Applied Veterinary Andrology and Artificial Insemination.

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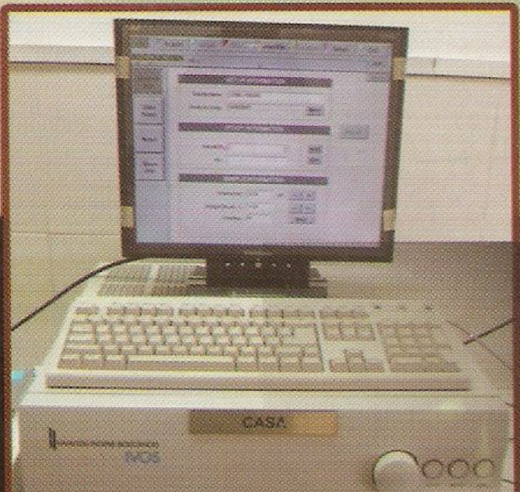
APPLIED

VETERINARY ANDROLOGY AND ARTIFICIAL INSEMINATION

Pradeep Kumar P.S. Yadav
R.K. Sethi



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Applied

Veterinary Andrology and Artificial Insemination

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Preface

The purpose of this book is to compile the information concerned to ‘Veterinary Andrology and Artificial Insemination’ in a manner that everyone can understand the facts in an interesting way. In this book, chapters are presented in concise format and almost all chapters start with very interesting and informative fascinating facts of reproductive world, therefore, the name of this section is ‘Digest’. Hence readers are suggested to read these facts very carefully and digest them. In order to make this book more interesting, each chapter is written in simple language, and in-between the text ‘Interesting Facts’, ‘Do You Know’ etc are given in boxes which are also very informative that create and maintain interest while reading the chapter. At the end of each chapter motivational quote is given which will build confidence in reader. The authors hope that the reader will find this book both interesting and useful for students, teachers and research workers. At last, let us turn the page and see if we have met those objectives. Constructive comments and valuable suggestions will be most appreciated.

Pradeep Kumar

P.S.Yadav

R.K.Sethi

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PART I

Male Reproductive Physiology

Development of Male Genitalia and Gonads

Digest

The sex can be reversed after birth in chicken, some ducks and doves. The removal of functional left ovary results in development of the non-functional right ovary into a testis or ovotestis. A gonad containing both follicle and seminiferous tubules is called ovotestis. The younger the bird when left ovary is removed, the greater the chance that the right gonad will develop into a testis. The older the bird, the greater the probability that an ovotestis will develop.

Most of the reproductive system is derived from the mesoderm. During the organ development period, formation of the reproductive system also occurs. In mammals, male differentiate early genotypically and females differentiate late. Sex determination is controlled by the genetic sex of the animal, which is fixed at the time of fertilization. In the process of sex differentiation, the genetic sex directs the development of either ovaries or testes, which then determines the phenotypic sex as the animal matures. In the undifferentiated stage of gonadal development, both Wolffian and Mullerian ducts are present and later these develop into the male or female accessory sex organs. The Mullerian ducts differentiate to form the Fallopian tubes, uterus and vagina, while the Wolffian ducts form the epididymis, ductus deferens, and seminal vesicles.

Development of testes

The development of testes occurs in the following ways:

- The **primordial germ cells** originate from the inner lining (endoderm) of the **yolk sac** of early embryo. Thus, **origins of primordial germ cells are extragonadal.**
- After origin, the primordial germ cells migrate by **ameboid movement** from yolk sac to the **genital ridges** (or gonadal ridges). A pair of genital ridges (undifferentiated gonads) is located on either side of the dorsal wall of abdomen of embryo near the embryonic kidney.
- The primordial germ cells undergo mitosis division and their numbers increase significantly.

- **Primordial germ cells** form **gonocytes** which further differentiate into **spermatogonia** just before puberty.
- The primordial germ cells also stimulate local connective tissue of genital ridge to proliferate.
- Now, the undifferentiated gonad (genital ridge) differentiates into **medulla (inner)** and **cortex (outer).**
- The proliferative genital ridge forms **primary sex cords.**
- **In the genetically male**, **primary sex cords** are found in the **medulla** which develops into the testes and **cortex regresses.**
- **In the genetically female, secondary sex cords** develop in the **cortex** which develops into the ovaries. **The primary sex cords and medulla regress**.

INTERESTING FACTS

In the avian species, H-Y antigen remains present in the female because females are heterogametic (ZW) and males are homogametic (ZZ).

- Now, **primary sex cords (medullary cords)** are differentiated into **seminiferous tubules** and **rete testis.**
- The Leydig cells arise from the intertubular element of developing gonad.
- The connective tissue of developing gonad forms **tunica albuginea.**

The genes for testis- determing factor (TDF) located on the short arm of the Y chromosomes induce the embryonic gonad to become a testis. Two antigens are assumed responsible for male gonadogenesis: the **minor histocompatability (H-Y) antigen** (recently no longer believed to be solely responsible) and the **sex determining region Y (SRY) antigen**.

SEX- DETERMINING GENES

The key sex-determining genes are *SRY* (sex determining gene on Y) and *SOX9* (SRY box) in mammals. These genes are involved in testis formation and in the development of the male phenotype. In the absence of the *SRY* gene, ovaries develop. *SRY* is located on the short arm of the Y chromosome (fig.1) and codes for a protein called the testis-determining factor (TDF). *SRY* is involved in the differentiation of Sertoli cells, the migration of primordial germ cells from the mesonephros to the genital ridges and the proliferation of cells within the genital ridges. The exact role of *SRY* in testis differentiation is not known, but *SRY* is thought to trigger each of these events, possibly by activating a secondary gene, such as *SOX9*. *SOX9* is up-regulated in the genital ridges of male embryos and down regulated in females. The *WNT* genes appear to play a role in the development of the ovaries and oocytes. WNT-4 may act as a suppressor of Leydig cell function in females and is necessary for the formation of Mullerian ducts. WNT-7α is involved in further development of the Mullerian ducts to form the oviduct and uterus.

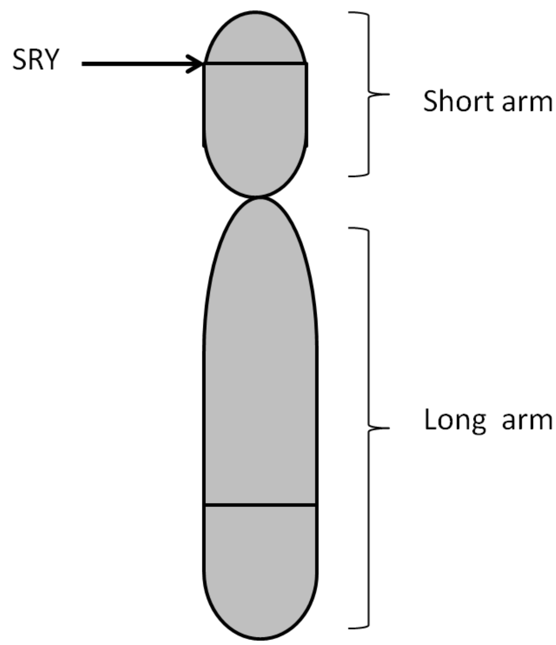

Fig.1: Y chromosome showing short and long arm

DO YOU KNOW?

The gene for the H-Y antigen is on the short arm of the Y chromosome whereas the H-Y antigen is found on the long arm of the Y chromosome.

Development of accessory genital tract and glands

After the fetal testis has been formed in response to the testis-determining gene or genes, male testicular hormone cause the development of the male phenotype. Immature Sertoli cells produce the **first testicular hormone**, mullerian inhibiting substance (MIS), which is responsible for the regression of the paramesonephric (Mullerian) ducts in the male fetus. Leydig cells secrete testosterone, the **second testicular hormone** that stimulates the development of the mesonephric

(Wolffian) duct and tubules. Differentiation of the external genitalia in the male occurs under the influence of dihydrotestosterone (DHT). The accessory genital ducts and glands in the male arise from the masonephric (Wolffian) ducts and tubules.

DO YOU KNOW?

MIS is also produced by the granulosa cells of the postnatal ovary in the female but physiologic role of MIS in the postnatal female has not been determined.

The reproductive system develops at the same time as the renal system is developing. During the development, embryo utilizes three distinct renal systems. The developments of renal system and accessory genital tract have been described below:

- The pronephros (pronephric kidney) regresses and is replaced by **mesonephros** (mesonephric kidney).
- The mesonephric kidney is closely associated with the undifferentiated gonad.
- The mesonephric kidney produces urine that is drained by a series of **mesonephric tubules** that merge into a larger **mesonephric duct**.
- The mesonephric duct extends caudally and empties urine into the **urogenital sinus.**
- When the mesonephric kidney is developing at the same time, a new pair of ducts begins to develop besides the mesonephric duct. Because these ducts are formed on either side of the mesonephric duct so these are called **paramesonephric ducts (Mullerian ducts).**
- During the first 10% to 15% of gestation peroid, the final form of kidney begins to appear.
- The final form of kidney is called **metanephros (metanephric kidney).**
- The **metanephric kidney** initially develops as a small bud.
- The mesonephric kidney begins to lose its function and decreases in size as the metanephric kidney increases in size.
- The gonad also continues its enlargement as does the metanephric kidney.
- The **metanephric kidney** becomes fully functional and the gonad becomes larger, while the **mesonphric kidney** was almost completely regressed.

- Now, some of the **mesonephric tubules** (5-15) penetrate into the **gonad** and make connections with the **primitive sex cords** via the rete testis.
- The **mesonephric tubules** are gradually transformed into the **efferent ducts.**
- The **mesonephric duct** will give rise to the **epididymis** and **ductus deferens (vas deferens).**
- Paramesonephric ducts degenerate in male
- Shortly after the virilisation of the mesonephric ducts, external genitalia develop from the urogenital sinus (inner genital folds).
- The **genital tubercle** becomes the **glans penis**.
- Fusion and caudal migration of the **labioscrotal swelling** form the **scrotum**.

Summary of development of male reproductive system

Embryological structure	**Mature structure**
Yolk sac	Primordial germ cells
Primary sex cords	Testes
Secondary sex cords	Ovaries
Mesonephric tubules	Efferent ducts
Mesonephric ducts	Epididymis, vas deferens and seminal vesicles
Urogenital sinus	Penile urethra, bulbourethral glands
Urethral folds	Prepuce
Genital tubercle	Glans penis
Genital swelling	Scrotum

*****

Give me six hours to chop down a tree and I will spend the first four sharpening the axe. - ***Abraham Lincoln***

Male Reproductive System

Digest

In the male cat, the glans is covered with 120 to 150 penile spines that are directed backward, away from the end of the glans. These penile spines start to appear at about 12 weeks of age and are fully developed at puberty. The spines rake the walls of the female's vagina, which is a trigger for ovulation. The female will utter a loud yowl as the male pulls his penis from her vagina. This act also occurs to clear the vagina of other sperm in the context of a 2nd (or more) mating, thus giving the latter males a larger chance of conception.

The male reproductive system consists of a pair of testis that produces sperm, ducts that transport the sperm to the penis and glands that add seminal plasma to the sperm. The various parts of the male reproductive system with their functions are discussed below (Fig. 1).

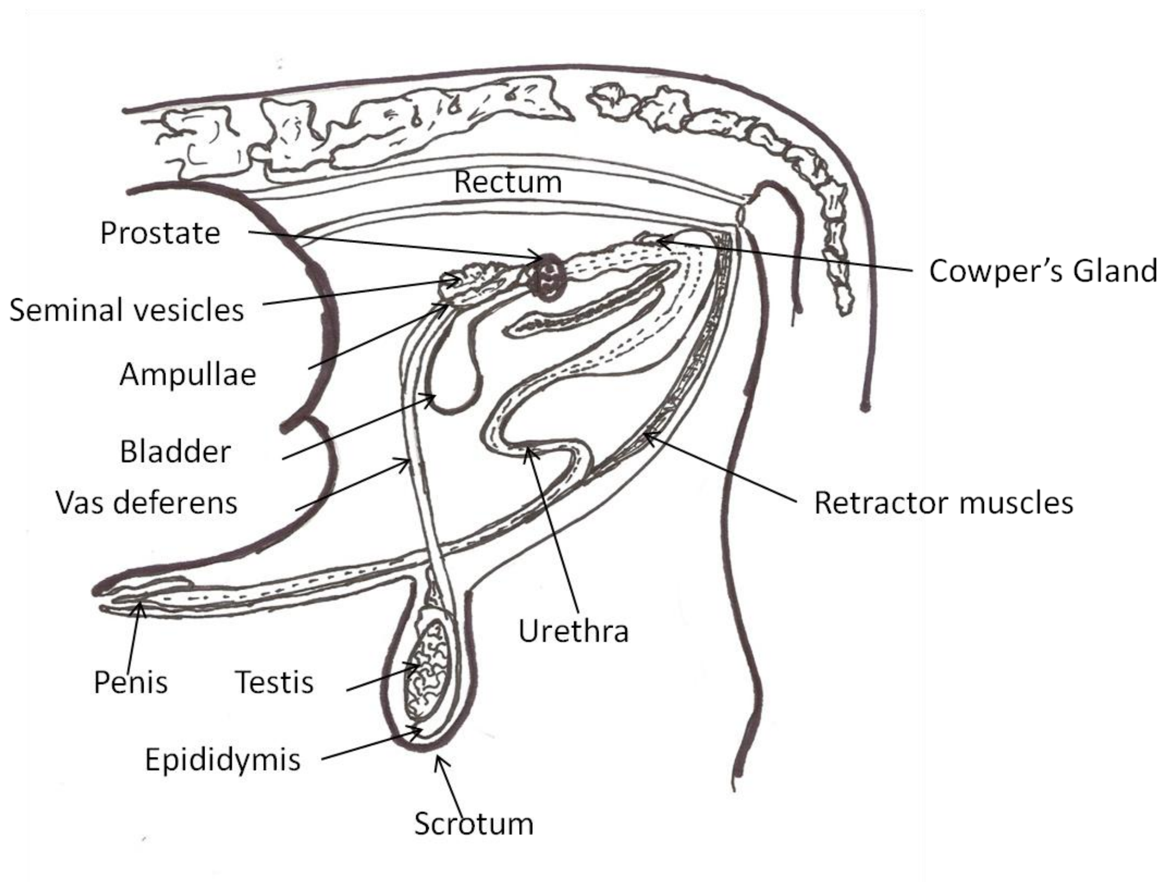

Fig. 1: Reproductive organ of bull

Primary sex organs	-	Testicles
Secondary sex organs	-	Epididymis, vas deference and penis.
Accessory sex glands	-	Seminal vesicles, prostate and bulbo-urethral glands.

Scrotum:

- The scrotum has the same embryonic origin as the labia majora in the female.
- It is a cutaneous pouch in which testicles are located.
- The scrotum of the domestic animals is located between the thighs except boar and cat. In the boar and cat the scrotum is located caudal to the thighs.
- The scrotum has the following layers from outside inwards (Fig. 2).
    - ✓ **Skin:** It is smooth and thinly covered by hair.
    - ✓ **Dartos layer:** It is made up of smooth muscle fibres and fibroelastic connective tissues. This layer divides the scrotum into two halves.
    - ✓ **Tunica vaginalis communis or parietal layer of tunica vaginalis:** It is a direct evagination of parietal peritoneum. It surrounds the two halves of the scrotum separately.

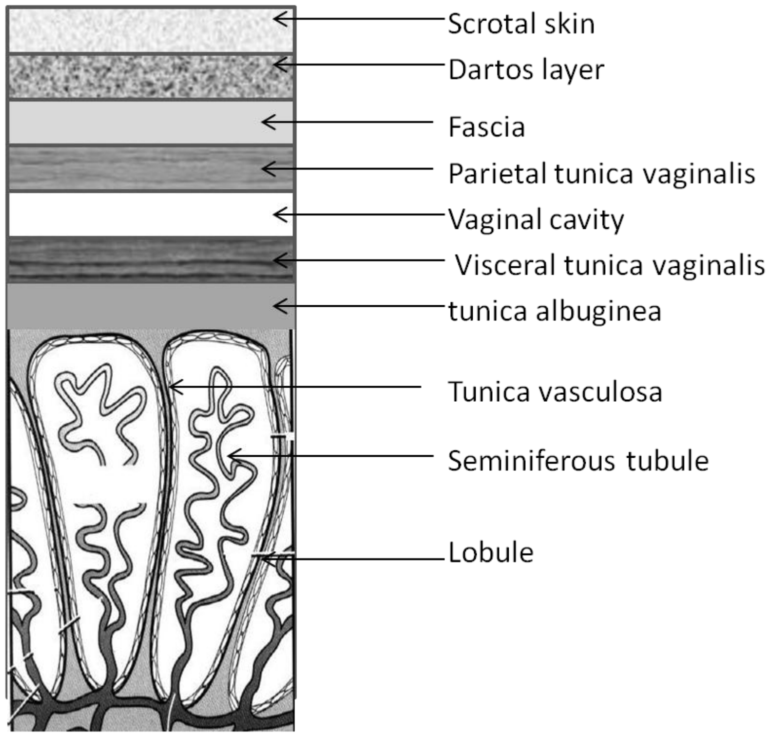

Fig. 2: Different layers of scrotum and testicle.

Functions

- It holds the testes.
- It regulates the temperature of testes by contractions, relaxations and through sweating.

DO YOU KNOW?

The scrotum of the horse is relatively short and non-pendulous in nature, when compared with that of other farm livestock such as the bull or the ram.

Testes

- They are two in number.
- They are suspended by spermatic cords.
- They are the primary organs of reproduction in males, just as ovaries in females.
- They are considered primary because they produce male gametes (spermatozoa) and male sex hormones (androgens).
- Each testicle has **two surfaces, two borders and two ends.**

- The **lateral surface** is convex and **medial surface** is flat.
- The **cranial border** is convex and free.
- The **caudal border** is less convex and is attached with the **body of the epididymis**.
- The **upper end** is occupied by the **head of epididymis.**
- The **lower end** is slightly thicker and is connected to the **tail of epididymis.**
- The testis is covered by **visceral layer tunica vaginalis, tunica albugenia and tunica vasculosa** from outside inwards (Fig. 2).
- The **tunica vaginalis** is derived from **peritoneum** during the descent of testis from the abdominal cavity.
- There is a narrow space filled with fluid in between parietal and visceral layers of tunica vaginalis. This fluid facilitates the movement of testis within scrotum.
- Inner to visceral layer of tunica vaginalis, a thick fibrous membrane covering the whole testis is known as **tunica albugenia** (Fig. 2)**.**
- Inner to tunica vaginalis, there is a membrane with plenty of blood vessels known as **tunica vasculosa** (Fig. 2).
- Along the caudal border, a mass of tissue projects from the tunica albugenia into the testis known as **mediastinum testis** (Fig. 3).
- From mediastinum testis numerous **fibrous septa** radiate and divide the testis into a number of chambers or **lobules** of testis (Fig. 3).

There are no well-developed septa to divide the testes into lobules in chicken.

- Each lobule contains some **seminiferous tubules** (Fig. 3).
- The seminiferrous tubule is made up of two types of cells: **Germ cells** (spermatogonia) which form spermatozoa and **Sertoli cells** which are also called **sperm mother cells or nurse cells** (Fig. 4)**.**
- **Leydig cells or interstitial cells** are found between the seminiferous tubules (Fig. 4).

- The Leydig cells produce male sex hormone testosterone.

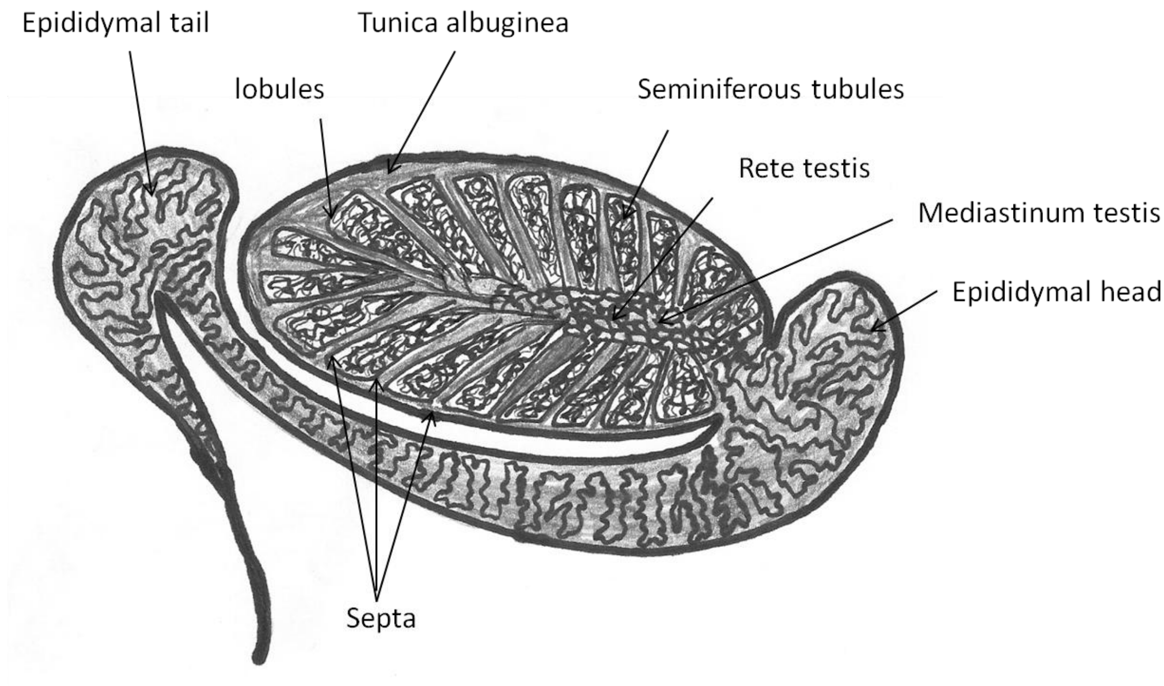

Fig. 3: Internal structure of testis and epididymis

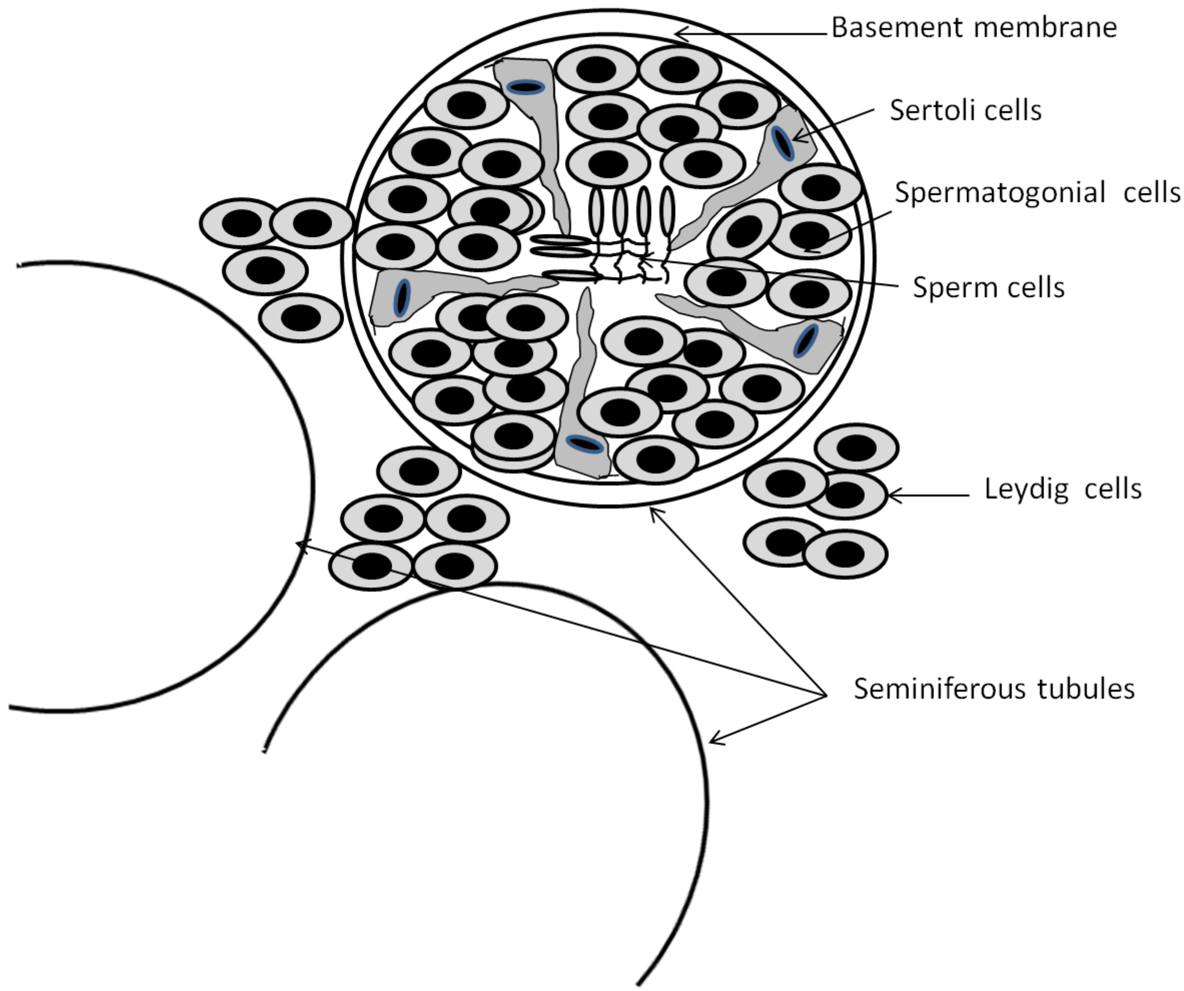

Fig. 4: Cross section though the testis showing arrangement of Leydig cells, Sertoli cells and spermatogonial cells.

INTERESTING FACTSS

It has been estimated that the seminiferous tubules from both testes of bull, if stretched out and joined end to end, it approaches 5 m in length.

- Seminiferous tubules join a network of tubules known as **rete testis.**
- About 12-15 tubules arising from rete-testis are called **vasa efferentia or efferent ducts.**
- These vasa efferentia unit in the head of epididymis and form a single duct called duct of **epididymis.**

Function

1.	Germ cells	-	produce sperm
2.	Leydig cells	-	produce testosterone
3.	Sertoli cells	-	Nutritive to spermatozoa. Protective to spermatozoa Supportive to spermatozoa. Phagocytose regressive spermatozoa Detach residual bodies of spermatids Produce androgen binding protein. Secrete intratubular fluid rich in potassium, inositol and glutamate.

Enrico Sertoli

Enrico Sertoli was an Italian physiologist and histologist. Sertoli is remembered for his discovery of the eponymous Sertoli cell in 1865.

Franz Leydig

Franz von Leydig was a German zoologist and comparative anatomist. Leydig shared both his father's Catholic religion and hobbies: his father was a keen gardener and beekeeper. Leydig himself recalled later that those childhood interests began his lifelong concern with botany and zoology. At age 12, he acquired a simple microscope, which he used in the majority of his free time. He is remembered for his discovery of the eponymous Leydig cells in 1850.

Epididymis

On the posterolateral side of each testis, there is an elongated structure called epididymis. It is differentiated into three parts: **head (caput), body (corpus) and tail (cauda).** The head of the epididymis is flattened at the upper end of the testis, where 12 to 15 vasa efferentia merge into a single duct of epididymis.

Functions

- **Transportation of spermatozoa:**

  The ciliated epithelium and peristaltic contraction of smooth muscle fibre of epididymis help in transportation of spermatozoa through epididymis.

- **Concentration of spermatozoa:**

  The head of the eididymis is the site of absorption of excess fluid. More than 90 % of fluid leaving testis is absorbed in the head of the epididymis

- **Storage**

  The cauda of epididymis is the site of storage of spermatozoa. The conditions of cauda of epididymis are optimum for preserving the viability of spermatozoa for an extended period.

- **Maturation**

  The freshly formed spermatozoa in testes have neither motility nor fertility i.e. they are immature spermatozoa. The maturation of spermatozoa occurs in the head and body of epididymis. The spermatozoa lose its protoplasmic droplet which forms during spermatogenesis.

Inguinal canal

It connects scrotal sac to abdominal cavity

Spermatic cord

It is an elastic cord which passes through inguinal canal and attached with testis. Spermatic cord is formed of spermatic arteries, veins, lymphatics nerves, cremaster muscles and vas deferens.

Vas deferens (plural vasa deferentia)

Vasa deferentia are a pair of ducts extending from tail of epididymis to the **colliculus seminalis** of the urethra. The terminal portion of the vas deferens is little dilated and is known as **ampulla.** Some have suggested that ampulla serve as a **short-term storage**

depot for semen. **Ampulla is absent in dog and cat.** The ampulla is **well developed** in **stallion** which secretes a sulphur containing base called **ergothionine** in the ejaculates.

Functions

Transport of sperm from epididymis to urethra through peristaltic movement during ejaculation.

Urethra

- It extends from the **neck of the bladder** to the **glans penis.**
- It serves as a common passage for the urine and semen.
- **Colliculus seminalis** is a small elongate or oval eminence structure on the urethra into which vas deferens and duct of seminal vesicle opens.
- During ejaculation in the bull, ram and doe, there is a complete mixing of spermatozoa with fluids from the accessory glands. **In stallion and boars, mixing is not as complete. Therefore, the ejaculates contain sperm-free and sperm rich fractions.**

Accessory sex glands

- The accessory sex glands are located along the pelvic portion of the urethra.
- They empty their secretions into the urethra.
- They include **vesicular glands**, **prostate gland**, and **bulbourethral glands.**
- The size of accessory sex glands vary in different species (table 1)

Vesicular glands or seminal vesicle

- These are two lobulated and elongated glands.
- They are situated adjacent to the neck of the urinary bladder and lateral to the ampullae.
- They open into the urethra just distally to the vasa deferentia.
- They secrete fluid that adds volume, nutrients and buffers to the semen.
- In all species its secretion contains large quantities of **citrate**.

- In the **ruminants** its secretion contains large quantity of **fructose** than other domestic animals.
- In **boar**, its secretion also contains **inositol.**
- The vesicular secretion of bull is **yellow in colour** due to presence of vitamin **riboflavin.**
- They contribute more than half of the total volume of semen in bull.
- **The gland is absent in dog and cat.**

INTERESTING FACT

The seminal vesicles in domestic animals except horse, is called vesicular gland because of its gland-like nature. In the horse, because this structure is more bladder-like with the glands in the walls, therefore, it is still called seminal vesicle.

Prostate gland

- It is a single gland located around and along the urethra just posterior to the ducts of the vesicular glands.
- It is **well developed in dog.**
- The secretions of the prostate are high in inorganic ions.
- It pours its **alkaline secretion** into urethra.

Bulbourethral glands or Cowper's gland

- These are two small glands on either side of the dorsal part of urethra at the level of ischial arch.
- They are about the **size and shape of walnuts** in bulls, but are much larger in boars.
- Secretion of the gland flushes the urine residue from the urethra before ejaculation in bulls. This can be seen as dribbling from the prepuce just before copulation.
- In boars, secretion of the gland account for that portion of semen which coagulates resulting prevention of back flow of semen through the cervix into the vagina of sows.

Table 1. Relative Size of Accessory Sex Glands in Different animals

Species	Ampulla	Prostate	Vesicular gland	Bulbourethral gland
Cattle/buffalo	(+)	++	+++	+
Sheep	(+)	++	+++	+
Horse	++	++	++	+
Pig	Absent	+	++	+++
Dog	Absent	+++	Absent	Absent
Cat	Absent	++	Absent	++

Ampullary glands are present in species marked (+) but are not anatomically prominent

Penis

- It is the organ of copulation in males.
- It is more or less cylindrical in form in all species.
- It extends from the ischial arch to near the umbilicus on the ventral abdominal wall except in **cat** in which the **penis points posteriorly** in the relaxed stage.
- It consists of three parts – **root, body and glans.**
- Root – It begins at the ischial arch.
- Body – It is the largest part of the organ between the root and the glans.
- Glans penis – It is the free conical end of the penis.
- It is formed by three cylindrical long erectile tissues: two dorsolaterally **corpora cavernosa** and one ventrolaterally **corpus spongiosum**.
- The erectile tissue is a sponge like system of irregular blood channels. When a male is sexually stimulated these erectile tissues become filled with blood under high pressure resulting in erection of penis.
- Towards the root of the penis the corpus spongiosum enlarge into a bulb called **bulbs of penis.**

- Towards the tip of the penis, the corpus spongiosum swells into a knob like structure called **glans penis.**

Copulatory tie or locking phase
The dog has a bulbus glandis at the caudal part of the glans penis. The enlargement of the bulbus glandis and the contraction of vestibular muscle of vagina are responsive for prolonged retension of the penis during coitus, commonly known as the copulatory tie.

- The glans penis is well supplied with the sensory nerves and is homologous to the clitoris in the female.
- Bull, buck, ram and boar have a **sigmoid flexure**, an **S-shaped** bend in the penis which permits it to be retracted completely into the body.
- In bull, buck and ram, the **sigmoid flexure** is **post-scrotal** while in boar is **prescrotal.**
- Two types of penis: **fibroelastic and musculocavernous penis** (table 2)

Table 2. Difference between Fibroelastic Penis and Musculocavernous penis

	Fibroelastic	**Musculocavernous**
1	Thick fibrous tunica albuginea	Tunica albuginea is less pronounced
2	Less erectile tissue	More erectile tissue
3	Sigmoid flexure - present	Sigmoid flexure - absent
4	Little blood enters the penis during erection	More blood enters the penis during erection
5	Lengthening of penis is achieved mainly by straightening the sigmoid flexure of the penis	Lengthening of penis is achieved entirely by vascular engorgement

6	It is found in ruminants and boar	It is found in stallion, dog and cat.

- **Three types of muscles are associated with penis**. These are described below.

Erector penis muscles or Ischio- cavernosus muscles

- They are **paired** muscles.
- They extend from ischial to the lateral surface of penis.
- They help in erection of penis.
- When these muscles contract, they pull the penis upward against the floor of the pelvis. Much of the venous drainage from the penis is obstructed and erection is thereby assisted.

Bulbospongiosus muscle

- It extends from ischial arch to the glans penis.
- At the root, it is bulky and then diminishes.
- It helps in empty the extra pelvic portion of urethra during ejaculation or urination.

Retractor penis muscles

- They are paired muscles.
- They extend from first and second coccygeal verterbrae to the distal end of the sigmoid flexure.
- The muscles pull the penis back into the sheath after ejaculation.

Stallion's Penis
• At the base of the glans, there is a border known as **corona glandis**. • The projected end of the urethra outside the glans is called **urethral process**. • The urethral process is situated at the **fossa glandis**.

Prepuce

- The prepuce is an invaginated fold of skin that surrounds the free extremity of the penis.
- It is **homologous to the labia majora** in female.

- The external opening of the prepuce is called **preputial orifice.**
- The orifice of the prepuce is surrounded by long and tough preputial hairs.

INTERESTING FACTS

- The stallion has a double folded prepuce. Some times in the outer fold, wax accumulates and it must be removed manually. The wax is called ‘beans’.
- The boar has a preputial diverticulum (pouch), which often contains decomposing urine and macerated epithelium. This fluid in the diverticulum also contains a pheromone that causes sows to assume the immobile mating stance.
- Swine and ruminants tend to urinate inside the prepuce, whereas horses, dogs and cat extend the penis beyond the sheath.

Muscles of male genitalia

1. External cremaster muscle

- It is formed from the caudal fibres of the **internal abdominal oblique muscle.**
- It passes through the inguinal canal and attaches to the outside of the parietal layer of tunica vaginalis.
- This muscle pulls the testis upward direction in cold weather.

INTERESTING FACT

The cremaster muscles are responsible for the testes being drawn into the abdominal cavity of the elephant, deer, and rabbit during times other than breeding season.

2. Internal cremaster muscle

It helps to hold the spermatic cord structures together.

3. Urethralis muscle

Peristaltic action of this muscle assists in the transport of urine or semen through the pelvic urethra. Thus it **aids in micturation and ejaculation.**

4. Bulbospongiosus muscle

It continues the action of the urethralis muscle in emplying the extrapelvic urethral content.

5. **Ischiocavernosus muscles:** described earlier.

6. **Retractor penis muscles:** described earlier.

Blood supply to male reproductive system

- Testicle - Internal spermatic artery
- Scrotum - External pudental artery
- Penis - Internal pudental artery – root of penis

  Obturatory artery - body of penis
- Accessory sex gland - Internal pudental artery (Large animal)

INTERESTING FACTS

- In all species, vaginal intromission of the penis requires full erection except dog. The penis of the dog contains a bone called os penis which facilitates vaginal entry without full erection. In fact, the fully erected dog's penis cannot enter the bitch's vagina.
- Spiral deviation of the bull's penis commonly occurs within the vagina during normal ejaculation.
- The penis of the cat is directed backward and downwards from the ischial area.
- The prostate gland of the dog, being the only accessory gland present, is well developed contributes a large volume of fluid to the ejaculate and is mostly delivered as part of the postsperm fraction of the ejaculate.

CLINICAL POINTER

The penis of ram and buck are characterized by a urethral process extending beyond the glans penis. The function of the urethral process is to spray the cervical area of the female with semen during ejaculation by moving it in a circular pattern. Sometimes urethral calculi become lodged in its narrow extremity which causes difficulty in urination. This can be corrected by amputation of the urethral process.

Table 3. Differential feature of scrotum, testis and penis in different species

	Cattle	**Buffalo**	**Sheep**	**Horse**	**Pig**	**Dog**	**Cat**
Scrotum	Between the thighs	Between the thighs	Between the thighs	Between the thighs	**Caudal to the thighs**	Between the thighs	**Caudal to the thighs**
Testis							
Shape	Oval	Oval	Oval	Oval			
Long axis	Vertical	vertical	Vertical	**Horizontal**	Vertical	**Oblique**	**Oblique**
Weight (gm)	350	168	275	180	**360**	7-15	-
Length of seminiferous tubules length (meters)	5000	3000	4000	-	**6000**	150	25
Length of epididymis (meters)	40	-	50	20	**50**	-	-

Epididymal journey of spermatozoa (days)	10	-	13-15	8-11	9-12	-	-
Penis							
Sigmoid flexure	Present	present	Present	Absent	Present	Absent	Absent
Position of sigmoid flexure	Post scrotal	Post scrotal	Post scrotal	-	**Pre-scrotal**	-	-
Glans penis	Present	Present	Present	Present	Absent	Present	Absent
Os penis	Absent	Absent	Absent	Absent	Absent	Present	Sometims present
Type of penis	Fibroelastic	Fibroelastic	Fibroelastic	Vascular or musculo cavernous	Fibrolastic	Vascular or muscular cavernous	Vascular or muscular cavernous

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Testicular Descent

Digest

A blow to the testicles is very painful and every man understands this thing at a young stage. During embryonic life testicles are formed near the stomach and kidney. During the foetal life, when testicles are descending from the abdomen to the scrotum, the nerves originating in this region also travell with the testicles. This might be the reason why the blow to the testicles feels like a punch in the stomach.

When the testis migrates from its position within the abdomen towards its definitive position in the scrotum it is called testicular descent. In most mammals, the testis must descend from the abdomen to scrotum to provide a lower ambient temperature for normal spermatogenesis. It is hypothesised that this is the result of evolutionary adaptation. In ancestral mammals hyperthermic metabolic environment were needed for the function of abdominal organs such as the liver and kidneys. Nevertheless, in some mammals testes are permanently in the abdomen. Thus mammals can be classified into two groups: scrotal and non-scrotal animals.

NON-SCROTAL ANIMALS

Some mammals such as the sloths, elephants, rhinoceros and seal have no scrotum and the testes are intra- abdominal. Many marine mammals, such as whales and dolphins, also have internal testes, which are kept cool by special circulatory systems that cool the arterial blood going to the testes by placing the arteries near veins bringing cooled venous blood from the skin. It is argued that those mammals with internal testes have lower core body temperatures than those mammals with external testes. However, the question remains why birds despite having very high core body temperatures have internal testes and did not evolve external testes. It was once theorized that birds used their air sacs to cool the testes internally, but later studies revealed that birds' testes are able to function at core body temperature. Some mammals which have seasonal breeding cycles keep their testes internal until the breeding season at which point their testes descend and increase in size and become external.

Mechanism of testicular descent

Testis descent is the process by which the developing testes move from its initial position in the abdomen into the scrotum. Initially, in both sexes the gonad is displaced caudally by the developing metanephros. This process is called nephric displacement. Subsequent descent of the testis can be divided into two phases, **transabdominal migration** and an **inguinoscrotal phase**. The transabdominal phase is completed early in fetal development, but the inguinoscrotal stage occurs late in fetal life or after birth. During the first or transabdominal phase, the testes migrate to the bottom of the abdomen. The second or inguinoscrotal phase involves movement of the testis from the abdominal bottom into the scrotum. Two structures other than the testes itself are involved in its descent, the **cranial suspensory ligament** and the **gubernaculum**. The cranial suspensory ligament (CSL) holds cranially both the male and female gonad to dorsal wall of the abdominal cavity or the caudal pole of the kidney. The cranio-suspensory ligament degenerates in the male and thereby allows the release of the testis to migrate across the abdomen. The cranio-suspensory ligament does not degenerate in the female thus the ovary is retained in pelvic cavity. The gubernaculum is formed behind the gonad in the inguinal area of the abdominal cavity. There is thickening of the gubernaculum in the male, which helps to anchor and retain the testis to the inguinal region while the abdominal cavity is enlarging. The gubernaculum does not enlarge in the female and is retained as the **ovarian round ligament**. During the transabdominal phase, the male gubernaculum is developing, whereas outgrowth of the CSL is lacking, resulting in migration of testis. During descent two layers of peritoneum cover the testis. These two layers, visceral vaginal tunic and the parietal vaginal tunic are separated by a vaginal cavity (vaginal process) that is continuous with the peritoneal cavity. These tunicae are slippery and allow the testis to move freely within the scrotum.

INTERESTING FACT

A key structure in the trans-abdominal phase is the gubernaculum (genitoinguinal ligament). It was named by the famous Scottish surgeon-scientist, John Hunter, because the term gubernaculum means helm or rudder. Hunter chose this name because his observations suggested that the gubernaculum steered the testis to the scrotum.

Hormonal control of testicular descent

The control of testicular descent is still controversial. It appears to involve at least four factors: **insulin-like factor 3 (INSL3)**, **calcitonin gene-related peptide (CGRP)**, **Müllerian inhibiting substance (MIS)** and **androgens**. Distinctly separate mechanisms are involved in controlling the two phases of testis descent. Androgen induces regression of the cranio-suspensory ligament to release the testis to descend. Otherwise, androgens are not required for the first phase of testis descent. Earlier mullerian inhibiting substance (MIS) (also known as antimullerian hormone [AMH]) was proposed as the key nonandrogenic hormone for transabdominal phase. However, there are a lot of other evidences suggest that MIS/AMH is not the major factor. In the late 1990s, a new testicular hormone was discovered, known as insulin-like hormone 3 (INSL3), which is related to insulin and relaxin and is also known as **relaxin-like factor**. The INSL3 is produced by the Leydig cells in the foetal testis. Thus, in first phase, INSL3 stimulates the gubernacular enlargement, augmented by MIS/AMH and androgen. The inguinoscrotal phase in which the gubernaculum migrates and elongates toward the scrotum is indirectly controlled by androgen via the **genitofemoral nerve (GFN)** and **calcitonin gene-related peptide (CGRP)**. GFN acts as a second messenger for androgen. The GFN releases the neuropeptide, calcitonin gene-related peptide (CGRP) which acts on gubernaculum.

Evolution of Scrotum

The significance of the evolution of the scrotum is an area of debate. It has been argued that migration of the testis into the scrotum helps to externalize the cauda epididymis, which needs a cool environment for sperm storage. However, if the temperature of scrotal testes is raised to body temperature, spermatogenesis is disrupted. It has also been proposed that testicular descent into a cool environment may have evolved to minimize the mutation rate in male germ cells. One of the more old outdated suggestions was that scrotal testes were a result of the animals jumping or leaping. Another eccentric suggestion is that scrotal testes are involved for stimulation of the clitoris during mating. These hypotheses are just imaginary without scientific proofs.

Important steps in descent of the testes

- Before descent occurs, the testis lies in a retroperitoneal position.
- At the caudal end of the testis, a structure known as the **gubernaculum** develops.
- The testis migrates in the abdomen partly due to differential growth between the pelvis, abdomen, kidney and testis, and partly due to the tension upon the testis by the gubernaculum.
- As the testis reaches near to internal ring of the inguinal canal, rapid growth occur in the caudal part of the gubernaculums dilating the inguinal canal and scrotum. Thus pulling of the testis across the abdominal cavity into the inguinal canal occurs due to rapid growth of the gubernaculum in scrotum.
- Once the testes come in the inguinal region, they are moved through the inguinal opening because of regression of gubernaculum. This can be understood by ball and flask model (Fig.1).
- Final passage of the testis is achieved by tension from the gubernaculums and pressure from the abdominal viscera.
- After the complete descent of testis, the gubernaculum is regressed to a small knot like structure and forms **ligamentum testis** and **scrotal ligament** that attaches the testis to the bottom of the scrotum.

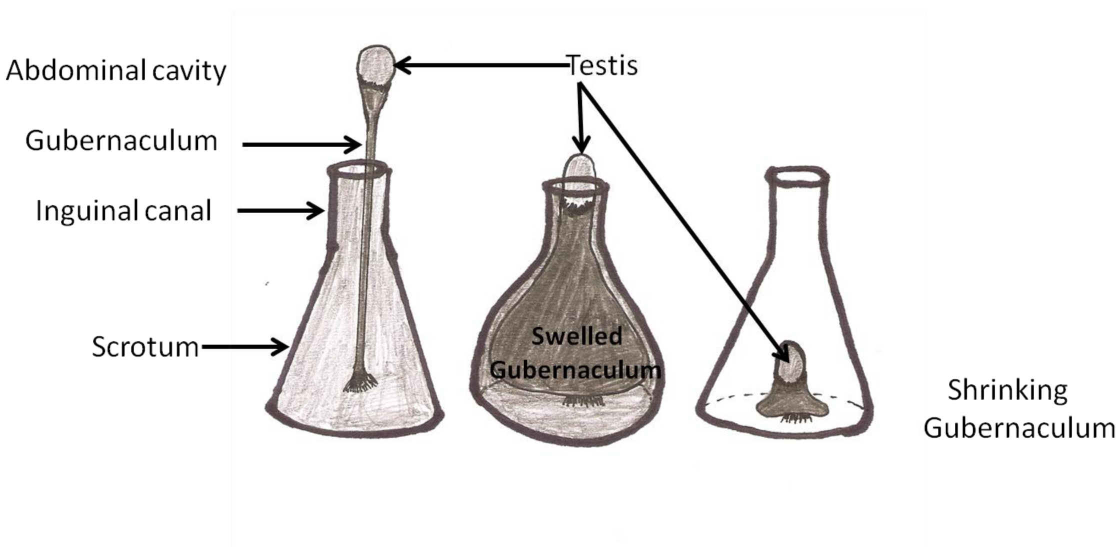

Fig.1: Ball and Flask Model of testicular descent

Age at which testes descend into the scrotum in different species

Species	**Time of testicular descent**
Cat	2–5 days after birth.
Dog	Between the last few days of gestation and the first few weeks after birth.
Stallion	Between 9 months of gestation and a few days after birth.
Cow bull	3.5–4 months of gestation.
Buffalo bull	2-4 month of age after birth, although may be present at birth in some animals.
Ram	Mid gestation (80 days).
Boar	After 85 days of gestation or 3 months of gestation.

DO YOU KNFOW?

- In elephants, the testes remain next to the kidneys indicating that germ cell development in these species occurs at typical mammalian body temperature.
- The elephant have straight testicular arteries and lack of pampiniform plexus.
- In elephants, there is no gubernaculum or inguinal canal.
- In whales and dolphins, there is a gubernacular primordium but this does not develop.
- In marsupials, young are born at an early stage of development before full differentiation of the gonads has occurred, and both phases of testis descent occur after birth. Testicular descent starts early in pouch life in these species.

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*Success is the good fortune that comes from aspiration, desperation, perspiration and inspiration.-**Evan Esar***

Thermoregulation of Testes

Digest

The word 'testis' is derived from Latin which means that 'witness' or 'spectator'. The English words 'testify' and 'testament' are taken from testis but the exact reason for this derivation is not known. It was said that testis were witnesses of virility. In Rome, earlier it was essential that witness should be an adult intact male. Therefore, pre-pubertal boys, women and intersex could not become witnesses. In some cultures, placing the hand on the testicles or someone else's testicles was essential while testifying.

Temperature control of testes is essential for optimum production of spermatozoa. If the testes are maintained at body temperature, as occur in bilateral cryptochidism, spermatogenesis does not occur. Testicular temperature in bulls must be 2 to 6° C cooler than core body temperature for effective production of fertile spermatozoa. Several mechanisms act to regulate the testicular temperature. However, testosterone production by interstitial cells or Leydig cells is not affected by temperature. Temperature of testis is maintained by various mechanisms. These are:

- **The skin of the scrotum** has both sweat and sebaceous glands which are more active during hot weather.
- The **dartos muscle**, the smooth muscle lining the scrotum, and the **cremaster muscle**, a smooth muscle around the spermatic cord, are sensitive to temperature. During cold weather, contraction of these muscles causes the scrotum to pucker and draws the testes closer to the body. During hot weather, these muscles relax to lower the testis. The lengthening of the spermatic cord during hot weather provides more surface area for the heat exchange.
- A little above the testicle, the **testicular vein** is convoluted (**pampiniform plexus**) and is in close association with the convoluted part of the testicles artery. Therefore, this interwining allows the cooler venous blood to cool the warmer arterial blood going to the testis.

INTERESTING FACTS

Dartos and cremaster muscles do not respond to changes in temperature before the age of puberty. These muscles must be sensitized by testosterone to respond to the change of ambient temperature.

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A wise man learns by the mistakes of others, a fool by his own.

Sexual Behaviour in Male Animals

Digest

The life of the adult elephant male is very different. As he gets older, he begins to spend more time at the edge of the herd, gradually going away from herd for hours or days at a time. Eventually, days become weeks, and somewhere around the age of fourteen, the mature male, or bull, sets out from his natal group for good. While the males do live primarily solitary lives, they will occasionally form loose associations with other males. These groups are called bachelor herds.

Reproductive or sexual behaviour is essential component of reproduction. The purpose of reproductive behaviour is to promote the opportunities for copulation. The female reproductive behaviour is relatively simple than male. They have to stand only so that male can mount. Sexual behaviour of buffalo bull is similar to cow bull but less intense. Sexual behaviour in male can be divided into three stages. These stages are precopulatory, copulatory and postcoplatory stage.

Precopulatory stage

1. Sexual arousal
2. Courtship
3. Erection
4. Penile protrusion

Copulatory stage

5. Mounting
6. Intromission
7. Ejaculation

Postcopulatory stage

8. Dismounting
9. Refractory period and
10. Memory.

Sexual arousal (Sexual excitement)

It is the arousal of sexual desires during sexual activity. Sexual arousal leads to physiological changes in the male.

Courtship (Sexual display)

It is the specialized behaviour in animals that leads to or initiates mating. The courtship is simple and shorter in mammals than birds, fish and arthropods. Once a sexual partner has been identified, a series of highly specific courtship behaviours begin. Courtship - specific behaviours include sniffing of the vulva, urination, flehmen reaction, chin resting, circling and increased phonation. In domestic animal the pattern of courtship varies with species. Sniffing and licking are the most common pattern of courtship. Sniffing to female genitalia and to urine is common in cattle, buffalo, sheep, and goat. Sniffing to female's head is common in swine and horse. After sniffing the male stands rigidly and holds his head in horizontal position with extended neck and raise upper lips. This reaction is called Flehmen reaction (means to curl the upper lip) (Fig. 1). This behaviour facilitates the transfer of pheromones into the vomeronasal organ. Flehmen reaction helps to the animal to determine presence or absence of estrus and the physiological state of the animal. **The flehmen reaction is found in all domestic animals except swine.**

Fig. 1: Flehmen reaction shown by a buffalo bull

The tactile stimulation of the female is made by nuzzling and licking in cattle, sheep and goat whereas in horse, the stallion bites the female's neck and in swine, the boar noses her flanks.

Frequent urination on foreleg during excitement is characteristic pattern of courtship of goat. Stallion marks with urine the place where a mare has urinated, whereas, rhythmic emission of urine occurs during sexual excitement in boar. Urination is not species-specific pattern of courtship in cattle, buffalo, and sheep.

The sound (vocalization) production during courtship is found in sheep and goat (bleating), boar (grunts) and stallion (neighing). Interestingly, no vocalization pattern of courtship is found in cattle and buffalo.

DO YOU KNOW?

Flehmen behaviour is occasionally found in the females during encounter with males. Cows generally show flehmen reaction while sniffing other cows that are in estrus or proestrus. Flehmen is frequently displayed by post-parturient female through which they identify their own neonates.

Erection and penile protrusion

Erection is defined as stiffening of the penis during sexual arousal. Following exposure of the appropriate stimuli, erection and protrusion of the penis occur. The anatomical structure of the male penis influences the precoital sexual behaviours of the animal. The vascular penis is found in stallion and dog which erects slowly. Therefore, foreplay before copulation is essential in these animals. The fibroelastic penis erects rapidly. Therefore, there is less foreplay and prompt mounting occurs in those species having fibroelastic penis. The fibroelastic penis is found in bull, ram and boar.

IMPORTANT POINTS

- Erection is predominantly under the influence of parasympathetic system and ejaculation is controlled by the sympathetic system.
- Successful penile erection requires a complex series of neural and vasomotor (blood vessel) reactions.
- The penis of the bull, boar and ram is fibroelastic in nature and therefore does not increase significantly in diameter during erection and protrusion. In contrast, the penis of the stallion increases significantly in diameter during erection.

Mounting

Mounting behaviour requires elevation of the front legs of the male to straddle the posterior region of the female. The purpose of mounting is to position himself so that intromission can occur. In the bull, dribbling of watery fluid occurs though penis during mounting which is derived from the Cowper's gland. In the presence of receptive female, the bull rests his chin on the female and she in turn responds by standing. The male then mounts, fixes his forelegs around the female, grasps her firmly and performs rhythmic pelvic thrusts. Some boars and stallions mount and dismount the female repeatedly before copulation, whereas other mount once and copulate. During mounting, the abdominal muscles, particularly the rectus abdominis muscles contract resulting the pelvic region of the male comes into direct opposition to the external genitalia of the female.

DO YOY KNOW?

Testosterone in male is aromatized to estradiol in the brain. This estradiol promotes reproductive behaviour in male. As we know that testosterone is continuously produced by Sertoli cells in every 4 to 6 hours. Thus, testosterone is continuously supplied to brain. This is why a male can initiate reproductive behaviour at any time. In contrast, in the female, high estradiol concentration is found during the follicular phase only. Therefore, a female shows sexual activity during estrus phase only.

Intromission

Intromission is defined as successful entrance of the penis into the vagina. In the boar, penis is partially out of the prepuce. In this position, boar thrusts his pelvis until the tip of the penis penetrate the vulva; only then, penis is fully unsheathed and intromission is completed. In the stallion, oscillation of pelvis occurs several times before intromission resulting engorgement of the penis with blood which causes maximum intromission. The duration of intromission varies widely between species.

IMPORTANT POINTS

- Animals such as the horse, dog and boar, which ejaculate large amount of semen, have a prolonged period of coitus. The bull and ram ejaculate small amount of semen and their period of coitus is short.

- In the bull and ram the warmth of the vagina is most important and pressure and friction are less important as stimuli for ejaculation. In stallions, boars and dog, pressure on the penis is relativly more important than temperature.

Ejaculation

Ejaculation is defined as the reflex expulsion of semen from the penis. Following intromission, ejaculation takes place in response to sensory stimulation of the glans penis. The time of ejaculation relative to intromission varies among species. In the bull and ram, ejaculation occurs within one or two seconds after intromission. In these species ejaculation is stimulated by the warm temperature of the vagina. In the stallion, ejaculation occurs between 30 seconds to one minute after intromission. In the boar, sustained ejaculation occurs for periods of up to 30 minutes. In the dog, copulation occurs continuously for up to 50 minutes.

In cattle and sheep, semen is ejaculated near the os cervix; in swine, into the uterus and in horses, partially into the uterus. In the bull, ejaculatory thrust is so strong that the hind legs of the male leave the ground. In the ram and buck, during ejaculation, the male’s head suddenly moves backward whereas in the bull it is pressed down on the female’s neck. During ejaculation, the boar is quiet, only a slight rhythmic contraction of the scrotum occurs and such periods of immobility are followed by some thrusts at irregular intervals.

‘FIRE HOSE’ EFFECT

In the ram urethral or vermiform process gets filled with blood at the time of copulation. At the time of ejaculation, semen is forced out with high pressure through the narrow orifice of the urethra. The urethral process and narrow orifice of the urethra produces spraying or ‘fire hose’ effect at the time of ejaculation.

Dismounting

After ejaculation, the male dismounts and withdraws his penis into the prepuce. Post coital displays are rare in domestic animals. The male goat usually licks the penis after ejaculation. The ram stretches his head and neck after ejaculation.

Refractoriness

The refractory period is a period of time during which a second copulation does not take place. Memory is important in both a positive and negative way. Positive mating experiences promote reproductive behaviour and negative inhibit reproductive behaviour next time. The duration of the refractory period depends on several factors. These factors are age, species, sexual rest, number of previous ejaculations etc. The postcopulatory behaviour is sometimes confused with sexual exhaustion. Restimulation may occur after the refractory period. **Exhaustion** is the condition in which no further sexual behaviour can be induced even if stimuli are present. **Sexual satiation** refers to a condition in which further stimuli will not cause immediate response under a given set of stimulus conditions. The **'Coolidge effect'** is defined as the restoration of mating behaviour in males (that have reached sexual satiation) when the original female is replaced by a novel female.

ORIGIN OF 'COOLIDGE EFFECT' TERM
The "Coolidge effect" name is given by an ethologist, Frank A. Beach in 1955. He credited the new term to an old joke about Calvin Coolidge when he was President of America. Once, the President and Mrs. Coolidge visited a poultry farm and took around the farm separately. Mrs. Coolidge surprised to see that a rooster was mating very frequently. She asked the attendant how often that happened. The attendant told, "Dozens of times each day." Mrs. Coolidge said, "Tell that to the President when he comes by." Upon being told, President asked, "Same hen every time?" The reply was, "Oh, no, Mr. President, a different hen every time." President: "Tell that to Mrs. Coolidge."

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Thinking is progress. Non-thinking is stagnation of the individual, organisation and the country. ***-Abdul Kalam***

PART II

MALE INFERTILITY

Forms of Male Infertility

Digest

Normally the females take care of their eggs in the oviparous species but in some species males take care of eggs until hatching. The female bell toad lays her eggs in water which is 3 to 4 feet long attached to each other by strings. The male wraps the egg string around his body making sure that egg are not exposed to adverse environment. He hides during the day and seeks water at night to moisten the eggs. At hatching the male sits in the water and the tadpoles swim away. The endocrine basis for this event is not exactly known.

Male infertility is defined as the inability of a male to achieve a pregnancy in a fertile female. According to Lagerlof the causes of sub- or infertility of the bull are classified under the three headings *Impotentia coeundi*, *Impotentia generandi* and miscellaneous causes. *Impotentia coeundi* is defined as the reduced to complete lack of sexual desire and ability of male to copulate. In other words the condition of male causing failure of normal service is called *Impotentia coeundi*. *Impotentia generandi* is defined as the inability or reduced ability of a male to fertilize after normal service. Most of the causes of infertility can be detected by systematic examination of the bull and by representative semen sample.

Causes of male infertility

Impotentia coeundi

- Lack of libido
- Failure of erection
- Diseases of joints, muscles, nerve, bone, and tendons
- Tumour of the penis and prepuce
- Diseases of the penis and prepuces

    Rupture of the corpus cavernosum penis

    Deviation of the penis

    Balanoposthitis

Pizzle rot

Phimosis

Paraphimosis

Diphallus

Priapism

Hypospadias and episapadias

- Other causes

Hernia

Premature erection

Loss of sensory innervations of the glans penis

Urinary calculi

Impotentia generandi

With apparently normal semen

- Inherited sperm defects

Diadem defect

Knobbed sperm

Enzymatic disturbances

Chromosomal defects

- Infectious diseases

With apparently abnormal semen

- Cryptorchidism
- Inguinal hernia
- Imperfect testicular descent
- Testicular hypoplasia

- Testicular degeneration
- Testicular fibrosis
- Testicular neoplasm
- Epididymitis
- Spermiostasis
- Segmental aplasia
- Pathology of accessory glands
- Congenital defects
- Scrotal torsion
- Orchitis
- Testicular calcification

Impotentia coeundi

1. Lack of libido

Libido is defined as sexual desire, while serving capacity is the ability to complete the act of mating. Libido (sex drive) is a critical component of fertility. It is independent of scrotal circumference, semen quality, body weight, growth rate or masculinity. Libido is mainly genetic in nature but the environmental influences play important role in modifying it. Nutrition, systemic diseases, age, management practices etc are environmental factors affecting libido and copulatory ability.

2. Failure of erection

Penis is composed of corpus cavernosum and ischiocavernosus spongy tissues which surround the urethra. For erection of penis, corpus cavernosum is mainly responsible due to its peculiar anatomy. During erection, blood is pumped into corpus cavernosum and ischiocavernosus muscles contract which occlude the veins thereby raising the pressure within the penis. The veins are unable to drain blood out of the penis and so the penis becomes rigid and erect. Due to this pressure, lengthening and stiffening of penis occur. Failure of erection occurs either due to **abnormal venous drainage** or **occlusion of the longitudinal canals of the penis.**

Abnormal venous drainage: This condition is most commonly seen in young bulls. These bulls have normal libido, eagerness to mount but never achieve erection or intromission. This is due to failure of closing the veins that drain the blood from the corpus cavernosum during the foetal life. This vein normally closes after foetal life. Thus blood is pumped into the spongy tissue but it is drained through this vein resulting failure of erection. Prognosis is poor in this condition.

Occlusion of the longitudinal canals of the penis: This condition occurs both in younger and older bulls. In the young bulls the condition is typically caused by a congenital failure of cannulation of the short segment of the single dorsal longitudinal canal. Older bulls can be affected with a very similar condition. Such animals generally have a history of a long period of normal service behaviour, which has latterly changed into failure of erection. Libido remains normal. In such older animals, the longitudinal canals are blocked by fibrinous material.

3. Diseases of joints, muscles, nerve, bone and tendons

Disease in these structures usually causes reduction or cessation of mating behaviour in the male depending upon the severity. **Coxitis** (inflammation of hip joints) is more commonly found in dogs and boars compared to bulls and stallions. **Gonitis** (inflammation of the stifle [femorotibial] joint) is common in bulls. It is characterized by a short, stifle gait and enlargement of the capsule of the stifle joint. Polyarthrits may occur in swine due to swine erysipelas, mycoplasma or other organisms. Over-grown claws or hooves, foot rot, tendonitis, arthritis, tarsitis, dropped hock (resulting from traumatic injury of peroneal nerve), myosotis etc cause animals to failure to mount. Similarly, any lesion of the trunk affects ability to mate. In young bulls that are more enthusiastic for mating, the lumbodorsal fascia may rupture, producing the so-called condition of **'honeymoon back'**. In this condition, the pain caused by the rupture of the fascia is such that the forelimb cannot be raised during mounting. This condition is diagnosed by palpation of crepitus in the lumbodorsal region or by the presence of swollen muscle masses protruding through the fascia. As bulls age, progressive deposition of new bone occurs around the intervertebral joints resulting in inability to copulate. In dogs (especially in Dachshund) prolapse of the intervertebral discs causes compression of the spinal cord resulting in inability to copulate. In bulls, **Spastic syndromes** (progressive hind limb paralysis) interfere with copulation due to involuntary muscle contraction or spasm. In most bulls the signs are mild and persist for the whole life with occasional period of occurrence. Spastic syndrome is progressive, and because of the possibility of genetic transmission, animals (particularly bulls used for artificial insemination) should be eliminated as soon as a positive diagnosis is made.

Prognosis: It depends upon the nature and severity of the condition and the species, age and value of the animal.

Treatment: Sexual rest and confinement are advised in most cases. In bulls or rams use of electro ejaculation might permit the collection of semen in precious animals. Pain killer, tranquilizers and glucocorticoids are advised according to conditions.

4. Tumors of the penis and prepuce

The three most common tumours of the penis are squamous cell carcinoma of horses, fibropapilloma of bulls and transmissible venereal tumour of dogs.

Squamous cell carcinoma

It mainly affects old horses and appears to occur with equal frequency in castrated horses and stallions. The etiology has been associated with carcinogenic properties of the smegma accumulations in the horse. They often ulcerate and usually bleed at the time of service and produce a fetid preputial discharge.

Fibropapilloma (fibroma)

Fibropapilloma of the penis is caused by the bovine papilloma virus and transmitted by coitus. This is a benign tumour of bulls that is mainly composed of fibroblasts. The tumours are usually less than 3 cm across but they are sometimes larger. Young bulls are most commonly affected. They are single or multiple, firm, cauliflower like growths. Tumours can be found in intact and castrated animals, but rarely persist beyond 3 years of age. Haemorrhage and ulceration are the most commonly found in penis. Their presence may seriously impede copulation because of pain or mechanical interference. Spontaneous regression is common.

Transmissible venereal tumour (TVT)

TVT is most commonly seen in sexually active dogs in tropical and subtropical climates. TVT is also known as **infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or Sticker tumor.** It is a benign tumour of the dog that mainly affects the external genitalia. The disease is spread when dogs mate. The tumour does not often metastasize (occurring in about 5 percent of cases), except in puppies and immunocompromised dogs. Metastasis occurs most commonly in regional lymph nodes, but can also be seen in the skin, brain, eye, liver, spleen, testicle and muscle. Transmissible venereal tumor cells contain an abnormal number of chromosomes ranging from 57 to 64 and averaging 59, in contrast to the normal 78 of the species.

Clinical signs: In males, lesions usually localize cranially on the glans penis, on preputial mucosa or on the bulbus glandis. The tumour often has a cauliflower-like appearance. Signs include haemorrhagic discharge from the prepuce and in some cases urinary retention due to blockage of the urethra. The discharge can be confused with urethritis, cystitis, or prostatitis. Tumoral masses often protrude from the prepuce and phimosis can be a complication.

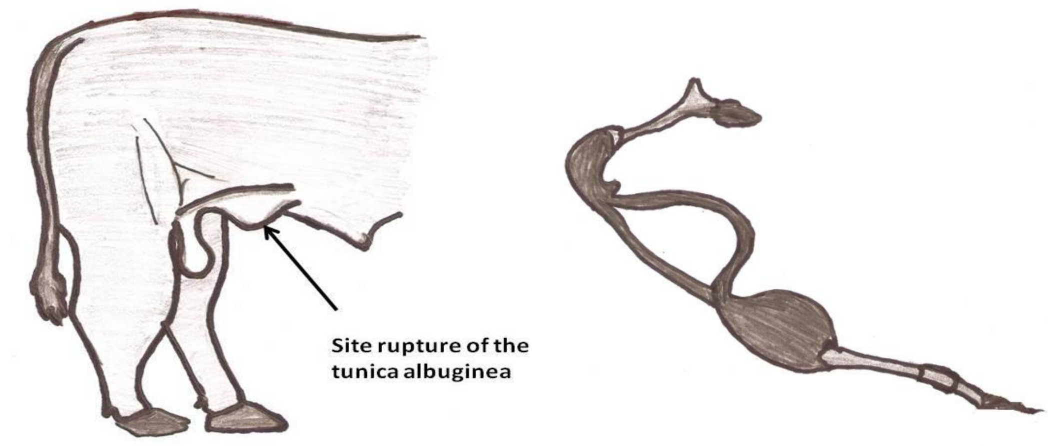
Treatment: Several treatments including surgery, radiotherapy, immunotherapy and chemotherapy have been applied for TVT. Surgery has been used extensively for the treatment of small, localized TVTs, although the recurrence rate can be as high as 50 - 68% in cases of large invasive tumours. Chemotherapy is the most effective and practical therapy. Vincristine sulfate is the most frequently used drug. Vincristine is administered weekly at a dose of 0.5 to 0.7 mg/m^2 of body surface area or 0.025 mg/kg, IV.

5. Diseases of the penis and prepuce

Diseases of the penis and prepuce are common causes for inability or difficulty in copulating.

Rupture of the corpus cavernosum penis (ruptured, fractured or broken penis)

The condition is known by many names, including rupture of the CCP, ruptured penis, fractured penis and broken penis. The corpus cavernosum is sponge-like regions of erectile tissue which contain most of the blood in the penis during erection. Rupture of corpus cavernosun is a common and serious condition of bulls. It also occurs sporadically in boars and rams. The tunica albuginea is the fibrous envelope of the corpus cavernosum. Rupture of the tunica albuginea occurs spontaneously if pressures within the CCP rise significantly above the pressures achieved during normal copulation (Fig.1 A).



A B

Fig. 1: A. Site of ruptured penis **B.** Haematoma ruptured penis.

This condition occurs when a cow move suddenly at the moment of ejaculation or the ejaculatory thrust being directed against the anus of the cow rather than the vagina. The condition is more common in younger than older bulls, due to the enthusiasm and inexperience. Immediately after rupture, the animal refuses to make further attempts to copulate. Other clinical signs include shortness of gait, preputial oedema and prolapse of penis. Haemorrhage occurs from the site of rupture and haematoma develop (fig.1B). The haematoma is initially soft and fluctuant, but later it becomes firm and hard. If untreated haematomata becomes infected, and abscesses and fibrous adhesions develop between the penis and prepuce. **Prognosis** is poor due to adhesion after treatment.

Treatment:

Conservative treatment

- Sexual rest for 90 days.
- Antibiotic therapy to prevent abscess formation in the haematoma
- Daily massage of the affected area to prevent formation of adhesions.

Surgical treatment

- Evacuation of the haematoma.
- At many times the site of rupture of the tunica albuginea is identified and closed.

- Sexual rest after surgery should only last for a few days, as long period of inactivity promotes adhesions.
- Affected bulls should be teased regularly.

Persistence of the penile frenulum

The literal meaning of frenulum is- bands of fibrous material that limits the movements of an organ or part. Persistent penile frenulum results from failure of the separation of the penis and prepuce during puberty. It is a band of tissue that extends from near the ventral tips of the glans penis to the prepuce. This band of tissue prevents normal extension and intromission. At birth the epithelial surfaces of the penis and prepuce of bulls are fused and ventrally they are united by a band of connective tissue called the frenulum. At approximately 4 months in bulls, the penis and prepuce begin to separate under the influence of male hormones. Separation should be completed by 8 to 11 months of age. The frenulum normally ruptures during separation of the penis from the prepuce.

Persistence of the penile frenulum is commonly found in young bulls. It has also been occasionally reported in boars and dogs. During erection, ventral bending of the penis occur in affected animals. **This condition prevents intromission in *Bos taurus* breed but may not interfere with breeding in *Bos indicus* bulls due to the length of the prepuce.** The anomaly is congenital and suspected to be a heritable condition. The condition can be easily corrected surgically but owners should be advised of the possibility that this is a heritable condition. To surgically correct this condition the bull is restrained in a chute and the penis is extended. The frenulum is infiltrated with 5 CC of 2% lidocaine at the penile and preputial attachments. It is then ligated at each end with #2-0 absorbable suture materials and excised. Sexual rest is allowed for 2 weeks. Healing occurs rapidly. However, surgical correction is not advisable because possibility of heritable condition.

Penile deviation (Phallocampsis)

Various penile deviations (Fig. 2) can develop which prevent penis from entering the vagina when bulls try to mate. These are:

Spiral or corkscrew

This is the most common deviation observed in penis. It occurs in bulls between 2 ½ and 5 years of age. After intromission, spiralling of the tip of the penis is a normal part of the process of ejaculation in the bull. If spiralling occurs prior to intromission, the latter cannot be achieved and the bull is described as suffering from a spiral deviation. It is caused by slipping off the **dorsal apical ligament** to the left hand side of the penis resulting in a **counter clockwise spiral** as viewed from the rear. But some studies indicate that cause is neural or behavioural rather than a defect of the architecture of the penis. It is not considered pathologic until its occurrence is observed on repeated natural breeding trials in which it occurs prior to entrance into the vulva and thereby prevents intromission. The condition can be alleviated by suturing the dorsal apical ligament to the tunica albuginea with alternating catgut and stainless steel sutures. It is questionable whether surgical correction is justifiable because some evidence indicate it is heritable in nature.

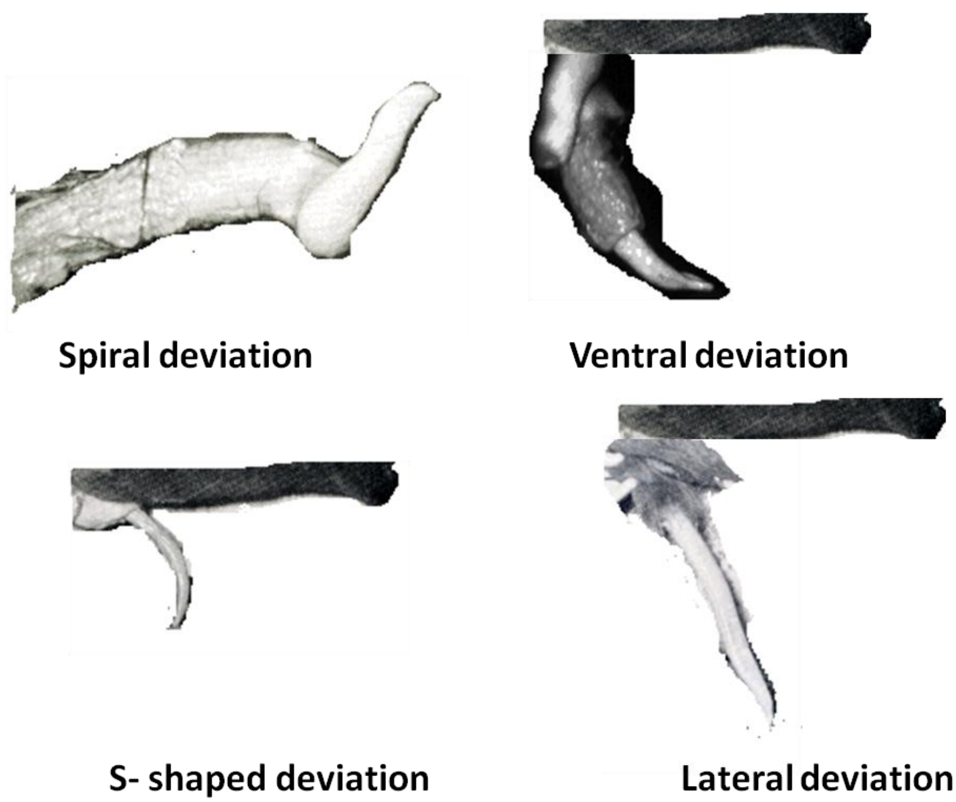


Fig.2: Different types of penile deviations in bull

Ventral or rainbow deviation

It is less common than spiral. It prevents intromission. This condition can be diagnosed by electroejaculation. It occurs when the ligament is thin and stretched to the point that it is incapable of holding up the distal portion of the penis during erection.

S-shaped deviation

It is relatively rare. It usually occurs in older bulls with an excessively long penis during erection. In S-shaped deviation, the apical ligament is sufficient in strength but insufficient in length. There is little importance of this deviation and some affected bulls breed successfully.

Lateral deviations

It may occur secondary to trauma of the penis or prepuce. Scars or adhesions of the elastic tissue pull the penis to one side or the other.

Apical ligament

The apical ligament of the penis is an extension of the tunica albuginea. This ligament provides dorsal strength and support to the erect penis to prevent downward deviation and to the right, which is the natural tendency during erection. If it is too short or the bull has an unusually long penis, then S-shaped deviation may occur. A spiral deviation occurs when the ligament slips off the dorsal surface to the left side.

Treatment: Fascia lata implant technique is used for repair of both spiral and ventral penile deviation. The objective of the technique is to create a firm union of the dorsal apical ligament to the tunica albuginea to prevent its slippage. This technique uses an autogenous or homologous strip of fascia lata from the cranial border of the biceps femoris muscle to provide dorsal reinforcement of the apical ligament and fixation of the ligament to the tunica albuginea. Homologous strips of fascia lata can be harvested from freshly dead cattle and stored in 70% ethyl alcohol until used.

Balanoposthitis

Balanoposthitis is an inflammation of the glans penis and the prepuce. It is common in the dog, bull and ram, occasional in the stallion, and rare in the boar and cat.

Many organisms including bacteria, moulds, protozoa, and virus are causative agent. IBR–IPV causes an acute, ulcerative inflammation of the penis and prepuce. Secondary bacterial infection of the ulcers results in a severe, purulent balanoposthitis. Affected bulls may exhibit swelling and pain in the region of the penis. Other symptoms are unwillingness to mate, adhesions between penis and prepuce, preputial stenosis, and peripenile adhesions. **Treatment** is symptomatic which consists of sexual rest and infusion of oily suspensions of antibiotics into the prepuce. Healing occurs over 2–8 weeks. In neglected cases, fibrinous adhesions may develop between adjacent ulcers on the penis and prepuce, eventually resulting in an impaired protrusion of the penis. The virus is transmissible in semen, which presents considerable risk if bulls that either have active infection or are seropositive are used in AI programmes. The most serious cause of balanoposthitis in the stallion is the notifiable disease of dourine. Dourine is caused by the protozoan parasite *Trypanosoma equiperdum.*

Pizzle rot

Pizzle rot or ulcerative posthitis is principally a disease of sheep but occasionally is seen in goats and cattle (usually in castrated males). Pizzle means the penis of an animal and 'Rot' means decaying caused by bacterial or fungal action. It is a contagious infection that most commonly affects castrated sheep because of the hypoplastic nature of the penis and failure of penile-preputial separation that leads to pooling of urine in the prepuce. While most common in wethers the infection also occurs in intact rams and in ewes. Other names for this infection are sheath rot, enzootic balanoposthitis and ulcerative vulvitis (when it occurs in ewes).

Etiology: Pizzle rot is the result of an interaction between a bacteria and some other factor. The bacterium is *Corynebacterium renale*. These bacteria have the ability to break urea using own enzyme, **urease**. The other factor is an increase in the protein level of the diet. Once the protein in the diet rises above 16%, urine will have more than 4% urea. This excess urea makes the urine alkaline. The bacterial urease breaks down the **urea** to release excess **ammonia** which causes a severe irritation and ulceration of the skin around the preputial opening. Once the skin is ulcerated, *C. renale* or other bacteria will infect it. The debris from the ulcer forms a crust which may block the opening to the prepuce. The infected ulcers can spread through the opening to the mucosa of the preputial cavity. Any scar tissue formed around an untreated ulcer can permanently constrict the preputial opening to prevent extrusion of the penis at breeding. Once the opening is blocked, urea dribbles out to stain the surrounding wool. Internal ulceration is painful. The prepuce becomes enlarged and swollen, containing old urine and debris. If there is severe interference with urination, the ram may become uremic and die. Animals grazing on rich green pastures or given extra feed are at risk. Therefore, the condition is seasonal in nature, coinciding with the growth of lush pasture land.

Symptoms: Mild cases of pizzle rot result in a swelling of the prepuce. However in severe cases, the swelling and inflammation may interfere with urination resulting in straining to produce urine. In very severe cases this urinary retention causes the animal to become uraemic and die.

Treatment and control: If possible, affected animals should be isolated from the rest of the herd and not fed a high-protein diet. Lesions should be examined to ensure that they do not interfere with urethral patency. Clipping and cleaning hair around the prepuce may be beneficial. Remove the dead tissue in the ulcer with a debriding agent, such as a dilute hydrogen peroxide solution. *C. renale* is usually sensitive to penicillins and cephalosporins. Apply an ointment containing penicillin, bacitracin or 5% copper sulphate at weekly intervals. If there is a suspicion that the ulcer has spread to the mucosa of the preputial sac, penicillin injections will help the healing. Implantation of wethers with 70-100 mg of testosterone every 3 month is effective in preventing ulcerative posthitis. A ram with pizzle rot should not restart breeding until the ulcers have completely healed.

Phimosis

The term 'phimosis' is derived from the Greek word for 'muzzling.' Phimosis is the inability to protrude the penis beyond the preputial orifice. This is a condition in which the opening of the prepuce is too small to extrude the penis. It has been found in most of the domestic species. Although it is usually a congenital defect but it can also be the result of neoplasia, oedema, or fibrosis following trauma, inflammation, or infection. **In dogs**, it is generally congenital defect, particularly in the **German shepherd and golden retriever breeds**. When the male gets an erection, the penis cannot pass through the preputial opening. During breeding, the male may make repeated and unsuccessful attempts to mate. The penis trapped in the sheath causes a large bulge under the skin. These animals frequently traumatize themselves during mating attempts resulting blood dripping from the preputial opening. In severe cases, it can cause problems with urination. Diagnosis is made by physical examination of the prepuce and penis. Surgical enlargement of the preputial orifice is indicated if the animal is to be used for breeding.

Paraphimosis

This condition occurs when the penis protrudes from the preputial sheath and cannot be replaced to its normal position. The condition is most common in the dog and the stallion, but is also reported in most domestic species. Paraphimosis following copulation or spontaneous erection is relatively common in the dog (Fig. 3). The skin at the preputial orifice becomes inverted, trapping the extruded penis and impairing venous drainage. Other causes of paraphimosis include mild phimosis, a constricting band of hair at the preputial orifice, or trauma. In stallions, prolapse of the penis is the sequel to many conditions. For example, it generally occurs transiently after the administration of phenothiazide tranquillisers. The symptoms are similar to that of the dog: namely, swelling, oedema and inflammation, followed by trauma, ischaemia and necrosis.

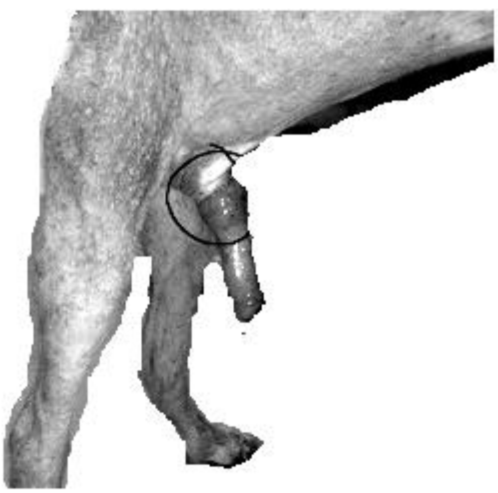


Fig. 3: Paraphimosis in dog.

Paraphimosis is a medical emergency. The exposed penis quickly becomes edematous because venous drainage is compromised. With continued exposure, it becomes dry and painful. If untreated, ulceration, ischemic necrosis, or gangrene may develop. If recognized early, before severe edema; paraphimosis can be easily treated. Treatment consists of gentle cleansing and lubrication of the exposed penis. In the early stages, oedema may be controlled by the use of cold water and ice packs, whereas in the later stages the use of anti-inflammatory drugs and diuretics is essential. The penis is replaced inside the prepuce by sliding the prepuce first in a posterior direction, extruding the penis further. This everts the skin at the preputial orifice; usually the prepuce then slides easily over the penis. The edema resolves promptly once circulation is restored. Where the condition fails to resolve, amputation of the penis may become necessary.

Diphallus

It is also called double penis. It is a rare congenital anomaly that occurs when two genital tubercles develop. The penis is partly or completely duplicated and may or may not be symmetrical. It is rare condition in bulls. It is often associated with urogenital or other anomalies. It prevents normal copulation.

Priapism

DO YOU KNOW?
The name comes from the Greek God Priapus (Fig. 2). In Greek mythology, **Priapos,** Latinized as **Priapus**, was a minor rustic fertility God, protector of livestock, fruit plants, gardens and male genitalia. Priapus was best noted for his large penis with permanent erection.

Fig. 2: Roman God Priapus, a god of fertility (particularly of farm animals) gifted with permanent erected penis.

Priapism is a potentially harmful and painful condition in which the erect penis does not return to its flaccid state, despite the absence of both physical and psychological stimulation. The pattern of blood flow to the penis that occurs during normal erection is altered in priapism resulting into edema, increased risk of abrasion, tissue drying and necrosis of the penis. The prognosis depends on the type of priapism and the amount of time that passes before therapeutic intervention. Surgical methods, such as aspiration and shunting procedures, have traditionally been used to treat priapism but carry a risk of postsurgical complications. Use of α-agonists for treatment of priapism in humans is often successful and avoids the risks of impotence and other surgical complications. Investigation of the use of α-agonists for treatment of priapism in animals should be considered.

INTERESTING FACT

There were two outbreaks of priapism in French soldiers eating frog legs in South Africa in the 19^{th} century. The French doctor observed that the symptoms were similar to those which are indulged in taking a drug called cantharidin. This drug is extracted from beetle fly. One of the attending doctors dissected the local frog and found that its gut was full of beetle which produce cantharidin. Recently, it has been seen that frogs eating this beetle have high levels of cantharidin in their thigh muscles that are enough to cause priapism.

Hypospadias and epispadias

Hypospadias and epispadias are birth anomalies that result from defective development of the penis during embryological development. Normally the urethra runs the entire length of the penis, forming an opening at the tip. However, the penis sometimes does not form correctly and the urethra fails to reach the tip of the penis. The incompletely-developed urethra may form an opening at the underside of the penis (hypospadias) or the upper side of the penis (epispadias). The consequences of such lesions depend upon the site of the urethral opening. In the mildest cases, the urethral opening forms just short of the tip of the penis. In more severe cases, the opening forms closer to the scrotum. When this is close to the distal end of the penis, the condition may be undetected and have no effect upon fertility. However, more proximal lesions adversely affect fertility.

Impotentia Generendi

Apparently normal semen production

Recent studies in cytogenetics have shown that gene or chromosome defects may occur at the time of meiosis and result in infertility. An apparently normal semen picture may accompany these defects. Affected spermatozoa may fertilize the ovum, the zygote will often lack of a balanced gene complement and death often results at an early stage of gestation.

Abnormal semen production

This may be due to diseases of testes, epididymis, vas deferens and accessory glands. All these diseases will be discussed in separate chapters. Due to these diseases, sufficient numbers of healthy fertile sperm cells are not produced and results in failures of fertilization or abnormal development of embryo. The abnormal sperm may be due to congenital defect, hereditary or acquired causes.

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Success does not consist in never making blunders, but in never making the same one a second time. ***- Josh Billings***

Testicular Hypoplasia

Digest

The female porcupines are interested in sex only about 8 to 12 hours in a year! Once mating begins, the female is insatiable: she forces the male to mate many times until he is thoroughly exhausted. If he gets tired too quickly, she will leave him for another male!

Testicular hypoplasia is a congenital failure in the development of the spermatogenic epithelium while the intersititial tissue and Leydig cells are developed normally. It is found in two forms: hereditary and non-hereditary.

Hereditary testicular hypoplasia: It is analogous to ovarian hypoplasia caused by a **recessive gene with variable expression and incomplete penetration.**

Non-hereditary testicular hypoplasia: It is due to one or more extra X chromosome (e.g. XXY). The best example is in the **male tortoiseshell cat** (see box) which has an XXY karyotype. Chromosomal testicular hypoplasia always leads to complete sterility probable as a consequence of disturbed meiosis.

Testicular hypoplasia may affect one testis (unilateral) or both testes (bilateral). The degree of hypoplasia varies from partial to complete and only in bilateral complete hypoplasia the animals are sterile. The affected testis is reduced in size depending on the degree of hypoplasia. The development of other genital organ is normal, sexual desire is not affected.

Note: Congenital characteristics can be either inherited or acquired.

Fertility of male tortoise shell cats

Most male tortoiseshell cats are infertile. Male tortoiseshell cats with the chromosome complement of 39, XXY are infertile because the extra X chromosome in spermaogonia blocks spermatogenesis and such animals are known as Klinefelter cats.

Incidence

- Incidence is low and condition is sporadic.

- Testicular hypoplasia is commonly observed in bulls and boars and is infrequent in rams, stallions, dogs and cats.
- **Unilateral testicular hypoplasia is more common than the bilateral hypoplasia.**
- **In bulls, left sided hypoplasia is more than the right sided.**

Etiology

- It is mostly caused by **autosomal recessive gene with variable expression and incomplete penetrance.**
- Partial or complete failure of germinal cells to develop in the yolk sac during fetal life.
- Failure of migration of germinal cells from yolk sac to gonadal ridge during fetal life.
- Failure of multiplication of germinal cells in gonad during embryogenesis.
- Degeneration of embryonic germinal cells
- Klinefelter is syndrome (Karyotype XXY).

Symptoms

- Lower conception rates in bilaterally affected males.
- The affected testes are usually firmer than normal.
- The affected testes are 1/3rd to 2/3rd of normal size.
- The scrotum of the affected male is smaller than normal male.
- The spermatic cords of hypoplastic testicles are shorter.
- A striking feature in partial hypoplasia is an **increased incidence of proximal protoplasmic droplets** in spermatozoa.
- An increased frequency of abnormal sperm head is another common signs.
- **Sexual desire:** In most cases sexual desire is excellent and coitus is prompt because Leydig cells remain unaffected.
- **Semen picture**

  Unilateral or partial

- ✓ Low concentration
- ✓ Low motility
- ✓ High incidence of proximal protoplasmic droplets
- ✓ Presence of giant cells and medusa cells

Bilateral and complete

- ✓ Clear and watery
- ✓ Contain few or no sperm.

Diagnosis

- **Size of scrotum:** If this value is below the acceptable limit for the species and breed, the male is suspected for severe testicular hypoplasia.

Scrotal Circumference and Testicular Hypoplasia
The society for theriogenology has published minimum standards for scrotal circumference in bulls. Scrotal circumference measurement of less than 30 cm or 32 cm in post pubertal bull has been considered for testicular hypoplasia in the bull.

- **Size of testis:** The hypoplastic testis is small and flabby but regular in outline and freely movable in the scrotum while atrophic testis feel more sclerotic at palpation and adhesions are frequently present.
- **Size of epididymis: small and hard**
- **Libido – Normal**
- **Semen picture:** see symptoms
- **Karyotype analysis:** Mild cases are difficult to diagnose on the basis of above criteria. For this karyotype analysis may aid diagnosis. Chromosomal secondary constrictions remain present in high incidence in leukocyte culture.

CLINICAL POINTER
The diagnosis of testicular hypoplasia should not be done before two years of age in the bull and horse and before one year of age in the boar, ram, dog and cat unless the male is well grown

because many times testicular hypoplasia may be erroneously diagnosed in young immature males that are underdeveloped or poorly fed or slow maturing males.

Prognosis: Poor

Treatment: Affected animal should not be used for breeding purpose because the condition is hereditary.

CLINICAL POINTER

Since libido is generally normal in testicular hypoplasia. Hence, the condition may escape the owner's attention until unsatisfactory pregnancy rates are noticed.

HYPO-ORCHIDISM

Mant times, it is clinically difficult to differentiate testicular atrophy from testicular hypoplasia. Therefore, the term hypo-orchidism has been coined to include both testicular atrophy and testicular hypoplasia.

Difference between hypoplastic testicles and atrophic testicles

Hypoplastic testicles	Atrophic testicles
Testes are firm	Testes are sclerotic
Freely movable in the scrotum	Adhesionss are frequently present.

DEFINITIONS

The definitions of some terminology are given below because these terms are used frequently but many times we do not understand the exact meaning.

Sex linked genes: The genes that are found on sex chromosomes are said to be sex linked. The term 'linkage' indicates where the gene is located; not about the function or action of the gene.

Sex- limited genes: Those genes whose expression is limited. The expressions of the genes depend on the sex of the animal and the genes are found on the autosomes. The term 'limited' refers to when the gene is expressed. For example some genes are associated with milk production. The genes are carried by both male and females but only the female can express the genes. Such genes are said to be sex limited.

Incomplete gene penetrance: The concept of incomplete gene penetrance refers to a group of animals carrying a particular gene but only some of which exhibit the trait or characteristic. Thus the genes 'penetrate' and get expressed in some animals but not all animals. This incomplete penetrance is due to **epistatis phenomena**.

Variable expressivity: It occurs when all animals carrying a gene and express the trait but to variable degree. Variable expressivity is due to polygene effect.

The concept of gene penetrance and variable expressivity are often confusing. Gene penetrance is an **all or none effect** i.e. the characteristic is either expressed or not, while in variable expressivity all animals express the gene but to variable extents.

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Competition is a by-product of productive work, not its goal. A creative man is motivated by the desire to achieve, not by the desire to beat other- ***Ayn Rand***

Testicular Degeneration

Digest

The annual mating of ***red-sided garter snakes*** *is a tourist attraction in Manitoba, Canada. This is because when a female garter snake emerges from hibernation, she releases a pheromone that attracts hundreds of male snakes in the vicinity to rush her and create a large squirming "mating ball."*

The literal meaning of degeneration is deterioration of a tissue or an organ in which its function is diminished or its structure is impaired. Testicular degeneration is defined as the partial or complete failure of epithelium of seminiferous tubules to proceed with spermatogenesis. It is an acquired disorder and is **most common cause of infertility in male domestic animals.** Testicular degeneration is an acquired condition in which testicles that were initially normal, undergo pathologic changes resulting in small testicular size and abnormal function. Testicular degeneration may develop very rapidly within hours or few days but its regeneration takes very long time (over weeks or months).

Testicular Cells and Degeneration

The seminiferous tubules of the testis are highly susceptible to damage from a wide variety of agents which cause reversible or irreversible degeneration. Differentiating germinal cells like primary spermatocytes, secondary spermatocytes and round spermatids are most susceptible to degeneration. Spermatogonia are the most resistant among all types of germinal cells. Sertoli cells are considered to be the most resistant to testicular degeneration. Therefore, the **ratio of Sertoli cells to germinal cells is used as an index to access the degree of testicular degeneration present**. Spermatogonia cells provide a foundation for the possible regeneration of degenerated testicles. If the basal layers of germinal epithelium including spermatogonia and Sertoli cells are destroyed, regeneration of the germinal epithelium is not possible and the animal becomes sterile.

Etiology

There is seemingly endless list of potential causes of testicular degeneration. Some of these are:

- **Hyperthermia:** It is the most common cause of testicular degeneration and can result from
  - ✓ Scrotal fat
  - ✓ Scrotal dermatitis
  - ✓ Orchitis
  - ✓ Periorchitis
  - ✓ High environmental temperature
  - ✓ Fever
  - ✓ Systemic diseases
  - ✓ Inflammation of scrotal skin
  - ✓ Toxaemia
  - ✓ Cryptorchidism
  - ✓ Inguinal hernia
  - ✓ Testicular trauma
- **Extreme cold** resulting in scrotal frostbite is reported to lead to testicular degeneration. This occurs due to ischemia in which blood flow to the inner parenchyma of the testis is progressively impeded with lowering temperature.
- Arsenical dips
- Parasitism
- Blockage of parts of the excurrent duct system such as epididymis.
- Advanced age: Most bull will undergo degenerative changes by 8 to 10 years of age. Increasing age is a major factor in testicular degeneration.
- Stress: Stress-related degeneration occurs due to the inhibition of LH secretion by the corticosteroids that are released during stress.

- Irradiation
- Auto-immunisation
- Hormonal imbalance
- Testicular neoplasms
- Nutritional disorders
    - ✓ Negative energy balance
    - ✓ Fatty acid deficiency
    - ✓ Hypovitaminosis A
    - ✓ Hypervitaminosis A
    - ✓ Hypovitaminosis B
    - ✓ Hypovitaminosis C
    - ✓ Hypovitaminosis E
    - ✓ Protein and amino acid deficiency
    - ✓ Zinc deficiency.

Incidence

- Testicular degeneration is **most common cause of infertility in male**.
- It may be unilateral or bilateral but **bilateral is most common** because it occurs mainly due to systemic problem while unilateral is the result of a local event.

Symptoms

- Testes that are undergoing degeneration are usually smaller and softer than normal. In chronic cases, there may be fibrosis and calcification that lead to a firmer and smaller testicle.
- The first symptom is a reduction in fertility but the libido of the bull is not affected.
- Clinical signs of infertility and abnormalities in spermatozoa are usually observed **4-8 weeks after the onset of the cause of the degeneration.**

- Adhesions between testis and scrotum are usually found.
- In case of testicular hypoplasia, the epididymis is not as well developed but in cases of testicular degeneration, the **epididymis remains disproportionately larger.**
- Fibrosis and calcification also occur in severe and late stage.
- Testicles become hard shrunken and irregular in late stage.

Semen picture

The semen picture ranges from nearly normal to watery depending on the degree and duration of involvement of testicular tissue.

- ✓ The first sign of degeneration is the **increase in the incidence of loose heads (early stage).**
- ✓ Presence of **immature spermatozoa is a typical characteristic of late stage.**
- ✓ The **tail abnormalities are not usually associated** with testicular degeneration.
- ✓ **Ejaculate volume is usually unaffected.**
- ✓ Sperm motility falls.
- ✓ Sperm abnormalities rise.
- ✓ An early sign of improvement is that abnormal spermatozoa are replaced by relative high percentages of distal protoplasmic droplets spermatozoa.

Diagnosis:

It is based on

i) History

ii) Careful examination of the scrotal contents.

iii) Scrotal circumference measurements

iv) Semen picture

v) Ultrasound examination

vi) Histology of testis

- ✓ Thickness of the germinal cells decrease.
- ✓ Presence of **pyknotic nuclei** in the germinal layer is very common.
- ✓ **Cypoplasmic vacuolation** of spermatocyte
- ✓ Presence of **giant cells** in the tubules.
- ✓ Hyaline thickening of the basement membrane.
- ✓ Fibrosis
- ✓ Apparent interstitial cell hyperplasia.
- ✓ Presence of lymphocytes and plasma cells in the tubules may be indicative of an immune response to degenerate sperm cells.

Prognosis

The prognosis of testicular degeneration is variable depending upon the causative agents, the duration and degree of the degeneration.

Mild cases	-	fair to good
Moderate	-	guarded to fair
Severe	-	poor

Treatment

- Correction or alleviation of causative factors.
- Supplementation of nutrition.
- Supplementation of vit. A.
- Animal should be kept in cool place.
- Sexual rest.

Giant Cells and Testicular Degeneration
• Two types of giant cells are produced during testicular degeneration: mononuclear giant cells and multinucleate giant cells.

- Mononuclear giant cells are derived from **pachytene spermatocytes** that fail to differentiate further.
- Multinuclear giant cells are derived from coalescence of identical spermatids.

INTERESTING FACT

Before the discovery of electron microscope and biochemical studies of seminiferous epithelium, it was thought that the germ cells were vulnerable to increased temperature whereas the Serotoli cells were relatively resistant since they remained present as a lot of germ cells degenerated. But recent studies indicate that the Sertoli cells are acutely sensitive to temperature rise, showing ultrastructural and functional abnormalities previously unsuspected. Sertoli cells may remain in the testes of severely damaged heat treated testes but their morphology and function is markedly altered. Why Sertoli cells survive heat treatment is unknown and conversely why most germ cells readily degenerate in response to elevated temperature is also not known. The exact mechanisms by which the seminiferous epithelium undergoes degeneration and the Sertoli cells become dysfunctional remain unknown.

Leydig Cells and Degeneration

The Leydig cells do not degenerate but become dysfunctional. These cells persist in heat-stressed testes of animal including man. They continue to secrete androgen, albeit at reduced or low-normal levels and libido is generally not markedly affected.

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Most X-rated films are advertised as "adult entertainment," for "mature adults," when in reality they are juvenile entertainment for immature and insecure people. - Zig Ziglar

Seminal Granulomas

Digest

Young elephants are blind at birth and rely upon their trunks and their mothers to help them.

Definition

Formation of granuloma around the head of the epididymis due to accumulation and inspissations of sperm at the blind end of efferent ducts is called seminal granulomas.

Species

Sperm granulomas are common in **buck**. It is frequently, associated with the **polled conditio**n but has also been observed in horned bucks.

Transmission

This condition is believed to be **heritable and recessive with incomplete penetrance**.

Physiopathology

The normal head of the epididymis forms from the union of approximately 16 to 19 efferent ducts. If one or more of the efferent ducts in the head of epididymis ends blindly, spermatozoa will accumulate into the blind segments. The accumulated sperm undergo degeneration and release mycolic acid. The ducts become distended with inspissated sperm until rupture occurs. After rupture, sperm are released into the stroma of the epididymis. A severe inflammatory reaction with lymphocytes and giant cells occurs in response to the sperm cells and eventually a granuloma form, which may calcify later. The sperm granulomas vary in size from pinhead to several centimetres in diameters. It causes back pressure which lead to degeneration of the testicular stroma. If all the efferent ducts of an animal are blind, the animal becomes sterile. In this condition normal libido persists.

Diagnosis

It can be confirmed by gross and histological examination after castration or slaughter.

Treatment

Correction of the defect is not possible.

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High achievement always takes place in a framework of high expectation. - ***Jack Kinder***

PART III

Artificial Insemination

Cryopreservation of Semen

Digest

The semen of alpacas and llamas is highly viscous and makes assessment of sperm concentration, morphology, and motility difficult. A comparatively high proportion of morphologic abnormalities (up to 40%) are common. Unlike the progressive motility of sperm seen in other domestic ruminants, only oscillatory movement is seen in the ejaculate of llamas and alpacas.

Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures. At these low temperatures, any biological activity is effectively stopped.

Principle of cryopreservation

Cryopreservation includes several steps such as, dilution, cooling, freezing and thawing. The phenomena which cause damage to sperm cells during cryopreservation are mainly **cold shock, solution effects** and **intracellular ice formation**. Egg yolk in the extender prevents the sperm from cold shock during cooling. The freezing rate is directly related to the water permeability of the sperm cell membrane. The cells that have higher water permeability require a higher freeze rate for survival. Ideally, extracellular ice formed during freezing will lead to increased osmotic pressure in the unfrozen medium, which in turn will pull more water out of the cell. The concentration of salts will thus increase both intra- and extracellularly as water freezes. Thus, too rapid freezing rate causes structural damage by intracellular ice formation, and too slow freezing rate causes salt damage to proteins (solution effect). In this way, the survivability of the spermatozoa depends upon the optimum-cooling rate, which results in less intracellular dehydration, less intracellular solute concentrations and less shrinkage of the cells (Fig.1). In addition to that it is very essential to add a cryoprotectant (glycerol etc) in the extender because they modify ice crystal formation during freezing and thawing.

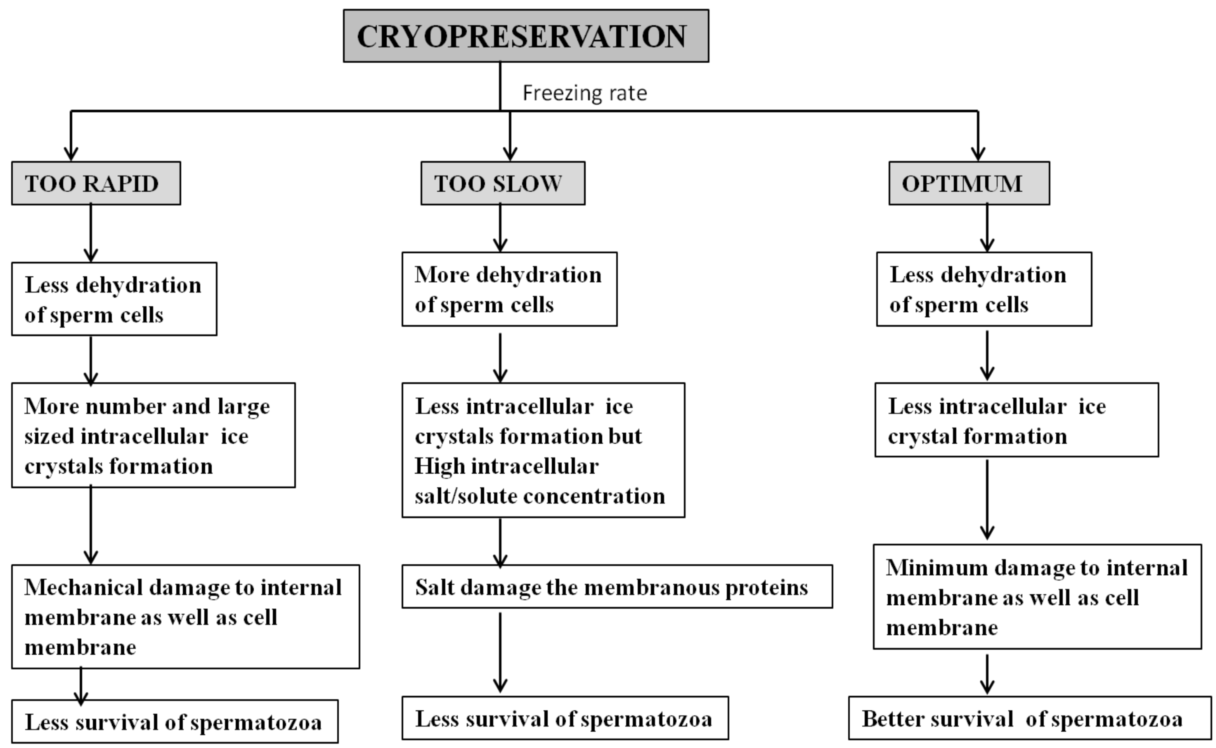


Fig.1: Flow diagram showing effect of different freezing rates.

Physiology of cryopreservation

There are two types of fluids involved in semen freezing- one is inside the sperm cells (intracellular fluid) other found surrounding of the sperm (extracellular fluid) is mixture of seminal plasma and extender. When temperature of semen falls below the freezing point ice crystal starts to form. During freezing, ice crystal is formed first extracellularly because it is more near to liquid nitrogen vapour than intracellular fluid. As we know that ice is pure water, as ice formation occurs, the concentration of unfrozen extracellular fluid will increase. Ice crystals do not extend intracellularly at this stage, as they are excluded by the cell membrane. Now the condition becomes hypertonic outside sperm cells, therefore, intracellular fluid comes outside by osmosis to maintain the equilibrium resulting in variable degree of sperm cell dehydration which is followed by the formation of intracellular ice crystals. Thus, during freezing, sperm cells are damaged by high concentration of solute (solution effect) inside the sperm cells and formation of ice crystal especially intracellular ice crystal. The spermatozoa find themselves confined to very narrower channels of un-frozen solution, squeezed in between growing masses of ice, which could also lead to mechanical stress in cells. During fast freezing, sperm are damaged mainly due to intracellular ice crystal formation because there is very little time for water to move from inside the sperm to outside through semi permeable sperm membrane to create equilibrium, and hence large intracellular ice crystals form, causing physical damage to cell membranes and components. Thus, too rapid freezing rates cause structural damage by intracellular ice formation. However, the problems of dehydration and solute concentration are less evident with rapid cooling. During slow freezing, sperm are mainly damaged by solution effect because sperm cells get enough time for water movement from inside to outside resulting in high concentration of solute inside the sperm cell but small intracellular ice crystals formed. Thus, extremely high concentrations of salts inside the sperm cells affecting the cell membrane or intracellular components. In this way, the survivability of the spermatozoa depends upon the optimum-cooling rate, which results in less intracellular dehydration, less intracellular solute concentrations and less shrinkage of the cells.

Bull semen freezing protocol

Freezing protocol may vary from one semen lab to other lab. These protocols are two step dilution and packaging at room temperature, one step dilution and packaging at 4^0C, and one step dilution and packaging at room temperature. Here, we will discuss one step dilution and packaging at 4^0C.

1. Preparation of extender

- Prepare the extender on the day of collection or on the previous day in the evening.
- Keep the extender in water bath at 32^0C before start of collection**.**

2. Semen collection

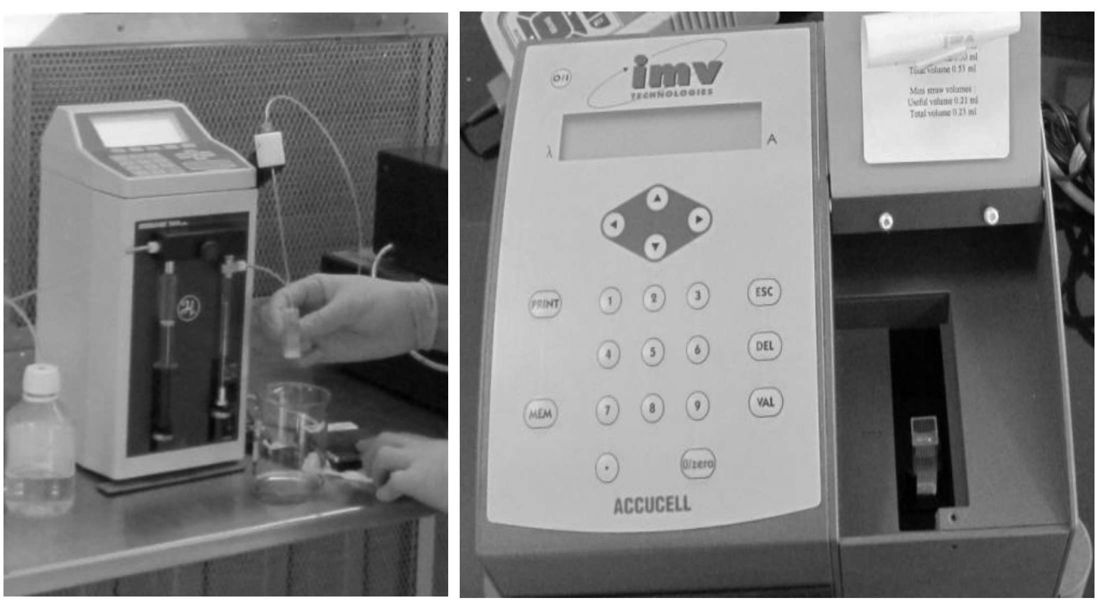
- Semen is collected in the early hours of morning before feeding.
- From each bull two ejaculate should be taken on the day of collection and the interval between the two collections should be 10 -12 minutes (as per MSP).

3. Semen evaluation

- As soon as the neat semen is received, it should be kept in a water bath at 32° C under Laminar Unit (Fig. 1), after recording the volume of semen.

Fig. 1: Neat semen is being kept in water bath under Laminar flow

- Immediately after collection, evaluate semen for volume, colour, consistency, presence of foreign matter, mass motility, individual motility and concentration.
- Place one drop of semen on glass slide and observe under microscope for mass motility. Ejaculates having more than +3 motility are selected for further processing.
- Sperm concentration and volume of extender to be added are determined with the help auto diluter and digital photometer manufactured by a reputed company (Fig. 2).



A B

Fig. 2: A. Auto diluter, B. Digital photometer.

4. Initial dilution

- Till the final dilution rate is decided, semen is diluted with an equal quantity of diluents and kept in the same water bath.
- The progressive motility of extended semen is assessed with the help of phase contrast microscope (Fig.3) and the ejaculate showing more than 70 percent of motility is taken for further processing.

Fig. 3: Progressive motility is being assessed by phase contrast microscope

5. Final dilution

- The diluter and semen ejaculate should be kept in water bath at same temperature (32^0C).
- As soon as final volume of diluted semen is calculated, the remainder of the diluents is added to pre diluted semen so that each straw should contain 20 million progressive motile spermatozoa (as per MSP).
- The flask/capped bottle/measuring cylinder containing diluted semen is kept in another large beaker containing water at 32^0C (the level of water should not be above the level of diluted semen) and is placed in cold cabinet (Fig. 4). Anyway, 4^0C temperature should be reached within 1- 1.5 hour. This slow cooling prevents the sperm from the cold shock and egg yolk in the extender plays important role to prevent cold shock. Lecithin and phospho-lipid in the egg yolk make a protective layer over sperm membrane which prevents the sperm from cold shock.

Fig. 4: The extended semen is being kept in cold cabinet.

6. Equilibration of semen

The pre- freeze storage of diluted semen is known as ‘equilibration’ of diluted semen. Equilibration period refer to the time from adding the glycerol fraction of extender until freezing. In order to extend the fertile life of the sperm, temperature is reduced to decrease the metabolic rate. A thumb rule is that metabolic rate doubles for every 10 degrees, so cooling semen from body temperature of about 39^0C to 4^0C reduces the metabolic rate to about 1/10 of that at body temperature. After the initial cooling period from 32^0 C to 4^0 C, the extended semen should be allowed to equilibrate at 4ºC for at least 4 hours. The changes occurring in the sperm cell membrane during this period increase sperm survival during freeze- thaw process. It was originally considered that this was the period over which glycerol penetrated the sperm, although more recent studies indicate that the penetration of glycerol is very rapid and most of the equilibration period is concerned with membrane stabilization during exposure to low temperatures.. During this time, the straws are filled with extended semen and sealed and placed on racks for freezing in cold cabinet.

7. Printing of straws

The name or number of bull, breed, name of semen bank and date of semen collection are printed on the straws (Fig. 5) by using automated straw printing machine (Fig. 6) before filling of straws. Thereafter, straws are sterilized in ultraviolet ray by keeping in UV switched on laminar flow for few minutes.

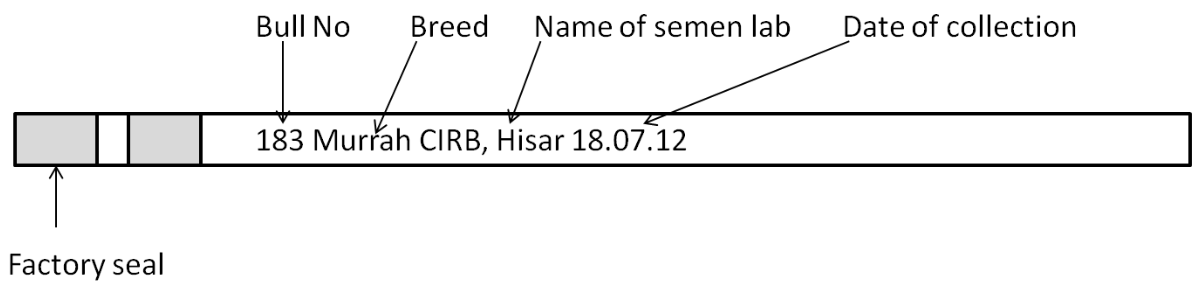


Fig. 5: Printed semen straw

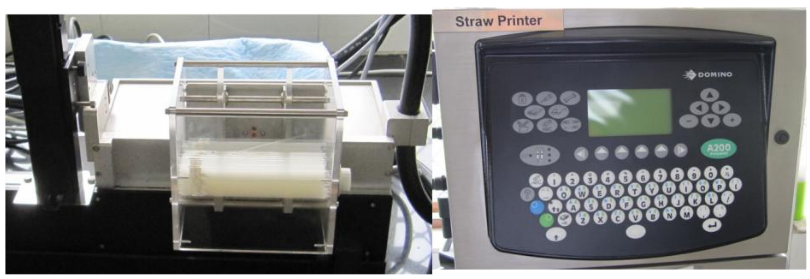


Fig. 6: Straw Printer

8. Pre- freeze semen evaluation

At the end of equilibration period, semen samples are evaluated for pre- freeze motility. The ejaculates showing ≥70% pre-freeze motility are selected for freezing.

9. **Filling and sealing of straws**

Manual as well as automatic filling and sealing machine are available for filling and sealing of semen in the straws. Nowadays, manual method of filling is not commonly used because automatic filling and sealing machine are more efficient and easy to handle. The diluted semen is filled either in mini or medium straws. In automatic filling and sealing machine (Fig. 7), semen is sucked into the straws and on touching the polyvinyl alcohol powder plug at one end and impervious seal is made. The other end is pinched together. Filling and sealing of straws are done in cold cabinet at 4^0C during the equilibration period.

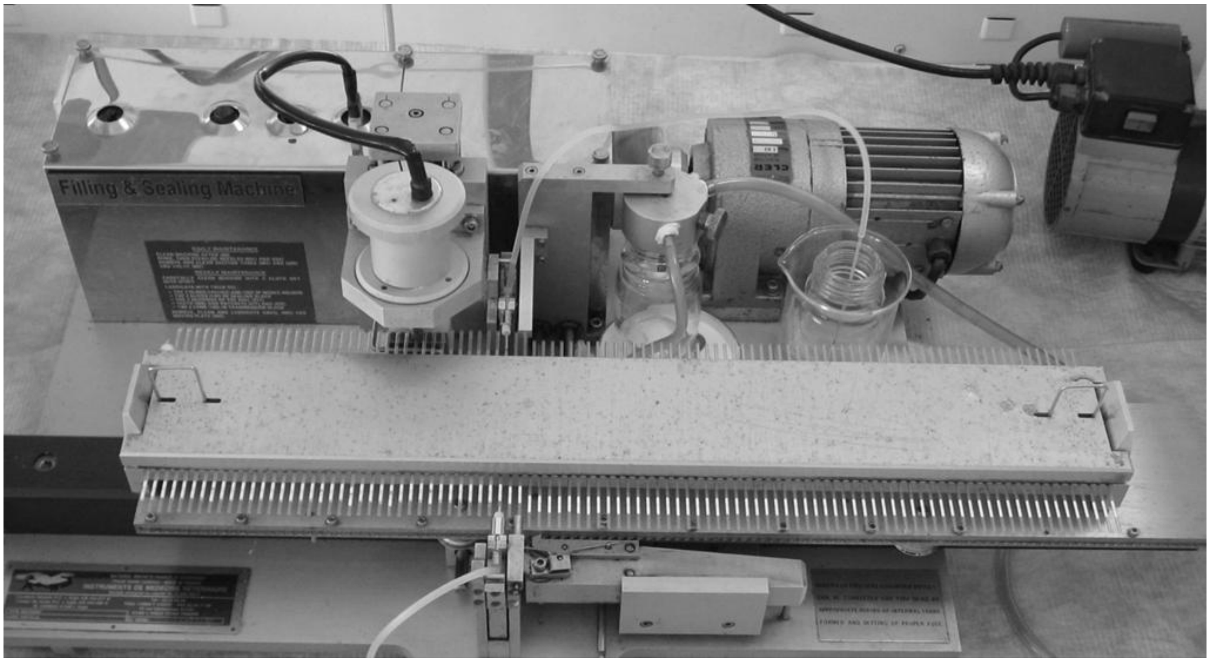


Fig. 7: Filling and sealing machine.

10. Racking

Once the filling and sealing is done, the straws are carefully spread over freezing rack (Fig. 8) inside the same cold cabinet at the same temperature. Thus, in the large cold cabinet, semen storing during equilibration period, filling and sealing of straws, and racking of straws are done.

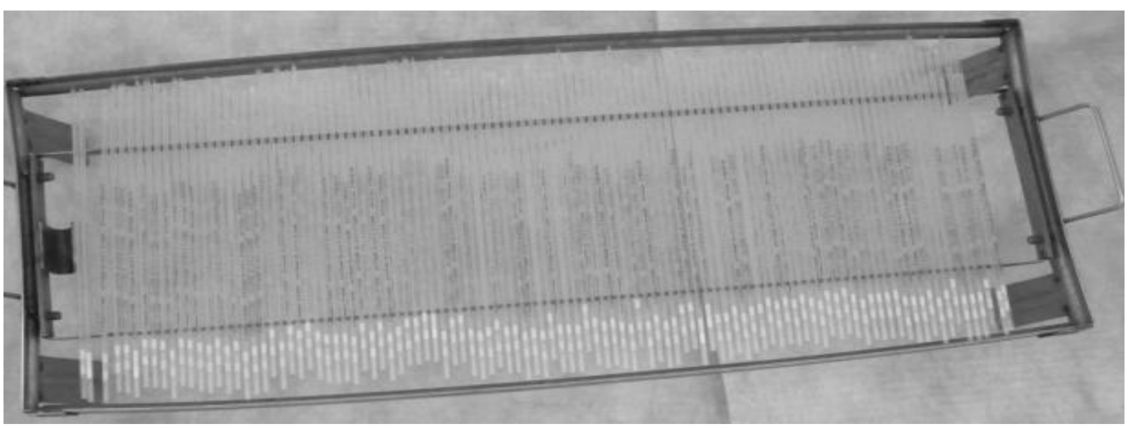


Fig. 8: Straws are spread over freezing rack

11. Freezing of straws

The filled and sealed straws can be frozen by two methods, namely:

1. Static vapour freezing

2. Forced vapour freezing

Static Vapour Freezing

Vapour freezing is carried out in a wide mouthed container (LR 320, LR 250) especially designed for freezing of straws or in the thermo cool boxes (Fig. 9) designed for this purpose. The equilibrated straws are placed 4-5 cm above the level of liquid nitrogen thereby exposing to the vapours of liquid nitrogen. Thereafter, lid is placed over the thermo cool box for settling the vapour. The temperature at this height is -180^{0}C. When the temperature of semen reaches -130^{0}C to -150^{0}C by about 10-15 minutes, straws are immediately transferred to pre-cooled goblet and the goblets are plunged into the other thermo cooled box containing adequate liquid nitrogen. These goblets are now carefully transferred into the desired LN_2 container for further storage.

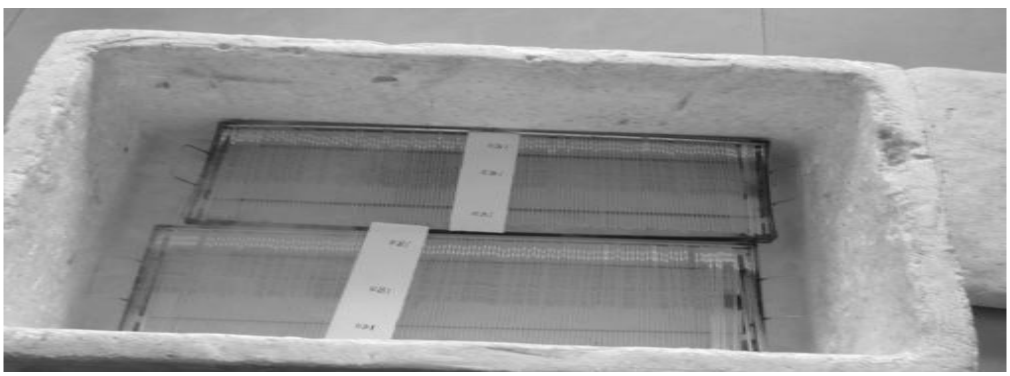


Fig. 9: Straws spread over freezing rack kept in thermo cool box containing liquid nitrogen.

Forced Vapour Freezing

Forced vapour freezing is carried out by **computerized programmable bio freezer**. The computerized programmable freezer has mainly three components: a **freezing chamber**, a **computer with a screen** and a **liquid nitrogen container** (Fig.10). Before placing the straws in freezing chamber, freezer is run to lower the temperature up to 4^{0}C. After reaching at the temperature 4^{0}C, the lid of the freezer is opened and the racks containing straws are transferred carefully from the cold cabinet to freezer. The lid is then closed and the desired freezing curve is chosen and command is given to the computer. The typical freezing curve for bovine semen is given below. The computer monitor displays the temperature curves for freezing chamber and for the straws being frozen. Temperature inside the freezer is controlled by the computer by allowing the appropriate amount of liquid nitrogen flowing to the freezing chamber from the liquid nitrogen container. When temperature reaches to -140^{0}C, the lid of the freezer is opened and immediately all straws are removed from the freezer and plunged into the liquid nitrogen in minimum possible time.

Typical freezing rate for bovine semen

Temperature fall	Rate of fall	Time taken
4^0 C to -10^0 C	@5^0 C/ min	1.8 min
-10^0 C to -100^0 C	@40^0C/ min	2.2 min
-100^0 C to -140^0 C	@20^0 C/ min	2.0 min

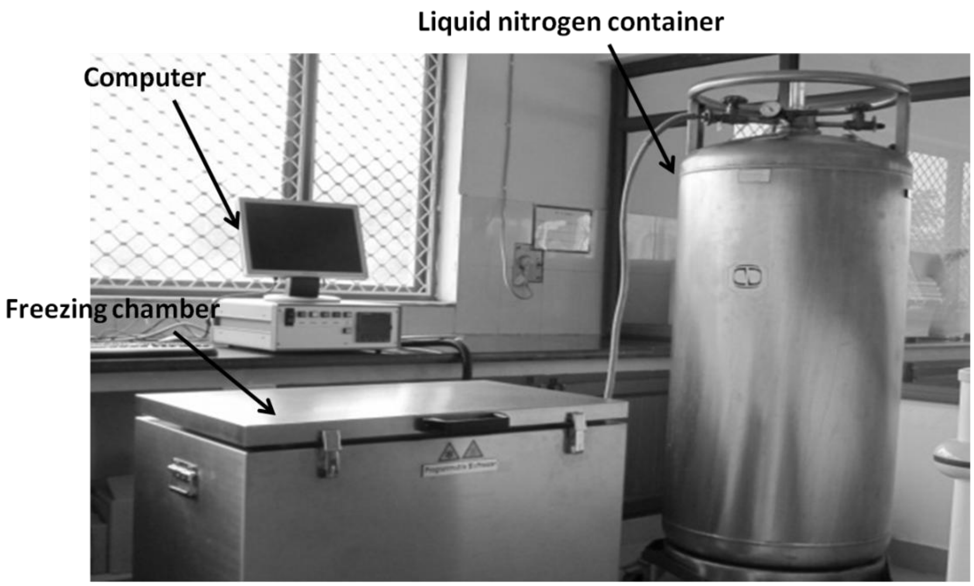


Fig. 10: Computerized programmable bio-freezers

Post-freezing examination

Post thaw motility of semen samples is assessed after 24 hours of storage. Samples having less than 50 percent progressive motility are discarded. Good samples are transferred to permanent storage containers.

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"Nothing splendid has ever been achieved except by those who dared believe that something inside them was superior to circumstances." ***Bruce Barton***

Liquid Nitrogen Containers

Digest

Polar bear cubs have no chance of survival without their mother. The family stays in the maternity den until early spring, and the mother doesn't drink, eat or defecate during that time. All she does is protect and feed her young. Even after they leave the den, cubs stay with their mom until they're about two years old, learning to hunt, clean themselves and survive in the harshest of habitats.

Liquid nitrogen (LN_2) is nitrogen in a liquid state at a very low temperature. It is produced industrially by fractional distillation of liquid air. LN_2 is inert, colourless, odourless, non-corrosive, non-flammable and extremely cold. At atmospheric pressure, liquid nitrogen boils at $-196\ ^0$C. Liquid nitrogen freezes at -210^0C. LN_2 has a specific gravity of 0.82 i.e. one litre weighs 0.82 kg. Thus, 1 kg of LN_2 measures 1.22 litres by volume. Further, its ability to maintain temperatures far below the freezing point of water makes it extremely useful in a wide range of applications. Liquid nitrogen is used as coolant in artificial insemination industry:

- Its -196^0C temperature is excellent for efficient storage of frozen semen.
- It is a relatively inexpensive refrigerant than other liquefied gases.
- It has excellent physical properties, being relatively inert, not supporting oxidation or combustion.
- It is a very safe substance, except for being extremely cold.

Liquid nitrogen container

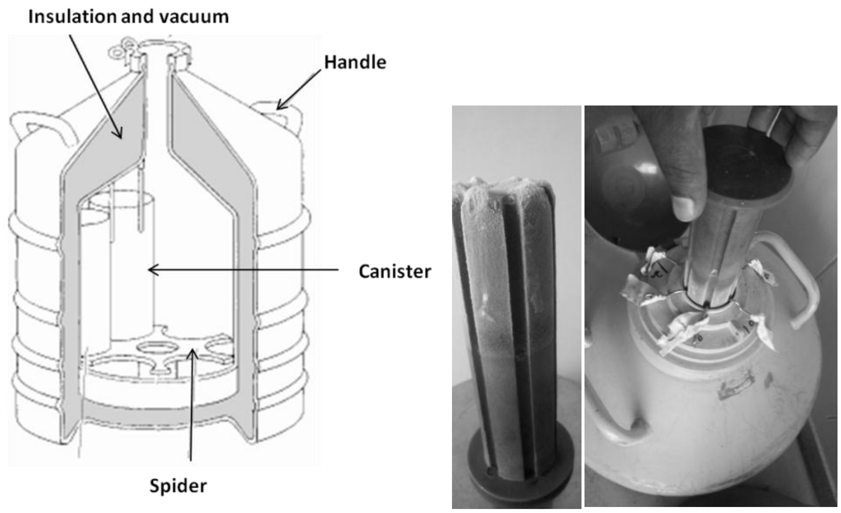
There are two types of containers commonly used to store liquid nitrogen. These are:

Pressurized liquid nitrogen cylinders: It is pressurized container specifically designed for cryogenic liquids (Fig. 1). This type of container has valves for filling and dispensing the cryogenic liquid, and a pressure-control valve with a bursting disk as backup protection. This container is used for only storing liquid nitrogen not semen straws.

Fig. 1: Pressurized liquid nitrogen container.

Dewar/nonpressurized liquid nitrogen container: The Dewar flask was named after its inventor, the Scottish physicist Sir James Dewar. Sir James Dewar discovered that, if a properly insulated vacuum space can be created between an inner and outer container, a liquid gas can be stored for long periods of time. Basically this same construction principle is used to build a liquid nitrogen container. The container is built on the same principle as a thermos bottle.

Dewar or LN_2 container is made of aluminium and has two separate chambers: an inner and an outer chamber (Fig. 2A). The inner shell is an unwelded, one-piece, aluminium container. The outer shell of a LN_2 container is made up of very tough aluminium. The space between the two chambers is filled with foil and special paper, and the air is removed to create a partial vacuum in this area. The vacuum increases the insulating effect. The neck of the container is made of epoxy-fibreglass. A specially designed stopper plug (Fig. 2B) is fitted to the neck which insulates liquid nitrogen and semen from outside air. The stopper plug is not airtight (Fig. 2C). A tight stopper plug may cause tank to explode. The entire inner wall of the container is supported by the neck. It hangs like a pendulum and can deflect during rough handling.



A B C

Fig. 2: A. Internal structure of LN_2 container B. Stopper C. Loose fitting stopper being placed in the neck of the container.

Sir James Dewar

About 1892 the idea came to the mind of Sir James Dewar for using vacuum-jacketed vessels for the storage of liquid gases - the Dewar flask (otherwise known as a Thermos or vacuum flask) - the invention for which he became most famous. The vacuum flask was so efficient at keeping heat out that it was possible to preserve the liquids for comparatively long periods. Dewar did not get profit from the widespread adoption of his vacuum flask because he lost a court case against Thermos concerning the patent for his invention. Though, Dewar was recognized as the inventor of thermos flask.

Different type of Dewar containers

Liquid nitrogen container: It is designed to store liquid nitrogen. These containers are available in two designs: one only store liquid nitrogen because it does not have groove for hanging canisters (Fig. 4A) and other has groove in the neck region to hang the canister thus it holds the straws of frozen semen at -196°C in liquid nitrogen (Fig. 4B). The LN_2 containers having wide mouth are also designed for bulk semen storage (Fig. 5 A & B).

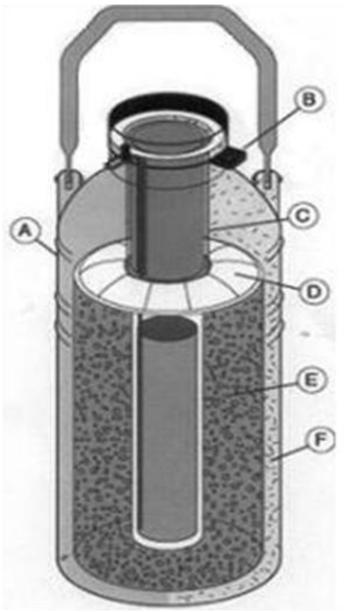
A B

Fig. 3: A. Liquid nitrogen container for liquid nitrogen storage only B. LN_2 container for storage of semen doses.

A B

Fig. 4: A. Wide mouth liquid nitrogen container B. Wide mouth liquid nitrogen container equipped with electric bulb to identify the canisters inside it.

Vapour shipper or dry shipper: Dry shipper is also Dewar (vacuum) flask that is designed for the safe shipment of semen at liquid nitrogen temperatures without the risk of spilling liquid nitrogen. When prepared correctly, a dry shipper does not contain any free liquid nitrogen. It has an absorbent material around the canister that absorbs the liquid nitrogen (Fig. 6). Storage temperature inside the shipping cavity remains at approximately -150°C for several days until the liquid nitrogen evaporates from the absorbent material. The dry shippers must be properly charged by filling with LN_2 to the point of saturation of the absorbing material and then removing the excess LN_2 to provide maximum holding time.



A- Aluminum wall

B- Locking tab

C- Neck

D- Vacuum retention system

E- Hydrophobic adsorbent

F- Insulation system

Fig. 5: Dry shipper (Courtesy MVE)

Filling of LN_2 container

- The empty LN_2 container is filled either by pouring liquid nitrogen from other container by using a funnel or LN_2 is transferred from pressurized liquid nitrogen container through a transfer tube (Fig. 7 A, B & Fig. 8).
- When filling a warm LN_2 container, add the liquid slowly at first to prevent losses until the inner shell is cooled down.
- First fill the container only about 3/4 and allow it to continue cooling for a few minutes. Then completely fill to the desired level.
- When LN_2 is filled in dry container, maximum insulation efficiency is not reached until about 48 hours after filling. Therefore, the LN_2 loss rate will be high for the first few hours.
- Once the container is filled, it should be visually checked every day, and the LN_2 level should be checked weekly with the help of dip stick.

A B

Fig.6: A. A funnel is placed into the neck before transfer of LN_2 B. LN_2 being transfered into the container.

Fig.7: LN_2 being transferred from pressurized LN_2 container into Dewar container through transfer tube.

Handling of liquid nitrogen container

Although semen storage tanks are well constructed, they are still susceptible to damage from mishandling. Therefore, very careful attention should be given for proper semen tank management. For proper semen tank management the following points should be kept in mind.

- It can cause asphyxiation in a confined area where there is no adequate ventilation. Therefore, semen tanks should be **kept in clean, dry and well ventilated areas.**
- Keep the tank in a **cool location away from direct sunlight.**
- **Avoid excessive movement** of the tank. The inner chamber, which contains the liquid nitrogen, is actually suspended from the outer shell by the neck tube. Any abnormal stress on the neck tube, which can be caused by sudden jarring or any excessive swinging motion, can possibly crack the tube and result in vacuum loss from the outer chamber.
- Protect the tank from corrosion by keeping it on the wooden boards or spongy sheet above concrete floors.
- Place the tank where it will be safe from children and vandals; but don't hide the tank. It must be placed where it can be seen daily and monitored for excessive nitrogen loss.
- Give particular attention to the neck and vacuum fitting. Frost over the container indicates that the vacuum insulation has been lost and liquid nitrogen is evaporating rapidly.
- Failure to secure the tank in a moving vehicle can result in not only increased probability of damage to equipment but also increased risk of injury to the driver or passengers.
- The liquid nitrogen level should be monitored weekly with a measuring stick.

Precautions during handling of liquid nitrogen

- Normally contact of LN_2 for short period does not cause frost bite. Accidentally excess amount of LN_2 or LN_2 vapours can cause a freezing injury similar to a burn. Therefore, handle the liquid so that it will not splash or spill.
- Protect eyes with safety goggles and wear protective gloves when large amounts of LN_2 has to be handled (Fig. 9 A & B).
- In the event of a burn from LN_2, treat immediately for frostbite.

A B

Fig. 8: A. Cryo gloves B. Handling of frozen semen with the help of cryo gloves.

DO YOU KNOW?

Rapid release of nitrogen gas into an enclosed space can displace oxygen in the air to levels below that required to support life and it may result into asphyxia. The human **carotid body**, located near the bifurcation of the carotid artery, is **an anatomical organ that measures changes in blood pressure and the composition of arterial blood including the partial pressures of oxygen and carbon dioxide.** Unfortunately, when somebody aspirates air with higher concentration of nitrogen gas than normal, the carotid body works relatively slow and this low-oxygen concentration sensing system (hypoxia) will not react efficiently to prevent asphyxiation. At low oxygen and high nitrogen concentrations, unconsciousness results and death may occur in seconds and without any warning. Because of this asphyxiation hazard, when working with LN_2 in a confined or enclosed space it is recommended to monitor oxygen concentration with specialized ambient oxygen monitor-alarms that are available in the market.

Problems in LN_2 container

Complete frosting of outer shell: When the entire outer shell of the LN_2 container frosts up, it indicates complete loss of vacuum. When this occurs, the semen should be immediately transferred into another LN_2 container.

Ice spot on outer shell: If an isolated ice or frost spots are found on the outer shell it means that a "short" has occurred between the inner and outer shell.

Ice ring around the cap and top of outer shell. When frosting occurs around the cap and outer neck area, it is a sure sign of a slow vacuum leak. This takes place in older containers (seven-10 years old) as a result of vacuum decay. It is also a symptom of a faulty vacuum in a newer tank.

Broken goblet or dislodged canister. Sometimes, due to careless handling, the AI worker drops goblet down inside of LN2 container. In that condition, transfer the semen into other temporary storage containers, empty the container and recover the lost article.

Measurement of liquid nitrogen level

- Accurate measurement of the volume of liquid nitrogen in LN2 containers can be made by dipping a graduated dip stick (Fig.10) of low thermal conductivity into the liquid nitrogen till it touches the bottom of the container.
- Keeps it undisturbed for 5-10 seconds, then stick is withdrawn and waved in the air.
- Frost will appear in the wetted section (Fig. 10 & 11). The atmospheric air condenses as a frost on the dip stick to the level of liquid nitrogen.
- Read the level, 1 cm below the top of the frost line because when dip stick is immersed the liquid nitrogen boils and frost appear about 1 cm above the actual level of liquid nitrogen. Once the length of frosted section is read the same can be converted into volume as the diameter of the containers is known.
- At least half the length of the straws (straw length -13.3 cm) should always be dipped in liquid nitrogen. Further lowering of liquid nitrogen level will lead to decline in post thaw motility and fertility of semen. Therefore, when 20 cm LN_2 is remaining in the LN_2 container, fill the container in advance so that it will not go down to the minimum level.
- ***Precaution:*** *Never use a hollow tube for measuring*

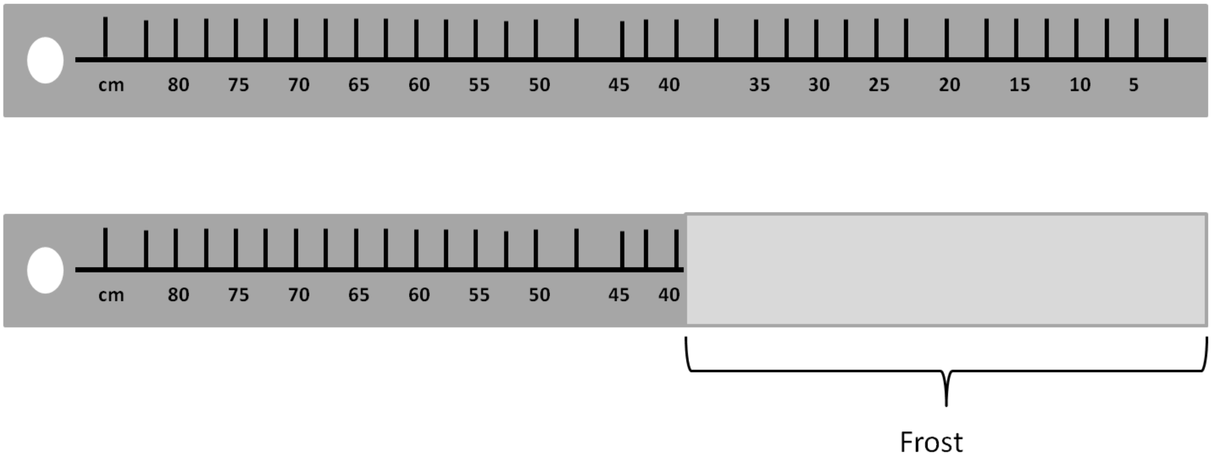


Fig. 9: Graduated dip sticks showing frost on the level of LN2 in the container.

A B

Fig. 10: A. Dipping of a graduated stick into the liquid nitrogen till it touches the bottom of the container. B. Frost appears in the wetted section

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Let us sacrifice our today so that our children can have a better tomorrow. ***Abdul Kalam***

Storage and Shipment of Frozen Semen

Digest

In rabbit, the doe may accept the buck at any time during pregnancy, except for a brief period of 30–40 hours post-mating. Fertilization can occur in such cases if a second ovulation takes place within 2–3 days of the first. This can produce litters of mixed buck.

Storage of frozen semen

Frozen semen is stored in liquid nitrogen at -196^{0}C in LN_2 container. The different sizes of liquid nitrogen containers are available in the market. These range from 1 litre capacity of nitrogen to 550 litres or more (Fig.1). At the semen freezing lab, large cryogenic containers are used for storing of semen straw in bulk undisturbed for longer period. Smaller containers are used to store semen from which straws have to be utilized frequently. Artificial insemination workers use small capacity of liquid nitrogen container ranging from one litre to 20 litre capacity. The recommended minimum volume of LN_2 present in the tank is at least 5 cm (2 inches) in order to prevent any damage to the stored specimens. Ideally, the LN_2 level should be monitored every week with a calibrated measuring stick.

Fig. 1: Showing different capacities of Dewar liquid nitrogen containers.

The straws are first placed in pre-cooled goblet. The goblet is kept in the canister (Fig. 2). The canisters are hanged inside the liquid nitrogen container with the help of their long handles.

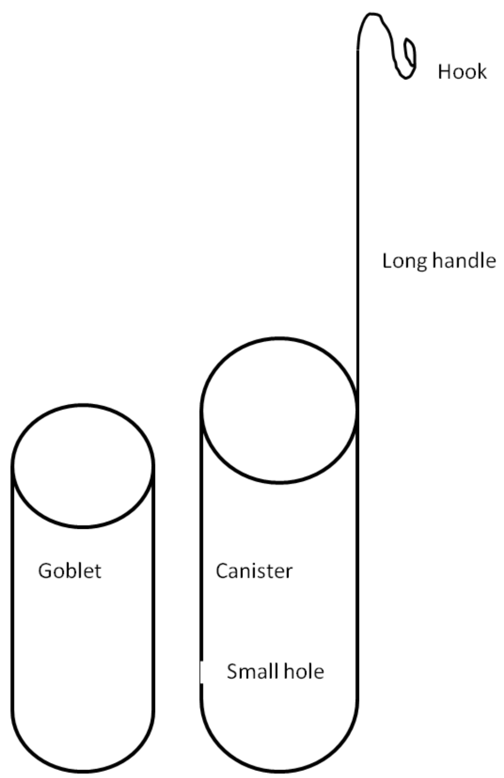

Fig. 2: Goblet and Canister.

Handling of semen in LN_2 container

In the upper half of the neck tube of LN_2 container, high temperatures exist. Time spent in removing semen from the liquid nitrogen (LN_2) tank must be kept to a minimum to reduce semen damage. The typical temperature range in the neck of the LN_2 container is -191^0C to $+2.2^0$C (Fig. 3) Temperatures can reach $+54^0$F (12.2^0C) in the neck of the container (1 inch from the top). If the entire canister of semen (10 straws) is withdrawn above the frost line (3 to 4 inches from the top of the tank) (Fig. 3& 4), all straws of semen will be damaged. Thermal injury to sperm is permanent and cannot be corrected by returning semen to liquid nitrogen.

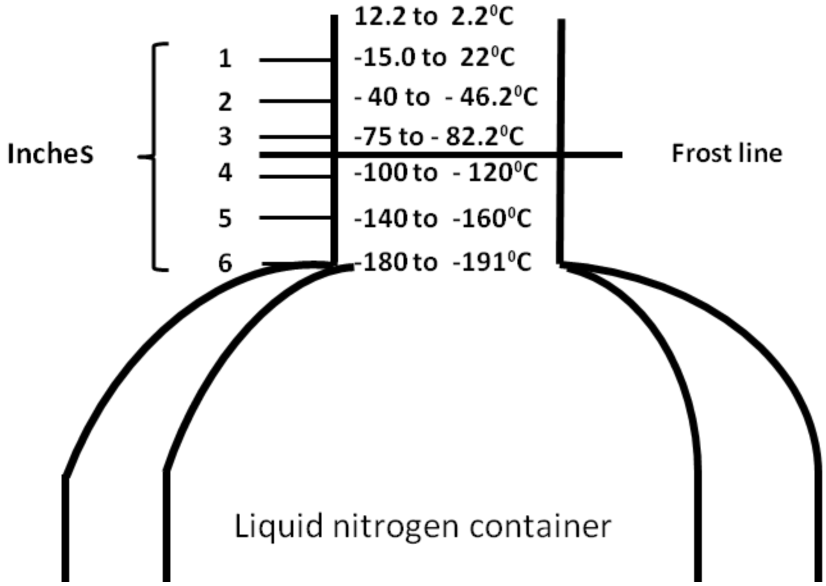

Fig. 3: Diagram showing temperature in the region of neck of the container

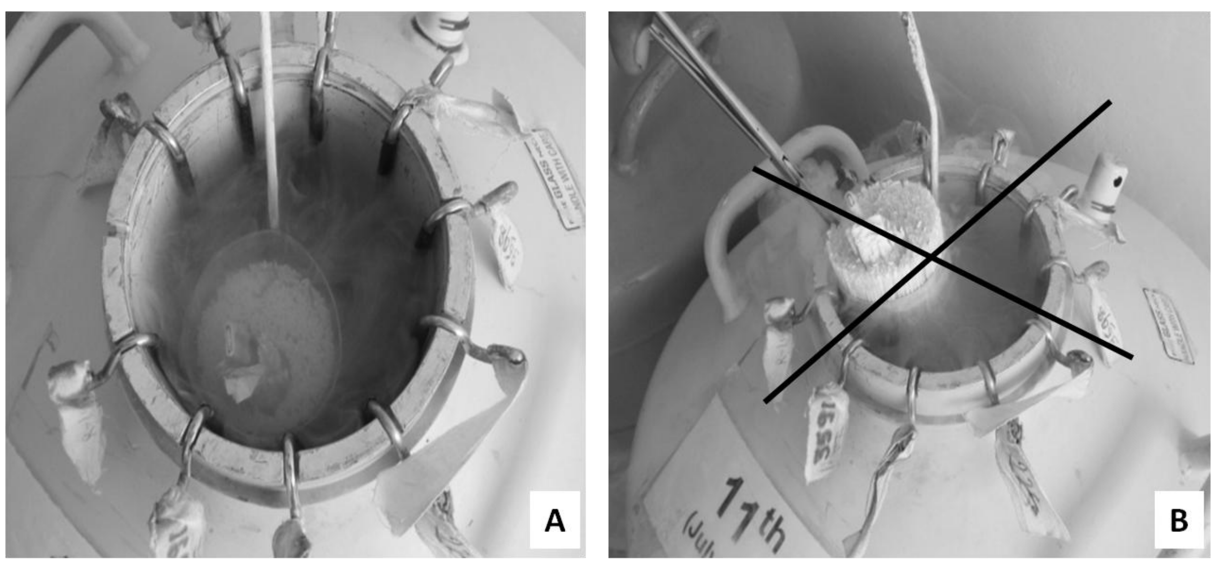

Fig.4: A. Right method to raise the canister **B.** Wrong method to raise the canister

Therefore, following steps should be remembered during the handling of semen.

- Identify which canister contains the desired semen with the help of attached tags to the canisters (Fig. 5A).
- Remove the canister from its storage position to the middle of the tank (Fig. 5b).

- Raise the canister just high enough in the neck region to grasp the desired straw (Fig. 5C).
- Grasp the straw and immediately lower the canister to the tank floor (Fig. 5D).
- Use tweezers or long pre cooled forceps to remove the straw (Fig. 6).
- The straw should be removed within 10 seconds from the time the canister is raised into position. Then immerse the straw in 37^0C water.
- Immediately, after the straw is immersed in water, the canister should be returned to its storage position.
- Any time if it takes more than 15 seconds to take out the desired number of straws, the canister should be lowered back into the tank to cool completely and again after sometime take out the canister.

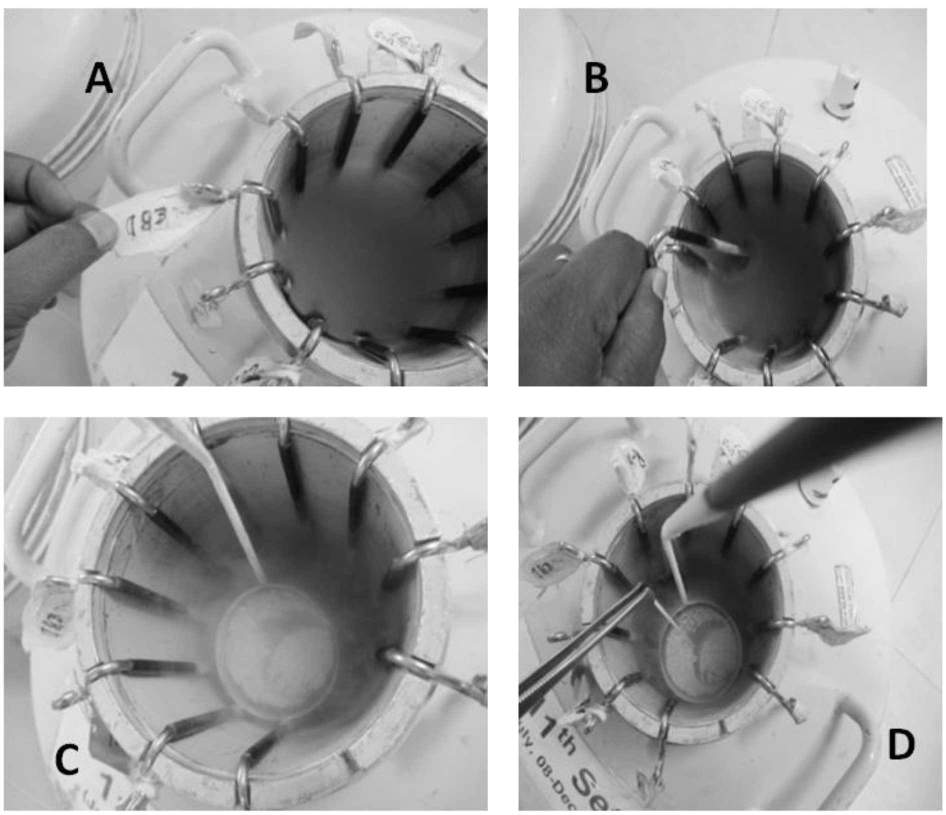

Fig. 5: Handling of semen in LN2 container A. Identify canister that contains the desired semen. **B**. Remove the canister from its storage position to the middle of the tank. **C** Do not lift the canister above the frost line to grasp the desired straw. **D.** Grasp the

straw with pre-cool tweezers or haemostats and immediately lower the canister to the tank floor.

Fig.6: Use tweezers or long pre cooled forceps to remove the straw **A**. Tweezers **B**. Forceps

Shipment of semen

Shipment of chilled semen

Use sterile plastic tubes for the semen which do not break during the transport. The chilled semen is usually sent in an ordinary thermos flask, or a styrofoam box with ice-packs. It is imperative that the temperature does not drop below 0°C to ensure that the semen does not freeze. Therefore, the tube containing the semen must be protected from direct contact with ice cubes or cold-packs, for this, the tube is wrapped in a piece of cotton wool.

Shipment of frozen semen

Although some distributors transport frozen semen in liquid storage containers but shipping of frozen semen is best accomplished using containers specifically designed for transport. The dry-shipper is specially designed for shipment of frozen semen. The various sizes of the dry- shipper are commercially available (Fig. 7). The shipper absorbs the liquid nitrogen into a porous material in its walls. These will not spill and therefore need not to be shipped as dangerous goods, which is more expensive. They should, however, always be sent as fragile goods, because they are easily broken by rough handling. The tank is usually shipped in a plastic box for protection.

Fig.7: Different sizes of cryoshipper (Courtesy of MVE Company)

Care should be taken while transferring frozen semen from storage to shipping containers. Exposure of frozen semen to room temperature should be not more than a few seconds; therefore, this should be performed with shipping and storage containers placed side by side. Always work within the neck of the storage container, below the frost line, while locating, identifying, and grasping straws.

Liquid shipper vs. vapour shipper

If liquid shippers' i.e. liquid nitrogen containers are tilted during transport, it will lose all liquid nitrogen and warm rapidly, destroying valuable semen. Also, liquid nitrogen container is a safety hazard and could cause serious injury to handlers. Because of this, liquid shippers must be classified as containing hazardous materials, which requires special paperwork and increases shipping costs. Vapour shippers, on the other hand, contain no "liquid" nitrogen per se. The liquid nitrogen vapour absorbed into the containers. Hence, vapour shippers are often referred to as "dry shippers." No liquid nitrogen means no hazardous materials classification and fewer shipping restrictions. Vapour shippers are generally lighter than liquid storage containers and are therefore also less expensive to ship. MVE manufactures a "mushroom" shaped protective shipping carton for all sizes of shipping tanks (Fig. 8). The carton has wide base with a rounded top that reduces the likelihood of the tank tipping over or being loaded upside down or on its side. The hard moulded plastic carton also helps prevent tank damage due to normal shipping use.

Fig.8: Diagram showing cryoshipper and its plastic box for protection (Courtesy of MVE Company).

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The first requisite of success is the ability to apply your physical and mental energies to one problem without growing weary.

Technique of A.I. in Bovines

Digest (Traumatic insemination)

The male bedbugs don't even bother with the female's sex organs. Instead, a male bedbug uses its sharp sexual organ to pierce the female bedbug's body and deposit its sperm! Scientists have given a cute name for this sort of thing: "traumatic insemination."

Several techniques have been used for inseminating cow/buffalo artificially. These are vaginal insemination, cervical insemination and recto-vaginal technique. Vaginal insemination requires fairly large quantities of semen and has low conception rate. Therefore, this technique has now been replaced by other methods. In the cervical insemination technique, semen is deposited in the cervix by using speculum. This method has also been replaced by recto-vaginal method.

Recto-vaginal method of AI

The recto- vaginal technique is the most efficient method for bovine artificial insemination and is in use worldwide. The AI is performed in relatively unhygienic surrounding; therefore, the responsibility of the inseminator is to clean in every possible aspect. Always use a glove while inseminating. Plastic disposable gloves are preferred but reusable rubber gloves may be used.

Artificial insemination guns

The AI gun is hollow stainless steel with a plunger to expel the semen. There are commonly three types of AI gun: **O-ring, spiral and universal guns** used in India. AI guns are available to accommodate specific straw sizes: French medium and mini straw; but universal guns accommodate both the straws (Fig. 1).

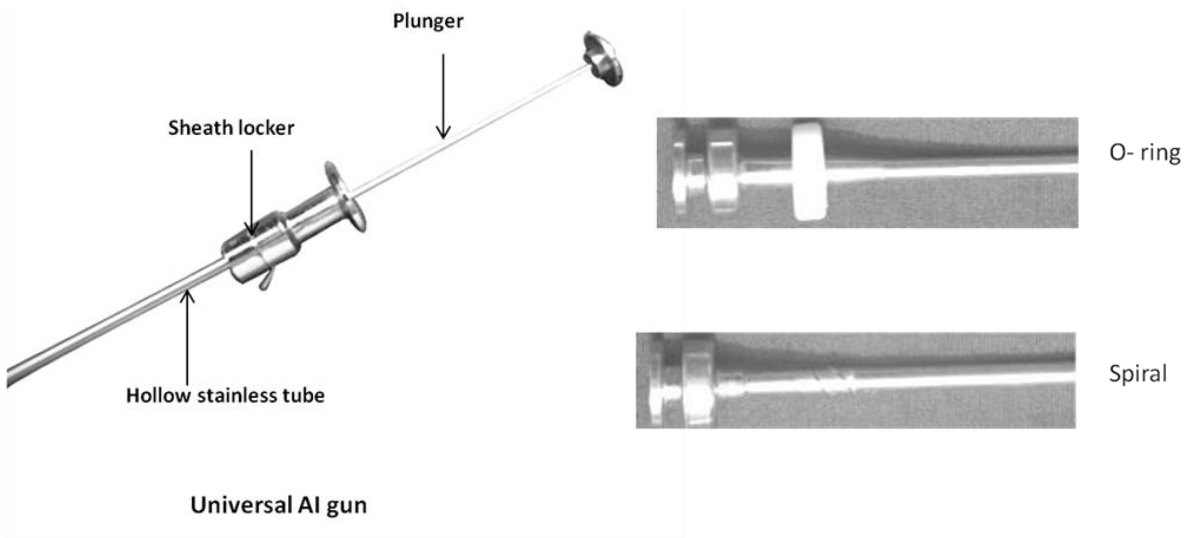
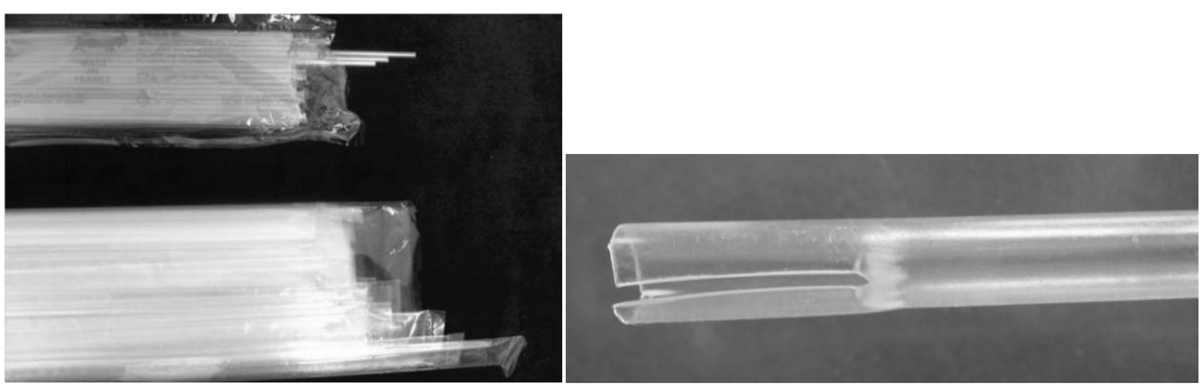


Fig. 1: Different types of AI guns.

Breeding Sheaths

It is disposal plastic tube specially designed to fit over the barrel of AI gun to secure semen straw and prevent transfer contaminants in the uterus during insemination (Fig. 2). During breeding, various contaminants can also be picked up by the open end of a sheath on the way to the uterus and released into the uterus. Therefore, the sealed sheath (i.e. sheath is individually covered with thin plastic) should be used. It keeps the inside of the breeding sheath clean until ruptured by depressing the plunger during breeding. The various types of sheaths are available for example:

- **Split sheaths** are designed for use with O-ring gun.
- **Non-split sheaths** are designed for use with spiral gun.



A **B**

Fig.2: A. Breeding sheaths in packet **B.** Split end of a sheath

Loading of A.I. gun

- Tear off approximately 20 cm of tissue paper.
- Remove the straw from the thawing tray using fingers and dry it with tissue paper.
- Drying is done by following steps:
  - ✓ Hold the straw by the laboratory seal end
  - ✓ Draw it through the paper once
  - ✓ Now, grasp the straw by the manufacture's end
  - ✓ Again draw it through the paper
  - ✓ Hold the straw by the manufacturer's end after drying is completed.

Don't dry the straw excessively as heat produced due to friction may raise the semen temperature.

- Check the name of the bull printed on the straw. If the wrong straw has been selected it must be discarded. **Never replace a thawed straw in liquid nitrogen**.
- Pull back the plunger of the AI gun.
- Holding the straw by the end, to avoid damage to the semen through temperature shocks, insert the manufacturer's end into the gun as far as it will go. There is an in-built stop preventing it going too far (Fig.3A).
- Prepare to cut off the laboratory end of the straw using thoroughly cleaned and dried the scissors.
- Hold the loaded gun vertically at eye level and using clean sharp scissors make a horizontal cut just below the laboratory seal end (Fig. 3B). The cut must be at right angle to the straw so that a perfect seal occurs between the straw and the sheath (Fig. 4).

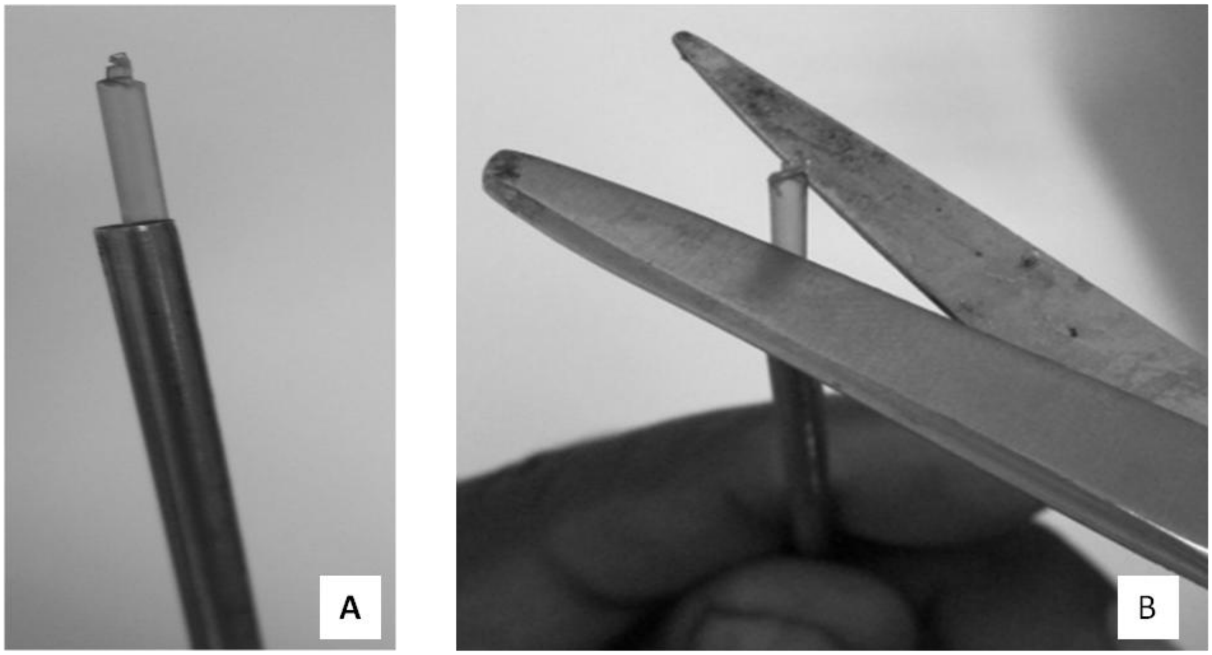


Fig. 3: A. Laboratory end outside the gun and inbuilt stopper prevent it going too inside.
B. Cutting of laboratory end of straw with scissor.

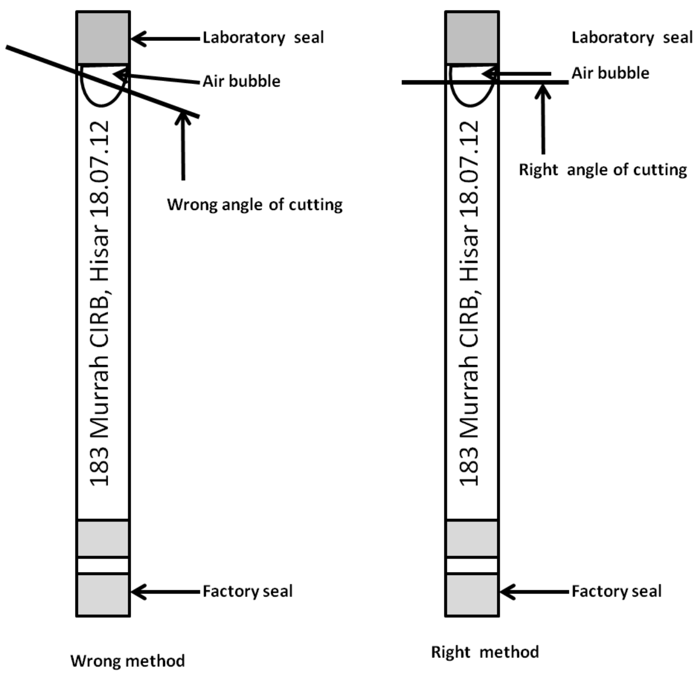


Fig. 4: Right and wrong methods of cutting straw.

- Clean and dry the scissors after cutting the straw and keep them to their correct location in the kit box.

- Place a sheath over the barrel of the gun. Handle and touch only the split end of sheath to keep the other end clean.
- Push the sheath through the central hole of the locking ring, twist it down on the conical seal of the gun and place O-ring over the sheath (Fig. 5A).

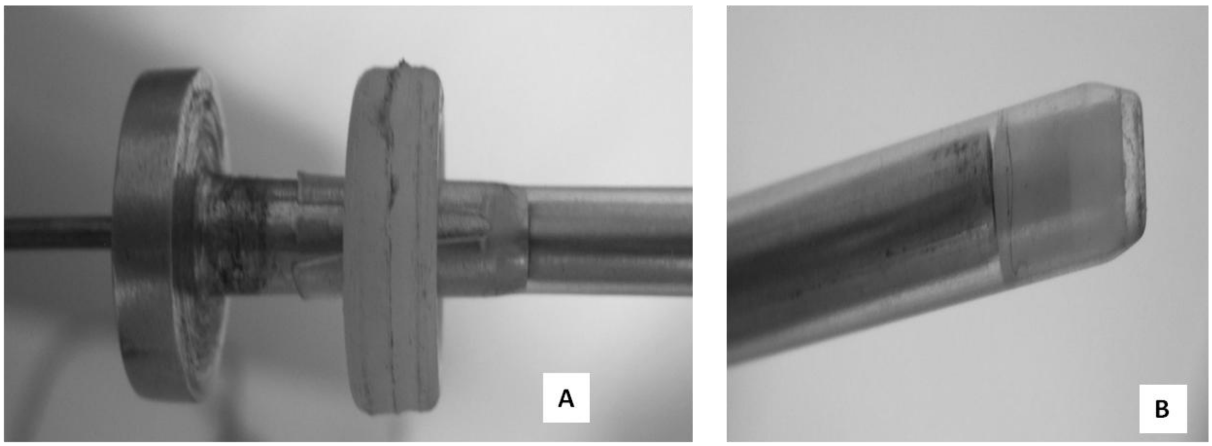


Fig. 5: A. Sheath passed through the O-ring and twisted on conical seal of the gun. B**.** Proper seal between sheath and laboratory end of straw.

- Seal between the sheath and the gun is essential otherwise semen will leak into the gun (Fig. 5B).
- Press the plunger of the gun until the semen is just visible at the end of the gun. This reduces the stretching of fingers needed during handling of the loaded gun.
- Now, loaded AI gun is ready to inseminate the animals.

Steps of artificial insemination

1. Properly secure the animal in the crate.
2. Wear the glove and wet it by dipping the gloved hand into a bucket of clean water, scooping water up in your hand and letting it run back down your arm. Apply a small quantity of lubricant to the back of your hand.
3. Make the cow/buffalo aware of your presence. A distressed animal may kick.
4. Ask to attendant to grasp the animal’s tail and lift it aside.
5. Smear lubricant across the cow’s anus, using the lubricated back of the gloved hand.

6. Form a cone of your gloved fingers and insert hand into the rectum. Pause at this stage and encourage the anus to relax by gently revolving your fingers. The wide part of the hand passes easily without rubbing the surrounding of anus and remove the faecal matter. This is best accomplished by first inserting two or three fingers into the opening of the anus and allowing air to enter the rectum, which usually allows the cow defecate. If the rectum doesn't empty, the dung should be removed manually. However, complete removal of dung is not possible.

   Avoid rough sudden entry which can abrade the anus and cause the release of adrenalin which reduces the conception rates.

7. Once most of the faeces are removed, relax constriction rings by placing two fingers through the rings and gently massaging back and forth (Fig. 6).

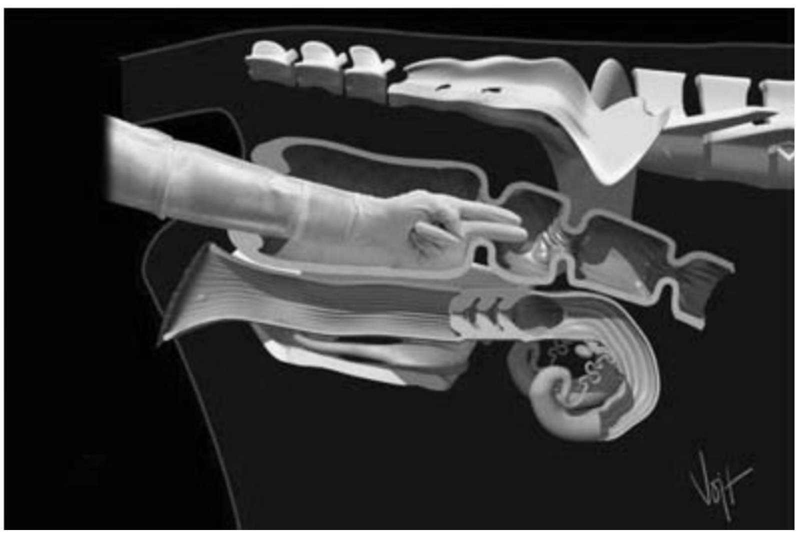


Fig. 6: To relax rectal constriction rings, insert two fingers through the centre of the ring and massage back and forth (courtesy: www.selectsires.com).

8. Thoroughly clean the vulva with water and wipe with tissue paper. Use a fresh piece if the paper is too soiled.
9. Give some pressure downwards with the wrist of the hand in the rectum which helps to apart the vulval lips presenting a clean area for inserting the gun or the vulval lips are pulled apart with the help of assistant.
10. Insert the gun clearly between the lips of the vulva into the vagina. Ensure the gun passes along the roof of the vagina thus avoiding the bladder. AI gun is passed at an angle of 45^0 through vagina.

11. Gently push the gun through the vagina up to external os of the cervix.
12. Now, hold the cervix between fingers and keep the thumb over the external os. Maintain a light forward pressure on the gun and manipulate the cervix so that the gun passes through the cervix canal.
13. Manipulate the AI gun so as to strike thumb placed over the external os and then pass the gun through the cervix.
14. While passing the gun through the cervix, feel the tip of the gun at internal os to ensure that the gun is in correct place (Fig. 7).

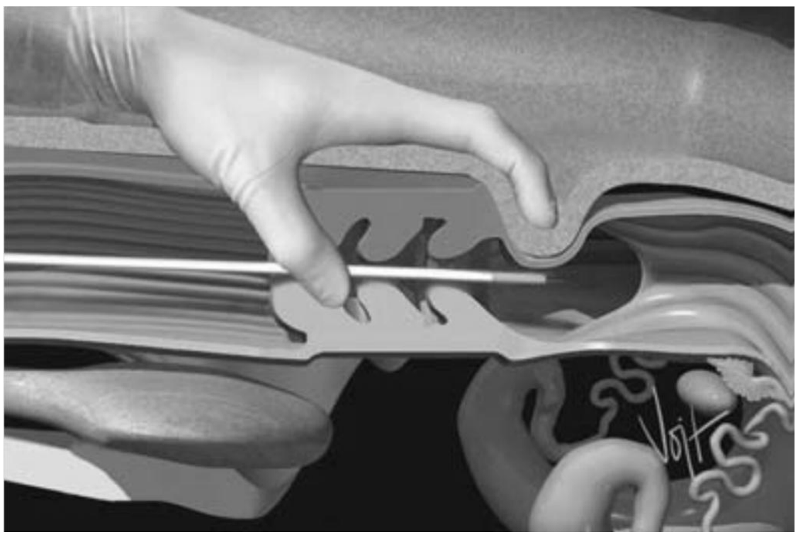


Fig. 7: Use index finger to assure the correct position of gun before depositing semen (courtesy: www.selectsires.com).

15. Gently deposit the semen in the body of the uterus being careful not to ‘spit’ it out (Fig. 8).

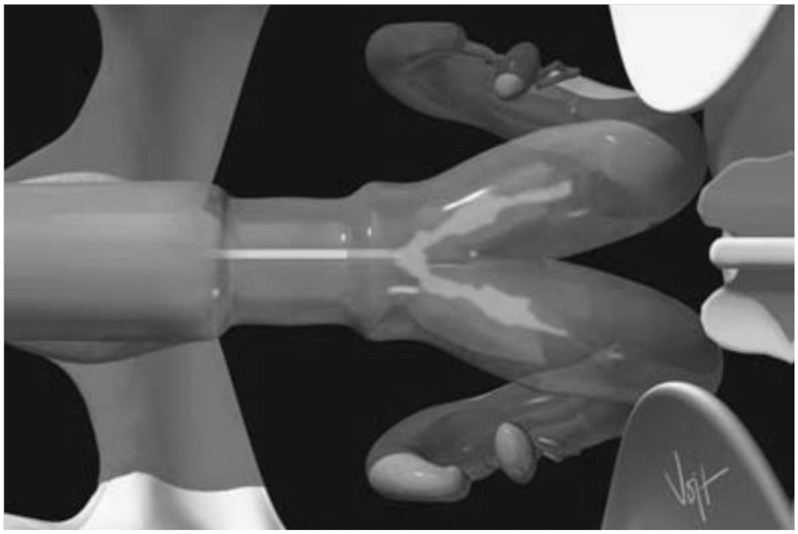


Fig. 8: Deposit semen in the uterine body and contractions will transport spermatozoa forward to both horns and oviducts (courtesy: www.selectsires.com).

16. Don’t pass AI gun beyond the uterine body otherwise all semen will be deposited into only one horn. (Fig. 9).

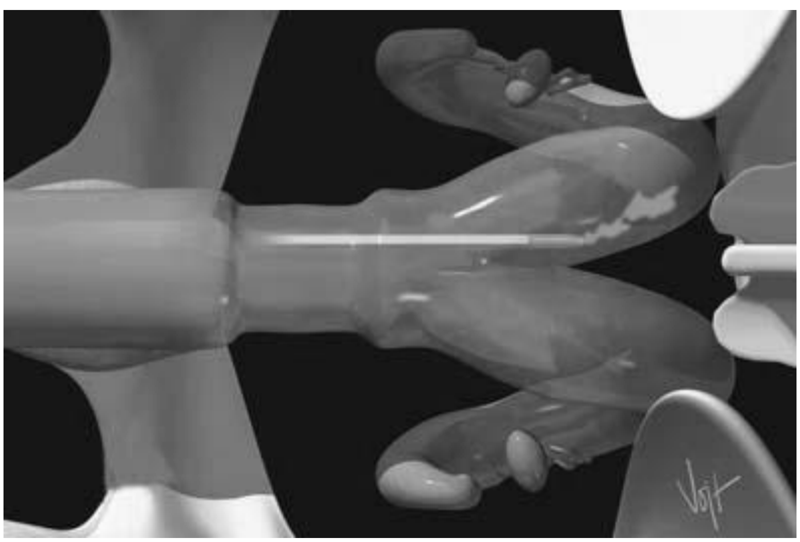


Fig. 9: Don’t pass AI gun beyond the uterine body (courtesy: www.selectsires.com).

17. Gently take out the gun and check for abnormal discharge and complete semen deposition.
18. Slowly withdraw the arm from the rectum of the cow.
19. Loosen the locking ring on the gun and remove the soiled sheath.
20. Dispose the straw, sheath and dirty glove properly.

Post Artificial Insemination

After AI, there are several clean - up steps. These are:

- Inspect the gun tip for sign of infection.
- Remove sheath and straw and bend at a 90^0 angle and dispose properly.
- Remove the glove and the used straw, sheath and manure put inside the glove. Tie a knot in the open end and dispose the glove.
- Clean the gun with 70% alcohol and dry it.
- Tighten the O-ring on the gun so it will not get lost.
- Clean and disinfect yours boots before leaving the farm.
- Wash your hands before leaving.
- After the last call of the day, wash outside of your AI kit.
- It is good practice to remove all equipment from AI kit and clean them thoroughly once a week.

CLINICAL POINTER

- Insemination is accomplished by pushing the plunger to deposit the semen slowly. This is accomplished by slowly counting up to 10 during expulsion.
- Clitoral massage immediately after insemination has been advocated to improve conception. In one study, a 10 second massage increases 6 percent conception rate over no massage (58 vs52 percent) in cows but there is no effect in heifers.
- Insertion of AI gun is most easily accomplished by passing the tip along the roof of the vagina for about 15 cm to bypass the **sub-urethral diverticulum** and **urethral orifice** on the floor. During the insertion into the vagina, the hand in the rectum should grasp the cervix and stretch the vagina forward for easy passage of the AI gun.
- Once the tip of the inseminating gun passes into the uterus it is easily detected through the relatively thin wall of the uterus.
- Avoid deep penetration of the uterus as the gun may cause damage and possibly infection thus reducing the chance of the conception or in the case of pregnant cow, an abortion (up to 5 percent of pregnant cows show some signs of heat) may occur.

- Occasionally the gun cannot be passed to the proper position. In this condition, avoid bruising and other injury to the cow by depositing the semen at the position reached after reasonable effort. Prolonged and forceful struggling will have a worse effect on conception rates than incorrect semen placement.
- Rapid withdrawal of the gun can suck semen back through the cervix into the vagina.
- Sometimes the cervical opening is not in the center of the cervix but is located to either side or on the upper or lower part of the cervix. Use the forefinger of the palpating hand to feel the tip of the AI gun and to determine when it has penetrated the cervical opening.

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Only very foolish people are convinced that they are never wrong. Confess often!

Breeding Soundness Evaluation of Bulls

Digest

For flatworms, sex is more like war than love. Flatworms are hermaphrodites (they have both male and female sexual organs). In this case, the male organ turns out to be two dagger-like penises that they use to hunt as well as to mate. During mating, two flatworms fight (i.e. "penis fence") to stab each other, while avoiding getting stabed. The "loser" who gets stabbed will absorb the sperm through its skin and then scoots off to bear the burden of motherhood.

The society of theriogenology developed a standardized system to evaluate breeding ability of bulls in 1976. Breeding soundness of bulls are measured by this standard system. Again, in 1993, this system has been slightly modified to include some current concept in bull fertility examination. According to this system bull must meet minimum standards in four categories.

- General and reproductive physical examination.
- Scrotal circumference
- Sperm motility and
- Sperm morphology.

In addition to the above four standards, pelvic measurement may be included in a breeding soundness evaluation.

Breeding soundness evaluation (BSE)

A breeding soundness is a prediction of a bull's potential reproductive capacity based on standard measurements and interpretations of certain selection criteria.

Classification of Breeding Bulls

After thorough examination, a bull is classified as:

- Satisfactory potential breeder
- Unsatisfactory potential breeder
- Deferred or questionable potential breeder.

This classification is based on scores of scrotal circumference, percent abnormal cells and motility.

Satisfactory potential breeder

A bull that fulfils all the four minimum standards is considered as satisfactory potential breeder. This bull scores 60 to 100 out of 100.

Unsatisfactory potential breeder

A bull that fails to meet all the four minimum standards is considered as unsatisfactory potential breeder. This bull scores less than 30 out of 100.

Deferred or questionable potential breeder

Any bull may be classified as deferred if it fails to fulfil minimum standard in critical areas. It is common for young bull due to immaturity. A second fertility examination is required to achieve satisfactory potential breeder. The bull scores 30 to 60 out of 100.

<u>Scoring system</u>

Parameters	**Score range**
Scrotal circumference	10 - 40
Percent abnormal spermatozoa	3 - 40
Motility	3 - 20
Total score	**16 - 100**

This presumes that the bull is physically normal, has satisfactory libido and is managed properly.

<u>Examination on the basis of score</u>

Grade	**Total score point acquired**
Satisfactory	60 to 100
Questionable	30 to 60
Unsatisfactory	Below 30

Minimum Scrotal Circumference (cm) at different ages of bulls

Age (months)	Minimum scrotal circumference (cm)
<15	30 cm
15-18	31 cm
19-21	32 cm
22-24	33 cm
>24	34 cm

Rating and Score Point for Scrotal Circumference (cm)

Rating	Age (months)				
	12-14	**15-20**	**21-30**	**>30**	**Score point**
Very Good	>35	>37	>39	>40	40
Fair	30-35	31-37	32-39	33-40	24
Poor	<30	<31	<32	<33	10

Rating and Score Point for Sperm motility

Rating	Gross	Individual	Score point
Very Good	Vigorous swirls	Rapid, straight	20
Good	Slow swirls	Moderate straight	12
Fair	No swirls, some oscillation	Slow, straight	10
Poor	Little movement, sporadic oscillation	Very slow, erratic	3

Rating and Score Point for Sperm morphology

Rating	Primary abnormalities	Total abnormalities	Score point
Very Good	<10	<25	40
Good	10-19	26-39	24
Fair	20-29	40-59	10
Poor	>29	>59	3

Note:

- *If a bull scores high on two criteria but has a serious problem with the third, the bull should be classified as questionable potential breeder, even if the score is above 60 points.*
- *The score should be used only as a guide to assist in making intelligent management decisions.*

Pelvic measurement

Another evolving technology is pelvic evaluation. Research studies indicate that pelvic area has heritability 0.55 which is higher than calf birth weight heritability (0.45). One study indicate that 0.6 genetic correlation between male and female pelvic area. This indicates selection for pelvic size in bull should result in increased pelvic size of daughter offspring. The suggested minimum pelvic areas for yearing and two-year old bulls are given below.

Age	Weight	Pelvic Area
Yearling	900-1000 lb	150 cm^2
	1000 – 1200 lb	170 cm^2
2 year old	1300 – 1500 lb	190-200 cm^2

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English is necessary as at present original works of science are in English. I believe that in two decades times original works of science will start coming out in our languages. Then we can move over like the Japanese. ***- Abdul Kalam***

PART IV

Semen Evaluation Techniques

Computer Assisted Sperm Analyser (CASA)

Introduction

Light microscope is the first generation device for visualization and analysis of sperm. This device was first used by von Leeuwenhoek in 1678 to visualize sperm. Phase contrast microscope is second generation device for semen evaluation. Such second-generation devices were first used by pioneering andrology labs in the mid- 1950s, and phase-contrast microscopes remains the primary instruments for observation of living sperm. Classical microscopic assessment has the disadvantage that sperm motility estimates can vary among examiners. In 1985 the first commercial CASA system developed specifically for evaluation of sperm motion was called the cell soft system. Thereafter many advanced versions of CASA have been launched by different companies. Today's CASA systems represent third-generation devices for visualization and analysis of sperm motion. Thus CASA evolved some 300 years after the first-generation device. Several brands of computer-aided sperm analysis (CASA) instruments are now available that operate on broadly similar principles.

What is CASA?

CASA refers to an automated system (hardware and software) to visualize and digitize successive images of sperm, process and analyze the information.

How CASA works?

The common components of CASA instrument are **microscope, video camera and computer** (Fig. 1). The CASA captures multiple "snapshots" extremely quick (e.g., 30 images in 0.5 second).The computer software is used to identify and follow all of the spermatozoa in the video images and to perform data calculations. First, the image of the microscope field is sent from the camera and is converted into a digital image.

DO YOU KNOW?

Earlier version of CASA instruments could not differentiate between sperm head and debris, if the size of debris equals to size range expected for a sperm head. The Hamilton Thorne system now uses the IDENT system, in which Hoechst dye binds to the DNA in sperm heads, which differentiate sperm head from the debris.

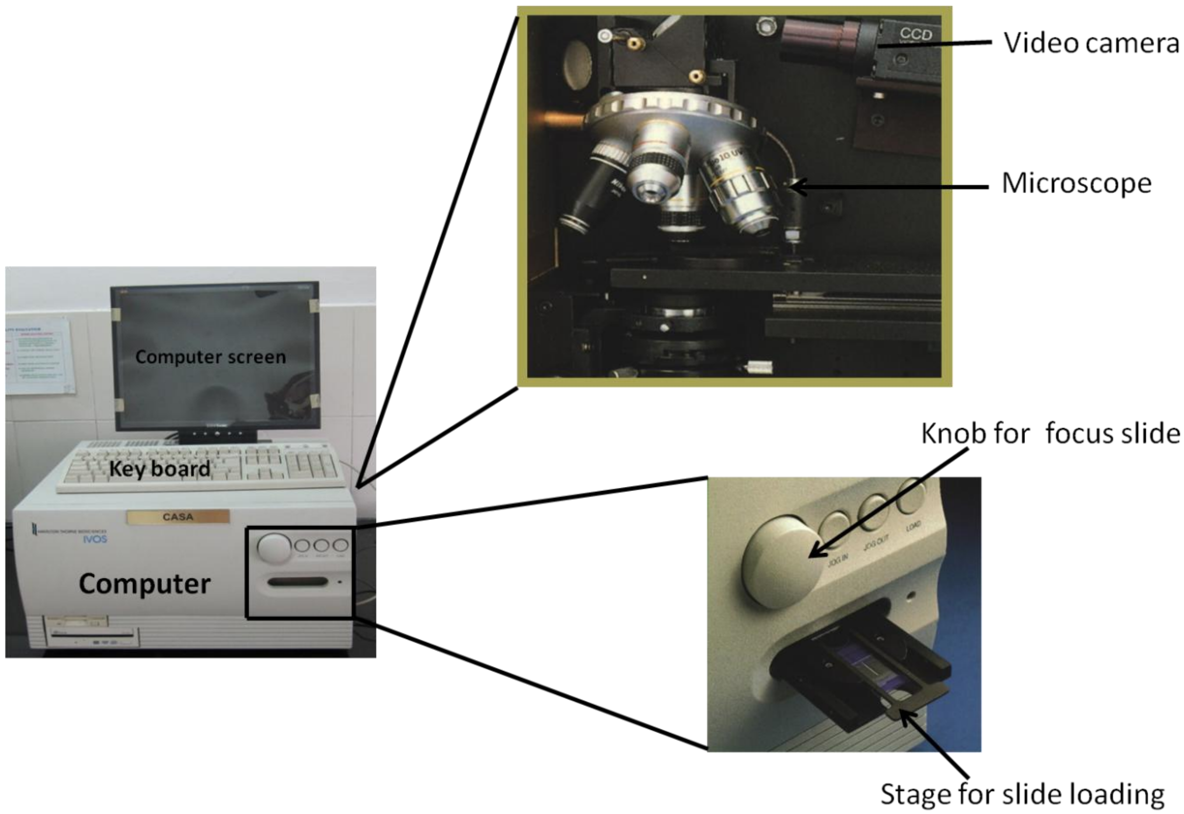


Fig. 1: Components of Computer Assisted Semen Analyser (IVOS).

Different Types of CASA

Different types of CASA are available in market but CASA of Hamilton Thorne, the leading manufacturer of CASA is very popular. There are two types of Hamilton Throne CASA:

IVOS Sperm Analyzer - The Integrated Visual Optical System (IVOS) is unique in that it is the only CASA system that integrates the optical system within the unit, so that an external microscope is not needed. Samples are loaded in a special type of slide called **Leija.** The slide is kept on the warm stage inside the CASA. The slide is focused with the help of the knob and image is analysed in just 0.5 second with a level of accuracy and repeatability which cannot be obtained by visual assessment.

Results obtained by CASA include (Fig.2&3) a number of parameters of sperm in seconds which is not possible with simple microscope. These results include

- Sperm count
- Sperm concentration

- Sperm motility
- Progressive motility
- Curvilinear velocity (VCL) of sperm
- Straight line velocity (VSL) of sperm
- Average path velocity (VAP) of sperm
- Linearity (LIN)
- Straightness (STR),
- Amplitude of lateral head displacement (ALH) and
- Beat cross frequency (BCF).

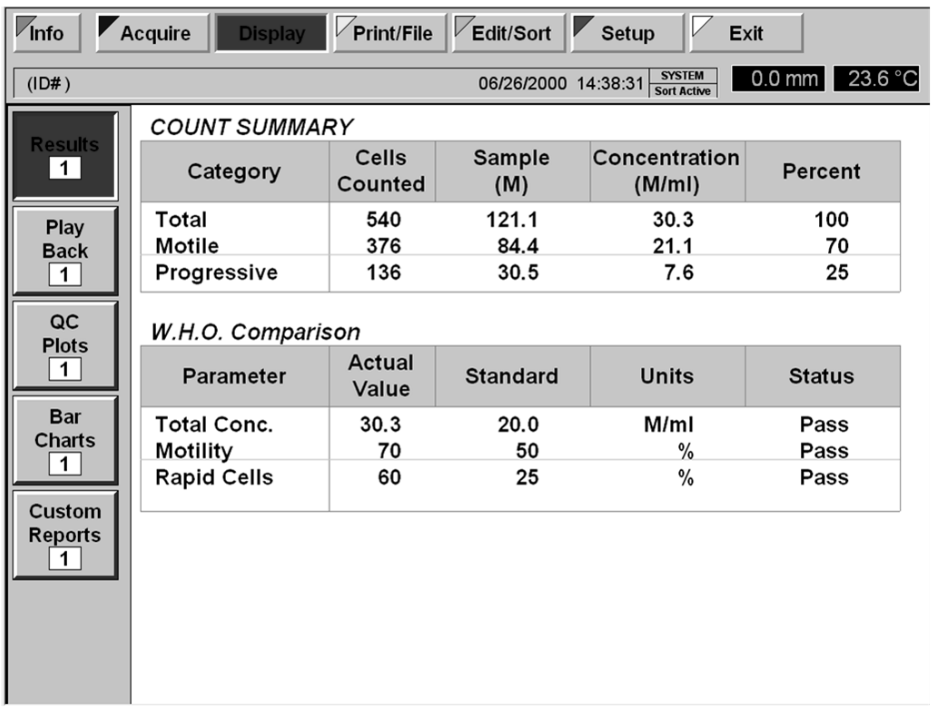
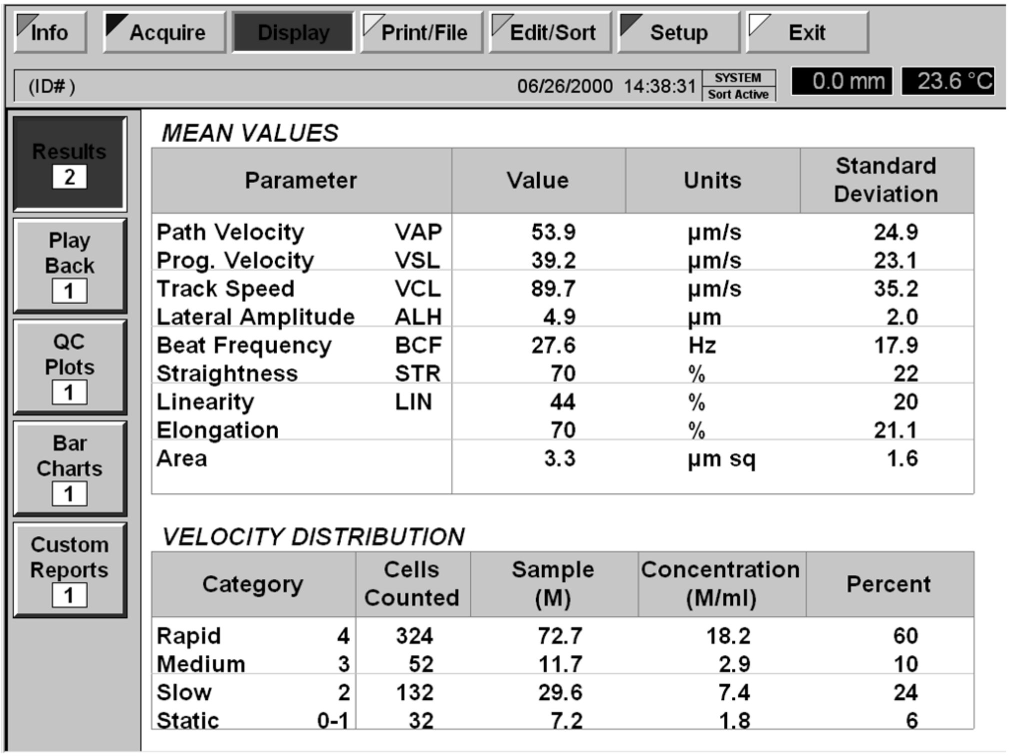


Fig. 2: Diagram showing results after analysis of semen sample by CASA. Total 540 sperm cells were counted by CASA among which 376 (70%) were motile and 136 (25%) sperm cells were progressively motile.



MEAN VALUES

Parameter		Value	Units	Standard Deviation
Path Velocity	VAP	53.9	µm/s	24.9
Prog. Velocity	VSL	39.2	µm/s	23.1
Track Speed	VCL	89.7	µm/s	35.2
Lateral Amplitude	ALH	4.9	µm	2.0
Beat Frequency	BCF	27.6	Hz	17.9
Straightness	STR	70	%	22
Linearity	LIN	44	%	20
Elongation		70	%	21.1
Area		3.3	µm sq	1.6

VELOCITY DISTRIBUTION

Category		Cells Counted	Sample (M)	Concentration (M/ml)	Percent
Rapid	4	324	72.7	18.2	60
Medium	3	52	11.7	2.9	10
Slow	2	132	29.6	7.4	24
Static	0-1	32	7.2	1.8	6

Fig. 3: Diagram showing results after analysis of a semen sample by CASA with their values and units.

For the study of morphology of sperm, thin smear is prepared on the normal slide and stained by special recommended stained. The stained slide is loaded in CASA. The CASA measures the following parameters (Fig. 4).

- Major axis of sperm head
- Minor axis of sperm head
- Length (elongation) of sperm head
- Area of sperm head
- Perimeter of sperm head
- Acrosomal percentage and
- Tail length

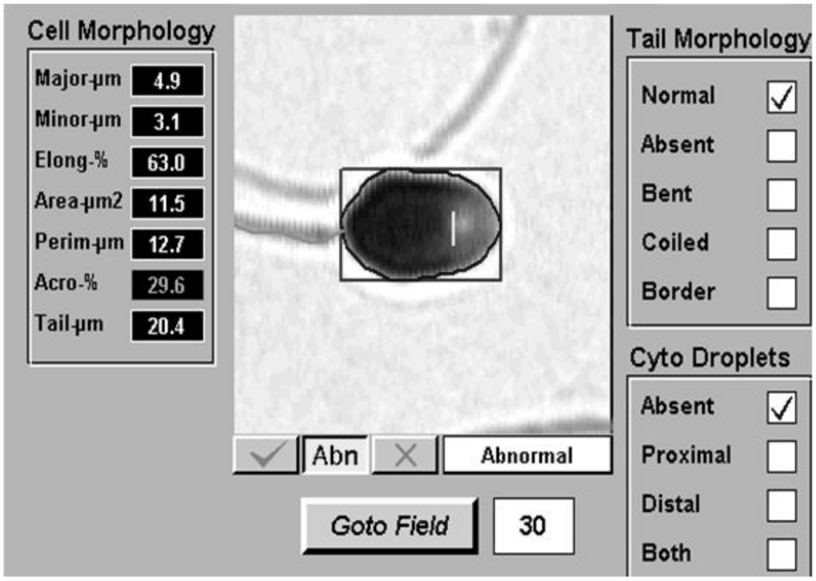


Fig. 4: Different parameters measured by CASA.

CEROS Sperm Analyzer - the CEROS offers the same level of accuracy and reliability for sperm analysis. The CEROS uses an external negative phase contrast microscope for image visualization and analysis. It is cost- effective instruments than IVOS.

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Always look at what you have left. Never look at what you have lost. - ***Robert H. Schuller***

Appendices

Normal Parameter of cow bull semen

Parameter	Normal value
Ejaculate volume (ml)	5 (1-10)
Concentration (million/ml)	1200 (300-2500)
Mass Motility (0-5)	≥3
Percentage motility (%)	≥70
Percentage live (%)	70
Abnormal sperm (%)	< 20 (Range for bulls with good fertility is 8–12)
pH	6.2-6.8

Normal Parameter of buffalo bull semen

Parameter	Normal value
Ejaculate volume (ml)	2.5 (1.5-5)
Colour	Milky white to creamy white
Concentration (million/ml)	900 (300-2200)
Mass Motility (0-5)	≥3
Percentage motility (%)	≥70
Percentage live (%)	70
Abnormal sperm (%)	< 20 (Range for bulls with good fertility is 8–12)
pH	6.8-7.2

Normal Parameter and limit value of boar semen

Parameter	Normal value	Limit value
Ejaculate volume (ml)	125-500	50
Concentration (million/ml)	25-1000 (150)	-

Total sperm per ejaculate (x 10^9)	10-100	10
Progressive motility (%)	70-95	62
Clumping (% coverage of microscopic field)	0-10	25
Curled tails (%)	1-2	10
Morphological abnormalities (%)	5-10	30
Acrosome abnormalities (%)	5-10	49
Protoplasmic droplets (%)	< 5	15

Normal Parameter of stallion semen

Parameter	**Normal value**
Volume (ml)	70 (30–300)
Concentration (million/ ml)	120 (30–600)
Morphologically normal (%) (minimum physiologically normal)	60-90 (65)
Live: dead ratio	6.5:3.5
Progressive Motility (%) (minimum progressively motile)	40-75 (40)
Longevity (at room temperature)	45% alive after 3 hr 10% alive after 8 hr

Normal Parameter of buck semen

Parameter	**Normal value**
Ejaculate volume (ml)	0.8 (0.1-1.5)
Concentration (million/ml)	2000-6000
Progressive motility (%)	60-80
Morphologically normal sperm (%)	80-95
pH	6.8 (6.2-7.0)

Normal Parameter of ram semen

Parameter	**Normal value**
Ejaculate volume (ml)	1 (0.8- 1.2)

Concentration (million/ml)	1500
Progressive motility (%)	40-75
Morphologically normal sperm (%)	65-90
pH	6.8 (6.2-7.0)

Normal Parameter of dog semen

Parameter	**Normal value**
Ejaculate volume (ml)	10(1- 25)
Concentration (million/ml)	125 (20- 540)
Progressive motility (%)	> 70
Morphologically normal sperm (%)	> 80
pH	6.7 (6.0- 6.8)

Normal Parameters of tom semen

Parameter	**Normal value**
Ejaculate volume (ml)	0.04 (0.01- 0.12)
Colour	Moderately milky or opalescent
Concentration (million/ml)	1730 (96- 3740)
Total number of spermatozoa in the ejaculate (million)	3.5 to 60
Progressive motility (%)	> 70
Morphologically normal sperm (%)	44-71
pH	7.4

Normal Parameters of camel semen

Parameter	**Normal value**

Volume (ml)	3.5 (1-10)
Colour	White
Appearance	Slimy
Concentration (million/ml)	140-760
pH	7.8 (7.2-8.8)
Live sperm (%)	55
Spermatozoa/dose	400 million

Normal Parameters of yak semen

Parameter	**Normal value**
Volume (ml)	2 - 5
Colour	Milky-White
Concentration (million/ml)	750-1600
Progressive motility (%)	70-85
Morphologically abnormal sperm (%)	5-10
Intact acrosome (%)	72
Viscosity (centipoise)	1.17

Age of puberty and maturity of male domestic animals

Species	**Puberty (months)**	**Sexual maturity**
Cow bull	10-12	2-3yrs
Buffalo bull	24-36	3-4 yrs
Stallion	18-24	3-4 yrs
Ram	3-6	8-12 months
Buck	3-6	8-12 months
Boar	5-6	8-9 months

Dog	6-12	12 months
Tom	6-12	9-12 months

Semen analysis nomenclature

Parameter	**Evaluation criteria**	**Nomenclature**
Volume	None	Aspermia
	Reduced	Hypospermia
	Increased	Hyperspermia
Sperm concentration	Zero	Azoospermia
	Reduced	Oligospermia
	Normal	Normozoospermia
	Increased	Polyzoospermia
Sperm motility	Decreased	Asthenozoospermia
Sperm viability	All dead	Necrozoospermia
Abnormal spermatozoa	High percentage	Teratozoospermia

Weight of testes, length of epididymis and length of penis in animals

Species	**Weight of testis (gm)**	**Length of epididymis(m)**	**Length of penis(m)**
Bull	350	40	102
Ram	275	50	40
Boar	360	18	55
Stallion	180	75	50

Biochemical Composition of Semen

Species	**Fructose** (mg/100ml)	**GPC** (mg/100ml)	**Inositol** (mg/100ml)	**Ergothionine** (mg/100ml)	**Citric acid** (mg/100ml)
Bull	**530**	**350**	35	0	**700**
Stallion	2	40- 120	30	**40-110**	25
Ram/goat	**250**	**1650**	12	0	**140**

Boar	13	110-240	**530**	6-23	80
Dog	0	180	-	-	Trace
Cat	-	-	-	-	-

Difference between X and Y DNA

Species	**Difference between X and Y DNA**
Cattle	3.8%
Sheep	4.2%
Pig	3.6%
Dog	3.9%
Horse	4.1%
Rabbit	3.0%
Human	2.9%
Turkey	0%
Ram	4.2%
Chinchilla	7.2%

Characteristics of mating in various species

Species	**Site of Semen deposit**	**Number of sperm at the ampulla-isthamus junction**
Cattle and buffalo	Vagina	Few
Ewe	Vagina	650
Sow	Cervix and uterus	1,000
Mare	Vagina and vaginal portion of the cervix	Few

Duration of copulation in the domestic animals

Species	Duration of copulation
Stallion	1-3minutes
Boar	4- 6 minutes
Dog	5- 45 minutes
Bull	5- 10 seconds
Tom	10 seconds
Ram	3- 5seconds
Buck	3- 5 seconds

Difference between cattle and buffalo sperm morphometry

	Cattle (μ)	Buffalo (μ)
Head length	9.1	7.4
Neck	0.65	0.44
Middle piece	12.5	11.6
Tail	46.2	42.8

Different courtship behaviour in the male animals

Behaviour	Cattle and buffalo	Sheep	Goat	Swine	Horse
Sniffing	Sniffing to female genitalia and urine				Sniffing to female's head
Flehmen reaction	Present	Present	Present	Absent	Present
Urination during sexual excitement	Absent	Absent	Frequent on foreleg	Rhythmic emission of urine	Marks with urine the place where a mare has urinated.
Vocalization during sexual	No	Present	Present	Present	present

excitement					
Tactile stimulation of female	Lick female genitalia			Noses female's flank	Bite back and neck
Postures during copulation	Presses head on female's back and leap during ejaculation	Rapid backwards movement of head during ejaculation		Motionless during ejaculation and scrotal contraction	Bites female's neck
Post coital reaction	Absent	Stretches head and neck	Lick penis	Absent	Absent

Standards for genetic merit of breeding bulls (as per MSP, Government of India)

Breed	**Dam's Lactation yield (Kgs)**				**Sire's daughters' averages of the bulls**		
	Farm born		**Imported (minimum)**	**Fat %**	**Min number of records**	**Average First Lactation yield (Kgs)**	**Reliability(%)**
	First	Best					
Pure HF	4500	5600	9000	3.5	30	4000	85
Pure Jersey	3000	3750	6000	5	30	2500	85
Sahiwal	2400	3000		4	20	1500	80
Red sindhi	2000	2500		4.5	20	1500	80
Gir	2400	3000		4.5	20	1500	80
Kankrej	2000	2500		4.5	20	1500	80
Tharparker	2000	2500		4	15	1400	75
Hariyana	1600	2000		4	15	1400	75
Rathi	1600	2000		4	20	1500	80
Ongole	1100	1600		4			
Deoni	800	1000	---	4	---	---	---
Khillar	380	500	---	4	----	---	---
Dangi	400	530	--	4	--	--	--
Amritmahal	400	500	--	4	--	--	--

Sunandini	2500	3000	--	3.5	30	3500	85
Murrah	2400	3000	--	7	30	1800	85
Mehsana	2400	3000	--	7	30	1800	85
Nilli Ravi	2400	3000	--	7	30	1800	85
Jaffrabadi	2800	3500	--	8	30	2000	85
Surti	1600	2000	--	7	30	1600	85

Abbreviations of breed on semen straws

As per the MSP of Government of India all semen stations have to follow strictly the following abbreviations on the semen straws

Breed	**Abbreviations**
Jersey	JY
Holstein	HF
HF cross	CB HF
Jersey Cross	CB JY
Sunandini	SUN
Sahiwal	SAH
Red sindhi	RS
Kankrej	KANK
Gir	GIR
Tharparker	THAR
Rathi	RATHI
Haryana	HAR
Murrah buffalo	MBF
Surthi buffalo	SBF
Jaffrabadi	JBF
Mehsana	MSNB

Colour of straws

As per the MSP of Government of India all semen stations have to use strictly the following colour of the straws for particular breeds:

Breed	**Colour of straws**
Jersey	Yellow
Holstein	Pink
Indigenous	Orange
HF crossbred	Pistachio Green(light green)
Jersey crossbred	Salmon
Sunandini	Blue
Buffalo	Grey

If any above colour is not available then transparent colour is used.

Summary of Sterilization (as per MSP)

Autoclave

Sr.No.	**Item**	**Pressure (p.s.i.)**	**Time (Min.)**
1.	Artificial vagina	5	20
2.	Buffer	5	15/20
3.	Plastic tips	5	20
4.	Filter papers	5	20
5.	Bull apron	5	20
6.	Thermo-resistant rubber wares	3-4	10
7.	Bacteriological media	15	15
8.	Distilled water	15	15
9.	Surgical equipment	10	10

Hot air oven

Sr. No	Item	Temperature	Time (min.)
1	Glass wares	160^0 C/ 180^0 C	60/30
2	Filling Nozzles	160^0 C/ 180^0 C	60/30

Semen stations, bulls and semen production in India (Annual Report 2011-2012, Department of Animal Husbandry, Dairying and Fisheries)

Agency	Semen Stations	No of Bulls	Semen Production (million)
Government	37	2029	31.59
NDDB, Dairy Coop, NGO and Private	11	1292	30.37
Total	48	3321	61.96

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*Success is the sum of small efforts, repeated day in and day out.-**Robert Collier***

GLOSSARY

GLOSSARY

Andrology (Greek Andros, man)

A branch of science concerned with function and diseases to male especially those affecting the male reproductive system.

Artificial insemination (AI)

It is a technique in which sperm are collected from the male, processed, stored and artificially introduced into the female reproductive tract at proper time for the purpose of conception.

Puberty

The onset of **puberty** can be defined as a gradual progression of a number of biologic events, such as the onset of spermatogenesis, appearance of sperm in the ejaculate, and capability of intromission.

Sexual maturity

It occurs when the development of both spermatogenesis and reproductive behaviour allow effective coordinated service and subsequent fertilization.

Impotentia coeundi

The reduced to complete lack of sexual desire and ability of male to copulate is called *Impotentia coeundi*. In other words the condition of male causing failure of normal service is called *Impotentia coeundi.*

Impotentia generandi

The inability or reduced ability of a male to fertilize after normal service is called *Impotentia generandi.*

Orchitis

Inflammation of **testis**.

Orchiepididymitis

Inflammation of both **testis** and **epididymis**

Periorchitis

Inflammation of **tunica vaginalis**

Periorchiepididymitis

Inflammation of tunica vaginalis, testis and epididymis.

Epididymitis

Inflammation of epididymis.

Balanoposthitis

It is an inflammation of the glans penis and the prepuce.

Posthitis (pizzle rot)

It is a chronic condition in sheep characterized by scabs and ulcers of the skin and mucosa of the prepuce and is caused by an interaction of a high protein diet and *Corynebacterium renale.*

Varicocele

It is a dilatation and tortuosity of the veins of the pampiniform plexus and the cremasteric veins.

Paraphimosis

This condition occurs when the penis protrudes from the preputial sheath and cannot be replaced to its normal position.

Phimosis

The inability to completely protrude the penis from the prepuce.

Diphhalus

It is also called double penis.

Priapism

It is a potentially harmful and painful condition in which the erect penis does not return to its flaccid state, despite the absence of both physical and psychological stimulation.

Honeymoon back

In young bulls that are more enthusiastic in their early attempts to mate, the lumbodorsal fascia may rupture during the mounting, producing the so-called condition of **‘honeymoon back’**.

Pederasty

The rectal copulation is called pederasty. It is more common in young boar.

Onanism

Masturbation is called onanism

Preputial diverticulum

It is a bilobed sac located on the dorsal wall of the preputial cavity of boar.

Balling up

The entrapment of the penis in the preputial diverticulum during attempts of copulation is called balling up.

Epididymectomy

The surgical removal of the caudal epididymis is a simple procedure for creating sterile male for use in estrus detection programs.

Persistent penile frenulum

It is a congenital band of tissue extending from the median raphe of the prepuce to the ventral side of the penis near the glans.

Teaser bull

A bull that detects heat in cows without being able to fertilize them is called teaser bull.

Hypospadias and epispadias

They are birth anomalies that result from defective development of the penis during embryological development. The incompletely-developed urethra may form an opening at the underside of the penis (hypospadias) or the upper side of the penis (epispadias).

Hydrocele

It is a pathologic accumulation of serous fluid between the visceral and parietal layers of the vaginal tunic.

Hematocele

It resembles a hydrocele but is a collection of hemorrhagic, rather than serous fluid within the vaginal cavity.

Testicular degeneration

It is defined as the partial or complete failure of epithelium of seminiferous tubules to proceed with spermatogenesis.

Testicular hypoplasia

It is a congenital failure in the development of the spermatogenic epithelium while the intersititial tissue and Leyding cells are practically normal.

Testicular descent

When the testis migrates from its position within the abdomen towards its definitive position in the scrotum is called testicular descent.

High flankers or inguinal cryptorchid

When the testes are located in the inguinal canal, the male is called high flankers or inguinal cryptorchid.

Low flankers

If the testes are located within the subcutaneous tissues dorsal and cranial to the scrotum, the male is called "low flanker".

Cryptorchidism

It is a developmental abnormality in which one or both testes fail to descend from the abdominal cavity into the scrotum.

Complete cryptorchid

If the testicles and entire epididymis are located within the abdominal cavity, the male is called complete cryptorchid.

Partial cryptorchid

If the testicles and majority of the epididymis are located within the abdominal cavity but vas deferens and tail of epididymis are located in the scrotum, the male is called partial crytorchid.

Unilateral cryptorchid or monocryptorchid

If one testicle fails to descend into the scrotum, the male is called unilateral crytorchid.

Bilateral crytorchid

If both testicles fail to descend into the scrotum, the male is called bilateral cryptorchid. A less common term for the condition is ectopic testis.

Monochidism

When only one testis is present, the condition is called monorchidism.

Anorchidism

When both testes are absent, the condtion is called anorchidism.

Seminal granulomas

Formation of granuloma around the head of the epididymis due to accumulation and inspissations of sperm at the blind end of efferent ducts is called seminal granulomas.

Intracytoplasmic sperm injection (ICSI)

It is one of the modified forms of IVF that involves a micromanipulator to inject a single spermatozoon into the cytoplasm of a mature metaphase II oocyte. The ICSI eliminates the need for the spermatozoon to bind to and penetrate the zona pellucida and oocyte plasma membrane.

Spermatogenesis

It is a highly synchronized and hormonally controlled sequence of events wherein the germ cells undergo a series of divisions and differentiation (spermatogonia, primary spermatocytes, secondary spermatocytes, early spermatids, and late spermatids) resulting in the formation of haploid sperm.

Spermatocytogenesis

It is the proliferative phase in which spermatogonial cells multiply by a series of mitotic divisions followed by the meiotic divisions which produce haploid spermatid.

Spermiogenesis

The round spermatids are transformed into elongated spermatozoa by a series of progressive morphologic changes is called spermiogenesis. This is also called differentiation phase.

Spermiation

The release of spermatozoa from the Sertoli cells into the lumen of seminiferous tubule is called spermiation. It is **analogous to ovulation** in the female except that spermiation occurs continuously throughout the testis.

Spermatogenic cycle

It is defined as the reappearance of the same stage or complete sequence of stages at any given site within a given section of the seminiferous tubule.

Spermatogenic wave

It is defined as a complete series of the stages along the length of a seminiferous tubule which is approximately 10 mm long in bulls.

Epididymal transit time

The interval between release and ejaculation of sperm. In bulls, the duration of this interval averages 8.3 days (range, 7 to 13 days), with shorter periods occurring in response to frequent ejaculation.

Daily sperm output (DSO)

The number of sperm harvested per day from a male. DSO is not only a function of sperm production, but it is also influenced by ejaculation frequency, bull stimulation, and collection method.

Daily sperm production (DSP)

The total number of sperm produced per day by the testes. DSP is usually assessed by histologic examination of testicular tissue or by preparing a homogenate and counting cells.

Sexual behaviour

It can be described as a pattern of well-defined steps, including actively seeking estrus females, courtship, mounting, intromission, and ejaculation.

Libido

Libido is defined as the "willingness and eagerness" of a male to attempt to mount and service.

Mating ability

It refers to the ability and competence of the bull in fulfilling this aspiration.

Serving capacity

It is a measure of the number of services achieved by a male under stipulated conditions and thus it includes both libido and mating ability.

Courtship (Sexual display)

It is the specialized behaviour in animals that leads to or initiates mating.

Flehmen Reaction

After sniffing the male stands rigidly and holds his head in horizontal position with extended neck and raised upper lips. This reaction is called Flehmen reaction.

Erection

An erection is the stiffening of the penis which occurs during sexual arousal.

Intromission

Intromission is the successful entrance of the penis into the vagina.

Ejaculation

It is defined as the reflex expulsion of semen from the penis.

Refractory period

It is a period of time during which a second copulation does not take place.

Sexual satiation

It refers to a condition in which after a certain copulation further stimuli will not make ready to a male for copulation.

Coolidge effect

It is defined as the restoration of mating behaviour in males (that have reached sexual satiation) when the original female is replaced by a novel female.

Mediastinum testis

Along the caudal border of testis, a mass of tissue projects from the tunica albugenia into the testis known as mediastinum testis.

Gabernaculum

It is fibro muscular tissue which is elastic in nature which binds testis with scrotal sac.

Primary sperm abnormalities

These are those sperm abnormalities which arise during spermatogenesis.

Secondary sperm abnormalities

These are those sperm abnormalities which occur after the sperm have left the seminiferous tubules and during their passage through the efferent ducts, epididymis and vas deferens.

Tertiary sperm abnormalities

These are those sperm abnormalities which arise during or after ejaculation.

Knobbed acrosome defect

This sperm defect was first reported in a sterile Friesian bull. The defect is generally described as an eccentric thickening at the apex of the affected spermatozoa.

Diadem defect

The sperm defect which represents pouches in the nuclear material.

Decapitated (disintegrated) sperm defect

In this sperm defect most sperm are decapitated and the detached tails are motile.

Dag defect

The term Dag defect was coined named after the Jersey bull (Dag) in which the defect was first identified. This defect is represented by strong coiled tail and fracture of the distal part of the sperm midpiece. It results from abnormal development of the axoneme and mitochondrial sheath of sperm.

Pseudo-droplet defect

The defect is characterized by a local thickening on the midpiece. Although they resemble to cytoplasmic droplets under normal microscope, they are found in regions where droplets are seldom encountered (e.g. the middle of the midpiece).

Corkscrew sperm defect

In this defect, irregular distribution (lumps) or loose arrangement of the helix of mitochondria is found on the middle piece of sperm which resembles to a corkscrew under light microscopy.

Tail-stump defect

In this defect normal heads are attached to a vestigial structure that appears like a protoplasmic droplet.

Breeding soundness

It is a prediction of a bull's potential reproductive capacity based on standard measurements and interpretations of certain selection criteria.

Breeding soundness evaluation (BSE)

It is an overall assessment of the male's capacity for serving and impregnating a number of females.

Post-legged

When an animal is post-legged, the leg joints are too straight with almost no bend in the legs.

Sickle-hock

When a bull is sickle-hock conformation, the hind legs are too curved (sickle-hocked).

Hocks-in or hocks-out

Hocks-in or hocks-out cause uneven weight bearing on the claws, which inevitably affects hoof growth. Hocks-out is more structurally damaging than hocks- in, causing persistent ligament damage.

Cryopreservation

It is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, such as (typically) −196 °C (the boiling point of liquid nitrogen).

Liquid nitrogen

It is nitrogen in a liquid state at a very low temperature. It is produced industrially by fractional distillation of liquid air. Liquid nitrogen is inert, colourless, odourless, non-corrosive, non-flammable and extremely cold.

Straws

These are thin-walled plastic tubes of different dimensions. Straws are now the most popular container in the world for semen packaging. They have replaced glass ampoules and pellets.

Recrystallization

It refers to the growth of larger crystals at the expense of smaller ones.

Dry ice

The solid carbon dioxide with a temperature of -77^{0} C.

Dewar flask

An insulated flask used for holding cryogenic fluids. The flask contains an inner and outer layer usually separated by an evacuated space. The inner surface of the flask is usually coated with silver or aluminium to minimize heat loss.

Freezing

The conversion of an unfrozen solution into the solid form.

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The ability to convert ideas to things is the secret to outward success.