Preface

Today, the artificial insemination has become a normal method of breeding quality cattle. A large number of cows and buffaloes are inseminated artificially. The technique of artificial insemination is particularly more useful in a country like Iraq where the paucity of quality sires has been the main hurdle in the way of cattle improvement. This booklet describes the process of artificial insemination in detail including methods or semen collection, semen dilution, management of sire for artificial insemination use and sterilization or apparatus to be used. AI revolutionized animal breeding in the 20th century, particularly in combination with sperm cryopreservation. The AI industry has developed dramatically in most domestic species in the last few decades and its use is now widespread in intensive animal production. The development of other associated technologies, such as sperm selection and sex selection, are predicted to create powerful tools for the future, both for domestic livestock breeding and for the purposes of conservation. AI will continue to play a role in fertility treatment for human patients, although it may be superseded by IVF or ICSI. It has been suggested that AI (in animals) is entering a new era where it will be used for the efficient application of current and new sperm technologies. Exciting possibilities are offered by emerging techniques, such as Single Layer Centrifugation, for improving sperm quality in AI doses as well as for increasing sperm survival during cryopreservation.

What is artificial insemination (AI)?

Artificial insemination (AI) is the manual placement of semen in the reproductive tract of the female by a method other than natural mating. It is one of a group of technologies commonly known as “assisted reproduction technologies”.

Artificial insemination (AI) is a popular, simple and inexpensive treatment of infertility in animals, in which the sperm from the male is collected and introduced artificially into the reproductive tract of the female for conception. It was in 1780 that the first scientific research in AI of domestic animals was carried out on dogs. Lazanno Spalbanzani, an Italian scientist, conducted experiments that proved the power of fertilization vested with the spermatozoa and not with the liquid portion of the semen. These studies spearheaded the commercial utilization of this technique for breeding across the globe. Today, AI has emerged as one of the best techniques devised for genetic melioration of farm animals. This is a remarkable method of
breeding quality cattle in the most natural way possible. AI is being carried out in a large number of cows and is extremely useful in countries like Iraq, wherein quality sires have been scarce. Artificial insemination in cattle has taken care of this major obstacle in the path of cattle improvement.

**Introduction**

**Artificial insemination** is the technique in which semen with living sperms is collected from the male and introduced into female reproductive tract at proper time with the help of instruments. This has been found to result in a normal offspring. In this process, the semen is inseminated into the female by placing a portion of it either in a collected or diluted forms into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions. The first scientific research in artificial insemination of domestic animals was performed on dogs in 1780 by the Italian scientist, Lazanno Spalbanzani. His experiments proved that the fertilizing power reside in the spermatozoa and not in the liquid portion of semen. Few further studies under research station conditions helped this technique to be used commercially all over the world including India. Artificial insemination is not merely a novel method of bringing about impregnation in females. Instead, it is a powerful tool mostly employed for livestock improvement. In artificial insemination the germplasms of the bulls of superior quality can be effectively utilized with the least regard for their location in faraway places. By adoption of artificial insemination, there would be considerable reduction in both genital and non-genital diseases in the farm stock.

**Advantages of Artificial Insemination**

Artificial insemination has following advantages over natural breeding

1. The main advantage of artificial insemination (A.I.) is that it increases the usefulness of superior sire to an extra ordinary degree. It makes available sires of inheritance for milk and butter fat production to all dairymen within a limited area. Previously only a few could get the advantage of good bulls.
2. The services of superior sires are greatly extended. By natural services, a bull can be bred to 50 to 60 cows per year. On the other hand, by artificial insemination technique thousands of cows can be sired in one year by one bull.
3. The breeder does not need to maintain a herd sire and thus can avoid the botherations accompanied with the management of a bull. It helps to regulate the breeding programme and the space between successive calvings without unnecessarily prolonging the dry period.
4. The dairyman does not have the problem of searching and purchasing a new herd sire every two years to avoid inbreeding.
5. The technique of artificial insemination can be made useful in cross breeding for hybrid vigor by quickly transporting the semen by air to different continents.
6. The intensity of the spread of genital diseases is minimized if artificial insemination is conducted under complete sanitary conditions by the specially trained persons.
7. It overcomes the difficulty of size and weight.
8. It increases the rate of conception in females.
9. Outstanding animals located apart can be mated.
10. It helps in better record keeping.
11. Old, heavy and injured sires can also be used with

The advantages of artificial insemination in cattle are as follows:

**Quality Sires:** During natural breeding, males deposit more than the theoretically required quantities of semen into the female's reproductive tract for conception. AI method involves dilution of collected semen so as to create hundreds of doses from one ejaculate. Thus, AI makes superior sire semen to be available to hundreds of female cows. Artificial insemination in dairy cattle leads to sires of inheritance for butter fat and milk production. Prior to AI, only few cows could have the advantage of good bulls.

**Decreased Costs and Increased Safety:** Bulls are bigger and stronger than cows and generally quite difficult to handle around the farm. Their aggressive nature can make them potential threats on the farm. However, AI eliminates the need to have a bull on the farm, as semen can be easily transported to various geographical areas. They can also be stored for a long period of time, which means the semen from a male can be used even after a bull's natural reproductive life ends. Since maintaining males costs quite a bit, AI decreases the overall costs on the farm.

**Reduction in Disease Transmission:** The transfer of venereal diseases is quite likely to happen during natural mating. Certain pathogens can be transferred via the semen into the female, during AI as well, however, the screening done after semen collection prohibits this transfer.

**Genetic Selection Improvement:** Since one male's semen is more than enough to produce hundreds of offspring, the best few males can be selected for breeding. This helps maintain the vigor of the cattle breed. Artificial insemination in beef cattle helps maintain the genetic pool, thereby obtaining the right strain of beef cattle required for meat production. Bulls of high genetic merit are available with AI.

Despite all the pros, AI do have its share of cons. Artificial insemination in cattle requires dexterity, patience, knowledge, experience as well as specialized equipments. Improper ways of carrying out AI in animal species, such as improper sterilization of equipments, insanitary conditions, etc. can nullify the efforts taken to obtain conception. The severe climatic conditions prevalent in most parts of India make transportation and preservation of semen difficult. Moreover, the need for superior germ plasm has reduced the market for bulls.

**Disadvantages**
However, despite a number of advantages over natural breeding processes, artificial insemination has certain limitations. These are as follows.
1. It requires well trained operators and special equipments.
2. It requires more time than natural services.
3. It necessitates the knowledge of structure and function of reproduction on the part of the operator.
4. Improper cleaning of the instruments and insanitary conditions may lead to lower fertility.
5. Market for the bulls is reduced while that for the superior germplasm is increased.
6. Selection of the sire should be very rigid in all respect.
7. Preservation and transportation of semen is difficult under severe climatic conditions like those prevailing in most parts of India.

ANATOMY AND PHYSIOLOGY OF REPRODUCTIVE ORGANS

The Testis
The testicles are two in number, which are carried, outside the body wall in the scrotum. The testicles have at least two functions. (1) The production of Spermatozoa is the primary function. (2) The production of endocrine substances which markedly affect the development and behavior of the male is complementary function. In the bull the testicles are elongated ovoid organs placed with their long axis vertical in the scrotum. In the mature animals they are 12-16 cm in length and 6 to 8 cm in breadth. Each testes weighs 300 to 500 g including epididymis depending upon the age, body weight and breed of the bull. Normally the testis in the bull are equal in size have a firm but not hard consistency and can be freely moved up and down with in scrotum. In buffalo bulls the size of the testicles is slightly different they are smaller 10 to 12 cm in length, 5 cm in width and 3 to 4 cm in thickness and weighs about 100 g. each. The testicles are made up of connective, interstitial and spermatogenic tissue. They are richly vasculated and have an intricate system of nervous tissue. The sperm forming tissue is found in seminiferous tubules. These tubules are highly convoluted with tremendous surface area. Laid end to end the tubules in one testicle of the bull would be some 10000 to 15000 feet in length in the interstitial tissue leydig cells are present, which are responsible for secretion of male hormones, which are intrinsically linked with the production of seminal plasma. The wall of the seminiferous tubules consists of a basement membrane and a multilayered sperm producing epithelium. This epithelium consists of two types of cells; a) Germ cells differing in age and morphology, generally arranged in concentric lay6rs. b) Sertoli cells which are slender pillar like structures perpendicular to the basement membrane, to which they are attached by a flat base. They are situated among the densely crowded germ cells to support and nourish them. The seminiferous tubules pass into the body of the mediastinum and unite with a net work of ducts, the retetestis, which is lined with a cubic epithelium. At the proximal end of the testes the net work of rete passes through a 4 to 5 cm wide opening in the tunica albuginea and
connects with the epididymis by the 13 to 15 tubules constituting the ductuli efferentes testis. Between the seminiferous tubules there is loose connective tissue containing blood and lymph vessels, nerves and isolated groups of polygonal interstitial cells (Leydig cells) with large spherical nuclei. Testicular tissue has prolific activity: In the bull 1 g of testicular tissue manufactures on an average $9 \times 10^6$ sperm per day (Willett and Ohms, 1957) i.e. 6000 per minute. In some mammals the testis and accessory sex organs function fully during the breeding season. In farm mammals sperm production is maintained throughout the year with some seasonal variations.

**The Epididymis**

Attached to each testicle is an intricately convoluted tube the epididymis, which extends down the outside of the testicle to its base. The epididymis consists of three parts caput, corpus and cauda. Histology: The 13 to 15 efferent ducts occupy about 1/3rd of the caput of the epididymis. They have a diameter of 100 to 300 microns, contain only a few sperm in the lumen and a very characteristic epithelium. Two types of cylindrical epithelial cells are found attached to the thin basement membrane of the ducts (a) Secretary cells with large cytoplasmic granules and (b) Ciliated cells with kinocilia, motile cilia, all beating outwards. Blom (1944) has found that these ciliated cells normally detach their ciliated borders and excrete them into semen at the rate of one per 10,000 sperm (i.e. about 500,000 in an average ejaculate). Near the centre of the caput epididymis the epididymal duct starts its long and tortuous course through the rest of the epididymis. The epididymal duct can be differentiated into 6 distinct regions histologically and cytochemically. The Lumen of the ducts is 1 mm in diameter.

Functions: The epididymis has four major functions. 1. Transport, 2. concentration, 3. Maturation and 4. Storage of sperm. The functions of epithelium are in part absorption and in part secretion. The epididymis behaves like Kidney by virtue of its embryonic characteristics.

1. Transport: After their release from the seminiferous tubules. The sperm pass rapidly into the epididymal duct proper via the tubuli recti, rete testis and ductus efferentes. From rete testis to efferent ducts sperm pass by fluid pressure in the testis. Their passage through the efferent ducts is assisted by the active onward beating cilia
of the ciliated cells and in the epididymal duct by the peristaltic movement of the musculature of the wall. The transport of the sperm from the germinal epithelium to the cauda of the epididymis takes 7 to 9 days in the bull to some degree depending upon the frequency of the ejaculation, (Knudson, 1954). The transport is also assisted by the continuous flow of fluids from the testis. The epididymal duct is a single unbranched, strongly winding canal, in bulls upto 50 mts. long. Transport of sperm along the duct is brought about by spontaneous rhythmic contractions in the layer of smooth musculature surrounding the duct, while the sperm remain in a quiescent state. Contractions in duct are dependent on androgen hormones. The contractions are regulated via autonomous nervous system but the reaction to nervous stimulation is not similar along the duct. Oxytocin which is released in connection with the sexual preparation of the male and at ejaculation will aid in strengthening the muscular contractions. Concentration: From the dilute sperm suspension originating in the testis, water is absorbed into the epithelial cells during the passage through the epididymis especially in caput and a highly concentrated suspension (4000000 or more/mm³) is left in the tail of the epididymis.

Maturation : The physiological maturation of sperm concerns their morphology, their biochemical and functional characteristics. When the sperm leave the testis they are provided with a cytoplasmic remnant (derived from Golgi complex) situated at the neck of the tail. (Proximal cytoplasmic droplet) When the sperm have passed the flexure of the epididymal caput and entered into the distal part of the caput, the droplet has moved to the distal part of the mid piece i.e. distal droplet. This maturation is achieved probably as a result of secretions from the epithelial cells. The membranes surrounding the head are loosely fitting and easily distinguishable in the sperm from epididymal caput (observed by electron microscopy) while the acrosome is very tightly contracted around the head of sperm from the cauda epididymis. Biochemically epididymal sperms differ in several respects from ejaculated ones, During epididymal transit the sperm become increasingly dehydrated, thus sperm from cauda have a higher specific gravity than those taken from the caput. Furthermore the spermatozoal content of phospholipids decreases as the sperm move from caput to cauda. It is well established that sperm from testis and upper parts of the epididymis are immobile and cannot be made mobile. The ability of the flagellar motility of the tail is acquired gradually during epididymal transit and only sperms from cauda are capable to show progressive motility similar to that of ejaculated sperm. The development of the fertilizing ability is also gradually acquired during the epididymal passage.
Sperm from the caput are not able to fertilize ovum, while cauda sperm exert a normal fertility. Extensive research for last 10 to 15 years has substantiated that the major factor in the maturation of sperm can be ascribed to a very active function of the epididymal epithelium. The secretary function of the epididymis is limited to a very few compounds. The most important of which is glycerophosphoryl choline which is present in fairly high amounts in the semen of both mammals and poultry. The much more conspicuous characteristic of the epididymis is its large number of cells typical for epithelia engaged in absorption phenomena such as epithelium in proximal tubules of kidney. The ability to absorb is not limited to fluid and electrolytes but also high molecular substances such as hormones (Testosterone) and other proteins. The epididymal fluid has a composition which could effect keeping the sperm immotile and reducing their metabolic activity to a minimum within the duct (Physiological anabiosis). The general features of epididymal plasma is as follows: Low pH (around 6), High partial Co2 pressure, Lack of metabolizable substrate, High osmotic pressure, Relatively high in Cauda Na+ Inhibitory effect on metabolism by testosterone. To these factors may be added that sperm are densely packed within the duct. These conditions help in conserving the life span of sperm in the duct (5-6 weeks) at a temperature only about 3°C below the body temperature.

4. Storage: The tail of epididymis is the sperm storage depot. The concentration of the sperm is very high and the lumen of the duct is relatively wide. It is not surprising, therefore, to find that half of the total numbers of sperm are stored in this part, which constitutes only about a quarter of the length of the epididymal length. The conditions in the tail are optimal for preserving the viability of sperm which are in quiescent state of metabolism. The sperm will remain alive and fertile till 60 days even if the epididymis is ligated. The fact that the spontaneous rhythmic contractions of the epididymal wall do not involve the cauda (except during sexual excitement) makes this compartment particularly suited for storage of sperm. While the sperm of a similar age are constantly carried into the cauda, this part of the duct will contain sperm of varying age as mixing of sperm inside cauda also takes place.

The size of the sperm stock is regulated in the first time by ejaculation, besides sperm are passed without ejaculation via excretory ducts. During sexual preparation of the bull sperm are carried from the distal cauda due to contractions in the wall into the ductus deference the so called emission. The better the bull is prepared the larger number of sperm will be expelled at ejaculation. The mixing of sperm with seminal plasma takes place in the pelvic part of genital tract. During extremely long periods of sexual rest, the first few ejaculates may contain non fertile sperm. Detachment of acrosome cap (galea capitis) is one of the first changes visible in the sperm (Blom 1945). Leading upward from the epididymis are the vasa deferentia.
slendertubes connecting with the urethra. The vasa deferentia enlarge to from the ampullae. The ampullae are located just above the exterior part of the pubis, where they join and progress forward as the urethra. Sperm are stored in the ampullae until the time of ejaculation. Lying on either side of the ampullae are the seminal vesicles. They are also double glands. They are about 2 to 3 inches long and approximately one inch wide. These are lobulated organs and secrete seminal plasma. These seminal vesicles empty into ampullae. Cowper's gland are also two in number located on either side of the urethra. These glands are deeply embedded in bulbourethral muscle (Fig. 1). It also secretes seminal plasma, which serves as a carrier for spermatozoa. Prostate gland is a single gland and is nng shape, located near the neck of the bladder, surrounding the urethra. The secretion of this gland is alkaline in nature, it also adds to seminal plasma. In addition to these, there are glands located in urethral musculature called urethral glands. Penis has the function of draining the bladder as well as it serves as a copulatory organ to introduce spermatozoa into vagina in natural course.

The Accessory Glands

The main accessory sex glands in the bull are ampullae, the seminal vesicles, the prostate and cowpers glands (also known as bulls urethral gland) all of which produce secretions which contribute to ejaculated semen. There are also other urethral glands and preputial glands. The ampullae appear as dilations of the urethral end of the ductus deferens. They are well developed in the stallion and absent in the boar. The vasa deferens transport semen from the tail of the epididymis to the urethra. The outer diameter is 2 mm and its thick muscular wall makes it feel firm and cord like. The two different ducts lying side by side above the bladder are gradually become thicker in diameter thus forming the ampullae. This thickening of the duct is due to abundant occurrence of the glands in the wall. The ampullary glands are tubular and histologically very similar to the structure of vesicular glands. The two ampullae pass under the body of the prostate and open together with the excretory ducts of vesicular glands in to the urethra. During courtship and precoital stimulation (teasing) the sperm are transported from the tail of the epididymis to the ampulla aided by the peristaltic movements of the vas deferens. The ampullar glands secret small amounts of fructose and citric acid.

Vesicular glands:

They are paired, markedly lobulated glands situated in the urogenital fold lateral to the ampullae. The vesicular glands may vary considerably in size and lobulations between individuals. The secretory ducts from the individual lobules form one main excretory duct which is located in the centre of the gland and extends posteriorly under the body of the prostate. Each excretory duct unites with the different duct at its outlet into the urethra. The secretion of the gland is faintly opalescent, sticky fluid. It contains high concentrations of proteins, potassium, citric acid, fructose. It is often distinctly yellow in colour because of high flavin content. The pH varies from 5.7 to 6.2. The secretion of vesicular gland constitutes 60% or more of the volume of normal
ejaculate in the bull. It also contains ascorbic acid, ergothionine, inorganic and soluble phosphorus, sodium, ATP and several enzymes.

Prostate:

The prostate gland which surrounds the urethra is composed of two parts: Body of prostate (corpus prostate) and the disseminate or cryptic prostate. The secretions of these two parts pass through the numerous small ducts which open into the urethra in rows. The prostate gland secretes citric acid, fructose ergothionine, acid phosphatase. It is a source of calcium, sodium, potassium chloride and bicarbonate. It is rather high in Zinc (9.4 to 11.3 mg/100 g of fresh tissue). The protein content is very low. The secretion is acidic pH 6.4.

Cowpers glands:

It is a paired glands. They are round compact bodies with a dense capsule and are about a size of walnut in the bull. They are located above the urethra near its exit from the pelvic cavity. The secretary ducts from each gland unite in one excretary duct which is 2 to 3 cm in length. The two excretary ducts of the gland have small separate openings in the margin of the mucosal fold of urethra. Both prostate and cowpers glands are lobulated, tubular glands and the thick septa between the lobules contain unstriated muscle as in the vesicular glands. These muscles enable the glands to discharge their secretion suddenly. The secretion is viscous rubber like white. Secretion is distinguished by a high content of a sialo protein. The viscous fluid plays an important role in formation of "gelation which occurs in Boar seminal plasma at the time of ejaculation and immediately afterwards. As the gel is formed the sialo protein swells and is incorporated in it leaving behind the liquid portion of the semen. The pH of the secretion is alkaline 7.0. The low protein and high chloride content of the secretion is rather hostile to sperm. The dribblings from the prepuce of the bull before mounting are secretions from cowpers' gland. Most probably their function is to flush urethra.

Epididymis:

In addition to its repository function for spermatozoa it also produces secretion and participates in production of seminal plasma complete absence of fructose or any other glycolysable substance is one of the interesting features of this secretion. Another interesting feature is its high potassium low sodium ratio. Salisbury and Cragle (1956) recorded 373 K/i 4 Na in head, 177 K/90 Na in tail of epididymis. 240 K! 160 Na in ampullae and 287 K/250 Na mg/i 00 ml in vesicular glands. Another interesting feature of epididymal gland is its high content of Glycerophosphoryl
choline. Amongst enzymes there are several active glycosidases including B glucoronidases and mannosidases, B galactosidases, B & a fucosidases and B N acetyl glucosaminidase. Some of these enzymes especially mannosidases and 13 N acetyl glucosamidase occur in extra ordinary concentrations in epididymal seminal plasma.

**Erection**

The penis is composed of three cylinders of erectile tissue. These consist largely of sinuses, which during erection become engorged with blood rendering the organ turgid. In the farm species there is a relatively small quantity of erectile tissue so that there is little increase in size of the penis during erection. Most of the elongation is brought about by a straightening of the sigmoid flexure. Erection is achieved by the action of the ischiocavernosus muscles pumping blood into the corpus cavernosum penis (CCP). Erection occurs following stimulation of the nerves of the parasympathetic branch of the autonomic nervous system which causes dilation of the arterial blood supply to the penis, thus increasing influx of blood. This also causes the contraction of the ischio-cavernosus (erector-penis) and bulbo-cavernosus muscles, which prevent venous drainage from the penile tissue. The pressure of blood in the sinuses of the penis can reach ten times that of normal peripheral blood pressure. Full extension of the penis in bulls may be up to 55 cm.

**Ejaculation**

The process of ejaculation occurs as a result of a wave of peristaltic contractions from the epididymis, along the vas deferens to the urethra. At the same time the walls of the accessory glands contract, forcing their secretions into the urethra. Ejaculation occurs on average approximately one second after contact of the penis and vulva. Full erection and ejaculation are almost simultaneous, with the ejaculatory thrust being accompanied by a slight twisting of the glans penis. Semen is deposited in the anterior vagina, just posterior to the external os of the cervix. There is some evidence that oxytocin release from the posterior pituitary gland may be involved in the ejaculatory process. After ejaculation, the penis is withdrawn into the prepuce by the action of the retractor penis muscle. The ejaculation reflex is stimulated by sensory nerves within the glans penis that transmit to the spinal cord through the dorsal nerve of the penis, a branch of the pudendal nerve. Thereafter, erection and ejaculation are primarily coordinated as spinal reflexes in the lower lumbar and sacral segments of the spinal cord. The integrity of this nerve is essential for the ejaculation reflex to take place and, if it becomes damaged, ejaculation, though not erection, becomes impossible. Erection of the penis depends on the general aspects of sexual behavior and is controlled by the nervous system. With erection, the penis begins to elongate and it becomes more rigid. Stimulation of the sympathetic and parasympathetic nerves is caused by sensory input and psychic stimuli. The sympathetic nerves inhibit vasoconstriction and the parasympathetic nerves affect the dilation of blood vessels and enlargement of the corpus cavernosum. In addition, in all farm animals there is a relaxation of the penile retractor muscle. Ejaculation involves muscle contractions of various parts of the male reproductive system that is mediated by the nervous system.
ERECTION. The normal flaccid state of the penis is maintained by tonic contraction of smooth muscle in the corpora cavernosa and spongiosum and arteries supplying them under the influence of sympathetic adrenergic input, involving norepinephrine as the neurotransmitter. Both $\alpha_1$-adrenergic and $\alpha_2$-adrenergic receptors are involved. Blood pressures within the erectile bodies in the flaccid state are actually much lower than are systemic blood pressures (Table 7.4). This is due to the tonic constriction and the use of numerous shunts that divert blood directly into draining vessels before it enters the penis (Andersson and Wagner 1995; Kandeel et al. 2001) (see Figure 7.5).

During erection, psychic stimuli acting on brain and tactile stimuli on penis (transmitted both to the local spinal cord reflex and to the brain) result in increased parasympathetic tone and relaxation of smooth muscle in arteries supplying the penis. Within the corpora, the increased parasympathetic tone results in the production of NO from endothelial cells lining the sinusoids and from the parasympathetic nerve terminals themselves. The NO then moves into smooth muscle cells of the surrounding trabeculae, where it causes relaxation by activation of guanylate cyclase and a subsequent increase in cGMP levels. The smooth muscle cells of the corpora are interconnected by gap junctions that enable a synchronized response. Arterial dilation and relaxation of the corporal sinusoids cause increased blood flow to the erectile corpora, and erection results (Andersson and Wagner 1995; Kandeel et al. 2001). Cyclic guanosine 3,5'-monophosphate (cGMP) within the smooth muscle cells is inactivated by the phosphodiesterase enzyme (PDE). In humans, the major subtype of PDE in the corpus cavernosum is PDE5, and it is this enzyme that is targeted for inhibition by the drug sildenafil (Viagra), resulting in increased cGMP levels, continued cavernosal smooth muscle relaxation, and erection (Kandeel et al. 2001).

Following increased compliance and blood flow into the vascular spaces of the penis, the venous drainage is compressed, partly passively by expansion of the corpora and partly by contraction of bulbospongiosus and ischiocavernosus muscles. Evidence from several species indicates that contraction of the ischiocavernosus muscles occludes venous outflow and compresses arterial input to the corpus cavernosum penis during peak erection. Effectively, this makes the cavernosa a temporarily closed system. Continued contraction of the ischiocavernosus muscles compresses the crura of the corpus cavernosum against the ischium, forcing this blood into the body of the penis, resulting in the extraordinarily high peak pressures (Table 7.4).
penis, accessory glands, and ductus deferens, and closure of the neck of the bladder, aid in the movement of sperm and seminal plasma and final expulsion of the semen through the penis. Some species such as the boar and stallion have a very prolonged ejaculation compared to other species. In the stallion and the boar, the penis is primarily responsive to pressure, whereas ruminants are more responsive to temperature. In these species the semen can be divided into pre-sperm, sperm-rich fraction, and post-sperm (gel/plug fraction). The site of ejaculation is also species-dependent. Ruminants deposit semen in the vagina, whereas the stallion deposits semen in the uterus. The pig deposits his semen in two locations—the cervix and the body of the uterus—largely due to the retention of the penis during copulation.

**Physiological significance of seminal plasma**

It is a natural diluent for spermatozoa. Spermatozoa could scarcely be expected to function without the provision of seminal plasma. It is vehicle for spermatozoa. It is the best medium since it is well buffered and contains energy source for spermatozoa. It exerts distinct stimulatory effect on spermatozoa motility. This is simply due to "dilution effect". It contains ready source of energy for spermatozoa.

**The penis**

The penis comprises three tracts of erectile tissue and the penile urethra. The urethra is surrounded by the corpus spongiosum penis (CSP), which arises at the bulb of the penis and terminates in the glans penis. The dorsum of the penis is made up of the paired corpora cavernosa penis (CCP), which arise in the two crura (roots) of the penis and terminate behind the glans. The blood supply of all three tracts is via branches of the pudendal artery, but the venous drainage of the CCP is markedly different from that of the CSP. The CCP is drained via the root of the penis, into the pudendal vein, whereas the CSP drains into the dorsal vein of the penis from its distal extremity. Thus, both the supply and drainage of blood to the CCP are via the root of the penis, whereas the supply of the CSP is through the bulb and its drainage from its distal part. The roots of the penis are surrounded by the ischiocavernosus muscles, which, on contraction, occlude the veins draining the CCP against the ischium of the pelvis, so that the cavernous spaces of the erectile tissue in the blind-ending CCP become engorged with blood, causing stiffening and lengthening of the penis. However, the detailed anatomy of the penis varies greatly between species and, as a result, details of the functional anatomy of erection are similarly variable.
The scrotum

The scrotum is an outpouching of the skin from the inguinal region. There are two sacs, one surrounding each testicle, and they are separated by a septum. The scrotum has four layers:

- The skin. This has minimal underlying fat and has an abundant supply of sweat glands, which are important in the thermoregulation of the testicles.

- The tunica dartos. This comprises smooth muscle fibers and connective tissue. In warm weather, the muscle cells within the tunica dartos relax and allow the testicles to drop away from the body wall; in cold weather, the dartos muscle contracts, helping to pull the testicles closer to the body.

- The scrotal fascia. This allows the testicles to move freely within the scrotum.

- The common vaginal tunic. Two layers of connective tissue, called tunics, surround the testicles themselves. The outer tunic, the tunica vaginalis or common vaginal tunic, is considered by some to be part of the scrotum and by others to be part of the testicles themselves. The tunica vaginalis is an invagination of the peritoneal lining and passes through the inguinal rings when testicular descent occurs. The tunica albuginea closely adheres to the testicle whereas the tunica vaginalis is separated from the inner tunic by a very thin layer of fluid. This fluid facilitates the movement of the testes in the scrotum.

The penis of the stallion is musculocavernous in nature. It is composed of three general areas

- The base or root. This is attached to the ischium by the paired crural muscles, the paired ischiocavernosus muscles and a pair of suspensory ligaments. There are paired retractor penis muscles, which relax to allow the penis to be extended and contract to pull the penis back into the prepuce.

- The shaft.

- The glans (free end).

The urethra runs through the center of the penis and is surrounded by the corpus spongiosum penis.

- The corpus spongiosum penis contains some erectile tissue, which becomes engorged when sexual stimulation occurs.
- The corpus cavernosum penis surrounds the corpus spongiosum penis.
- The corpus spongiosum penis is not surrounded by any connective tissue and continues distally as the glans (free end) of the penis.
- The corpus cavernosum penis is an intricately sinusoidal structure, which becomes engorged with blood during sexual arousal. A connective tissue covering called the tunica albuginea surrounds the corpus cavernosum penis.
- The glans (free end) of the penis will increase 300-400% in size with engorgement and ejaculation. There are many nerve endings in the glans penis. There is a prominent rim of tissue on the glans (free end) of the penis called the corona glandis, which becomes firm and prominent with penile engorgement.

**PUBERTY**

Puberty in bulls is a process that implies the attainment of functional sexual organs and behavior. Sexual maturity occurs when the development of both spermatogenesis and reproductive behavior allow effective coordinated service and subsequent fertilization. However, such definitions obscure the fact that puberty is a continuous and dynamic process, which commences prior to birth and is mediated through the hypothalamic-pituitary axis. At birth, the testes are small, solid, and composed of cords of gonocytes and undifferentiated supporting cells. In prepubertal bulls, there is an early rise in gonadotropins (in particular LH and less consistently FSH) between 10 and 20 weeks of age. The earlier this rise in gonadotropins occurs, the earlier the onset of puberty.
Spermatogonia start to appear in tubules within approximately 8 to 14 weeks after birth, with spermatocytes appearing shortly afterward. Seminiferous tubules form lumens between 15 and 40 weeks. Sequential maturation of spermatogonia through primary and secondary spermatocytes to spermatids and spermatozoa is achieved between weeks 32 to 44 in well-fed *Bos Taurus* breeds. Testicular growth is very rapid between 7 and 10 months of age. Blood concentrations of androgens (initially androstenedione and then testosterone) start to increase at about 6 months of age and continue to rise through puberty (until at least 13 months of age). Puberty is often defined as the first time a bull produces an ejaculate with at least 50 × 10^6 spermatozoa per milliliter with at least 10% progressive motility. Increases in sperm concentration and the proportion of morphologically normal spermatozoa occur in conjunction with decreasing proximal droplets for at least 4 months after puberty.

Although breeds vary in both testicular development and body weight, puberty is quite consistently achieved when scrotal circumference reaches approximately 25 to 27 cm. Therefore, scrotal circumference is generally a better predictor of puberty than either age or body weight, regardless of breed. However, breed differences do occur in the pattern of testicular development. Bull age strongly influences scrotal circumference in young well-grown *Bos taurus* breeds, whereas body weight tends to be more predictive in *Bos indicus* breeds, especially when nutrition is suboptimal.

Postweaning average daily gain does not appear to be highly related to scrotal circumference in bulls. However, high-energy diets may depress both libido and spermatogenesis in young bulls, and an association has been reported between excessive backfat and lowered fertility. Prepuberal scrotal circumference measures in bulls have not proved to be reliable predictors of subsequent postpuberal testicular size, although use of minimum thresholds show promise. For example, one study estimated that, to obtain a 30-cm scrotal circumference by 365 days of age, Angus, Simmental and Zebu-derived bulls needed to have a scrotal circumference of 23 cm at start of a 140-day growth test, while other continental breeds and Polled Herefords required 26 cm. Favorable associations with scrotal circumference in beef bulls include age at puberty in related heifers and improved semen quality.

**Physiology of the testis**

All aspects of male reproductive physiology are under the endocrine control of the two major gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The secretion of LH is pulsatile, with irregular episodes of secretion occurring every 2–4 hours. The actions of LH are primarily upon the Leydig cell, where, acting through adenylate cyclase, it promotes steroidogenesis by regulating the rate-limiting step of steroidogenesis; namely, conversion of cholesterol into the testosterone precursor, pregnenolone. Peak testosterone concentrations follow those of LH by about 40 minutes and decline back to prestimulation values over a further 40–80 minutes (D’Occhio et al., 1982a; Figure 29.6). Testosterone is required for the production of sperm in the testis and their subsequent maturation in the epididymis, for the function of the accessory sex glands and for the development of masculine secondary sexual characteristics. After aromatization into oestrogen within the brain,
testosterone is also responsible for negative feedback regulation of LH secretion and for male behaviour. Curiously, in long-term castrated animals, neither negative feedback nor libido can be restored by testosterone administration, for brain aromatase activity is eventually lost, and oestrogen itself has to be given for the restoration of these effects (D’Occhio et al., 1982b). Within the lumen of the seminiferous tubule, testosterone is converted by 5-reduction into 5-dihydrotestosterone (DHT), which is not susceptible to aromatization and is a more potent androgen than testosterone itself. Both testosterone and DHT are bound within the tubule lumen by the secretory product of the Sertoli cells, androgen binding protein (ABP). The role of ABP therefore appears to be to maintain high androgen concentrations in the lumina of the seminiferous tubule and epididymis. The main target of FSH is the Sertoli cell, where it also acts through adenylate cyclase-linked enzyme systems. Under the influence of FSH, Sertoli cells secrete ABP (Gunsalus et al., 1981) and aromatize testosterone into estrogen (Setchell et al., 1983). Adequate FSH stimulation is also required to permit Sertoli cells to support spermatogenesis.

Some evidence suggests that the production of pyruvate and lactate, which act as energy substrates for germ cells, may be a key role of the FSH-stimulated activity of the Sertoli cell in maintaining spermatogenesis. Debate remains over the pattern of secretion of FSH; some consider it to be pulsatile, a manner analogous to LH, while others consider its secretion only to exhibit longer-term fluctuations. FSH secretion is regulated by both gonadal steroids and inhibin, the regulatory protein secreted by Sertoli cells (Baird et al., 1991). The actions of the gonadotrophins are, however, not limited to the somatic cells of the testis, as both LH and FSH regulate aspects of germ cell activity. For example, experiments upon hypophysectomized rams indicated that the rate of division of stem spermatogonia is controlled by LH, while the rate of subsequent divisions and the ability of cells to undergo meiosis are regulated by FSH (Courot and Ortavant, 1981). Whilst these actions upon germ cells are, in part, mediated through the stimulation of activity in the somatic component of the testis by the gonadotrophins, circumstantial evidence indicates direct actions upon germ cells themselves. A schematic summary of the endocrine relationships of the testis is given in Figure 29.7 (Amann and Schanbacher, 1983).

**Spermatogenesis**

Spermatogenesis is the basic process of male reproduction, resulting in the production of spermatozoa. It is carried out in the seminiferous tubule of the adult testis and comprises three main processes. Initially, the relatively undifferentiated spermatogonia undergo a period of mitotic multiplication, divisions, followed by the meiotic reduction of the diploid to haploid genome. Finally, the postmeiotic cells undergo the morphological transformation of spermiogenesis, resulting in the release of formed spermatozoa into the lumen of the tubule. These processes of spermatogenesis are reflected in the functional morphology of the seminiferous tubule (see Courot et al., 1970). The basement membrane of the seminiferous tubule is surrounded externally by fibroblasts and myoid cells. The blood supply is limited by the basement membrane and does not pass into the tubule itself. Within the tubule
there are somatic Sertoli cells and the various stages of the seminiferous cell line, which together form the seminiferous epithelium. Sertoli cells rest upon the basement membrane, but extend through the entire thickness of the seminiferous epithelium, so that the germinal cells in all stages of spermatogenesis are in contact with the plasmalemma of Sertoli cells. Sertoli cells are irregularly cylindrical in shape, with large, variably shaped nuclei situated close to the basement membrane. They multiply during fetal and prepubertal life, with the full complement being present at the time of puberty. Until recently it was considered that Sertoli cell numbers were fixed at the time of puberty, but it is now evident that there is an annual cycle of loss and regeneration in at least some seasonally breeding species (Johnson and Thompson, 1983; Hochereau-de-Reviers et al., 1987). Sertoli cells secrete oestrogens, inhibin, a gonadotrophin-releasing hormone (GnRH)-like peptide, proteins (including ABP), lactate, pyruvate and tubule fluid. The cells are joined by specialized tight-cell-like junctions, so that the seminiferous epithelium is separated into apical and basal compartments by the blood–testis barrier formed by these junctions (see Hochereau-de-Reviers et al., 1990). During early fetal life, primordial germ cells enter the body from the yolk sac. In the gonadal ridge these cells differentiate into gonocytes, which undergo mitosis throughout fetal and prepubertal life. Gonocytes in turn differentiate into spermatogonia, at which stage development in the seminiferous cells is arrested until the onset of puberty. In the mature animal, spermatogonia are divided into A, intermediate and B classes, with each class further subdivided according to morphology and degree of differentiation. Thus, in the ram, A0, A1, A2, A3, intermediate, B1 and B2 spermatogonia occur (Hochereau-de-Reviers, 1976). A-series spermatogonia are the least differentiated and form the reservoir of stem cells within the seminiferous tubule. It is likely that stem cells are regenerated by asymmetrical divisions of early A-series spermatogonia; with one daughter cell
remaining as an uncommitted stem cell, the other being committed to undergo further mitotic and meiotic divisions. All spermatogonia remain in contact with the basement membrane, but, as the final meiotic division of spermatogonia gives rise to the primary spermatocytes, the cytoplasm of the Sertoli cells starts to intervene between the basement membrane and the primary spermatocytes. DNA synthesis occurs during mitotic divisions and then, to its greatest extent, during the formation of tetraploid nuclei during meiosis (for a review, see Hochereaude-Reviers et al., 1990). RNA synthesis occurs during preleptotene and late pachytene (Kierszenbaum and Tres, 1974). The first meiotic division then proceeds through the highly sensitive zygotene and pachytene stages. The pachytene stage is particularly sensitive to noxious damage, such as by high testicular temperature and inadequate maintenance of spermatogenesis by inappropriate gonadotrophin levels. During the first meiotic division, the cells move deeper into the seminiferous epithelium, and the tight cell junctions of the Sertoli cells form beneath the spermatocytes and degenerate above them (Russell, 1977, 1978), so that the cells effectively pass through the blood–testis barrier. Thus, the progeny of the first meiotic division, the secondary spermatocytes, move from the basal to the apical compartment of the seminiferous epithelium and are thereafter separated from the general tissue fluid compartment. The second meiotic division produces spermatids, which do not divide further. The spermatids thereafter differentiate into spermatozoa (Figures 29.8 and 29.9). At the end of meiosis, spermatids are round cells with round nuclei, which have to then undergo the very marked changes in cell function and morphology that occur during spermiogenesis. Immediately after completion of meiosis, the spermatids undergo a period of RNA synthesis, which is then followed by the beginnings of nuclear chromatin condensation (Monesi, 1971). Simultaneously, acrosomal contents are synthesized in the Golgi, whose vesicles progressively fuse to form the acrosome. As the nucleus condenses and elongates, the acrosome forms over the basal pole of the nucleus (Courten, 1979), while at the opposite pole the flagellum starts to form from one of the centrioles. A transient microtubular structure, the manchette, appears during the formation of the flagellum in the postnuclear cytoplasm of the elongating spermatid. The function of the manchette is unknown and it disappears after the flagellum is formed (Fawcett, 1970; Zirkin, 1971). The last stage of flagellum formation is the development of the midpiece, when a helix of mitochondria condense around the proximal part of the flagellum. During formation of the acrosome and flagellum, the cytoplasm of the spermatid is deeply invaded by a process of the Sertoli cell that extends between the forming flagellum and the residual cytoplasm. It is suggested that this process is responsible for the reduction in cytoplasmic volume of the spermatid that occurs during spermiogenesis. Finally, most remaining cytoplasm is engulfed by the Sertoli cell as the formed spermatozoon, with its remnant cytoplasmic droplet, is expelled from the crypt of the Sertoli cell into the lumen of the seminiferous tubule (see Fouquet, 1974). The duration of spermatogenesis, i.e. the time between spermatogonial divisions and the release of the spermatozoan, is approximately 60 days in most domestic animals. Epididymal transit takes a further 8–14 days. Thus, the interval between the most sensitive stage of spermatogenesis, meiotic prophase, and ejaculation, is approximately 30 days (see Amann and Schanbacher, 1983).
Hence, the interval between damage to the testis and the appearance of abnormal spermatozoa in the ejaculate is generally between 30 and 60 days, depending upon the site of damage. The seminiferous epithelium appears as concentric layers of spermatogonia, spermatocytes and spermatids, with characteristic associations between generations of cells throughout the depth of the seminiferous epithelium.

Each generation of seminiferous cells is linked by cytoplasmic bridges, so that developmental stages are synchronous within each generation, and substantial areas of seminiferous epithelium exhibit cells at a similar stage of development. Cellular associations are generally classified into type I, where two generations of primary spermatocytes and one of spermatids are present, and type II, where there is only one generation of primary spermatocytes but two of spermatids (see Hochereau-de-Reviers et al., 1990). Transition between type I and type II occurs after the maturation divisions, while type II changes into type I with the release of spermatozoa and the arrival of a new generation of spermatocytes from the last spermatogonial division.

**Structure and function of spermatozoa**

Spermatozoa are divided into three main segments:

- the head, midpiece and tail (Figure 29.10). The head consists of little other than the condensed nucleus and the overlaying acrosome. Of the enzymes contained within the acrosome, the main two are acrosin and hyaluronidase (Morton, 1977). During the acrosome reaction, the outer acrosomal membrane fuses with the plasmalemma, under the control of intra- and extracellular calcium, whereupon exocytosis of the contents of the acrosome occurs (see Harrison and Roldan, 1990). The main functions ascribed to the acrosomal enzymes are dispersal of the cumulus oophorus and local lysis of the zona pellucida; although it has been questioned recently whether the latter function is indeed a function of the released acrosomal enzymes per se. The inner membrane of the acrosome is relatively stable and remains intact after the acrosome reaction has occurred, and some of the acrosomal enzymes are probably bound to the inner acrosomal membrane. Penetration of the zona pellucida and fusion with the oolemma are both receptor-mediated events, with specific areas of the sperm head binding to target components of the oocyte (see Wassarman, 1990). The midpiece and tail of the sperm may be considered to form a single functional entity. The tail itself consists of a central axoneme, which, in the region of the midpiece, is sheathed in a helix of mitochondria (reviewed by Bedford and Hoskins, 1990). Sperm metabolize simple molecules, principally sugars and their derivatives (e.g. fructose, glucose, mannose and pyruvate), by both aerobic and anaerobic pathways, to provide energy for motility and the maintenance of ionic gradients across membranes (see Harrison, 1977).

Forward motility of sperm results from coordinated waves of flagellar bending progressing from neck along the length of the tail. Bending of the tail occurs as the result of forces generated between adjacent peripheral doublets of the axoneme. The dynein arms of the doublet, which in the resting state are bound to the adjacent doublet, unbind, elongate and then bind to a new site further along the filament. The unbinding process, which is the ATP-using step, is then repeated, resulting in a progressive bending of the flagellum. The doublets on one side of the axoneme work
in opposition to each other, providing the alternating beat of the tail. After capacitation, the rate and amplitude of the flagellar beat greatly increase, and the rate of energy usage by the sperm is correspondingly elevated (Yanagimachi, 1981). The motility of the cell itself probably has little role in the movement of spermatozoa through the cervix and uterus, for this is accomplished mainly through contractions of the female genital tract (Hunter, 1980). However, passage through the uterotubal junction and within the oviduct does require sperm motility, while the enhanced, whiplash motility of the capacitated sperm is necessary for penetration of the cumulus and zona pellucida.
Properties of Semen:

Semen or germplasm is the complete-discharge of the male genital tract occurring at the time of ejaculation by the male. It is a white, opaque, creamy fluid, occasionally yellowish green due to the pigment carotene. Semen consists of cellular part spermatozoa or sperms and the fluid parts, known as seminal plasma. A spermatozoon is a male germ cell apportioned into three regions: head, middle piece and tail. The shape of the head of the sperm in the bull, ram, boar and rabbit is a blunt ovoid. In fowl, the sperm head appears as elongated cylinder. In the bull, the spermatozoon measuring 80 microns in length resembles an agile tadpole. The head is a blunt ovoid structure known as acrosome.

The liquid portion of semen i.e. seminal plasma is nothing but the secretions of accessory sex glands such as the prostrate, seminal vesicles and cowper's glands. Seminal plasma presents an ideal medium for the viability and mobility of the male germ cells. The volume of single ejaculate in the bull ranges from 2-10 mL Variations in quantity may be due to breed differences and also age, frequency of service, season and nutritional status of the individual.

Spermatogenesis

The process of germ cell development from spermatogonia to spermatozoa is termed spermatogenesis, and this progression occurs within the convoluted seminiferous tubules.

Spermatogenesis is further divided into three phases:

spermatocytogenesis, meiosis, and spermiogenesis.

Spermatocytogenesis

The end product of spermatocytogenesis is the primary spermatocyte cell. The primordial germ cells give rise to type A spermatogonia, which are diploid. These cells remain close to the basement membrane and will continue to divide (see Figure 3.14). Type A spermatogonia are extremely resistant to toxic insult and if need be, can repopulate the germ cells within the seminiferous tubules. Some type A spermatogonia eventually differentiate into diploid type B spermatogonia, which further differentiate.
into diploid primary spermatocytes.

**Meiosis**

Meiosis is the process by which the diploid primary spermatocytes eventually give rise to haploid round spermatids. The primary spermatocytes (2N) undergo DNA duplication, and thus they are considered 4N in terms of DNA content. However, most individuals use N to refer to chromosomes rather than DNA. These cells will enter the first round of meiosis and one primary spermatocyte will give rise to **two secondary spermatocytes** (1N in terms of chromosomes and 2N for DNA content). These cells almost immediately initiate meiosis II, and thus they are transient and difficult to see in most histological sections of the testes. Two secondary spermatocytes will yield four **round spermatids** in the process of meiosis II (1N chromosomal content, 1N DNA content). The round spermatids are the end product of meiosis.

**Spermiogenesis**

The final stage of spermatogenesis is called **spermiogenesis**, and this process includes the development of the haploid **round spermatids** into haploid **elongated spermatozoa** that are ready to be released from the convoluted seminiferous tubules. Four main events occur as part of this stage: **acrosome formation**, **condensation of the chromatin**, **flagellar formation**, and **the excess cytoplasm is shed as the residual body**. The **acrosome** is a glycoprotein cap of digestive enzymes overlying the nucleus that is thought to aid the sperm in penetration through the corona radiata and zona pellucida surrounding the oocyte. Rough endoplasmic reticulum (RER) and the Golgi body are essential for the synthesis of this region. Initially the acrosome starts out as a granule, but as the cell develops
further, the acrosome will expand to cover the nucleus. The chromatin must undergo condensation to permit the spermatozoa to travel rapidly within the male and female reproductive tracts. The large spherical nucleus of the round spermatid becomes streamlined, but not pyknotic. The sperm must develop a flagellum for it to become motile within the male and female reproductive tracts. This structure will develop from one of the centrioles, and thus it is composed of microtubules in the classic 9 + 2 arrangement. The mitochondria orient alongside the flagellum to generate ATP needed for flagellar movement. Finally, the round spermatids must shed their excess cytoplasm, as the residual body. The Sertoli cells then phagocytose the residual body.

**Sperm transport in the female genital tract:**

Passage of sperm through the female reproductive tract is regulated to maximize the chance of fertilization and ensure that sperm with normal morphology and vigorous motility will be the ones to succeed. The site of semen deposition is not easy to establish in many species because it must be determined by examining the female immediately after coitus and by considering the anatomy of the penis, vagina and cervix during coitus.

**Vaginal defenses against infectious organisms may affect sperm:**

The vagina is open to the exterior and thus to infection, especially at the time of coitus; therefore, it is well equipped with antimicrobial defenses. These defenses include acidic pH and immunological responses and can damage sperm as well as infectious organisms. To enable fertilization to take place, both the female and the male have adopted mechanisms for protecting sperm. In humans, semen is deposited at the external os of the cervix so that sperm can quickly move out of the vagina. Human sperm must contend, however briefly, with the acidic pH of vaginal fluid. The vaginal pH of women is normally five or lower, which is microbicidal for many sexually transmitted disease pathogens. Evidence indicates that the acidity is maintained through lactic acid production by anaerobic lactobacilli that feed on glycogen present in shed vaginal epithelial cells. Lowering pH with lactic acid has been demonstrated to immobilize bull sperm. The pH of seminal plasma ranges from 6.7 to 7.4 in common domestic species and has the potential to neutralize vaginal acid. Vaginal pH was measured by radio-telemetry in a fertile human couple during coitus. The pH rose from 4.3 to 7.2 within 8 s of the arrival of semen; whereas, no change was detected when the partner used a condom. Vaginal washings of women with high levels of detectable seminal antigens had a median pH of 6.1, whereas the median pH of washings lacking detectable antigens was 3.7. Contraceptive gel designed to maintain a low vaginal pH after coitus has been shown to immobilize human sperm in vitro and in vivo. In additions to pH buffers, seminal plasma contains inhibitors of immune responses, including protective components that coat sperm. These are most effective when sperm are bathing in seminal plasma and may be gradually shed when sperm leave the seminal plasma behind. Males may also overcome female defenses by inseminating many sperm. This strategy is particularly effective for overcoming cellular immune responses. In the rabbit, deposition of
semen results in an invasion of neutrophils into the vagina. This invasion takes time, however, to build to an effective level. Numerous leukocytes, many containing ingested sperm, were recovered from vaginas of rabbits 3–24 h post coitus. By that time, however, thousands of sperm had already reached the Fallopian tubes.

**Sperm transport through the cervix:**

In some species, the cervical canal widens under the influence of estrogen. Sperm of humans and cattle enter the cervical canal rapidly where they encounter cervical mucus. The extent of hydration is correlated with penetrability to sperm. Coitus on the day of maximal mucus hydration in women is more closely correlated with incidence of pregnancy than coitus timed with respect to ovulation detected using basal body temperature. Cervical mucus presents a greater barrier to abnormal sperm that cannot swim properly or that present a poor hydrodynamic profile than it does to morphologically normal, vigorously motile sperm and is thus thought as one means of sperm selection. The greatest barrier to sperm penetration of cervical mucus is at its border, because here the mucus microarchitecture is more compact. Components of seminal plasma may assist sperm in penetrating the mucus border. More human sperm were found to enter cervical mucus *in vitro* when an inseminate was diluted 1:1 with whole seminal plasma than when it was diluted with Tyrode's medium, even though the sperm swam faster in the medium. Like the vagina, the cervix can mount immune responses. In rabbits and humans, vaginal insemination stimulates the migration of leukocytes, particularly neutrophils and macrophages, into the cervix as well as into the vagina. Neutrophils migrate readily through midcycle human cervical mucus. In rabbits, neutrophils were found to heavily infiltrate cervices within a ½ h of mating or artificial insemination. Interestingly, it was discovered that if female rabbits were mated to a second male during the neutrophilic infiltration induced by an earlier mating, sperm from the second male were still able to fertilize. Thus, although the cervix is capable of mounting a leukocytic response, and neutrophils may migrate into cervical mucus, the leukocytes may not present a significant barrier to sperm. It has been demonstrated that neutrophils will bind to human sperm and ingest them only if serum that contains both serological complement and complement-fixing anti-sperm antibodies is present. This can happen *in vivo* if the female somehow becomes immunized against sperm antigens. Altogether, the evidence indicates that leukocytic invasion serves to protect against microbes that accompany sperm and does not normally present a barrier to normal motile sperm, at least not shortly after coitus. Immunoglobulins, IgG and IgA, have been detected in human cervical mucus. Secretory IgA is produced locally by plasma cells in subepithelial connective tissue. The amount secreted increases in the follicular phase but then decreases at about the time of ovulation. The immunoglobulins provide greater protection from microbes at the time when the cervical mucus is highly hydrated and offers the least resistance to penetration. However, when there are antibodies present that recognize antigens on the surface of ejaculated sperm, infertility can result. Complement proteins are also present in cervical mucus, along with regulators of complement activity. Thus, there is a potential for antibody-mediated destruction of sperm in the cervical mucus as well.
as leukocytic capture of sperm. Some anti-sperm antibodies are not complement-activating; however, they can still interfere with movement of sperm through cervical mucus by physical obstruction.

**Sperm transport through the uterus:**

At only a few centimeters in length, the human uterine cavity is relatively small and could be traversed in less than 10 min by sperm swimming at about 5 mm/min, which is the swimming speed of sperm in aqueous medium. Transport of sperm through the uterus is likely aided by proovarian contractions of the myometrium. Waves of uterine smooth muscle contractions that increase in intensity during the late follicular phase. Myometrial contractions may be stimulated by seminal components. When vasectomized male rats were mated with females, the incidence of strong uterine contractions declined, indicating that sperm or testicular or epididymal secretions have stimulatory activity. Removal of the seminal vesicles significantly reduced the pregnancy rate in mice. In boars, there is evidence that estrogens, which may reach 11.5 μg in an ejaculate,(262,501),(812,516) increase myometrial contraction frequency. Since boar semen is deposited directly into the uterine cavity, the uterus is exposed to the full amount of estrogens in the semen. There is evidence that the estrogens enhance contraction by stimulating secretion of PGF-2α. Rapid transport of sperm through the uterus by myometrial contractions can enhance sperm survival by propelling them past the immunological defenses of the female. As is the case in the vagina and cervix, coitus induces a leukocytic infiltration of the uterine cavity, which reaches a peak several hours after mating in mice. The leukocytes are primarily neutrophils and have been observed phagocytizing uterine sperm in mice, rats and rabbits. This phagocytosis was observed several hours after insemination and therefore might be directed primarily against damaged sperm. However, normal sperm may also be attacked, particularly in vaginal inseminators like humans, because their sperm have lost much of the immune protection afforded by seminal plasma constituents. When sperm first enter the uterus, they outnumber the leukocytes. As time passes, the leukocytes begin to outnumber the sperm. Also, as sperm lose protective seminal plasma coating, they may become more susceptible to leukocytic attack. At some point, even undamaged sperm may fall victim to the leukocytes. Probably, to ensure fertilization, sperm should pass through the uterine cavity before significant numbers of leukocytes arrive.

**Transport through the utero-tubal junction:**

The uterotubal junction presents anatomical, physiological and/or mucous barriers to sperm passage in most mammals. Anatomically, the lumen in species as distantly related as dairy cattle and mice is particularly tortuous and narrow. Within the lumen of the junction, there are large and small folds in the mucosa. In the cow, mucosal folds form cul-de-sacs with openings that face back towards the uterus. This arrangement of folds seems designed to entrap sperm and prevent further ascent. A vascular plexus in the lamina propria/submucosal layer of the wall may create a physiological valve. When engorged, the plexus can compress the lumen. This plexus has been well described in cattle. The walls of the bovine junction and adjacent tubal...
Isthmus also contain a thick muscular layer that could further constrict the lumen. The bovine utero-tubal junction is sigmoidal in shape and supported by muscular ligaments that appear capable of increasing the flexure of the curve and thus compressing the lumen. The narrow lumen of the uterotubal junction may be filled with viscous mucus that can impede the progress of sperm. Mucus has been found in the uterotubal junction in humans, as well as in rabbits, pigs, cattle.

**Rapid sperm transport:**

Sperm have been recovered in the cranial reaches of the tubal ampulla only minutes after mating or insemination in humans and several other species of mammals. Rapid transport of sperm into the Fallopian tube would seem to counter the proposed model of sperm swimming one-by-one through the utero-tubal junction. However, when rabbit sperm recovered from the cranial ampulla shortly after mating were evaluated, they found that most were immotile and damaged. They proposed that waves of contractions stimulated by insemination transport some sperm rapidly to the site of fertilization, but these sperm are mortally damaged by the associated shear stress and do not fertilize. Later, motile sperm gradually pass through the uterotubal junction to establish a tubal population capable of fertilizing. The contractions may serve primarily to draw sperm into the cervix but result in overshooting of some sperm. As described above, motile human sperm have been recovered from Fallopian tubes within an hour of insemination; however, it is not known whether function was normal in these women.

**A sperm reservoir in the Fallopian tube:**

As sperm pass through the uterotubal junction and enter the tubal isthmus, they may be trapped and held in a reservoir. The Fallopian tube provides a haven for sperm. Unlike the vagina, cervix and uterus, the tube does not respond to insemination with an influx of leukocytes. In addition to providing a haven, the storage reservoir maintains the fertility of sperm until ovulation. *In vitro*, sperm fertility and motility are maintained longer when sperm are incubated with endosalpingeal epithelium. Entrapment and storage of sperm in the initial segment of the tube may serve to prevent polyspermic fertilization by allowing only a few sperm at a time to reach the oocyte in the ampulla. Sperm numbers have been artificially increased at the site of fertilization in the pig by surgical insemination directly into the ampullar lumen. by resecting the isthmus to bypass the reservoir or by administering progesterone into the muscularis to inhibit smooth muscle constriction of the lumen. In each of these cases, the incidence of polyspermy increased.

**Preserving sperm fertility during storage:**

Sperm–endosalpingeal contact somehow preserves sperm during storage. Human sperm incubated with epithelium *in vitro* remain viable longer than when they are incubated in medium alone, as do sperm from other mammals. Viability of human sperm and other species can be extended by incubating them with vesicles prepared from the apical membranes of the endosalpinx, indicating that the epithelium can
produce the effect by direct contact rather than by secretions. It was reported that equine sperm binding to epithelium or membrane vesicles maintain low levels of cytoplasmic Ca2+, compared to free-swimming sperm or sperm incubated with vesicles made from kidney membranes. Human and equine sperm incubated with endosalpingeal membrane vesicles capacitate more slowly than sperm incubated in capacitating medium alone. Possibly, viability is maintained by preventing capacitation and its concomitant rise in cytoplasmic Ca2+. The mechanism for preventing rises of cytoplasmic Ca2+ in sperm are not known, but one suggestion is that catalase, which has been detected in the bovine tube, serves to protect against peroxidative damage to the sperm membranes, perhaps preventing inward leakage of Ca2+. The endosalpingeal binding protein on bull sperm, PDC-109, probably acts to stabilize sperm membranes. PDC-109 reduces membrane fluidity and immobilizes cholesterol in phospholipids membranes, including those of epididymal sperm. PDC-109 can also contribute to membrane stability by inhibiting the activity of phospholipase A2. Thus, PDC-109 may play a role in preserving bull sperm fertility while they are stored in the reservoir.

Hyperactivation of sperm and the final stages of transport:

At some point in the female tract, most likely in the Fallopian tubes, sperm become hyperactivated. In aqueous media in vitro, hyperactivated sperm swim vigorously but in circular or erratic patterns. In vivo, the physical environment encountered by sperm is quite different and evidence indicates that hyperactivation is required by sperm to progress towards the oocyte and penetrate its vestments. As discussed above, hyperactivation may assist sperm in detaching from the endosalpingeal epithelium. In addition, hyperactivation enhances the ability of sperm to swim through viscoelastic substances such as mucus in the tubal lumen and the extracellular matrix of the cumulus oophorus. Mucus fills the utero-tubal junction and extends into the isthmus in humans, rabbits, pigs and dairy cattle. Hyperactivated sperm penetrate artificial mucus, such as viscoelastic solutions of long-chain polyacrylamide or methylcellulose, far more effectively than non-hyperactivated sperm.

Taxis of sperm towards oocytes:

Although the existence of a guidance system to help mammalian sperm reach the unfertilized oocyte has been debated over the years, stronger evidence for such a system has surfaced recently. There is evidence for the existence of two complementary guidance mechanisms operating within the Fallopian tube. The first (long-range) mechanism is where capacitated sperm—released from intimate contact with the endosalpinx are guided by thermotaxis towards the site of fertilization. A temperature difference of up to 2°C between the cooler tubal isthmus and the warmer tubal ampulla has been detected in rabbits and there are indications that capacitated rabbit sperm tend to swim towards warmer temperatures. Once in the tubal ampulla, and at a closer proximity to the oocyte, a second (shortrange) chemotactic mechanism may guide sperm closer to the oocyte. Sperm are equipped with a mechanism for turning towards the oocyte in response to chemotactic factors; that is, they can switch
back and forth between symmetrical flagellar beating and the asymmetrical flagellar beating of hyperactivation. Hyperactivation is reversible, so sperm can alternate between turning and swimming straight ahead. Mammalian sperm have been reported to turn towards, or accumulate in, a gradient of follicular fluid, which could accompany the oocyte into the Fallopian tube. Nevertheless, the chemotactic agent in follicular fluid has not been identified, nor has its presence in the Fallopian tube been detected. Odorant receptors unique to sperm have been localized to a spot on the base of the flagellum of human, canine and rat sperm. Placing human sperm in a gradient of the odorant bourgeonal caused them to orient into the gradient and triggered a calcium and cAMP-mediated signalling cascade. Nevertheless, a chemotactic odorant has yet to be identified in humans or other mammals. If one were found, it could have vast implications for the development of contraceptives, as well as assessment and treatment of infertility.

The fate of non-fertilizing sperm:

After fertilization, any sperm remaining in the female reproductive tract may be phagocytosed by isthmic epithelial cells or may be eliminated into the peritoneal cavity where they are phagocytosed. Phagocytosis within the Fallopian tubes may be primarily employed by species, such as mice, which have an extensive ovarian bursa that would limit passage of sperm into the peritoneal cavity. In species where the passage of sperm into the peritoneal cavity is possible, this does not quickly render sperm non-functional as evidenced by the numerous case reports of human tubal pregnancies that arose in spite of lack of access of sperm from the uterus into the oviduct on the side of ovulation. In these cases, the only route available to the sperm was through the peritoneal cavity.

Fertilization and conception:

Before spermatozoa are able to fertilize the ovum, they have to undergo a further series of maturational changes in the female tract. These processes are known as capacitation and the acrosome reaction and are thought to require about six hours in the cow. This requirement for maturational changes is the main reason why it is preferable to inseminate cows several hours before ovulation. The precise changes involved in capacitation are not fully understood, but they involve enzymic and structural modifications to the acrosome and anterior part of the sperm head membrane. These include:

(1) an increase in membrane permeability to calcium
(2) modification of the membrane structure
(3) activation of the enzyme adenyl cyclase
(4) conversion of the protein proacrosin to acrosin.

The process of capacitation is stimulated when sperm enter the female reproductive tract. The acrosome reaction follows capacitation and involves the fusion of the sperm
cell membrane and the acrosome and the formation of gaps through which the acrosome contents can diffuse. The acrosome reaction is necessary to allow penetration of the oocyte by the sperm. Capacitation and the acrosome reaction are very closely linked and therefore it is not always possible to distinguish between the two processes. The presence of ovarian follicular fluid and the cumulus oophorus have a stimulatory effect on the acrosome reaction but do not appear to be essential for it. On reaching the ovum, the sperm penetrates any remaining cumulus oophorus by the action of the enzyme hyaluronidase from the acrosome and comes into contact with the zona pellucida. The sperm nucleus possesses a cytoskeletal coat, the perinuclear theca (PT), which is removed from the sperm head at fertilization. The PT contains an oocyte-activating factor. This has not been characterized, but is thought to be responsible for triggering the signaling cascade of oocyte activation (Sutovsky, 2003). Mobility of the spermatozoa is also important in the process of sperm penetration. Normally, only one sperm is able to pass through the zona, but when more enter, a process known as polyspermy, the resultant embryo is non-viable. Following fusion of sperm and egg, the contents of the cortical granules in the egg release into the perivitelline space (the cortical reaction), causing the zona pellucida to become refractory to sperm binding and penetration (the zona reaction). Several ways in which the cortical reaction may be mediated. It was demonstrated that the ability of the zona pellucida to prevent the entry of another spermatozoon after fertilization persisted through to the blastocyst stage. The fusion of the sperm and ovum cell membranes begins at the middle of the sperm head region. The sperm head becomes engulfed by the ova with the loss of the tail. The sperm’s nuclear membrane disappears and the male chromatin comes into contact with the ova cytoplasm. Penetration by the fertilizing sperm (pronucleus) stimulates the resumption of the second meiotic division of the oocyte and the extrusion of the second polar body. Fertilization is completed with the fusion of the haploid male and female pronuclei, a process known as syngamy.
During estrus secretion of submucous from the glandular portion of the cervix produces prostatic sheets of mucous mucus. Secretion is toward the lumen and in a caudal direction. Less viscous submucous is produced in the basal crypts of the cervix. Speciﬁcations found in the basal regions are obliterated in the same way. The submucous from the basal regions is surfaced toward the uterus through these “privileged pathways” (PP) of low viscous submucous (adapted from Melara and Scardina 1985, Am J Reprod 87:199).

**Cow**

- **Semen**
  - Inseminating pipelet
  - Hand grasping cervix
- **Uterus**
  - Left Uterine Lumen
  - Right Uterine Lumen
  - Left ovary
  - Right ovary
- **Vagina**

- **Mare**
  - **Vagina**
  - **Uterine body**
  - **Pipette**

The radiographs above are from vasectomized cow reproductive tracts (dorsal view). In normal insemination, one half of the semen is deposited in each uterine horn. In both examples, the insemination is caudal. Correct insemination minimizes the possibility of seminal deposition that results in signiﬁcant stereotypic loss of spermatozoa (see Figure 12.8).

In the mare, the gloved lubricated hand is inserted directly into the vagina and the index ﬁnger is used to guide the insemination pipelet into the cervical lumen. A marker (shovel) is used to gauge the depth of insemination.

**Fertilization**
- Acrosome reaction
- Spermatozoon penetrates egg
- Male and female pronuclei form

**Immediate Transport**
- Retrograde passage
- Phagocytosis
- Entrance into cervix/uterus

**Oviduct**
- Capacitation completed
- Hyperactive motility

**Cervix**
- “Privileged pathways”
- Removal of non-motile sperm
- Removal of some abnormalities

**Uterus**
- Capacitation initiated
- Phagocytosis

Three leukocytes (A, B and C) phagocytizing sperm. Sperm heads (SH) can be observed in the cytoplasm of the leukocytes. A sperm tail (ST) can also be seen protruding from the leukocyte. (Micrograph courtesy of R.G. Saacke, Virginia Polytechnic Institute and State University, Blacksburg)
Figure 12-10. Zona Binding by Sperm and Initiation of the Acrosomal Reaction

Proposed model for zona binding and the initiation of the acrosomal reaction in mammalian spermatozoa. The sperm plasma membrane overlying the acrosome contains two receptor-like regions. The first, called the zona binding region (ZBR), reacts with ZP3 to cause physical attachment of the sperm to the zona pellucida. A second membrane region, the acrosome reaction-promoting region (ARPR), also binds to ZP3 and initiates the acrosome reaction by causing the sperm plasma membrane to fuse (arrow) to the outer acrosomal membrane.

Figure 12-11. Schematic Illustration of the Acrosomal Reaction

Before acrosomal reaction: Before the reaction begins, all membranes of the head are intact.

During acrosomal reaction: During the reaction, the plasma membrane overlying the acrosome membrane begins to fuse with the outer acrosomal membrane. The fusion of the two membranes leads to vesiculation that creates pores through which the acrosomal enzymes can pass. This allows sperm to penetrate through the zona pellucida.

After acrosomal reaction: After the reaction, the vesicles are sloughed, leaving the inner acrosomal membrane, the equatorial segment, and the post-nuclear cap intact.

Figure 12-12. Illustration of Sperm-Oocyte Fusion

When the spermatozoon completely penetrates the zona and reaches the perivitelline space, it settles into a bed of microvilli formed by the oocyte plasma membrane. The cortical granules migrate to the periphery of the oocyte.

The plasma membrane of the oocyte fuses with the equatorial segment and the fertilizing spermatozoon is engulfed. The cortical granule membrane fuses with the oocyte plasma membrane and the cortical contents are released into the perivitelline space by exocytosis.

After the fusion between the membranes of the equatorial segment and the sperm plasma membrane occurs, the nucleus of the spermatozoon is within the cytoplasm. The sperm nuclear membrane disintegrates and the nucleus of the sperm decondenses.
In some animals (cow, sheep, rabbit, primates, dog and cat), the male ejaculates the semen into the cranial vagina. In others, (pigs, horses and camellids) semen is either deposited directly into the cervix (pig) or is squirted through the cervical lumen during copulation (horse). In the dog, pig and the horse most of the ejaculate gains entrance into the uterine lumen.

The stallion ejaculates in a series of “jets” in which a sperm-rich fraction is ejaculated first in a series of 3 to 4 high pressure squirts. This fraction contains about 80% of the spermatozoa. The last 5 to 8 “jets” are of lower pressure and contain fewer sperm. The seminal plasma in the final “jets” is highly viscous and may serve to minimize retrograde sperm loss from the mare’s tract.

Because of the large volume (200 to 400 ml) of boar ejaculate, most of the semen flows from the cervix into the uterine lumen. As in the stallion, the boar ejaculates a series of seminal fractions with different characteristics as ejaculation progresses. The first fraction consists of accessory fluids and gelatinous pellets. This fraction contains few sperm. The second fraction is rich in spermatozoa and this sperm-rich fraction is followed by a final fraction that forms a coagulum that resembles rice pudding. This coagulum reduces retrograde sperm loss. Immediately after insemination, semen undergoes varying degrees of retrograde transport (from the cervix towards the vulva).

In the dog semen is ejaculated in three fractions. The first, is a pre-sperm fraction that is thought to originate from the prostate. The volume of the pre-sperm fraction is usually small but can range from 0.5 to 5ml. This pre-sperm fraction (clear and acellular) is ejaculated in conjunction with pelvic thrusting by the male during “first stage coitus.” The second, a sperm rich fraction, is between 1 and 4 ml and is opalescent in color and contains between 300 million and 2 billion sperm. The final fraction originating from the prostate ranges in volume from 1 to 80ml. The first two fractions are ejaculated without visible force. However, the third fraction is ejaculated in surges of prostatic fluid that squirt into the vagina of the bitch during “second stage coitus.” Because of the “tie” (See Chapter 11) most of this fraction is forced cranially into the uterus and is believed to “push” the sperm-rich fraction ahead of it into the uterus.

Ejaculate volumes in the tom average only 0.2 to 0.3ml with a range of 0.1 to 0.7ml and it is therefore difficult to evaluate whether the ejaculate consists of multiple fractions.

The degree to which spermatozoa are lost from the female tract depends upon the physical nature of the ejaculate and the site of seminal deposition. In some species, the seminal plasma contains coagulating protein(s) that form a conspicuous vaginal plug to prevent spermatozoa from undergoing retrograde flow to the exterior. Female rodents (mice and rats) have a relatively solid vaginal plug that is externally visible following copulation. The presence of the vaginal plug can be used to determine when mating occurred. Domestic animals do not have a conspicuous vaginal plug.

**Spermatozoa are lost from the female tract by:**
- **phagocytosis by neutrophils**
- **retrograde transport**

When the female reproductive tract is under the influence of estradiol during estrus, neutrophils (powerful phagocytic white blood cells) sequester in the mucosa of the tract, especially in the vagina and uterus. These neutrophils are poised to attack foreign materials that are introduced into the female reproductive tract at insemination. It should be recognized that, in addition to spermatozoa, microorganisms are introduced into the tract during copulation. Thus, the neutrophil population is important in preventing these microorganisms from colonizing the female tract. From an immunologic perspective, spermatozoa are foreign to the female. As a result, neutrophils actively phagocytize spermatozoa. They do not discriminate between live and dead sperm. In fact, a single neutrophil is capable of engulfing several motile spermatozoa (See Figure 12-2).

Studies have shown that within 6 to 12 hours after the introduction of spermatozoa into the uterus, there is a large migration of neutrophils from the uterine mucosa into the uterine lumen (See Figure 12-2). While leukocyte infiltration is an important contributor to post-insemination spermatozoal losses, this infiltration is important for the prevention of reproductive tract infection.

**Spermatozoal transport consists of a rapid phase and a sustained phase.**

Among the least understood phenomena in reproductive physiology are factors that regulate loss of spermatozoa from the female tract. The ability of the female to retain viable spermatozoa may influence the fertility of a given mating. Transport of spermatozoa following copulation can be divided into two phases. These are the rapid transport phase and the sustained
As you already know, estradiol is high during the follicular phase when insemination occurs. Estradiol stimulates contractions of the muscularis, particularly the myometrium. Also, prostaglandins in semen (PGF sub 2 alpha and PGE sub 1) cause increased tone and motility of the uterus and/or the oviduct. Intermittent contractions of the muscularis propel spermatozoa in both a cranial and a caudal direction. Fluids secreted into the lumen of the female tract also serve as a vehicle for transport. Control of directionality, while not understood, is probably under the collective influence of muscular contractions and fluid distribution and characteristics.

In addition to alteration of tract motility, seminal plasma from boars has been shown by German researchers to advance the time of ovulation in gilts. For example, when seminal plasma was infused into the right uterine horn, ovulation occurred about 11 hours earlier in the right ovary than in the left ovary. The left uterine horn did not receive seminal plasma. The specific material in boar seminal plasma inducing early ovulation has not been identified, but it appears to be a protein. Identification of these factors could provide an avenue to control more precisely the time of ovulation in swine. A similar phenomenon occurs in camels where seminal plasma components have been shown to cause ovulation.

A logical approach to alter or to minimize the retrograde sperm loss that accompanies natural and artificial insemination would be to treat the female with pharmaceuticals that alter smooth muscle motility in the female tract and thus alter sperm transport. Pharmacological manipulation of the uterine myometrium may represent an avenue whereby fewer numbers of spermatozoa can be inseminated with acceptable fertility.

In one test, when rabbits were injected with phenylephrine or ergonovine, two smooth muscle stimulants, the number of sperm reaching the oviducts was significantly elevated. In does inseminated with very low sperm numbers (around 90,000), an intramuscular injection of either phenylephrine or ergonovine resulted in a significantly increased fertilization rate when compared to controls that received neither phenylephrine nor ergonovine (See Figure 12-4). This finding suggests that a reduction in numbers of spermatozoa, if accompanied by proper stimulators of uterine smooth muscle motility, can result in acceptable fertilization rates. This would be particularly important if pharmaceuticals could be added to semen used in artificial insemination. If “transport conservation” could be accomplished, fewer sperm could be used in each dose of semen and spermatozoa from genetically superior sires could be distributed on a wider basis. Further, the problem of low sperm numbers following X-Y cell sorting described in Chapter 10 might be minimized using this approach.
The cervix is a major barrier to spermatzoal transport and it can also serve as a reservoir for spermatozoa.

Following natural service in the cow and ewe and, to some degree, the mare, spermatozoa must negotiate the highly convoluted system of grooves within the cervix (See Figure 12-5). During estrus, the cervix produces mucus. In the cow, cervical mucus consists of two types. One type is a sialomucin, a mucous of low viscosity. It is produced by cells in the basal areas of the cervical crypts (See Figure 12-5). A second type, sulfomucin, is produced in the apical portions of the cervical epithelium covering the tips of the cervical folds. This type of mucus is quite viscous. The production of two types of mucus (one of low viscosity and one of high viscosity) creates two distinct environments within the cervix. Spermatozoa encountering the viscous sulfomucin are washed out of the tract. Those that encounter the low viscosity sialomucin in the environment of the crypts of the cervix swim into it. Thus, the low viscosity environment of the deeper cervical crypts creates “privileged pathways” through which spermatozoa can move.

The ability of spermatozoa to traverse these “privileged pathways” is believed to depend on their ability to swim through the basal channels (crypts) of the cervix. In this context, the cervix may be a filter that eliminates non-motile spermatozoa. The time required for motile sperm to gain access and traverse these “privileged pathways” probably contributes significantly to the time required for the sustained phase of sperm transport. The specific role of the cervix in spermatzoal transport and/or retention awaits further clarification in the sow and the mare, where a high proportion of spermatozoa are ejaculated into the uterus.

Delivery of Semen to the Proper Anatomical Region of the Female Tract is Required for Successful Artificial Insemination

Artificial insemination technique requires that spermatozoa be deposited in the reproductive tract of the female by artificial means. In general, semen is delivered using a pipette to penetrate and bypass the cervix (See Figure 12-6). This type of insemination is referred to as transcervical insemination. In the sow, the insemination pipette is positioned within the cervix and semen is delivered into the cranial half of the cervix and flows directly into the uterine horns. This type of insemination is referred to as intracervical insemination (See Figure 12-7). In dogs and cats semen is deposited in the cranial vagina. This type of insemination is referred to as intravaginal insemination (See Figure 12-7).

In cases where sperm are in very limited supply, surgical insemination can be performed by exteriorizing the reproductive tract and injecting sperm directly into the uterus or utero-tubal junction region. Also, use of laparoscopy enables insemination to be performed without laparotomy (an abdominal incision). In bulls, X-Y sorted semen are in short-supply. Therefore, a technique has been developed to “thread” the tip of an insemination pipette near the utero-tubal junction. Such a technique has been reported to generate excellent results.

Spermatozoa must reside in the female tract before they acquire maximum fertility.

As you recall from Chapter 3, spermatozoa acquire maturity during epididymal transit. However, the maturational changes that occur in the epididymis do not render spermatozoa completely fertile. For maximum fertility to be achieved, spermatozoa must reside in the female reproductive tract for a minimum period of time. During the time in the female reproductive tract, some spermatozoa will undergo changes that allow them to become fertile. These changes are referred to as spermatozoal capacitation. The site for capacitation varies among species. In species where spermatozoa are deposited in the cranial vagina, capacitation may begin as sperm ascend and pass through the cervix. In species where semen is deposited into the mid-cervix (sow) or caudal cervix (mare) and immediately enters the uterus, capacitation is probably initiated within the uterus and completed in the isthmus of the oviduct. All spermatozoa are not capacitated at the same rate. Instead, they are capacitated over a relatively long period of time (several hours) and this reflects individual sperm differences as well as location within the tract. Capacitation can occur in fluids other than those found in the luminal compartment of the female reproductive tract. For example, in vitro capacitation has been accomplished in a wide variety of species using blood serum, a variety of commercial tissue culture media, Krebs Ringer solution and Tyrodes solution. No single in vitro environment will support capacitation for all species.

There is little doubt that the plasma membrane of the sperm (particularly the head) undergoes marked biochemical changes during capacitation. One way to envision the process of capacitation is presented in Figure 12-8. During mixing of sperm with seminal plasma the sperm become coated with various proteins. The coating of seminal plasma proteins is
“stripped” away by the female tract environment. The exact nature of the “stripping process” of capacitation is not understood.

An important concept with regard to capacitation is that the process can be reversed by returning capacitated spermatozoa to seminal plasma. For example, when capacitated spermatozoa are removed from the female reproductive tract and returned to seminal plasma, they become decapacitated and require additional capacitation time in the female reproductive tract before they can regain their fertility. It appears that the seminal plasma components coat the plasma membrane with surface substances that prevent or inhibit interaction of spermatozoa with the egg.

**Fertilization is a Complex Process and Involves a Cascade of Events**

The process of fertilization involves a series of specific interactions between spermatozoa and the oocyte. These are outlined in Figure 12-9.

**Acquisition of hyperactive motility occurs in the oviduct.**

In the oviduct the motility patterns of spermatozoa become hyperactive. The motility pattern changes from a progressive, linear motility in which they swim in a relatively straight line (like an Olympic swimmer), into a frenzied, dancing motion that is not linear and is localized in a small area (like dancers in a disco). In general, hyperactive motility occurs in the ampulla of the oviduct and is believed to be brought about by specific molecules produced by the epithelium there. Hyperactive motility is believed to facilitate sperm-oocyte contact.

**Binding to the zona pellucida requires specific zona-binding proteins on the spermatozoal membrane.**

Spermatozoa are known to contain specific proteins on their plasma membrane surfaces overlying the acrosome that bind specifically to zona pellucida proteins. These zona binding proteins on the plasma membrane must be exposed during the capacitation process before binding to the zona pellucida can occur. Before zona binding can be understood fully, the molecular makeup of the zona must be described.

**Figure 12-8. Conceptual Version of Mammalian Capacitation**

The plasma membrane of epididymal spermatozoa contains a complement of surface molecules (proteins and carbohydrates) illustrated here as yellow T's.

The surface molecules in epididymal sperm become coated with seminal plasma proteins (orange halos) that mask portions of the membrane molecules.

When sperm are exposed to the female tract environment, these seminal plasma coatings, along with some of the surface molecules, are removed, thus exposing portions of the molecules that can bind to the zona pellucida of the oocyte.
In the oviduct the motility patterns of spermatozoa become hyperactive. The motility pattern changes from a progressive, linear motility in which they swim in a relatively straight line (like an Olympic swimmer), into a frenzied, dancing motion that is not linear and is localized in a small area (like dancers in a disco). In general, hyperactive motility occurs in the ampulla of the oviduct and is believed to be brought about by specific molecules produced by the epithelium there. Hyperactive motility is believed to facilitate sperm-oocyte contact.

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The zona pellucida of the oocyte consists of three glycoproteins. These glycoproteins have been named zona proteins 1, 2 and 3 (ZP1, ZP2 and ZP3). Zona proteins 1 and 2 are structural proteins providing the structural integrity of the zona. Zona protein 3 is much like a receptor for a hormone. It binds to proteins on the spermatozoal membrane. Binding of spermatozoa to the zona pellucida is believed to require between 10,000 and 50,000 ZP3 molecules. The current understanding is that the sperm plasma membrane contains two zona binding sites. The first binding site, referred to as the primary zona binding region is responsible for adherence of spermatozoa to the zona pellucida. The second binding site on the spermatozoal plasma membrane is believed to be acrosome reaction promoting ligand. When binding occurs between this region and the ZP3 molecule, a signal transduction occurs. This is much like a typical hormone-receptor binding complex. Binding initiates the acrosomal reaction. The relationship between ZP3 and the spermatozoal plasma membrane during binding is illustrated in Figure 12-10.
The acrosomal reaction is an orderly fusion of the spermatozoal plasma membrane and the outer acrosomal membrane.

The purpose of the acrosomal reaction is two-fold. First, the reaction enables spermatozoa to penetrate the zona pellucida. Second, it exposes the equatorial segment so that it can later fuse with the plasma membrane of the oocyte.

The acrosomal reaction begins when the plasma membrane of the spermatozoon forms multiple fusion sites with the outer acrosomal membrane. When the two membranes fuse, many small vesicles are formed (See Figure 12-11) and this process is called vesiculation. After vesiculation has occurred, the acrosomal contents are dispersed and the sperm nucleus is left with the inner acrosomal membrane surrounding it. Vesiculation characterizes the acrosomal reaction and morphologically distinguishes it from a damaged acrosome. Damage to the acrosome membrane and plasma membrane is irreversible. Damage to these membranes is brought about by changes in osmotic pressure, sudden cooling, sudden heating or marked changes in pH. Damage to the membranes causes premature loss of acrosomal contents and such sperm cannot accomplish fertilization. Damaged acrosomes do not vesiculate in an orderly fashion, but rupture all-at-once.

Release of acrosomal enzymes allows the spermatozoon to digest its way through the zona pellucida.
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Release of acrosomal enzymes allows the spermatozoon to digest its way through the zona pellucida.

The penetration of the zona pellucida by a spermatozoon is believed to be a rapid process and probably takes no more than a few minutes. Following attachment to the zona pellucida, the acrosome reaction allows the release of a variety of enzymes. Acrosin is one enzyme that is released from spermatozoa during the acrosomal reaction. It hydrolyzes zona proteins as well as enhances the sperm’s ability to bind to the zona. In the inactive form, acrosin is known as proacrosin which has a strong affinity for the zona. Thus, proacrosin aids in binding the spermatozoon to the zona as the acrosomal reaction proceeds. As proacrosin is converted to acrosin, the sperm begins to penetrate and make its way through the zona pellucida. The mechanical force generated by the flagellar action of the tail may be sufficient to push the sperm through the zona. It is important to note that the acrosomal reaction allows the spermatozoon to digest a small hole through the zona through which it can pass. Placing a hot marble on the surface of a block of chilled butter would be an appropriate analogy. The hot marble would move through the butter in a small regional hole, but the butter in most of the block would be unchanged. This small regional dissolution leaves the zona predominately intact. Maintenance of an intact zona pellucida is important because it prevents blastomeres in the early embryo from separating during embryogenesis.

Fertilization requires fusion of the equatorial segment and the oocyte plasma membrane.
When the spermatozoon completely penetrates the zona and reaches the perivitelline space (the space between the zona and the oocyte plasma membrane), it settles into a bed of microvilli formed from the oocyte plasma membrane. The plasma membrane of the oocyte fuses with the membrane of the equatorial segment and the fertilizing spermatozoon is engulfed. The actual fusion of the oocyte plasma membrane with the equatorial segment is believed to be brought about by a so-called fusion protein located on this portion of the membrane. Prior to the acrosome reaction, this fusion protein is inactive. After vesiculation and release of the acrosomal contents, the fusion protein is activated, enabling the sperm membrane to fuse or bind with the oocyte membrane. This process is illustrated in Figure 12-12.

**The cortical reaction prevents penetration by additional spermatozoa.**

After membrane fusion, the oocyte undergoes a series of changes that prepare it for early embryogenesis. The most easily recognizable is the cortical reaction. During the first and second meiotic divisions of oogenesis, small, dense granules called cortical granules move to the periphery of the oocyte cytoplasm. The contents of the cortical granules consist of mucopolysaccharides, proteases, plasminogen activator, acid phosphatase and peroxidase. After membrane fusion between the oocyte and spermatozoon, the cortical granules undergo exocytosis and their contents are released into the perivitelline space (See Figure 12-12). Exocytosis of the cortical granules results in the zona block, a process whereby the zona pellucida undergoes biochemical changes so that further sperm cannot penetrate it. Polyspermy is prevented by the zona block.

**Pronuclei formation allows the male and female DNA to form a single nucleus.**

Polyspermy is the fertilization of an oocyte by more than one spermatozoon which results in embryo death. In addition to alteration of the zona pellucida, the cortical reaction is believed to reduce the ability of the oocyte plasma membrane to fuse with additional spermatozoa, thus causing the vitelline block, another mechanism that prevents polyspermy. Some species have both a zona block as well as a vitelline block, while others have either a zona or a vitelline block.

After the sperm nucleus has entered the cytoplasm of the egg, it becomes the male pronucleus. Before the pronucleus can be formed, however, the nucleus of the sperm must undergo marked changes within the oocyte cytoplasm. As you will recall, one of the mutational changes that occurs in the epididymis is the acquisition of large numbers of disulfide cross-links in the sperm nucleus. Thus, the nucleus of the mammalian sperm is almost inert. The keratinoid-like quality of insobility is considered to be important during exposure to the female tract environment, during sperm transport and during penetration through the zona pellucida. After the fertilizing spermatozoon enters the oocyte cytoplasm, the nucleus must "decondense" so that the male chromosomes may pair up with the chromosomes of the female pronucleus. The decondensation of the sperm nucleus requires the reduction of the many disulfide cross-links. In the cytoplasm of the oocyte, disulfide cross-links in the sperm nucleus are reduced quickly. The primary reducing agent is glutathione. When disulfide bond reduction occurs, the sperm nucleus decondenses and the nuclear material is available for interaction with the female nuclear material. The final step of fertilization is the fusion of the male and female pronuclei. This fusion is referred to as syngamy. Following syngamy, the zygote enters the first stages of embryogenesis that are described in Chapter 13.

**The Fertile Period Varies Significantly Among Mammalian Females**

The fertile life-span of sperm after deposition in the female reproductive tract varies immensely among species. For example, fertility of spermatozoa is retained for four to five years in certain reptiles. Among mammals, bat spermatozoa remain viable after insemination in the female tract for up to 4-5 months before the female ovulates. In general, retention of fertilizing capacity among domestic animals and humans lasts only a few days. Values in Table 12-1 document.

<table>
<thead>
<tr>
<th>Species</th>
<th>Fertile Life (days)</th>
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<tr>
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</tbody>
</table>
Figure 12-13. Probability of Conception When Copulation Occurred on Specific Days Relative of Ovulation in Women
(From Wilcox et al. 1995. NEJM 333:1517)

Conception can occur within a 6-day window prior to ovulation. At 5 days prior to ovulation, the probability of conception was 0.11 and the probability increases to about 0.33 two days before ovulation.

- The variation in fertilizing ability in the female tract among various domestic species and women.

In most domestic species the period of estrus is less than 24 hours. In other words, copulation must take place within a time-period that is close to ovulation. In contrast, sperm can remain viable for as long as 5 to 6 days before ovulation in women. Another example of a sustained fertile period is the bitch. Ovulation takes place over about a three day period after the onset of sexual receptivity. Fertilization can be accomplished as long as six days after the onset of sexual receptivity. It should be pointed out that in a multiparous species like the dog, several males can sire offspring because the bitch may be bred by several males during her relatively long estrus. Spermatozoa from all males are eligible to fertilize oocytes. This phenomenon is called superfecundation. Thus, it is not uncommon to observe litters that have different breeds of puppies.

It should be emphasized that the long fertile period in women coupled with a high frequency of copulation predisposes humans to unwanted pregnancies and a high global birth rate. Since the woman does not have a definite period of sexual receptivity, copulation taking place within 5-6 days of ovulation can result in a pregnancy. Where a poor understanding of the cycle exists, the probability of pregnancy becomes quite high because almost one-fourth of the menstrual cycle has the potential to generate a pregnancy.

The question is often asked as to whether the number of copulations can influence the chance of pregnancy within a given mating period. In spontaneous ovulators the answer is "probably not". In induced ovulators (especially in felids), there appears to be a threshold number of copulations required to optimize the chance of ovulation and therefore pregnancies. The probability of conception (pregnancy) is about 0.33 per cycle in women. This means if mating takes place among fertile individuals there is a one-in-three chance that the woman will become pregnant every cycle (if mating takes place within 2 days of ovulation as Figure 12-13 shows). It is like a batting average. If your favorite baseball player had a batting average of 0.333 for the season, he had a one in three chance to get a base-hit during each at-bat. Each at-bat is equivalent to the fertile period of an estrous or menstrual cycle. On average, your favorite hitter needs 3 at-bats to get a hit (a pregnancy). It makes no difference how many times the batter swings (number of matings) during each "at-bat," his batting average will still be 0.333. Similarly, assuming a threshold number of sperm are deposited during the first copulation, the number of matings during each fertile period (an "at bat") will not influence the probability of pregnancy.

Batting Averages and Pregnancies are Similar:
- Each "at-bat" = 1 opportunity to achieve pregnancy
- The batting average = probability of becoming pregnant
- A swing = 1 mating
- A good "at-bat" = many swings (but depletes extragonadal reserves)
SPERM MOTILITY

The pacemaker for initiation of sperm motility is located at the base of head "centriole". The rhythmic beats are propagated along the whole length of flagellum towards the tip of the tail. The entire flagellum participates in the whip like movement. The speed of spermatozoa moves in a fluid medium is 0.15 mm per second at body temperature. The axial filament complex which forms the core of the flagellum consists of number of fine fibrils which run through the entire length of the middle piece and tail. They represent the main: contractile element of the sperm cell. The contractions are excited by the rhythmic impulses which occur first in neck and then transported to each fibril in turn. The arrangement in outer bundle of fibrils govern the three dimensional wave like movements. Certain researchers have demonstrated the resemblance of certain proteins extracted from spermatozoa to those of myosin which is responsible for muscular contraction. ATPase activity is in the nine outer most axial fibrils of rat spermatozoa. There is also chemical evidence that like in muscular activity adenosine triphosphoric acid is involved in motion and metabolism of spermatozoa. Studies have demonstrated by using immunochemical techniques the presence of actin and myosin in axial fibrils. The progressive motility is promoted by flagellator movements of tail established through contractions of the contractile fibers of the axial filament in a rigid pattern. The sperm head rotates along its axis during progression. Isolated tails are able to move independently. The sperm will move against the stream rheotaxis. The active movement is of importance for transportation of sperm through cervix and for distribution of sperm at the site of fertilization and collision with the ovum; but is otherwise probably of no importance for transportation in the female genital tract. Sperms have been found in the oviducts of females of many species including cattle, sheep within few minutes after mating.

Initiation of motility:

Spermatozoa are immotile or nearly so while in the mate reproductive tract although they readily show motility as soon as they are taken out from the tract and examined under the microscope. The quiescence in the tract is because of low partial pressure of oxygen combined with deficiency of glycolysable sugar, inhibition of motility could be produced for several hours by with-holding both sugar and oxygen. Active motility resumed on supplying either oxygen or fructose, but maximum motility occurred only when both oxygen and fructose were supplied together. Epididymal spermatozoa are active in presence of oxygen only while stallion epididymal spermatozoa are active in presence of glycolysable sugar only. There is a strong evidence that potassium/sodium ratio is high in epididymis as compared to ejaculated semen. Potassium is more in epididymal fluid while sodium is in higher amounts in ejaculated semen. The epididymal sperm gets activated on dilution to normal saline. it is quite clear now that principle activating agent is, increased availability of oxygen and the action of inhibitory agent is reduced in high partial pressure of oxygen. The exogenous source of glycolysable sugar increases the motility and dilution of semen in sodium containing fluid increases its activity.
Factors affecting sperm motility:

Environmental factors influencing the active movements of sperm are temperature, pH, osmotic pressure and viscosity of the medium. Sperm are reversibly immobilized when cooled to 6°C. Temperature up to 42°C will increase their activity to a maximum while temperature above 46°C will kill the sperm. At pH 6 sperm are immobilized but are revivable even after a short exposure to pH 5. From pH 8 and upwards increasing damage occurs. Both hypo and hypertonic conditions are toxic to sperm. The former is being more hostile. High viscosity inhibits progressive movements (e.g. gelatin in semen diluent). At ejaculation the immobile sperm become actively motile as they are exposed to 1) sufficient O2 and a reduced CO² partial pressure 2) contact with metabolizable substrate 3) Reduced K and increased Na concentrations 4) Optimal pH, 5) Reduced viscosity and 6) Enzymes and coenzymes necessary for their metabolism. Energy for flagellar movement is derived from splitting of ATP to ADP + free energy and ADP to AMP + free energy. If the energy rich bonds are exhausted motility will cease. The energy necessary for re-synthesis of ADP and ATP is above all derived from enzymatic break down of fructose (fructolysis), under anaerobic conditions leading to an accumulation of the resulting lactic acid, which if sufficient oxygen is present will be broken down to CO² and H²O via kreb's cycle. The sperm are able to retain their motility and fertilizing ability in vitro provided the biochemical reactions of release and synthesis of energy are controlled. Many factors influence these reactions:

Temperature: Sperm are susceptible to sudden cooling (cold shock) which is prevented at semen collection, (collection tube placed in felt cap and before placing it in felt cap the tube is warmed up to 35°C by placing it on warming table), at dilution (the diluent preheated to 35°C is added equal to semen quantity and allowed to cool down slowly before final dilution with cooled diluent).

PH: Reversible reduction of sperm metabolism by moderate lowering of the pH (Citric acid or bicarbonate and saturation with CO² as in the IVT diluent). Toxic compounds such as disinfectants of all kinds and heavy metal ions and other cell poisons should never get into direct contact with semen. Protection against ultra violet light (direct sunshine) is essential to prevent toxic metabolic products to be produced (H²O₂ for example). Measures adopted to control the motility of sperm in vitro are not necessarily beneficial to the conservation of their fertilizing ability.

Depolymerisation of the DNA protein complex is almost impossible to counteract and is probably the cause of the gradual decrease in the fertilizing ability of sperm in diluted semen stored in vitro unless deep frozen.

COMPOSITION OF SPERMATOZOA AND SEMINAL PLASMA

Spermatozoa

The spermatozoon possesses two highly specialized functions motility and fertilizing capacity, which are located in two different parts of sperm structure. The sperm head which incorporates the nucleus carries deoxyribonucleoprotein, which is the most important constituent's in. so far as the fertilizing capacity of spermatozoa is
concerned. The sperm flagellum which comprises the midpiece and tail is the organ of motility and carries all the enzymes and coenzymes needed for metabolic activity. Sperm DNA when separated from nuclear protein is composed chiefly of four nucleotides each consisting of one molecule of phosphoric acid, one molecule of sugar, deoxyribose and one molecule of purine or pyrimidine base (Adenine, guanine, cytosine or thymine). Within species all spermatozoa carry constant amount of DNA, while RNA is virtually absent from mature spermatozoa. The protein conjugated with DNA is of basic type and have been shown to be either protamines or histones. In addition to DNA the sperm head carries several other important constituents including some carbohydrates in acrosome. A lypoglycoprotein, composed of phospholipid and glycoprotein, several amino acids and carbohydrate components are present. The chief among these may be glutamic acid, aspartic acid, leucine, alanine, serine, glycine and proline and mannoses, galactose, fucose, glycosamine, galactosamine and sialic acid. The whole lipoglycoprotein complex as prepared from ram and bull spermatozoa exhibit characteristic proteolytic and hyaluronidase activity and is capable of dispersing cumulus oophorous and carona radiata. In addition the sperm head contains several other -residual proteins with in the category of soluble non basic nuclear protein containing tryptophane and another much less soluble sulphur rich, keratin like protein, which is believed to constitute the sperm membrane. The middle piece and tail together constitute the sperm flagellum. The mitochondrial sheath is rich in phospholipid, most of it is bound to protein, some phospholipid is also present in other part of spermatozoa which appears as choline plasmologen. Other important components of the mitochondrial sheath are the complete cytochrome system and a number of enzymes viz. succinic, malic, isocitric, 6 phosphoglucose and lactic enzymes, oxidizing enzymes like sorbitol dehydrogenase,lactic dehydrogenase, ATPase etc.

Seminal Plasma

The extra cellular fluid, which provides the medium---vehicle for spermatozoa is a composite mixture- of secretions, which comes from the male accessory organs, of reproduction. The seminal plasma in higher mammals is proportionately more than the mass of spermatozoa. It is the secretion from epididymis, vasdeferens, ampullae, seminal vesicles, prostate, cowper's glands and urethral glands.

Composition of seminal plasma

The average specific gravity of seminal plasma is 1.03 that of sperm is 1.075 while semen has 1.035 dependent on sperm concentration. Osmotic pressure of the seminal plasma corresponds to that of blood plasma i.e. freezing point depression = 0.53°C. Freshly collected semen has a pH of 6.4 to 6.8 dependent upon the relative amount of partial secretions. Lower values are seen upon standing at room temperature (production of lactic acid under anaerobic metabolism). Higher pH values are found in semen having got contaminated with bacteria, (NH3 formation from deamination processes) in successively collected ejaculates, incomplete ejaculates (in bulls inadequately prepared, faulty collection technique), and in inflammatory conditions of
testis-epididymis. The seminal plasma has a poor buffering capacity. The composition of bovine seminal plasma is very complex and can be divided into 1) Inorganic substances 3) Nitrogenous bases 4) Proteins, enzymes, amino acids 5) Vitamins 6) Organic acids and 7) Lipids.

Inorganic Substances:
Sodium, potassium, calcium, magnesium, chloride, CO2, (hydrogen carbonate) phosphorus and sulphur mostly bond in organic compounds and Iron, copper, zinc mostly bond to enzymes. Seminal plasma has higher sodium than potassium while the opposite is true for spermatozoa. Exchange of Na+ and K+ between sperm and seminal plasma is an active transport across the cell membrane and is of importance for contractile mechanism of the sperm tail fibrils.

Carbohydrate:
Fructose is the major carbohydrate in bull semen (average 0.5%) and is formed from blood glucose. Two more polyols viz. inositol and sorbitol are found. The latter can be oxidised to fructose when sufficient oxygen is present inositol is possibly helping in maintaining the osmotic pressure.

Nitrogenous Bases:
Glycerophosphoryl choline is the most important and (0.4 to 0.5%), is not metabolized by sperm, but can be split by the enzyme present in the female genital tract (diesterase) into choline, and phosphoglycerol. The latter is metabolizable by sperm.

Protein, amino acids etc:
90% of N in seminal plasma is bound to protein. Only minute amounts are present as NH3, uric acid and urea. Total protein contents in bovine semen is 7% mainly as globulins. 18 amino acids have been demonstrated. Most of them in their free form. Glutamic acid constitutes more than 50%. Enzymes in semen are mainly bound to sperm. Those found in seminal plasma have leaked out from damaged or dead sperms. Proteolytic enzymes (e.g. Trypsin like) phospholipases, Transaminases e.g. Glutamic oxaloacetic transaminase (GOT used as test for sperm membrane damage) glycosidases (e.g. B. glucoronidase) a complete set of cytochromes, hexokinases and dehydrogenases (necessary for metabolizing fructose), a number of phosphatases (Acid and Alkaline). ATPase (Through splitting of adenosin triphosphate furnish direct energy for sperm movement) 5 nucleotidases (splits ribose 5 phosphates) DNAase (Possible functioning by splitting DNA in dead/degenerating sperm in vivo) and hyaluronidase (active at sperm penetration into the ovum by splitting hyaluronic acid) are examples, Catalase is not present in sperm or seminal plasma (used as a test for contamination with pus and bacteria). Mucoproteins (e.g. sialo-mucoprotein as such or split off sialic acid are of importance for adhesion of sperm to ovum at fertilization.

Organic acids:
Most important is lactic acid (as intermediary step in the anaerobic fructose breakdown). Acetate, Pyruvate, citrate succinate (the two former metabolizable by sperm) Prostaglandins (in some species possibly of importance for semen transport in female genital tract through their effect on myometrial contractions) are also present in bull semen.

Lipids:

Mainly phospholipids (above all plasmalogen and some lecithin) and small amounts of cholesterol, di and tn glycendes. Bull semen also contains androgens (Testosterone, dehydro androsterone) and minute amounts of estrogenic hormones.

The composition of seminal plasma is dependent on testosterone. Fructose and citric acid do not appear until puberty and disappear after castration but will reappear in castrated males after administration of testosterone (Fructose test and citric acid test have been utilized for studying androgen production by leydig cells).
# SEMEN CHARACTERISTICS OF DOMESTIC ANIMALS

*(From Roberts 1971)*

<table>
<thead>
<tr>
<th>Semen Constituents</th>
<th>Buffalo</th>
<th>Bull</th>
<th>Stallion</th>
<th>Ram/Goat</th>
<th>Boar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>2.5</td>
<td>4.0</td>
<td>70.0</td>
<td>1.0</td>
<td>250.0</td>
</tr>
<tr>
<td>(2-6)</td>
<td>(1-15)</td>
<td>(30 to 250)</td>
<td>(0.7 to 3.0)</td>
<td>(125 to 250)</td>
<td></td>
</tr>
<tr>
<td>Spermatozoan concentration</td>
<td>800</td>
<td>1200</td>
<td>120</td>
<td>3000</td>
<td>150</td>
</tr>
<tr>
<td>millions/ml.</td>
<td>(300 to 2200)</td>
<td>(300 to 2500)</td>
<td>(30 to 500)</td>
<td>(1000 to 6000)</td>
<td>(25 to 1000)</td>
</tr>
<tr>
<td>pH</td>
<td>8.6</td>
<td>8.6</td>
<td>7.4</td>
<td>6.6</td>
<td>7.4</td>
</tr>
<tr>
<td>(8.6 to 7.0)</td>
<td>(8.2 to 7.5)</td>
<td>(7.0 to 7.8)</td>
<td>(6.2 to 7.0)</td>
<td>(7.0 to 7.8)</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>450</td>
<td>530</td>
<td>2</td>
<td>250</td>
<td>13</td>
</tr>
<tr>
<td>Mg/100 ml.</td>
<td>(300 to 900)</td>
<td>(150 to 900)</td>
<td>(2 to 6)</td>
<td>-</td>
<td>(3 to 50)</td>
</tr>
<tr>
<td>Fructolytic Index</td>
<td>1.44 ± 0.11</td>
<td>1.99 ± 0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyceryl phosphoryl choline Mg/100 mol.</td>
<td>-</td>
<td>350</td>
<td>-</td>
<td>1650</td>
<td>-</td>
</tr>
<tr>
<td>(100 to 500)</td>
<td>(40 to 120)</td>
<td>(1100 to 2100)</td>
<td>(110 to 240)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>140</td>
<td>60</td>
<td>90</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>(80 to 210)</td>
<td>(50 to 140)</td>
<td>(80 to 380)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mg)/100 ml</td>
<td>230</td>
<td>70</td>
<td>190</td>
<td>650</td>
<td></td>
</tr>
<tr>
<td>(140 to 280)</td>
<td>(120 to 250)</td>
<td>(290 to 850)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inorganic Phosphorus</td>
<td>8.40 ± 0.50</td>
<td>5.90 ± 0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus total (mg)</td>
<td>103.20 ± 8.90</td>
<td>60</td>
<td>17</td>
<td>375</td>
<td>66</td>
</tr>
<tr>
<td>Organic phosphorus</td>
<td>68</td>
<td>59.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>489</td>
<td>700</td>
<td>25</td>
<td>140</td>
<td>80</td>
</tr>
<tr>
<td>mg/100 ml.</td>
<td>(300 to 1100)</td>
<td>(8 to 50)</td>
<td>(110 to 260)</td>
<td>(60 to 100)</td>
<td></td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>35</td>
<td>30</td>
<td>12</td>
<td>530</td>
</tr>
<tr>
<td>mg/100 ml.</td>
<td>(25 to 46)</td>
<td>(20 to 47)</td>
<td>(7 to 14)</td>
<td>(380 to 830)</td>
<td></td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
<td>40</td>
<td>92</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>(mg/100 ml.)</td>
<td>(10 to 140)</td>
<td>(20 to 40)</td>
<td>(76 to 120)</td>
<td>(8 to 18)</td>
<td></td>
</tr>
<tr>
<td>Ergothioneine</td>
<td>-</td>
<td>0</td>
<td>(40 to 110)</td>
<td>0</td>
<td>(8 to 23)</td>
</tr>
<tr>
<td>Ca</td>
<td>40.50 ± 2.10</td>
<td>44 (35-60)</td>
<td>20</td>
<td>11 (6-15)</td>
<td>5 (2-4)</td>
</tr>
<tr>
<td>Mag</td>
<td>-</td>
<td>9 (7-12)</td>
<td>3</td>
<td>8 (2-13)</td>
<td>11 (5-14)</td>
</tr>
<tr>
<td>Chloride</td>
<td>369</td>
<td>180</td>
<td>270</td>
<td>86</td>
<td>330</td>
</tr>
<tr>
<td>(110-290)</td>
<td>(90 to 450)</td>
<td>-</td>
<td>(260-430)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein g/100 ml.</td>
<td>-</td>
<td>6.8</td>
<td>1.0</td>
<td>5.0</td>
<td>3.7</td>
</tr>
</tbody>
</table>

All the figures except otherwise mentioned are in mg / 100ml.

Protein, amino acids etc:
90% of N in seminal plasma is bound to protein. Only minute amounts are present as NH₃, uric acid and urea. Total protein contents in bovine semen is 7% mainly as globulins. 18 amino acids have been demonstrated. Most of them in their free form. Glutamic acid constitutes more than 50%. Enzymes in semen are mainly bound to sperm. Those found in seminal plasma have leaked out from damaged or dead sperms. Proteolytic enzymes (e.g. Trypsin like) phospholipases, Transaminases e.g. Glutamic oxaloacetic transaminase (GOT used as test for sperm membrane damage) glycosidases (e.g. B. glucoronidase) a complete set of cytochromes, hexokinases and dehydrogenases (necessary for metabolizing fructose), a number of phosphatases (Acid and Alkaline). ATPase (Through splitting of adenosin triphosphate furnish direct energy for sperm movement) 5 nucleotidases (splits ribose 5 phosphates) DNAase (Possible functioning by splitting DNA in dead/degenerating sperm in vivo) and hyaluronidase (active at sperm penetration into the ovum by splitting hyaluronic acid) are examples, Catalase is not present in sperm or seminal plasma (used as a test for contamination with pus and bacteria). Mucoproteins (e.g. sialo-mucoprotein as such or split off sialic acid are of importance for adhesion of sperm to ovum at fertilization.

Vitamins:

Only water soluble vitamins are found in semen. Vitamin B complex (riboflavin, the major component responsible for yellow semen) and ascorbic acid (partly responsible for reducing properties) are the main vitamins components.

Organic acids:

Most important is lactic acid (as intermediary step in the anaerobic fructose breakdown). Acetate, Pyruvate, citrate succinate (the two former metabolizable by sperm) Prostaglandins (in some species possibly of importance for semen transport in female genital tract through their effect on myometrial contractions) are also present in bull semen.

Lipids:

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**COLLECTION OF SEMEN**

One of the most important aspects of artificial insemination is the proper and clean collection of semen. This involves the proper scheduling of bulls and sexual preparation as well as techniques of semen collection. Frequency of semen collection should be determined depending upon the age and body size of the bulls. Very young bulls to start with should be collected only once in fortnight. Slightly older bulls
between 2 to 3.5 years) of age should be collected once in a week or even twice a week if they maintain the quality of semen and the bulls above 3.5 years until 7 years should be collected only once in a week. This schedule is applicable to exotic or cross-bred bulls but tropical breeds have late maturity and therefore, the bulls and buffalo bulls donate semen around 36 months some of the bulls of dairy breeds. Preparation of bulls for collection consists of washing and grooming of bulls, which may form the source of conditioned reflexes for collection. Tying of aprons to the bulls (the apron is 65 x 62 cm. with semicircular cut at one end by leaving 12 cm. on either side and fixing slightly heavy 2 to 3 metal pieces on opposite side to keep it straight and to avoid folding of apron). The apron cloth should be of coated rexin. The rexin side should face the penis of bulls. The apron should have two metallic rings on each corner fixed up and rubber strap going round the heart girth of a bull. The apron should be fixed behind the elbows and should hang so much so that it does not touch ground while standing. The tuft of hair in the preputial opening should not be clipped and should be allowed to grow since this is the natural barrier which will prevent entry of foreign particles in prepuce. The clipping of hair will cause irritation and stimulation for glans penis resulting in habitual masturbation. The bull is allowed to mount the teaser with the apron on so that it prevents penis coming in contact with the hind portion of the teaser thereby preventing contamination. The bull should work on the teaser and then slowly dismount. Two to three false mounts in 10 minutes improves the ejaculate volume and number of sperm per ejaculate.

Advantages of using bull apron

* It prevents cross infection from bull to bull, which otherwise may take place through contact of penis to the rump of the teaser.

* prevents losses of semen ejaculates which happen in certain bulls by tactile stimulation because of the penis rubbing against the skin of the rump of teaser and bulls throwing semen outside before penis is directed in A.V.

* The Apron prevents the direct contact with the skin of teaser and thereby more hygienic ejaculates can be collected.

Various methods of collection of semen have been devised from time to time. The older unsatisfactory methods have been gradually replaced by the newer modern techniques. The three most common methods are: (i) use of an artificial vagina, (ii) by electro-stimulation technique, and (iii) by massaging the ampulae of the ductus difference through rectal wall. Suitability of a particular method depends on the type and condition of a species, e.g. artificial vagina method is applicable to almost all of our domestic animals but certainly not on poultry, where the massage method is the only practical solution to get ejaculation.

The ideal method of semen collection will be one that is safe for the sire and the semen collector, the semen obtained is really a representative of a normal ejaculate, free of contamination and lastly it is protected from thermal shock.
Artificial vagina technique:

The best method of semen collection is with artificial vagina. Walton (1945) preferred short artificial vagina. There are three universal sizes of artificial vagina 25, 30, and 40 cm. The artificial vagina is a rubber cylinder with a water inlet and air inlet valves. The rubber cylinder has rims on either side combined water and air inlet is provided about 8 cms. from one end. The rubber lining either of latex rubber or PVC is mounted on the cylinder and is fixed with the help of rubber strap. Latex rubber cone is mounted on the cylinder to the end where water and air inlets are closer. The glass semen collection tube is fixed to the conical end of rubber cone and a insulation bag is mounted on glass tube and rubber cone and fixed on the rubber cylinder with strap. The water of 55°C is filled in through the inlet valve. The AV is then shaken and the water is discarded (pre-warming). The water of correct temperature 48°C is filled in. The A.V. is now ready for semen collection. No lubricant should be applied to AV.

The air should be pumped to give a star like appearance. At BAIF two types of AVs are used one smaller 25 cm and longer 30 cm. Smaller AVS are used for young bulls and buffalo bulls while longer AVs are used for adult cattle bulls. Normally the AV's final temperature is kept at 48°C with little variation in summer and winter. In winter the temperature is raised to 49°C while in summer it is reduced to 47°C. Some bulls always desire to have higher temperature. Two types of rubber linings are used. With old bulls not donating semen in smooth rubber lining rough linings are used. Troublesome bulls refusing to donate semen do donate semen easily in artificial vagina with rough lining. In young bulls usually smooth rubber lining AVs should be used. Polyethylene linings and cones for AV proved to be the most useful from the point of view of quality of semen. Since the semen comes out of reproductive tract of male at certain specific temperature (i.e. 35°C) the collection tube should be kept at 35°C so that freshly ejaculated spermatozoa should not be put to thermal shock. This can be affected by providing a small plastic jacket for collection tube containing warm water at 35°C or felt cap. As soon as the semen is collected it should be then placed in a water bath of 35°C. The collection tubes are kept warm at 35°C by use of warming table and the felt cap is mounted on the tube to retain the warmth of tube. The insulation bag is also mounted on the cone and tube. On an average 50 mm Hg pressure is given in AV, using cycle pump for pumping air in AV. Some bulls like to have more pressure. The pressure ranges from 45 to 55 mm. Hg. The method of collection The semen collector should have a blue greenish or green overall on himself. He should wear gum boot with uncrushable toes. He should also wear hand gloves. He should hold the artificial vagina with water and air inlets pointing to the ground in a inclined position of 45°. When the bull mounts the teaser, he should push the bull with his shoulder catch hold of sheath and allow the protruded penis to search the A.V. As soon as the penis touches the warm surface of A.V. he will give a strong thrust by virtually lifting his hind legs and bringing his hind portion in opposition to the teasers hind portion and ejaculates. The A.V. should not be removed immediately but the bull should be allowed to dismount and then the A.V. should be removed. Immediately the A.V. should be held horizontally and not vertically to avoid the contamination of semen by preputial exudates and flora. The AV should not be
carried inside the lab, but only semen should be delivered in and A.V. should go for washing. Some scientists like small A.Vs to be used (so that ejaculates can be directly had in rubber cone). It avoids:

* Warm surface of A.V. and thus thermal shock

* Preputial contamination from A.V. - generally small young bulls should be collected in small A.Vs while large bulls should be collected in bigger size A.V. The bulls prefer thick lining and pressure. The thin smooth linings do not last long on giving pressure and on sterilizations and they give way on thrust by the bull. Thus the bull gets his penis burnt by warm water in addition to this the semen gets spoiled and the bull gets shy for mounting second time. A.V. method of semen collection holds good for bucks, Rams, Boars and Stallions. The size of A.V. differs from species to species with little modification in construction of A.V. e.g. Bucks require A.V. of small size 20 cm., the diameter also is small, linings and cones also are short to the size of A.V. The A.V. for stallions is 54 cm. long and diameter is 13 cm. The diameter is reduced at distal end and is only 8.5 cm. To distal end rubber cone and semen collection bottle is fixed. In Denmark and other Scandinavian countries condom has received a support. For Boars, short A.V. which is used for goat can be used with intermittent air pumping to supply full pressure simulating the locking device. Only cone holding the protruded penis with fingers will also allow the boars to ejaculate since the finger can act as screw type locking device. It has been proved to be the best method and is now most commonly used. There are different kinds of artificial vagina for different classes of animals. The one for cattle and buffaloes consists of (i) an outer heavy rubber cylinder, (ii) inner sleeves of rubber, (ii) the semen receiving cone, (iii) semen collecting vial made of glass or plastic which may be graduated. Prior to collection all these parts are cleaned, sterilized and assembled into artificial vagina. The inner sleeve is put into the outer cylinder and both the ends of inner sleeves are reflected over the cylinder forming a watertight space between them and water at 40 –50°C is filled in there. The cone along with the attached vial is then slipped over one of the ends of this water jacketed barrel then tightly secured with the help of rubber bands. The vial and cone are then invaginated into the artificial vagina and 3" to 4" at the open and are lubricated with sterilized jelly. The temperature of the water to be taken depends upon the liking of the bull, and season of the year. The modern artificial vagina types are also provided with an air screw along with the water screw which can be used for blowing the air between the two layers to create the desired pressure. The temperature of the artificial vagina is more important and should always be checked before making the collection. Too high a temperature may cause injury to the penis and the bull may refuse to serve in future. On the other hand at lower temperature, there may not be complete ejaculation and the whole ejaculate may be contaminated with urine. Prior to collection, bulls are usually allowed to become excited by bringing the bull to cows or other dummy when it will smell and also will make attempts to ride. During collection, the artificial vagina thus assembled, is held at an angle of 55 degree with the horizontal plane of the cow or dummy. It is so because the penis of the bull enters
the vagina of the cow at that angle. When the bull mounts, the penis is quickly guided into the artificial vagina with the operator's hand. Care is taken not to touch the exposed part of the penis, as this stimulates the bull to ejaculate. After the bull dismounts, the artificial vagina is taken off the penis and kept in an upright condition after releasing the pressure by draining water and air out of it. This allows the ejaculate to flow from the cone of the vial which is then detached from the cone and taken to the laboratory for examination. The size of the artificial vagina to be used depends upon the development of the penis. The artificial vagina should be long enough for the ejaculate to be deposited near the mouth of the collection tube in order to reduce sperm cell losses from cells remaining on the collection funnel and to prevent injury to the penis by entry into the collection tube. Generally mature bulls require an outer rubber casing 40 cm in length and 6.4 cm in diameter. Yearling bulls required a casing 30 -35 cm in length and 5.1 -5.7 cm in diameter. In case of goat and sheep the outer tube should be about 20 cm long and 5 cm in diameter.

**Advantages of Artificial Semen Collection through artificial vagina method**

The artificial vagina method of semen collection has following advantages.

1. Practically the whole ejaculate is collected in uncontaminated and natural stage.
2. It is free from the extraneous secretions.
3. Sterile conditions of the apparatus ensure disease control.
4. The viability of the sperm is better.
5. No female is needed if dummy is a success.

**Disadvantages**

The technique of artificial vagina for semen collection has the following disadvantages. Occasionally it is difficult to get the males to serve the artificial vagina.

**Electro – Ejaculation**

This method is preferred in males that refuse to donate semen in artificial vagina or when injuries or infirmities make this impossible. This method of semen collection is justified in cases where the cause of inability to mount is non-genetic. The electro-ejaculator is run on 110 volts as well as 12 volts dry cell. The new models are transistorized and portable. For bulls the rectal probe is either ring or straight electrodes. The bull has to be backtracked before this is applied since feces in the rectum prevent proper stimulation. The erection is usually present with electrode method and semen can be collected with contamination from prepuce. Secretion from accessory sex glands takes place at lower voltage level and ejaculation at higher voltages. By this method of ejaculation of semen is brought about by inserting a probe or electrode in sires rectum and stimulating nerves of the reproductive system by gradually increasing voltage in rhythmic fashion with a rheostat for a short period. Successful use requires skill, experience, patience and the knowledge of individual requirement of the stimulation. At present, there are a number of electro-ejaculators in the market of the world which are either operated only on battery or battery cum-electric transistorized circuits. The method is used on males of certain species where
the use of artificial vagina is not possible or not practical. The widest use of electro-ejaculators has been observed in obtaining semen samples from large number of bulls and rams during examination for breeding soundness. The method is also used to collect semen from bulls for artificial insemination when the bull is extremely slow in serving the artificial vagina or physically incapable of mounting; the method of semen collection in bull is as follows

1. At the beginning, the rectum is washed with 6% sodium chloride solution.

2. The probe is then inserted up to about 12 inches and held in a position of rectal floor.

3. Alternate current increasing in voltage gradually from 0 to 5 volts and returning again to zero within every 5-10 seconds is initially passed.

4. The subsequent stimulations made progressively higher so that at about 5th stimulus a maximum of 10 -15 volts is reached. Erection and ejaculation occur at 10 -15 volts when 0.5 to 1 ampere current is flown. The home consumption of electrical current in India is between 200 -250 volts. The voltage is reduced by a step down transformer between 10 -15 volts and low voltage current can further be made as desired by the operator. This is done by varying the resistance without the circuit.

**Advantages**
The advantages of this method are as follows.

1. Semen can be collected from males that are too young or old or unable to mount due to weak or injured legs.

2. No female or dummy is required for collecting the semen.

3. Less chance of contamination.

**Disadvantages**
The important disadvantages of this method are as follows.

1. The method is highly technical and needs considerable skill and practice.

2. The semen generally gets contaminated with urine.

3. Some males resist too much to this method and refuse collection.

4. Sciatic nerves are temporarily affected during the operation but is relatively minor if the electrodes are kept over the ampullar region.

**Massage Method**
This method involves the simplest technique of semen collection by massaging the seminal vesicles and ampulæ. Undoubtedly the collectors have a considerable
training to adopt the skill. This method is commonly used to collect semen from cock, turkey and dog.

1) Is AI For You?
If you have a few backyard does that you enjoy as a hobby, with little concern for genetic improvements of their offspring, then artificial insemination (AI) is probably not for you, assuming a suitable buck can be located for servicing the does. The expense of purchasing the necessary equipment and learning to do AI are likely not worthwhile. However, if there is an experienced inseminator in the area who is willing to work with your goats, then this may prove to be a viable alternative and certainly is much simpler than hauling your does in heat to the buck's home.

2) AI has some key advantages over natural breeding.

- 1) It eliminates the necessity of keeping one or several bucks on the farm (depending on herd size). Costs of feeding, housing, separate fencing and labor are eliminated. However, heat detection may be more difficult in the absence of a buck.

- 2) AI can increase the rate of genetic improvement in an herd, as long as superior bucks are consistently selected. In natural service, the prospective breeder has only the buck's pedigree to rely on, whereas AI bucks should be progeny tested for their transmitting ability of milk and fat percentage, weight gain, type conformation, etc.

- 3) AI allows breeding of different portions of the herd to different bucks. Young does may be bred to not yet proven but high potential bucks, while the majority of the herd can be bred to proven high quality bucks.

- 4) AI permits breeding of many does on one day when synchronization is practiced. No long drives to top bucks are involved.

- 5) The danger of transmission of diseases or parasites is greatly reduced. (The transmission of diseases through frozen semen needs further study.)

- 6) The time of breeding can be more carefully regulated, and the owner knows exactly when the doe was bred, as opposed to pasture servicing by a buck that is allowed to run with the herd.

- 7) AI induces good recordkeeping of dates of heat, breeding, pedigrees, etc. This will aid in herd improvements and enable the owner to make better culling decisions.

3) Once the decision to use AI has been made, the next step is to determine whether to do the inseminating yourself or pay someone else to do it. If there are only a few does in your herd, and an experienced inseminator of goats is available, then it may be more practical to pay to have the service done. However, if the number of does in the
herd is rather large, or an experienced inseminator is nowhere to be found, then its probably time to learn how to practice AI techniques yourself.

4) AI technicians of the cattle industry may not necessarily be of much help when it comes to inseminating goats, for the modern method of inseminating cattle (rectal palpation) differs from that of breeding goats (speculum method) considerably. The speculum was used on cattle early in AI history, and some cattle inseminators may be capable of teaching goat insemination.

5) The cost of getting started in AI, not including semen purchases, will generally run around $500, of which $400 to $450 is tied up in the liquid nitrogen tank, which is necessary for storing semen any length of time. Temperatures must be kept at -320F (-196C) for sperm survival to be maximized at breeding time. It may be possible to share the cost of the tank with neighboring goat owners or dairy farmers, thus alleviating some initial costs of an AI program.

6) If AI is to be used with any hope of achieving a good level of success must be known and well understood by the prospective inseminator.

1) basic knowledge of the doe's reproductive organs and their functions;

2) understanding of storage and handling of semen;

3) ability to use, in a proper and sanitary manner, the equipment required for inseminating goats;

4) ability to accurately detect heat at an early stage;

5) necessity of keeping accurate, up to date records of heat cycles, breeding, kidding, reproductive problems, treatments, and any other pertinent information that may reflect on the goat's reproductive patterns.

7) Reproductive Organs and Functions

The two ovaries are the sites of egg formation. They produce estrogens and progesterone, and as such are determining factors of heat cycle, ovulation and pregnancy. Basically the estrus (heat) cycle in goats operates as follows:

- 1) Proestrus is the time of follicle growth. As an egg (ovum) begins to mature in an ovary, it becomes surrounded by a fluid filled sac on the outside of the ovary, much like a blister forms on the skin. This growth is accompanied by increasing levels of estrogen in the blood.

- 2) Estrus - As estrogen levels peak, the doe will come into heat. This can be observed by changes in behavior (increased bleating and restlessness), willingness to be bred, and the swelling of the external genital area. The period of "standing heat" (acceptance of the buck) will generally last for 24 to 36 hours.
3) Ovulation, or the release of the egg, is accomplished by the rupturing of the follicle, expelling the egg from the ovary, and receiving it into the oviduct via the fimbria funnel. This occurs very near, or soon after, the end of standing heat (6 hours before to 12 hours after). Egg life is 12 to 24 hours, while the sperm lasts 24 to 48 hours.

4) Metaestrus - in this stage, the ruptured follicle is undergoing cellular differentiation to form a functionally important tissue mass, the corpus luteum (yellow body). This structure is responsible for the secretion of progesterone, a hormone which prevents the development of another follicle and prepares the uterus to receive a fertilized egg.

5) Diestrus - is the longest period of the estrous cycle in does. During this period of corpus luteum influence, two events may happen:

   o a) if fertilization of the egg occurred, the corpus luteum will persist for the entire gestation period, preventing follicular development and keeping estrogen levels low.

   o b) if no fertilization took place, the progesterone secretions of the corpus luteum gradually lessen, allowing a new cycle of follicular development to begin, with a corresponding increase in estrogen levels. The length of time required for one estrous cycle without fertilization, ranges from 17 to 24 days in goats, with the majority taking 21 days. Shorter cycles are not uncommon (5-10 days).

8 The egg, after being expelled from the ovary, passes into the oviduct via the infundibulum, and toward the cornua (horns) of the uterus. This movement is produced by wave-like motions of the ciliated (hair-like projections) cells of the oviduct. Sperm and eggs meet in the oviduct and fertilization occurs in the middle to upper one third of the duct.

9 The egg continues into the horn of the uterus, where, if it has been fertilized and undergone several cellular divisions, it will become attached to the uterine wall. If no fertilization has occurred, the egg will degenerate and the cycle goes on.

10 The cervix of the uterus plays a key role in artificial insemination, as it is the external entrance to the uterus which must be located and penetrated with the inseminating instrument. The cervix is normally tightly closed, except during periods of heat or kidding. Semen is deposited on the vaginal side of the cervix in natural services, but AI requires the deposition of semen in the uterine side of the cervix. This is because of the greatly reduced volume of semen that is used in AI. If the 0.5 to 1 cc of semen in AI were deposited on the vaginal side of the cervix, there is a good chance that none of the sperm would reach the egg.

11) The vagina serves as the connecting tube between the uterus and the outside opening, the vulva. It is part of the birth canal, and also contains the urethral opening, from which urine will pass during emptying of the bladder.
12) Purchase and Preparation of Semen

In most cases, the inseminator will acquire the semen needed by direct purchase from a commercial operation, in which case it will be shipped to the inseminator. It is of the greatest importance that the semen be transferred to permanent storage (the liquid nitrogen tank) without exposing it to anything approaching air temperature. Generally, this means transferring the container element which houses the semen directly to the liquid nitrogen tank. Here it can be safely stored for long periods of time, since biological activity practically stops at liquid nitrogen temperatures (-320F). Semen is generally to be used within 6 months, but conceptions have resulted from semen stored for several years, although sperm survival is decreased, resulting in lower conception rates.

13) Semen Collection

Bucks are handled basically the same way as bulls for semen collection. Three basic methods may be employed, but all three require an artificial vagina, a double walled device with an opening at one end and collection tube at the other. The inner lining holding warm water should be coated with a light application of water soluble lubricating jelly. The three methods are:

- 1) A buck may be allowed to mount a doe, with the semen collector manually diverting the buck's penis into the artificial vagina (ram or dog size). Don't touch the penis directly, instead direct the penis into the artificial vagina by grasping the buck's sheath. After ejaculation (usually 0.5 to 1.0 cc) has occurred, remove the artificial vagina and tip it so that the semen will all run into the collection tube. This method may require practice and adjustment by both the buck and the collector before good samples are collected.

- 2) A buck is trained to mount a dummy instead of a live doe. The same procedures are followed for sample collection. Mounting may be facilitated by applying vaginal mucus scrapings of a doe that is in heat to the dummy, at least during the training process.

- 3) Use of electro-ejaculation. The buck is not required to mount an object, although an artificial vagina should still be used for semen collection. An electrode unit, which has a number of contact rings, is inserted into the buck's rectum. Slight electric stimulation brings on ejaculation. This technique generally results in good samples in quantity and quality. However, the sperm concentration of the sample will be lower. This method does not require extensive training, and will allow collections from bucks that may refuse or are unable to mount and serve an artificial vagina.

14) Semen, once collected, may be used in one of three different ways:

- 1) As liquid semen, directly or on the same day one ejaculate can serve 3 to 5 does. If kept at body temperature, the semen may be good for three hours.
• 2) Semen may be stored 24 to 48 hours by placing the collection tube in a container of water and putting this unit in a refrigerator. No diluter is needed, although plain egg yolk can serve as simple extender to double the number of does that can be served.

• 3) Semen that is to be stored for longer periods of time must be mixed with a diluter and very carefully frozen. A commercially prepared diluter extender, such as Ortho Semen Diluter is desirable, although plain milk can be used successfully also. Following are steps in semen extending:
  
  o a) with a commercial preparation, use a diluter to semen ratio of 19:1, adding the semen to the diluter, and rolling the bottle gently to achieve a thorough mixing. The semen and diluter should be at the same temperature. This mixture can be stored in the refrigerator and used for a week, or slowly cooled and stepwise frozen for storage in a liquid nitrogen tank for later insemination.

  o b) for a homemade milk diluter, it is best to use fresh 3.5 pasteurized, homogenized whole milk. It must be heated and held at 210°F for 10 minutes in a glass boiler, keep the lid in place so that no moisture is lost. Next, the milk is cooled in a water bath with the lid on. When the milk is in equilibrium temperature with the water bath, the water condensation on the inside of the lid is shaken back into the milk. To every 400 cc of milk, add 100,000 units of potassium G crystalline penicillin and 500 mg crystalline di-hydrostreptomycin sulfate, mixing well. Warm this diluter to about body temperature before adding the fresh semen at 19:1 ratio. Place the diluted semen in a water bath at body temperature of 10°F and allow to cool slowly. Semen may be frozen, if the extender contains an antifreeze compound, slowly, stepwise for storage on dry ice or in liquid nitrogen.

15) A microscope, capable of 900x magnification is an essential tool when doing your own semen collection in order to determine semen quantity and quality. First, place a semen sample on a clean slide and cover with a coverslip or another slide. Set the magnification to 400x and observe the appearance of dark patches or spots thru the scope; four dark areas or more per microscope field represent high concentrations of sperm, a really good sample. Three dark areas is somewhat chancy for use at a diluted service, but is good enough for natural service. Two dark areas should be used only for natural services and one dark area means that the concentration of sperm is too low for even natural service.

16) Switching to 900x, the sperm cells can be individually observed for normal structures. Diluting in warm saline is helpful. Coiled tails, broken tails, absence of tails and abnormal shapes all constitute deficient sperm cells. Sixty to 70 2.256835e+199ood motility before freezin should be observed in a good sample, with a minimum of 30motility after freezing and thawing. Any insemination program, no
matter how carefully carried out, will yield poor results if the concentration and quality of the collected sperm is not of high standards. Sophisticated techniques of washing the sperm free of seminal plasma before extending and freezing will improve post-thaw viability.

17) The concentration of a buck semen ejaculate can be determined accurately by using a red blood cell diluting pipette and standard hemocytometer techniques. Typical results during the breeding season are 3 to 5 billion sperm per cc. Optical density can also be used to estimate sperm concentration if the photometer has been calibrated for buck semen. A simpler technique involves the determination of a spermatocrit using microhematocrit pipettes. The aliquot of semen is centrifuged for 10 minutes; for each percentage point of packed sperm, approximately 200 million sperm cells per cc are present. Correction is made for the percent motile sperm, after which the ejaculate can be diluted appropriately to supply a minimum of 125 million motile sperm in each breeding dose. It is often difficult to introduce more than 0.2 ml of semen into the cervix, so dilution to a final concentration of 600 million to 1.2 billion live sperm per cc has been recommended. When no laboratory support is available, fresh semen for immediate use may be diluted up to 5 times in extender if it is yellowish and 10 times if the ejaculate is white. A straw holding 0.5 cc of this diluted semen will provide adequate sperm if excessive reflux does not occur.

18) Storage and Removal of Semen from the Liquid Nitrogen Tank
A liquid nitrogen tank is basically a very large thermos-bottle in which liquid nitrogen is placed to keep the inner temperature near -320F (-196C). The spacing between the inner and outer walls is insulated and under vacuum. The temperature in the tank is maintained uniformly at -320F up to the bottom of the tank neck until the liquid nitrogen level gets down to around 5". To measure liquid nitrogen, use a piece of black metal rod that is long enough to hold and touch the bottom of the tank. Dip the rod to the tank bottom and remove after 30 seconds. By waving it in the air, a white frost line will appear on the rod. This line indicates the liquid nitrogen depth of the tank. Levels nearing 5" require a refill. The only real differences between tanks is their storage capacity (number of ampules or straws that they will hold) and their length of holding time (liquid nitrogen evaporation rate). The neck diameter varies somewhat also, with wider openings being easier to work with, but an increased evaporation rate usually results.

19) When working with semen in the liquid nitrogen tank, it is important to keep the racks below the frost line in the neck of the tank. Removal of semen from the tank for periods as brief as 10 seconds, such as for identification, before replacing it to the tank will often result in lowered fertility levels. If the right rack can't be located in 5 seconds, lower the canister back to the bottom of the tank for at least 30 seconds before trying again. Also, when handling semen, try to stay out of any direct sunlight, as ultraviolet light has a spermicidal effect.

20) The semen comes in two basic types of packaging: ampules (1 ml) and straws (0.5 or 0.25 ml). The ampule is the most common type of packaging for buck sperm. Both
ampules and straws are stored in racks (canes), which are aluminum pieces that hold a vertical row of ampules, usually six, or two g ++++MISSING DATA++++

21) A few key reminders concerning semen storage:

- 1) Always keep the liquid nitrogen level above 5".
- 2) Never lift a canister above the frost line of the tank.
- 3) When the semen is removed with a forceps from the tank it should be placed immediately in the thaw box.
- 4) Never expose semen to direct ultraviolet light.
- 5) Never refreeze semen that has been thawed as it will be destroyed.
- 6) Check for proper identification on ampule or straw.
- 7) A defective ampule may blow up after it is removed from the tank. This is due to a small leak that allows nitrogen to enter the ampule. When removed from the tank, the gas expands too rapidly to vent back out the hole and it explodes the glass. A hissing sound is usually audible when it is removed. Keep your hand between the ampule and your face when putting it into thaw box.
- 8) Always wear gloves and goggles for your own protection when working inside a liquid nitrogen tank.

22) Thawing Procedures

Methods for semen thawing vary among manufacturers, and it is best to follow their recommendation. The thawing procedure for 1cc ampules, the most common for goat semen, is generally the ice water bath:

- 1) Ice water (38-42F) is placed in a styrofoam box long enough before-hand to allow temperature to equilibrate.
- 2) Remove the ampule from tank and place immediately into thaw box. Ampule may be placed in a small plastic cup with holes in the bottom. This prevents ice from coming into direct contact with ampule.
- 3) Ampule should thaw in 3 to 5 minutes. Check for slushiness and allow more time if needed.
- 4) Ampule may sit in ice water for as long as 30 minutes with no damage. Once removed, the semen must be used right away.
- 5) The layer of ice on the ampule must be peeled off before opening to avoid possible contamination.
23) The ice water thaw method is especially good during winter breeding of does because of low risk of cold shock to thawed and exposed semen. Thawing of semen can be done from -320F rapidly, but any subsequent exposure to lower temperatures after thawing will kill many or all of the sperm.

24) The warm water method of thawing is more exact than the ice water method, but probably will not work in cold weather, although it may give somewhat better results the rest of the year. The procedure is basically the same as for the ice water thaw except that:

- 1) The water must be maintained at 92 to 98F. This requires a source of warm water and an accurate thermometer.
- 2) Thawing will be complete in about 1 minute with no ice layer formation of the ampule.
- 3) Ampules thawed with the warm water method should be used within 5 minutes.

25) Straws (0.5 or 0.25 ml) can be thawed by either of the previous two methods. A given amount of semen in a straw will take about one half as long to thaw as an equal amount in an ampule. Many inseminators simply thaw straws by placing them into their shirt or pants pocket.

26) Inseminating Procedures
All the care in handling, storage and preparation of semen will be useless if the inseminating process is not done carefully and cleanly. Hygienic practices at this point cannot be over-emphasized. All reusable items such as inseminating guns (for straws), scissors for cutting straws, scribe for cutting ampules, etc. must be wiped clean with 70% isopropyl alcohol and allowed to dry before reuse. Disposable items should be kept in their sealed packages until they are to be used. The speculum should be sterilized after each use (this is one reason why the cattle industry discontinued the speculum method; the inseminator would have to carry a few dozen specula on his daily rounds, sterilizing them each night). This is best accomplished by boiling for 10 minutes, allowing to air dry. Then place inside a sterile container or wrapping, such as a new plastic AI glove. Disposable plastic type specula for goats can be obtained from mail order companies, eliminating the need for constant resterilization.

27) Materials needed for artificial insemination:

- 1) Speculum, Pyrex 22 x 175 mm for doelings; 25 x 200 mm for adult does; or stainless steel human vaginal speculum; or plastic disposables; with a small clip-on flashlight.
- 2) Sterile lubricating jelly (K-Y)
- 3) Thaw box
4) a. Inseminating pipette with bulb or syringe (ampules only) or b. Inseminating gun (straws only)

5) Paper towels

6) Facility for securing doe (stanchion, fence, rope hoist)

7) Recording journal for breeding dates, buck's name, etc.

28) Preparing Ampules:

1) Partially remove an inseminating pipette from its plastic bag.

2) Place bulb or syringe on exposed end.

3) Thaw ampule according to the described methods.

4) Dry ampule after thawing, hold in paper towel and scribe (with proper tool) one side of ampule collar. Some ampule types do not need to be scribed, but can be snapped open.

5) Pull syringe back 1/2 cc on plunger or squeeze bulb closed before placing pipette into ampule.

6) Tip ampule to slight angle and maintain constant suction on pipette while it is slowly inserted into the ampule. Try to get all the semen into the pipette, keeping the semen column down near the end of the pipette.

7) When filled, the pipette should have a semen column with no air spaces, with the bottom of the column being 1 to 2" from the pipette tip. Do not draw semen into the syringe or bulb.

8) Keep the ampule for information to complete breeding records.

9) Keep the pipette away from sunlight or cover with paper towels.

10) The semen is now ready to be placed into the doe in estrus.

29) Preparing Straws:

1) An inseminating gun, designed for your type of straw is needed, obtainable thru farm supply houses or the local cattle AI technician. Have cover sheath available, sealed until needed.

2) Place straw in thaw box.

3) Remove when thawed, wipe dry. Check buck information.

4) Pull plunger on gun back 4 to 6" and insert straw into gun, cotton plug end first (towards plunger).

5) Hold gun in upright position, allowing air bubble to rise to the sealed end.
6) Cut sealed end of straw with scissors. Take care to cut straw squarely for proper seating.

7) Install the sheath over the gun, fastening it down with the provided O-ring. Install it so that the wider side of the ring faces the straw, with the narrower side facing the syringe end.

**30) Insemination:** Assuming that the doe has been observed in heat, has been suitably restrained (i.e. in stanchion) and the steps for preparing the ampule or straw have been followed. The next steps are:

1) Position doe on milk stand. The inseminator places his left foot on the stand and drapes the hindquarters of the goat across his horizontally positioned thigh. The goat is allowed to stand as long as she does not struggle or collapse. The vulva is cleaned.

2) Hold pipette or inseminating gun, wrapped in a paper towel, in your mouth; or let someone else hold it if extra hands are available.

3) Turn head light on and insert lubricated speculum in a slow and gentle manner. Begin entrance at a somewhat upward angle for the first several inches. This is to prevent the speculum from scraping across the vaginal floor, possibly doing damage to the urethral opening.

4) Complete insertion of speculum and locate cervix. Center the end of the speculum over the os uteri (entrance to cervical canal).

5) Cervix should be of a red-purple coloration with a viscous whitish mucus present if doe is truly in heat.

6) Insert pipette or inseminating gun into speculum to the cervix. Gently manipulate the instrument through the cervical canal (cervix is 1 to 2" long) to the 4th or 5th annular ring.

7) Deposit semen near the uterine end of the cervix or just inside the uterus. Do not enter too far into the uterus as the semen will then tend to be dumped into one horn or the other. If the semen is pushed into the wrong horn (i.e. egg produced in left ovary, semen dumped into right horn) then fertilization may not occur.

8) Deposit semen slowly, taking at least five seconds.

9) Slowly withdraw instrument without release of syringe or depressed bulb, then carefully remove the speculum.

10) Record all pertinent breeding information.

11) Carefully discard all disposable materials. Arrange to sanitize reuseable items and sterilize the speculum (if it is a non-disposable type).
31) Frequently, the pipette cannot be passed all the way through the cervix even though the doe is in heat. If it has penetrated deeply into the cervix (3 to 4 cm, as determined by laying another pipette alongside the first and observing the distance by which the outer ends are offset), cervical insemination will provide a conception rate almost equal to that of intrauterine semen deposition. The conception rate expected from intra-vaginal insemination, however, is less than 30. If semen is very valuable, it may be advisable to pass a trial pipette to determine patency of the cervix before thawing the semen unit.

32) In France, a doe is usually restrained by a second person who straddles the doe's neck and elevates the hindquarters to a vertical position while holding the hind limbs tightly flexed. The inseminator is free to stand in a comfortable position. He holds the speculum and the goat's tail in one hand and the pipette or straw gun in the other hand. If excess mucus is a problem, the assistant lowers the goat's hindquarters almost to the ground; if the mucus does not run out of the speculum, the latter is removed and shaken to clear it. The goat is then lifted to its former position. If many goats are to be bred, the assistant may tire using this technique. If the doe is not held in a vertical position, it is often impossible to adequately visualize and penetrate the cervix. Various slings have been devised to suspend the goat in the appropriate position.