

Animal Production

Level – III

**Based on March 2022, Version-4 Occupational
Standard**



**Module Title: Performing Artificial Insemination for
Livestock**

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Introduction to the Module

Artificial insemination is the technique in which semen with living sperms is collected from the male and introduced into female reproductive tract at proper time with the help of instruments. Artificial Insemination has been defined as the transfer of male gametes to the oocyte by means other than natural mating. This has been found to result in a normal offspring. In this process, the semen is inseminated into the female by placing a portion of it either in a collected or diluted forms into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions.

This includes insemination of the female and in vitro fertilization of oocytes with fresh or frozen semen. Frozen semen in 0.5 ml or 0.25 ml straws has become the universally accepted unit of storage and transfer of bovine genetics to cattle producers. The efficiency of cow insemination depends on the collection and deposition of appropriate numbers of normal spermatozoa near the oocyte, and also on the ability of the inseminator to deliver the semen to the appropriate site in the reproductive tract at the appropriate time of oestrus. AI is still the main method of genetically improving the quality of livestock breeds on a worldwide scale.

LG # 38

LO # 1- Prepare Animals for Insemination

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Addressing history of the animal for the insemination
- Identifying and restraining animals for insemination
- Considering body condition and body frame of animal
- Addressing physiological status and signs of heat
- Scheduling time of insemination
- Carrying out estruses synchronization
- Identifying and preparing animals in heat for insemination

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Address history of the animal for the insemination
- Identify and restrain animals for insemination
- Consider body condition and body frame of animal
- Address physiological status and signs of heat
- Schedule time of insemination
- Carry out estruses synchronization
- Identify and prepare animals in heat for insemination

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the information Sheets
4. Accomplish the Self-checks
5. Perform Operation Sheets
6. Do the “LAP test”

Information Sheet – 1

1.1. Addressing history of the animal for the insemination

The first successful experiment with artificial insemination in animals was performed by Italian physiologist Lazzaro Spallanzani, who in 1780, while investigating animal reproduction, developed a technique for artificial insemination in dogs. Dogs were confined in Spallanzani's house, and after a lapse of 20 days the bitch manifested obvious signs of being in heat. Then with semen at body temperature she was artificially inseminated, the semen being deposited directly into the uterus with a pointed syringe. 62 days following insemination, the bitch gave birth to three pups, all of which resembled not only the mother but also the dog from which semen had been taken. In 1782 Spallanzani's experiment was successfully repeated by P. Rossi and checked by professor Branch. These experiments proved the feasibility of inducing pregnancy by artificial insemination, with the resultant birth of normal offspring.

Spallanzani (1803) also contributed to the knowledge of the effect of cooling on the prolonging of sperm life. He observed that freezing stallion semen in snow or winter cold did not necessarily kill the “spermatic varmiculi” but held them in a motionless state until exposed to heat, after which they continued to move for seven and a half hours.

In 1899, Ivanov of Russia pioneered AI research in birds, horses, cattle and sheep. He was apparently the first of successfully inseminate cattle artificially. Mass breeding of cows via AI was first accomplished in Russia, where 19,800 cows were bred in 1931. Denmark was first to establish an AI cooperative association in 1936. E. J. Perry of New Jersey visited the AI facilities in Denmark and established the first United State AI cooperative in 1938 at the New Jersey State Collage of Agriculture.

Sorensen in 1937 (Danish Veterinarian), developed the recto-vaginal technique of insemination (used worldwide today). Cassou, from France in 1960s brought semen processing technology by designing the straw system of packaging deep frozen semen, culminating in the development of the mini-straw in 1969.

Reproductive technology revolutionized dairy production during the past century. Artificial insemination was first successfully applied to cattle in the early 1900s. The next major developments involved semen extenders, invention of the electro-ejaculator, progeny testing,

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addition of antibiotics to semen during the 1930s and 1940s, and the major discovery of sperm cryopreservation with glycerol in 1949. The 1950s and 1960s were particularly productive with the development of protocols for the superovulation of cattle with both pregnant mare serum gonadotrophin/equine chorionic gonadotrophin and FSH, the first successful bovine embryo transfer, the discovery of sperm capacitation, the birth of rabbits after in vitro fertilization, and the development of insulated liquid nitrogen tanks. Improved semen extenders and the replacement of glass ampules with plastic semen straws followed.

Artificial insemination (AI) was the first great biotechnology applied to improve reproduction and genetics of farm animals. It has had an enormous impact world-wide in many species, particularly in dairy cattle. The acceptance of AI technology worldwide provided the impetus for developing other technologies, such as cryopreservation and sexing of sperm, estrous cycle regulation, and embryo harvesting, freezing, culture and transfer, and cloning. New, highly effective methods of sire evaluation were developed. The history of development of AI is reviewed, particularly in dairy cattle, in which the impact on genetic improvement and control of venereal diseases has been greatest.

Artificial insemination is the implanting of live spermatozoa into the genital tract of the female. The diluted or treated semen is usually deposited in the body of the uterus because a higher fertility rate is obtained, but insemination into the uterine, cervix or even the vagina may be practiced. It is simply define as a process by which sperm are collected from the male, processed, stored and artificially introduced into the female reproductive tract for the purpose of conception.

Artificial insemination, or AI, is the process by which sperm placed into the reproductive tract of a female for the purpose of impregnating the female by using means other than sexual intercourse or natural insemination. Artificial insemination (AI) is the process of collecting sperm cells from a male animal and manually depositing them into the reproductive tract of a female. Artificial insemination is commonly used instead of natural mating in many species of animals because of the many benefits it can reap.

Advantages of AI;

- Extensive use can be made of superior sires.
- Semen can be stored, when frozen, for many years after the sire is dead.

- Semen can be used from sires after they have been progeny tested.
- Venereal diseases can be controlled, provided that there is careful screening and monitoring of sires at AI centers.
- Farm safety is improved because potentially dangerous dairy bulls need not be kept.
- The need to rear and feed sires on the farm is removed/save feed cost
- It overcomes the difficulty of size and weight.
- It increases the rate of conception in females.
- Outstanding animals located apart can be mated.
- It helps in better record keeping of farm.
- Old, heavy and injured sires can also be used with AI technique

Disadvantages of AI;

- Estrus needs to be detected and insemination timed accurately in order to obtain good pregnancy rates so that it is difficult.
- Dystocia can result if semen from exotic breeds is used on immature heifers or local breed.
- There is a possibility of inbreeding if there is extensive use of a limited number of sires.
- There is a possibility of extensive transfer of undesirable genetic traits if sires are not carefully monitored.
- It requires well trained operators and special equipment.
- It requires more time than natural services.
- It necessitates the knowledge of structure and function of reproduction
- Market for the bulls is reduced while that for the superior germ plasm is increased.
- Selection of the sire should be very rigid in all respect.

1.2. Identifying and restraining animals for insemination

Female animals with observed heat signs and are ready to be inseminated. The identified animal must be restrained for proper AI procedure. The first rule to keep in mind when handling any kind of animal is that the simplest and suitable restraint is often the best restraint. This does not mean that you give up your control, just that you use as little restraint as necessary while maintaining control of the situation. Every animal and every situation is different so there are no hard and fast rules as to what method work best in which situation.

For insemination process the animal handled properly in AI crushes.

Restraining methods can be:

- Psychological /Behavioural/ Verbal-commands
- Physical – by using equipment and materials
- Chemical – by means of drugs

But for insemination process the animal should handle be properly in AI crushes. Area should be safe, clean and free from any stressors. Attention must be given to the possible development of lesions or illnesses associated with the restraint including contusions (bruises or discoloration), decubital ulcers, dependent oedema, and weight loss. If these or other problems occur, prompt veterinary care must be provided. This may require temporary or permanent removal of the animal from the restraint device depending upon advice of the attending veterinarian.

1.3. Considering body condition and body frame of animal

Body condition scoring is an easy and efficient tool to assess the energy reserves of livestock which reflects their recent nutritional management. It is used to evaluate energy reserves of the animals based on their body fat and muscle reserves and is a better predictor of body fat than is live weight. The assessment of body condition score (BCS) should not simply reflect the live weight of the animals nor be influenced by factors like gut-fill, mature size or weight of the concepts in pregnant cows. BCS can be used to evaluate the body reserves of cows at crucial time points throughout the year to improve feed planning, which is known to influence reproductive performance. Typically, BCS is evaluated through visual assessment that is easily implemented on-farm, without the need for any off-site training or further equipment. Reproductive performance has been reported as being influenced by BCS, in terms of pregnancy rates or inter-calving interval. BCS before calving and at the start of mating are the main factors influencing pregnancy rates in beef cows.

BCS is the tool to measure the status of energy balance in the cow. The severity and length of negative energy balance (NEB) can be estimated through changes in body condition score. BCS (<2.5) have a prolonged anoestrous period due to low LH pulse and concentrations of oestradiol, which are ineffective to induce an LH surge. High BCS (>3.5) which results in a subsequent prolonged period of negative energy balance (NEB) and low reproductive efficiency.

Precondition the cow to moderate changes in NEB and minimal BCS loss (<0.5 unit) to overcome massive changes in metabolites and metabolic hormones during early postpartum period is very essential.

Feeding high roughage diets at the start of the dry period to minimize BCS gain and maintaining change in BCS score of 0.5 during the transition period are important for high conception rates and herd pregnancy rates. To achieve this important target, cows should be maintained at a BCS of 2.5–3.0 in association with maintenance of proper rumen function through adequate dietary fibre, shortening the dry period (6–8 weeks maximum), reduction in the incidence of subclinical metabolic disorders, particularly hypocalcemia and minimizing mobilization of body reserves in the early postpartum period.

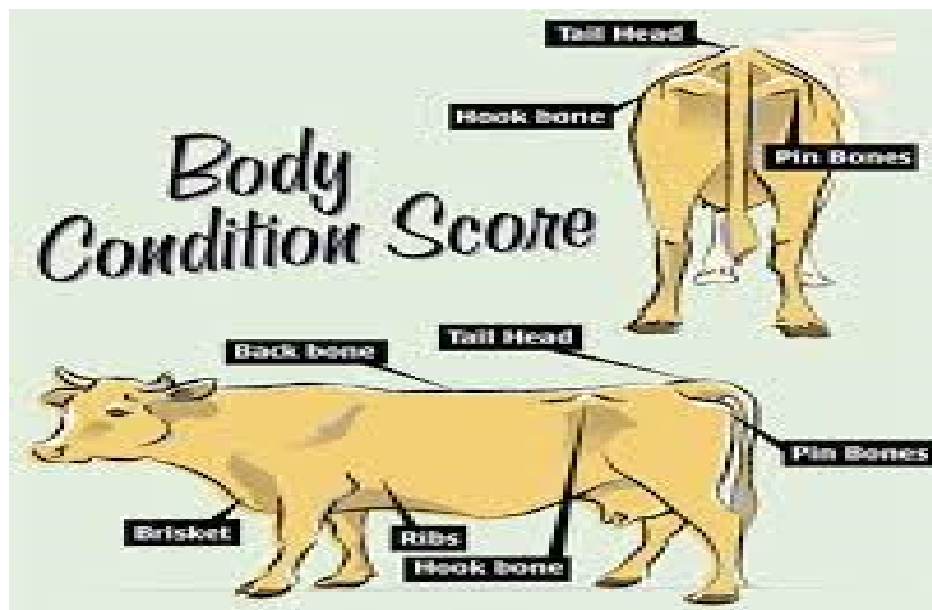


Figure 1.1: Body condition scoring areas

1.3.1. Identifying anatomy and physiology of reproductive organ of animals

The Primary significance of the reproductive system lies in continuation of species of animal. In higher animals sexual dimorphism is present and sexes are separate. The male possess male reproductive system and females' possess female reproductive system. Successful artificial insemination programs are based on a clear understanding of the anatomy and physiology of reproduction in cattle. Before attempting to inseminate cows, you must develop a mental picture of the anatomical parts that comprise the female reproductive tract.

In order to understand why an animal displays the many signs of estrus, when she should be inseminated, and how the pregnancy develops, you must clearly understand the hormonal mechanisms controlling the estrous cycle in cattle.

Benefit of knowledge of anatomy

- To identify the location & the structural status of the organs
- To understand & determine the developmental stage of reproductive organs for breeding purpose
- To determine whether the cow/heifer is in heat or not
- To manipulate for AI service & perform skillful insemination procedures
- To perform pregnancy diagnosis
- To differentiate the difference b/n normal & abnormal /diseased/ conditions
- For treatment purpose

Male reproductive System

It consists of primary **sex organs, testes and accessory sex structures** - the vas deferens, the vesicular glands, the ejaculatory ducts, prostate, bulb urethral glands and penis.

The testes: are encapsulated by inelastic capsule of dense connective tissue called tunica albuginea. The visceral layer of vaginal tunic immediately overlies the tunica albuginea and is bound to it by transversal fascia. The tunica albuginea is continuous with connective tissue structure of testis at mediastinum testis from which connective tissue trabeculae ramify to form the septulae testis. The mediastinum testes contain blood vessels, nerves, lymphatics and rete testis.

The Scrotum: The scrotum is a cutaneous sac having thin, pliable sac. A layer of fibro elastic tissue with smooth muscle fibres called tunica dartos. It helps in maintenance of testicular temperature. The scrotum actually represents a fusion of 2 scrotal pouches. Internally separated by septum Scroti. The scrotum connects abdominal cavity by inguinal canal.

The canal remains open in those species in which testes periodically with draw into body cavity. Sometimes a loop of intestine may pass through the canal into scrotum and be caught and produces inguinal hernia. When testis fails to descend down into scrotum is called Cryptorchid testes, condition is Cryptorchidism. It is a sterile condition.

An animal in which testis descends into inguinal canal but not into scrotum is called high flanker. Generally the 3⁰c below body temperature is required for development and maintenance of sperms. Spermatozoa pass from rete testis through efferent ductless to the epididymis. The smooth muscle functions to move materials through the efferent ductules into the epididymis. The Epididymis is temporary store house of sperms. Epididymis is extremely tortuous. Spermatozoa are propelled through the epididymis by the activity of the smooth muscle coat.

The epididymis: is composed of head, body and tail. The physiological maturation takes place in epididymal duct. Sperms are usually stored within tail of epididymis. In this region they are not motile. The epididymis is continuous with the ductus deferens which carries spermatozoa and suspending fluid to prostatic urethra. The major functions of the ductus deferens (vas deferens) are the storage of spermatozoa and transport of spermatozoa from the epididymis to pelvic urethra.

The vesicular glands: are present at distal extremity of the ductus deferens. They are absent in carnivore but present in other domestic animals. The addition of fructose to seminal fluid by ampulla and vesicular glands provides a source of energy. The secretions of vesicular glands contribute a major portion of the volume of semen available at each ejaculation. These secretions of the bull empty into prostatic urethra through a common opening with ductus deferens. These structures form a true ejaculatory duct.

The prostate gland: is associated with initial portion of urethra. It is a branched tubule alveolar gland organized into lobules. The prostate gland consists of two parts. 1. Body – dorsal to urethra. 2. Pars disseminate – under lying at urethral is muscle. The prostate of the ram is composed only pars disseminate. The prostate of the dog is relatively large composed to that of other domestic animals.

Spermatogenesis: Spermatozoa are formed in seminiferous tubules. The cells lining seminiferous tubules which give spermatozoa are called spermatogonia by series of cell divisions. It consists of two stages.

- Spermatocytogenesis proliferation of cells
- Spermiogenesis maturation of spermatid spermatozoa

Structure of Mature Spermatozoon: The spermatozoa consist of three parts

- a) Head: It is the principal piece. It consists of acrosomal cap and nucleus.
- b) Neck: It connects the head with tail. It contains two centrioles.
- c) Tail

In domestic animals the testis is always in scrotum descended to outside. The other cells present in seminiferous tubules are sertoli cells which have considerable importance in spermatogenesis and normal maturation of germ cells. They act as supportive cells. They provide mechanical support for developing germ cells and also supply nutrition

Erection and Ejaculation: The penis composed of root, body and glands portions. The root attaches the penis to ischial arch. The body of pens consists of 2 cavernous structures known as corpora cavernosum. One which surrounds urethra is called corpus cavernosum urethra. The other is dorsal to urethra and is known as corpora cavernosum penis. Engorgement of Venus sinuses of corpora cavernosum penis and corpus cavernosum urethrae results in rigidity of penis known as erection. The movement of semen from duct system into urethra is known as emission and further movement from male reproductive system is known as ejaculation.

Female Reproductive System

The female reproductive organs consist of a pair of ovaries, a pair of oviduct (Fallopian tubes), uterus and vagina.

The cow's reproductive system has four basic functions.

- To produce ova (eggs) which provides half of the eventual offspring's genetic makeup
- To provide an environment and conditions for the fertilization of those ova
- To provide a place following fertilization for the nourishment and fetal development of the calf
- To provide a mechanism for the birth of the calf

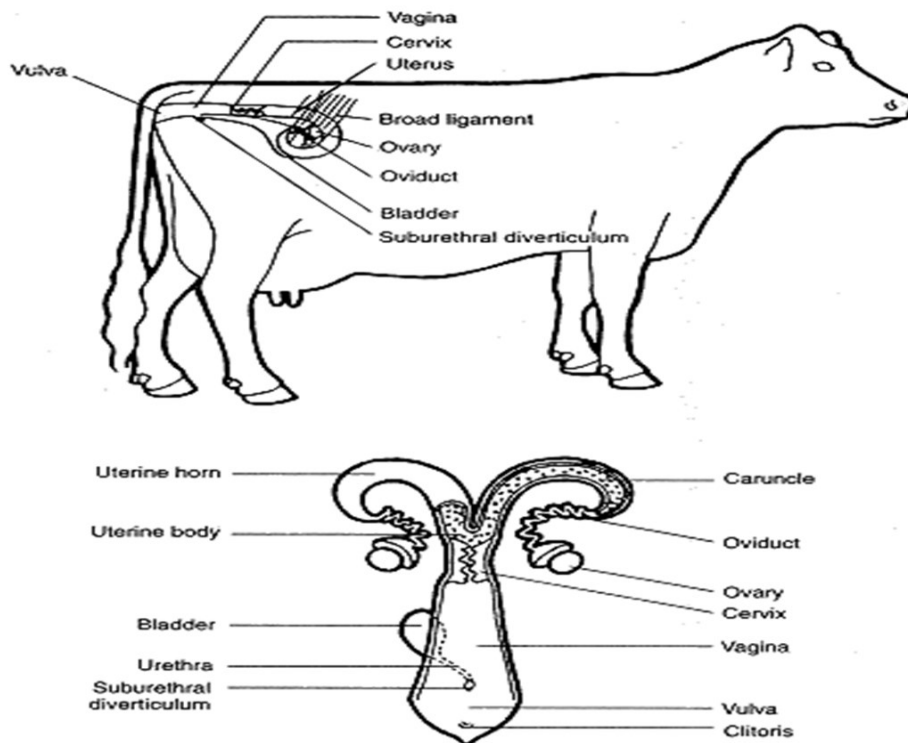


Figure 1.2A: Female reproductive organs

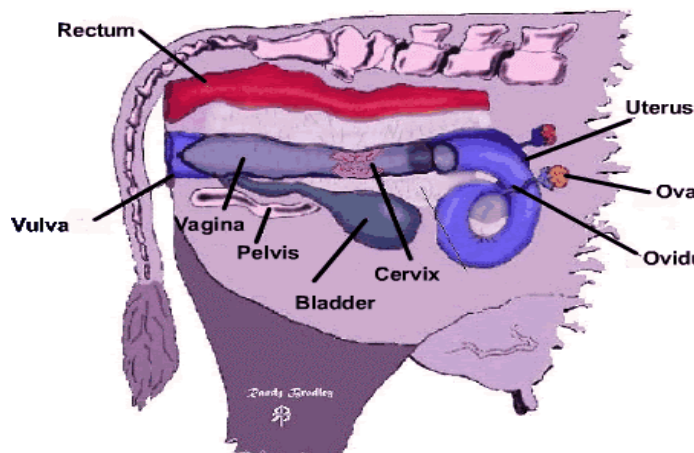


Figure 1.2B: Female reproductive organs

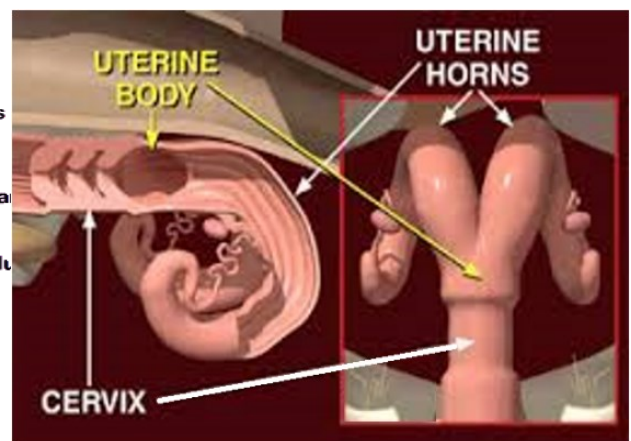


Figure 1.2C: Female reproductive organs

Ovaries: They are female reproductive organs and also act as endocrine organs. The mammal's ovaries are solid structures lying on sides of vertebral column behind kidney in pelvic cavity. In the ovaries germinal epithelium is present. From these cells some cells enlarge and differentiate to become ova. Each ovum is surrounded by a granulose membrane. The ovum with granulose is called primordial follicle.

Oviducts / Fallopian tubes: These are a pair of tube like structure. Inside it is lined with ciliated epithelium which helps in movement of ovum. The end of the tube is wide and funnel like structure having finger like structure called fimbriae. When ovum is released into body cavity it is normally directed into funnel of fallopian tube. Then it passes through oviduct and gets fertilised before it reaches uterus. Its motility is increased by hormones, oestrogen but depressed by progesterone. The ovarian end of the oviduct is funnel shaped and called the infundibulum. The infundibulum catches the egg as it is released from the ovary at ovulation and moves it to the enlarged upper end of the oviduct called the ampulla. Fertilization occurs here within 12 hours of ovulation.

Uterus: After fertilization, the fertilized ovum is transported to the uterus in a process requiring 3 to 4 days. It is a highly muscular sac like structure which houses the developing embryo until the time of birth. The uterus has 3 parts. The broad apical portion is Fundus, uterine cavity is body, and neck is Isthmus. The uterus wall consists of three layers (Epimetrium, Myometrium and Endometrium)

The uterus consists of a “body” and two “horns”. It is attached to the broad ligament and suspended within the pelvic cavity and posterior portion of the body cavity. The body of the uterus is adjacent to the cervix. In a non-pregnant state it extends less than 2 inches before it divides into two separate horns. The uterine body is the major site of semen deposition during AI. If the tip of the inseminating rod is inserted too far into the uterus, semen is deposited in only one of the uterine horns. If the egg was released from the ovary on the other side, there is little chance that sperm and egg would unite.

Remember, the body of the uterus is less than 2 inches long and caution must be used to correctly deposit semen into this region. The uterus has many functions. Its walls are composed of several layers of muscle which aid in transport of sperm to the oviduct following insemination and in expulsion of the calf at birth. Certain glands within the walls of the uterus secrete fluid, uterine milk, which provides nutrients to the developing embryo before and after its attachment to the uterine wall.

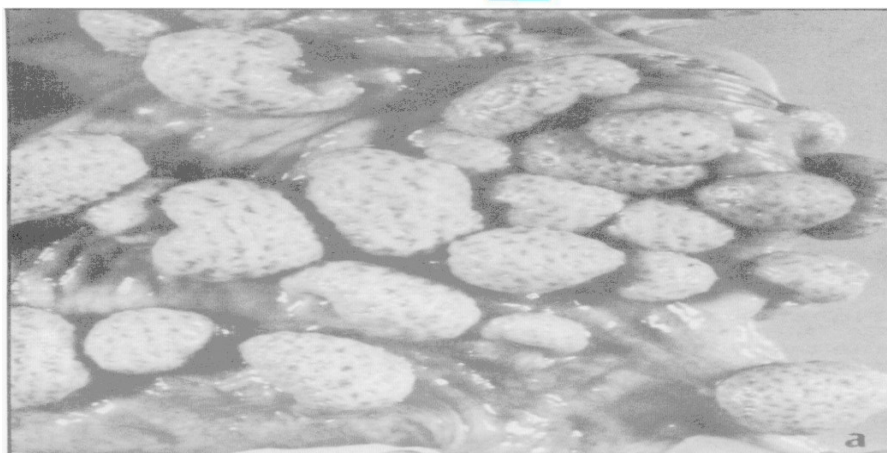


Figure 1.3: Lining of uterus showing caruncles on its surface

The uterus also develops the maternal side of the placenta to nourish and protect the developing fetus. Its surface contains many specialized areas called caruncles. Cotyledons of the fetal placenta interlock with caruncles on the uterus to provide a passageway for the exchange of nutrients and waste between fetus and cow. After calving, if the caruncles and cotyledons fail to unlock, the placenta cannot be expelled and a retained placenta results.

Cervix: The uterus enters into short a muscular tube called cervix. The opening through which it opens into cervix is called internal openings. The cervix passes to short distance and opens into vagina by external openings. The cervix is a unique structure within the reproductive tract that structure is designed to restrict access to the uterus. The area around the opening of the cervix actually protrudes back into the vagina. Three or four ridges or rings within the body or the cervix, called annular folds, can be distinguished by rectal palpation. The folds must be manipulated rectally while an inseminating rod is passed through to the uterus. The anterior cervix may serve as a site for semen deposition during artificial insemination (AI).

This occurs on services where the cycle length is not 21 days and pregnancy from a previous service is possible. Whether by deposition following AI or by migration from the vagina after natural service, the cervix acts as a reservoir for semen. The cervix provides a favorable environment for sperm survival. Secretions of the cervix are usually thick, but these fluids thin at the time of estrus to facilitate transfer of sperm to the uterus. Some of the mucus may be seen as discharge from the vulva around the time of estrus. The cervix, or fluids of the cervix, act as a physical barrier and protect the uterus from any foreign material or bacteria during pregnancy.

Vagina: It is a birth canal located within the pelvis between the uterus anteriorly and vulva caudally. It is a long muscular tube, it serves both as receptacle for sperm and accommodates the penis and as birth canal. Near the opening of vagina, bulbo urethral glands present. They secrete lubricant secretions to neutralise acid condition similar to male. The vagina is located between the opening to the bladder and the cervix. Approximately 8 inches long, it is the site semen deposition during natural service. The vagina also serves as an unrestrictive passageway for the calf at time of birth. One important function of the vagina is as a line of defense against invasion by bacteria. The epithelium of the vagina secretes fluids which combine with cervical fluids to inhibit growth of undesirable bacteria.

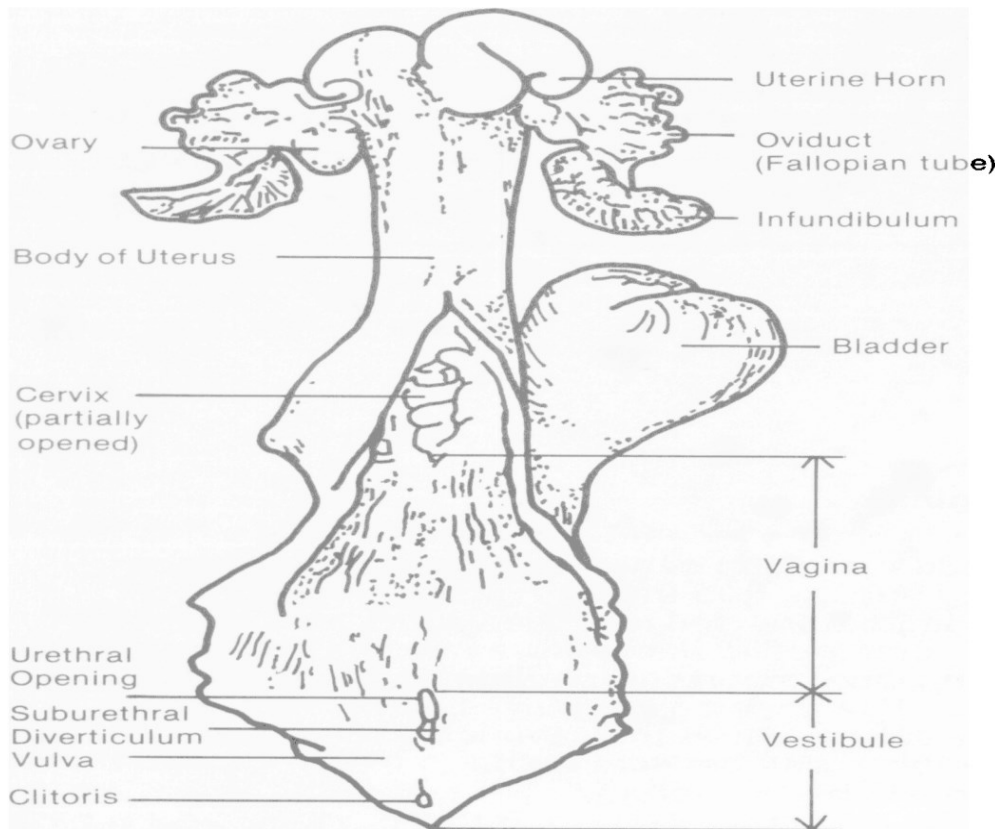


Figure 1.4: Parts of a cow's reproductive tract

Vulva: The vulva is external portion of genitalia of female that extends from vagina to exterior. The vulva is the external part of the reproductive tract. The thickened folds of skin of the structure are sensitive to changes in estrogen, the hormone responsible for estrus (heat). Swelling and redness of the vulva, due to increased blood flow, can be useful in estrous detection when coupled with other signs.

The two folds of skin covered with hair, the labia majora extend back and down to enclose the opening of the vagina. Inside the labia majora are 2 other folds of skin called is a sensitive, erectile organ called Clitoris. It is homologous to penis. It contains spongy tissue which becomes engorged with blood during sexual excitement and nerve endings that responds to erotic stimulation.

Vestibule: is a part of the reproductive tract shared with the urinary system. It is approximately 4 inches long. Openings from the urinary bladder and a blind sac located below the opening of the urethra called the suburethral diverticulum are located on its floor.

The hormones of female reproduction

- Reproduction in the female is controlled by numerous hormones secreted from specialized glands called endocrine glands. These secretions are produced in the glandular cells and pass into the blood and lymph systems for transport to specific parts of the body where they produce their function.
- The female hormone, estrogen, is produced by the Graafian follicle. Estrogen has among its varied effects
 - ✓ The development and function of the secondary sex organs
 - ✓ The onset of behavioral estrus, i.e., the period of sexual receptivity
 - ✓ It prepares the prepuberal heifer and post-partum cow for onset of cyclic sexual activity
- Any condition that prolongs the period of time that blood levels of progesterone remain high will have the same effect as pregnancy in stopping the regular 21-day cycle. Occasionally the CL does not regress normally (persistent CL) even though the animal does not become pregnant. This requires diagnosis and treatment by a veterinarian.
- An estrous cycle can be shortened intentionally by injecting a hormone called prostaglandin, which causes the CL to regress. Prostaglandin injection is one method used in estrous synchronization.

Ovulation: When graffian follicle is ripe, it is about the size of a pea and projects from ovary. At that point, ovary thins and finally ruptures releasing ovum to extrude into body cavity, a process known as ovulation. Ovum is a spherical mass of protoplasm with a central nucleus. The ovum is carried with fluid and is subsequently drawn into fallopian tube through funnel like structure.

In monotocous animals (horse and cow) at each heat period one follicle usually develops more rapidly than others, so that when it ruptures only one ovum is released. In polytocous animals (carnivores) at each heat period several follicles develop and rupture during ovulation.

Corpus luteum: After discharge of ovum, the blood filled cavity is called corpus hemorrhagicum. Then after, a yellow coloured corpus luteum appears replacing clotted blood. It stays for 14 days only if no pregnancy occurs. If pregnancy occurs, corpus luteum pregnancy continues to grow for several months and degenerates after 6 months.

Progesterone, secreted by the corpora lutea, suppresses the further development of follicles and the secretion of estrogen. High levels of progesterone and low levels of estrogen prevent a cow from coming into heat. Progesterone is necessary for preparing the uterus to receive the fertilized egg and maintains the proper uterine environment for continuation of pregnancy.

The production of ovarian hormones is under direct influence of gonadotropic hormones produced by the anterior pituitary. Follicle stimulating hormone (FSH) and luteninizing hormone (LH) are secreted from the pituitary and travel through the blood to the ovary. FSH and LH are mediated by gonadotropic releasing hormone (GnRH) coming from the hypothalamus to signal their release from the pituitary. FSH stimulates the growth, development and functions of the follicle, while LH causes the follicle to rupture during ovulation and causes the subsequent development of the corpus luteum.

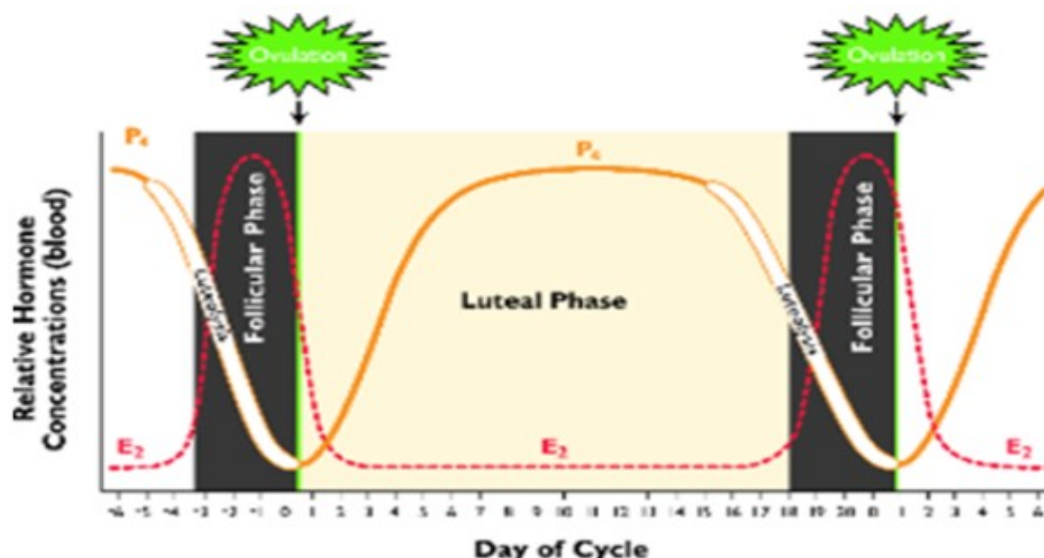


Figure 1.5: Stages of follicle development in dairy cow

The ovarian changes and sequence of events in a typical 21-day cycle in which pregnancy does not occur;

Days 0-1

The cow is in for estrus (standing heat) on Day 0 for an average of 18 hours (range 12 to 24 hours). About 12 hours after the end of the standing heat, the mature Graafian follicle ruptures (ovulates) in response to a surge of LH released by the pituitary gland.

Days 1 and 2

The cells that formerly lined the follicle change and become the lutein cells of the corpus luteum. This change in cell forms is caused by hormonal action, primarily that of LH.

Days 2 to 5

The corpus luteum grows rapidly in both size and function. Numerous follicles may be seen on the ovary at this stage, but by Day 5 they have begun to regress.

Days 5 to 16

The corpus luteum continues to develop and reaches its maximum growth and function about Day 10. It secretes the hormone progesterone which inhibits (blocks) LH release by the pituitary gland. During this period, the ovaries are relatively inactive except for the functional corpus luteum. No follicles reach maturity and/or ovulate because of the existence of the high levels of progesterone.

Days 16 to 18

The corpus luteum regresses rapidly due to some luteolytic activity of the uterus. Evidence is increasing that this may be a prostaglandin.

Days 18 to 20

The corpus luteum is almost non-functional and this releases the blocking action of progesterone. Of the several follicles that commence growth, one becomes more prominent by a surge in rapid growth and activity. As the Graafian follicle grows, it secretes increasing amounts of estrogen. The remainder of the follicles regress.

Day 21 or 0

With the increase in estrogen release by the Graafian follicle and a corresponding decrease in progesterone by the regressing corpus luteum, estrus or heat will occur (cycle has now returned to Day 0). The high estrogen level in the blood triggers a release of LH near the end of heat.

1.3.2. Identifying nutrition, animal health and abnormalities in reproduction

1.3.2.1. Identifying nutrition in reproduction

Nutrition plays a major role on enhancing reproductive efficiency in all animals. Nutrition has an important effect on reproductive potential of all living species. Body condition is a useful indicator of nutritional status and when used in conjunction with body weight change can provide a useful method to assess reproductive potential. Nutritional considerations and impacts on reproduction have primarily focused on postnatal development; however, prenatal nutrition appears to have potential effects on subsequent reproductive performance in dairy animals. Poor nutrition during the dry and early postpartum periods results in reduced glucose, insulin, insulin-like growth factor (IGF-I) and low LH pulse frequency. High nutrition can also increase metabolic clearance rate of steroid hormones such as progesterone or oestradiol. Excessive loss of BCS and excess protein content of the ration can reduce conception while supplemental fats that attenuate the production of $\text{PGF}_2\alpha$ can improve conception rate. Feeding diets that promote increases in plasma glucose and insulin may improve the metabolic and endocrine status of cows in early lactation.

Major nutrients and their effect on reproduction and fertility

- **Energy and protein**

Energy and protein are the major nutrients required in the greatest amounts and should be in the topmost priority in order to optimize reproduction in dairy cattle. Energy and protein are the nutrients required in the greatest amounts and should be first priority in developing nutritional programs to optimize reproduction. Excessive dietary nutrients during the last trimester of pregnancy may negatively influence calf birth weights and dystocia. Feeding excessive amounts of energy or protein before or after calving is not only costly, but animals with BCS >6 have lower reproductive performance and more calving difficulty than animals in moderate BCS 4-5. Excessive protein and energy both can have negative effects on reproduction. Overfeeding protein during the breeding season and early gestation, particularly if the rumen receives an inadequate supply of energy may be associated with decreased fertility. This decrease in fertility may result from decreased uterine pH during the luteal phase of the estrous cycle in cattle fed high levels of degradable protein. During early postpartum, high-producing dairy cows undergo a period of extensive tissue catabolism because of negative nutrient balance.

Table 1.1: Nutrient consumption and its consequence

Nutrient Consumption	Reproductive Consequence
Excessive energy intake	Low conception, abortion, dystocia, retained placenta, reduced libido
Inadequate energy intake	Delayed puberty, suppressed estrus and ovulation, suppressed libido and spermatozoa production
Excessive protein intake	Low conception rate
Inadequate protein intake	Suppressed estrus, low conception, fetal reabsorption, premature parturition, weak offspring

- **Fat**

Fatty acids and cholesterol are substrates for hormone synthesis, increasing fat in the diet may increase levels of reproductive hormones (progesterone, prostaglandins) or fats may act directly on the reproductive axis. Effects of fat may be independent of or additive to those of increased energy availability. Supplementing fat to improve reproduction was initially attempted to increase the energy density in the diet. High fat diets for cattle contain 5 to 8 % fat. Exceeding these dietary fat levels impairs rumen function. Lactating cows are the primary animals to be supplemented because of their increased energy requirements. Feeding high fat diets to cycling heifers and postpartum cows increased progesterone production and the lifespan of the corpus luteum (CL). Higher progesterone levels during the luteal phase generally result in improved fertility. Increasing dietary fat also results in increased follicular growth.

- **Vitamins**

Major vitamins essential for proper reproductive behaviour are Vitamin A, D and E.

Deficiency of Vitamin E

- Depression in ovulation rate, uterine motility, sperm motility, conception rate, fetal membrane expulsion, embryo survival. Vitamin E+ Se injections during perpartum period can reduce the incidence of RFM from 17.5 to 0% and reduced metritis for about 40% in the risk group when compared to the control.

Deficiency of Vitamin A

- Late maturity

- Late conception
- Abortion
- Foetal deformities
- Retained placenta
- Uterine infection
- Delayed uterine involution
- Reduction in libido
- Increased incidence of cystic ovaries

1.3.2.2. Abnormalities of the Reproductive Tract

Abnormalities may be classified as structural or functional and are estimated to account for 10 - 20% of infertility in dairy cattle. A structural abnormality could be the result of abnormal embryonic development while functional abnormalities could be due to hormonal imbalances. The birth of a heifer co-twin to a bull results in abnormal development of the heifer's reproductive tract. This condition results when the placentas of the embryos unite during pregnancy, allowing the two embryonic circulations to combine. As a result, a substance responsible for organizing the male reproductive system crosses into the female and inhibits the development of the ovaries. In addition, the development of the oviducts, uterus, cervix and part of the vagina is blocked by a substance produced by the developing testis of the male. The degree of inhibition is related to the stage at which the placentas joined. The earlier the fusion, the more complete the inhibition.

Other abnormalities such as two cervixes, absence of one uterine horn, or blockage of the oviducts also occur. Functional abnormalities such as cystic ovaries or infections in the oviducts or uterus leading to the distension with pus are also common. While theories exist to explain the causes of these conditions, treatment consists of hormone or antibiotic therapy.

1.3.3. Identifying disease transmission through semen

Thoughtful and systematic control procedures can ensure the safety of introducing new bulls and cryopreserved semen into cattle production systems. As a result of a careful analysis of the characteristics of infections that may cause transmission of disease through semen, effective control procedures can be identified that provide minimal constraint to the introduction of new bulls into herds for natural breeding and importation of valuable novel genetics through AI.

Diseases spread by semen

- Semen has great potential for spreading infectious diseases
- Semen can be infected from
 - ✓ Testes & Accessory sex organs
 - ✓ Preputium
 - ✓ Circulatory system
 - ✓ Tissue fluids entering urogenital system
 - ✓ Microorganisms in the atmosphere
 - ✓ Teaser animal
 - ✓ Unsterilized equipment
- The most important diseases spread (transmitted) by semen are;

Viral diseases

- Infectious Bovine Rhinotracheitis-Infectious Pustular Vulvovaginitis (IBR-IPV)
- Bovine Viral Diarrhoea (BVD)
- Foot and Mouth Disease (FMD)
- Bluetongue (BT)
- Bovine leukosis (BL)

Bacterial diseases

- Brucellosis
- Bovine Tuberculosis (TB)
- Bovine genital campylobacteriosis
- Leptospirosis
- Johne's disease

Protozoal disease

- Trichomonosis

1.3.4. Carryout Pregnancy testing

Early pregnancy diagnosis is critical for maximizing herd productivity in the cattle industry. Pregnancy testing is one method of monitoring reproductive efficiency and detecting any problems early in the breeding cycle.

Major changes in the ovaries, uterus and cervix occur during pregnancy. The presence of the CL on the ovary during pregnancy prohibits development of mature follicles. A thick mucus plug forms in the cervical canal and is thought to protect the uterus from infections. The walls of the vagina and vulva are dry and white because of the lack of estrogen. The relative position of the reproductive tract within the pelvic arch also changes. As the embryo increases in size, the pregnant horn begins to drop over the rim of the pelvis into the body cavity and displaces the intestines.

The birth process may be divided into two parts: the delivery of the calf and the subsequent delivery of the placenta or after birth in a process referred to as “cleaning.” It must be remembered that the attachment sites of the placenta in the uterus consist of the maternal contribution, the caruncle, and a fetal side called the cotyledon. During pregnancy these tissues interlock with each other forming tight attachments. When the calf is born, the fetal placenta loses its source of nourishment, blood pumped by the calf heart. The loss of nutrients coupled with changes in the caruncle brought about by decreases in progesterone lead to a loosening of the attachment sites. The placenta is then passed by strong uterine contractions elicited by prostaglandins from the uterus and oxytocin released by nursing or milking. Failure of the release mechanisms, due to hormonal imbalance, nutritional imbalances (Vitamin E & Selenium) or infections that cause swelling of the tissues, results in a retained placenta. After “cleaning” occurs it takes the uterus 30-40 days to return to its non-pregnant size and condition.

- Several methods are used to do pregnancy diagnosis for cattle;
 - a) Direct methods of pregnancy diagnosis include trans-rectal palpation and ultrasonography
 - b) Indirect methods for early pregnancy diagnosis include measurement of endocrine hormones, early pregnancy-associated protein (pregnancy specific proteins) and ultrasound examination
 - c) Visual method is far from perfect such as development of udder, non-return to heat....

Rectal palpation/Trans rectal palpation of the uterus for pregnancy diagnosis in cattle was first described in the 1800's (Cowie, 1948) and is the oldest and most widely used method of pregnancy diagnosis up to now.



Figure 1.6: Rectal pregnancy diagnosis

Manual rectal palpation is a proven effective and reliable technique. Using this method pregnancy can be reliably detected as early as 6 weeks.

Ultrasound has also become commonly used. The main advantage of ultrasound pregnancy diagnosis is it reduces operator fatigue and injuries. It is important to recognize that females identified as non-pregnant by ultrasound must then be confirmed by rectal palpation.

Non-return rate to first insemination;

- This is the percentage of cows or heifers, in a particular group over a specified period of time, which have not been presented for a repeat insemination within a specified period of time. The period is usually 30-60 days.
- Used to monitor:
 - ✓ Fertility of bulls
 - ✓ The performance of inseminators
- Figures of 80% are frequently obtained, which is often 20% better than the calving rate to first insemination.

First insemination (80%-non return rate) (Calving rate 60%)

The discrepancy is due to: -

- Failure to identify, record and report if the cow returns to estrus
- Culling of the cow after she has returned to estrus

- Subsequently using natural service
- Prenatal death
- Estrus may not be detected
- Luteal cysts
- Some cows may abort

There are **four** positive signs of pregnancy that are detectable by palpation per rectum, and the operator must detect at least one of these four signs before giving a pregnant diagnosis;

Palpation of the amniotic vesicle - The amniotic vesicle is palpable from days 28 (heifers) and from day 32 to 35 (pluriparous cows) after service until approximately day 65 of pregnancy. The palpation of amniotic vesicle is similar to that of allantochorion. The uncoiled horn can be gently palpated along the entire length between the fingers. The amniotic sac can be felt as a spherical, turgid, fluid-filled structure 1-2 cm in diameter floating in the allantoic fluid. It is most commonly found at the cranial edge of the intercornual ligament. The vesicle should not be compressed directly but gently pushed backwards and forwards.

Palpation of the allantochorion (foetal membrane slip) - From day 35 to 40 (up to day 95) of gestation the allantochorion membrane can be palpated using the membrane slip technique. After locating the bifurcation of the uterine horns, the enlarged gravid uterine horn is picked up between thumb and middle finger, just cranial to the bifurcation, and the whole thickness of the horn may be gently squeezed. The allantochorion can be eventually identified as a very fine structure as it slips between the fingers. From day 60 of gestation, the examination of the non-gravid uterine horn is recommended because the allantochorion membrane can be felt more easily.

Palpation of the foetus - The foetus can be palpated when the amniotic sac becomes less turgid, i.e. day 45 to 50 of gestation or day 65 until term. During the first 4 months of pregnancy, the foetus may be grasped directly, and later it is felt as a free-floating firm structure on rocking of the uterine wall. Between months 5 and 7 of gestation, the foetus can be palpated in 40 to 80% of cows; and in more than 95% during months 3 to 4 and months 8 to 9 of gestation.

Palpation of the placentomes - Placentomes (caruncles/cotyledons) can be palpated per rectum from approximately day 90-100 of gestation, particularly in the uterine body and base of the gravid horn close to the midline, cranial and ventral to the pelvic brim. Palpation of the pregnant

uterus can be likened to palpating a sack full of small potatoes. Between months 5 and 7 of gestation, when the uterus has sunk into the abdomen, palpation of placentomes is successive of pregnancy.

1.4. Addressing physiological status and signs of heat

1.4.1. Physiological status of female animals

Estrus or “heat” is a period during the reproductive cycle when female animals become sexually receptive, signalling they are ready for mating. In most cases, this can also be referred to as “standing heat” because the female will stand to be mated by the male. Estrus is caused by estrogen being produced within developing follicles on the ovary, and ovulation usually occurs after the initial signs of estrus are detected. Duration of estrus and the time of ovulation in relationship to the onset of estrus vary with the species. If behavioral or physical signs are not obvious, estrus may even pass unnoticed. Successful recognition of the signs of estrus for mating, just prior to the time of ovulation, can result in increased conception rates for the herd or flock.

Sexual cycle: It is a common phenomenon among mammals. It is more marked in female than male. A brief period of pronounced sexual activity, when it occurs in male is known as rutting season. The period corresponding in female’s sexual arousal activity is called period of heat or physiologically estrus. The females of most species will accept the males in copulation only during estrus period. In cats and cows, the period of estrus is accompanied by period of restlessness and sexual receptivity. The estrus cycle is marked not only by change in intensity of sex urge but by changes in living of vagina and uterus which prepare the later to receive fertilised egg. Most wild animals have one estrus cycle and the condition is called mono estrus. Cats and dogs have about 2 estrus periods each year called diestrus condition. Poly estrus condition repeats the cycle more than twice a year. Non primate cycle: Non primates like pig-estrus cycle 21 days.

Table 1.2: Normal ranges of reproductive behavior

Animal	Type of estrous cycle	Length of estrous cycle (days)	Duration of estrus	Time of ovulation
Cattle	Polyestrous	19-23 Average: 21	6-30 hours Average: 18 hrs	12 hours after end of estrus
Goat	Seasonal polyestrous in Fall	12-24 Average: 20	1-4 days Average: 39 hrs	30-36 hours after start of estrus
Sheep	Seasonal polyestrous in Fall	14-20 Average: 17	20-42 hours Average: 30 hrs	At or near the end of estrus
Horse	Seasonal polyestrous in Spring	10-37 Average: 21	2-6 days Average: 4 days	24 - 48 hours before the start of estrus to 24 hours after the end of estrus
Swine	Polyestrous	18-24 Average: 21	1-2 days Average: 36 hours	8-12 hours before the end of estrus or 37-40 hours after start of estrus

Estrus consists of following phases.

- 1) **Proestrus:** 1st phase. Ovarian follicle and ovum increases in size. Secrete estrogens into blood. Copulation is not permitted.
- 2) **Estrus (heat period):** Copulation is allowed only at this time. Vaginal epithelium thickness increases. Graafian follicles come to maturity at about the middle of this period and at that point ovulation occurs. Follicular rupture occurs spontaneously in most animal sps. However in cat, rabbit and some animals rupture is possible only if coitus occurs. This estrus period lasts for 9-15 hrs while in dog it last for 9 days. The conception (pregnancy takes place in estrus period only).
- 3) **Metestrus:** is the period of functional development of corpus luteum. This occurs shortly after ovulation, it is also known as post ovulatory phase. In this period decrease in estrogen and increase in progesterone occurs. The ruptured follicle changes into organized structure called corpus luteum (yellow body).
- 4) **Diestrus and Anestrus:** is resting stage between estrus cycles in poly estrus animals.

It shows preparatory change for initiation of second estrus. Anestrus is the resting asexual period. In mono estrus animals it lasts up to next mating season.

1.4.2. Signs of heat

Female animals with observed heat signs are ready to be inseminated. Animals in heat can be identified by observing heat signs or by palpation of reproductive organ e.g. if you palpate the uterus and cervix the animal will discharge mucus. Other signs of heat include:

- Sniffing and being sniffed by others
- Moist , red and swollen vulva
- Enlargement of cervix and uterine horn
- Mounting other animals (frequent head or tail mounting)
- Discharge of stringy mucus through vulva
- Decreased milk yield and loss of appetite
- Being restless with dilated pupil
- Standing heat (willingness to be served) is reliable sign of heat
- Frequent urination



Figure 1.7A: Signs of estrus



Figure 1.7B: Signs of estrus



Figure 1.7C: Signs of estrus



Figure 1.7D: Signs of estrus

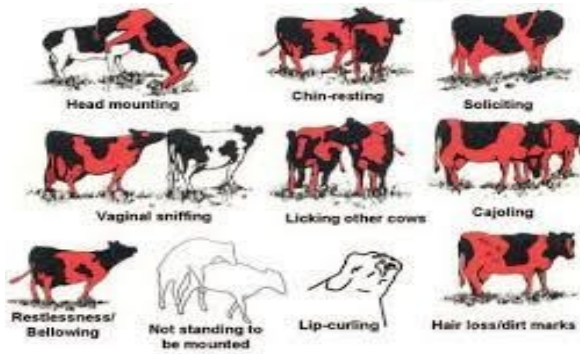


Figure 1.7E: Signs of estrus

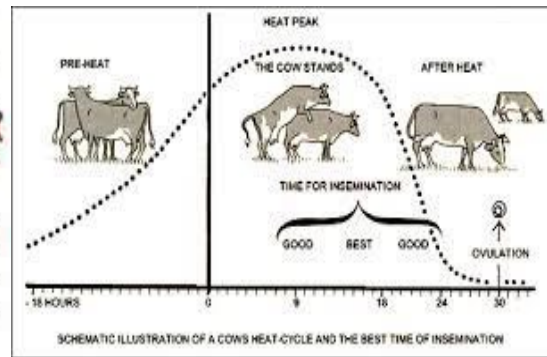


Figure 1.7F: Signs of estrus

1.5. Scheduling time of insemination

Timing is important to ensure good pregnancy rates and it is depend on time of insemination, semen quality, experience and skill of inseminator, etc. The animal should be inseminated within 24 hours as heat sign observed. In most cases, one insemination/day is adequate. Try to set up a schedule that ensures repeat inseminations will occur less than 24 hours apart. For the sperm, the uterus is a battlefield, so it's vital to help as many as possible to survive. They don't complete the journey on their own. They need a strong, powerful current pulling them up into the uterine horns. This cannot be accomplished well without good, face-to-face exposure of the male with the female.

The chance of conception is depend on time of insemination. The ideal time to inseminate is 12 hours after the onset of heat. The average cow is on heat for 18 hours and releases the egg from the ovary about 12 hours after the end of heat. The egg must be fertilized within 10 hours of ovulation, otherwise it dies. Sperm must spend several hours in the reproductive tract of the female before they are capable of fertilizing the egg. During this time, the sperm undergo chemical changes, called capacitating. When the sperm are deposited either at the cervix by the bull or into the uterus by AI they are rapidly transported up to the oviducts (fallopian tubes), arriving there in three to four minutes.

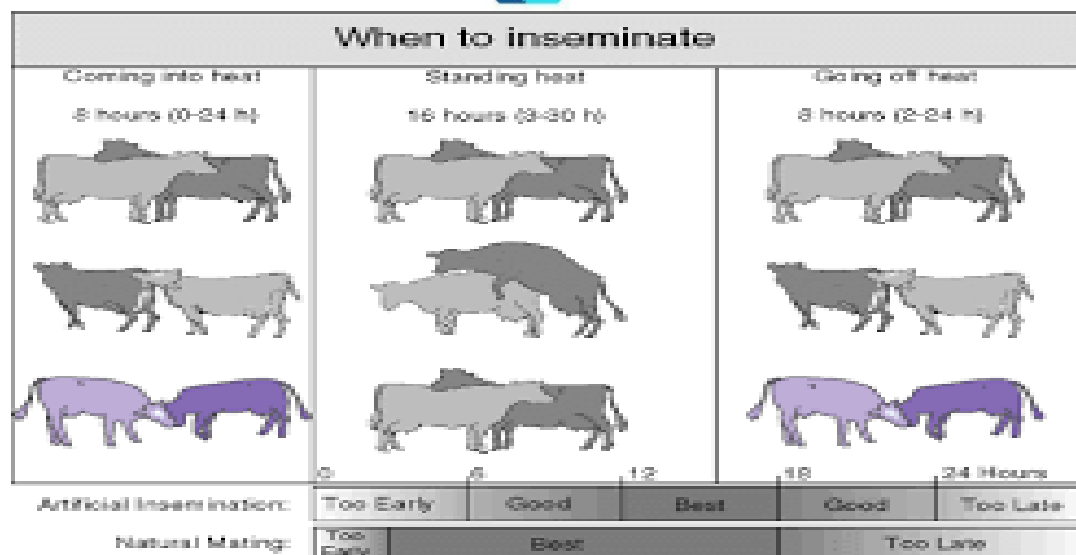


Figure 1.8A: Scheduling time of insemination

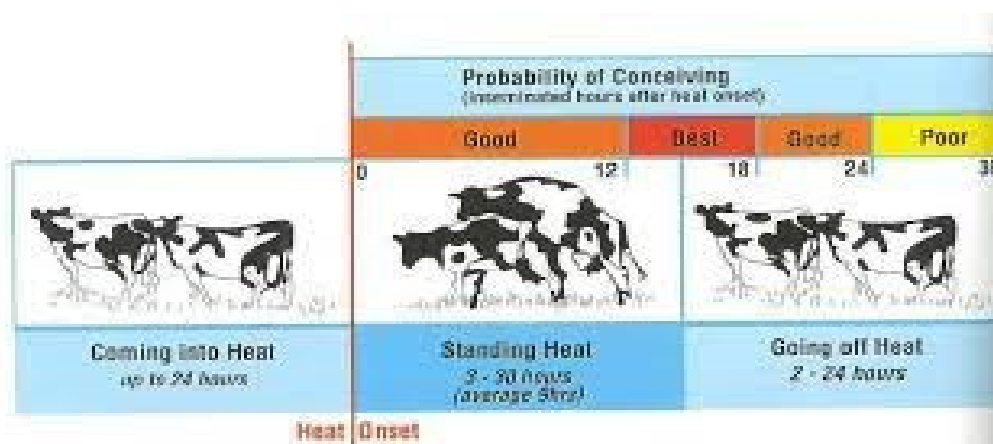


Figure 1.8B: Scheduling time of insemination

Transport of the sperm is achieved by wave-like contractions of the uterus, caused in part by the hormones prostaglandin and oxytocin. Rough handling of the cow and stress can cause the release of adrenalin, which stops these contractions occurring and may result in a lower conception rate. In most cases, one insemination per day is adequate. Try to set up a schedule that ensures repeat inseminations will occur less than 24 hours apart.

1.6. Carrying out estruses synchronization

The added requirements for implementing AI are increased labor and training in heat detection and semen handling and deposition. Heat detection is often the most limiting factor in an AI program.

Proper timing of insemination depends largely on effective heat detection and is critical to achieve high conception rates.

Estrus synchronization tools

The approved drugs for estrus synchronization in the world; elicits one of the following actions:

1. Keep animals out of heat and extend the oestrous cycle (Progestin)
2. Bring females into heat and shorten the oestrous cycle (Prostaglandins)
3. Cause ovulation or start development of a new follicular wave (GnRH)

Estrus synchronization systems often use two or three of these tools to synchronize estrus and ovulation.

Purpose of estrus synchronization

- Group females for parturition:
 - ✓ decrease labor, decrease calving period
 - ✓ reduce calving season
 - ✓ More uniform weaning weights.
- Reduce or eliminate estrus detection.
- Needed for artificial insemination

A working knowledge of estrous synchronization is also valuable for most AI programs. Because of the challenge of heat detection, estrous synchronization protocols involving a fixed-time insemination have been developed. Estrous synchronization protocols specify the appropriate timing of heat detection and insemination. In the absence of using an estrous synchronization protocol, many producers rely on the “AM/PM rule” to decide when to AI cattle. According to this rule thumb, females first observed in heat in the morning should be inseminated that evening. Similarly, females first observed in heat in the evening should be bred the next morning. Proper heat detection is important when using the AM/PM rule.

Timing insemination as close to 12 hours after females first show heat is important when using this rule. Waiting until the next day to breed a female observed in heat in the morning is too late for good results. Inseminate the cow from the middle to the last of the standing heat period; egg is released (ovulation) about 10 to 14 hours at the end of standing heat with the range of 3 to 18 hours after heat. Breed cows which comes heat at that day morning. And when heat is observed at afternoon breed the cow on the next morning, it is better to breed late (up to 6 hours after standing heat) than to bred in the first half of the heat period.

Isolate the cow in quite area away from the herd at the first sign of standing heat. Again after breeding, keep cow away from the herd until heat is over.

1.7. Identifying and preparing animals in heat for insemination

Female animals with observed heat signs are ready to be inseminated. The longer the time interval from calving until insemination is, the higher the cow's chance of becoming pregnant. In seasonal calving herds, once mating has commenced every cow on heat should be joined regardless of how long she has calved. For example, a cow calved for twenty-eight days may only have a 27% chance of becoming pregnant. This is better than a zero chance if she has not joined at all.

Accurate heat detection is necessary to maintain a 12-month calving interval, where at least 90% of cows should show standing heat within 60 days after parturition (i.e. 60 days after calving). Animals in heat can be identified by observing heat signs or by palpation of reproductive organ. Successful heat detection involves looking for one or more of the following signs at least twice a day:

- Stand to be mounted by other cows
- Have a swollen vulva and clear mucus on the tail
- Have signs of being mounted including ruffled tail hair, mud (dirt or mucus) on flank (side body) and on the back
- Show unusual behaviors' such as holding milk, bellowing (shouting), fence prowling, frequent urination, aggressiveness, restlessness and licking, sniffing and rubbing against other cows.
- Records of dates and services can be used to calculate the date of the next expected heat

Self-check - 1	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below.

Test I: Multiple choices

1. A cow showed an estrus (heat) at AM. What is the best time to inseminate the cow?(2 pts.)
A. at AM B. at PM C. as soon as estrus stands D. unknown
2. The technique of keeping animal under control is called as (2 pts.)
A. artificial insemination B. estrus C. restraining D. crush
3. The release of ovum from ovary to oviduct is called as (2 pts.)
A. Ovulation B. Estrus cycle C. Estrus D. Fertilization
4. The only reliable estrus signs in cattle are (2 pts.)
A. Bellowing B. frequent urination C. Standing to be mounted D. nervousness

Test II: Short Answer Questions

1. Write down at least four different types of female reproductive organs (4 pts.)
2. List three estrus signs that a cow shows during heat period. (3 pts.)
3. Mention six different types of artificial insemination equipment & tools (3 pts.)

Satisfactory rating - 15 points

Unsatisfactory - below 15 points

You can ask you teacher for the copy of the correct answers.

Operation Sheet -1

1.1. Technique of preparing animal for insemination

a. Tools and equipment's

- PPE
- Recording book
- Paper and pencil

b. Procedures

- Put on necessary clothes
- Join the nearest dairy farm
- Observe changes in behavior of the animal
- Interview herds man on changes in animal behavior
- Observe reproductive organ of female animal
- Identify animal on heat
- Report to dairy farm inseminator

1.2. Technique of construction of cattle Crush for insemination

a. Tools and equipment's

- PPE
- Rope
- Pegs & nails
- Meter
- Pole

b. Procedures

- Select appropriate site for construction
- Prepare all the necessary materials and equipment
- Layout the length, width & height of the crush depending upon the number of animals
- Put peg in each corner
- Level the area that are already selected
- Construct the gun pole and other parts of the crush
- Finally Check the strength of the crush

1.3. Technique of Pregnancy diagnosis by rectal palpation

a. Tools and equipment's

- PPE
- Record book
- Wheelbarrow
- Meter
- Crush

b. Procedures

- Put on PPE
- Assemble necessary materials
- Present the animal to be examined
- Restrain the animal
- Wear gloves
- Remove manure from the rectum
- Retract the uterine and cervix
- Try to palpate the foetus in the uterus based on key indicators of pregnancy
- Keep and report record

LAP TEST-1	Performance Test
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Name..... ID.....

Date.....

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within **3 hours**. The project is expected from each student to do it.

Task 1: Prepare animal for insemination

Task 2: Construct cattle Crush for insemination

Task 3: Carryout pregnancy diagnosis by rectal palpation

LG #39

LO # 2- Handle Semen

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Using required materials, tools and equipment
- Undertaking artificial insemination work (activities)
- Handling semen properly and periodically top-upping

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Use required materials, tools and equipment
- Undertake artificial insemination work (activities)
- Handle semen properly and periodically top-upping

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the information Sheets
4. Accomplish the Self-checks
5. Perform Operation Sheets
6. Do the “LAP test”

Information Sheet - 2

2.1. Using required materials, tools and equipment

Semen collection tools, materials and equipment must be cleaned and stored properly. The most important equipment, tools and materials that used in semen handling are including;

- Artificial vagina (AV) and Electro ejaculator
- Conical centrifuge tubes with stoppers
- Liquid nitrogen container
- Thermos flask
- Pasteur, capillary and graduated plastic pipettes
- Vortex mixer
- Microscope, Microscope slides and cover slips
- Warmer tray, Water bath
- Centrifuge
- Dead-Alive stain
- Sodium citrate solution (98.6 mM)
- Egg yolk extender (98.6 mM Na citrate; 20% egg yolk)
- Skim milk extender (98.6 mM Na citrate; 20% pre-heated skim milk)
- Spectrophotometer
- Frozen straws of bull semen
- 95°F water in container (thermos wide mouth)
- Stethoscope
- Thermometer
- Ultrasonography (optional)
- Vaginal speculum (optional)
- Insemination gun
- Containers
- Scissors

- Canister (removable cylinder with a mesh or solid bottom to hold semen in the tank. It has a long hooked handle to permit straw identification and access from the mouth of the tank)
- Towel or tissue paper
- Gas sterilizer (a ventilation hood is a useful option)
- Balance
- pH meter
- Osmometer (optional)
- Refrigerator with freezer
- Still or deionizer (unless water is purchased)
- Bunsen burner or alcohol lamp
- Large bacteriological filter unit
- Personal protective equipment
- Electronic photometer
- Liquid nitrogen
- Electronic thawing device or insulated water bath
- Plastic sheaths
- Record-keeping supplies
- Cattle handling facilities (including breeding box or squeeze chute with head catch and palpation cage and protection from weather)



Figure 2.1: Basic equipment and material for AI

2.2. Undertaking work in safe and appropriate manner

2.2.1. Semen collection

Sires should be ready for semen collection and freezing procedures after they have passed a complete physical examination, the required federal health tests and a breeding soundness evaluation.

Semen pre-collection works: This includes

- **Testing male animals for fertility:** the animal which is free of disease must be evaluated for reproductive performance.

Special physical examination: this focus on reproductive organs and accessory activities.

- The scrotum checked for size, symmetry, circumference, elasticity
- Palpation of prepuce and penis for deformities and infection
- Locomotors system and body condition hook, bowling leg, sickle, etc.
- Serving behavior libido, erection, mounting and dismounting, etc.

Table 2.1: Scrotum circumference of bull at different time of age

Category	Threshold
Scrotum circumference of bull	30cm at 15 month age
	31cm at 15-18 month age
	32cm at 18 to 21 month age
	33cm at 21 to 24 month age
	34cm at 25 month age

▪ **Preparing male animals for semen collection**

- ✓ Live mount such as teaser female or castrated male are best for routine semen collection.
- ✓ Sexual preparation of sire is better to increase quantity of spermatozoa.
- ✓ The male which fulfill criteria for semen collection must be restrained properly.

▪ **Preparing all the necessary materials, equipment, tools and utilities**

All necessary materials which enable semen collection, technicians, restraining chutes must be prepared to carry out semen collection procedures properly.

For semen collection the bull selected, the selected bull trained for semen collection procedures, after which semen is collected once or twice a week. Gentle and quite treatment of bull is important. The bull must not become nervous by people clothes, by noise from dogs, motors cars, visitors or handlers.

If the bull has served the teaser cow repeatedly, it is preferable to attempt semen collection after a 7-days sexual rest.

The most common semen collection methods: Artificial vagina, Electro ejaculation, and Massage method.

a) Artificial vagina (AV)

The best and commonly used method; Consists of outer support with inner jacket containing temperature controlled water and pressure, and collecting funnel a container. When the male mounts the female, the sheath is grasped and the penis is directed in to the artificial vagina for ejaculation. Prior to semen collection all parts of AV should be cleaned, sterilized and assembled to AV. Mature bull require an outer casing 40cm in length and 5.1-5.7cm in diameter. In case of shoa it should be 20cm long and 5cm in diameter.

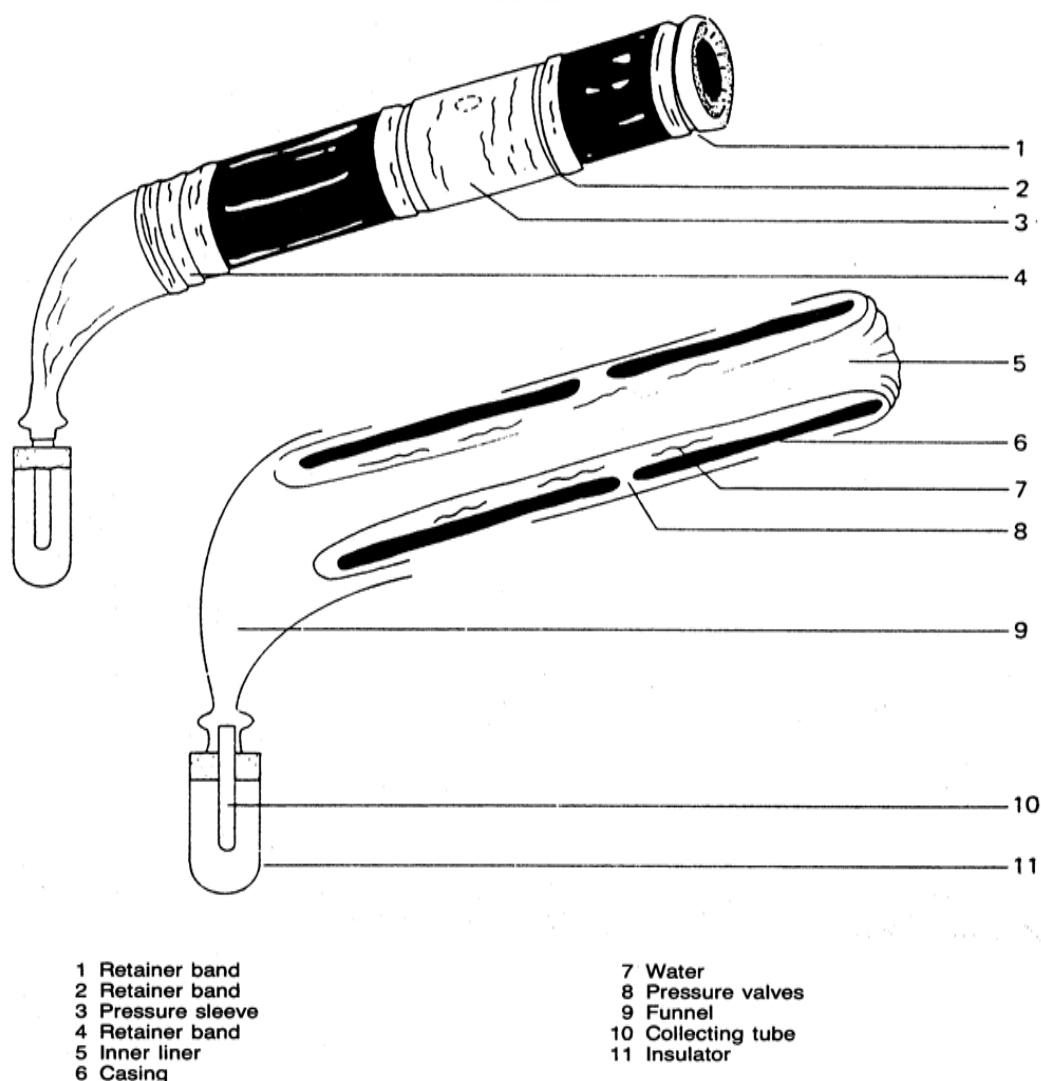


Figure 2.2: Artificial vagina

Advantage of AV

- Uncontaminated sperm and natural stage
- Free from extraneous secretion
- Viability of sperm is better
- No female is needed if dummy is success
- A sterile condition of the apparatus ensures disease control

Disadvantage of AV

- Difficult to get males that serves AV
- The apparatus involved is slightly costly and requires technical hands

The following Precautions should be taken while collection of semen is made by using AV;

- Protect the collection vial from sunlight by placing a black cover immediately after collection.
- Avoid contamination of the semen from water, lubricating jelly or other harmful substances.
- Avoid cold shock by providing adequate protection for the collection tube and funnel.

b) Electro ejaculation (EE)

- Electro-ejaculators are designed to stimulate the pelvic sympathetic and parasympathetic nerves with pulses of low voltage and amperage to induce penile erection and ejaculation.
- An electro-ejaculator set has the following components: carrying case, rectal probe, control unit, battery charger, power cord, probe cord, semen collection handle, collection cone and a collection vial.
- Electrode or probe inserted into rectum to stimulate ejaculation through stimulation of reproductive system by gradual increase of voltage, not widely used with boars or stallions. . Electro ejaculation is usually used only with bulls and rams which are unable or unwilling to mount and to serve an artificial vagina. Results are rather variable as to the quality of the ejaculate collected, and are dependent upon the skill of the technician making the collection, as well as individual animal variability.
- The method is used on males of certain species where the use of AV is not possible not practical. The widest use of electro-ejaculator has been observed in obtaining semen samples from large number of bulls and rams during examination for breeding soundness. The method is also used to collect semen from bulls for AI purpose when the bull is extremely slow in serving the AV or physically incapable of mounting.
- Modern models of electro-ejaculators consists of a single rectal probe equipped with bipolar electrodes capable of producing ejaculation by electrical stimuli of the vesicular glands, ampulae of the vas deferens, accessory sex glands, and the hypogastric plexus.

The method of semen collection in bull is as follows:

- At the beginning, the rectum is washed with 6% sodium chloride solution.
- The probe is then inserted up to about 12 inches and held in a position of rectal floor.

- Alternate current increasing in voltage gradually from zero to 5 volts and returning again to zero within every 5 to 10 seconds is initially -passed.
- The subsequent stimulation made progressively higher so that at about fifth stimulus a maximum of 10-15 volts is reached. Erection and ejaculation occur at 10 to 15, volts when 0.5 to 1 ampere current is passed. The source of electric current is AC/220-250 volts/single phase/50 cycles

Advantages of EE

- Collect semen without sexual response from the male
- Collect from males unable to copulate (Semen can be collected from males that are too young or old or unable to mount due too weak or injured legs).
- Female in estrus not needed (No female or dummy is required for collection).
- Less chance of contamination.

Disadvantages of EE

- Equipment cost
- Possibility of misuse
- Not advisable for repeated use
- Make discomfort to bulls/rams/stallion/bucks...etc.

c) Massage method

This technique requires two people, one to do the massage and one to collect the semen. The bull is held in a chute. After removal of feces from the rectum, a longitudinal back and forth massage is applied mainly over the ampullae, drawing semen toward the pelvic urethra. When the urethral muscle begins to pulsate the massaging action should be in synchrony with the pulsations. The semen collector must collect the cloudy fluid into a warm receptacle as it dribbles from the penis or prepuce.

The extended penis may be held by the semen collector during rectal massage to facilitate collection of a clean semen sample. The simplest method of semen collection by massaging seminal vesicle & ampulae, that commonly used to collect semen from turkeys, ducks and dog.

Advantages massage method:

- No expensive equipment is required.
- The technique avoids the potentially painful aspects of electro-ejaculation.
- No need dummy or real animal

Disadvantages massage method:

- A skilled palpate is required.
- Libido, mating ability, penile erectile function and the ability to ejaculate are not evaluated.
- Semen samples may be contaminated with epithelial cells, bacteria, and dirt especially when it dribbles through the prepuce and off perpetual hairs.
- Semen volume and concentration are very variable.

2.2.2. Processing semen

Semen processing includes the activities are; **Semen collection, Semen evaluation, semen dilution and extension, packing, printing and preservation.**

• Properties of Semen

Semen is the complete-discharge of the male genital tract occurring at the time of ejaculation by the male. It is a white, opaque, creamy fluid, occasionally yellowish green due to the pigment carotene. Semen consists of cellular part spermatozoa or sperms and the fluid parts, known as seminal plasma.

Spermatozoa are a male germ cells apportioned into three regions: head, middle piece and tail. The shape of the head of the sperm in the bull, ram, boar and rabbit is a blunt ovoid. In fowl, the sperm head appears as elongated cylinder. In the bull, the spermatozoa measuring 80 microns in length resembles an agile tadpole. The head is a blunt ovoid structure known as acrosome.

The liquid portion of semen i.e. seminal plasma is nothing but the secretions of accessory sex glands such as the prostate, seminal vesicles and Cowper's glands. Seminal plasma presents an ideal medium for the viability and mobility of the male germ cells.

2.2.3. Evaluation of Semen quality

Semen evaluation must be rapid and effective to reject poor quality semen.

Minimum standard set to fertile bull sperm are:

- Over 500 million sperm/ml of concentration
- Over 50% motile spermatozoa
- More than 80% normal morphology.

Appearance and volume

The capacity to produce spermatozoa per gram of testicular tissue (Daily Sperm Production; DSP) is well correlated to scrotal circumference measurement in young bulls.

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In bulls on a regular semen collection schedule, volume and sperm concentration indicates that the bulls capacity to produce sperm and also allow an AI center to monitor testis function. It should relatively opaque, uniform and creamy appearance and 6ml (ranges 2 to 10 ml). Volume of ejaculate and concentration per unit volume determines the total number of cells in the semen. If no sperm are present, male is sterile. The volume of single ejaculate in the bull ranges from 2-10 ml. Variations in quantity may be due to breed differences and also age, frequency of service, season and nutritional status of the individual.

Table 2.2: Semen volume and sperm density and count in different animal species

S.No	Species	Semen volume (ml)	Range (ml)	Sperm concentration (Million/ml)	Range (Million/ml)	Total sperm ejaculate (Million/ml)
1	Man	3.5	2.0 - 6.0	100	50 - 150	3500
2	Bull	8	0.5 - 12.0	1200	300 - 2000	9600
3	Buffalo	2.5	0.5 - 4.5	600	200 - 800	1500
4	Ram	1.0	0.7 - 2.0	3000	2000 - 5000	3000
5	Goat	1.0	0.2 - 2.5	3000	1000 - 5000	3000
6	Boar	215	125 - 500	250	25 - 1000	53750
7	Stallion	125	30 - 320	120	30 - 800	15000
8	Jack	50	10 - 15	400	95 - 600	20000
9	Rabbit	0.5	0.2 - 2.0	300	100 - 700	150

Density

Density may be classified as follows:

- **Very Good (VG):** creamy, grainy semen with 750 - 1 billion or more spermatozoa per ml
- **Good (G):** milk-like semen with 400 to 750 million spermatozoa per ml
- **Fair (F):** skim milk-like semen with 250 to 400 million spermatozoa per ml
- **Poor (P):** translucent semen with less than 250 million spermatozoa per ml

Semen collected by AV may be more concentrated, and cleaner, than samples collected by electro-ejaculation or by massage. With the latter two methods, volume and density may not be representative of a bull's normal capacity to produce sperm; however, with good technique, clean

ejaculates with good concentration are often possible when the bulls are sexually rested and handled in a calm way.

Motility

A 5 mm diameter drop of the semen is placed on a warm glass -slide and mass motion is observed under bright field microscopy at 40X magnification with the field diaphragm closed. Factors that affect mass motion of the spermatozoa include concentration, percentage of progressively motile cells and the speed/vigor of sperm motion. If one or more of these factors is compromised the swirling of mass motion will be suppressed.

Descriptive assessment of gross motility:

- **Very Good (VG):** rapid dark swirls and eddies
 - **Good (Good):** slower swirls and eddies
 - **Fair (F):** no swirls, but prominent individual cell motion
 - **Poor (P):** little or no individual cell motion
- **Motility and mass activity: Progressive motility:** (Note: Other types of motility which involve circling or jerking without forward motion are undesirable. Motility is best determined by putting a thin, diluted drop of semen on a slide under a microscope on low power, 100X.)
1. Acceptable samples should have a progressive motility exceeding 50 percent
 2. Ratings

Grading Point %age range of quality

- a) Very good -5 - 80 -100% motile sperm cells
 - b) Good - 4 - 0 - 80% motile sperm cells
 - c) Fair - 3 - 40 - 60% motile sperm cells
 - d) Poor - 2 - 20 - 40% motile sperm cells
 - e) Very poor - 1 - 0 - 20% motile sperm cells
- **Concentration:** measured by electronic photometer. E.g. Bull 5-6ml/ejaculation, average concentration of sperm 800- 1200million/ml these can serve about 300- 500 cows by means of AI.
- **Morphology:** abnormal morphology does not affect fertility unless it exceeds 20%. E.g. of abnormality (detached head, coiled or bent tails).
- **Color:** creamy yellow to milky white.

- **Foreign material**--blood or pus in the semen indicates a serious problem and is unacceptable; bedding, dust or fecal material should be disregarded
- **Wave pattern** (Note: This is best determined by placing a thick drop of semen on a slide under a microscope on low power and with reduced light.)

Acceptable

- Very good - Dark, distinct waves moving rapidly
- Good - Waves apparent, but with moderate motion
- Very poor - No waves and no sperm motility

2.2.4. Semen extender/diluents

Semen Dilution

Semen is extended with an appropriate diluent to increase its utility in fertilizing more females. Besides, semen diluents provide buffer action against the lactic acid produced due to the metabolism of sperms; provide nutrients to the sperms, protection against cold shock, reducing substances for preventing the activities of certain enzymes and in some case CO₂ gas which will retard metabolism of spermatozoa. Many diluents have been tried for various species of farm animals.

In essence, all diluents will have three major categories of constituents;

- (i) Nutrients or extenders like egg-yolk, boiled milk or whey which provided nutrients to the spermatozoa and increase the volume of diluted semen.
- (ii) Buffers which maintain an optimum pH
- (ii) Bacteriostatic or bactericidal agents like antibiotics to suppress the deteriorating and disease producing effect by bacterial contamination.

Table 2.3: Composition of some important semen diluents for cattle

S.No	Diluent ingredients	Modified egg-yolk citrate	C.U.E	IVT for preservation at room temp.	For deep freezing
Buffer					
1	Sodium citrate dihydrate (g)	1.8	14.5	2.000	2.4
2	Glycerine (g)	2.0	9.37	-	-
3	Sodium bicarbonate (g)	-	2.1	0.210	-
4	Potassium chloride (g)	-	0.4	0.040	-
5	Glucose (g)	-	3.0	0.300	-

6	Sulphanilamide (g)	-	3.0	0.300	-
7	Citric acid (g)	-	0.87	-	-
8	Distilled water to (ml)	100	1000	100	100
9	Gased with CO ₂ for (mts)	-	-	10.15	-
10	Fructose (g)	-	-	-	2.0
11	Glycerol (ml)	-	-	-	8.0
Extenders					
12	Buffer (% by volume)	50	80	90	-
13	Egg-yolk (% by volume)	50	20	10	25.0
Antibiotics					
14	Pencillin (per 100 ml)				
15	Semen diluent (units)	100,00	100,0000	100,000	50,000
16	Dihydrostreptomycin (mg per 100 ml semen diluents)	100	100	100	50

Table 2.4: Composition of semen diluents for diluting ram and boar semen

S.No	Ingredient	Ram semen	Boar semen
1	Heat treated cow's milk	100 ml	-
2	Penicillin	100,000 units	500,000 units
3	Dihydrostreptomycin	100 mg	500 mg
4	Sodium citrate dihydrate	-	20.0 g
5	Sodium bicarbonate	-	2.1 g
6	Potassium chloride	-	0.4 g
7	Sulphonilamide	-	3.0 g
8	Glucose	-	3.0 g
9	Glass distilled water	-	1000.0 ml

It is used to increase viability in vitro (out of the body), increase volume of semen, and provide protection. Commonly used extenders for frozen semen are:

- Tri glycerol diluents
- Glycerol egg yolk- citrate
- Milk glycerol
- Commercial diluents

2.2.5. Semen conservation

Deep freezing of semen for the successful preservation of spermatozoa for long periods is of great importance to livestock business. It has opened new vistas in animal breeding enabling great improvement to be made in the conservation and distribution of semen; full use of outstanding sires and effective application of progeny testing practice. Dilution for deep freezing is done in special glycerol diluent. The addition of glycerol and provides nutrients. Frozen semen is packed in single dose glass vials or plastic straw at $+5^{\circ}\text{C}$. Quick freezing is done at a rate of 1°C per minute from $+5$ to -15°C , 2°C per minute from -15 to -31°C and to 4 to 5°C per minute from -31°C to -75°C , thus taking 40 minutes in total. Further cooling to -196°C can be done quickly, as it is not very critical. After freezing ampules or pailletes (plastic straw) are removed from the racks and stored in special liquid nitrogen containers. Just before insemination, the ampules or pailletes are taken out from the liquid nitrogen refrigeration, immediately wrapped in a towel and kept for a minute. This is then thawed either by running cold water, or in cold water contained in a vessel, keeping the ampules or pailletes upright. Thawing takes several minutes. Thawed semen in the ampule is inseminated in the same way as semen stored at 40°C , while an inseminating gun is used in the case of pailletes. Thawed semen should be inseminated immediately.

Freezing of semen: semen is maintained at 34°C before and after dilution.

- Every insemination dose contain at least 15-40 million sperm
- Straw marked with bull ID and date of ejaculation
- Semen is frozen in liquid nitrogen at (-196°C)

2.3. Handling semen properly and periodically top-upping

Semen Handling

Sperm are motile and vigorous cells, but also fragile and susceptible to damage and killing by several environmental conditions. For transport of liquid fresh semen to a farm or within a farm, it is necessary to have a thermos flask and ice cubes covered with cotton. During transportation the temperature of container in which the semen packed must be close to 5°C . Semen can stored in liquid nitrogen container at (-196.5°C) which is also known as deep- freezing. Immediately after collection the semen can placed in a water bath at 25°C .

Once semen is considered acceptable it is diluted at 34.5°C. Generally AI requires preservation of semen outside the body for certain time or several years. Sperm survival prolonged by diluting semen with certain extenders. Commonly used extenders are tris- glycerol, egg yolk, milk glycerol etc. During transportation the temperature of semen should maintained between (0 to 5°C). The room temperature is maintained between (10 - 25°C).

When collecting and handling semen it is critical to avoid exposing sperm to **two** types of insults:

- **Exposure to toxic chemicals:** Keeping collection equipment clean and disinfected is important, but soap and disinfecting chemicals are quite potent spermicides. Take great care to rinse the inner liners of the artificial vagina and collection tubes with deionized water to remove such agents. Finally, be certain to lubricate artificial vaginas with a lubricant known to be non-toxic to sperm.
- **Thermal stress:** sperm are sensitive to both heat and cold. Rapid chilling of semen results in a phenomenon called "cold shock" that is often manifest by abnormal sperm motility and morphology. Short periods of exposure to temperatures just a few degrees above body temperature will usually kill large numbers of sperm.

As long as semen remains submerged in liquid nitrogen, the condition of the sperm and its fertility remains unchanged. Problems can arise when straws are exposed to elevated temperatures before they are actually needed for A.I. This may occur if a tank is allowed to run out of liquid nitrogen, if straws remain out of a tank too long when being transferred, or if too much time is taken when inspecting or selecting straws in the neck of the nitrogen tank. Semen from most species is not damaged by exposure to room temperature (20-22°C) for an hour or two. If a longer period of maintenance is required, it is best to dilute the raw ejaculate in a buffered nutrient solution - usually called an extender - and cool it slowly to refrigerator temperature (4-5°C). A large number of extenders have been developed, usually for use in freezing semen.

Extended, chilled semen is frequently transported for insemination, providing a useful alternative to either freezing or immediate use. Damage can occur even if the semen does not completely defrost or thaw, as physical changes in the frozen medium at sub-freezing temperatures will result in reduced sperm motility and fertility. Semen must be stored at temperatures below - 112°F at all times.

Semen can be permanently damaged by even very short exposures to elevated temperatures. By maintaining storage conditions of consistently very low temperatures, bull semen can be stored indefinitely. Semen storage tanks are large vacuum-sealed metal bottles. They are extremely well-insulated. These tanks can maintain internal temperatures of -320°F (liquid nitrogen temperature) as long as they contain at least 2 inches of liquid nitrogen.

The amount of time that a semen storage tank can hold adequate quantities of liquid nitrogen before needing to be refilled varies. Many older tank models must be refilled every 6 to 8 weeks. Newer, more technically advanced tanks may have nitrogen holding times as long as 6 to 9 months. It is critical to schedule timely nitrogen fills to maintain both semen and tank integrity. Make sure the nitrogen is refreshed before transferring semen to the tank. Tank nitrogen supplies may also need refreshing after extensive use from insemination sessions. Keep semen storage tanks in locations where they can be viewed daily. Make sure that tanks lids are secured, and closely monitor the amount of liquid nitrogen. Flexible plastic measuring sticks are available for this purpose. Wooden yardsticks may also be used to determine nitrogen depth in the bottom of a tank.

Self-Check – 2	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below.

Test I: Multiple choice

- Which one of the following AI materials used for covering AI gun? (2pts.)
A. Semen straw B. AI bag C. Thermo-flask D. AI sheath
- Which one of the following AI equipment used to hold semen straw in the liquid nitrogen container? (2pts.)
A. thermo-flask B. thermometer C. AI sheath D. canister
- Bulls selected for semen collection should not be (2pts.)
A. In poor sexual desire and performances.
B. Free from previous disease problems and any recent stressful conditions.
C. Good conformations and body condition
D. Free from any reproductive abnormalities
- After completion of Artificial insemination work, one the following is not disposed as waste; (2pts.)
A. AI gun B. AI gloves C. AI sheath D. Semen straw

Test II: Short Answer Questions

- List 10 (ten) the most important equipment, tools and materials that used in semen handling (10 pts.)
- What are minimum standard set to evaluate semen quality of a bull? (3pts.)
- Discuss the difference between semen volume and density (4pts.)

Note: Satisfactory rating - 20 points Unsatisfactory - below 20 points

You can ask you teacher for the copy of the correct answers.

Operation Sheet -2

2.1. Technique of testing male animals for fertility by physical examination

a. Tools and equipment's

- PPE
- Recording book
- Paper and pencil
- Meter
- Bull

b. Procedures

- Check scrotum size, symmetry, circumference, elasticity

Category	Threshold
Scrotum circumference of bull	30cm at 15 month age
	31cm at 15 - 18 month age
	32cm at 18 - 21 month age
	33cm at 21 - 24 month age
	34cm at 25 month age

- Palpate prepuce and penis for deformities and infection
- Locomote system and body condition hook, bowling leg, sickle, etc.
- Serve behavior libido, erection, mounting and dismounting, etc.

2.2. Technique of semen collection and handling

a. Tools and equipment

- PPE
- Recording book
- Artificial vagina
- Bull
- Teaser bull or female
- Microscope

b. Procedures

- Schedule male for semen collection
- Present and maintain temperature of (AV, electro ejaculator, thermos flask)
- prepare male sexual
- Live mount such as teaser bull or female
- Held AV parallel to teaser bull and slant to the path of bull penis
- Allow bull to mount
- Grasp the sheath of penis and guide to AV
- Take semen immediately to lab after ejaculation

LAP TEST-2	Performance Test
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Name..... ID.....

Date.....

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within 2 **hour**. The project is expected from each student to do it.

Task-1: Test male animals for fertility by physical examination

Task-2: Collect and handle semen

LG #40

LO # 3 – Perform Insemination Procedures

Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Preparing necessary materials and equipment for insemination
- Using Personal protective clothes and equipment
- Selecting and thawing semen
- Carryout work according to occupational health and safety (**OHS**) requirements
- Carry out insemination

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Prepare necessary materials and equipment
- Use personal protective clothes and equipment
- Select and thaw semen
- Carryout occupational health and safety (OHS) requirements
- Carry out insemination

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the information Sheets
4. Accomplish the Self-checks
5. Perform Operation Sheets
6. Do the “LAP test”

Information Sheet-3

3.1. Preparing necessary materials and equipment for insemination

AI Supplies and Equipment needed or useful for AI programs include these:

- Semen, Semen storage tank
- Liquid nitrogen
- Electronic thawing device or insulated water bath
- Thermometer
- Timer
- Straw-cutting device
- Insemination rod or gun
- Plastic sheaths
- Plastic obstetrical sleeves, Obstetrical lubricant
- Paper towels
- Record-keeping supplies
- Cattle handling facilities (including breeding box, squeeze chute with head catch and palpation cage and protection from weather)
- PPE (boots, suites, gloves...etc.)
- Insemination gun
- Containers , Thermos flask , Scissors
- Canister (Removable cylinder with a mesh or solid bottom to hold semen in the tank. It has a long hooked handle to permit straw identification and access from the mouth of the tank.)

Tank: Aluminum vacuum-insulated vessel used to hold semen and liquid nitrogen

Canister: Removable cylinder with a mesh or solid bottom to hold semen in the tank. It has a long hooked handle to permit straw identification and access from the mouth of the tank.

Mini-goblet: Plastic cylinder with a sealed base and which fits into the canister. It will hold up to twenty-five straws in a bath of liquid nitrogen.

Straws: Each straw contains enough semen to inseminate a cow once. The volume of semen in the mini-straw is 0.25 ml, which normally contains 20 million sperm cells with a usual

minimum of 40% live at thaw. Semen produced in medium straws containing 0.5 ml of semen (also with 20 million sperm cells) is sometimes available. These straws should be treated in the same way as mini-straws. An appropriate 0.5 ml insemination gun is required.

3.2. Using personal protective clothes and equipment

All equipment (including clothing affording protection against the weather) which is intended to be worn or held by a person at work protects against one or more risks to health and safety. Personal protective equipment (PPE) refers to equipment used as a barrier between an individual and a hazard that could result in an injury or occupational illness.

Personal protective clothes and equipment may include:

- Boots
- Overalls
- Gloves
- Sun protection (sun hat, sunscreen)
- Apron

3.3. Selecting and thawing semen

The procedure for thawing semen is equally important and potentially just as lethal as the freezing process. As a rule of thumb, rapid freezing rates require rapid thawing. Typically, straws of bull semen are thawed rapidly, either in a warm water bath or by a “pocket thaw”. The relative effectiveness of each method depends upon the extender composition and freezing procedure. During semen selection it is not good to select bull that related to cow. Unfortunately, recommended procedures for thawing semen are not the same for all AI companies. Dairy producers usually use semen from several companies but use only one protocol for thawing semen. Calibrate the thermometers used for monitoring thaw water temperature on a regular basis to insure accuracy of temperature measurements.

With any thawing procedure, the recommendations of the semen supplier should be followed. Regardless of the thawing procedure, sperm injury can occur if semen is mishandled post-thaw. Straws thawed in warm water should be dried thoroughly to avoid the possibility of water contacting the semen when the straw is opened; fluctuations in the temperature of thawed semen should be avoided to minimize the risk of chilling injury; and the interval from thawing to

insemination should be kept to a minimum because post-thaw survival of sperm is short in the straw.

Thawing methods varied among AI organizations, each of which has a specific method for diluting, cooling, packaging, and freezing semen in straws. Semen should be thawed according to the recommendations of the organization supplying that specific unit of semen. Thawing semen at about 34-35°C for at least 15-30 seconds is a Comprehensive method. Thawing semen between these temperatures allows more semen to survive the thawing process. Never thaw semen straws in your pocket or in the cow.

3.4. Carryout occupational health and safety (OHS) requirements

OHS requirements should be kept in a location central to the work being performed and readily available to the work force. Some safe work practices will require specific job procedures, which clearly set out in a chronological order each step in a process. But safe work procedures should be included in the company's "worker orientation" program.

OHS may include:

- Safe animal handling systems and procedures including zoonosis control, Identify hazards, assess and report risks.
- Safe manual handling systems and procedures.
- Safe systems and procedures for outdoor work including protection from solar radiation.
- Appropriate use of personal protective clothes and equipment.

All workers should be aware of the fact that safe job procedures have been established, are in effect, are written down and must be followed. In order for communication be effective between those involved in occupational health and safety, the safety profession and other professions, it is important that we use common words such as 'accident, injury, hazard, safety, health and risk' with some consistency. Unless these words are given specific definitions in legislation; some effort must be made to give these words acceptable meanings.

Accident: An unplanned event that may or may not result in damage, loss or injury.

Injury: Damage to the body resulting from a delivery of energy to the body above the capacity of the body to cope with that energy or an interference with the normal function and systems within the body.

Hazard: A source of unwanted or excess energy with the capacity to cause damage, loss or injury.

Safety: an individual's perception of risk. Two alternative definitions are 'safety is a state of mind where by workers are made aware of the possibility of injury at all times. Safety is a state in which the risk of harm (to persons) or damage is limited to an acceptable level. Some would argue for 'tolerable' not 'acceptable', saying no risk is acceptable.

Health: the degree of physiological and psychological well-being of the individual.

Risk: The combination of the likelihood that a hazard will actually result in an accident and the consequences of that accident, often expressed as the product of the two.

3.5. Carry out insemination by maintaining veterinary sanitation procedures

All apparatus used in collection, storage and insemination must be completely dry before use and dispose the disposable ones. All known disinfectants are to some degree toxic to spermatozoa and they are, therefore, not to be used for sterilization. Most of the metals and the use of metal containers must be avoided. Soaps have deleterious effect and should not be used for the cleaning of apparatus.

The most effective and practicable forms of sterilization are, therefore, dry heat for glassware and boiling for rubber lining of artificial vaginas. The inner rubber linings and the cones of artificial vagina should be boiled for 30 minutes when first received from the manufacturers. They may be then allowed to dry in air and stored till required. Before use on the first occasion, they should be swabbed with 65% alcohol and allowed to dry.

After use of artificial vagina, the interior of the lining should be scrubbed out with boiling water to remove all lubricant. The linings should be boiled for 30 minutes each time after use for complete sterilization or by autoclaving at 10 pounds for 20 minutes. The lining and cones quickly deteriorate with repeated boiling or autoclaving. To avoid this trouble, and to ensure complete sterilization, the use of sterilized plastic lining and cones is fast coming into practice which is discarded after one use only.

Glass inseminating pipettes should be rinsed out with cold water after use, and then washed in hot water till quite clean, rinsed twice with glass distilled water, then dried and sterilized in a hot air oven at 1600°C for 60 minutes. If diluted semen is allowed to dry inside the pipettes, it may not be possible to remove it with hot water.

Pipettes should then be soaked in a mixture of 6% potassium bicarbonate and 6% of sulphuric acid solution for 24 -48 hours before washing them in the way described. Other glass for storage and manipulation of semen, should be cleaned, washed, rinsed and sterilized in the same way as inseminating pipettes.

Carrying out Insemination

The success of insemination depends mainly upon

- (i) The inherent fertility of the sperm
- (ii) Proper handling of the semen prior to insemination
- (iii) Insemination at right time during oestrus
- (iv) Proper semen deposition

Methods of Artificial insemination

- Speculum method
- Vaginal method
- Recto-vaginal method

Early methods of AI / speculum & vaginal methods/ involved deposition of the semen in the vagina, as would occur in natural mating. Those methods are not satisfactory. Hence fertility is low and greater numbers of sperm are required. Now a day's insemination with recto-vaginal method is the safe and best that becomes a popular and widely used technique

Recto vaginal method;

The recto-vaginal technique is the most commonly used method to artificially inseminate cattle.

The first step in the insemination process is to restrain the animal to be inseminated. There are several things to keep in mind when choosing a location for inseminating cattle including;

- Safety of both the animal and the inseminator
- Ease of use
- Shelter from adverse weather

Regardless of whether you are left or right handed, it is recommended that you use your left hand in the rectum to manipulate the reproductive tract and the right hand to manipulate the insemination gun. This is because the rumen or stomach of the cow lies on the left side of the abdominal cavity, displacing the reproductive tract slightly to the right. Thus, you will find it much easier to locate and manipulate the tract with your left as opposed to right hand.

A gentle pat on the rump or a soft-spoken word as you approach for insemination, it will help to avoid startling or surprising the animal. Raise the tail with your right hand and gently massage the rectum with the lubricated glove on your left hand. Place the tail on the back side of your left forearm so it will not interfere with the insemination process. Cup your fingers together in a pointed fashion and insert your hand in the rectum, up to the wrist.

Gently wipe the vulva with a paper towel to remove excess manure and debris. Be careful not to apply excessive pressure, which may smear or push manure into the vulva and vagina. With your left hand make a fist and press down directly on top of the vulva. This will spread the vulva lips allowing clear access to insert the gun tip several inches into the vagina before contacting the vaginal walls. Insert the gun at a 30-45° upward angle to avoid entering the urethral opening and bladder located on the floor of the vagina. With the gun about 6 to 8 inches inside the vagina, raise the rear of the gun to a somewhat level position and slide it forward until it contacts the external portion of the cervix. You will note a distinct gristly sensation on the gun when it contacts the end of the cervix.

These procedures incorporates semen thawing, bringing parts of AI gun (plunger & barrel) together, inserting semen straw into the barrel, cover the barrel with AI sheath and fix the sheath with ring to the barrel.

Insemination Equipment

The following equipment is needed to insure proper semen storage, straw handling, and insemination procedures. This equipment should be stored in a clean, dry, stainless steel breeding kit.

- Forceps: for removing straws from the tank
- One-pint, insulated, wide-mouthed thermos with a dial thermometer: for thaw water
- Sharp stainless steel scissors for cutting straws
- Paper towels
- Straw gun for insemination
- Disposable plastic breeding sheathes
- Disposable plastic insemination gloves

Preparations for insemination and sanitation

- Ensure that the cow to be bred is truly in heat. Research studies indicate that 7- 20% of the cattle inseminated are not in heat.
- Restrain the cow first and then thaw the semen.
- The restraint area should be familiar to the cow and free of stressful conditions. Unnecessary excitement may interfere with physiological mechanisms important to achieving a good conception rate.
- Develop good sanitary procedures and insemination practices. It is easier to learn good habits than to break bad habits.
- Insemination supplies should be kept dry and clean at all times. Breeding sheaths should be stored in the original package until used.
- Once the insemination device is assembled it must be protected from contamination and cold shock temperatures.
- Materials used to lubricate the rectum should not come in contact with the vulva region. Lubricants are generally spermicidal. Avoid using products that are irritating.
- The vulva region must be thoroughly wiped clean with a paper towel. This is important in helping prevent the interior of the reproductive tract from becoming contaminated and possibly infected. A folded paper towel can be inserted into the lower portion of the vulva. The insemination rod can then be placed between the folds of the towel and inserted into the vagina without contacting the lips of the vulva.
- Protective rods or sheaths are used in herds or for specific cows where vulvo-vaginal infection is a problem. When this system is used, the standard insemination rod and plastic sheath are inserted into the larger protective rod or sheath. This double rod combination is passed through the vagina to the external cervical opening. At the cervix, the tip of the protective device is punctured by the insemination rod, which is then threaded through the cervix. This technique should only be used following the recommendations of a veterinarian, extension specialist, or AI representative and only when specific diseases have been diagnosed or suspected.

In short, insemination procedures are

The recto-vaginal technique is the most commonly used method of AI cattle.

- clean the vulva
- put your hand in the rectum and remove manure
- locate the cervix and uterus with your hand in the rectum
- grasp the cervix with your hand & straighten any vaginal folds that encounter with tip of gun
- open the cervix in the center if not opened
- pass the gun in the cervical folds
- the gun Slip forwards easily and reach uterine body
- make sure the semen is deposited in the uterine body

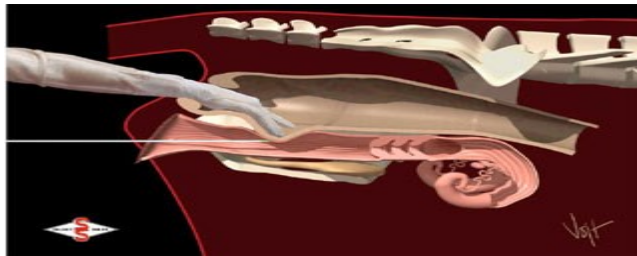


Figure 3.1: Keeping the gloved hand even with the tip of the inseminator gun

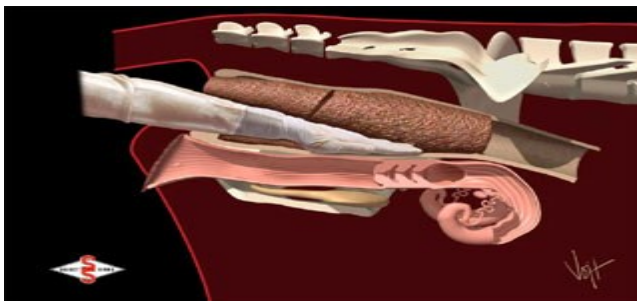


Figure 3.2: Allowing manure to pass over the top of the hand and arm

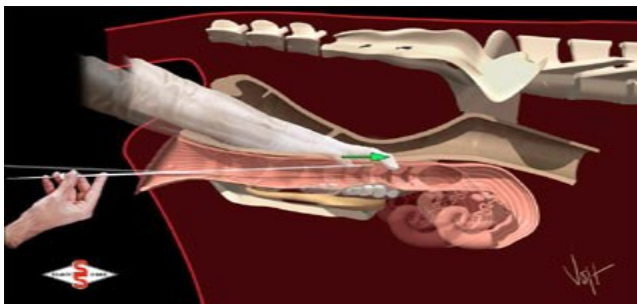


Figure 3.3: Grasping the cervix and gently moving it forward.

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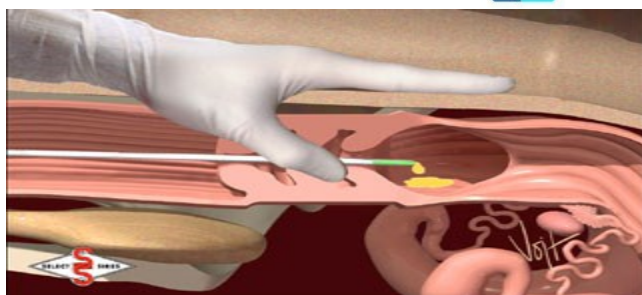


Figure 3.4: Depositing the semen in the body of the uterus.

Or

Inseminating the cow

Always use a glove when inseminating. Plastic disposable gloves are preferred but reusable rubber gloves may be used.

- Don 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 glove lubricant to the back of your hand.
- Carry the loaded gun in your mouth ensuring that the cow end does not become contaminated by contacting walls etc.
- Make the cow aware of your presence. A startled cow may kick.
- Retrieve the paper from your pocket.
- Protect your hand from contamination with a piece of paper toweling, then grasp the cow's tail and lift it aside.
- Using the lubricated back of the gloved hand with the gloved hand, smear lubricant across the cow's anus. Form a cone with the gloved fingers and insert your hand into the rectum. Pause at this stage and encourage the anus to relax by gently revolving your fingers. The wide part of your hand can then be eased in without dragging the rough dry skin surrounding the anus. Avoid rough sudden entry which can abrade the anus and cause the release of adrenalin which reduces conception rates.
- Thoroughly clean the vulva of all soap, dung and dirt by wiping it with the piece of paper used on the tail. Use a fresh piece if the paper is too soiled.
- Bear downwards with the wrist of the hand in the rectum which helps to part the lips of the vulva presenting a clean area for inserting the gun.

- Insert the gun cleanly between the lips of the vulva into the vagina. Take care to ensure the gun passes along the top of the vagina thus avoiding the bladder.
- Gently push the gun through the vagina until you reach the surface of the cervix which has a ‘grating’ feel about it.
- When the gun reaches the mouth of the cervix, hold the cervix in the finger tips. Maintain a light forward pressure on the gun and manipulate the cervix so that the gun passes through the cervix canal. If you are unable to hold the cervix you may pin it against the pelvis.
- While passing the gun through the cervix locate the forward end of the cervix with the middle or index finger. Gently push the gun forward but only as far as this locating finger. This is important when doing a repeat service as the cow may be pregnant. Avoid deep penetration of the uterus as the gun may cause damage and possibly infection thus reducing the chance of conception or in the case of a pregnant cow, an abortion (up to 5 per cent of pregnant cows show some signs of heat).
- Begin to express the semen at this position ensuring that most of the semen (two-thirds) is expressed in the body of the uterus. The remaining one third should be placed near the uterus in the front 1 cm of the cervix. Take care not to draw the gun too far back. It is easy to pull back too far at this stage, even as far as the vagina. Occasionally the gun cannot be passed to the proper position. 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.
- Gently express the semen being careful not to ‘spit’ it out.
- Pause before withdrawing the gun allowing the semen to get away, then slowly withdraw the gun from the cervix. Rapid withdrawal of the gun can suck semen back through the cervix into the vagina.
- Remove the gun slowly from the vagina.
- Slowly withdraw the arm from the rectum of the cow.
- Loosen the locking ring on the gun and remove the soiled sheath. The empty straw should come out with the sheath.
- Dispose of the straw, sheath, paper and dirty glove in a waste bin.
- If the unprotected parts of the insemination gun become soiled with the dung or mucus the gun should be thoroughly cleaned before it is returned to the kit box.

General tips for insemination technique

To avoid the possibility of entering the urethral opening on the floor of the vagina, the insemination rod should be inserted into the vulva upward at a 30° to 40° angle. The anterior portion of the vagina, termed the fornix vagina, tends to stretch rather easily when the insemination rod is pushed forward and beyond the cervix. This may give the false impression that the rod is advancing through the cervix, when indeed it is above, below, or to either side of the cervix. The inseminator should be able to feel the rod within the vaginal fold, but unable to feel the rod tip within the cervix.

Maintain slight forward pressure on the rod while manipulating the cervix slightly ahead of the rod. The target for semen deposition, the uterine body, is quite small. Accurate rod tip placement is probably the most important skill involved in the whole AI technique. Inseminators generally identify this target area by feeling for the end of the cervix and the tip of the rod as the rod emerges through the internal opening. Depositing the semen in the cervix or randomly in the uterine horns may result in lower conception rates. Semen deposition should take about five seconds. Slow delivery maximizes the amount of semen delivered from the straw and minimizes the unequal flow of semen into one uterine horn. During the process of semen deposition, take care that the fingers of the palpating hand are not inadvertently blocking a uterine horn or misdirecting the flow of semen in some manner.

Some of the most important aspects to remember when inseminating a cow include;

- Be gentle (do not use too much force)
- Insemination is basically a two-step process: get the gun to the cervix, and then place the cervix over the gun.
- Deposit the semen just through the cervix into the uterine body
- Take your time
- Relax

Veterinary sanitation procedures during AI work includes:

- Washing and hygienic preparation of the vulva area
- Disinfection and cleaning of AI equipment
- Preventing zoonosis and venereal diseases by wearing gloves and other appropriate protective materials.

Self-Check - 3	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below.

Test I: Short Answer Questions

- Equipment used as a barrier between an individual and a hazard that could result in an injury or occupational illness is called----- (2pts.).
A. Safety B. Personal protective equipment C. Hazard D. Risk
- Thawing semen at ----- for at least 15-30 seconds is a Comprehensive method (2pts.).
A. 10 – 15 °C B. 5 – 10 °C C. 34 – 35 °C D. 15 - 30 °C
- The most commonly used method to artificially inseminate cattle is----- (2pts.).
A. Speculum method B. Vaginal method C. Recto-vaginal method

Test II: Short Answer Questions

- List methods of artificial insemination (3pts.)
- What are the considerations in artificial insemination? (5pts.)

Note: Satisfactory rating – 12 points Unsatisfactory - below 12 points

You can ask you teacher for the copy of the correct answers

Operation Sheet -3

3.1. Techniques of Semen Thawing

a. Tools and equipment's

- PPE
- Recording book
- Forceps
- Breeding guns
- Thermos
- Thermometer
- Disposable sheaths
- Straw cutter
- Paper towels

b. procedures

- Fill thermos with 95-degree water ½ inch from top or Assure the water for thawing is at 34-35°C
- With forceps, remove one straw from the goblet of the sire chosen, and place in the thaw water with crimped end up or Choose the semen you want to use and remove it from the canister with forceps
- Thaw the frozen semen at 34-35°C for 15-30 (40) seconds
- Remove a straw of semen from the thaw water and wipe it completely dry with a paper towel (Do not thaw more than one straw at a time and take care to not elevate canister beyond the neck level of the container)

2.3. Technique of Performing insemination

a. Tools and equipment's

- PPE
- Recording book
- Forceps
- Breeding guns

- Thermos
- Thermometer
- Disposable sheaths
- Straw cutter
- Paper towels

b. Procedures

- Check identity of cow
- Check thermos temperature
- Select semen not close to cow
- Place straw in thermos for 15 second
- Clean straw by tissue paper
- Cut the plug at the end of straw
- Put the straw in the insemination gun sealed end first
- Push the plunger of insemination gun until the semen is visible
- Keep the insemination gun in your hand or between your teeth
- Clean the vulva
- Put your hand in the rectum and remove manure
- Locate the cervix and uterus with your hand in the rectum
- Grasp the cervix with your hand and straighten any vaginal folds that encounter with tip of gun
- Open the cervix in the center if not opened
- Pass the gun in the cervixale folds
- The gun Slip forwards easily and reach uterine body
- Make sure the semen is deposited in the uterine body

LAP TEST-3	Performance Test
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Name: _____ Date: _____

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within 2 hours.

Task- 1: Thaw semen

Task- 2: Perform Artificial insemination

LG #41	LO # 4 – Record data and clean up on completion of work
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Instruction sheet

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Recording insemination and breeding data and evaluating AI efficiency
- Cleaning and maintaining work area
- Cleaning and returning reused materials and equipment
- Disposing wastes

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Record insemination and breeding data and evaluate AI efficiency
- Clean and maintain work area
- Clean and return reused materials and equipment
- Dispose wastes

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the information Sheets
4. Accomplish the Self-checks
5. Perform Operation Sheets
6. Do the “LAP test”

Information Sheet-4

4.1. Recording breeding data and evaluating AI efficiency

4.1.1. Recording insemination and breeding data

AI is essential information among those used for genetic evaluation purposes. It comes just after identification and before parentage recording. Without accurate AI recording, records on percentage are hardly precise and then accuracy of genetic evaluations is bad. Besides, AI records are important for quality controls in AI stations and reproduction management of herds, along with other important data.

Data that recorded and used for evaluation of AI efficiency includes: number of services per conception, pregnancy rate, calving interval, none return rate. These parameters in turn may indicate the breeding efficiency of the animal, the efficiency and ability of the inseminator, and the fertility and quality of the semen. This information was important for generation of ideas for farm productivity and further research works.

Records that inseminators are to keep and have available;

- The number of cows inseminated with semen from approved bulls.
- Name and address of the cow's owner.
- Total number of doses used.
- Name & address of any other person supplied with semen and number of Doses
- The premises from which the semen has been received and the quantity of Semen received.
- Identity of cows inseminated.
- The dates of insemination.
- Identity of the bull used.
- Batch number of the semen. These records could at any time is requested by the chief veterinary officer.

4.1.2. Evaluating AI efficiency

Accurate insemination technique requires concentration, attention to detail, a clear understanding of reproductive anatomy and the ability to identify the target area and properly position the insemination rod.

Inseminators should calculate the first-service conception rate for their herds during a 6-month interval. They should review breeding charts and consider only those cows that have been bred long enough to have been pregnancy checked. Strive for a goal of 45 percent first-service conception rate. In smaller herds there may not be enough first service during a 6-month period to determine the conception rate accurately. In that case, inseminators should summarize first services over 12 months or calculate the percentage of cows pregnant after three breeding. In very large herds, calculate conception rate more often than every 6 months.

In any size herd, service per conception is another index of breeding performance related to the effectiveness of insemination technique. A reasonable goal is to maintain a rate of fewer than 1.8 services for pregnant cows. Livestock producers must realize that other factors in addition to AI technique can affect conception rate and services per conception. If an evaluation of your records indicates that your insemination technique may be a problem area, then you should consider attending an AI retraining session. The number of services per conception, pregnancy rate, calving interval, non-return rate is parameters in turn may indicate the breeding efficiency of the animal, the efficiency and ability of the inseminator, and the fertility and quality of the semen.

4.2. Cleaning and maintaining work area

After insemination, there are several clean-up steps you will want to follow.

- Inspect the gun tip for signs of infection. Make a note for your veterinarian if you see any.
- Bend the sheath and straw tip at a 90° angle and remove these from the gun.
- Tighten the O-ring back on the gun so it will not get lost.
- Double-check the bull's identity on the straw.
- Reverse strips your glove so the straw, sheath, and manure are trapped inside. Squeeze out any excess air; tie a knot in the open end and dispose of the glove.
- Wipe the gun clean and dry it before returning it to the insemination kit.
- If the gun becomes dirty after use, take it completely apart, wash with soapy water and rinse with clean water. Shake the gun to remove excess water, then allow it to completely dry before reassembly

4.3. Cleaning and returning reused materials and equipment

At the end of AI procedures the animals must be cleaned if there is any defecation on it. The working materials also cleaned and stored properly.

4.4. Disposing Wastes

Waste materials should be disposed properly in a designated place. The waste materials also disposed in the designated pit. You can dispose by burning or by collecting and placing a waste material in designated pit. Waste materials may include:

- Gloves
- AI Sheath
- Straws
- Pipettes
- Packaging materials
- Dead animals
- Mud or defecation
- Paper based materials

Self-Check - 4	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below.

Test I: Short Answer Questions

- Records that inseminators are to keep and have available (2pts.)
 - The number of cows inseminated
 - Name and address of the cow's owner
 - The dates of insemination
 - All
- Which one of the following is a waste disposing method except one? (2pts.)
 - Burning
 - burying
 - leave in the crush
 - putting in designed pit

Test II: Short Answer Questions

- List data that recorded and used for evaluation of AI efficiency (4pts.)
- List wastes produced during AI work (5pts.)

Note: Satisfactory rating – 10 points Unsatisfactory - below 10 points

You can ask you teacher for the copy of the correct answers

Reference Materials

- Pathogens that cause infertility of bulls or transmission via semen (Givens & Marley, 2008)
- Risks of disease transmission through semen in cattle (Givens. 2018)
- Prevention of disease transmission by semen in cattle (Wentink et al., 2000)
- Hygienic aspects of storage and use of semen for artificial insemination (Thibier & Guerin, 2000)
- Disease risks to animal health from artificial insemination with bovine semen (Eaglesome & Garcia, 1997)
- Semen processing and artificial insemination in health management of small ruminants (Cseh et al., 2012)
- Battaglia, Richard A. Handbook of Livestock Management. 3rd ed. New Jersey: Prentice-Hall, Inc., 2001.
- Hafez, E.S., editor. Reproduction in Farm Animals. 2 nd ed. Philadelphia: Lea & Febiger, 1968

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