of tissue from the side of the penis opposite the plaque, which cancels out the bending effect. The first method can involve partial loss of erectile function especially the rigidity. The second method, known as the Nesbit procedure, causes a shortening of the erect penis.

Some practicing urologist recommend a rigiscan to compare the base to the tip erection. If the penis is severely bent, but the erection is firm, only a straightening procedure is recommended. If it is so severely bent that the tip is soft, surgically straightening the penis would require an additional method to improve erections such as a vacuum erection device or penile injections. A penile implant is recommended in men, who do not wish to have additional treatments.

In simple terms, surgery for Peyronie's disease is not a curative measure. By the time surgery is contemplated, the scarring has run a fair distance with irreversible loss of elasticity. Notwithstanding the benefit of surgery in restoration of sexual function in most cases, there are specific risks involved in any surgical procedure, however effective and reliable they may be. Each patient should be apprised of the compromise inherent in this approach, as different operative procedures do not guarantee restoration of the length of the erected penis to its normal predisease state, because of the loss of elasticity.

Some procedures shorten the penis more than others; and in real terms, there is always a risk of less than perfect straightening. Moreover, at times, there may be some degree of temporary loss of sensation due to the anatomical location of sensory nerves in the penis. There is also an inherent risk of interference with the blood inflow or outflow, causing either loss of erectile rigidity (hardness) or inability to maintain erection.

Before embarking on the surgical treatment of Peyronie's disease, a surgeon must take into consideration, four basic criteria—severity of the disease, adequate time for resolution, adequate trial with medical therapy, and stable state of the disease. Firstly, the risks and expense of an operation only make sense, when bending or deformity is severe enough to seriously interfere with sexual function. Secondly, natural healing or spontaneous resolution should have been given an opportunity. This is usually accomplished by deferring the decision for surgery at least twelve months from the time of onset. During this time, the third criteria can be satisfied—an adequate trial with some form of medical therapy. Finally, no operation should be done on a man, whose condition is changing, either for the better or the worse. The best surgical outcomes are seen in men with stable condition.

Surgical Options

Nesbit Procedure

This operation or its various modifications, corrects bending by plicating (gathering) the convex or outer side of the bend. Counteracting the relative shortage of tunica albuginea on the concave side straightens the penis, though length is reduced slightly as a consequence. Still, this procedure is less likely to cause ED than tissue grafting, and remains the first choice for moderate bends without associated diameter reduction. It is the best way to surgically correct any form of congenital curvature.

Tissue Grafts

These procedures involve the replacement or expansion of scarred tunica albuginea with grafts of healthy tissue from another site. Originally, grafts were used to repair the defect that remained after excision of a Peyronie's plaque. More recently, surgeons have been using grafts to expand the contracted scars (or plaque) without excising them. Though some calcified plaques still require removal, this nonexcisional approach seems less disturbing to erectile function, and less likely to cause postoperative

ED. Despite this risk, grafting remains the most versatile reconstructive technique, particularly suited to correcting severe bending and/or diameter constriction.

Implant Surgery

Penile implants are biocompatible plastic cylinders, either solid or inflatable, that are surgically implanted into the corpora cavernosum to produce a functional erection. Penile prosthesis implantation is a safe, simple, effective technique for straightening penile curvature in patients with Peyronie's disease. It causes low morbidity in restoring potency in men with ED.[113] Prior to the advent of newer medical remedies for ED, the implants were the first choice for Peyronie's patients, who had trouble keeping an erection. Presently, they are used less frequently. However, in men, who do not respond to drugs, penile prosthesis implantation remains an excellent option. (See Chapter 5 on penile implants).

Conclusion

Variety of agents and methods used, and lack of consensus amongst workers in the choice of any proven treatment are ample testimony to the enigmatic status of Peyronie's disease. Investigators should now seriously look for new insights into the wound healing process to innovate methods for treatment of idiopathic fibrosing conditions. This knowledge will undoubtedly open new vista to establish precise etiology and widen scope for a definitive treatment of Peyronie's disease.

BICYCLE INJURIES

Classic biking-related perineal injuries may cause urethral bleeding and stricture formation, perineal numbness, pudendal neuropathy, scrotal injuries, testicular trauma, prostatitis and ED.[114, 115] In particular, the association between biking and ED has been suggested by case reports, observational studies, case-control studies, mechanistic studies and community-based population studies. Although several case reports relate biking to ED, there are limited case-control epidemiologic studies on the health effects of bicycle riding, especially on any associated risks of permanent sexual and urinary tract dysfunctions.

Whenever the bicycle rider is supporting body weight on the narrow straddle-type bicycle seat, the perineum is compressed between the seat and the ischiopubic ramus portion of the bony pelvis. The male perineum contains important anatomical structures, such as the penile crurae, the common penile and cavernosal arteries, the scrotal sensory nerves, and the bulbous and membranous urethra. In particular, the penile crurae are directly attached proximally to the bony ischiopubic rami (*see* Fig. 4.1).

Bicycle riding has been associated with two types of blunt perineal traumatic injuries. A perineal crush injury occurs, when the bike rider's perineum suddenly hits the bicycle top tub or seat, due to a fall from a certain height. During this crush injury, the bicyclist with a specific mass and is moving at certain velocity, thus possessing a determinable momentum. Since the bicycle seat and the top tub are hard surfaces with little padding, in a fall, there is abrupt stoppage of momentum for a short period of time. The result is a large force applied to the perineum.

Blunt chronic traumatic perineal injury following bicycle riding is more controversial and is associated with chronic compression injuries to the perineal contents without a recognised crush accident. In particular, the crural erectile tissue, common penile/cavernosal arteries and pudendal nerves may be prone to injury from chronic compression by virtue of their anatomical relation to the bony ischiopubic rami. Straddling a narrow bicycle seat differs substantially from an individual supporting body weight while seated on a chair. In this case, compression is applied to the gluteal muscles and buttock fatty tissues between the chair surface and the ischial tuberosity portion of the bony pelvis. Furthermore, no organs have direct anatomical attachment to the bony ischial tuberosities. Avid cyclists, who experience problems should change to a padded seat and take shorter, but more frequent, bike rides. New bicycle seats are being designed to minimise the risk of ED.[116]

Environmental Causes

These have already been discussed in Chapter 6.

EJACULATORY DYSFUNCTIONS (see also Chapters 4 and 5)

Ejaculation is a complex neuromuscular mechanism to deliver the sperm to the female reproductive system. The normal ejaculatory process requires coordination and integration of neurologic, physiologic, anatomic, and psychologic events.[117,118]

Ejaculatory pathways have been mentioned in Chapter 4 under "Ejaculatory dysfunctions" (Fig. 4.15). A concurrent sympathetic stimulation causes

closure of bladder neck, and contractions of prostate, ampullae of vas and seminal vesicle to propel semen into the prostatic urethra. Opening of urogenital diaphragm and rhythmic contractions of bulbocavernosus, ishiocavernosus and pelvic floor muscles under somatic control of pudendal nerve ($S_{2, 3 \text{ and } 4}$), finally expels the semen from the prostatic to the anterior urethra.

The role of nerve transmitter nitric oxide (NO) in the regulation of smooth muscle contractions of vas, seminal vesicles and prostate involved in ejaculation is possibly very important. Recent studies have shown that the enzyme nitric oxide synthase (NOS) catalyses the production of NO, which is abundantly present in prostate, vas, seminal vesicles and epididymis (see Chapter 4). Any breakdown of the ejaculatory pathways due to any abnormality (congenital or acquired) that disturbs the delicate coordination would result in ejaculatory failure and subsequent infertility. Ejaculatory dysfunctions may present in any of the following forms:

1. An-ejaculation (AE) is defined as complete absence of antegrade ejaculate.
2. Retrograde ejaculation (RE) is defined as ejaculate entering the bladder instead of coming out through its normal passage. Presence of sperms in the microscopic examination of urine after masturbation or intercourse confirms the diagnosis.
3. Premature ejaculation is inability to control ejaculation for sufficient length of time for intravaginal containment.
4. Ejaculatory incompetence or delayed or retarded ejaculation.

   These conditions have been discussed in details in Chapter 4.
5. Ejaculatory obstruction.

Causes of Ejaculatory Dysfunctions

These are mentioned in Table 10.8.

Table 10.8: Causes of ejaculatory dysfunctions

1. Congenital—anatomical abnormalities
2. Acquired:
Traumatic—accidental or iatrogenic
Metabolic
Pharmacological or drug induced
Inflammatory or infective
Functional

Traumatic

Accidental spinal cord injuries (SCIs) are the commonest form of trauma, where ejaculatory failure occurs in 90% of cases. Ejaculatory failure is almost invariably seen in a complete upper motor neuron (UMN) lesions, but its restoration is possible in some cases of incomplete UMN lesions. In partial or incomplete lower motor neuron (LMN) lesions majority of patients eventually are capable of erection and ejaculation, but only a few with complete LMN lesions recover completely to have satisfactory ejaculation.

Iatrogenic

Iatrogenic or postsurgical trauma can occur after retroperitoneal gland dissection, abdominal aneurysm repair, anterior resection of colon and abdominoperineal resection of rectum, transurethral or radical surgery on the bladder neck or prostate and posterior urethroplasty.

Metabolic

Diabetes is the commonest disease that causes ejaculatory dysfunctions such as anejaculation, retrograde or premature ejaculation (see Chapter 4). Infertility assumes importance in juvenile diabetes, as young persons are involved unlike the old age diabetics, who most probably have completed the family. Although the autonomic neuropathy is the principal underlying cause of the ejaculatory problem in diabetes, associated vascular abnormalities also play an important role. Recently, changes in the endothelial smooth muscle and depletion of 'NOS' in the cavernosal tissues in diabetics have been cited as incriminating factors.

Pharmacological (Drug induced)

Several drugs such as antihypertensives (beta blockers, calcium channel blockers, methyldopa), diuretics taken for heart disease and hypertension and digoxin for heart failure can cause erectile and ejaculatory problems. Besides, psychotrophic drugs acting on the sympathetic nerves may disrupt ejaculatory function.

Obstructive

Clinical diagnosis of ejaculatory duct obstruction rests on triad of small ejaculate volume, azoospermia and absence of seminal fructose. It is singularly important not to omit examination of the scrotum for the presence of vas. Testes, epididymis and serum levels of testosterone and FSH are usually normal. The

diagnosis ultimately is confirmed by the presence of normal spermatogenesis in a testicular biopsy, TRUS and MRI.

Treatment of Ejaculatory Dysfunction

Conservative

The medicines that are known to cause ejaculatory dysfunction should be withdrawn. Ejaculation with full bladder may help in some cases of retrograde ejaculation. Use of α-adrenergic sympathomimetics, which act by increasing the closure pressure of the internal urethral sphincter through release of norepinephrine, has been effective in some cases. Sperm quality in patients with retrograde and AE is often impaired. Compared with ephedrine, imipramine and chlorpheniramine with phenylpropanalamine showed significantly higher reversal rates. For the reversal of AE, the alpha agonistic drugs were significantly inferior to treatment with parasympathetic drugs. Of the different alpha agonistic medical treatments for the reversal of AE, milodrin showed significantly better results than imipramine, pseudoephidrine and ephedrine. Other medicines used are tricyclic antidepressants, which block re-uptake of norepinephrine to increase the adrenergic activities.[119] Neostigmine, an acetylcholine esterase inhibitor injected intrathecally acts directly on the anterior horn cells. Intracavernosal injection of papaverine, phentolamine or prostaglandin E_2 has also been used with success in many cases of ejaculatory dysfunction.

In AE, medical treatment cannot be recommended generally as the treatment of first choice. It shows low overall success rates compared with electrovibration stimulation and electroejaculation. In contrast, medical treatment for reversal of RE offers a realistic chance of conceiving offspring naturally and should be the first choice treatment modality. However, with the advent of assisted reproductive technology (ART) many cases of RE can be treated successfully by extracting sperms from the urine and then by sperm processing. For the collection of sperms for IVF or IUI, buffer solution like Ham's F-10 solution is put into the bladder prior to ejaculation to obviate the deleterious effect of acid urine on the sperms. Functional cases need to be treated with psychotherapy.

Surgical

Plastic reconstruction of the bladder neck to restore proper its functioning may be necessary for extensive scarring. However, results are not very satisfactory. Resection by de-roofing or resection of verumontanum for cystic lesions of ejaculatory ducts is one of the methods that have been used in the past with indifferent results. However, it may be combined with vaso-epididymal anastomosis after such resection with some success. Any epididymal anastomosis is always a challenging proposition as epididymal mucosa lacks the muscular support to give strength at the site of anastomosis.

Electromedical (Penile Vibratory Stimulation and Electroejaculation)

Penile Vibratory Stimulation

Ejaculatory dysfunction is almost universal sequela of spinal cord injury (SCI) in men and the majority of these men are not able to produce antegrade ejaculation by masturbation or sexual stimulation (see Chapter 4). Besides neurological conditions such as diabetes mellitus, and transverse myelitis, multiple sclerosis, psychological overlay may be associated with ejaculatory dysfunction. Penile vibratory stimulation (PVS) and electroejaculation (EEJ) have been tried extensively in the animal husbandry. In recent years, both these procedures have been in successful clinical use in men with ejaculatory dysfunction, who are unable to ejaculate and to impregnate the female partners.

Penile vibrator is used to collect ejaculate for subsequent artificial insemination with intrauterine insemination (see Chapter 13). The vibrator is moved gently after placing it on the under surface of the glans till a trigger point is found to initiate ejaculatory sequence to express the semen. Approximately 80% of all SCI men with an intact ejaculatory reflex arc (above T_{10}) can obtain antegrade ejaculation with PVS. However, it often fails during initial six months of spinal injury and in lesions below T_{10}. Its efficacy can be tested by noting penile tumescence and contraction of external urethral sphincter (EUS) and bulbocavernosus evidenced by increase in the EMG activity. According to Shafif,[120] the rhythmic EUS activity at ejaculation might act as a suction ejection pump, sucking the genital fluid into the posterior urethra while being relaxed, and ejecting it into the bulbous urethra upon contraction.

The vibratory stimulation is very simple in use, noninvasive and does not require anaesthesia. The patients also show preference for PVS, when compared

with EEJ. Moreover, the semen quality is better when obtained by PVS compared with EEJ.[121] Consequently, PVS is recommended to be the first choice of treatment in spinal cord injuries.[122, 123]

Electroejaculation

Electroejaculation (EEJ) works by stimulating the sympathetic efferent nerve fibres. Best result is obtained with the stimulator placed between the bifurcation of the aorta between the rectum and the obturator nerves, as this area coincides with abundance of both pre- and postganglionic sympathetic nerve fibres. In practice, the rectal EEJ probe is positioned inside the anal ring with the electrodes placed against the prostate gland and seminal vesicles. However, with this method, the semen often comes in dribbles instead of a spurt, as the closure of bladder neck does not occur concurrently.[124]

Electroejaculation may be successful in obtaining ejaculate from men with all types of SCI irrespective of its duration and the site, including men, who do not have major components of the ejaculatory reflex arc.[125]

Furthermore, EEJ has been successfully used to induce ejaculation in men with multiple sclerosis and diabetic neuropathy. Any other conditions, which affect the ejaculatory mechanism of the central and/or peripheral nervous system including surgical nerve injury, may be treated successfully with EEJ. This technology is considered the best approach in patients with SCI below T_{10} or in other patients where PVS fails.

Presently, refinement of PVS and EEJ methods has significantly enhanced the prospects for treatment of ejaculatory dysfunction in men with SCI. It has been used at Tulane University (Louisiana, USA) with Perkash et al[126] using computer programme to control the rhythmic delivery of semen. Presently, the fertility potential of majority of men has been further enhanced with combined use of either PVS or EEJ with assisted reproduction techniques such as intrauterine insemination or *in vitro* fertilisation with or without intracytoplasmic sperm injection (ICSI).[127] These methods certainly have provided hopes for these men to have their own biologic children.

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APPENDIX 1

DNA, RNA, Gene, Genome and Gene therapy [128, 129]

(Fig. A10.2, Plate 4)

DNA

DNA is composed of two side-by-side chains of nucleotides. It is shaped like a twisted ladder into a double helix. The double helix structure was discovered by Dr. Watson in 1953, and consists of two intertwined anti-parallel and complementary nucleotide chains each consisting of a sugar-phosphate backbone with bases hydrogen-bonded with complementary bases of the other chain. DNA and proteins are key molecules of the cell nucleus. A half DNA ladder is a template for copying the whole. Chromosomes carry genes. RNA is an intermediary between DNA and protein synthesis. Approximately, there are 30,000 genes in human DNA.

Every cell in the body has the same genetic makeup. Consequently, the genetic material must be faithfully duplicated at every cell division that is ensured by the structural feature of DNA. The genetic material must have informational content, since it must encode the constellation of proteins expressed by an organism. The structure of DNA must be relatively stable, so that a particular species or organism can rely on its encoded information. However, on rare occasions it also allows the coded information to change. These changes called *mutation* provide the material for genetic variation. The observed effect of an abnormal gene, which leads to a disorder or dysfunction, is called the abnormal phenotype.

GENE

A *gene* is a discrete sequence of DNA nucleotides. One gene codes for one protein, and is made of DNA. A gene is a chromosomal region capable of making a functional transcript. Genes come in pairs, do not blend and get shuffled, when chromosomes exchange pieces. A gene is a region of the chromosomal DNA that can be transcribed into a *functional RNA* at the correct time and place during development.

RNA only becomes properly functional, if it is also made appropriately at that correct time and place during the development of the organism. This function is only achieved as the gene has a *regulatory region,* a segment of DNA with a specific nucleotide sequence that enables it to receive and respond to signals from other parts of the genome or from the environment.

GENOME

A *genome* is an entire set of genes and is composed of one or more DNA molecules, each organised as a chromosome. The nuclear complement of the genome consists of one or two sets of linear chromosomes.

GENETIC INHERITANCE

Genes, which are carried on chromosomes, are the basic physical and functional units of heredity, and are the specific sequences of bases that encode instructions on how to make proteins. There are only four basic patterns of single gene inheritance: autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive.

In *autosomal dominant* inheritance, the abnorma-lity or abnormalities appear in every generation.

In *autosomal recessive* inheritance, the parents of an affected individual may not express the disease. For a child to have symptoms of an autosomal recessive disorder, the child must receive the recessive gene from both parents.

In *X-linked recessive inheritance*, the incidence of the disease is much higher in males than females. Since the abnormal gene is carried on the X chromosome, males do not transmit it to their sons, but do it to all their daughters. The presence of one normal X chromosome masks the effects of the X chromosome with the abnormal gene. So almost all of the daughters of an affected man appear normal, but are all carriers of the abnormal gene.

In *X-linked dominant* inheritance, the presence of the defective gene makes itself manifest in females even if there is also a normal X chromosome present. Since males pass the Y chromosome to their sons, affected males will not have affected sons, but all of their daughters will be affected.

GENE THERAPY

Gene therapy is a technique for correcting defective genes responsible for development of disease. Researchers use one of several approaches for correcting faulty genes. Gene therapy, by replacing a defective gene or altering it, have the advantage of actually treating the cause of an illness, not just the symptoms.

In most gene therapy, a normal gene is inserted into the genome to replace an abnormal disease-causing gene. The most common approach involves insertion of a normal gene into a nonspecific location within the genome to replace a nonfunctional gene. In other two methods, either an abnormal gene could be swapped for a normal gene through homologous recombination, or the abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function. In the fourth method, the regulation (the degree to which a gene is turned on or off) of a particular gene could be altered. The risk of stimulating the immune system that reduces effectiveness of gene therapy is always a potential risk.

(Fig. 10.8, Plate 4)

Courtesy- FDA Consumer Magazine -healthfinder@nhic.org

CHAPTER 11

Medical and Nonsurgical Management in Male Infertility

INTRODUCTION

When one deals with a complex subject like male infertility, where the result of the surgical treatment is sometimes unpredictable and capricious, one naturally looks for other alternatives. Scope of the non-surgical or medical treatment for male reproductive disorders like infertility thus needs a looking into for discussion.

SURGICAL INTERVENTION

Indubitably, surgical intervention has its role in correcting some of the abnormalities; but when the causative factors are due to abnormalities of hormones, defective immune mechanism, nutritional deficiency, or toxicity caused by the environment, the role of medical treatment cannot be overlooked; and probably, they have a definite role. Although many medical therapies have been advocated, no regimen has proved to be consistently effective in the treatment of male infertility. Broadly, the non–surgical or medical management can be grouped under three broad heads (Table 11.1).

Table 11.1: Medical (non-surgical) management

1. Preventive—Smoking, alcohol, occupational and environmental hazards.
2. Curative-
   a. Hormone—Hormone replacement or manipulation (TRT or Anti-estrogen receptors).
   b. Erectile dysfunction and Immunological abnormalities.
   c. Infections of the urogenital tract
   d. Sperm Nutrients.
   e. Sperm motility enhancer.
   f. Miscellaneous—TCM, acupuncture, ayurvedic preparation and herbs.
3. As an adjunct to surgery.

Preventive Measures

Smoking and Alcohol

Deleterious effects of smoking and alcohol have been discussed in Chapter 6. One of the common problems that an andrologist faces is patients' propensity to cite instance of their friends and associates indulging in smoking and alcohol, and still being able to have biological offspring. It is important to explain to them that effects of smoking and alcohol cannot be quantified or qualified, as they are person-specific. Close association between smoking and low sperm count, poor sperm motility, and abnormal sperms are now well established. Alcohol consumption beyond certain limits can lead to premature ejaculation, even anejaculation, and is associated with increased number of defective sperms. Consequently, strict restrictions on smoking and alcohol should be one of the first steps in preventive measures to be advocated.

Occupational and Environmental Hazards

Male reproductive system is known to be highly sensitive to some physical and chemical agents (see Chapter 6). Unlike the small laboratory animals, human spermatogenesis is more vulnerable to the

toxic actions of some chemicals. Numerous drugs and medications have been shown to have adverse effects on male fertility, acting through diverse mechanisms. Simply discontinuing the offending agents can reverse most adverse effects from drugs and medications.[1] Occupational exposure in work environment (occupational hazards) to many chemical argents or substances may damage the sperm-producing testicular germ cells causing infertility. Potential toxicants that have been detected in semen include: lead, cadmium, hexachlorobenzene, hexachlorocyclohexane, dieldrin, dibromochloropropane (DBCP), other pesticides, organic solvents, and polychlorinated biphenyls. Thus, environmental pollution has been often and justifiably incriminated for the deterioration of sperm functions. A list of chemical agents and substances known to cause injurious effects on sperms have already been mentioned in Chapter 6. In addition, there are many medicines or drugs that can interfere with the reproductive functions in male. Chelation therapy (therapy for metal poisoning) with EDTA (ethylenediaminetetraacetic acid) or BAL (2,3 dimercaprol: British anti-lewisite) may be helpful for the systemic effects of lead toxicity, although reproductive benefits have yet to be documented.

HORMONAL CORRECTIONS

Endocrinal defects account for 10% of male reproductive disorders from developmental or chromosomal defects.[2] The role of hormones in male reproduction (see Chapter 3) mainly has two components—testicular and extratesticular (Table 11.1).

Effective therapies are available for the treatment of infertility owing to specific causes. Most hormonal imbalances can be readily identified and successfully treated, but treatment with gonadotrophin-releasing hormone (GnRH) analogues, gonadotrophins, androgens, antiestrogens, aromatase inhibitors, growth hormone and prolactin-suppressing drugs is ineffective in unselected infertile men. The endocrine conditions having effects on the male reproductive dysfunctions are listed in Chapter 3.

Adequate testicular androgen from Leydig cells is required to coordinate the intratesticular control mechanism and cell interactions. Effective spermatogenesis can only be achieved only, if there is high intra-testicular testosterone, which is dependent on adequate follicle-stimulating hormone (FSH). Normal pituitary function ensures adequate secretions of FSH and luteinising hormone (LH), which regulate the activities of Sertoli and Leydig cells of the testis.

There remains a potential for hormonal methods to improve sperm quality or ultrastructure in subgroups of infertile men more responsive to hormonal manipulation or using novel protein gene-targeted therapies or biochemical approaches based on post-hormonal receptor mechanisms that stimulate spermatogenesis. Hormonal therapy appears to be a logical approach for empirical drug therapy given the fundamental role of hormones in regulating spermatogenesis. Endocrine therapy for male reproduction dysfunction is formulated under two heads—hormone replacement and manipulation.

HORMONE REPLACEMENT

Hormone replacement therapy involves replenishment of endogenous production of hormones. Various hormone products or derivatives of androgen, gonadotrophin, GnRH or thyroxine are used in different forms for therapeutic purpose.

Androgen Derivatives

Pure androgen is not absorbed orally, so the structures in synthetic androgen derivatives are modified to facilitate oral absorption. These substances cause decrease in aromatisation of estrogen and thus increase the concentration of 5-alpha dihydrotestosterone (5αDHT). Orally, either Mesterolone (75 mg/day) or testosterone undecanoate (Andriol or Nuvir -120 mg/day) are used. The latter is preferred as it has minimal suppressive effect on the hypothalamus-pituitary function.

Gonadotrophin Derivatives

Two common fertility drugs used are human chorionic gonadotrophin (hCG) and human menopausal gonadotrophin (hMG). These are used to treat men with hormone deficiency of the pituitary gland. The hMG, injected two to three times a week, prompt the pituitary gland to secrete more LH and FSH. Exogenous gonadotrophin therapy is indicated in infertile men with hypogonadotrophic hypogonadism (secondary hypogonadism), who are appropriate candidates for the initiation of spermatogenesis. However, LH must be given to stimulate the Leydig cells to produce high intratesticular testosterone levels.[2]

hCG (Pregnyl or Profasi) 2000 IU intramuscularly three times a week, is usually effective in stimulating adequate production of testosterone for full virilisation. Once the patient is fully virilised and 8 to 12 months of hCG therapy have not led to the production of sperm, FSH therapy should be initiated. FSH is available as hMG in the commercial preparation Pergonal (Metrodin) containing 75 IU of FSH and 75 IU of LH per vial. The usual dosage is ½ to 1 vial intramuscularly three times weekly. Since hCG and hMG are compatible in solution, the same syringe may be used. It takes months for sperms to appear in the ejaculate after initiation of FSH therapy. With the normal response, most patients achieve a sperm count of 3 to 5 million sperms per ejaculate, which is the minimal requirement for some form of impregnation (see Chapter 13). Once the pregnancy has occurred, the FSH therapy can be stopped. Later spermatogenesis can be maintained with hCG alone. One uncommon, but treatable cause of male infertility is gonadotrophin deficiency in which gonadotrophin replacement therapy is highly effective at inducing spermatogenesis and fertility.[3]

GnRH Derivatives

In theory, GnRH should achieve a more physiologic pattern of gonadotrophin stimulation, but in practice, combined treatment with hCG and hMG is often necessary. An alternative to exogenous gonadotrophin usage is use of GnRH to stimulate LH and FSH endogenously. GnRH must be given in a pulsatile manner as continuous administration down-regulates the pituitary. The initial dosage is 25 to 50 ng/kilogram every two hours by a small infusion pump.[2] Gonadorelin is the common GnRH preparation that is used. It comes in two forms—0.8 and 3.2 mgm in powder form in a 10 ml vial. The pulse is set at 5 microgram per minute with one pulse at 90 minutes, and is given for 21 days in a cycle (usual dose is 1020 microgram). Both gonadotrophins and GnRH are expensive.

Although GnRH achieves a more physiologic pattern of gonadotrophin stimulation, its superiority is yet to be proved. For pituitary disease, combined treatment with hCG and hMG is necessary, as it is not often amenable to GnRH therapy alone.

Individuals of fertile eunuch syndrome (partial LH deficiency) may respond to hCG therapy alone.[2] Acetate form of GnRH is available as Lutre-Pulse, and its hydrochloride form as FACTREL. GnRH antagonists such as Cetrorelix, Ganirelix, etc., are used in IVF methods. GnRH-analogues such as Lupron, Leuprolide, Goserelin, Histrelin, Nafaerelin, Triptorelin, etc., are used in prostate cancer.

Thyroxine Derivatives

Endogenous depletion of thyroxine (hypothyroidism) can alter spermatogenesis. Commonly used preparation in the subcontinent is eltroxin, which is started with a minimum dose of 25 microgram daily and later it is varied by monitoring of TSH, T3 and T4, but a maintenance dose must be continued.

HORMONE MANIPULATION

In hormone manipulation, antagonists are used to counteract the effects of a hormone, and are indicated when there is excess of estrogen, prolactin, corticosteroids or thyroxin.

Antiestrogens

It is important to know the role of estrogen in spermatogenesis. In men, estrogen is formed either from the testosterone by the Sertoli cells through aromatisation (see Chapter 3) or from androstanediol in other tissues of the body, especially the adrenal cortex and liver (the latter accounting for as much as 80 per cent male estrogen production). Androgen-binding proteins secreted by the Sertoli cells bind both testosterone and estrogen, and carry these products into the seminiferous tubular fluid to play an important role in spermatogenesis (see Chapters 2 and 3).

Use of the antiestrogen becomes a necessity, when the estrogen is far in excess to its normal requirement, thus causing antagonist effect on the testosterone. Two types of drugs are used—one that interferes with synthesis of estradiol and the other that acts on the receptor cells.

Drugs interfering with estradiol synthesis are mainly of two types—one group known as aromatase inhibitor (testolactin) that inhibits synthesis of estradiol, and the second group includes drugs that block the effects on target cells (clomiphene citrate—a mixture of two isomers, and tamoxifen). Pharmacologically, tamoxifen is superior as it is devoid of any intrinsic estrogenic activity.

Rationale of Using Estrogen-receptor Antagonist (anti-estrogens) in Male Infertility

Presently, there is a growing apprehension that a segment of infertile males may have an unacceptably high level of circulating estrogen. The source may be

endogenous from decreased hepatic degradation or exogenous from the environmental sources such as foodstuff, cosmetics, pesticides, environmental pollution, etc. In an American study, it has also been established that smoking also raises the serum estradiol level. High estrogen level may have induced corresponding antitestosterone effects in patients of male infertility to cause oligospermia and related abnormalities.

It is theoretically a sound hypothesis that the spermatogenesis can be increased by indirectly stimulating FSH and LH secretions from the pituitary gland. But for this to fructify, it requires the use of testosterone antagonist. This would nullify the negative feedback effect of circulating testosterone on the release of FSH and LH, thus augmenting the secretion of testosterone and spermatogenesis. Unfortunately, a testosterone antagonist will be unacceptable to males, as it may reduce secondary sexual functions including erection and ejaculation that is vital for the successful fertilisation.

To prove the hypothesis that the high estrogen level induces antitestosterone effect in patients of male infertility to cause oligospermia and related abnormalities, a detailed clinical study was undertaken in oligospermic patients. From October 1996 till March 2000, serum estrogen (SE) and serum testosterone (ST) were studied in 333 oligospermic subjects. Results (Fig. 11.1 and Table 11.2) showed definite evidence of higher than normal values of estrogen with corresponding low serum testosterone levels in significant number of patients against age-matched controls. These patients were chosen for treatment with either clomiphene or lately tamoxifen.

Table 11.2: Total of 333 patients had SE and ST estimated against 30 subjects for age-matched control

Out of 333 patients—
SE levels
• 103 had between 40-60 pg.
• 202 had values above 60 pg (64 above 80 pg and 138 between 60-80 pg)
• 28 had below normal value of 40 pg
Correspondingly all 333 patients
ST levels
• 239 had normal value between 3-15 ng.
• 23 had values above normal.
• 71 had value below normal

Clomiphene and tamoxifen are two common drugs that are used in male infertility. Occasionally, hCG is also used. Clomiphene increases the FSH and LH levels, which are regulated in the pituitary, and the sperm production. Combination of tamoxifen with testo-sterone undecanoate has also been found to be improving significantly important seminal parameters and this is favourably compared with the single treatments used. According to Adamopoulos et al, this combination deserves a place as a first line of treatment in idiopathic oligozoospermia.[4] Drugs acting through testosterone rebound action that have an inherent risk of permanent azoospermia is not an acceptable initial therapy.[5]

Clomiphene citrate (Clomid or Serophene) acts by competitive inhibition of estrogen receptors within the cells, especially at the hypothalamic level by preventing the negative feedback action of the estrogen. It thus increases the synthesis and release of pituitary gonadotrophins—FSH and LH. However, at the pituitary level the clomiphene also has an estrogen agonistic action. Its response in different individuals thus remains unpredictable due to its dual antagonistic and agonistic actions at various levels that cannot be quantified beforehand in an individual.[6-8] This results in an increase in GnRH that stimulates gonadotrophin secretion. The resulting elevation in levels of LH and FSH stimulates intratesticular testosterone synthesis to improve spermatogenesis. When empiric therapy is decided upon clomiphene citrate is given in a dose of 25 to 50 mg a day for 3 to 6 months. Siddiq recommends that the dose may be increased to 50 or 75 mg to raise testosterone levels to the upper normal range.[9] The antiestrogenic effect of clomiphene causes increased production of A and B types of spermatogonia, primary spermatocytes and spermatids. However, at a daily dose higher than 100 mgm, the spermatid production is inhibited. This is the rationale behind keeping the daily dose to 50 mgm a day for oligospermic patients.

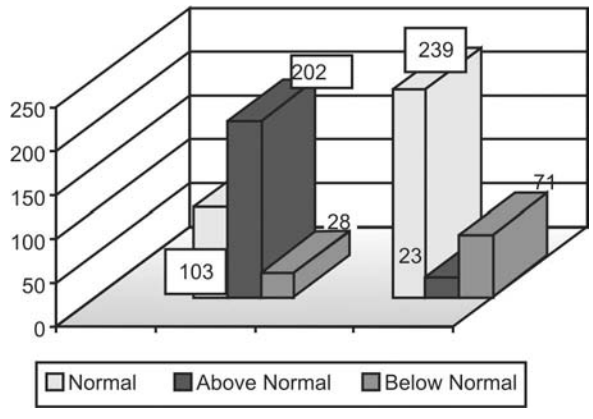

Fig. 11.1: Levels of ST and St in 333 patients

Tamoxifen blocks the action of the female hormone that is produced by the testicle in small amounts. Tamoxifen supposedly improves (less than 0.05) the progressive motility in all patient groups, where there is reduced pretreatment motility. In patients with normogonadotrophic oligozoospermia, tamoxifen citrate may be offered as a practical and economic alternative before using any assisted reproduction techniques (ARTs).[10] Sperm density also significantly improves in oligozoospermic patients.[11] In another study, tamoxifen treatment led to significant increase in the spermatozoa concentration and the total sperm count, but this could not be confirmed with the double-blind set-up.[12] Clomiphene citrate significantly decreases cervical mucus production, whereas tamoxifen significantly improves the total score. Tamoxifen is a better drug than clomiphene for ovulation induction in women with poor cervical mucus quality.[13] Multivariate analysis shows that the basal serum testosterone level is the only variable in predicting the probability of response to the antiestrogen therapy like clomiphene.[14] According to AinMelk Y et al, tamoxifen appeared to be no more effective than the placebo in the treatment of idiopathic oligozoospermia.[15]

There are also opposing views that the use of estrogen-receptor antagonists does not have any effect on the ultimate improvement in the prognosis of infertile males. The role of empirical treatment with antiestrogens like clomiphene for unexplained infertility continues to be debated with some studies stating that the presented data are inconclusive.[16] Vandekerckhove et al[17] are of the opinion that the anti-estrogens appear to have a beneficial effect on endocrinal outcomes, but there is not enough evidence to evaluate the use of antiestrogens for increasing the fertility of males with idiopathic oligoasthenospermia.[17]

Notwithstanding these reports, we have found that quite a few cases show improvement in their semen-analysis, thus enhancing their chances of fathering. In fact, 12.8% patients treated accordingly had successful pregnancy in our series. The overall pregnancy rate was 72 out of 657 (10.9%) with all types of medical treatment (see later). This record is substantiated by reports quoting 10 to 15 per cent success rate for this type of medication.[18]

We usually start patients with clomiphene 25 mg per day and have them checked in six weeks for the current testosterone, estradiol and FSH levels, and then adjust the doses accordingly. Paulson, however, advocated a dose of 25 mgm a day for twenty-five days followed by a gap of 5 days for 12 or more months.[19] It is hard to find any rationale of using such dose schedule as spermatogenesis is a continuous process and does not get interrupted for a few days every month.

Any positive effect is usually perceived after 4 to 6 months of treatment. The patients, who do not present a normal increase in ST with antiestrogen receptor antagonists, may possibly benefit from gonadotrophin treatment. It is hypothesised that the hypothalamic–pituitary functions of these patients are impaired. Pure FSH (Metrodin) can be used in these cases. FSH is not likely to suppress pulsatile release of LH. A few instances are on record of both clomiphene and hMG causing temporary blurred vision, weight gain, and temporary breast enlargement and tenderness. In rare cases, clomiphene has also been found to cause liver damage.

Prolactin

The precise role of prolactin (PRL) in males is not fully understood, but secretion of adequate amount of PRL seems to be necessary for normal testosterone production. Elevated PRL is associated with depressed testosterone production with its attendant effects on fertility (see Chapter 3). Men with severe hyperprolactinaemia frequently show mild hypogonadism, and many complain of loss of libido and erectile dysfunction (ED).

Table 11.3: Hyperprolactinaemia in male

1. Certain medications.
2. Hypothyroidism.
3. Prolactinoma

There are three likely causes of a high prolactin level in a blood sample (Table 11.3). Certain medications such as metoclopramide (Maxolon), tranquillisers like chlorpromazine (Largactil) and the antidepressants like amitriptyline and fluoxetine (Prozac) are known to cause high PRL level, when used for a period. Hypothyroidism, which has now become endemic in the subcontinent, also causes hyper prolactinaemia. Lastly, there may be increased production of prolactin due to a[20] prolactinoma or prolactin-producing tumour of the pituitary gland in the form of a macroadenoma or microadenoma. These tumours grow very slowly, but are one of the

commons types of hormone producing pituitary tumour (see also Chapter 3).

Withdrawal of offending medications and correction of hypothyroid state by daily administration of thyroid hormone easily set the prolactin level right. If a prolactin macroadenoma is ruled out, bromocriptine is used for patients with higher than normal prolactin. Initially, the dose is 1.25 mg daily after night meal, which is gradually increased every week to 2.5 mg twice and then to thrice daily. Serum prolactin needs to be monitored periodically to adjust the dose schedule. In my series 54 out of 657 patients were treated for hyperprolactinaemia with bromocryptine. Surgery is indicated for macroadenoma, especially if there is a supracellar extension of the tumour. Initially dopamine agonist therapy may be tried for a while, unless there is drug resistance or intolerable side effects like vomiting, headache and postural hypotension. At present, amongst other drugs used, the long-acting cabergolin has shown the best promise (Table 11.4).

Table 11.4: Other alternative dopamine agonists

1. Quinagolide has a faster action than the bromocryptine and is used at a dose of 75 mcg/ day.
2. Pergolide and Hydergine can be used for patients, who cannot tolerate bromocryptine.
3. Cabergolin (an ergot derivative) is the newest and long-acting dopamine agonist that has replaced the other drugs. It is used at a dose of 0.5 mg once or biweekly and often for long-term therapy for microadenomas.

Corticosteroid and Thyroxin

Sometimes glucocorticoid excess is encountered from exogenous cortisone therapy in ulcerative colitis, asthma, or rheumatoid arthritis with resultant decreased spermatogenesis. The elevated plasma cortisone level depresses LH secretion and can cause secondary testicular dysfunction. Correction of the glucocorticoid excess results in improvement in spermatogenesis. Hyperthyroidism affects both pituitary and testicular functions with alterations in the secretion of releasing hormones and increased conversion of androgens to estrogens (see Chapter 3). Drugs (Neomercazole or Propanolol) that decrease the levels of thyroid hormones are commonly used as treatment.

IMMUNOLOGICAL DEFECTS

Immunological response occurs both at cellular and humoral levels. Immunological factors may also play a role in the pathogenesis of 10% cases of unexplained infertility.

Genesis of immunological response is traced to two facts. Firstly, the spermatozoa come into existence only at about puberty and have different chromosomal structure from the rest of the somatic cells. With the immune system of the body developing much earlier, the sperm specific antigen is viewed as foreign intrusion to the system and it creates a situation to activate the protective anti-sperm antigen reaction. Secondly, nature protects the sperms from this possibility under normal conditions by organising barriers at the testicular level through blood-testis barrier (see Chapters 2 and 3) and similarly at the epithelial level of the genital tract of the male. The latter is achieved either through a local cellular immunosuppressive barrier of the epithelial lining or through activation of the suppressor "T" lymphocytes inhibiting the activation of the antisperm immune response.

The origin of antisperm antibody (ASA) is traced to breaking down of these "blood-testis" and the epithelial barriers as a result of injurious effects on the testis, which releases excessive sperm antigens overriding the natural immune mechanism. Commonly, this occurs in any form of testicular damage that releases the testicular cells outside. ASA is also formed after breaking down of the "blood-testis" and the epithelial barriers. Observance of agglutination and estimation of ASA titre in semen and in blood form the basis of treatment. Detectable ASA are clinically treated mostly by use of immunosuppressive agents or by sperm manipulation (Table 11.5).

Table 11.5: Clinical treatment for antisperm antibodies (ASAs)

1. Use of immunosuppressive agents such as corticosteroids to abort or modulate the production of antibodies.
2. Several innovative methods of semen manipulation like:
   a. Immediate dilution and washing of the semen following ejaculation,
   b. Use of sperm surface fragments as immunoabsorbants to remove "unbound" antibody,
   c. *In vitro* cleavage of sperm-bound antibodies with proteases,
   d. Absorption of sperms with bound ASA in different types of columns to allow separation and capture of the unbound sperms.

When detectable ASAs are clinically relevant, treatment is difficult. A common form of therapy was the use of immunosuppressive agents such as corticosteroids to abort or modulate the production of antibodies.[2] Men and women, who have high levels of ASA, may benefit from taking the drug corticosteroids. In low doses, corticosteroids suppress the

sperm antibodies and improve sperm function.[18] One of the recommended regimes is dexamethasone 0.5 mg for three weeks for the female partner starting on the first day of cycle, and for the male partner to start it on 22nd day for the rest of the cycle.

However, there is no universal acceptance of any recommended regimen of steroid therapy that really lessens either the production or the clinical effects of ASA in the male. Sometimes, the risks of this therapy must be weighed against its benefits. When taken for extended periods of time adverse side effects develop and so it may only be used for a few months.[18] Even though complications were generally mild and self-limited, aseptic necrosis of the femur was reported in a few patients. This is obviously a devastating consequence of yet-unproven therapy.

In one series in 48 subfertile couples the male partner with motile spermatozoa bound to antibodies of immunoglobulin IgG, IgA or a combination of both, were treated with prednisolone 40 mg a day for the first 10 days, then 5 mg on days 11 and 12 of the partner's cycle for 9 months. The prednisolone caused a significant increase in grade I motility and there was suppression of the total ASA concentrations.[21] However, prolonged high-dose glucocorticoid therapy for sperm autoimmunity may improve pregnancy rates modestly, but the risks are generally unacceptable.[3]

With uncertainty of the outcome of various treatment modalities, use of semen processing and intrauterine insemination (IUI) have become increasingly used for male factor cases with immunological infertility. Use of donor sperms for severe male factor infertility is an alternative in some rare cases of failures, provided it has the explicit sanction of the partners. However, this form of treatment is possibly done in India surreptitiously without the legal sanction.

Several innovative methods of semen manipulation have also been attempted. These include immediate dilution and washing of the semen following ejaculation, use of sperm surface fragments as immunoabsorbants to remove "unbound" antibody, *in vitro* cleavage of sperm-bound antibodies with proteases, and the absorption of sperms with bound ASA on different types of columns to allow separation and capture of the unbound sperms. Unfortunately, most of these techniques have not proven to be routinely effective. Most patients end up trying superovulation with sperm washing and IUI. Despite the theoretical advantages of the above, IUI pregnancies for antibodies have not exceeded 20% (see Chapter 13).

INFECTIVE LESIONS

It is essential to manage every attack of urinary tract infections (UTIs) with adequate and aggressive treatment to prevent it from turning into chronic, which may eventually lead to irreversible sperm damage. Presence of pus cells in semen and the urine remains the main plank for the diagnosis of genitourinary infections. Concomitant presence of round cells from different origins are common findings in semen analysis of these patients. However, the majority of patients with excess round cells in the semen do not have pyospermia and, therefore, empiric antibiotic therapy without further testing will likely be unproductive. Appropriate antibiotic treatment based on microbiological tests should always be for extended periods till the clinical and investigational parameters show eradication of the disease.[22] Antibiotic therapy improves sperm parameters by increasing antioxidant activity.

Ofloxacin has been proved to be an effective medication for the treatment of these infections. But it still remains controversial, whether the improvement in sperm motility is primarily related to the ofloxacin therapy. Andreessen et al[23] reported significant initial decrease in the sperm concentration and the motility after one-month therapy with ofloxacin. But three months later, these parameters again reached the starting values and after 6 months, there was a significant improvement. For chlamydial infection, doxycyline therapy has proven success, but it must be given to both the partners for at least for 3 to 4 weeks.

SPERM NUTRIENTS

The importance of a healthy diet cannot be overstated. To function properly, the reproductive system requires the proper vitamins and minerals.

Table 11.6: Sperm nutrients

1. Vitamin C.
2. Zinc.
3. Vitamin B_{12}.
4. L-arginine
5. Vitamin E.
6. Selenium
7. L-carnitine

Nutritional deficiencies can impair hormone function, inhibit sperm production, and contribute to the production of abnormal sperms. Male fertility is affected by foods and nutrients taken—although some of the specifics are different (Table 11.6).

Fertility Enhancing Nutrients

Following is a review of the fertility enhancing factors that seem most important for men. These simple, inexpensive and safe nutritional interventions should be routinely considered in all infertility treatment programmes.

Vitamin C

Vitamin C protects the sperms from endogenous oxidative DNA damage that could affect sperm quality and increased risk of genetic defects, particularly in population with low ascorbic acid (like smokers) against free radical damage.[24] Studies show that a daily dose of 1000 mg showed statistically significant improvement of sperms.[25-27]

Zinc

Zinc is the most important nutrient mineral influencing male fertility. Zinc level in the seminal plasma is directly related to sperm motility. Dietary zinc restriction reduces both sperm count and seminal plasma volume. Skandhan[28] reported that the zinc levels in seminal plasma of normal, oligospermic, asthenospermic and azoospermic subjects show that a linear direct relationship seems to exist between zinc in seminal plasma and motility of spermatozoa.[26] This is corroborated by another study, where those with oligospermia had decreased seminal zinc levels.[27] The study by Abbasi has demonstrated that dietary restriction of zinc can affect testicular function adversely. This effect of zinc deficiency, however, is a reversible process and can be corrected by proper supplementation with zinc.[28-30]

The serum testosterone concentration and seminal volume are most sensitive to zinc depletion in men in the reproductive period. Netter found that zinc therapy is more relevant, if the testosterone level is low. Testosterone and dihydro-testosterone rose significantly after oral administration of zinc, as did the sperm count.[30-32] Takihara found that administration of 440 mg of zinc sulphate therapy might be an effective treatment for infertile patients especially after varicocelectomy.[33] In a study conducted in the subcontinent, infertile men with unexplained low sperm counts received 220 mg of zinc sulphate daily. After four months, there was significant improvement in sperm count and in the number of progressively motile and normal spermatozoa and the wives of two men conceived.[32-35]

Vitamin B_{12}

Vitamin B_{12} deficiency also plays a role in fertility. "Intrinsic factor" is necessary for the proper absorption of B_{12} and its deficiency is one of the causes of secondary infertility in male or female.[36] However, this is reversible with treatment with vitamin B_{12}. It is not necessary to obtain symptomatic or laboratory evidence of vitamin B_{12} deficiency, because, among infertile men, even those without evidence of B_{12} deficiency may respond to supplementation. In one double-blind study, 375 infertile men received daily injections of 1,500-6,000 mcg of mecobalamin, (a common form of the vitamin), although none of the participants had been evaluated for vitamin B_{12} deficiency.

L-arginine

The biochemical and physiological relevance of L-arginine lies in its role as the precursor in the synthesis of polyamines and testosterone. The polyamines putrescine and spermidine are organic components important to sperm motility.[37] Arginine metabolism is a factor in normal sperm production being involved as a source of nitric oxide within spermatozoa. Nitric oxide (at endogenous concentrations) appears to be necessary for adequate sperm motility. The endothelial (eNOS) and brain (bNOS) nitric oxide synthases are abundant in normozoospermic samples, but is low in asthenozoospermic patients. Consequently, an adequate dietary amount of L-arginine is necessary for normal spermatogenesis, especially for the sperm motility and arginine aspartate (9 g daily) has been found to be effective in some cases of asthenospermia. L-arginine, 4 gm daily has been shown to improve sperm counts in men with oligospermia. Obviously, these simple inexpensive and safe nutritional interventions can often transform infertile couple into proud parents and need to be considered routinely in infertility treatment programmes. Most researchers recommend a daily dose of 4 grams to improve sperm counts in men with oligospermia. Nuts, oilseeds, flesh foods, pulses and legumes are common sources of L-arginine.

Vitamin E

The membranes of the germ cells and spermatozoa are very sensitive to oxidation because of their high content of PUFA (polyunsaturated fatty acids). Vitamin E is a major lipophilic chain-breaking antioxidant, which protects tissue PUFA against peroxidation, a property that is beneficial in the male reproductive physiology. Oral administration of vitamin E significantly improves the *in vitro* function of human spermatozoa as assessed by the zona-binding test. Vitamin E antioxidant therapy is, however, dependent on the dosage or the *in vitro* concentration of the vitamin. Vitamin E-in a dose of 200 IU twice daily acts an antioxidant and improves sperms' ability to impregnate.[38-40]

Selenium

In a double-blind study of 64 infertile men with reduced sperm motility, supplementation with selenium (100 mcg per day for three months) significantly increased sperm motility, but it had no effect on sperm count. Liver, kidney, sea-foods (0.4-1.5 mg/kg wet weight), muscle meats (0.1-0.4 mg/kg), cereals and cereal products (<0.1->0.8.), dairy products (<0.1-0.3), and fruits and vegetables (<0.1) are common sources of selenium. Selenium is one of the important ingredients that is very often lacking in older men and can be found in horsetail, which has been used with success in ED following prostatic enlargement.[41]

L-carnitine

L-carnitine is a substance made in the body. It is also found in supplements and normally taken up from food. Carnitine is essential for mitochondrial energy production. It is thus necessary for normal functioning of sperm cells and disturbance in mitochondrial function may contribute to abnormal spermatogenesis[42]

L-carnitine is a carrier molecule for medium and long chain fatty acids and is essential in the transportation of these fatty acids into the mitochondria, where they can be utilised. It is likely that even marginal deficiencies contribute to the clinical manifestations.

L-carnitine is a component of both seminal plasma and sperm cells, and it plays a critical role in sperm maturation and potential sperm motility.[18,42,] Supplementing diet with L-carnitine 3 to 4 grams per day for four months helped to normalise sperm motility in men with low sperm quality in one study. Sperm motility also increased both in quantitative and qualitative manners. In a multicentric study, an increase in the sperm motility was also observed in terms of both rapid linear progression and linearity index along with that of the sperm output after oral administration of L-carnitine in patients with idiopathic asthenozoospermia.[43-45]

Animal foods are generally good sources of L-carnitine, whereas plant foods, vegetables, contain only traces or no carnitine at all. Meat is by far the richest source. Among meat from different animals, it is mutton, which shows the highest content. A diet, which is based on mutton, can provide an intake of about 1000 mg L-carnitine per day, while vegetarian diet is low in L-carnitine, as latter is often low in the two amino acids—lysine and methionine that are necessary for the biosynthesis of L-carnitine in the body.

SPERM MOTILITY ENHANCER

Sperm motility being a very important factor for fertilisation methods for its enhancement have been tried for some time. Pentoxifylline had an additional effect on the motility rather than placebo and was useful treatment in these cases of male factor infertility.[46]

MISCELLANEOUS METHODS

A comprehensive list of medical or nonsurgical treatment option would not be complete without mention of, traditional Chinese medicine (TCM), acupuncture and herbs.

Traditional Chinese Medicine

Traditional Chinese medicine (TCM) is an ancient system of health care that is based on the concept of balanced vital energy (qi), which flows throughout the body. Among the components of TCM are herbal and nutritional therapies, restorative physical exercises, acupuncture, acupressure, and remedial massage. A meridian is a TCM term for the 20 pathways throughout the body for the flow of qi, or vital energy, accessed through acupuncture points.

TCM is based on more than 2,000 acupuncture points on the human body, and that these connect with 12 main and 8 secondary pathways called meridians. Chinese medicine practitioners believe these meridians conduct energy, or *qi* (pronounced

"*chee*"), throughout the body. Qi is believed to be influenced by the opposing forces of yin and yang. According to traditional Chinese medicine, when yin and yang are balanced, they work together with the natural flow of qi to help the body achieve and maintain health. Acupuncture is believed to balance yin and yang, keep the normal flow of energy unblocked, and maintain or restore health to the body and mind.[47]

In China, practitioners of TCM regard it as the primary treatment modality, with acupuncture relegated to second place. Clinical and experimental studies using TCM in the treatment of male spermatogenetic disorder have been on record for number of years. Use of Chinese herbal medicines like composite wuzi dihuang liquorm shengjing pill, tripterygium wilfordii (GTW, Tripterygium hypoglaucum, sairei-to (an herbal medicine), etc, have been documented with great success in Chinese medical literatures. A few of these reports have also been recorded in the contemporary western medical journals. TCM may be used alone as a treatment for infertility or combined with Western medicines. Recent research in China on the fertility-enhancing effects of TCM has helped it to become an important complementary treatment modality in the United States.

One paper analysed the deleterious effects of some Chinese herbs and concluded that high concentrations of St. John's wort, echinacea, and ginkgo had adverse effects on oocytes. This data also suggested that St. John's wort, ginkgo, and echinacea at high concentrations damage the reproductive cells. St. John's wort was found to be mutagenic to sperm cells. However, there is always an argument in favour of trying these alternative measures, when the scientific medical treatment has not been an unqualified success in some idiopathic cases of male infertility. If the cause of male infertility is a congenital or structural abnormality, TCM usually will not prove effective.[48]

Acupuncture

Acupuncture is one of the oldest, most commonly used medical procedures in the world. Originating in China more than 2,000 years ago, acupuncture became better known in the United States in 1971, when New York Times reporter James Reston wrote about how doctors in China used needles to ease his abdominal pain after surgery. Research shows that acupuncture is beneficial in treating a variety of health conditions. Acupuncture is growing in popularity in most Western countries and continues to be a major form of medicine in China and Japan. In the USA, an estimated ten million consultations for acupuncture take place each year. But in spite of its success many scientists "regard acupuncture, like many other 'alternative' therapies, as an over-dressed placebo that fulfils a need for mysticism and ancient ritual".[49]

Acupuncture points are believed to stimulate the central nervous system (the brain and spinal cord) to release chemicals into the muscles, spinal cord, and brain. These chemicals either change the experience of pain or release other chemicals, such as hormones, that influence the body's self-regulating systems. Studies have shown that acupuncture may alter brain chemistry by changing the release of neurotransmitters and neurohormones. Preclinical studies have documented acupuncture's effects, but they have not been able to fully explain how acupuncture works within the framework of the Western system of medicine.[49-56]

The use of acupuncture, like the use of many other complementary and alternative medicine (CAM) treatments, has produced a good deal of anecdotal evidence. Much of this evidence comes from people, who report their own successful use of the treatment. If a treatment appears to be safe and patients report recovery from their illness or condition after using it, others may decide to use the treatment. However, scientific research may not support the anecdotal reports.

Acupuncture treatment for infertility focuses on a strategy of attacking the overall disharmony before treating the special areas. Firstly, the overall pattern of disharmony is treated. Secondly, the local points are treated. Siterman et al from Israel[57] reported patients exhibiting a low fertility potential due to reduced sperm activity might benefit from acupuncture treatment.

Acupuncture can improve the sperm quality and fertilisation rates in ART and seems to be a useful tool for improving pregnancy rate after the ART.[58] A controlled study was undertaken to assess the effect of acupuncture on the sperm quality of males suffering from subfertility related to sperm impairment. Semen samples of 16 acupuncture-treated subfertile patients were analysed before and one month after treatment. The results showed that the patients exhibiting a low fertility potential due to reduced sperm activity might benefit from acupuncture treatment.[57] In another study, it is concluded

that acupuncture may be a useful, nontraumatic treatment for males with very poor sperm density, especially those with a history of genital tract inflammation.[59]

Herbal Medicine

Treatment with herbs has gained prominence in recent years and is said to be useful in treating male infertility. Herbal medicine like acupuncture treats infertility on the basis of a careful differential diagnosis and combines herbs into formulas to resolve underlying pathological process. Records from China have demonstrated that some of the herbs have ability to aid infertility (see TCM). These herbs have been carefully used to match as exactly as possible the patient's individual signs and symptoms, in various formulas in combination with other herbs. Specific herbs also may be used based on findings of conventional medical examination.

Helpful herbs include ginger, prickly ash bark, chickweed, sarsaparilla, garlic, turmeric and pumpkinseeds. For men with low testosterone levels, Korean and Siberian ginseng may be able to enhance virility. Korean red ginseng can be as effective alternative for treating male erectile dysfunction.

According to advocates of the herbal medicine, these medicines rarely have significant side effects, when used appropriately and at suggested doses. The clinical trial results are suggestive of efficacy of some herbal therapies for some conditions. German Commission E, a regulatory body that evaluates the safety and efficacy of herbs on the basis of clinical trials, cases, and other scientific literature, has established indications and dosage recommendations for many herbal therapies.[60] Occasionally, herb at the prescribed dose causes stomach upset or headache. This may reflect the purity of the preparation or added ingredients, such as synthetic binders or fillers. For this reason, it is recommended that only high-quality products be used. As with all medications, more is not better and overdosing can lead to serious illness and death.

Contrary to these claims a few disquieting articles have appeared in the western literature. These articles have sent a word of caution in the use of herbal products, especially when used along with other medicines. These workers contend that none of these herbal medicines is free of adverse effects. As the evidence is incomplete, risk-benefit assessments are not completely reliable, and much knowledge is still lacking. Interactions between herbal medicines and synthetic drugs exist and can have serious clinical consequences. Healthcare professionals should ask their patients about the use of herbal products and consider the possibility of herb-drug interactions.[61,62]

Commonly used Herbs

Following herbs are commonly used.[63-65]

1. Ginseng (Panax ginseng)—known as a male tonic (an agent that improves general health) and used to increase testosterone levels and sperm count. Siberian ginseng (Eleutherococcus senticosus) may also be used.
2. Astragalus (Astragalus membranaceus)—increases sperm motility.
3. Sarsaparilla (Smilax)—known as a male (and female) tonic.
4. Saw palmetto (Serenoa repens or whole berry and extract) is used for overall male reproductive health. Randomised trial lends an element of support to the hypothesis that inhibition of the enzyme 5-alpha reductase is a mechanism of action of this substance.

Herbs or other botanical medicines such as saw palmetto, which was originally used for enlarged prostate, possibly has some effect on testosterone levels in the body. Saw palmetto helps to maintain the proper hormone balance of prostate, which is needed, for sexual function especially in older men. An optimally functioning prostate gland not only produces and delivers semen, but is also part of the biochemical and mechanical processes involved in achieving an erection.

We do not have any experience of herbal treatment and personally have doubts about the efficacy without documented scientific evidence in medical journals.

Electromedical (Electroejaculation and Vibratory Stimulation)

Ejaculatory dysfunction is almost universal sequela of spinal cord injury to men (see chapter 10). Besides neurological conditions such as diabetes mellitus, transverse myelitis, multiple sclerosis, psychogenic disorders may be associated with ejaculatory dysfunction.

Electroejaculation (EEJ) and penile vibratory stimulation (PVS) are methods used to collect semen from men, who are unable to ejaculate and to impregnate the female partner. These methods have been described in Chapter 10.

OUR EXPERIENCE

A total of 2061 patients of male infertility were investigated and managed in the infertility clinic during the period from 1990 January till March 2001. Various methods of treatment were used—some were treated by surgery, but quite a few by conservative medical treatment.[66] 657 patients were treated by various conservative methods (hormonal manipulation, immunological and nutritional) in preventive and therapeutic forms, as well as adjunct to surgery. In this series, routinely patients had 500 mg of vitamin C (Celin or Redoxan), 220 mg of zinc in the form of zinc sulphate (Zincolak), and 200 IU of vitamin E (Evion). In patients, where no perceptible improvement was noticed in 3 months, Vitamin B_{12} was added. All patients were encouraged to take dietary supplements for selenium in the form of meat products, seafood, cereals, and dairy products and similarly for arginine, nuts, oilseeds, pulses and legumes. Animal foods are good sources of L-carnitine. For vegetarians, milk remains the only source of L-carnitine as plant foods and vegetables contain only traces or none at all.

Advice and guidance of endocrinologists for hormone replacement therapy (HRT) was particularly useful; and consequently, these patients sent to them for primary care. Should be hormone manipulation therapy was carried out in the infertility clinic for all patients and in 62 out of 484 patients achieved pregnancies (12.8%). Antiestrogen receptors (clomiphene and tamoxifen) were used in 432 patients, anti-PRL therapy in 54 patients and immunological treatment in 36 patients (Table 11.7). Routinely, various food supplements for sperm nutrients and preventive measures (avoidance of smoking, excess drinking of alcohol, etc, were advised in all patients routinely.

Table 11.7: Medical management of infertility in the series

A. 657 patients were treated by nonsurgical methods (hormonal manipulation, immunological and nutritional) out of 2061 patients of male infertility from 1990 January till March 2001.**
B. Treatment modalities –
   1. Hormone replacement: Mostly treated by endocrinologists.
   2. Hormone manipulation: 62 couple out of 484 had conception (pregnancy rate 12.8%).
      a. Antiestrogen receptor = 432
      b. Antiprolactin = 54
      c Immunological = 36
   3. Sperm nutrients and Preventive measures – for all patients routinely.

** Further 117 patients were added since April 2001 till August 2002 making the total of 2178.

CONCLUSION

It is a common fact that the male factor infertility results from many different causes. It is, therefore, important for couples seeking infertility treatment to have thorough and frank discussions with their health care providers to understand fully their conditions, recent clinical research and treatment options. The treatment of men with unexplained idiopathic infertility remains unpredictable. Often infertile men are described as 'idiopathic oligo/asthenospermic without a precise medical diagnosis and hence, any specific medical treatment is not possible. Availability of a multitude of agents ranging from hormones to nutritional supplements is a testimony to the fact that none are consistently effective. However, effective therapy is available for the treatment of infertility owing to specific causes, especially the hormonal imbalances, which can be readily identified and successfully treated.

Many forms of therapies have been advocated in the treatment of idiopathic male infertility, but no specific regimen has proved to be consistently effective. The medical therapy should always be given a chance notwithstanding its inconsistent and often-low conception rates, [5] but the patients should be apprised of the uncertain outcome in idiopathic male infertility.

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APPENDIX 1

Dietary sources of various products

SELENIUM- Liver, Kidney, Seafoods: 0.4-1.5 mg/Kg wet weight, Muscle meats: 0.1-0.4 mg/Kg., Cereals and cereal products: < 0.1-> 0.8. Dairy products: < 0.1-0.3, Fruits and vegetables: < 0.1.

ARGININE- Nuts and Oilseeds, Flesh foods, Pulses and legumes

L-CARNITINE- L-Carnitine is normally taken up from food. Animal foods are generally good sources of L-Carnitine, whereas plant foods - vegetables - contain only traces or no carnitine at all. Meat is by far the richest source. Among meat from different animals, it is mutton, which shows the highest content. A vegetarian diet is low in L-Carnitine. Such a diet often is also low in the two amino acids - lysine and methionine - that are necessary for the biosynthesis of L-Carnitine in the body. A diet, which is based on mutton, can provide an intake of about 1000 mg L-Carnitine per day.

Total content of L-Carnitine in mg/100 grams of raw foods:

1. Sheep (meat) = 210
2. Lamb (meat) = 78

3. Beef (meat) = 64
4. Pig (meat) = 30
5. Rabbit (meat) = 21
6. Chicken (meat) = 7.5
7. Cow's milk = 2.0
8. Eggs = 0.8
9. Peanuts = 0.1
10. Cabbage, orange juice, barley seed, spinach leaf, potatoes, etc. do not have any.

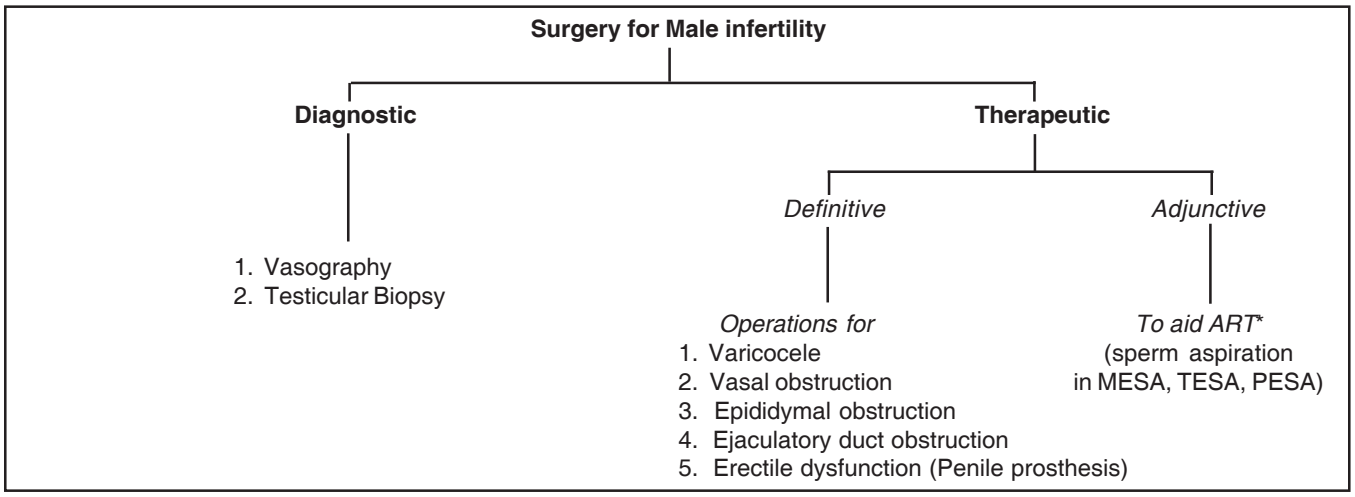
CHAPTER

12 Role of Surgery in Male Infertility

INTRODUCTION

In present-day marketing jargon, the male infertility metaphorically is either due to a manufacturing defect or a marketing incompetence. Some of these manufacturing defects or spermatogenetic abnormalities can be corrected by surgery. So are some of the marketing incompetence or transporting defect. Surgery is contemplated both for the diagnostic and the therapeutic purposes. Surgery in the form of the testicular biopsy with or without vasogram in azoospermia is performed for confirming the diagnosis. When the surgery is done for the treatment of male infertile patients with varicocele or obstructive lesions, it is for a definitive therapeutic purpose (Fig. 12.1). Infertility affects 15 per cent of couples, and 50 per cent of male infertility are potentially correctable.[1]

The erectile dysfunction (ED) in real terms is an inability to effect transport of the sperms. So, any corrective surgical treatment for the ED should be classified under the therapeutic category. The surgery for this condition had a distinct role before the PDE inhibitors like Viagra brought revolution in the management of erectile dysfunction. But these drugs are not panacea for the treatment of this condition and are not very effective in patients with diabetes, after prostatectomy and with complete organic ED, where surgical treatments like penile prosthesis and vascularisation still have their utilities.



*** ART =** Assisted Reproductive Technology

Fig. 12.1: Surgery for male infertility

TESTICULAR BIOPSY, VASOGRAPHY AND PENILE PROSTHESIS

These subjects have been discussed in Chapters 5 and 8.

SURGICAL TREATMENT FOR VARICOCELE

This has been discussed in Chapter 9.

SURGERY FOR OBSTRUCTIVE LESIONS

Obstructions to the ejaculatory duct and vas are common conditions causing azoospermic condition. Apart from these, any lesion in the sperm pathways can also lead to infertility in males. A reparative bypass operation is indicated for such obstructive lesions, as excisional surgery is often not possible. Two common obstructive conditions, where surgery has been successfully performed, are congenital anomalies causing obstruction in the passage of the sperms, and vasectomised persons seeking re-canalisation of the vas (Fig. 12.2).

As production of sperms is a continuous process by the testes, backpressure is induced by the accumulated sperms to cause rupture of tubules of the epididymis at any point. The resultant extravasation of sperms would cause a local inflammatory reaction to block effectively the movement of sperms. Favourable preconditions for all these operative options are spermatogenetic normalcy and accessibility of the site of the obstructive pathology as determined by the transrectal ultrasonography (TRUS), magnetic resonance imaging (MRI) and testicular biopsy.

There are several options available for doing the corrective surgery for obstructive lesions. It was originally performed by an open surgery using magnifying loupe relying entirely on the dexterity of the surgeon. But advent of the operating microscope has now put a new dimension to these reconstructive operations. For any obstructive lesion causing azoospermia, the microsurgery presently is the best choice.[2] With vastly superior magnification available with an operating microscope, the microsurgery has ensured near perfect anatomical union and greatly minimised the incidence of sperm granuloma at the site of anastomosis. With unprecedented success of ICSI (intracytoplasmic sperm injection) and other micromanipulation methods (see Chapter 13), the microsurgery perhaps may have lost some ground, but it is still an important armamentarium in the treatment of obstructive lesions causing male infertility.

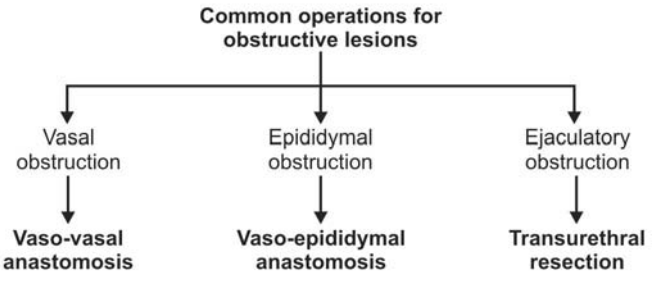


Fig. 12.2: Common operations for obstructive lesions

VASOVASOSTOMY

The vasovasostomy implies joining the ends of the vas to restore its anatomical continuity. It is the most commonly performed operation for the restoration of fertility after vasectomy and also indicated in other acquired obstructions caused by severing of the continuity of vas following childhood inguinal/juvenile hernia repair and other similar kinds of surgery. Other than the post-vasectomy, the herniorraphy is one of the most frequent causes of such obstruction. The incidence of vasal obstruction was found to be as high as 26.7% in subfertile patients with a history of childhood hernia by Matsuda.[3, 4]

The vasovasostomy is most rewarding in terms of success. With increase in the numbers of vasectomies and divorces, especially in the Western world, more men with renewed wish for a child after vasectomy, are seeking restoration of the vas. But with the advent of several methods of microsurgical and micromanipulation techniques, there are now a few alternatives. Nevertheless, the vasovasostomy would probably still remain the approach of first choice.

After an anatomical restoration of vas, one of the hindrances of getting motile sperms to achieve subsequent pregnancy is high titre of sperm antibodies. According to Meinertz,[5] the antibody reaction after vasectomy could be an important factor, but many researchers hold contrary views that the presence of these antibodies in the ejaculated sperm certainly does not have a great effect on the chance of pregnancy. Moreover, a vasovasostomy should not be considered in isolation without taking into consideration the role of female factor in achieving conception.[6] Most of the female partners of the patients, who come for this operation, are found to be not so young probably in the post-thirty age groups, and even subfertile. As soon as the ejaculate becomes positive

for spermatozoa, a complete gynecological investigation and appropriate treatment of any female factor of infertility are mandatory to optimise the chance of pregnancy.

Procedure

An adequate exposure of the cut ends of the vas deferens is an essential prerequisite for this operation. So, the incision for a vasovasostomy should be large enough to allow good exposure and to ensure full mobilisation of both ends of the vas. The surgeon may need to extend the incision well into the inguinal region from the scrotum. Often, the distal or the prostatic stump may be embedded in fibrous tissue in the lower inguinal region. In most cases, the vasectomy ends can be felt and it is not too difficult to identify the site of vasectomy, although it may be tricky in some cases.

The surgeon should always look for the unfavourable prognostic factors such as the sperm granuloma around the proximal or the testicular stump, as this may affect the patency of the vas. Once the ends are dissected and freed, they should be kept constantly wet by irrigation with warm saline. Using a 23-gauge Teflon catheter, the distal prostatic end is then flushed with saline to assess its patency. The patency of the epididymal tract may be assessed by observing fluid leaking from the proximal testicular end and examining it under a microscope. The presence of intact preferably motile sperm is a favourable finding.[7]

Sometimes, fluid starts to ooze spontaneously during operation probably reflecting intermittent ductal contractions. But, at times, it is seen only after a gentle massage of the cauda of the epididymis. If no fluid can be obtained, one should perform a *vasoepididymostomy* linking the vas to the transition zone between the coils of the caput and the corpus. If there are no signs of obstruction, the etiology could often be an underlying (perhaps unknown) testicular factor. A careful and precise documentation of the tubules and a detailed drawing of the epididymal appearance should be included in the operation note for any future exploration, if necessary.

Unfortunately, even a technically perfect anastomosis is probably not enough to restore the contractile function of the vas deferens. The surgeon should endeavour to preserve the intactness of all vascular, nervous and fibrous (containing lymphatic) elements that are come across at the operation. This important step prevents necrosis of the testicular end of the anastomosis and the epididymis, and maintains the vascular supply to the lower epididymal segment for keeping it functional. After the tension-free approximation of the stumps complete haemostasis must be ensured.

Some authors perform a *side-to-side* anastomosis, while others like Jerris and Devroey[7] prefer to do it *end-to-end*. There are options to do either a two-layer or one-layer anastomosis. Most surgeons prefer the two-layered technique for perfect approximation. However, single layer technique is quicker to perform (the mean operative duration was 96 min for a modified one-layer vasovasostomy and 167 min for the two-layer). Fisher et al [8] preferred the modified one-layer anastomosis and claimed that it is simpler and faster with satisfactory postoperative patency rate.

If the difference in diameter between two ends is considerable, a two-layer technique of anastomosis is normally recommended (Figs 12.3A to D). Slight difference in diameter may often occur between the narrower punctiform prostatic end and the wider testicular end that is caused by the backpressure. Symmetry of the stitches and prevention of rotation of the ends are some of the important points to be taken care of. Usually Prolene '6'o will suffice for a good anastomosis in the one-layer technique. In the two-layer technique, '8'o is preferable for the mucosal anastomosis. At the end, the surrounding fibrous sheet and loose adhesive tissues are used to create a protective and supporting peri-anastomotic covering for the site of anastomosis.

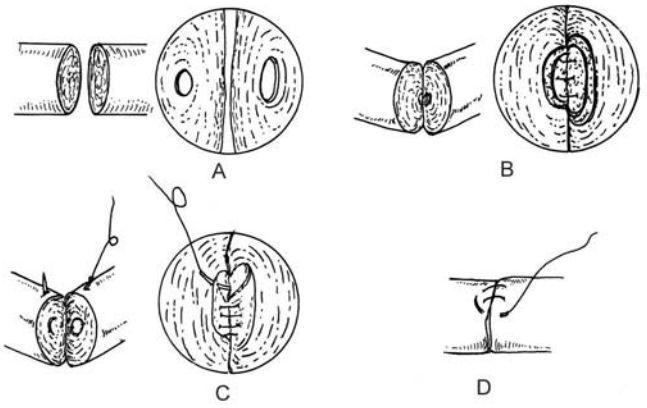


Fig. 12.3: Steps of vasovasostomy

Occasionally, one encounters a patient, who has an iatrogenic injury to the vas deferens from childhood inguinal herniorraphy.[3, 4, 7, 9-12] In such cases, if the vassal ends are not easily identified, complicated surgical techniques like retroperitoneal mobilisation of the vas deferens may have to be taken recourse to. The data obtained from the recent autopsy subjects by Buch[10] reveal that a mean length of 5.83 +/- 0.65 cm can be gained from a retroperitoneal mobilisation of the vas deferens.

Modifications

Occasionally, three modifications of the standard method have been tried. Some used *stents*,[10] while others fibrin glue instead of stitches.[12] The *glue* is used in addition to stitches, where the operator has doubts about the technical perfection of the anastomosis. According to Silverstein[13] the use of fibrin glue allowed the performance of a sperm tight patent anastomosis that had the advantages of reduced incidence of sperm granuloma formation, shortened operating time and lesser microsurgical skill required to perform the anastomosis. The nonabsorbable stent that is removed within the first week of the operation could still adversely affect the results of vasovasostomy. Instead the use of the hollow absorbable stent lately has been showing promising result. The *laser welding* after the preliminary microsurgical suturing of the ends of the vas has been successfully used both experimentally and clinically. The laser perhaps shortens the operating time, but it is the most expensive of all methods used till now. However, these techniques such as laser and fibrin glue applications are not routinely used in humans.[9]

A complete haemostasis of the microscopic anastomotic site and the operation wound must be ensured to prevent postoperative haematoma and infection. Postoperatively, the patient should guard against any excessive movement of the operating area by wearing a scrotal suspensor. Ejaculation is encouraged and a postoperative sperm sample is tested after 10 to 14 days to assess the patency of the anastomosis. A report of azoospermia at this stage does not exclude the chance of patency later on, but presence of sperms is always reassuring to the surgeon that at least the anastomosis is functioning, and the prognosis is favourable.

Results

Recent advances in microsurgical technique[14] have radically altered the prognosis for the treatment of obstructive azoospermia. Before the advent of the microsurgery Kessler [15] reported a pregnancy rate at 45% at the Stanford University Medical Center's Division of Urology and found a statistically significant correlation between postoperative motility and pregnancy rate, and none between the sperm count and pregnancy rate. The microsurgical repair of vasal obstruction is associated with patency rates greater than 90% (Paick—92%, Mathew/Goldstein—99%,) and pregnancy rates averaging 50% (Paick—57%, Jerris et al—54%, Mathew/Goldstein—52%, Kabalin —52%, Shaban—50-60%).[6,7,16-18]

Up to 8-10 years postvasectomy, the incidence of successful re-approximation with presence of sperm in the ejaculate is 80 to 90% with a functional success or pregnancy rate of 50 to 60%.[8,17] Assessment of actual rate of pregnancy is often difficult in the subcontinent, where many patients do not turn up for a regular follow up. Surprisingly, Comhaire et al[19] have also made similar observation for the patients in developed countries. Unless the surgeon is in touch with the gynaecologist attending the female partner for the record of subsequent pregnancies, the cumulative pregnancy rate could never be known.

However, macroscopic vasovasostomy as an effective means of re-establishing fertility in vasectomised men still has advocates in spite of success of microsurgical technique. Mason et al compared the results of macro- and microsurgical techniques. They claimed patency rate of 74% and pregnancy rate of 41% using micro method while Jokelainen et al reported overall pregnancy rate at 56%. These results are similar to those found by others using a macroscopic reversal of vasectomy, where the operator does not rely on the use of a microscope, which requires an added cost and extra expertise.[20, 21]

Failures of vasovasostomy may be attributed to anastomotic stenosis in about 10% of patients. Other causes of failures are due to antisperm antibodies (ASA), epididymal dysfunction, or an unrecognised epididymal tubule "blow-out" with subsequent obstruction. Important prognostic factors appear to be the duration of obstruction, sperm granulomas and length of the testicular end of the duct. Two-layered technique has the advantage over the single-layered technique and is considered gold standard.[9] Observations made by Fischer et al and Shaban.[8,18] on vasovasostomy are shown in Table 12.1.

Table 12.1: Facts about vasovasostomy (Fischer et al and Shaban) [8,18]

1. If surgery is undertaken with vassal obstruction lasting < 36 months, there is no difference between results of one- or two-layered anastomosis.
2. The modified one- and two-layer vasovasostomy has equal patency rate (88% and 90%, respectively), when undertaken after an obstructed interval of 36-96 months.
3. Outcome is generally poor for the surgeries performed after 96 months.
4. When clear copious fluid with motile sperms is found, the prognosis for postoperative pregnancy is in the 60-70% range.
5. If no fluid is found or it is thick and "toothpasty", an epididymovasostomy should preferentially be performed.

Even though ASA can be found in the serum of almost 50% of men,[18] who have undergone vasectomy, their presence in sperm of the ejaculate post-operatively is considerably less. According to Meinertz,[5] the antibody reaction after vasectomy with a pure IgG response, the conception rate does not have much adverse effect on the conception, but IgA-ASA bound to the sperm membrane can cause pregnancy rate to drop dramatically. Unfortunately, it is not possible to use preoperative serum antisperm antibody results to predict the success of vaso-vasostomy in a patient.

Postoperative Semen Analysis

Postoperative semen analysis can present with three different problems (Table 12.2).

Persistent azoospermia: There seems to be agreement among authors that no more than 10% of all vasovaso-stomies would have persistent azoospermia. This is usually due to ruptured epididymal tubules secon-dary to pressure build up. Precise assessment of the presence of spermatozoa in the testicular stump will allow the surgeon to detect such situations during the operation and use the option of either going ahead with vasovasostomy or doing a vasoepididymo-stomy. If no sperms are found, vasoepididymostomy should be the preferred choice. A re-operation after a vasovasostomy is extremely difficult, and less successful in best of hands.

Table 12.2: Postoperative semen analysis after vasovasostomy

1. Persistent azoospermia.
2. Delayed azoospermia.
3. Poor sperm characteristics

Delayed azoospermia: A few men show spermatozoa initially in their ejaculates, but azoospermia after-wards. The commonest cause of such situation is closing up of the anastomosis. According to Buch,[10] compromised anastomosis after previous surgery is the most common cause of failed vasovasostomy and a microsurgical revision vasovasostomy should be performed in these cases. Other workers recommend that these cases are best treated with vasoepididy-mostomy.

Poor sperm characteristics: When sperm concentration, motility and morphology conform to the WHO norm, intrauterine insemination (IUI) after "percoll treatment" of the ejaculate is the first choice. However, if high antibody titres and poor sperm characteristics are found in the ejaculate, and the pregnancy does not occur for one year, assisted reproductive technology (ART) should be resorted to.

Obviously, the surgeon's experience with micro-surgical vasovasostomy is an important factor for success. In most centres, there is coordination between the gynaecologist treating the female partner and the surgeon to optimise the female factor. If one looks carefully at the reasons of failure to conceive in couples after the man had a positive ejaculate after vasectomy repair, one frequently finds incidence of gynaecological disorders. Although microsurgical anastomosis of the spermatic cord results in high impregnation rates among partners, presently patients can still choose their preferred treatment, either microsurgical anastomosis or the ART using epididymal or testicular sperms.

VASOEPIDIDYMOSTOMY
(Vasoepididymal Anastomosis)

Vasoepididymostomy involves a surgical reconstruc-tion of joining the vas with the epididymis bypassing the obstruction. This has been performed in the beginning with the aid of a magnifying loupe till the microsurgical technique replaced it in the recent years. An epididymal obstruction can result from the con-genital anatomical abnormalities and post-inflam-matory cicatrisation of the vas or epididymis.

Repair of the epididymal obstruction remains problematic for several reasons. Unlike the vas deferens, the epididymis has little or no muscular support for the delicate mucosa. Vasoepididymal anastomosis is technically one of the most challenging microsurgical repairs. Furthermore, the epididymis has an important role in the acquisition of sperm

motility and fertilising capacity. Although in a obstructed system, the sperms can acquire motility and fertilising capacity with little or no exposure to the epididymal environment,[5, 22] more segments of the epididymis the sperms are exposed to, greater is their fertilising capacity.

Preoperative evaluation of the seminal vesicles with TRUS (see Chapter 8) is an absolute necessity in all cases, especially in patients with congenital absence of vas. Preoperative TRUS is also important to determine which testis should be exposed first. As there is 20% incidence of an absent kidney associated with congenital absence of the vas deferens, renal ultrasound should be performed as well.[23, 24] It is normally believed that men with congenital absence of vas are associated with absence of seminal vesicles as evidenced by a negative semen fructose. But Goldstein et al [12] found computerised tomographic scans more informative in this situation, as negative semen fructose and low ejaculate volume in men with absence of the vas deferens may well be due to agenesis or obstruction of the ejaculatory ducts rather than absent seminal vesicles.[12] Moreover, men with more seminal vesicle tissue on one side are likely to have a more complete epididymis on that side. It is generally agreed that the sperm quality would be better, if the epididymal volume is more. Men with severely impaired spermatogenesis expectedly have poorer outcomes than those with normal or mildly impaired spermatogenesis.

Neiderberger and Ross[25] showed that the best predictor of successful microsurgical vasoepididymostomy is presence of sperms preferably motile, in the epididymal fluid tested at the time of surgery. The presence or absence of sperms on intraoperative touch preparation is the only significant prognosticator of a successful microsurgical epididymovasostomy. This finding is consistent with the data published in large series of vasovasostomies. Far better outcome can be expected, when sperms are found in the fluid sampled from the testicular end of the vas.[26]

Vasoepididymostomy should preferably be performed at the lowest level in the epididymis at which sperms and good flow are found, regardless of the motility. It is important to preserve as much length of the epididymis as possible to ensure optimal motility and fertilising capacity of the sperms. Some workers have postulated that although normal maturation of sperms involves use of the full length of the epididymis, their experience has shown that after a period of obstruction, the epididymal function seems to be taken over by the more proximal segments allowing pregnancies even in the case of high anastomosis.[5,27,28]

Delayed appearance of sperm after end-to-side vasoepididymostomy is one of the common possibilities. The prognosis for patients with delayed appearance of sperm is not significantly worse than that for patients with presence of sperms in the initial semen analysis. Jarrow[29] has postulated that the postoperative patency rate should be accepted, if there is greater than 1 million sperms in the ejaculate.

Procedure

Like vasovasostomy, full mobilisation of the vasal ends to allow for a tension free anastomosis is the most important step for a successful outcome of the operation. Originally, a latero-lateral anastomosis (*side-to-side*) between the cut epididymal tubular system and the vas used to be done. This has now been replaced either by the termino-terminal (*end-to-end*) or the latero-terminal technique (*end-to-side*). The issue of end-to-end versus end-to-side anastomosis is probably not important. An end-to-end technique is useful in distal epididymal obstructions associated with a short vas, a situation frequently encountered at vasectomy reversal.[5,7,27,28] Otherwise, the end to side technique is preferred. Neiderberger and Ross used microsurgical end-to-side technique described by Wagenknecht et al and Thomas.[25, 30, 31] (Fig. 12.4)

The distended end of the epididymis is cut at its lower end, and dissected higher up in the direction of the caput until motile sperms are found. Micropuncture of the epididymal tubule,[32] as opposed to cutting the tubule and aspirating the effluxing fluid,

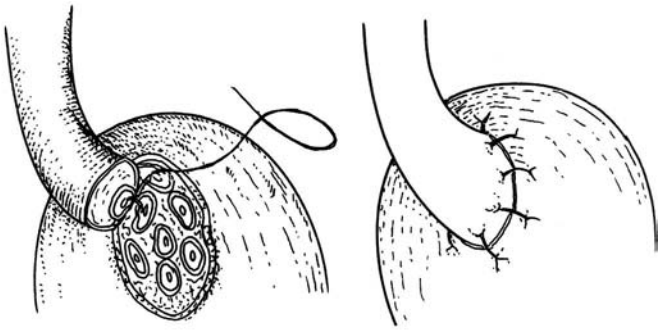
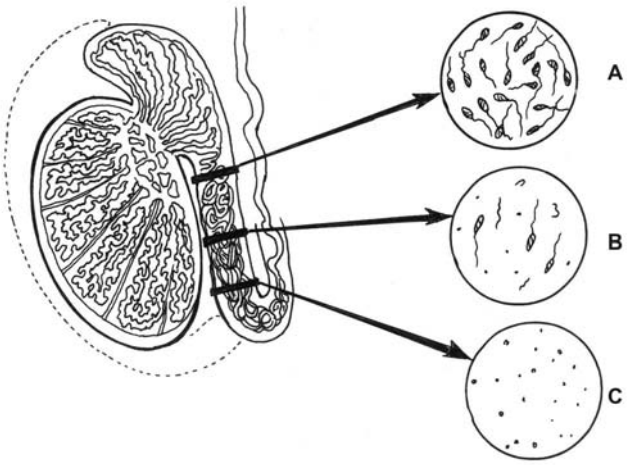


Fig. 12.4: End to side anastomosis

resulted in contamination with blood and tissue fluids. In the latero-lateral variant, a lateral incision is made in a visibly distended tubule and the oozing liquid is examined for the presence of sperm. One must then wait for some time to make sure that the liquid continues to ooze out intermittently from the tubular lumen. Sometimes, the surgeon needs to go all the way up to the rete testis before finding spermatozoa and a very high anastomosis has to be made (Figs. 12.5A to C). Once the right tubule is found, one may stain the area to be anastomosed using micro-cotton swabs drenched in methylene blue. This is especially useful for identifying the tubular mucosa, when applying the second, third and, if possible, fourth stitches with '8'o to '10'o prolene sutures. The operation is carried out under continuous irrigation of warm saline and high optical magnification. Ideally, the anastomosis should be as leak proof as a vascular anastomosis. It should be protected by supporting stitches through the epididymal and the vasal serosa. Repositioning of the testis should be done gently, and the patient should rest for 48 hours, avoid strenuous effort and wear a suspensory bandage for about two to three weeks.

Most surgeons advocate a two-layer anastomosis. Some surgeons modified the surgical technique to perform the second layer anastomosis between the vasal sheath and the epididymal tunic, instead of using the muscular layer of the vas.[33] Evaluation of the outcomes of three intussusception vasoepididymostomy techniques,[34] namely three-suture triangulation, two-suture transverse and a two-suture longitudinal technique concluded that the transverse two-suture vasoepididymostomy had a patency rate similar to that of the three-suture technique. But the new two-suture longitudinal technique, which allows a larger opening in the epididymal tubule for anastomosis, is claimed to be superior to other methods. The use of fibrin glue simplifies this procedure and provides patency rates comparable to microsutured anastomoses.[35]

A modification for the end-to-end type of vasoepididymostomy (known as the sling and blanket) has been tried by Marmar et al.[36] They used the differences in size between the vas and epididymis to gain a mechanical advantage. When the epididymis is transected, the redundant tunic is preserved. The sling is created from the tunic, which is drawn, forward and sutured to the vasposteriorly about 1 cm from its cut end. The sling provides support so that the end-to-end anastomosis between the vasal lumen and the specific epididymal tubule should be completed in one plane and without rotation. The blanket is created from the remaining epididymal tunic, which is sutured anteriorly to cover the anastomosis and stabilise the vas. Marmar et al claimed a patency rate was 50%, and the pregnancy rate was 25% in primary epididymal obstruction.[36]



Figs 12.5A to C: Dissection levels in vasoepididymal anastomosis
A. No sperms
B. A few sperms
C. Adequate sperms

Results

There is probably no operation in urology, where technical perfection on achieving an accurate mucosal approximation and a watertight tension-free anastomosis, assume so much importance in determining the ultimate results. Success of the operation is assessed in terms of patency rate, appearance of motile sperms and partner pregnancy rate. Mathew/Goldstein[16] reported a patency rate following vasoepididymostomy at 65%. It takes 12 or more months following vasoepididymostomy for the patency to acquire. The motile sperms are observed mostly after six months (5.8 ± 0.8 months with standard error) following vasoepididymostomy. Results of Jarrow demonstrated that the delayed appearance of sperms after vasoepididymostomy is common. The prognosis for patients with delayed appearance of sperms is not significantly worse than that for patients with sperms in the initial semen analysis.[29, 37]

The result of a particular operation is mostly dependent on the intrinsic local factors. In an ideal case, one encounters a cigar-shaped or cylindrical block in the corpus or the cauda. More often one finds a partially or completely diseased epididymis and the anastomosis has to be performed higher up. Results are better, when the block is well localised with dilatation of epididymal tubules as is seen in now relatively uncommon epididymitis of gonorrhoeal origin. In more commonly occurring chlamydial infection,[38] vasoepididymostomy is likely to be less successful, as there are often multiple incomplete blocks with less pronounced tubular dilatation.

The results obtained by Paick et al[39] in microsurgical single tubular epididymovasostomy are comparable to those obtained with the use of IVF with ICSI, which also involves complex invasive treatment for both female and male partners.[39]

Patency rates have been reported to be between 50% and 70% with pregnancy rates varying between 15 and 30%. Results expressed as patency and pregnancy rates published for the latero-lateral, termino-terminal and latero-terminal techniques are 62% and 15%, 82% and 28% and 82% and 42% respectively.[40] Pasqualotto[41] reported an overall patency rate at 66.7% and found that the patency rate is more, when the anastomosis is done at the level of cauda than those at the caput or corpus. The level at which the epididymis is obstructed plays a crucial role with regard to sperm maturation and the potential for fertilisation.[31]

Marmar et al[23] and Jerris et al[7] have emphasised that best results of microsurgical vasoepididymostomy are obtained with a team effort. With experience, surgeon's expertise also improves as there is a long learning curve. Once sperms are found in the epididymal tubule, a good outcome depends on accurate mucosal approximation and a watertight, tension-free closure around it. Pentoxifylline stimulation, mini-Percoll filtration and incubation with human follicular fluid provide better quality sperm. Adding oocyte micromanipulation to these measures can result in pregnancy rates greater than 30%.[42] Ability to visualise the 0.2 to 0.4 mm lumen of the opened epididymal tubule exuding sperms and accurate approximation of the vasal lumen are two essential and critical factors that determine the ultimate success of a vasoepididymal anastomosis.

Late failure rates for vasoepididymostomy have been reported to be 21%.[16] This can be accounted for by the epididymal blowouts with extravasation of sperms from the epididymal tubule into the interstitium and causing secondary obstruction and epididymal dysfunction, or partial obstruction causing poor sperm quality. Aspiration of motile sperm and cryopreservation should be recommended, in case azoospermia persists or occurs after repeat vasoepididymostomy.

Improved stimulation protocols, clean microsurgical sperm aspiration technique and refined sperm processing have yielded better partner pregnancy rate. However, even in experienced hands vasoepididymostomy frequently fails. In these cases, and in men with unreconstructible acquired or congenital lesions, micro-surgically aspirated epididymal sperm (MESA-see later) can be used in conjunction with *in vitro* fertilisation to salvage couple's fertility.

CROSSED TRANS-SEPTAL VASOVASOSTOMY

The procedure of crossed trans-septal vasovasostomy (transvasovasostomy) has lost much of its relevance after the advent of micromanipulation and *in vitro* fertilisation techniques. This operation is indicated in an acquired obstructive azoospermia following an irreparable obstruction of one vas deferens and severe damage of the contralateral testis. Lizza et al, Hamidinia and Belker et al[43-45] have claimed isolated successes with this rare operation. Gerris[7] claimed successes in cases with unilateral agenesis of the vas deferens and contralateral 'Sertoli cells-only syndrome', with unilateral complete absence of the vas and contralateral partial absence of the vas allowing a crossed anastomosis, and one with an inguinal vasectomy performed simultaneously with hernia repair and a contralateral 'Sertoli cells-only syndrome'. Sabanegh[46] performed crossover transseptal vasoepididymostomy for solitary functioning testis with irreparable ductal obstruction or agenesis, and claimed patency in most men, but pregnancy was achieved in two out of twelve patients.

Technically speaking, one should take care to free up the prostatic end as much as possible, and supporting sutures should reinforce the anastomosis. In all cases, micromanipulation technique has a definite role in achieving conception.

OPERATIONS FOR OBSTRUCTION OF EJACULATORY DUCT

A partial obstruction of the ejaculatory duct is an infrequent cause of infertility. With advancement of

the diagnostic measures, more cases have come to light in recent years. These patients may present with azoospermia and sometimes oligo-asthenospermia with normal sized testes and normal testicular biopsy result.[47,48] TRUS findings reveal dilated ejaculatory ducts. A transurethral resection of the ejaculatory ducts can result in marked improvement of semen quality with subsequent pregnancy amongst selected patients. The orifices of the ejaculatory ducts at the prostatic urethra just lateral to the verumontanum are endoscopically identified, and then they can be incised or unroofed.

Transurethral resection of ejaculatory duct is indicated[19] under the following situations as shown in Table 12.3. The orifices of the ejaculatory ducts exit within the prostatic urethra just lateral to the verumontanum (See Fig. 2.4 in Chapter 2). Under anaesthesia, they are inspected endoscopically, and then incised or deroofed. In selected cases, transurethral resection of the ejaculatory ducts has resulted in marked improvement in semen parameters, and pregnancies have been achieved.[18]

Table 12.3: Indications for transurethral resection of ejaculatory duct

1. Patients with azoospermia and even sometimes oligo-asthenospermia.
2. Normal sized testes.
3. Normal testicular biopsy.
4. Transrectal ultrasound findings of dilated ejaculatory ducts.

An extensive scarring of the bladder neck area may interfere with its proper functioning and subsequent infertility in male. A reconstruction of the bladder neck can be of help, but the results are not very satisfactory. Resection by deroofing or resection of verumontanum for cystic lesions of ejaculatory ducts is one of the methods that have been used in the past with indifferent results. In these cases, additional procedure of vasoepididymal anastomosis after such resection had some success.

ADJUNCTIVE OPERATIONS TO AID AND ART (MESA, PESA, TESE and TESA)

Sperm aspiration is a procedure to obtain viable sperm from the male reproductive tract. Main indication for this procedure is severe forms of male infertility caused by obstructive and nonobstructive azoospermia, sperm production severely impaired or nonexistent, severe oligospermia and necrospermia. Sperm aspiration (when performed using the appropriate technique) is usually a very successful and minimally invasive procedure that allows even men, who have only a few sperms to get a biological children of their own. Sperm harvesting techniques used to obtain sperms from men with obstructive azoospermia include MESA, PESA TESE and TESA. The subject has been dealt with in details in Chapter 13.

ALLOPLASTIC SPERMATOCELE

In the past, an artificial or alloplastic spermatocele was used to create storage of sperms for IUI. The resultant pregnancy rate was dismal at less than 5%. There are only a few reported pregnancies in the world literature. Poor or absent sperm motility in the epididymal fluid during planned alloplastic spermatocele implantation predicts a poor postoperative result and, therefore, contraindicates implantation of the prosthesis. Belker reported that pregnancy occurring postoperatively in 7 of 91 wives, ended in spontaneous abortion in 3 and progressed to full-term delivery only in 4.[45] This technique has now been abandoned with the advent of microscopical puncture of the distended epididymal tract and subsequent micromanipulation techniques.

CONCLUSION

1. Successful surgery and subsequent spermatogenetic normalcy do not guarantee pregnancy in all patients. Sometimes, this ambiguity can possibly be explained by the variable female factor.
2. The surgery for varicocele still gives the most encouraging results in terms of restoration of spermatogenic function of the testes and more importantly, occurrence of pregnancy. Microsurgery has a special place for a recurrent varicocele and on men with previous inguinal exploration (see Chapter 9).
3. The surgical correction of obstruction has achieved greatly improved results in recent times with the advent of microsurgery. The microsurgical reconstruction remains the treatment of choice for men with reconstructable obstructive azoospermia. The microsurgical vasovasostomy yields very satisfactory result, if it is done within five years of vasectomy. In comparison, other procedures such as microsurgical vasoepididymostomy are less successful. However, the enigma of ASA can stall success in terms of pregnancy in spite of achieving anatomical restoration.[49]

4. Best results for microsurgical techniques are obtained, when a surgeon has considerable experience having gone through the long learning curve. Notwithstanding its technical difficulties, microsurgical procedure has consolidated its place in the treatment of male infertility. The success of microsurgical repair can be furthered by various micromanipulation techniques especially ICSI, combined with Pentoxifylline stimulation, mini-Percoll filtration and incubation with human follicular fluid and oocyte micromanipulation.
5. However, macroscopic vasovasostomy for re-establishing fertility in vasectomised men is still recommended by some uro-andrologists.[20, 21] Cost effectiveness of macro method is certainly an important consideration in developing countries.
6. Overall results of surgery for the male infertility, even with improvement of surgical skill and the availability of sophisticated equipments still remain capricious and unpredictable. However, adjunctive operations such as MESA (microsurgical epididymal sperm aspiration) and TESE (testicular sperm extraction) have shown great promise, and are now considered one of the standard and fundamental procedures in obstructive or testicular azoospermia, where reconstruction operation has failed or not feasible.

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CHAPTER 13

Role of Assisted Reproduction in Male Infertility

INTRODUCTION

Procreation is a natural process and a normally functioning sperm is expected to complete its path culminating in fertilisation. Yet there is no guarantee that every time intercourse takes place a pregnancy would result, as human conception is a difficult and complex process, even under best conditions. Normal process of fertilisation involves sperms first breaking through cervical mucus, travelling up the length of the uterus and entering the fallopian tube. Once in the fallopian tube, a sperm must meet the ovum, penetrate the ova's protective coating and the inner membrane, and finally fertilise the ovum. To reach the target, not only must the sperms be morphologically normal, but they must also have the motility and the energy to penetrate the tough zona pellucida. In such a complex process, it is obvious that any reduction in the quality, motility and morphology of the sperms will drastically reduce the chance of fertilisation *in vivo.*

When a conception in normal course of event does not result, manipulation of nature is an option to assist the reproductive process. Probably, the simplest method of such manipulation was started with practicing intercourse during most fertile period of a woman, so that the sperms are conserved to meet the ovum at the most optimum period. Gradually, in quest of getting a solution to this vexed problem, various methods such as instillation of husband's semen directly into the cervix or uterus were innovated. With empirical medical treatment of male factor infertility meeting limited success, the semen processing and the intrauterine insemination (IUI) became increasingly used to overcome the problems of reduced sperm number and motility.

The principles of *in vitro fertilisation* (literally "in glass") was put altogether on a different height with successful culmination of efforts to achieve conception outside the human body in 1978 with the birth of the first test tube baby. With the advent of the micromanipulation and the assisted reproduction, the hit-and-miss experiment with fertilisation, where success was left to the caprice of nature, was transformed into a real time science. Great strides have since been made in the development of the appropriate culture media with careful establishment and maintenance of a well-controlled sterile environment for the normal physiology of fertilisation and early development of healthy embryos after its eventual transfer back into the body. Newer methods have enabled embryos to be grown for extended periods of time in culture. Surplus embryos and possibly eggs can now be routinely cryopreserved in liquid nitrogen for their use in subsequent attempts of pregnancy.

Artificial insemination or simple injection of sperms into the cervix was one of the first methods to manipulate natural course of fertilisation to achieve pregnancy. Its aim was to bypass the first part of the female reproductive passage to overcome the cervical and vaginal hostility factors. However, when the sperm quality or quantity was found to be inadequate, corrective measures like semen processing to modify

the sperms and its environment was the next course tried before the artificial insemination was taken recourse to.

The semen processing is simply an *in vitro* manipulation of semen to enhance sperm function. It is done in an attempt to remove adverse seminal fluid factors and to extract more normal and motile sperm population. To ensure success, it is imperative to test the semen in advance for the most appropriate preparative method, as often the survival of patient's sperms may be poor with some of these procedures. Simple processing methods rely on dilution of the semen and centrifugation followed by resuspension of the sperm pellet in culture medium such as Ham's F10.

ARTIFICIAL INSEMINATION

Artificial insemination is a rapidly advancing science used to improve sperm impregnation. A number of different techniques may be employed depending upon the needs of the patient. Artificial insemination involves physical use of the sperm to be instilled into the female genital passage with donor being either the husband (AIH) or someone else (AID). The chance of successful fertilisation is enhanced, if improved quality of sperms is used.

Artificial Insemination is done in either of the two ways—intracervical or intrauterine (IUI) with latter yielding better results. Most important and logical principle in an IUI is to overcome hostile female cervical factor. For idiopathic or unexplained and immunological cases of male infertility, IUI is a commonly tried method. AIH with IUI is particularly useful with low semen quality or in cases where repeated postcoital tests have shown cervical hostility. But the success rates have not often been predictable in individuals with oligospermia or asthenospermia in spite of coinciding the procedure with the ultrasonographic evidence of ovulation and the LH surge in female partner (Table 13.1).

Table 13.1: Indications for artificial insemination (AIH and AID)

1. To overcome hostile female cervical factor
2. Oligoasthenospermia or presence of immotile sperms incapable of fertilisation.
3. Neurological disorders causing ejaculatory disorder.
4. Immunological abnormality in the sperms that require prior sperm wash.
5. Azoospermia from primary testicular failure, after chemotherapy or radiation,
6. Husband being a carrier of dominant hereditary disease.
7. Failure of advanced treatment modalities and severe Rh isoimmunisation.

In addition to the tests done on a woman, it is mandatory to perform semen analysis to make sure that her husband has sufficient normally functioning sperms for fertilisation. For an optimal semen specimen, the husband should refrain from ejaculation for at least 48 to 72 hours prior to providing a specimen, and also not to abstain for longer than 5 days, as the quality of sperm decreases with prolonged storage in the body (See Chapter 7).

A private room should be provided near the reproductive laboratory for the collection of semen specimen. As many factors affect the quality of sperms produced by an individual at any given time, the husband should be asked to complete a brief reproductive history, where a note is made of any temporary conditions such as viral fever, any medication, prolonged illness or extreme stress within the last three months, etc. which may affect sperm quality.

In the USA, over 15% of couples experience difficulty in conceiving a child after attempting for one year[1] and approximately 10% of couples are infertile. Of these, approximately 50% are due to low male fertility. In India, comparable figures are not available, but the overall incidence of causative male factor is certainly 30% or more. Artificial insemination utilising donor semen has become an acceptable alternative to many of infertile couples, as it is by far the most successful and cost-effective form of therapy. It is estimated that in 1983 over 20,000 births in the United States were the results of donor insemination.

Artificial insemination utilising an anonymous donor or AID may be necessary for a number of reasons. Azoospermia is by far the commonest indication. In other cases, husband may have a very low sperm count or immotile sperms incapable of fertilisation. Neurological disorders may render some men unable to ejaculate. Donor insemination may also be indicated, when the husband is a carrier or a victim of a serious inherited disease. In India, AID or artificial insemination with donor sperm does not have any legal sanction. Child born out of this method would be without a legal father, unless the couple goes through all the legal formalities of adoption.

Sperms are prepared for the IUI by the standard swim-up technique or the density gradient centrifugation using 0.5 ml of frozen thawed semen, In the subcontinent, sperm banks are almost nonexistent and mostly fresh semen is obtained from the husband or the donor.

The American Society of Reproductive Medicine (formerly American Fertility Society) has a well-documented protocol, which has an ethical acceptance for the artificial insemination by donors (AID). Main indications are idiopathic azoospermia, primary testicular failure, after chemotherapy or radiation, carrier of dominant hereditary disease, failure of advanced treatment modalities and severe Rh iso-immunisation.[2] To prevent transmission of infectious disease, the quarantining of the human sperm is a prerequisite, and its preservation must be for six months for this purpose (See 'Donor' later).

Sperm Washing

Sperm washing selects the best sperms for subsequent use in artificial insemination and for *in-vitro* fertilisation. The technique of sperm washing removes sperms from its natural fluid in the semen and places it in an artificial fluid to improve the sperm motility, longevity and its ability to penetrate the ovum. Sperm washing not only dispenses with the relatively less fertile sperms, but also removes the sperm antibodies and even some microbes.[3-5] Moreover, washed and incubated sperms improve the sperm velocity and therefore, their fertilising capability.[6] Simple processing methods rely on the dilution of the semen nd the centrifugation, followed by re-suspension of the sperm pellet in culture medium such as Ham's F10. Other culture media used are HTF SPM, EBSS SPM with pentoxyphylline and human serum albumin.

Success of any ART procedure greatly depends on the quality of the processed semen. Two important methods are washing by simple *swim-up migration* or by *density gradient centrifugation.* Sperm washing and swim up were the first methods used. Later as the technology developed, several modifications with refinement came into existence. The sperm wash is done on the day of the insemination.

In a swim-up procedure, adequately buffered physiological saline is layered on top of an aliquot of whole semen. Most vigorously active sperms enter the medium, while others with lesser motility and viability remain with the sediment. Swim up procedure takes about fifteen minutes after the semen is liquefied. It is also the most helpful process to handle fragile frozen-thawed sperms.

In density gradient centrifugation procedure, the sperms are washed, and then separated by putting it through two different concentrations of a colloidal solution. One of the common methods used is *Percoll density centrifugation*. This separates the sperm with the best movement from those with poor or no movement. It also helps to reduce or eliminate the presence of white blood cells. The procedure takes about one or two hours to complete. Occasionally, this technique cannot be used due to too low sperm count or motility. The collection of semen samples for the sperm analysis or the therapeutic use should be performed in glass containers, as the glass appears to be superior to polystyrene and polypropylene containers in maintaining various semen parameters like motility, linearity and ALH.[6]

Relative Merits of Different Methods

The general consensus is that the swim-up is preferred for semen with relatively higher sperm content, while Percoll method is probably better for oligospermic men. However, there is some difference of opinion regarding the relative merits of different methods.

Bongso et al[7] compared three sperm-washing methods—direct layering without centrifugation (Swim-up or DL), Ficoll entrapment (F) and Percoll gradient (P). Percoll separation without layering was found to be the best washing method for both normospermic and oligospermic patients.

Chen et al (1995)[8] are of the opinion that the Percoll gradient technique although by recovering more motile sperms may be used to prepare oligospermic samples, the swim-up method often becomes the standard choice to prepare normal semen due to its simplicity. Moreover, the latter method could obtain sufficiently motile sperms, and the recovered sperms have motility of superior quality. Evliyaoglu[9] et al are of the opinion that the Percoll gradients produce final sperm pools with higher proportion of motility within all concentration ranges. Their results suggest that the Percoll gradient centrifugation should be the preferred selection method regardless of the initial fresh sample concentration. Ng et al (1992)[10] opined that the Percoll (P) and Mini Percoll (MP) gradient methods result in greater yields of motile spermatozoa than the swim-up preparation. But latter selects higher proportions of spermatozoa with improved characteristics (like velocity, intact acrosomes and normal morphology), which help in fertilisation rates *in vitro*. They conclude that P and MP methods are not superior to swim-up method.

Somfai et al (2002)[11] found the use of Percoll gradient resulted in a significantly higher percentage

of living sperms with an intact acrosome (88.2%) than the swim-up method (69.4%), and the concentration of spermatozoa after Percoll separation was higher than that after the swim-up method. Although the swim-up isolates the sperms with greater ability to fertilise, it does not change the acrosome reaction rate.

Modifications used

Chen et al (1996)[12] used the combination of the direct swim-up technique and discontinuous Percoll gradient centrifugation with greater recovery of motile sperms in oligoasthenospermic samples. Percoll density centrifugation has shown some adverse effects due to its silicon contents recently. Newer development includes use of polysaccharide based sperm media product containing arabino-galactan, which is relatively non-toxic and has high solubility and low viscosity.[13]

Various modifications have been tried like the selective fractionation of sperm subpopulations with the use of silica-bead suspensions especially in density-gradient centrifugation. Similarly the differential filtration based on adherence to glass or self-migration swim-up through hyaluronic acid[14] has been tried. Johnson et al (1996)[15] tried modification of using glass wool filtration that yielded more functionally intact spermatozoa than the mini-Percoll gradient processing.[16]

However, the washing procedures *per se* in most cases had an immediate and extended harmful effects on the sperm motility, especially after the second washing. Addition of human albumin could protect the sperms from this deleterious effect.[17] The pH of fresh ejaculates ranged from 7.2 to 8.2. Gradually changing the pH and osmolarity to either side of normal values led to progressive loss of sperm motility.[18]

Penetration Enhancing Treatment

If the sperm penetration assay suggests a capacitation or acrosome reaction defect, a sperm penetration enhancing treatment may be employed.[19] The technique involves obtaining two sperm samples about 24 hours apart. The first sample undergoes refrigeration in a capacitation-stimulating medium; the second sample is incubated with the chemical heparin. Both these treatments help to improve the sperm's penetration capacity increasing its ability to fertilise.[2] The treatment of such recovered sperm with a motility-enhancing agent such as pentoxyfylline may yield optimum fertilisation rates. Mortimer (1994)[20] opined that the pharmacological stimulation of sperm motility may increase yields, but for *in vitro* fertilisation the sperms must be used to inseminate oocytes as soon as its removal from the frozen state to maximise the fertilising capacity.[21, 22]

Intrauterine Insemination

The procedure of intrauterine insemination or IUI is relatively simple. This involves passing a small flexible catheter through the cervical canal into the uterus and then injecting the sperms into the uterus. Either the husband or the donor (AIH or AID) produces through masturbation a semen specimen. Using different techniques, the specimen is processed in the laboratory to remove the sperms from the semen. The best and most normal sperms are concentrated into a droplet, which is injected through the catheter (Fig. 13.1). For most women, it is almost a painless procedure except slight discomfort, but some may experience a slight cramp, while the catheter is introduced into the uterine cavity. Intrauterine insemination is less invasive and less costly than other assisted reproductive techniques. Sperms survive in the female reproductive tract for up to 72 hours, and an egg can be fertilised for up to 24 hours after ovulation. Increasing the frequency of intercourse during this period increases the chances for conception.[23]

Intrauterine insemination have been performed since the beginning of this century for treatment of infertility. Indications for IUI include male factor,

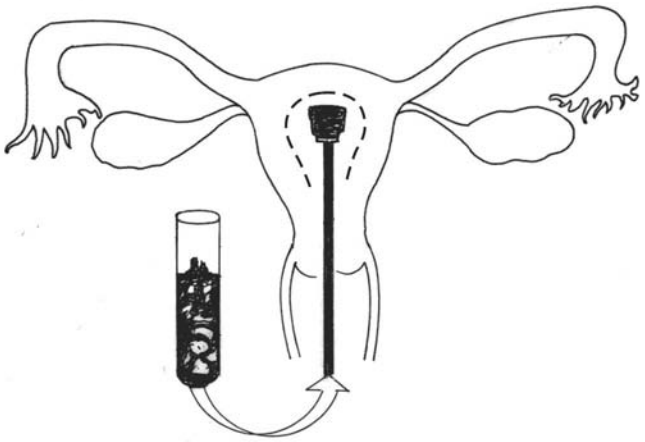

Fig. 13.1: Intrauterine insemination

cervical factor, immunological and unexplained infertilities and infertility due to ejaculatory disorders.

IUI is superior to intravaginal or IVI or intracervical insemination or ICI.[24] In most centres, the intrauterine insemination is tried at least for three treatment cycles of ovulation, whenever good progressive motile sperms are obtained after suitable preparation.[25] However, others recommend a minimum of four cycles to compensate for low pregnancy rates due to age, semen quality, or reduced follicle number in patients with ovulation dysfunction.[26]

Centola[27] found that an acceptable pregnancy rate could be achieved with IUI even in severe oligozoospermic patients with proper sperm washing and concentration method. Although the husband or the donor should have at least 3-5 million sperms available for IUI to work, there are exceptions to the rule. Occasionally, a pregnancy may occur after an insemination with only a few hundred sperms. However, with persistent counts of less than 3 million sperms in samples, the couple should really consider alternatives, because IUI is mostly unsuccessful. Under these circumstances, instead of trying multiple attempts of IUI with its attendant expense in terms of money and time, other assisted reproductive methods are expected to give better success rate.

Ovarian stimulation further improves pregnancy rates achieved by insemination. The best method to prepare the ova is to have induction of ovulation and its careful monitoring using combination of clomiphene citrate and human menopausal gonadotrophin (hMG). Insemination should be aimed on the day of luteinising hormone (LH) peak. Use of the human menopausal gonadotrophin for ovarian stimulation seems to be superior to that of clomiphene citrate. With sperm manipulation along with properly timed insemination by identifying the LH surge the chances of a pregnancy through six cycled attempts are between 15 and 40 percent. In a natural LH surge, LH value attains 10-20 times the basal level. There is often a disruption of normal feedback mechanism or inadequate amplitude of LH surge with pharmacological doses of FSH or combinatioan of FSH and LH. Consequently, for a surrogate LH surge administration of hCG has almost become a standard procedure.

The cases with immunological infertility as diagnosed by a positive mixed antiglobulin reaction (MAR) test are associated with low spontaneous conception rate. Assisted reproduction techniques are useful in the management of these cases, but the sperm characteristics required for a successful IUI are much higher than those needed for successful IVF.[28] Some information regarding IUI collated from different sources are shown in Tables 13.2 and 13.3.[29,30]

Table 13.2: Indications of IUI

Male
1. Severe oligoasthenospermia
2. Ejaculatory incompetence of various types.
3. Immunological cause with persistently high antisperm antibody titre.
4. Idiopathic infertility, where other conservative measures have failed.
Female
1. Cervical hostility evidenced by postcoital test
2. Cervical injuries or cervical anomalies causing obstruction to the sperm passage.

Table 13.3: Some information regarding IUI[29,30]

1. More than one insemination per cycle may increase chances of conception.
2. Some women experience severe cramping during insemination.
3. There is a slight risk of puncturing the uterus or cervix during IUI.
4. For unexplained infertility, IUI, artificial insemination (AI), and well-timed intercourse appear to produce similar pregnancy rates of about 8% per cycle; one study reports that IUI results can be as high as 15% per cycle.
5. Pregnancy rates with superovulated IUI have been reported as high as 23%, though overall they are generally lower.
6. For male infertility, IUI has doubled the pregnancy rate (6.5%) of that of AI (3%).

There is a tendency amongst some of the female infertility specialists to treat an infertile couple using IUI with sperm washing for subfertile semen in a male partner without seeking an andrologist's opinion. Ideally, the female and male infertility specialists should always form a team. In the subcontinental set up, cost-effectiveness of bypassing an andrologist could be cited as a factor, but such practice indubitably is against the guiding principle in the management of infertility. Incidentally varicocele, which is one of the common causes of male infertility, should always be looked into, as treatment of varicocele improves pregnancy and live birth rates among couples undergoing intrauterine insemination for male factor infertility.[31]

Sperm Cryopreservation

To do away with the disadvantage of having to repeat the process to get the sperms with optimum

density, motility and morphology, storage of sperms becomes an important consideration. Cryopreservation in an atmosphere of liquid nitrogen has provided a convenient option for the purpose. Sperm cryopreservation allows men with threatened fertility to preserve their progenitive potential.[32] However, the cryopreservation and subsequent thawing of semen for assisted reproductive procedures have some adverse effects on the sperm parameters. Cryopreservation decreases the number of living sperms as assessed by bis-bezimide. It further causes decline in both in the number of motile sperms and their velocity, and extensive alteration of acrosome. The motility often gets reduced further, when the cryoprotectant medium is removed, because of the osmotic shock and centrifugation done for the the preparation of sperms.[33] Insemination with cryopreserved sperms can thus result in a lower pregnancy rate with AID and *in vitro* fertilisation. According to a study in Cambridge, UK, conventional approaches to cryopreservation impose a linear change of temperature with time; however, the stress that the cells encounter during cryopreservation are all nonlinear with time.[32]

Procedure of Cryopreservation

The semen sample after its collection is placed on a warming block at 37°C till it is liquefied. It is then mixed in a 1:1 ratio with a freezing medium, and the mixture is divided into one-millilitre set and stored in cryovials. The freezing medium contains cyroprotectants, which are salts or chemicals that help to remove water from the cells while frozen.

Most commonly used cryopreservation is based on the technique recommended by Sherman, and involves dilution of the fresh semen in a HEPES buffer containing 15% glycerol. After thorough mixing, the mixture is aspirated in 0.25 ml straw and left for 10 minutes at –80°C in liquid nitrogen vapour. It is then submerged in liquid nitrogen in a very low temperature of –196°C, which halts all metabolic activities in the cell. If this process of anhydration is not carried out, the ice crystals formed inside will break the cells resulting in cell death.[1,34]

When the semen sample is needed for artificial insemination or other assisted reproductive methods, gradual thawing is done by removing it from the liquid nitrogen and placing it in water bath at 37°C for not more than 60 seconds for rendering the specimen to reach a liquid state. The liquid is allowed to warm to attain normal body temperature prior to being prepared for use. By slow thawing in stages, the sperms are protected from sudden swelling, and rupture of the sperm heads as the water moves back into the cells.

Computer-controlled freezing ensures higher survival and higher penetration rate and less ultrastructural head damage.[35] In addition to the conventional sperm characteristics, the ATP content of the thawed semen should be taken into account in making the final preparation for the assisted reproduction (Table 13.4).[36]

Table 13.4: Sperm cryopreservation

1.	Sperms are successfully cryopreserved but currently, there is no satisfactory method for oocytes.
2.	Loss of viable sperms is significant in currently available methods.
3.	Although sperms can be cryopreserved for indefinite period, only the stronger sperms survive.
4.	Freezing itself does not cause DNA damage to the sperm. Again, the effects of cryopreservation are variable between samples and patients.
5.	Pregnancies from semen samples older than 15 years have not been reported, though the sperms are certainly capable of surviving longer period.

Arrangements should be made by the reproductive physician to obtain frozen semen specimens preferably from a local sperm bank. The bank should have an experienced physician or pathologist directing the operation, and assisted by a staff with special interest in male infertility to maintain the highest possible professional standard. At the appropriate time, the andrology laboratory prepares it by carefully thawing and washing the semen sample to remove the cryoprotectants and seminal fluid. It then concentrates the sperms into a volume appropriate for insemination. In the subcontinent, sperm banks are almost nonexistent, so mostly the donor's semen is collected a few hours before the IUI or IVF is planned. Sperm concentration lower for all cryostorage groups compared with healthy sperm donor controls.

Elective sperm cryopreservation is an effective form of fertility insurance for men whose fertility is threatened by medical treatment.[36]

Donor

Donors should be carefully selected to obtain the their background taking a careful history to rule out hereditary diseases, chronic infections, or any

venereal diseases. Persons, who have demonstrated previous fertility, should preferably be chosen as donors. The race, blood type, physical characteristics, eye and hair colours, complexion and the height of the husband should be matched. All donors should always be screened for AIDS, hepatitis and STD. (Table 13.5).

Table 13.5: Pre-conditions for sperm donation

1. Strict secrecy to ensure no contact between the donor and recipient.
2. Written consent from both husband and wife.
3. Undertaking from husband to go through legal process for naturalisation of the child.
4. Couple made aware of inherent risks of pregnancy and congenital abnormalities in the child.
5. Donor to be screened for AIDS, hepatitis, STD and hereditory diseases.
6. Donor to be matched for blood group and physical characteristics of husband.

Maintenance of strict secrecy of the procedure is mandatory to make it impossible for any future contact between the donor and the recipient. Both husband and the wife must be asked to sign a consent form prior to the insemination process. Not only does the husband gives consent to the artificial insemination of his wife, but agrees to the process of law and to treat child borne by his wife after an artificial insemination as his natural child. He should be fully apprised that any such pregnancy would carry the same risks and all possible complications as is natural in general population, including chances of having child with congenital and genetically transmitted diseases.[37]

In order to obtain an optimal semen specimen, the same protocol or drill should be followed as in AIH to enable the physician to evaluate the condition for potential adverse effects upon the sperm production at the time of the ovulation. Follicular maturation studies need monitoring, so that the sperm insemination coincides with the ovulation to maximise the chance of fertilisation. Probability factor of conception would naturally increase with one or more "fresh" semen specimens.

Artificial insemination has become an acceptable and recognised practice throughout the world. Adoption can be an attractive alternative, but there has been a growing scarcity of babies for adoption and prospective parents may have to wait a long time before adoption becomes possible. In India, there is much greater remand for a male child to adopt, while a female child is relatively easy to get.

Artificial insemination in selected cases may have certain advantages. It has been found that the child conceived by artificial insemination is often biologically closer to the parents both physically and in some cases, emotionally. The wife has an opportunity to experience pregnancy, birth, nursing, and all the other roles of motherhood she wishes. But each couple on an individual basis must consider the moral, philosophical, and religious aspects. A detailed discussion between the physicians, the couple and if necessary, a counsellor should thus be mandatory before the process is initiated.[38]

NEWER METHODS OF ASSISTED REPRODUCTION

Sperm Aspiration

Sperm aspiration is a procedure to obtain viable sperms from the male reproductive tract. Main indication for this procedure is severe forms of male infertility caused by obstructive and non-obstructive azoospermia. It is also indicated in patients with severely impaired or almost non-existent sperm production as in severe oligospermia and necrospermia. Sperm aspiration (when performed using the appropriate technique) is usually a very successful and minimally invasive procedure that allows even a men with only a few sperms to get his own biological child (Fig. 13.2).

The collected sperms are intended specifically for use in ART or ICSI (see later), and sometimes for IUI. Usually, enough sperms are obtained with this method for storage and freezing. An acceptable prerequisite for IUI is at least 3 million (preferably 5 million) mature motile sperms with normal

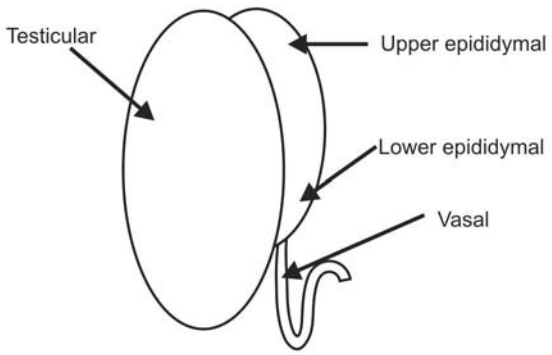

Fig. 13.2: Sites for sperm aspiration

morphology that have passed through the epididymis.[37] Sperm harvesting techniques used to obtain sperms include the following procedures.[35-37]

Microsurgical Epididymal Sperm Aspiration (MESA)

The MESA (also described as sperm microaspiration retrieval technique or SMART) is an adjunctive treatment method for an obstructive azoospermia[39] to obtain sperms from the epididymis using an operating microscope. The epididymal sperm can be obtained by several procedures other than MESA through mini-MESA (modified MESA), macroscopic epididymal sperm aspiration (MaESA) and percutaneous epididymal sperm aspiration or PESA.[40] The first pregnancy with MESA and IVF was recorded in 1985.[41] At present, several groups of workers have reported pregnancies using microscopic puncture of an obstructed vasoepididymal tract in acquired and congenital azoospermia, as well as in anejaculation.

MESA is indicated for all those obstructive disorders that are not surgically correctable, and where the results of repeat surgery are poor. The common indications for MESA are congenital bilateral absence of the vas deferens, bilateral ejaculatory duct obstruction not corrected by transurethral surgery, obstructive azoospermia secondary to surgical removal of the vasal ampullae and seminal vesicles during cystoprostatectomy or radical prostatectomy, and failed vasoepididymostomy. However, it is contraindicated for the nonobstructive lesion with normal ejaculation and for severely oligospermic patient with primary testicular dysfunction. MESA is also not preferred option for the vasectomised patient without a trial of a first-time vasectomy reversal (Table 13.6).

Table 13.6: Indications of MESA

1. Congenital absence of vas.
2. Bilateral non-constructible ejaculatory duct obstruction.
3. Obstructive azoospermia following operations like cystoprostatectomy, radical prostatecotmy, etc.
4. Failed vasoepididymostomy.

In MESA the sperms are aspirated either by an *epididymal* or *deferential* puncture. An epididymal puncture is indicated in congenital agenesis of the vas deferens, where only the head of the epididymis is available. A deferential or lower epididymal sperm aspiration can be used in an anejaculatory or in a failed vasovasostomy patient (Fig. 13.2).

There are qualitative differences between the sperms from a high epididymal punctures and those from low epididymal or deferential punctures. In a normal individual, the sperms acquire forward motility and fertilising ability after they have travelled through the distance of the epididymis.[42,43] On the contrary, in an abnormal epididymis, motile sperms may be found in the transition zone between caput and corpus of epididymis.[44] Probably, the sperm maturation takes place in the proximal part of the epididymis in this situation.[45] In case no or insufficient spermatozoa are obtained, one is left with option of using a donor sperm or to skip the cycle.

Procedure

There is a similarity in the steps of MESA and that of vasoepididymostomy, once the testes are exposed. A standby arrangement for a full microsurgical procedure must be made available before one performs MESA.

Identification of the areas with dilated tubules is the first important step. Using the operating microscope, dilated tubules are opened and the oozed out fluid is sent for the presence of sperms. As far as possible, one should endeavour to get good sperms to perform a conventional IVF. Accordingly, one logically tries to obtain sperms from the tubules located as low as possible along the epididymis. However, in the backdrop of experience gathered from vasoepididymostomy and MESA, sperms with better motility are not necessarily encountered there.[42,43] One may have to go higher, even up to the *rete testis* for harvesting motile sperms.

Once the dilated tubule is chosen, a tiny window in the overlying epididymal serosa of the tubule is made. Using microscissors, the tubule is then opened. Concurrently microhaemostasis is performed whenever necessary. After examining the oozed out fluid for the motile sperms, glass capillaries of three different diameters (100, 200 and 500 micron) are used to aspirate the fluid as quickly as possible.[46] The size of the capillary is so chosen that the tips enter snugly into the opening of the tubule. When one is filled, successively, one by one several capillary tubes are used. From then on the reproductive scientist or physician takes over. The contents of the capillary tubes are dissolved in the culture medium, and the sperm contents are meticulously checked.

There are two technical options. In the first, one tries to obtain as many sperms as possible through one single puncture site. The tubule and the

overlaying serosa are then closed leaving the epididymis intact for any subsequent trial. In the other option, one can enter at multiple different sites of the epididymis in order to obtain as many sperms as possible, but risking injuries to the whole epididymis. Some researchers have successfully enhanced the motility of frozen thawed epididymal sperms using pentoxifyllin.[47] There are now some instances, where pregnancy was successfully achieved with sperms obtained from puncture of the epididymis, thus avoiding scrotal exploration under general anaesthesia.

It is important to preserve extra sperms to obviate the patient from having to undergo multiple future procedures with an inherent chance of failure in the first cycle of ICSI. Moreover, the sample can consequently be used for the couple thinking of having more children later.

Advantages

1. MESA allows for the recovery of the best quality and highest quantity of sperms compared with the other techniques.
2. It is also the safest procedure and produces the least amount of complications, discomfort and swelling.
3. Each step of the procedure is clearly visualised under the microscope and the testicle itself is not entered.
4. Any bleeding during the procedure can be quickly stopped.
5. Most patients recover from MESA within a day and can return to work and daily activities.
6. MESA is more cost-effective than other techniques, because it is usually a one-time expense, whereas other sperm retrieval techniques may need to be repeated.

Disadvantages

1. Relative unavailability of MESA, as it can be offered only at centres specialising in the treatment of male infertility.
2. Need for the specialised equipment, an operating theatre and a trained infertility microsurgeon.
3. MESA is still very costly in the subcontinental set up and is affordable only to a few.

Percutaneous Epididymal Sperm Aspiration (PESA)

PESA is a simpler convenient, inexpensive and effective technique. It is an outpatient procedure for retrieving sperms, where they are sucked out from the epididymis by puncturing it with a fine No 6 needle. Microsurgery also can be used to find epididymal sperms. To improve the quality of sperms, Yamamoto suggested a modification, where the epididymal micropuncture was done with nerve stimulation to yield higher fertilisation and pregnancy rates than the conventional MESA for ICSI.[48]

Advantages

1. PESA is less expensive than MESA
2. It is conceptually more appealing than MESA.

Disadvantages

1. PESA is a blind procedure. Surgeon cannot see, where he is placing the needle.
2. Chance of formation of a haematoma because of accidental injury to the underlying blood vessels.
3. In the practical field, it often yields a few usable sperms and rarely, enough sperms are obtained for freezing.
4. Consequently, a second PESA procedure is needed to save the IVF cycle.

Male infertility experts rarely recommend PESA, because of relatively uncertain and often poor outcome and higher rate of complications. Sperms are rarely present in the epididymal tissue of patients with non-obstructive azoospermia. In an obstructive azoopsermia, where sperms cannot be found in the epididymis, the logical next step is to trace them in the testis itself, as the epididymal procedures such as MESA or PESA are inappropriate for retrieving sperms from these men.

Testicular Sperm Aspiration (TESA)

The TESA involves sucking out the testicular tissue through a fine needle under local or general anaesthesia. The sperms are then isolated from the testicular tissue. A second PESA procedure may be performed in case of failure to achieve partner pregnancy before performing a testicular sperm aspiration or extraction.[49] It is an office procedure performed under local anaesthesia. A small incision is made in the scrotal skin and a spring-loaded needle is fired through the testis and a small piece of testicular tissue is removed.

In certain situations like maturation arrest, a large amount of testicular tissues has to be removed, as foci of spermatogenesis are not easily discernable.

Extensive multiple biopsies from several areas of testis may need to be performed in TESA in an effort to get sufficient number of sperms. However, it may also cause damage to the testis and limits the patients to only one chance, as no worthwhile testicular tissue survives the procedure.

In a TESA dissection, closure of the tough inelastic tunica albuginea after the removal of the testicular tissues may also cause increased intratesticular tension from the microscopic and minute bleeding points in the testicular tissues. Consequently, it leads to further damage to the sparse testicular tissues. One way to obviate this situation is to use fine "9"o absorbable interrupted suture like vicryl instead of running stitches and to use microsurgical technique with microcoagulation of the bleeding vessels. Fine-needle TESA is more likely to get representative testicular tissues in patients with an obstructive rather than a nonobstructive pathology because of large variations and sparseness of spermatogenesis in the latter.[50]

Disadvantages

1. While it is possible to retrieve sperm using TESA, the volume of tissue is often small, because the needle cuts only a thin sliver of tissue.
2. Many embryologists find this small amount of tissue difficult to work with, as not enough sperms to freeze for future use are found.
3. The potential for complications is higher with testicular sperm extraction (TESA) than with TESE for two important reasons.
   a. TESA being a blind procedure, bleeding cannot be stopped, when any vessel is accidentally encountered.
   b. The needle is larger in diameter than the intratesticular artery, and can sever the artery potentially cutting off the testicular blood supply with eventual testicular atrophy.
4. Men producing very few sperms may need to have multiple areas of the testis sampled before enough usable sperms are found.
5. Needle biopsy does not yield as much tissue as open biopsy and, therefore, it yields fewer sperms.

TESA procedure is not advocated in most centres of excellence, because of the reasons mentioned above. If no sperm is found on a needle biopsy, an open biopsy should be performed before cancelling an IVF cycle. PTESE or TESE is the better alternative as the optimal technique for obtaining testicular sperms for men with nonobstructive azoospermia. Several studies have demonstrated that TESE is superior to TESA in all regards.

Testicular Sperm Extraction (TESE)

TESE stands for testicular sperm extraction. In men with persistent nonobstructive azoospermia,[49,51] TESE with ICSI has provided encouraging results. It is an open procedure performed under direct vision under mild sedation and, therefore, it minimises potential complications. A small piece of testicular tissue is removed through a ½-inch skin incision. The tissue is placed in a culture media and morselised into tiny pieces. Sperms within the seminiferous tubules are then extracted from the surrounding testicular tissue. This can be an exhaustive process depending on the degree of sperm production.

Percutaneous testicular sperm aspiration (PTESE) or the TESE is performed under general or local anaesthesia on patients with nonobstructive azoospermia in the upper and lower poles of the testis. Experienced andrologists perform this operation under local block like in MESA. The PTESE is followed by immediate microscopic search of the aspirate to confirm the presence of spermatozoa.

In the absence of spermatozoa an open excisional biopsy may need to be performed at the same setting. A conventional or a microdissection technique for the testicular sperm extraction is performed as a day-case using a biopsy needle (Hepafix Set B Braun). At first, the conventional open biopsy TESE is attempted in all patients. If the sperm is retrieved successfully, the procedure is terminated. Micro-dissection TESE is indicated only for the patients, where the conventional sperm retrieval is unsuccessful.[52]

The freshly aspirated spermatozoa are used either immediately after aspiration or frozen in cryo-preservative for later use.[53] Antibiotics and common analgesic drugs are used routinely after the puncture. PTESE can cause possible testicular injury in a blind or even in an ultrasound-guided puncture[54] and the resultant morbidity of the procedure should be borne in mind.[53] The effectiveness of obtaining sperms for micromanipulation is 100% in ICSI-PESA and 75% in ICSI-TESE.[55]

Advantages

1. TESE is the procedure of choice for men with non-obstructive azoospermia.
2. It can be performed in an operating room or office procedure room using a local anaesthetic.
3. Open biopsy TESE is more effective and potentially safer than the needle biopsy (TESA). It is the second best way to collect sperms.
4. Sperm harvested using TESE can be frozen and stored for later use.

Disadvantages

1. The amount of sperms obtained from the testicular tissue is not nearly as much as obtained with MESA.
2. Testicular sperms do not freeze and thaw as well as epididymal sperms.
3. Many andrology laboratories find it difficult to work with the TESE sperms.

OUTLINE OF IN VITRO FERTILISATION

(See also Appendix)

In vitro fertilisation or IVF was first used for the female factor infertility. The initial cases of IVF performed in the late 1970s and early 1980s were limited to women with fallopian tube obstruction. The techniques have since evolved to assist infertile men due to irreparable obstruction, such as congenital absence of the vas deferens or poor sperm production. Essentially, IVF involves extraction of human ova from the ovaries and then achieve the fertilisation in the laboratory. The fertilised ova is then transplanted to the uterus for the continuation of the pregnancy. Male factor infertility patients have a lower success rate compared to other groups. But once the fertilisation has taken place, there is not much difference in the pregnancy rate. Not only can IVF be useful in circumventing various obstructions to sperm pathways in the female genital tract, but also it can ensure fertilisation of ova even with concentrations of 20,000 to 100,000 motile sperms.

Fertility Drugs

Fertility drugs are the primary treatment for infertile women with ovulation disorders. These medications use different mechanisms to regulate or induce ovulation. In general, they exert actions designed to work like natural follicle-stimulating hormone (FSH) and luteinising hormone (LH). These fertility medications are listed in Table 13.7.

Table 13.7: Fertility medications

1. Clomiphene citrate (Clomid, Serophene).
2. Human menopausal gonadotrophin (hMG).
3. Human chorionic gonadotrophin (hCG).
4. Follicle-stimulating hormone (FSH).
5. Gonadotrophin-releasing hormone (GnRH).
6. GnRH analogues.
7. Bromocriptine.

1. *Clomiphene citrate (Clomid, Serophene):* This drug is used to stimulate ovulation in women, who have polycystic ovary syndrome (PCOS) or other ovulatory disorders. It causes the pituitary gland to release more FSH and LH, which stimulate the growth of an egg follicle.
2. *Human menopausal gonadotrophin (hMG):* This medication is prescribed for women, who do not menstruate on their own due to the failure of the pituitary gland to stimulate ovulation. Unlike clomiphene, which stimulates the pituitary gland, hMG and other gonadotrophins directly stimulate the ovaries.
3. *Human chorionic gonadotrophin (hCG):* This is used in combination with clomiphene, hMG and FSH. This drug stimulates the follicle to release its egg.
4. *Follicle-stimulating hormone (FSH):* This is essentially hMG without the LH. Like hMG, it works by stimulating the ovaries to mature egg follicles.
5. *Gonadotrophin-releasing hormone (GnRH):* This medication is used in women, whose hypothalamus does not properly release the natural hormone GnRH, which stimulates the pituitary gland's release of FSH and LH. GnRH is administered in a pattern that mimics the natural rhythm.
6. *GnRH analogues.* This drug is used to treat women with irregular ovulatory cycles, who ovulate prematurely (before the lead follicle is mature enough) during hMG treatment. GnRH analogues deliver constant GnRH to the pituitary gland, which alters hormone production, so that physician can induce follicle growth with FSH.
7. *Bromocriptine:* This medication is used to treat women, whose ovulation cycles are irregular due to elevated levels of prolactin. Bromocriptine inhibits prolactin production.

Fertility drugs are occasionally responsible for multiple births. While clomiphene citrate only rarely causes pregnancy with more than twins, hMG and

FSH carry a greater risk of causing multiple births in about 15 to 25 percent cases. As a rule, the greater is the number of foetuses, the higher the risk of premature labour with medical and developmental problems in newborns.

Some steps are available to reduce the risk of multiple pregnancies. If a woman requires an hCG injection to trigger ovulation, and the ultrasounds show that too many follicles, her physician can decide to withhold the hCG injection. For many couples, however, the desire to become pregnant overrides concerns about conceiving multiple babies. If too many babies are conceived, the removal of one or more foetuses (multifoetal pregnancy reduction) can offer improved survival odds for the surviving foetuses. However, this presents serious emotional and ethical challenges for many couples. Those considering fertility drug treatment should discuss this possibility with their doctors before starting therapy.

Some studies have suggested a link between fertility drugs and an increased, long-term risk of developing ovarian cancer. However, in 1998, a large study showed no association between the fertility drugs and ovarian cancer. Infertility due to endometriosis often is more difficult to treat. Although hormones, such as birth control pills, are effective for treating endometriosis and relieving pain, they have not been useful in treating infertility.[23]

Several modifications of IVF evolved with time. In gamete intrafallopian transfer (GIFT), the ova retrieved and the sperm are mixed together. They are then injected in a way, so that the fertilisation can occur in the fallopian tube. GIFT ensures the fertilisation to occur at the normal site in the fallopian tube, but makes its occurrence unknown till a pregnancy actually takes place. Presently, several variations of IVF and GIFT such as pronuclear stage tubal transfer (PROST), zygote intra-fallopian transfer (ZIFT), tubal embryo transfer (TET) and tubal embryo stage transfer (TEST) have been tried. All these techniques involve the IVF fertilisation of human ova and subsequent transfer of the early-stage embryo back into the fallopian tube. Using these methods, a pregnancy rate of 10 to 35% have been reported by various workers for the infertile couples.

Despite the success with IVF and GIFT, additional refinements were necessary for males with extremely poor concentration of functional sperms. This was achieved through various methods of micromanipulation of gametes, sperms and ova. The methods utilised include partial zona dissection (PZD), subzonal sperm injection (SUZI) and intracytoplasmic sperm injection (ICSI-pronounced "*eeksee*"). In recent times, advent of these methods has improved the overall fertilisation rates to about 20 to 40% and the clinical pregnancy rates to as high as 30% in some IVF laboratories, but the rate has only been close to 10% for the severe male factor problems.

In vitro, the zona pellucida is the most important barrier for penetration of the egg by the sperms. Using micromanipulation, this barrier can be overcome to achieve fusion of the ova and the sperm. Results of experimental studies for several decades by Lillie, Hiramoto, Lin, Uehera and Yanagimachi[56-60] provided ample evidence that activation of the oocyte and formation of male pronuclei are possible without the normal sperm-ova fusion inside a species. Mitha et al[59] first reported clinical application of microinjection in 1985 and 1987. Since then, the method made rapid strides and different micromanipulation techniques were developed. However, all these techniques require a skilful reproductive physician and laboratory technician.

The first method used, to address the problems of male factor infertility, was called zona drilling and partial zona dissection (PZD). Using PZD, the zona pellucida, or shell, surrounding a woman's egg was opened, using either chemical dissolution or a sharp instrument to file through the shell.

Initially, the zona was opened by digestion using acidic tyrode solution that was gently released at the zona with microneedle. But in human context, digestion of the inner layer took longer as the solution itself was found to be more toxic and the incidence of polyspermia was nearly 50% of the fertilised oocytes.

In 1988, the first pregnancy was reported, when Cohen et al[62] developed the mechanical method. By this method, abrasive effect of the micropipette created a slit in zona for the sperms and the polyspermia rate was brought down to 24%. PZD appeared to be simple and attractive idea for male infertility, but potential invasion of microorganism and protrusion of blastomeres through the gap may inhibit development of the embryo. This process, while certainly a step forward in the relatively new field of micromanipulation, was considered rather passive, because even with the zona opened, there

was no guarantee the sperm would enter and fertilise the egg. With PZD, frequently too many sperms would enter the egg causing genetic abnormalities and arrested development of the zygote. These problems related to the PZD process limited its success to achieve pregnancy.

The latest modification is the laser beam in a specific configuration that is able to induce minimal superficial damage to the zona pellucida of oocytes. This manipulation is aimed at increasing the fertilisation rate following insemination with low-quality spermatozoa. Another intracellular application of the laser beams is the destruction of extra pronuclei in polyspermic fertilised human oocytes.[63]

The next step was a process called subzonal insertion (SUZI), which was similar to PZD, but more aggressive (Fig. 13.3). In SUZI, once the shell was punctured, the sperm was injected into the area between the zona and the ovum instead of being left to find its own way. Its chances were greatly increased with this specific placement, although the sperms still had to enter the ovum. This process dramatically increased the success rate of IVF, by partially overcoming the poor motility and the low count of sperms.

In subzonal insertion, zona is not opened; but after the aspiration of sperms, the pipette is passed through the zona with the first polar body at 6 o'clock or 12 o'clock. Only a limited number of sperms are consecutively injected into the perivitelline space. After SUZI only sperms, which have undergone capacitation and acrosome reaction, are capable of fusing with oocyte. Acrosome-inducing agents such as strontium chloride may be used. However, one of the concerns was that arbitrarily chosen sperms caused increased incidence of chromosomal abnormalities. Injection of 10 to 15 sperms was found to give optimal success in fertilisation. Using this method Laws-King,[64] reported fertilisation in 1987 and Ng et al,[65] reported first pregnancy in 1988.

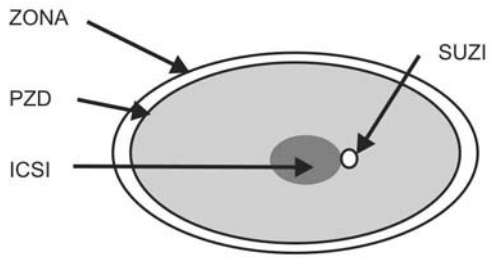

Fig. 13.3: Methods of micromanipulation of gamete

Although there was clear benefit of SUZI on the 2PN fertilisation, the results still remain lower than those obtained after IVF with normal semen. However, polyspermia (more than one sperms entering ovum) was still a problem. It was still not possible to control the number of sperms entering the egg in the SUZI process. Use of the ICSI has dramatically changed the overall situation.

INTRACYTOPLASMIC SPERM INJECTION (ICSI)

ICSI is a technique in which a single sperm is injected into the centre of the cytoplasm of the egg to achieve fertilisation. Since the sperm is being injected directly into the egg, all that is needed to achieve fertilisation are a few live sperms. In experimental studies, injection of single sperm into the oocyte cytoplasm resulted in fertilisation with formation of a male pronucleus.[57] In human beings, Lanzendorf[66] and co-workers made first successful attempt of single sperm injection into the cytoplasm of ovum in 1988. Palermo et al reported the first pregnancy with ICSI in 1992.[67,68]

With ICSI, the latest in the evolution of micromanipulation, the equation "*one egg plus one sperm = one embryo*" became possible. Sperms of virtually any quality and from any level of the male reproductive tract may be used, provided the sperm is alive, even if it is not motile. Dead sperms may even be able to achieve fertilisation. However, the DNA or genetic materials from such sperms are incapable of forming a viable embryo. Immature sperms from the testis, or the epididymis can be retrieved for use in ICSI for men with azoospermia.[49] An obstruction in the tract (obstructive) or extremely low production in the testis itself (nonobstructive) could show absence of sperms in analysis of semen. In certain cases men may produce sufficient sperms, but they are dead by the time they are ejaculated (*necrospermia*). Consequently, instead of using nonviable sperms from the ejaculate, testicular tissue can provide a ready source of freshly produced viable sperms.

Several authors,[69] who presented papers in the 90th Annual Meeting of the American Urological Association, in 1995 at Las Vegas, agreed, "ICSI has provided an important approach to the treatment of severe male factor infertility." The results demonstrate that this technique significantly enhances fertilisation rate compared with routine IVF for the male infertility.[70] A diode laser can be used for the immobilisation of sperm and for opening a hole in

the zona pellucida (ZP) to facilitate ICSI,[71] and assisted hatching. A focused laser beam is applied *in vitro* to form a channel or trench in the ZP.[69]

There is a debate, whether the acrosome reaction is necessary for sperm incorporation after ICSI. The acrosome reaction, when it occurs, is preceded by acrosome swelling and is followed by vesiculation of surface membranes exposing the inner acrosome membrane. This is observed on the surface of the zona during IVF or in the perivitelline space after subzonal sperm injection. In ICSI, the physiological sperm acrosome reaction is prevented and often the acrosome is totally deleted from the head, before sperm is incorporated. It is conjectured that the sperm head expansion could be delayed or abnormal without this process. Ultrastructural evidence shows that the acrosome reaction could occur in the ooplasm before the sperm incorporation in mature human oocytes, or the acrosome could be discarded intact before sperm incorporation in immature oocytes, matured *in vitro*. These sperms probably get capacitated at the time of ICSI. These facts lead to the conclusion that both the acrosome reaction and its deletion are possible prerequisites to sperm incorporation after ICSI. Unlike the IVF, in ICSI the process of sperm outer plasma membrane is not incorporated into the oolemma or oocyte outer membrane. [71-73]

Direct intracytoplasmic sperm injection (DICSI) has significantly decreased the minimum sperm density requirements for the assisted reproduction. DICSI with ejaculated sperm produces fertilisation and pregnancy rates equal to that of conventional IVF.[74] Using DICSI in conjunction with parenchymal sperm retrieval (PSR) or MESA has improved fertilisation rates per cycle to 100% and pregnancy rates per cycle to 50%.

The indications for PSR are bilateral congenital absence of the vas deferens and failed vasal reconstruction. Induction of ovulation is carried out using a combined treatment of GnRH agonist and hMG in a short protocol. Sperms are retrieved by micromanipulation after incubation in human tubal fluid of 0.5 gram of testicular parenchyma obtained by excision biopsy. The retrieved sperms were sequentially washed and concentrated into microdroplets and then injected.

Approximately 24 hours later, the eggs are observed for changes suggesting that fertilisation has taken place. Mainly the presence of two early (pronuclear masses (*2-PN stage* of fertilisation) within the egg is looked for. Next few days are very critical, as this phase constitutes the weak link in the process. In this phase, the egg is nurtured in the tubal fluid containing many substances that normally sustain an egg and early embryo in a woman's body. In natural fertilisation, the fertilised egg enters the uterus approximately four days after ovulation and fertilisation, and then get actually physically implanted in the uterine wall after another two days or so. Until recently, most laboratories could not sustain embryo development for this duration, so clinics were forced to place embryos in the uterus two or three days after the egg retrieval.

For several reasons, an embryo is less likely to survive, when placed in the uterus at this young age. Innate gene factor of the embryos do not take control until four cells have developed. At three-day stage period of the embryos, 20 to 30% are actually genetically imbalanced, although it appears apparently normal under the microscope. This is tantamount to nonsurvival after implantation in most cases. This necessitates implantation of several embryos in the hope that one will survive. Unfortunately, this has an attendant complication of high multiple pregnancy rates in 25-40% cases.

Indications

In the past, the only available source of sperms for these male infertility patients was the man-made reservoirs (artificial spermatoceles, see Chapter 12), which were used to collect sperms for artificial insemination. The resultant pregnancy rates were dismal at less than 5%. Indications for ICSI has been standardised by the American Society of Reproductive Medicine (Table 13.8).[75]

Table 13.8: American society for reproductive medicine [75] (Indications for ICSI)

1.	Very low numbers of motile sperm.
2.	Severe teratospermia.
3.	Problems with sperm binding and penetrating the egg.
4.	Antisperm antibodies thought to be the cause of infertility.
5.	Prior or repeated fertilisation failure with standard IVF methods.
6.	Frozen sperm limited in number and quality.
7.	Obstruction of the male reproductive tract not amenable to repair.

Procedure of ICSI

The ICSI is done in a superovulated cycle during which fertility drugs hMG are administered to the

wife to aid in the production of multiple eggs. A long, thin needle is inserted through the vagina using ultrasound guidance to target the needle to the ovary and the eggs are collected with the needle. In normal circumstances, all of the cumulus-corona, the cluster of cells that surrounds the ovum, must be removed. If the cumulus is not removed, it could create a shadow that may impair viewing and jeopardise the injection. This removal also allows the embryologist to assess the maturity of the ovum.

These cumulus cells are removed by repeated passage into the oocyte cumulus corona complex through fine pipettes, and by treating them with hyaluronidase, so that these cells are stripped off. The denuded eggs are examined, and only mature eggs (eggs in metaphase II) are used for ICSI. The metaphase II oocyte has extruded its first polar body and displays a sunburst array of cumulus-corona cells. The cumulus cells must be removed before the ICSI procedure can take place.[2]

Sperms are collected from the male partner, usually through masturbation. For men with severe oligospermia, sequential ejaculates are used. Even though the first semen sample may not contain any sperm, motile sperms in the second (or even the third sample) are often found as the later samples often contain "fresher". Since these samples contain only a few sperms, the subsequent processing needs to be done very carefully, so that all the sperms in the sample are recovered in the culture medium, and can be used for ICSI.

For men with variable sperm count, which vary from zero to a few thousands, it may be helpful to freeze a sample containing sperms in advance. For patients with azoospermia, sperm harvesting techniques need to be used to retrieve the sperm. For obstructive azoopsermia, the simplest technique such as PESA is conveniently used, but MESA or TESE is a better alternative treatment for all obstructive disorders that are not surgically remedial.

MESA is performed in conjunction with either IVF or GIFT and/or assisted fertilisation through gamete micromanipulation. Highest level of expertise and dedication from a team of andrologist, gynaecologist and reproductive physician is expected to get the optimal chance for conception. In this complex procedure, the level of sperm attainment, quality of the sperm and female factor are all quite important parameters in achieving fertilisation and pregnancy. Pregnancy rates from different groups vary widely ranging from 0 to 20% per attempt. Indubitably, improvement in the pregnancy rate is expected in future with refinement of the process of manipulation of sperm and the ovum.

In obstructive azoospermia, where sperms cannot be found in the epididymis, the logical next step is to trace them in testis itself through TESE or TESA. Even with very small testes, defects in sperm production are "patchy" and do not affect the entire testis uniformly. Production of sperms is obviously absent in certain areas, yet there may be other areas in the testis, where it is normal. Consequently, enough sperms are available for ICSI in over 50 percent of patients with testicular failure. It requires only a few sperms (theoretically one single sperm) for fertilisation. However, in patients with nonobstructive azoospermia, rate of fertilisation, quality of embryo and pregnancy rates are lower than those with obstructive azoospermia. This is accounted for by more severe defects in spermatogenesis leading to poor gamete quality in nonobstructive azoospermia.

Once ovum and sperms are collected, the actual process of injecting a single sperm into the ovum is carried out in a laboratory using a petri dish or a glass slide with a well in the centre. A glass holding pipette 40 to 50 micron in diameter is used to secure the egg, usually at its left side. An injection needle with an outer diameter of roughly five to six micron and an inner diameter of three to four micron is used to pierce the ovum membrane at its right side at about 3 o'clock position. The injection needle has an extremely sharp and bevelled ends, one that will most easily pierce the egg membrane. For embryologists using a glass slide, the arm of the injecting needle can be straight or only slightly bent at the end. For those using a petri dish, the arm of the needle must be angled about 40 degrees to ensure manipulation without interference from the lip or side of the petri dish.

Active sperms are chosen and placed in a drop of polyvinyl pyrrolidone or PVP solution. This solution is dropped onto a viscous medium such as mineral oil to slow down the activity of the sperm, and also to serve as a cleanser. Restraining the movements of active sperms ensures their detailed viewing and prevents damage, while being drawn into the injecting needle. Ironically, while the active sperms are chosen prior to being placed in the PVP, once in the solution, the sperms with the least amount of activity become the best candidates for injection. In fact, the best

choice is to use those sperms sticking to the bottom of the well by their heads. Actively moving sperm tails can whip around inside the egg to cause its damage or even its eventual destruction. Immobilising the tail of the sperm by pinching, before the sperm is put in the ovum, obviates this.

Commonly, the sperms are placed in a drop in the middle of the dish or slide surrounded by eggs that have also been placed in a viscous medium. In some cases, only one egg is placed in a droplet around the sperm in order to preserve its individuality. Some embryologists or reproductive physicians prefer all sperms in one drop and all eggs in another. Both these methods have been used with success.

In experienced hands, it takes less than 60 seconds for the sperm to be injected directly into the centre of the ovum, once the holding pipette secures the ovum. However, successful fruition of ISCI depends on precise penetration of the egg membrane ensuring that the sperm, not being redrawn back into the pipette upon removal from the egg. Remarkably, once the injecting pipette is withdrawn, the ovum will close and assume its original shape within 60 seconds. Injection of too much medium into the ovum along with the sperm needs to be avoided. In the ultimate analysis, the skill of the reproductive physician is singularly important critical factor in the success of the ICSI process.[2, 37]

Approximately 14 hours after the injection of the sperm, the physician looks through the microscope for the occurrence of fertilisation, and after 24 hours to observe whether the egg has cleaved. To ensure proper cleavage of eggs, removal of enucleated fragments located between the cell divisions is performed at times. This process is described as *assisted hatching*. If all these steps of ICSI process go as planned, implantation of the fertilised egg into the female patient can be completed within 72 hours of the initiation of the injection of sperm. The number of embryos to be implanted is often decided by the age of the female in most cases and varies from one laboratory to the other.

ICSI for Nonmale Factor Infertility

Whenever there is a possibility that conventional IVF may fail, ICSI has become a common feature of human IVF therapy. It is fast becoming the insemination technique of choice. ICSI has also made it possible to rescue cases following completely failed conventional fertilisation. For some conditions such as idiopathic or unexplained infertility caused by reduced production of quality eggs due to hyper-responsive ovarian stimulation, ICSI is now being routinely used.[76] Indeed, ICSI is being tried, even if there is an extreme need to maximise normal fertilisation. ICSI for oocyte pathology may represent the solution in case of zona pellucida anomalies, deficiency of the fusion ability or absence of cortical reaction. However, these indications remain scarce.[37]

Results of ICSI

According to the American Society for Reproductive Medicine, fertilisation occurs in 50 to 80% of injected ovum. In USA, 30% of all ICSI procedure resulted in live birth in a rate comparable to standard IVF. The overall fertilisation rate was significantly lower after standard IVF than after ICSI: 37.4% vs 64.3%.[73] ICSI also does not increase the incidence of multiple gestations as compared to standard IVF. Younger the patient more is the chance of having a favourable result. Advanced maternal age and poor quality of ovum, on the other hand may result in lower rates of success. Unfortunately, the process of ICSI may damage a small percentage of eggs. Some studies reported increased incidence of hypspadius in newborns, and with the familial incidence in male infertility, the defect may be passed on to the male offspring in some cases. Overall, fertilisation rates ranged from about 20 to 40%, with clinical pregnancy rates reported as high as 30% but average for severe male factor is close to 10%.[37]

ROUND SPERMATID NUCLEUS INJECTION (ROSNI)

Recent research has made another step forward and brightened the prospect of another exciting breakthrough in male factor infertility through a process called round spermatid nucleus injection or ROSNI.[77,78] This micromanipulation is specifically designed for men, who are not manufacturing mature sperms, and showing azoospermia.[79] ROSNI process involves taking immature cells (round spermatids) directly from the testicle, removing the nucleus containing the genetic material, and injecting the nucleus into the female partner's ovum removed during an IVF cycle.

In the hamster and mouse, the nuclei of round spermatids were found to be capable of participating in syngamy, when incorporated into homologous

mature oocytes by microsurgical ICSI.[80] Human spermatids from the ejaculate and testicular tissue have now been utilised for evaluating human fertilisation by ICSI.[81] Successful fertilisation and normal embryonic development in vivo after ROSNI into rabbit oocytes and embryo transfer procedures[82] have already been reported.

Round spermatids (RS) were preferred to elongated spermatids, because of very poor viability status of elongated spermatids present in the ejaculate. Next step was to perfect the *in vitro* culture technique of spermatogonia, spermatocytes and spermatids, which can then be used as male gametes.

Antinori et al[83] reported successful human pregnancy with normal karyotype after using round spermatids. Barak et al[84] also reported a normal pregnancy and childbirth from a single-nucleated zygote in a round spermatid injection treatment cycle with ejaculated spermatids. So have Gianaroli et al.[85] These results substantiate that ROSNI and subsequent embryo transfer can provide a great hope for azoospermic men to father biological children of their own.

These findings further indicate that post-meiotic modifications of the human RS are not required for the pairing of male gamete chromosomes with those of the oocyte. However, one has to accept the fact that the injection of round spermatids from patients with complete failure of spermiogenesis results in a significantly lower fertilisation rate and a higher developmental arrest compared with the injection of mature spermatozoa.[86, 87]

ASSISTED HATCHING

Assisted embryo hatching is a technique, which involves removing a portion of the zona pellucida with an acidic solution prior to embryo transfer in IVF. Hatching is performed potentially to increase the chance of implantation of the embryo. It has been observed that a few otherwise viable embryos having reached the blastocyst stage, are unable to get implanted, as they are unable to break free from the surrounding jelly coat of zona pellucida that protects the early pre-implantation embryo initially.

An unfertilised egg with a transparent glycoprotein coat around, is protected to regulate the normal fertilisation by any other penetrating sperms. When the embryo reaches the stage of blastocyst, it starts filling itself up with fluid like a water-filled balloon. It continues to expand itself larger and larger, till it ruptures and "hatches" from the zona pellucida to set the stage for the embryo to make direct contact with the endometrium, and get implanted. The procedure is also called *zona hatching*. This enables it to "hatch" free from the zona pellucida more easily, as a blastocyst in the uterus (Table 13.9).

Table 13.9: Methods of assisted hatching

1. ***Mechanical method (PZD):*** Embryo is held in position with gentle suction through the micropipette, while microneedle is pushed tangentially through the zona into the largest perivitelline space of the embryo.
2. ***Chemical method:*** Here zona drilling or thinning is carried out using a stream of acid Tyrode solution filled microneedle 180° from the point, the zona is stabilised using the suction through the micropipette.
3. ***Laser method:*** It has the advantage of making a standardised hole in the zona and can be used for both thinning as well as for drilling a hole. Moreover, the embryo need not be held with the suction pipette. Two or three minute holes close to each other are normally made instead of a large single hole.

   Zona thinning is more natural and thus advantageous as even the hatching can be done in the blastocyst stage as seen in vivo. As there is no hole made in the thinning method, chance of embryo being exposed to bacteria or toxic substances are greatly lessened.

The zona pellucida gets thick and tough due to advanced maternal age or other factors that distort the follicular environment following inappropriate ovarian hormonal milieu as is evidenced by increasing basal FSH and preovulatory estradiol levels.[88] When the embryo develops in the laboratory, the outer membrane may become thicker and harder than under normal conditions to impede the ability of the embryo to break through its wall for successful implantation in the uterus. In these circumstances the form of micromanipulation known as *assisted hatching* can extend benefit to the IVF process.

This process is normally carried out by purposefully weakening the membrane or zona pellucida. Holes in the zona pellucida may be made mechanically, chemically, or by laser (Table 13.9). Currently, however, assisted hatching can be easily performed on blastocysts by shrinking the embryo in a harmless sugar solution, and then drilling a large hole in the zona either by applying an acidic solution at one point, or making a small hole with a tiny glass needle or special laser. However, this method is still in experimental stage.

With the advent of more routine transfer of blastocyst-stage embryos, past technique of carrying

out transfer on day-three of developing embryo is fast changing. Indeed, at the blastocyst stage *in vitro*, it may be most appropriate to dissolve off the entire zona pellucida prior to transferring embryos into the uterus.[34,89, 90]

Authorities in the field do not universally accept assisted hatching truly beneficial, but some feel that it may be helpful, especially in women over 35. However, a lot of refinement of assisted hatching is required. It thus, still remains essentially an experimental procedure, as it has not been proved unconditionally effective in any well-defined group of IVF patients (Table 13.10).

Table 13.10: Common selection criteria for assisted hatching

1.	Older patients above 35 years especially with high basal FSH level.
2.	Zona thickness—more than 17 mm.
3.	Embryo with slow cleavage rate with number of blastomeres less than five on the day-three.
4.	Repeated failures with standard IVF, ICSI
5.	After cryopreservation

Another assisting or aiding technique used in IVF is *co-culture*. Co-culture refers to adding live cells grown in tissue culture from the tube, uterus or sometimes kidney of human, primate, or bovine sources in order to supply hormones, growth factors and nutrients to the embryo, while in the incubator. But with expected improvement of the media that is expected in future, this method will probably be used less often.

EMBRYO BIOPSY

In any discussion on the merits of the micromanipulation techniques, there is a relevance of potential of biopsy of both eggs and embryos to forewarn any genetic mutations or gross chromosomal errors. Technology of the screening of the unfertilised egg by removal of the first polar body, or the fertilised multicellular embryo by removal of one or more cells either at the 6 to 12-cell stage or from the trophoectoderm of the blastocyst, remains one of the options.

Indubitably, this can be of profound importance clinically for cases with very clear medically defined needs. The biopsy procedure requires very exacting skills of the IVF laboratory.[91]

PGD (preimplantation genetic diagnosis), a powerful new tool that enables diagnosis of genetically damaged embryos, is recommended by the Utah Medical Center in the USA upon reaching the eight-cell stage. A single cell is then removed from the embryo and analysed for chromosomal abnormalities prior to transfer. This can then be used to separate individuals, who are carriers of genetic anomalies such as translocations, and to select healthy genetically normal embryos.[34]

Notwithstanding the risk to the egg or the embryo during the procedure, couples with high probability of inheriting a genetic disorder should have their embryos screened. Women with risk of generating eggs with chromosomal anomalies can benefit from having their eggs or embryos screened for chromosomal abnormality. While sex determination of embryos can be done through this procedure, it is not appropriate to do so except in cases of sex chromosome-linked disorders.[1]

CONCLUSION

Introduction of microinjection technology such as in *in vitro* Intracytoplasmic sperm injection (ICSI) in *in vitro* fertilisation laboratory has revolutionised treatment of infertile men.[92] With almost unlimited potential to achieve some level of fertilisation regardless of sperm quality, it would seem that male factor infertility would now be much less of a concern. It promises the possibility for every man to father his own biological child irrespective of the gravity of his problem. There no longer seems to be any categories of male factor infertility that cannot be treated with ICSI. Even for men with azoospermia caused either by obstruction or by germinal failure, ICSI may be performed successfully.

Failures are expected in azoospermic men, who have neither spermatozoa nor spermatids retrievable from the testis. The age of the wife and, to a considerably lesser extent, her ovarian reserve would determine its success.[93] It must be noted, however, that subfertility in men can be related to certain numerical and structural defects of the chromosomes. Therefore, there is a strong argument in favour of all couples to undergo prenatal screening before the process of ICSI is tried.

Researchers expect that the current average fertilisation rate of 65 percent will continue to improve. Presently, the sperm selection for the ICSI process focuses on the availability of motile sperms, but research is on to use living, but nonmotile sperms for the purpose to improve the odds for more couples seeking to have a baby. Currently, results are poor

with nonmotile sperms, as we do not have precise technique and knowledge, whether they are really alive or dead.

Moreover, even the staining method itself that are in current use to determine whether nonmotile sperms are dead or alive, often can kill the living sperm. It is imperative to develop in future a foolproof staining technique precisely to determine, which sperms are alive and viable for the ICSI process without killing them with the stain. With advancement and further refinement of the ROSNI technique, it would without doubt be one of the great advancements in micromanipulation therapy for the infertile couples.

Transmission of genetic defects through ART can have serious long-term implications. ART should not be initiated in men with a possible or known genetic cause of infertility without prior genetic counselling and risk assessment. Clinicians and researchers involved in reproductive medicine must recognise that, although these techniques have revolutionised the treatment of male infertility, they carry the risk of passing genetic abnormalities to the progeny.[94, 95]

In certain cases of obstructive azoospermia, there is a higher incidence of cystic fibrosis in males. Some studies report that the incidence of a congenital malformation like hypospadias is increased in babies conceived through ICSI. This is an area of ongoing investigation. Often, there is a familial component in male infertility due to related to genetic problems, and the male offspring may develop similar reproductive problems, if the unknown genetic defects are passed on to him from the azoospermic male. Hence, before embarking upon treatment of the more extreme forms of male factor infertility, it is advisable to have some cytogenetic screening performed.

Incidentally, very subtle compromise in sperm quality may well be responsible for a lower embryonic viability rate, and a higher early miscarriage rate, even if such embryos get implanted. Such observations have led to the suggestion that the technique of ICSI itself is at fault; but ICSI *per se* is not causing the problem and merely facilitating the use of sperm, in situations, which otherwise would never have achieved fertilisation.

Mostly micromanipulation technique have used mechanical pipettes, but future lies in developing microinjection pipettes with lasers, especially the Excimer Lasers that can be used for microinsemination and also for the destruction of an extra pronucleus.[61,93]

With refinement of techniques, success of ART has shown quantitative and qualitative improvement over the last decade. Jain and colleagues from Boston's Brigham and Women's Hospital analysed data on ART reported to the CDC from 1995 until 2001 by fertility clinics in the US (see Appendix). They found that the percentage of pregnancies achieved at each attempt at assisted reproduction increased from 24% in 1995 to 33% in 2001 and the percentage of live births occurring per assisted reproduction attempt increased steadily from an average of 19% in 1995 to roughly 27% in 2001. The increase in pregnancy rates and live births occurred even though the average number of embryos transferred per ART attempt declined from roughly four in 1995 to three in 2001.[96]

Although the IVF pregnancy rates have been improved over the last two decades, but a lot needs to be achieved to improve individual embryo selection to the point, where we can routinely transfer only one embryo at a time, while being able successfully and consistently to freeze all surplus embryos of good quality for later use.

Scientists or clinicians with an access to the earliest stages of human development through culturing gametes and embryos *in vitro* are privileged individuals. A high degree of ethics is justifiably expected of them as they are put on a high pedestal as a demigod presiding over creation.

Germ cell implantation (GCI) is the latest method suggested for the treatment of male infertility. Research is on for its use for males, who had sperm defects following chemotherapy, and those with nonobstructive azoospermia.

Medical and scientific advances in ART and AID have created twelve different pregnancy-producing options for infertile couples. An offspring born out of ART and AID possibly could have as many as five parents (i.e. a donor father, a donor mother, a surrogate or gestational mother, and the mother and father actually rearing the child). As an attendant complication of these scientific and medical advances, myriad legal complexities have arisen. Accompanying technical, legal and moral complexities of anonymous donors versus those with identification disclosed, parental rights, grandparental rights, and the rights of siblings and of the extended families have surfaced, especially in the Western countries like USA.[97]

The new situations like "ownerships of sperm, ovum and embryo"; custody, visitation and inheritance rights and multiple other issues pose a challenge to the existing law. The enactment of rules and laws is known to be tardy, and it often does not keep pace with the realities of the today's scientific world witnessing astounding technological advances.

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APPENDIX 1

Outline of IVF Procedure[98-105]

The first step in IVF involves hormonal injection, so that woman can produce multiple eggs in each menstrual cycle instead of only one. Serial ultrasonography is done to determine the readiness for the egg retrieval.

Prior to the retrieval procedure, woman is given medications or injections (see Table 13.7) to ripen the developing eggs and to start the process of ovulation. The eggs must be retrieved just before they emerge from the follicles in the ovaries. Too early or too late retrieval could interfere with the normal development of the eggs. An ultrasound (at times hormonal assay as well) is done to ascertain the right stage of development before their retrieval.

During the procedure, the right and the appropriate follicles in the ovary are located with the ultrasound and the eggs are removed with a hollow needle. Immediately, the eggs are fertilised in the laboratory with the partner's sperm donated on the same day (at times using cryopreserved sperms). The fertilised eggs are kept in the laboratory under observation to ensure optimal growth till the embryo reaches a more advanced blastocyst stage.

Once the embryos are ready, they are transferred into the uterus inserting a flexible catheter passed through the vagina and the cervix. Most IVF experts recommend transferring three or four embryos at a time taking a calculated risk of multiple pregnancies. A pregnancy test is usually done in about two weeks after the embryo transfer. In cases, where the man's sperm count is extremely low, doctors may combine IVF with ICSI. Any embryo that is not used in the first IVF attempt, can be frozen for later use.

Success Rates

The CDC. USA compiles national statistics for all assisted reproductive technology procedures performed in the U.S. The most recent report from 2000 found:

- Successful pregnancy was achieved in 30.7% of all cycles.
- About 69% of the cycles carried out did not produce a pregnancy.
- Less than 1% of all cycles resulted in an ectopic pregnancy.
- About 11% of these pregnancies involved multiple foetuses.
- About 83% of pregnancies resulted in a live birth.
- About 17% of pregnancies resulted in miscarriage, induced abortion, or a stillbirth.

2001 ART DATA—OVERVIEW

- 421 ART clinics in the U.S. in 2001
- 384 ART clinics submitted data
- 107,587 cycles reported
- 28,344 live-birth deliveries
- 40,887 live bables born

CHAPTER

14 Psychological Impact of Infertility

INTRODUCTION

Infertility, the inability to conceive or carry a pregnancy to live-birth, will be experienced by 10% of the population of childbearing age. One couple in six in the United States has to deal with issues of infertility. It is assumed that worldwide rates are comparable. Infertility is caused by male and female reproductive problems approximately in 70% of cases, in almost equal proportion of 35% each.[1] The rest 30% cases are attributed to multiple factors or unknown causes.

Infertility is a global problem from time eternity but with different cultural consequences. By and large, infertile couples represent a neglected and silent minority group irrespective of the difference in the culture of the subcontinent, the Middle East or the West.[2-4] Indubitably, medical treatment in recent years has made great strides in the diagnosis and the treatment of problems of infertility. But like everything in this world, it has a price tag attached to it, as the newer treatment modalities are costly in terms of tremendous drain on the physical, emotional and financial energies.

Two primal and basal instincts of any living creature are eating and mating—first is for its survival and second for continuance of progeny. Echoing the same "Sir William Osler", a famous physician, once said that human beings have two basic desires—to get and to beget. When the universal dream of having one's own family is threatened or extinguished, it would inevitably cause painful and difficult emotions.

Infertile couples show various emotional reactions such as sadness, depression, anger, confusion, desperation, hurt, embarrassment, and humiliation. Disorganisation, distractibility, exhaustion, moodiness and obsessive thoughts and behaviours—are often the manifestations of the reactions to infertility. The infertile couples often have varied and different sources of stress.[2] Their strategies and means to cope with such situations also differ.

It is generally believed that the women and men experience infertility differently.[5] Meta-analytic procedures were used to review the empirical evidence (1966-1995) on gender differences in coping with infertility among heterosexual married couples.[5] These literatures suggest that infertility is more stressful for women, although many studies have excluded men/husbands. All the same both partners seem to have psychological problems, irrespective of whether one or both contribute to infertility. Without doubt psychological distress makes important contribution to infertility.[6,7]

STRESS AND INFERTILITY: CAUSE OR THE EFFECT?

Most physicians and mental health professionals in this field have encountered sizable impact of stress in reproductive life. Several studies have been undertaken to establish causative or reactive relation between stress and the infertility. Whether stress causes infertility or infertility causes stress is still debated. Contradictory results of various studies

have not provided any clear-cut answers to this vexed question. Several studies indicated that stress has a negative impact on sperm parameters.

Occupational parameters should be an important part of history-taking among patients attending infertility clinics.[8] Among the causes of stress are the couple's isolation, life with unrealised potential and unborn child, and disruption of day-to-day life during infertility evaluation and treatment, and the couple's feeling that they do not have control of their own lives.[9]

Most of the investigations that were performed during the last two decades show that in the majority of cases stress is the result and not the cause of infertility.[9] The biological interaction between stress and infertility is the result of the action of stress related hormones at the brain level, especially on the hypothalamus and pituitary, and on the female reproductive organs. These hormones such as catecholamines (adrenalin, noradrenalin and dopamine) and the hypothalamic-pituitary-adrenal axis interact with hormones responsible for normal ovulatory cycles. Gonadotrophin-releasing hormone (GnRH), prolactin (PRL), luteinising hormone (LH) and follicle-stimulating hormone (FSH), endogenous opiates and melatonin are altered by stress and interfere with ovulation. Sympathetic innervation of the female reproductive system provides routes by which stress can influence fertility at the level of the sex organs.

There is a significant gender difference in coping with infertility both at the individual and the couple levels.[5,6] It is thus important that the gender be considered before planning treatment of infertile couples. Male factor infertility often produces a state of erectile dysfunction (ED) making the union meaningless, while women may lose interest in sexual intimacy after a diagnosis of female factor infertility. Indubitably feelings about fertility are entwined in that about sexuality. Many women with infertility often feel that they are not "real women" having been denied the membership of the privileged class, who have experienced pregnancy and childbirth. They feel like outsiders at social functions, when children related topics are discussed. Men have a feeling of inadequacy, when they hear remarks like "he shoots blanks". [1] This feeling gets compounded with some men having an erroneous belief that virility is equated to normal sperm function, not knowing that impotence and male infertility are not the same.

Stress is also known to have a negative influence on the libido. It operates in a cyclical manner. Stress leads to decreased libido with resultant failure to consummate marriage. This naturally leads to more intense stress with frequent attribution to other sources or the partner for its causation. Aggressive behaviour is not an infrequent outcome, especially in highly educated and professional males.

This relationship of stress and infertility makes it a very difficult field to study. In real life, compared with other stresses such as illness, family problems or unemployment, infertility possibly plays a less significant role. No doubt some persons perform better under stressful conditions. But one needs to consider all factors such as innate personality, styles of coping with stress, the degree of stress and support systems in the environment from the family and friends.

When working with individuals going through infertility treatment, it is common to come across male sentiment and desire to be able to direct their wife's pregnancy. This desire may make a man feel responsible, if he is unable to get his wife pregnant. On the other hand, he becomes too uptight due to his work environment, spends too many hours in his place of work, and not relaxed enough at home. The problem gets complicated, when the real issue is conveniently side-tracked, if the man is not sufficiently relaxed for the end game, notwithstanding that sexual pleasure and pregnancy are not interdependent.

Treatment of infertility is a time-consuming affair as appointments, blood tests, ultrasound examinations, medications and doctored sex all take a lot of time. For working couples, doctor's appointments, phone calls to and from the doctor's clinics, procedures, consultations and counselling can take a serious toll on their working schedule. During some procedures or monitoring, men and women may be in the clinics or hospitals for the best part of the day. To maintain privacy, they may have to invent excuses to avoid prying eyes of their colleagues, and at times even of their family, especially if they belong to a joint family in the subcontinent. Certainly, there are employment situations, where an infertile couple's desire for pregnancy may have an impact on his or her promotions while trying to balance privacy, demands of treatment and work obligations.

Review of literature on the psychological background of infertility reveals two possible hypotheses.[10] One group of articles explored the possibility

that infertility may have psychological causes (*psychogenic hypothesis*) and others examined the psychological consequences of infertility (*psychological consequence hypothesis*). Most researchers now reject the psychogenic hypothesis. More in-depth research is needed to prove this relationship between the stress and the infertility.

Literatures on the psychological consequences of infertility present it as a devastating experience, especially for women, although the flaws like over-sampling of women, small sample size, non-representative samples, and failure to study those who have not sought treatment, primitive statistical techniques, and an over-reliance on self-reports are evident in these articles.[10,11] However, most researchers conclude that infertility is a more stressful experience for women than it is for men irrespective of which partner has the reproductive impairment. While stress has been identified in both sexes, depression is more common in women and men show a tendency towards repressed anxiety. In general, the literature shows little regard for the societal construction of infertility. Finding an appropriate set of "controls" is a particularly intractable problem for this area of research.[10] Attempts to test the psychological consequences hypothesis have also produced more equivocal results. In general, studies, which look for psychopathology, have not found significant differences between the infertile population and others. But according to our basic (psychosomatic) tenet, every somatic problem has its emotional side.

IMPACT OF INFERTILITY ON SELF-ESTEEM

Studies employing measures of stress and self-esteem have found significant differences.[1] People's self-esteem often is the commonest casualty in a case of infertility. Feeling of inadequacy as a man or as a woman naturally affects their self-image and stress is a natural outcome of this state of mind. Infertile women and men often feel that their bodies do not work right or are defective. Phrases such as "everyone else can get pregnant" or "I must have done something wrong to deserve this" reflect how badly these individuals view themselves. Unknowingly, family or friends can reinforce this image. Snide remarks like "I just had to look at my husband to get pregnant" coming out of conversation can be very hurtful. Nor does the simple sayings from even mothers telling their daughters "I don't understand—I never had a problem getting pregnant." It probably meant to reassure the daughter of no major cause for concern, as otherwise, it would have been known previously in the family.[1] To the contrary the infertile daughter feels inadequate or estranged even from her mother. The same remarks from a mother-in-law in the subcontinent could easily snowball into marital or family discord.

A man's self-esteem takes a beating, when a younger brother in a joint family of the subcontinent beats him in the race of becoming a father and the brother's family naturally becomes the cynosure of all members of the family. In large joint families, this stress can be stifling. Fertile daughters-in-law are given special privileges from which infertile women are excluded. It is impossible not to feel stress as months pass by and the diagnosis of infertility is eventually confirmed.

One study has revealed that women going through infertility rated themselves as having higher levels of depression than women going through cancer treatment. It is indeed strange that the infertile women can go into greater levels of depression than cancer patients. Perhaps, its explanation lies in the loss of self-esteem that infertility causes. For an infertile couple, hope of pregnancy goes through a roller coaster ride waxing and waning with each new menstrual cycle or treatment schedule. It is not common that they try a new doctor with a different treatment schedule with renewed hope only to despair with subsequent failure. It would seem impossible for stress not to enter into this situation in infertile couple's life.

EFFECTS OF INFERTILITY ON SEXUALITY

Infertility often demands a change in sex life. Scheduled sex often replaces sexual intimacy. Men may jokingly complain that they have become "sperm donors" during sex, as they get into a state of mind that their wives need their sperms and not them. Sex lives of the infertile couple may change as sex and pregnancy cease to have emotional relations, especially in artificial inseminations or assisted reproductive technologies. Women may also feel that their bodies are changed for the worse by the medications. Certainly, with wife having an ultrasound every morning or to rush for postcoital test, the partner may feel less sexy or sexual than usual.

IMPACT OF MEDICATION

The medications for most infertile men and women are not without side effects. These side effects in women include headaches, fatigue, and premenstrual symptoms such as feeling irritable, sad or moody. For those women, who need to take injections, the stress of mixing oral medications and the injections can cause a great deal of stress. For men, it could be loss of libido. Herein lies the importance in couple showing empathy for the stressful state of the spouse. This can help to reduce their feelings that the couple is going through treatment together.

Table 14.1: Emotions of infertility

Shock	Sadness and depression
Denial	Hopelessness
Fantasising	Loss of self-control
Guilt	Anger
Bargaining	Isolation
Blame	

EMOTIONAL ASPECTS OF INFERTILITY

Response to infertility is different depending on individual situations, emotional strengths, coping methods and personality. It is better to prepare for these difficult periods, so that with emotional support and mental preparation, the potential pain of infertility is dealt with pragmatism and fortitude. The news of infertility almost always comes unexpected. It not only involves the couple but the whole family, if they are closely knit.[12]An infertile couple naturally experience uncomfortable emotions. Some of the most common emotions[1,2] are mentioned in Table 14.1.

Shock

In most cases, infertility is not diagnosed until after one year of unsuccessfully trying to conceive. For many couples, infertility is very difficult to accept and initial response of most of them is feelings of shock and disbelief. After planning for months or years to have a pregnancy, one is suddenly confronted with a possibility or even reality, that life's plan has been put on hold. Fortunately, these feelings generally last a short while. Once it is properly recognised and addressed to, it ceases to cause any emotional harm.[13]

Denial

Another component of the emotional process is often denial. Rather than confronting infertility, a couple may choose to deny the problem itself. Denial is unhealthy, if it lasts for a prolonged period and prevents the couple from accepting the reality of infertility. However, this phase serves an important purpose. It allows adjustment to an overwhelming situation as one works to resolve the problem of infertility.

Fantasising

For some women, denial also leads to fantasising—and they dream of what life would be like with a child. They feel that all their problems would be solved, if they got pregnant. They lose touch with reality and every time they start treatment, they think they are going to conceive. They find it difficult to cope when it fails.

Guilt

Guilt is an unfortunate, but a common response to infertility. In an attempt to find the cause of infertility, many men and women often delve deep into their past socially unacceptable misdeeds as the cause of their problem. Some individuals may feel, that they are being punished for past premarital or extramarital sexual activities, or for undergoing an elective abortion. I find many educated men even blaming excessive masturbation as the cause of their infertility. Nothing can be further from the truth, as excessive masturbation can never be a cause of infertility. Often, infertile partners may feel that they are depriving fertile partners of the opportunity to have children. Inability to produce a baby may also make him feel, that he has let his family down, as he has not been able to fulfill what is expected of him. On the other side, the Sanskrit saying *Putrarthe Kreote Varja* (a Sanskrit saying—which literally means wife is for creation of progeny) is often mentioned to the wife to put an enormous pressure on her plight, especially when she is the so-called offender. Guilt complex assumes a sizable and real significance, especially if the husband happens to be the only son of their parents.

People are conditioned to assume that they are born fertile and could control the event of pregnancy at will, just as they could postpone having babies by taking contraceptive measures. Depression and hopelessness naturally emerge from the situation that dispossesses their so-called control and choice, and may lead to feeling of guilt.

Bargaining

This is a common subcontinental response especially, when a strong belief in God has been inculcated in the individual. One promises to fast, do penance or offer money, and to be good for the rest of the life, if the almighty (be it God, Christ, Allah, family deity, Guru as the case may be) blesses the couple with a pregnancy. Many infertile patients visit an endless number of temples and "holy men". They perform *yagna* and *tapasya* (Hindu religious rituals practiced through mediation of a God-man or *Purohit*) in order to conceive, often at a considerable expense. Frequently, these God-men literally cheat these hapless couple to exploit their sentiments.

Blame

It is very common for the couple to blame each other for the inability to conceive, especially when only one member is infertile. As the response of each individual is different as regards the emotional aspects of infertility, one of the spouses may be less concerned about having a child. Wives used the "increasing space and sharing the burden" strategies to a greater degree than their husbands.[4] Either partner may grow resentful for the other not experiencing the same emotions at an equal level. Even adultery may result from such resent.

Sadness and Depression

Depression makes a very common response with infertility or loss of baby. It is tantamount to the loss of fulfilling a dream and the end of a possible relationship that one might have had with a child. This type of sadness is often hard to deal with as one mourns the inability of having an experience. Unfortunately, near and dear ones and friends are often unaware of the degree of emotional impact of infertility, which deprives the couple of having a meaningful communication with others. In addition, often the nature of infertility could at times be ambiguous not revealing definitely, whether future pregnancy is impossible (no definite cause is known in 15% of infertility). The continual hope that "this will be the time" can leave a couple's emotions painfully suspended, creating a continual "hoping against hope" attitude. A death brings family and friends together to grieve and gives loss a healing touch. In contrast, infertility is a very private form of grief and often-infertile couple grieves alone mostly without social support. Here grief has nothing to focus on as the loss is hidden.

Moreover, traditional rules seem to impose a special burden on people, especially coming from the rural areas of India. The cursed word *Baanjh* or *Baanjha* (infertile woman) in rural surroundings of India could inflict a devastating psychological trauma, as they are not infrequently ostracised from social and religious congregations. However, as mentioned in Chapter 6, the onus is always first on the woman to go through the rigours of the fertility tests. Not infrequently, husbands find excuses to evade any form of investigations, and the wives silently and sadly bear the stigma of being infertile.

Studies have shown that there is an association between depression and erectile dysfunction (ED). This relationship between depressive symptoms and ED in middle-aged men is independent of demographic, anthropometric and lifestyle factors, health status, medication use, and hormones.[14]

The incidence of conflictual adjustment is often seen in the subcontinent. In this situation, one (mostly the woman) is forced to compromise with choice of partners due to either familial or social pressures or due to some guilt feeling. The stress is a natural culmination of such state of affairs, and it invariably has reflection on the conjugal life.

Hopelessness

Depression and hopelessness are mostly interrelated. Fertility potential is a quotient of male and female fertility. Most fertile period of female (midcycle) is often looked upon as the most likely period to achieve conception. Thus, with each period, the couple's hope surges up; but if the cycle is unsuccessful, hopelessness is a natural outcome. To maintain an upbeat mood after a series of failures becomes a difficult proposition for both males and females. Each failure rakes up the old wounds. As is the wont, couple keeps on trying a new type of therapy or a treatment with a new doctor. It is the hope that "this time it is going to work" keeps them going. If they did not have this hope, no matter how little, no one would ever start treatment at all!

Loss of Control

In the urban environment of the subcontinent, highly ambitioned couples (I describe them as career couples) probably plan to start a family at the most favourable time. They practice birth control for years until their careers are established. There is a common belief from the tenets of religion (as practiced in the subcontinent)

that everything is possible, if one works hard enough. But there is another saying that *Karmanne Vadhikaraste Ma faleshu kadachana* (you have only control over your work, but not in its successful fruition). When infertility eavesdrops into their life, there is an inevitable realisation that certain aspects of their life are beyond their realms. During treatment, they are prone to put parts of lives on hold. This might include postponing moving to a new home, changing jobs, or not taking holidays. The more they give up, the less control of their emotions they are likely to have. Each treatment cycle can become a roller coaster of emotions with its ups and downs—the hopes of success and the frustration of failure.

Barbara Eck Menning, is the founder of "Resolve",[4] a non-profit group dedicated to the support, education and advocacy of people with infertility. She describes infertility like being in "limbo." Infertile couples are living in limbo, not knowing what the future holds. They also live in limbo, because everything revolves round having a baby. The Vacations are postponed, the social gatherings avoided and women postpone buying clothes with the hope that they would be pregnant and not need them. The work schedule of infertile couple is disturbed for ready availability for ovulation monitoring or timed intercourse to provide a specimen for postcoital test.

Anger

Anger results from continued disappointments after trying various forms of treatment. Anger is roused from being deprived of having his or her own child. Thus not infrequently, couple (especially women) resents pregnant women. Friends and family often do not seem to understand the emotional tension associated with infertility, and they may also not be spared. Even the doctors may not escape from this type of anger. Consequently, frequent change of doctors is common with infertile patients. Several cross-sectional studies have reported associations between ED and depression, anger, and dominance. ED in men is much more likely to occur among men with submissive personality.[15]

Isolation

Isolation or loneliness is another common experience among infertile couples. As most people have children, they are incapable of comprehending the complex mindset of an infertile couple. Often remarks not factual, such as "relax and you'll get pregnant," or "after you adopt you'll have a child of your own," inflict a great deal of anguish and pain to the infertile couple. As times by, the infertile couple gets isolated, as they have only a few friends and relations left, who have an empathy with their feelings and support them emotionally.

It is the grief that comes to surface with the knowledge that infertility is a fact in the life of an infertile couple. Stages of grief may take various forms. It may be shock and denial with "not me", or anger with "why me". It then takes on to bargaining or leads to depression. In the end thee is always some amount of acceptability.[16]

CONCLUSION

Psychological aspect of infertility is a complex phenomenon and only a very few medical personnel or psychologists are trained and experienced enough to deal with this problem. In the USA, there are at least two renowned establishments-Alice Domar at the "Mind Body Program" at Harvard,[4] and "Resolve" run by Barbara Menning. Relationship between the stress and the infertility is still debatable, but the singularly important goal of its management should be aimed to cope with stress effectively.[17]

Infertility is an experience that continually fluctuates in intensity and direction. An infertile couple has different needs and experience different emotions at different times. There are many ways with no set pattern to cope with this sort of stress. The stress-related anger and depression often lead to ED. Sometimes a person's emotions can be mystifying and frighteningly intense, and at another time, he or she may simply feel numb. Time is needed to explore the right choice for easing the emotional burden of an infertile couple.

It is also important to acknowledge the differential emotional responses to infertility in individuals and that the gender reactions are different. Its management needs different strategies as the methods of coping with such situations vary with people.[15] One particular strategy may help in some cases and for some time, but later it may fail. The painful experience of infertility elicits destructive reactions in some, while in others it may act as a useful motivating force in life. Ultimately, each person has to find his or her own way of coping with the infertility situation, and sometimes they would benefit from professional help and support, when working through their emotional crisis. In most developing countries, there are no

known organisations specialising in this field. If the patients consciously try to overcome the tragedy of infertility, they will turn to their basic emotional needs and perspectives. In the West, approximately 30% conceive later either by chance or with help from the advanced technologies, while approximately 20% opt for adoption.[11] However, half of these patients take no positive action, neither giving up nor continuing with serious effort for a child. They also refrain from seeking any counselling. Analysis of many reports argues in favour of an adoptive model for coping with reproductive failure. Individuals and couples would certainly benefit from professional help and support, when working through their emotional crisis.

Infertility is multilayered and unique to every person challenged with it. There is no "right" or predictable way to feel when emotions of dealing with infertility.[18] Psychological impact on infertility elicits different response depending on the cultural, ethnic, and religious norms. Detailed study in China bears ample testimony to difference between Chinese and Western societies.[19] Consequently, subcontinental mindset would evoke response different from one is expected to see in other societies. In the Indian subcontinent, strong religious bias and probable help of a joint family often steer infertile couples to cope with the situation somewhat better than their Western counterparts. But I wish that more of these couple would go for adoption in the overpopulated subcontinent, where supply line is never empty, while the cost of availing the advanced technologies is prohibitive.[20]

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