TVET CERTIFICATE V in ANIMAL HEALTH

ASSIST REPRODUCTIVE TECHNOLOGY

ANHRT 502 **Perform Artificial Insemination**

Competence

Credits: 7 **Learning hours: 70**

Sector: Agriculture and Food Processing
Sub-sector: Animal health

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Purpose statement

This core module provides the skills, knowledge and attitude for a learner to be competent in a range of routine tasks and activities that require the application of practical skills in artificial insemination. Upon completion of this module the learner will be able to:

- Identify artificial insemination tools and equipment
- Collect and process sperms
- Apply artificial insemination techniques

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Acronyms and abbreviation

1. AI: Artificial insemination
2. SC: scrotal circumference
3. BSE: Breeding Soundness Exam
4. CSS: Certified Semen Services
5. CL: Corpus luteum
6. **GnRH: gonadotropin-releasing hormone**
7. **LH: luteinizing Hormone**
8. **PGF2α:** Prostaglandin **F2α**
9. **IU: international unit**
10. **Kg: kilogram**
11. **MGA: Melengestrol acetate**
12. **SMB: Synchro-Mate-B**
13. **Ml: mille liter**
14. **IBR: Infectious Bovine Rhinotracheitis**
15. **BVD: Bovine Virus Diarrhea**

Introduction to reproductive technology

Reproductive techniques

Reproduction techniques are becoming more and more important and necessary for modern animal breeding. Reproduction techniques are used to make safe and efficient breeding possible.
Breeding program can be improved and optimized through the use of reproductive techniques.
Reproductive techniques allow the dissemination of genes of interest and increased the reproductive capacities of animals. These technologies can bring a lot of advantages, but can also evoke strong emotions from the society.

Over the years different techniques are invented, developed and exploited for the breeding sector:
1. Artificial insemination (AI): semen is collected from an adult male and injected into the uteri of a fertile female. Collected semen is often stored or transported in frozen form, which makes this technique suitable for global use or storage. AI makes it possible to rapidly produce a large number of offspring from a genetically excellent male.

- **Definition**: Artificial insemination is the technique in which semen with living sperms is collected from the male and introduced into female reproductive tract at proper time with the help of instruments. This has been found to result in a normal offspring. In this process, the semen is inseminated into the female by placing a portion of it either in a collected or diluted forms into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions.
- **Advantages of artificial insemination**

There are several advantages by artificial insemination over natural mating or servicing.

- ✓ There is no need of maintenance of breeding bull for a herd; hence the cost of maintenance of breeding bull is saved.
- ✓ It prevents the spread of certain diseases and sterility due to genital diseases.
- ✓ By regular examination of semen after collection and frequent checking on fertility make early detection of interior males and better breeding efficiency is ensured.
- ✓ The progeny testing can be done at an early age.
- ✓ The semen of a desired size can be used even after the death of that particular sire.
- ✓ The semen collected can be taken to the urban areas or rural areas for insemination.
- ✓ It makes possible the mating of animals with great differences in size without injury to either of the animal.
- ✓ It is helpful to inseminate the animals that are refusing to stands or accept the male at the time of estrus.
- ✓ It increases the rate of conception.
- ✓ It helps in better record keeping.
- ✓ Old, heavy and injured sires can be used.

- **Disadvantages of Artificial insemination**

There are few disadvantages of artificial. The most noted are:

- ✓ Requires special equipment.

- ✓ Requires more time than natural services.
- ✓ Necessitates the trained personnel to perform the technique.
- ✓ The livestock managers must spend a great deal of time checking females for estrus
- ✓ Improper cleaning of instruments and in sanitary conditions may lead to lower fertility.
- ✓ If the bull is not properly tested, the spreading of genital diseases will be increased.
- ✓ Market for bulls will be reduced, while that for superior bull is increased.

2. Embryo transfer is the transfer of an embryo from a superior donor female and implanted in a surrogate mother. In this technique disease transmission is even more minimized, compared to AI. Equivalent to the AI semen, the embryos can be frozen, so they can be widely distributed or stored for future use.
Embryo transfer can be combined with super ovulation. This method stimulates female animals to produce many more ova and thus embryos, than they would naturally do. This is economically very valuable for the breeder, as more embryos can be taken away from the female.

Embryo Transfer is used to disseminate desirable genes from superior female animals from various species (horses, cattle, sheep, goats, and pigs).

- **Main advantages Embryo Transfer:**

✓ Increase in the number of offspring per female
✓ Easier and more rapid exchange of genetic material between countries
✓ Less transport of live animals, thereby reducing risks of disease transmission
✓ Storage and expansion of rare genetic stock.

- **The main disadvantage**:

✓ The high cost of the technique.

3. **Cloning:** another method of multiplying improved animals, currently not used for animal production. Potentially cloning can be used to produce genetic copies of individuals and for dissemination of genetic progress but it may also be used to introduce a new or endangered breed into a country without risk of disease transmission by transport of animals. Today cloning in farm animal breeding is done mainly for research purposes and not for production of milk, meat or eggs.

Learning Unit 1 Identify artificial insemination tools and equipment

LO 1.1- Identify tools and equipment for sperm collection

- Topic 1: Tools and equipment for sperm collection, processing, storage and transport

a. Seminal Collection Area

The collection area must provide by an earthen floor in the immediate collection area. Means to restrain the teaser animals to minimize lateral as well as forward movement must be provided.

b. Teaser

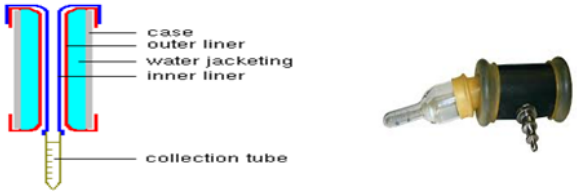
An animal used to sexually tease but not to impregnate the members of the opposite sex. In cattle and sheep vasectomized animals or castrates injected with testosterone are used; in horses an entire or cryptorchidic is used but at a distance so that no act of mating can take place.

Figure 1: teaser

c. Artificial vagina

Artificial vaginas (AVs to the initiated) are used to collect semen from many species, most prominently cattle and horses

Figure 2 artificial vagina



d. Electro ejaculator set

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.

Figure 3 electro ejaculator set

LO 1.2- Identify tools and equipment for semen storage and transport

a. Thermos

Thermos is bottle for keeping semen at constant temperature and away from sunlight during transportation.

Figure 4 Thermos

b. Thermometer

Thermometer is designed to measure the temperature of the Artificial Vagina or Water Bath.

Figure 5: Thermometer

c. Microscope

Individual progressive motility of spermatozoa can be assessed under a bright field or phase contrast microscope preferably equipped with a warm stage or other means of preventing cold shock of spermatozoa.

Figure 6: microscope

d. Incubator or Water Bath

As sperm is very heat (or cold) sensitive, you will need an incubator or water bath. This piece of equipment is used to keep anything that is going to come into contact with the semen at body temperature (38° Celsius).

Figure 7: incubator

e. Sperm Counting Device

Figure 8: Semen packaging and labelling machine

LO 1.3-Identify artificial insemination kit components

- **Topic 1: Artificial insemination kit components**

Figure: 9 AI kit

a. Semen storage tank

Semen storage tanks are large vacuum-sealed metal bottles .They are extremely well-insulated. These tanks can maintain internal temperatures of -320 °F

Figure 10: liquid nitrogen tank

b. **Forceps** for removing straws from the tank, one-pint, insulated, wide-mouthed

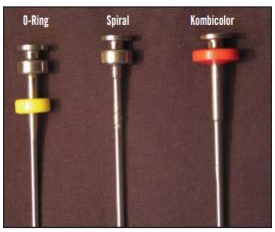
c. **Thermos with a dial thermometer** for thaw water

d. **Sharp scissors** for cutting straws

e. **Paper towels**: to Clean the vulva and surrounding area prior to insemination

f. **Straw gun for insemination**: The straw gun is hollow with a plunger to expel the semen Insemination guns are available to accommodate specific straw sizes: ½ cc straw guns, ¼ cc straw guns, and universal straw guns that accommodate both ¼ and ½ cc straws.

Figure 11: straw gun



g. Disposable plastic breeding sheathes

Breeding Sheaths not only vary in the straw size they were designed to accommodate, but also in the locking method for securing the gun and sheath together

h. Disposable plastic insemination gloves: This Long veterinary artificial **insemination gloves** disposable is mainly used for ***cattle***, buffalo, horses and donkeys etc… rectal palpation, ovarian pregnancy test, ...

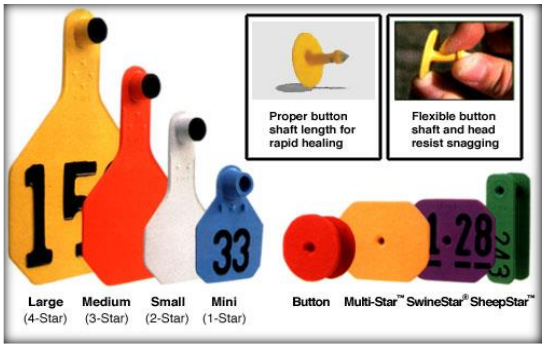
i. Speculum: A speculum is a duck-bill-shaped device. One common use of the speculum is for vaginal exams

Figure 12 speculum

j. **Lubricant***:* A lubricant is a substance which you put on the surfaces or parts of something, to make the parts move smoothly

k. **Ear tags**: An ear tag is a plastic or metal object used for identification of domestic livestock and other animals

Figure 13: ear tags



I. **Ear tags applicator**: pliers for quick and easy installation of various types of ***ear tags*** for animals.

Figure 14: ear tag applicator

Learning Unit 2 – Collect and process sperms

LO 2.1-Manage sire (males)

- **Topic 1: Selection of sires (males)**

For production and distribution of quality semen, it is most important that the bulls used in AI program satisfy quality norms, bulls are disease free and semen is harvested and processed in accordance with the standard protocols

Before procuring new bull calves/bulls for a semen station, a thorough physical examination shall be conducted by an accredited Official / Veterinarian to ensure that the bulls are free from abnormality and do not display clinical symptom(s) of any infection or any contagious diseases.

The Breeding Soundness Exam is a prognostication of a bull's ability to get cows pregnant and is typically performed on bulls that are used for natural service. The Breeding Soundness Exam consists of three major examination categories:

- ✓ Overall physical exam.
- ✓ Examination of the reproductive genitalia.
- ✓ Semen evaluation.

A. Physical Examination

1. Health

- Eyes are clear of any lesions – pink eye and squamous cell carcinomas.

- Oral examination - adequate number of teeth to graze and eat forage.
- General health should be good.

2. Body Condition

- Excessively fat bulls lack vigor and the excess fat may negatively affect sperm production.
- Excessively thin bulls may not have the stamina to service cows during the breeding season.
- Therefore, bulls must carry some extra weight at the start of the breeding season.

3. Conformation and Body Structure

- Rear leg conformation is very important.
  - ✓ Rear legs are load bearing during mounting and copulation.
  - ✓ Necessary for proper mobility around the breeding pasture.
- Hooves are in good shape and don't need trimming.
- Remember that structural faults are heritable.

B. **Genital Examination**

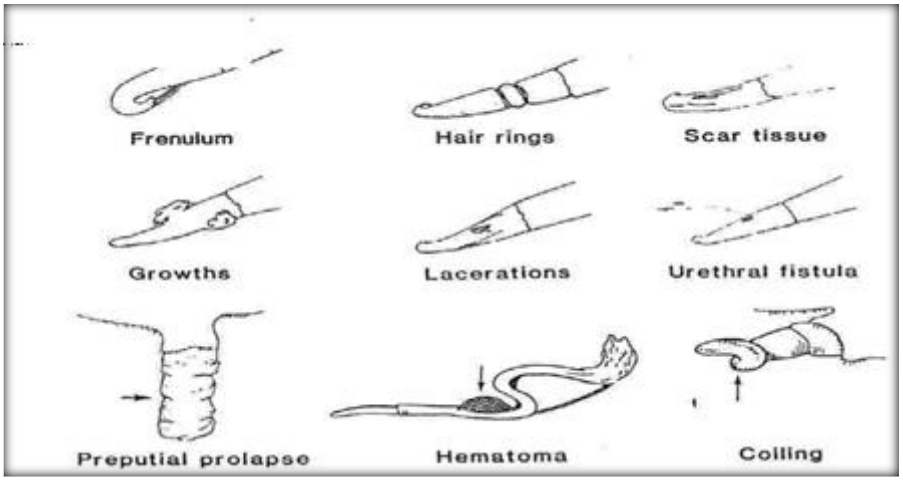
1. Prepuce

- Should be palpated for adhesions, inflammation, and abscesses.
- Preputial problems lead to:
  - ✓ **Phimosis** – inability to extend penis through the prepuce.
  - ✓ **Paraphimosis** – inability to retract penis through the prepuce.

2. Penis

- Penile frenulum: Developmental characteristic observed in virgin bulls
  - ✓ Penile deviations:During extension, penis should be parallel to the body of the bull.
- Other penile abnormalities.

Figure 15: penile abnormalities



3. Scrotum

The scrotum is a sack of skin that hangs from the body at the front of the pelvis, between the legs. The scrotum contains the testicles.

Variations in normal scrotal conformation include:

- Abnormal scrotal conformations,
- Scrotal circumference variation,

• **Cryptorchidism** is the failure of testis to descend into scrotum (unilateral or bilateral).

- ✓ Unilateral - one testicle is retained and bulls are generally fertile.

This is a heritable trait and bulls are usually not used for breeding.

- ✓ Bilateral - both testicles are retained and bulls are infertile.

4. Testes & Epididymis

• Palpate the testes and epididymis for size, tone, and symmetry.

- ✓ Testes should be firm; but, they should not be too soft or too hard.
- ✓ Symmetrical shape is normal. Any deviation in size, shape and/or position should be viewed with suspicion.
- ✓ Caput, corpus, and cauda are palpated to determine if there are lesions or enlargements.

5. Accessory Sex Glands

- Examined via rectal palpation.
- Vesicular glands, prostate gland, pelvic urethral muscle, and ampulla.
- Seminal vesiculitis is a common problem.
- Condition characterized by firm vesicular glands accompanied by inflammation of vas deferens, ampulla, epididymis, or testes with increased white blood cells in the semen.

6. Measuring & Importance of Scrotal Circumference (SC)

- Scrotal tape is used to measure area at the largest diameter of the scrotum
- As SC increases, sperm motility and normal sperm numbers increase, while abnormal sperm concentrations decrease.
- The following table lists the minimum recommended SC measurements that a bull must have, within an age category, to pass a BSE.

Age	**Scrotal Circumference (cm)**
< 15 months	30
> 15 < 18 months	31
> 18 < 21 months	32
> 21 < 24 months	33
> 24 months	34
Minimum Recommended SC for BSE.	

Table 1: scrotal circumference

Training the sires

Ideally, the sires should be trained for semen collection when they are still young. The training will depend on which type of semen collection will be used to it. It should be trained for mounting dummy, it should be trained for ejaculate within artificial vagina, as it should be trained for rectal massage or electro-ejaculator.

The training will also depend on the type of animal: the training of boar differ from training of bull etc…

- **Topic 2: Management of sires(males):**

1. Daily care of bulls

The objective of daily care of bulls is to ensure a satisfactory state of cleanliness. For proper management of bulls, the following points shall be considered:

- ✓ The bulls shall be kept under hygienic conditions at all times.
- ✓ The coat of the bulls shall be kept clean and generally short.
- ✓ The hooves shall be regularly trimmed.
- ✓ The length of the tuft of hairs at the Preputial orifice, which is invariably soiled, shall be cut to about 2 cm. The hair would not be removed altogether, because of its protective role. If cut too short, it may cause irritation of the Preputial mucosa.
- ✓ Bulls shall be brushed and groomed regularly, and where necessary, special attention shall be given to the underside of the abdomen, a day prior to semen collection.
- ✓ Cleaning of the prepuce with sterile normal saline solution may be done every ten days if the microbial load is within the prescribed limits.

1. Housing

Bull sheds shall have spacious individual pens with adequate loafing area, manger and drinker with access to drinking water all time. Adequate shade around the bull shed shall be provided. The roof shall be made of suitable materials.

Bulls are adaptable to a wide range of environmental conditions. Basic criteria for a satisfactory environment include physical comfort, disease control, access to adequate nutrition and water, and safety for both bulls and handlers

The housing system selected should be considered acceptable if the basic criteria listed above are satisfactorily met

- Each bull should have adequate space to move, and a dry area in which to comfortably lie down
- In "tie stall" facilities bulls may be tethered, but the animal must be able to lie down, stand, have limited movement, and to eat and drink unhindered
- Stalls should be regularly cleaned and suitable bedding materials should be used
- Cattle can tolerate a wide range of ambient temperatures; however, housing should be designed to protect bulls from extreme heat or cold
- Bulls housed outdoors should have access to shelter for protection from sun and severe weather
- . Enclosed structures should be properly ventilated or air conditioned to help provide a comfortable environment
- Ventilation can be provided actively by mechanical systems or passively with windows and vents, depending on the building design

- Internal surfaces of the housing and pens should allow for effective cleaning and disinfection
- To avoid injury, internal surfaces and fittings of buildings and pens should not have sharp edges or projections
- Any toxic paint and/or preservative should not be used on any surfaces the animals may contact
- It is recommended that provisions be made for the segregation of sick or injured animals

2. Diseases control

The bull attendants, labourers and veterinary officers who are in close contact with the bulls would need to follow certain hygienic practices that would minimize the spread of infection.

Vaccination Schedule

The bulls shall be vaccinated against FMD, HS, BQ, Theileriosis and Anthrax. However, vaccinations against bacterial diseases shall be done only if there is an outbreak or prevalence of a particular disease.

3. Sperm collection frequency

Normal bulls produce and ejaculate tremendous numbers of sperm. Most bulls have a sufficient libido for routine sexual activity, but become satiated to predictable stimulus situations.

Frequent changes to the novelty should allow weekly harvest of four to six ejaculates per week for most bulls.

Utilizing the physiological characteristics associated with each ejaculate to establish the collection frequency of each bull, and empowering an integrated collection and laboratory staff to monitor and make adjustments to the ejaculation frequency are necessary in maximizing the sperm harvest.

Young bulls can ejaculate 10 to 20 billion sperm per week, and mature bulls should ejaculate 40 to 60 billion sperm per week. Semen collection management procedures should be reviewed when bulls do not meet production goals.

LO 2.2-Collect sperms

Seminal collection procedures normally include; sexual stimulation, sexual preparation and collection of the semen.

- **Topic 1: Sexual stimulation and Sexual preparation techniques**

1. Sexual Stimulation

- The stimulation process starts by exposing the bull to a mount animal in a collection environment.
- The presence of other animals in this environment and various visual, olfactory, and auditory stimuli, sexually arouse the bull.
- When the bull is sexually aroused he will have an erection of the penis and will want to mount other bulls and/or a mount animal.

- Depending upon the libido of the bull and the frequency of collection, the stimulation may be accomplished in a matter of minutes or it may take much longer.

2. Sexual Preparation

- Sexual preparation is the intentional prolongation of sexual stimulation.
- It is achieved through a series of false mounts (allowing the bull to mount, but not ejaculate) and restraint, and ultimately
- Results in an increase in the quantity and quality of sperm ejaculated.
- For all false mounts, the semen collector should be at the bull's side to hold the sheath aside so the penis does not come in contact with the rear quarters of the mount animal.
- This diminishes the chance of contaminating the penis and also serves to prevent possible injury.

• Topic 2: Collection methods of the semen:

There are three commonly-used techniques for collecting semen:

- Use of an artificial vagina,
- Digital manipulation,
- Electro ejaculation.

The technique used depends on the species being collected and the disposition of the individual male.

1. Semen Collection by Artificial Vagina (AV)

- ✓ This method is used almost exclusively in AI centers for practical reasons and because it produces physiological semen samples.
- ✓ After all the AV components are put together, the space located between the rigid casing and the inner rubber liner is filled with warm water. Bulls for semen collection require an AV temperature of 42 - 50°C.
- ✓ AV pressure is also an important factor for obtaining ejaculates of optimum quality.
- ✓ Pressure is adjusted by adding air through a valve in the rigid casing until the AV liner protrudes slightly from the ends.
- ✓ A small amount of non-spermicidal lubricant is applied to the inner liner before the collection is performed.
- ✓ Two people are required for bull semen collection by AV, the bull handler and the semen collector who carries the AV.
- ✓ Since semen collection by AV imitates natural breeding, the bull must mount a cow in heat, a steer, a dummy cow, or a bull while the collection is being performed.

- ✓ If cows are available, but not in heat, a tranquilizer may be administered to facilitate bull mounting and semen collection
- ✓ When the bull has mounted, the collector must grasp the penis through the sheath and direct it to the opening of the lubricated AV.
- ✓ The bull will make seeking motions and thrust into the AV.
- ✓ The thrust should be vigorous to ensure an ejaculate has been collected.
- ✓ The operator should evince care so as not to touch the exposed part of the penis.
- ✓ After the bull dismounts, the artificial vagina is taken off from penis and the air vent is opened to release the pressure from the jacket.

Figure16 artificial vagina collection

2. Semen Collection by Trans-rectal Massage

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 ampulla, 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.

Advantages:

1. No expensive equipment is required.

2. The technique avoids the potentially painful aspects of electro ejaculation.

Disadvantages:

1. A skilled palpator is required.

2. Libido, mating ability, penile erectile function and the ability to ejaculate are not evaluated.

3. Semen samples may be contaminated with epithelial cells, bacteria, and dirt especially when it dribbles through the prepuce and off Preputial hairs.

4. Semen volume and concentration are very variable.

3. Electro ejaculation

Ideally, bulls are restrained in a chute with good footing without the head caught in a head gate. If they are caught by the head, they are more likely to lie down during the procedure. In rare instances, it may be necessary to prevent a bull from lying down during electro-ejaculation by applying a restraining belt under the bull's chest. A 30 inch (76 cm) wide chute can accommodate most bulls. It is extremely important to locate at least one strong pole behind the bull. With only one restraining pole, a height of 28 - 30 inches (71 - 76 cm) may be preferred if the scrotum and testicles are to be examined.

Technique:

- ✓ Move the bull to the chute and position the pole behind.
- ✓ Set up the electro-ejaculator beside the bull leaving it turned on and ready to be used.
- ✓ Using a palpation sleeve, the rectum is emptied of feces and a longitudinal massage is applied over the ampullae and urethralis muscle for 1 - 2 minutes.
- ✓ A lubricated probe is introduced into the rectum with the electrodes facing ventrally.
- ✓ Make sure the electro-ejaculator is turned on before performing this step. If it is turned on after inserting the rectal probe, the bull may receive a strong electrical pulse that will increase the level of stress in the animal.
- ✓ Electrical stimulation is begun slowly until the bull shows a minimal response. Consecutive stimuli are then given, each increasing in intensity a small amount. Stimuli should last 1 - 2 seconds and then be discontinued for 0.5 - 1 second before the next one starts.
- ✓ After several stimulations, clear pre -seminal fluid begins to flow from the protruded penis. This clear pre-seminal fraction should not be collected.
- ✓ As soon as the cloudy sperm rich fraction begins to flow from the penis, a collection cone with the test tube is placed over the penis and the sample is collected.
- ✓ After collecting a suitable sample, the stimulation is stopped and the rectal probe is removed. The semen sample is then taken to the lab for evaluation and processing

Figure17 electro ejaculation

- **Topic 3: Collection of sperm for boar**

Use a shady, draught-free area when collecting semen since exposure to ultra violet light, sudden temperature changes and water contamination lower the spermatozoa's viability.

When the boar is mounted on the dummy, grasp the spiral end of the penis with the hand. Allow the boar to thrust through the clenched hand a few times before applying pressure. Hand pressure on the spiral part of the penis imitates that of the oestrus sow's cervix, stimulating ejaculation.

When the penis is 'locked' in the hand and the boar relaxes, a four-phase ejaculation follows in a few seconds, taking 5 to 10 minutes to complete.

The first phase, called the pre-sperm fraction, has clear seminal fluid, some gel and dead sperm cells and is heavily contaminated with bacteria. It should not be collected.

The next phase is the sperm-rich fraction, easily recognized by its creamy-white colour. Although only 50 ml in volume, it contains the greatest density of spermatozoa. Because spermatozoa are very sensitive to rapid temperature change, a warm, dry, insulated collecting flask is required to safeguard semen fertility.

The third fraction, greyish because of lower density of spermatozoa, accounts for about 80 ml of the collection.

Fractions two and three only are collected when semen is to be diluted for storage over a few days.

The fourth phase or post-sperm fraction provides the large semen volume peculiar to pigs. Up to 250 ml of clear seminal plasma free of spermatozoa plus gel is secreted from the accessory glands.

The gel portion apparently has no physiological significance. It is separated from the collection by several layers of gauze or a similar filter fastened over the collection flask. Filtering particles of gel from the semen prevents catheter blockage during insemination. Hair, skin or dust particles from the boar or dummy must also be excluded from the flask during collection.

Large amounts of gel signal the end of ejaculation. When it is clear the boar's erection is fading, a second ejaculation can be stimulated with brief, firm, pulsating hand pressure applied to his penis.

Figure 18 semen collection in pig

Semen collection in buck

The collection of buck semen is accomplished, most commonly, by the use of an artificial vagina (AV). To assist in the buck's collection, a "teaser doe" (doe showing evidence of estrus) is restrained so that the donor buck becomes aroused and can mount her. Once he mounts the teaser doe, an AV is placed over the penis as it is extended. The stimulation provided by the AV's warm water bladder, lubrication, and pressure produce an ejaculation.

Figure19 semen collection in ram

LO 2.3-Process sperm and semen

- **Topic 1: Semen evaluation:**

1. **Visual Examination of Semen**

✓ Volume ranges between 1 to 6 ml.

✓ Gross appearance should be creamy white, which is indicative of high sperm concentration.

✓ Free from contaminants including blood, urine, dirt, or pus.

2. **Motility**

✓ Gross motility - mass swirling movement of semen.

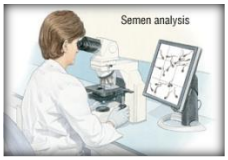
✓ Individual motility - strong forward progressive movement as a percentage of the sample. Sample diluted with physiologic saline or sodium citrate. Numerous factors can influence motility including

fractionation of semen sample, presence of urine, environmental insults, inadequate dilution techniques and temperature.

- ✓ Because so many different factors can influence motility, it is the least predictable, and therefore carries less influence on the final outcome of the BSE.

Gross Motility	Rating	Individual
Rapid swirling	Very good	≥ 70%
Slower swirling	Good	50 - 69%
Generalized oscillation	Fair	30 - 49%
Sporadic oscillation	Poor	≤ 30%
Minimum recommended motility is 30% or Fair for BSE.		

Figure20 microscopic semen examination

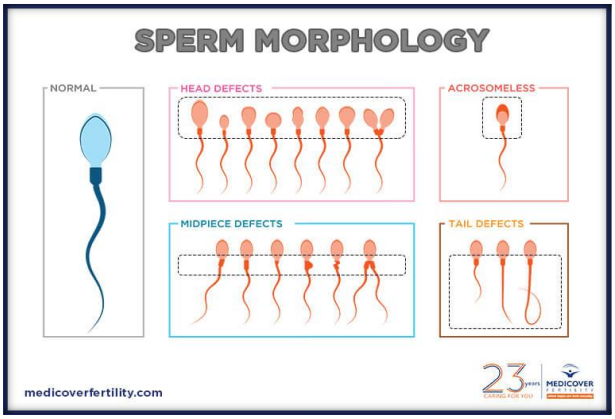


3. Morphology

Normal sperm have oval head shapes, an intact central or "mid" section, and an uncoiled, single tail.

- ✓ 800 - 2,000 million sperm/ml of ejaculate or 5 - 15 billion sperm/ejaculate. Determined by evaluating stained semen smears under a microscope.
- ✓ Minimum recommended normal sperm in an ejaculate is 70% to pass a BSE.
- ✓ Sperm abnormalities

Figure21 semen morphology



4. Density

Density may be classified as follows:

- ✓ Very Good (VG): creamy, grainy semen with 750 to 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.

Normal value of parameters evaluated for collected semen

Parameter	Normal Values
Ejaculate volume	5 ml (range 1-15 ml)
Sperm concentration	1200 million/ml (range 300-2500 million/ml)
Total sperm per ejaculate	Typically 4-5 billion
Progressive motility	Greater than 30%
Morphology	Greater than 70% normal

5. Semen acid-base balance (pH)

The pH of semen is measured using a specially treated paper blot that changes color according to the pH of the specimen that it is exposed to. The pH of normal semen is slightly alkaline ranging from 7.2 to 7.8.

- **Topic 2: Semen dilution**

a. Semen dilution:

The ejaculate is diluted with an appropriate medium (extender) to begin the cooling process. Besides the development of semen storage technology, the quality of semen extender is also a key point of artificial insemination. Good semen extender should provide **nutrients, protects the sperm cells from cold shock and osmotic shock during the chilling and shipping process, and inhibit bacterial growth**. The macromolecules are typically provided by including yolk from hens' eggs or heat-treated whole milk. This is why frozen thawed semen appears either yellow or “milky.”

- 1ml original semen of a bull can be diluted to 65 folds of its volume.
- Equine semen can be diluted up to 40 folds,
- Swine semen can be diluted up to 40 folds.

b. Cooling

In order to extend the fertile life of the sperm, temperature is reduced to reduce the metabolic rate.

A rule of thumb is that metabolic rate doubles for every 10 degrees, so cooling semen from body temperature of about 39 C to refrigerator temperature of 4 C reduces the metabolic rate to about 1/10 of that at body -temperature.

Unfortunately, bull sperm are sensitive to cooling to refrigerator temperature, and especially sensitive to rapid cooling. Egg yolk and milk permit a faster cooling rate with reduced damage, but do not prevent damage altogether.

c. Addition of the Cryoprotective Agent – Glycerol

Once semen is partially extended and cooled to 4 C, a final extension with medium containing glycerol is required. Glycerol is required to protect the sperm during the freezing process.

- **Topic 3: Semen Packaging**

After glycerol is added, extended semen is equilibrated for a few to several hours at 4 C.

During this period, semen is packaged in 0.5 ml or 0.25 ml plastic straws.

Straws are then positioned in a freezer or liquid nitrogen tank and frozen in liquid nitrogen vapor at an optimal rate selected to minimize freezing damage to the sperm.

Straws of frozen semen are then stored in liquid nitrogen until needed.

Freezing process

Cryoprotectant is added to the semen to control sperm damage caused by freezing. Cryoprotectant are low-molecular-weight and highly permeable chemicals used to protect sperm from freeze damage by ice crystallization.

There are two cryopreservation techniques:

- **Slow freezing**, which involves progressive sperm cooling over a period of two to four hours in two or three steps. The specimen is then plunged into liquid nitrogen at minus 196 degrees Celsius.
- **Rapid freezing** requires direct contact between sterile straws holding the samples and nitrogen vapors for eight to 10 minutes, followed by immersion in liquid nitrogen at minus 196 degrees Celsius.

Information appears on a straw of semen

a) Collection code – Date of semen collection

b) Name of bull – Full registration name

c) Bull registration number consisting of:

- Breed of bull
- Country of origin
- Unique herd book number

d) Stud code – Codes the semen collection center

e) NAAB code of bull, which consists of the following:

- A marketing code (sometimes the same as the stud code) – What kind of semen is in the straw and whose product is it (up to three numbers)
- A breed code – What breed of bull is the semen from (two letters)?
- A bull unique number (up to five numbers)

f) The CSS logo – for CSS certified semen.

The CSS logo is not mandatory. If a purchased semen straw does not contain a logo, then it's recommendable to ask your semen sales rep if the semen is CSS certified to ensure it's controlled. The other information is mandatory under CSS regulations

i. Transfer and Storage

Frozen semen is transported and stored in liquid nitrogen tanks designed specifically for this purpose. Frozen semen is stored in liquid nitrogen at -320^{0}F (-196^{0}C) or in LN vapor at about -292^{0}F (-180^{0}C). 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.

Learning Unit 3 – Apply artificial insemination techniques

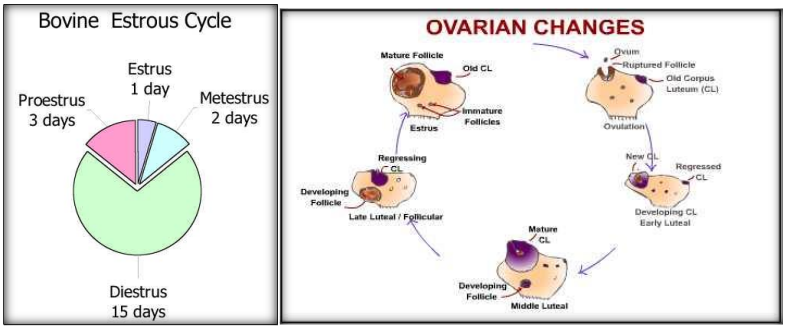
LO 3.1-Detect heat

- **Topic 1: Heat cycle for: Cows, doe, ewe, sow**

The estrous (heat) cycle can be divided into four periods: proestrus, estrus, metestrus, and diestrus.

- ✓ **Proestrus** is the period when progesterone declines with regression of the CL,estrogen increases, and secondary signs of estrus begin to occur.
- ✓ **Estrus** is characterized by standing behavior (true heat).
- ✓ **Metestrus** begins immediately after estrus when ovulation and early development of the CL occur, a period that lasts three to five days.
- ✓ **Diestrus** is the time when the CL is functional, the longest phase of the estrous cycle.

Figure22 estrous cycle and ovarian change



Heat signs for: Cows

1. **Primary sign**

- A cow standing to be mounted is the most accurate sign of estrus. *Standing heat* is the most sexually intensive period of the estrous cycle. During this period, cows stand to be mounted by other cows or move forward slightly with the weight of the mounting cow. Cows that move away quickly when a mount is attempted are not in true estrus. In order for standing behavior to be expressed, cattle obviously must be allowed to interact.
- The average duration of standing heat is 15 to 18 hours, but heat duration may vary from 8 to 30 hours among cows.
- An estrous cow usually stands to be mounted 20 to 55 times during her estrous period. Each mount lasts three to seven seconds.

2. **Secondary signs**

Secondary signs vary in duration and intensity. These signs may occur before, during, or after standing heat and are not related to time of ovulation. Farmers should use these signs as clues or watch the specific cow more closely for standing behavior.

- Mounting other cows
- Mucus discharge
- Swelling and reddening of the vulva
- Bellowing, restlessness, and trailing
- Rubbed trailhead hair and dirty flanks
- Chin resting and back rubbing
- Sniffing genitalia
- Head raising and lip curling
- Decreased feed intake and milk yield
- Metestrous bleeding

Some cows and most heifers have a bloody mucus discharge one to three days after estrus, but onset of this symptom, called metestrous bleeding, is quite variable. High estrogen levels during estrus cause blood to leak from vessels near the surface of the uterus. This discharge indicates that the cow was in heat and does not mean that she failed to conceive. However, such animals should be watched closely for a return to heat in 18 or 19 days.

- **Topic 2: Heat sign in doe, ewe, sow**

a. Signs of Heat in doe

Some does show little or no signs of estrus, a phenomenon known as silent heat. It's a fact about goats that most does show some signs, but each has different signs or different combinations of signs. Along with the length of each doe's estrous cycle, note the signs she displays so you will know what to look for in the future. Here are ten ways to recognize goat heat:

- ✓ **The doe gets talkative:** Most goats don't make much noise, but a doe in heat may vocalize more than usual.
- ✓ **The doe wags her tail:** A doe in heat usually wags her tail, like a dog, a behavior known as flagging. She may willingly let you handle her tail, while at other times she'll take exception to any attempts to touch her tail.
- ✓ **The doe's personality changes:** Raging hormones can cause a doe's personality to change. A normally submissive doe may become aggressive toward her sisters, while a normally aggressive doe may allow other goats in the herd to boss her around without resisting.
- ✓ **Her tail gets sticky:** The area under the doe's tail may become red, swollen, and wet with a gel-like vaginal discharge.
- ✓ **Milk volume changes:** If you are milking a doe that comes into heat, she may resist getting on the milk stand.
- ✓ **Your does act bucky:** If no buck is present when a doe comes into heat, she may mount other does in the herd or allow them to mount her. When other does notice her unusual odor and try to sniff at her tail, like a buck would, she may lift her tail to accommodate them.

- ✓ **The doe urinates often:** An interesting fact about goats is a doe in heat usually urinates more often than usual.
- ✓ **The buck acts goofy:** When a doe comes into heat while she's with a buck, or if a buck is housed nearby, you will have no doubt the doe is in heat. The buck will wag his tongue, slap a front hoof against the ground, urinate on his own face, and otherwise act the fool. If the buck cannot see the doe, he will go into his goofy routine when he smells the doe's odor on you.
- ✓ **The doe stands for mating:** A doe that is not in standing heat will move away from a buck that tries to mount her, while a doe in standing heat will remain still while the buck mounts her, or may urgently push her body against his.
- ✓ **The buck rag trick:** If no buck is close by, you might trick the doe into displaying signs of goat heat by using a buck rag. Rub a piece of cloth on the forehead of a mature buck, then place it in a sealed container. When you open the container in front of a doe in estrus, she will show clear signs of excitement

b. Cycles and Heat Determination in sow

- Sows and gilts have an average 21 day heat cycle, although this can range from 17 to 25 days. An average animal in heat today will be in heat again in three weeks.
- Gestation length of the pig is 114 days, so average farrowing will occur 114 days after breeding. An easy way to remember this is "3 months (90 days), 3 weeks (21 days), 3 days" (90 + 21 + 3 = 114).
- After weaning, sows will come into heat again as quickly as 3 days but 4-7 days is more typical with an average return to heat of 5 days after weaning.

Signs of heat

- **Unusual Noise and Activity**: Sows and gilts that are coming into heat may "chant" or make other unusual noises. You may hear squealing as animals in heat attempt to ride other sows. Watch for animals that sniff, nuzzle or ride other sows or walk the fence line. Many animals show less interest in feed when they are coming into heat. Nearly all sows and gilts show increased interest in boars as they are coming into heat.
- **Reddening and Swelling of Vulva**: Swelling of the vulva is perhaps the most easily recognized physical sign that an animal is coming into heat. In many animals the vulva swells and reddens 2 to5 days before she is ready to breed. Some sows (and gilts) show little or no swelling. To detect heat in these animals, compare the appearance of their vulva with those of other sows and gilts.
- **Engorged Clitoris/ Sticky Mucous Discharge**: When a female is in standing heat, the clitoris is engorged with blood, causing it to protrude outward and have a bright red color. The lining of the vulva may also be red at this time. When females are not in standing heat, the clitoris is flat and has the same light pink color as the lining of the vulva
- **Roaring**: Some sows and gilts "growl" or "roar" when they reach standing heat. A sow or gilt that gives repeated deep-throated growls should be checked immediately for standing heat.
- **Signs of Standing Heat**: Once signs of heat are noticed, the sow or gilt should be checked morning and night for standing heat. This is best done after the animal has finished eating. Check for standing heat by pushing down on the animal's loin with both hands, or by sitting on the loin. A female in standing heat will stand still and rigid, and often "push back" by arching her back slightly when weight is applied to the loin. This is an instinctive response that braces her to support the weight of the boar. The classic sign of standing heat is "ear popping," in which the female holds her ears erect with the tips nearly touching when weight is applied to her loin.

c. Heat in sheep

Young ewes generally reach puberty between seven and 12 months of age, although there are differences between breeds, and environmental conditions such as feeding and care also play a role.

Ewes this age are not suitable for breeding immediately, however; they should have a body mass of at least 40kg or two-thirds of adult mass before they are paired for the first time.

Signs of heat

Animals pair during a period of 'ruttishness' (feeling great sexual desire; "feeling horny").

- The ewes are restless,
- Shake their tails repeatedly and
- Have a slightly swollen vulva.

This is followed by a **'breeding rest'**, during which pairing does not take place. These periods follow a cycle controlled by hormones. The cycle is repeated every 14 to 19 days.

- Ruttishness(feeling great sexual desire; "feeling horny") lasts for an average of 27 hours, but can be as short as three hours in the case of young ewes.

Observation of heat

One of the best indicators of ruttishness is the presence of rams among ewes. Ewes on heat seek out rams and stay close to them, repeatedly rubbing their necks or bodies against the rams. Teasers, or rams with aprons, which prevent penetration, can be used to identify ewes on heat.

- Topic 3: Heat detection

a. Estrous Detection Program

- Use your time efficiently, observing for heat when cattle are likely to mount.
- Allow cows to interact, especially during the evening and early morning hours, when most of the mounting activity occurs.
- Even though loose housing systems provide more time for cow interaction, be sure to observe the cattle frequently.
- Move pastured cattle to an area where they easily can be observed. In conventional housing systems, turn cows out twice daily for 20 to 30 minutes. Be sure to turn cows out when time can be spent observing them.
- Avoid scheduling observation periods at feeding time or during the warmest hours in summer.
- Slippery and muddy conditions severely inhibit mounting activity. Provide an area with a good footing surface where cattle are free to interact and where few obstacles hinder movement.
- Moving cattle to a separate area for heat detection may stimulate estrous behavior.
- When cows have sore feet and legs, heat detection is more difficult. Minimize this problem by trimming hoofs periodically, and treat infected feet as soon as a problem is apparent.
- When several people are working with the herd, assign one person to be responsible for heat detection, and allow time for employees to do the job properly. Train employees to recognize signs of heat and promptly report this information to the responsible person
- Record all heats, whether the animal is to be inseminated or not. Heat detection will improve if future heats can be anticipated. Use a pocket notebook to record heats and other information.

Transfer information to a heat expectancy chart and to the permanent individual cow record. This permits monitoring of abnormally long cycles and long intervals from freshening to first service.

- Consider using heat detection aids to help increase the number of heats detected. Detection devices and detector animals should supplement routine visual observation.
- Isolate the cow thought to be in heat with a sexually active cow or heat-detector animal. Heat may not be detected in some cows in a large group situation, but when isolated with an active cow or heifer, a cow possibly in heat may exhibit standing behavior.
- Watch for sexually active groups of cattle. Cows in proestrus or estrus tend to congregate and stay together.
- Adjust the feeding program so that cows calve in proper body condition and weight loss is minimized during lactation.

b. Estrous Detection Aids

- **Records**

No matter which record system is used, the information should be posted and available to all farm employees.

- **Heat expectancy chart**

Special calendars are available from artificial insemination organizations. Most charts are organized on a 21-day cycle so that future heats can be anticipated. Some herd managers mark day 19 following insemination so that the expected heat can be anticipated several days in advance.

- **Computer generated action lists**

Some dairy management computer programs can generate listings of cows that require special attention or action on a specific day. Action lists indicate cows to watch closely for return heats or cows that have not yet been observed in heat.

- **Prostaglandins**

If more than one animal is in proestrus or estrus simultaneously, mounting behavior increases and standing behavior is more likely to be observed. Depending on herd size, it may be worthwhile to inject one or more cows with prostaglandin at various intervals during the week to induce more estrous behavior in the herd.

- **Vasectomized or surgically altered bull**

A vasectomized bull or a bull with a surgically altered penis can be an effective heat detector. With either method of preparing a bull, the animal will seek out cows in proestrus and estrus and possibly stimulate the overall estrous activity in the herd.

- **Testosterone-treated female**

Testosterone, a male hormone, causes increased sexual aggressiveness when injected or implanted into cows or heifers. Potential non lactating cull cows or heifers, even freemartin heifers, are candidates for masculinization.

LO 3.2 – Induce and synchronize heat

- Topic 1: Heat induction and synchronization

The problem of anoestrus causes a huge economical loss to the farmers or producers. So, it needs to be solved immediately. The oestrus inducers can grossly be divided into two parts, that is, non-hormonal and hormonal. Non-hormonal treatments include plant-derived heat inducers, mineral supplementation, uterine and ovarian massage, and use of Lugol's iodine. The hormones that are used in oestrus induction are oestrogen, progesterone, GnRH, prostaglandin, insulin, and anti-prolactin-based treatment.

1. Non-hormonal treatment
   - **Plant-derived heat inducers**

Different plant extracts are being used for the treatment of anoestrus traditionally. An examples include Aloes, Heat-Up, Fertivet, Heat-raj, etc. These can be applied in delayed puberty, postpartum anoestrus, and other problems. Feeding the leaves of these plants individually or combined can help in starting the cascade of reproductive cycle

- **Mineral supplementation**

Minerals have an important role in the reproduction of domestic animals, and their deficiency can cause several reproductive disorders. Deficiency of calcium is very common in postpartum cattle. Any alteration in Ca:P ration can affect the pituitary secretion and subsequently ovarian function . This can cause delayed puberty, irregular estrus, etc. The optimum ratio of Ca:P should be within 1.5:1–2.5:1. Excess calcium is also harmful as it can disturb the absorption of other minerals. Phosphorus is a very important mineral for the normal reproduction. In the case of phosphorus deficiency, several disorders can be observed like delayed maturity, low conception rate, inactive ovary, etc. Minerals should be supplemented in optimum quantity.

- **Uterine and ovarian massage**

It is the most economical method for the treatment of anoestrus. In this method, gentle massage of the uterus and ovary is done per rectally. There is no clear mechanism of action of this method. Possibly, it can be attributed to increased blood circulation on the surface of the ovary and stimulation of ovarian intrinsic factors

Application of this method needs experts who have a good idea about the anatomy of female reproductive system.

- **Lugol's iodine**

Intrauterine application of Lugol's iodine can effectively induce estrus in cattle, buffalo, etc. A dose of 20–30 ml is sufficient for treatment. It also shows a good conception rate with cost-effectiveness. It actually

acts as uterine irritant and increases blood supply there. It can also stimulate the hypothalamus for the secretion of GnRH, and thus the reproductive cycle is regained

2. **Hormonal treatment**

- **Estrogen**

Estrogen is a very important hormone for the reproductive cycle of the animals. Administration of estrogen can help the animal to come into estrus , though it may be ovulatory or anovulatory. If a dominant follicle is present in the ovary, there will be ovulation. If no dominant follicle is present, it can be anovulatory. Estrogen promotes the ovulation through LH surge as estrogen shows a positive feedback effect toward the pituitary at this time. Use of estrogen is limited nowadays due to its side effects. Prolonged administration of estrogen can cause cystic ovary, peristalsis of the oviduct, etc. These can also lead to several infections like ovaritis, adhesion, etc.

- **Progesterone**

Progesterone is secreted from the corpus luteum in a normal estrus cycle. With the decrease in the progesterone level, the follicles start growing. The same situation can be mimicked externally. Progesterone can be administered externally for certain duration, and its withdrawal can cause induction of estrus.

- **GnRH**

GnRH and its analogues can be successfully used to induce estrus in animals. It induces ovulation, if mature follicle is present by inducing the LH surge. GnRH can improve conception at the timed artificial insemination (Al) after estrous synchronization with prostaglandin F2α.

- **Prostaglandin**

For persistent corpus luteum and sub estrus, PGF2α is the treatment of choice. Successful management of silent estrus in cattle and buffaloes can be done by the natural or synthetic analogue of PGF2α as a single dose with a reasonable degree. PGF2α is only effective between days 6 and 16 of the cycle and in the presence of active corpus luteum. Administration of 25 mg of natural PGF2α intramuscular or 250–500 µg of synthetic ones is required to regress the CL in both cattle and buffaloes.

- **Insulin**

The recommended dose is 0.25 IU/kg body weight subcutaneously for 3–5 days. Insulin enhances the follicular growth in true anestrus buffalo which is a prerequisite for GnRH to be effective

- Topic 2: Oestrous synchronization methods and techniques

The manipulation of the estrous cycle or induction of estrus brings a large percentage of a group of females into estrus at a short, predetermined time. One of the advanced managemental processes through which the humane errors and managemental costs could be minimized is synchronization of estrus. It is predominantly useful in large herd of cattle. It helps in fixing the breeding time within a short predefined period and thereby scheduling the parturition time at the most favorable season in which newborns can be reared in suitable environment with ample food for augmenting their survivability. As timely breeding of the animals is possible with this technique, fertility in farm animals may be expected toward the upper side.

Synchronization can shorten the breeding period to less than 5 days, instead of females being bred over a 21-day period, depending on the treatment regimen. Production of a uniform group of calves for the future replacement in the animal farm is another important benefit of this program. The current and future aspect of estrous synchronization is to focus on combining traditional methods of controlling cycle length with the follicular development manipulation.

Basic approach for estrus synchronization

To control the timing of the onset of estrus by controlling the length of the estrous cycle is the basic approach for the estrus synchronization. Various approaches for controlling cycle length are as follows:

- Prostaglandin administration to regress the corpus luteum of the animal before the time of natural luteolysis
- Progesterone or synthetic progestin administration to suppress ovarian activity temporarily

- Creating estrous synchrony by using gonadotropin-releasing hormone or an analogue, which causes ovulation of a large follicle, helps in synchronizing estrous cycle in anestrous female.

Methods of estrus synchronization

- **<u>Prostaglandin treatment</u>**

Luteolytic agent such as prostaglandin F2α, or an analogue, which causes the regression of the corpus luteum can be used to synchronize estrus. Administration of PGF2α is only effective from 8 to 17 days of the estrous cycle when functional corpus luteum is available in one of the ovaries. Fertility is high after prostaglandin synchronization. Synchronization of estrus and fertility with this product are good in cyclic females but not in non-cycling cows.

1. One-shot prostaglandin: In this method a single injection of prostaglandin is given to cyclic females, and then these females are bred as they express estrus.
2. Two-shot prostaglandin: In this method two injections of prostaglandins are given at an interval of 10–14 days. Once the stage of estrous cycle in the cows is unknown and detection of estrus is not required before or between injections.

- **<u>Progesterone treatment</u>**

High levels of progesterone in the female's system are maintained with the help of progestogens even after the regression of the corpus luteum. After the progestin removal, synchrony of estrus occurs up to 2–5 days. Melengestrol acetate (MGA) (oral feeding), Syncro-Mate-B (SMB) (ear implant), and CIDR (intravaginal device) are the commercial products which fall into this category.

Techniques of progesterone treatment

- ✓ MGA feeding: MGA is added to feed such that females received 0.5 mg/head/day for 14 days and if MGA is administered, cyclic females begin to show estrus. This estrus is sub fertile, and it is recommended that females should be bred on the second estrus following MGA removal.
- ✓ Syncro-Mate-B (ear implant) treatment late in the estrous cycle (>14 days) in cow gives lower conception rates. The ideal time for SMB treatment to begin is between the 8th and 12th day of the estrous cycle to maximize estrus response
- ✓ Application of CIDR: The CIDR is easily inserted into the vagina and has good retention capacity (2.5% loss rate is normal); a flexible nylon tail is attached to it for easy removal. The CIDR provides an exogenous source of the progesterone, and its removal on treatment day 7 results in a rapid fall in plasma progesterone levels, which results in estrus synchronization in those animals responding to treatment.

- **GnRH-based treatment**

Estrus synchronization and fertility with a combination of GnRH and prostaglandin F2α are good for cyclic females, and this combination may induce cyclicity in cows experiencing postpartum anestrus.

Administration of GnRH during the estrous cycle in bovines causes regression or ovulation of the dominant follicle and initiates the emergence of a new wave of follicular growth.

Ovsynch, CO-Synch, Select-Synch, and Hybrid-Synch are the four systems for synchronization of estrus with GnRH-PG combinations.

At day 1 GnRH injection is used to program follicle growth in cyclic females and to induce ovulation in anestrous females, and PGF2α on day 8 induces regression of CL that is present to cause a decline in progesterone.

Then on days 10–11, the second GnRH is given which induces ovulation of dominant follicles that have been pre-programmed by the first GnRH treatment.

The major GnRH programs that do not involve use of the CIDR are described as follows:

1. GnRH-PGF system: This represents the simplest GnRH-based system. A common name for this system is "Select-Synch." In this system a single dose of GnRH and prostaglandin is injected on day 1 and day 8, respectively. Some cows (8%) exhibit estrus up to 48 hours before PGF (day 6). The early estrous are fertile and cows can be inseminated 12 hours after detection. The peak estrous response occurs 2–3 days after PGF with a range of 1–5 days. With this system, a minimum of 5 days of estrous detection after PGF and 2 days prior PGF is required to detect most heats.

2. GnRH-PGF + GnRH system: This system is a GnRH-PGF system in which second GnRH injection is given to all or some cows between 48 and 72 hours after PGF (days 2–3), with timed AI on all or a portion of the herd.

- ✓ In Ovsynch program, an injection of GnRH on day 1, an injection of prostaglandin on day 8, a second injection of GnRH on day 10, and then a timed insemination on day 11 are given
- ✓ In CO-Synch program, an injection of GnRH on day 1, an injection of prostaglandin on day 8, and then a second injection of GnRH with breeding on day 10 are given.
- ✓ . The Hybrid-Synch program is applied with an injection of GnRH on day 1, an injection of prostaglandin on day 8, and then estrous detection and breeding from day 8 to 11. Second injection of GnRH was given to the females which were not observed in estrus from day 8 to 11 and were bred on day 11.

LO 3.3 – Practice artificial insemination

The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception. Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates.

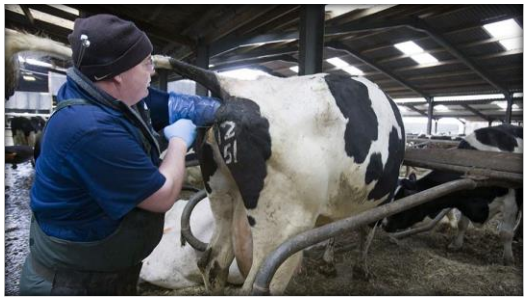
- Topic 1: Artificial insemination methods

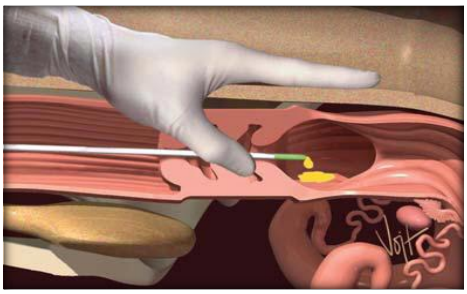
A. Recto vaginal method

In cattle the safe and best method of insemination is "Recto vaginal method of insemination". Cow which is in heat is well controlled placing it in a Travis. The inseminator will get ready by wearing a plastic apron, gumboots and gloves. The semen straw after thawing (keeping the semen straw in warm water for a minute to convert the freezed semen into liquid and the sperms become motile) is loaded in a sterilized A.I. gum and is covered with a plastic sheath.

The inseminator will insert the gloved left hand into the rectum after applying the soft soap or other lubricant on the glove and back racked the animal, and the hand is further inserted and will catch hold the cervix through rectal wall. The A.I gum loaded with semen straw is passed.

Figure24recto vaginal insemination





B. Speculum method

In this method spectrum is placed in the vagina of the cow, which provides passage outside to the site of insemination, then inseminating tube is passed through the speculum and semen is deposited at the cervix.

C. Vaginal method

Hand is passed through the vagina and the inseminating tube is guided by hand to the site of insemination and semen is deposited. Here there is a risk of contamination and injury of female genitalia.

Steps of artificial insemination in cow

1. **Positioning the cow**

The cow should be appropriately restrained.

Figure25 positioning cow

2. **Thawing the straw**

Before thawing the straw, check the water temperature; it should be at 35C, or as instructed by the semen company.

Particular care should be taken with sexed semen; for optimum results it may require slightly longer and warmer thawing temperatures.

The straw should be removed from the flask with forceps and submerged in the water. Leave it in for 20-30sec for a 0.25ml straw and 40sec for a 0.5ml one.

After withdrawal, wipe it dry and place it in the gun, which should have been pre-warmed by rubbing between the hands. Only thaw one straw at a time.

Cut the crimped straw end at a 90deg angle, then slide on to the plastic sheath and secure with the collar. Hold the gun vertically and gently press the plunger upwards, until the semen rises to the top. The gun is now primed.

Figure 26 thawing semen

3. Preparing the cow

Clean the cow's vulva with a paper towel and put on a full-arm glove and lubricant.

Insert your arm into the cow, by forming a cone with your fingers while keeping the tail aside with your other hand.

Gently work out any excess dung and if the rectum becomes distended with gas or the cow strains excessively, withdraw the arm and consider re-serving a few hours later.

The cow must be relaxed during the procedure to avoid injury, as the rectum wall is a delicate structure.

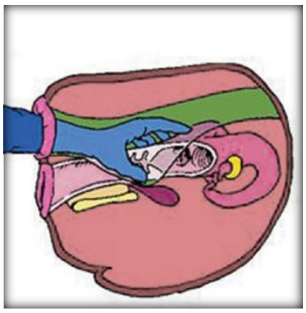
If the bladder is full, wait and try again once the cow has urinated.

4. Finding the cervix

The initial landmark is the cervix and this should be located before inserting the gun.

The cervix is normally found on the pelvic rim, but in older cows, it may have moved slightly to one side.

Figure: 27 fixation of cervix



5. Inserting the AI gun

To avoid the opening to the urethra, run the gun tip gently along the roof of the vagina, until the cervix is reached. The gun should be inserted almost vertically and finish horizontally.

The gun should be inserted as soon as possible after priming, to preserve semen quality. Insemination is a two-stage process:

- Guide the AI gun, so that it is engaged in the cervix
- Pass the cervix over the AI gun.

6. Lining up the AI gun

Line up the gun with the cervix and pass it through the canal, manipulating the cervix back over the tip of the gun.

7. Reaching the cervix

Once the gun is just through the cervix, you should feel a release in resistance to the gun.

The semen should be deposited into the short chamber of the uterine horns, which are located on the other side of the cervix.

If you put your index finger over the end of the cervix, you will feel where the top pokes through and this is the area where the semen should be deposited.

Deposit the semen slowly, by counting 5,4,3,2,1.

8. Maintaining equipment

Do not reuse sheaths; splitting straws increases the risk of disease spread and can reduce pregnancy rates.

Soiled insemination guns should be cleaned after use and treated with boiling water every few weeks. Disinfectant can damage sperm.

Use clean protective clothing and a fresh disposable glove for each insemination.

- Topic 2: artificial insemination in ewe doe and sow

a. AI in ewe

An artificial insemination pipette with a 1–2 ml syringe attached is placed deep into the vagina. This method is quick and involves minimal restraint of the ewe. For cervical insemination, the ewe is restrained to limit movement and to present the hindquarters at a convenient height for easy access to the vagina. After cleaning the vulvar region, the cervix is located with the aid of a speculum and suitable illumination, and the insemination made as deeply as possible into the cervical canal. A long, thin inseminating tube with attached syringe or a semiautomatic inseminating device can be used.

Figure 28:insemination in ewe

b. Artificial insemination in doe

- The first step is to restrain the doe to be inseminated. This can be done with a breeding stand or any other satisfactory facility.
- After the doe is restrained, the semen is thawed and the insemination gun is prepared
- dry the straw thoroughly with a paper towel. Semen must be kept warm and must not be exposed to sunlight or water during the thawing and inseminating process to prevent damaging or killing sperm cells.

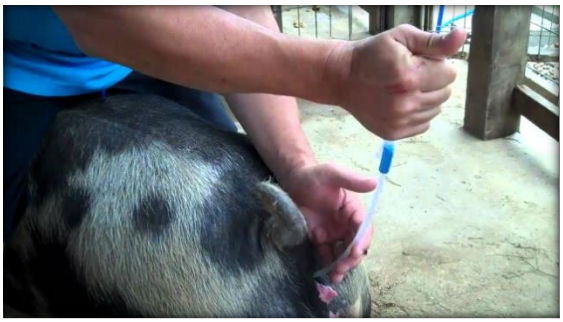
- Pull the plunger back 4 to 6 inches on the insemination gun and place the straw into the gun with the cotton plug toward the plunger.
- After the straw has been secured in the gun, the sealed end of the straw must be cut off with the straw cutter. The cover sheath should now be placed over the insemination gun and secured with an O ring.
- The next step is the actual insemination process. It may be necessary to lift the doe's hindquarters if she will not stand
- Lubricate the speculum with a non-spermicidal lubricant.
- Clean the doe's vulva with a dry paper towel and insert the lubricated speculum slowly into the vulva. Insert the speculum at an upward angle to prevent vaginal irritation.
- Once the speculum has been inserted, visually locate the cervix. The cervix should have a red-purple color, and white mucus will be present if the doe is in heat. Center the speculum over the opening of the cervix
- Insert the insemination gun into the speculum and thread it into the opening of the cervix.
- Use a circular motion and slight pressure to work the insemination gun through the rings of the cervix. Do not penetrate the cervix more than 1.5 inches
- Deposit the semen slowly by pushing the plunger forward.
- Remove the insemination gun slowly and remove the speculum.
- Record all important information in a breeding journal.

c. <u>Artificial insemination in sow</u>

- The first thing you'll do is clean around the vulva with the damp paper towels. Make sure that you DON'T put soap on the paper towels, only water. The soap will kill sperm cells.
- Put a generous amount of the lubricant on the end of the AI rod. This helps the rod go smoothly into the reproductive tract.
- insert your AI rod at a slightly upward angle to avoid the urethra.
- Once the rod is in, you'll gently push the rod until you hit the cervix
- you will gently push the rod into the cervix. You will know that you have entered the cervix properly when you gently pull back on the rod and feel slight resistance.
- Once the rod is in place, you can attach the bag of semen to the end of the rod.
- If your sow or gilt is in heat, her reproductive tract will begin contracting and will pull the sperm into her body.
- Once the bag is empty, you can remove the bag, leaving the rod in place. You want to give the sperm in the rod time to leave the rod and empty in to her.
- The tube of the rod is clear, so you will be able to see if there is fluid or air in the tube. Once you can no longer see sperm in the end of the AI rod, wait another minute or two to make sure that the entire rod is empty.
- If you used a foam tipped rod, you can gently pull it back out of the cervix and pull it out. If you used a corkscrew rod, you can twist it clockwise to remove it from the cervix. You will feel slight resistance as you back it out of the cervix. Once it feels like it is out of the cervix, you can gently remove it.

Artificial insemination in pigs really isn't complicated. It also offers so many benefits that you can't get with natural breeding.

Figure 29: insemination in saw



LO 3.4: Make follow-up

- Topic 1: Pregnancy diagnosis methods

The assessment of pregnancy diagnosis can be conducted at varying intervals after service. The methods used can have varying degrees of accuracy. However, pregnancy diagnosis only gives an assessment at a particular point of time, after which undetected loss of pregnancy may occur and give a false result. The various methods of assessing the pregnancy status are:

- ✓ failure to return to heat;
- ✓ manual rectal palpation;
- ✓ hormonal changes;
- ✓ Outward changes to appearance.

1. Failure to Return to Heat

Observation for service returns tends to be concentrated around the normal estrous cycle length of 18-24 days. The absence of a return to estrus at this time or around 42 days after service may indicate that the cow is pregnant. However, this method has inaccuracies in that:

- ✓ It is dependent upon efficient and accurate heat detection;
- ✓ It assumes that non-pregnant cows return to heat at regular intervals;
- ✓ It assumes that cows with a cessation of estrous cycles are pregnant. Up to 7% of pregnant cows can show signs of heat, usually between the fourth and eighth month of pregnancy.

These cows (false negatives) could abort following repeat insemination or may be unnecessarily culled.

2. Manual Rectal Palpation

This has been the standard method of pregnancy diagnosis for many years, and is still a valuable exercise. It is conducted through rectal palpation and provides a safe and accurate diagnosis based on changes to the uterine tract involving assessment of:

- ✓ the size of the uterus;
- ✓ any discrepancy in the diameter of the uterine horns;
- ✓ Blood flow to the uterus and the presence of fluid, membranes or cotyledons.

Rectal palpation can be carried out from around six weeks onwards

While pregnancy diagnosis through rectal palpation is generally very accurate, error is possible in some cases. Examples of this include:

- ✓ large cows that have had a number of calves and whose uterus has grown out of reach;
- ✓ cows inseminated early after calving and whose uterus has not fully involuted or shrunk may appear to be pregnant;
- ✓ A uterus filled with pus or mucus could be mistaken for a pregnancy.

3. Hormonal Changes during Pregnancy in Cattle

Pregnancy can be diagnosed by monitoring changes in the concentrations of various hormones, mainly progesterone, but also early pregnancy factor (very early pregnancy) or estrone sulfate (late pregnancy).

Progesterone: This can be tested in milk samples with the test being relatively simple and easy to use. Progesterone concentrations increase and decrease during the course of the normal estrous cycle

A milk sample taken 21 days after insemination can be used an early indicator of pregnancy status, based on the concentration of progesterone it has. If a cow has not become pregnant, concentrations of progesterone fall from around day 16 of the cycle and are low at day 21, while in a cow that has become pregnant, concentrations remain high. Therefore, milk progesterone testing at day 21, together with good herd records can be useful and give a fairly high level of accuracy:

- ✓ Low progesterone = Not pregnant (100% accurate);
- ✓ High progesterone = Pregnant (85% accurate).

Approximately 15% of cows with high progesterone concentrations may not be pregnant as a number of factors can influence its concentration, including:

- ✓ False heat detection and incorrect timing of service/insemination, leading to incorrect timing of milk sampling;
- ✓ Persistent corpus luteum or luteal cysts which continue to produce high levels of progesterone (similar to pregnancy);
- ✓ Short inter-heat intervals where the cow may have had a heat within 15 days of an earlier heat, giving rise to high progesterone again 21 days after service;
- ✓ Embryo mortality, where the cow may have conceived but the embryo has subsequently died, and the cow will return to heat. Embryo mortality is estimated to be approximately 6% between 21 and 42 days, and 5% after 42 days.

Estrogen Sulfate: This hormone is produced later in pregnancy (after day 100). A simple blood sample sent to a laboratory will confirm pregnancy if this cannot be ascertained by other means. However, the late diagnosis of pregnancy makes this test less useful for the subsequent treatment of non-pregnant cows.

Early pregnancy factor: Proteins secreted by the embryo at around day 7-8 of its development are a very early indicator of conception, and tests are being developed to test for these and improve their reliability. However, considerable embryo loss can occur before the embryo implants and the cow conceives so it is likely to be of limited use for pregnancy diagnosis.

4. Outward Signs of Pregnancy

In late pregnancy, animals develop various outward changes to their appearance. These include:

- ✓ Increased abdominal size: Occurs particularly in late pregnancy, but this can be difficult to determine in old or fat animals;
- ✓ Udder development: Most obvious in animals during their first pregnancy. Filling of the udder occurs in late pregnancy but other conditions such as mastitis may also cause filling of the udder;
- ✓ Vulval swelling;
- ✓ Slackening at the tail-head ('springing'): Occurs in the last two weeks of pregnancy as a sign of imminent birth.

These changes are too late to be of much value in management, with non-pregnant cows at this stage being generally destined for culling.

Regular pregnancy diagnosis is a key element of dairy herd management and is an important aspect of the routine fertility visit by your veterinary practitioner. Cows should be presented for diagnosis from approximately six weeks after service, allowing early identification of non-pregnant cows and timely remedial action to be taken.

While pregnancy diagnosis by rectal palpation and ultrasound scanning is extremely accurate, approximately 5% of cows in calf at 42 days subsequently lose the pregnancy to late embryo mortality so continued close observation of the herd is necessary.

- Topic 2: Causes of infertility

2.1. Types of infertility

a. Anestrous

- Anestrous is considered as a problem when cows are not seen in heat. Failure to observe heat and heat detection must always be ruled out as the primary problem..
- Other reasons for anoestrous are ovarian aplasia. which is a rare condition when one or both ovaries are absent. It is caused when the gonadal ridge does not form correctly.
- **Ovarian Hypoplasia**: Where one or both ovaries are smaller than normal.
- **Ovarian Atrophy** : Ovarian atrophy is caused from nutritional problems and is most often seen in dairy cows with high production.

- **Mummy :** A mummy in the uterus simulate a pregnancy and prevent the normal luteolytic mechanism. This results in anestrous

b. Non-Infectious Infertility

1. Management Causes

Breeding health can be judged from the conception rate within the herd. High conception rates within the herd can be maintained to certain extent by making adequate provisions for timely services from high fertile bulls over a suitably long period of time to give enough chance to the cows to express their fertility at proper time.

2. Nutritional infertility

Post partum nutrition is most important for fertility. If TDN is low both prepartum and postpartum, fertility suffers. Vitamin A had no effect on fertility, but may cause irregular cycles. Vitamin D deficiency suppresses signs of estrus and delays ovulation. Vitamin E deficiency may cause reproduction problems.

3. Hormonal infertility

a) **Silent Heat** : Silent heat is generally not a problem and usually is manifested by unobserved heats by farmer..

b) **Delayed Ovulation:** If a cow ovulates more than 18 hours after end of heat, then ovulation is said to be delayed. This may be diagnosed by palpation and can be treated with GnRH.

4. Cystic Ovarian Disease or Follicular Cysts :

c) The definition of a follicular cyst is: A fluid filled structure on the ovary greater than 2.5 cm in diameter. The cyst may persist for more than 10 days or regress and be replaced by another cyst. There are two types of cysts, follicular and luteal.

Etiology : It is a hereditary disease. The cysts are caused by a lack of LH surge. The LH may actually be present, but may not be released. This results in no ovulation, minimal luteinization.

5. Repeat breeder

A repeat breeder is defined as a cow that has calved before, is less that 10 years old, has normal heat cycles, has no palpable abnormalities has been bred 3 or more times and is not pregnant

6. Fertilization failure

Release of PGF from inflammatory conditions such as mastitis can cause luteolysis and pregnancy loss. Inability to prevent PGF release causes return to estrus.

. Salpingitis, metritis and cervicitis can cause a change in the uterine environment that leads to infertility.

c. Infectious Infertility

Diseases resulting in bovine reproductive failure can be an infection with a bacterium, virus or parasite

1. Metritis

Inflammation of the uterus is known as metritis. Cows normally have a red-to-brown discharge during the first two weeks after calving. If discharge persists beyond 2 weeks or if the discharge is foul-smelling, this is evidence of metritis. Possible factors involved are retained placenta, injury to the reproductive tract can occur due to a difficult calving or excessive force used to assist at calving. Injuries can also occur at the time of breeding or uterine treatment

2. Pyometra

A pyometra is a uterus filled with pus that has a closed cervix and a corpus luteum on the ovary. The pus prevents the normal luteolytic mechanism from happening. This results in anestrus.

3. Abortions

a. Definition and incidence

Abortion is defined as fetal death and expulsion between 42 (an estimated time of attachment) and 260 days (the age at which a fetus is capable of surviving outside the uterus) of gestation. The condition does not include fetal maceration and mummification. Pregnancies lost before 42 days are usually referred to as early embryonic deaths, whereas a calfthat is born dead between 260 days and full term is defined a stillbirth.

b. Infectious causes of abortion

i. Bacterial

Bacterial abortions result from brucellosis, leptospirosis, campylobacteriosis (vibriosis), listeriosis, Haemophilus somnus complex, and ureaplasmosis. Bacteria like Salmonella, Actinomyces, Escherichia coli, Streptococcus, Staphylococcus, Bacillus, Pseudomonas, Proteus, Pasteurella, Nocardia, and chlamydia species, as determined by the microbiological findings, can cause abortion. All these organisms and few others that are not listed have been isolated from sporadic cases of abortion. These are secondary to either a septicemia in the dam or ascending infection through the vagina and cervix or due to persistent endometritis.

ii. Viral

a. IBR (Infectious Bovine Rhinotracheitis or "Red Nose") :

Infectious Bovine Rhinotracheitis virus is the cause of respiratory disease of cattle. However, in cows and heifers, this virus can also cause vulvovaginitis (inflammation of the vulva and vagina) and abortion. Abortion typically occurs about 20 to 45 days after infection. The control of IBR infections can be accomplished by the use of vaccines.

b. BVD (Bovine Virus Diarrhea) :

Bovine Virus Diarrhea virus infection can cause abortion, weak calves at birth, calves with brain damage (cerebellar hypoplasia) or other abnormalities of fetal development. Clinical signs in newborn calves infected with BVD can include fever, nasal discharge, diarrhoea and inability to move about normally (ataxia).

iii. Protozoal :

Protozoal diseases causing abortion are Trichomoniasis, Sarcocystosis ("Sarcosporidiosis") and Neosporosis.

2. Non-infectious causes of abortion

- **Nutritional:** Starvation may result in placental insufficiency and abortion, however, it rarely occurs in a modern dairy practice. Vitamin A deficiency has been suggested to result in thickening and degeneration of placenta and abortion in late gestation. Iodine deficiency has also been suggested as a cause of abortion.
- **Chemicals, drugs, and toxins :** Toxic agents may also cause abortions or early embryonic deaths. Cattle are susceptible to fertilizer nitrites and nitrates or the nitrates found in plants under certain conditions (e.g. drought-stress). If a cow is exposed to sufficiently high levels of nitrates/ nitrites (-.55 % or greater nitrate in forage), abortions may occur, especially in late gestation.

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