CHAPTER-13

Artificial Insemination –

importance, advantages and disadvantages, semen composition, collection, processing, preservation, storage and transportation, A.I technique, time of insemination, factors affecting semen quality an quantity, conception

Objectives

1. To know the importance of artificial insemination in dairy animals.
2. To understand the process of artificial insemination.
3. To learn the semen evaluation, preservation and AI techniques.

Introduction

Artificial Insemination (AI) is the process by which semen is deposited inside the reproductive tract of the female by means of instruments. AI is the first great biotechnology applied to improve the reproduction and genetics of farm animals. AI is the major tool in the hands of animal breeders to propagate superior germplasm in quicker and efficient way. AI technology is the prime fact behind the success in dairy industry and India's top position in milk production.

Advantages of AI

1) AI with frozen semen allows transportation of semen to any corner of the world instead of purchasing superior bulls; its semen can be procured and taken to any country for improving the genetic of the local animal.
2) In AI, one bull can replace the use of at least 10 bulls. The number of spermatozoa in a single ejaculate is enough to cover 100 females in AI against a single female in natural service. Thus, an outstanding bull can be utilized maximum during his productive life span.
3) AI facilitates accelerated entry of new genetic material in a particular herd and thus an advantageous character is quickly propagated among the individuals in a herd.
4) In the routine examination of bulls under AI programme, it is possible to identify the bulls with poor sex drive or semen quality and helps in culling undesired bulls.

5) During processing of semen for AI, the procedures like filtration and incorporation of additives increases the fertility of semen and such processed semen gives good conception when inseminated. Thus, AI also helps in preventing unnecessary culls of high quality animals due to inferior semen quality.

6) As many contemporary females become available in shorter time, the progeny testing becomes easier and the indexes become more precised.

7) Recto-vaginal method of insemination provides an opportunity to evaluate the reproductive status of the female so that corrective measures can be employed timely.

8) AI may be used to overcome problems that may preclude a female from natural service. In animals with problems like skeletal abnormalities, weakness, laminitis, nervous temperament, natural service is difficult and AI provides an opportunity to breed these female, however, possibilities of inherited defects should be kept in mind while breeding such animals.

9) AI allows cows and bulls isolated due to health restrictions (for example FMD) still to be bred at the planned time, as no direct contact between stocks is required.

10) As the bull is routinely examined for major infectious diseases in AI program, it helps in reducing the transmission of diseases to the females. Further addition of antibiotics to semen extender also reduces the chances of venereal transmission of bacterial diseases.

11) AI also permits use of injured males and also permit more precise use of fixed time insemination

12) Mating of unequal sized animals and interspecies breeding is possible in AI.

13) AI provides employment opportunity to several unemployed youths

Disadvantages of AI

1. Artificial insemination of pregnant female results in abortion.

2. Trained person is required for the process of semen collection, preservation and insemination. Insemination may spread infection from animals to animals, if it not careful in all the steps in maintaining hygiene.

3. Artificial insemination requires special facilities like good laboratory and equipment etc.

4. Sometime there is failure of A.I. may be due to inappropriate time of AI. So, it requires more time than natural mating or services.

5. It requires a person who has sufficient knowledge of the structure and function of reproductive part of female animals.

6. Improper cleaning of instruments and in sanitary condition may lead to lower fertility.

Semen collection

Semen from a bull can be collected approximately 2 – 4 times a week. Various methods of collection of semen have been devised from time to time. The older unsatisfactory methods have gradually replaced by the new modern techniques. From bulls, semen is collected by three methods viz Artificial vagina (AV) method, Electro-stimulation method and by massaging the ampulae of the ductus difference. AV method most commonly used to collect the semen from bulls.

Assembling AV

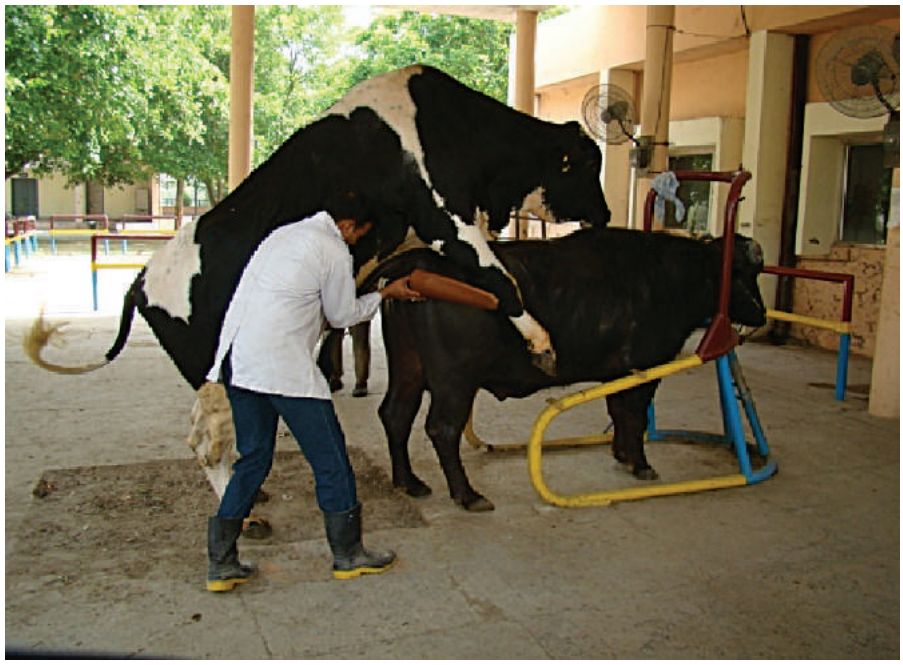
Artificial vagina consists of a hard spongy rubber cylinder or hose, latex liner, neoprine liner or other suitable material which is non toxic, non irritant sterilizable can be used, director cone, collection tube and tube cover. The rubber cylinder is open both the ways and has a ventil at its distal third part on the surface. The ventil is used to pour hot water in the compartment between the latex liner and rubber hose. The ventil is also provided with a valve to pump air. Latex liner or sleeve is used as inner lining of the cylinder, so that a vacant space is created between two components. The sleeve is inverted over both the ends of the cylinder. Latex cone is attached at the distal end of the cylinder over the turned up part of the latex liner. A graduated semen collection tube is attached to the narrow opening of the cone. The size of AV for cattle and buffaloes is 40 x 6.5 cm and 30 x 6.5 cm. respectively. The cone is 10 - 13 cm in diameter at proximal end and at distal end it is 1.5 to 2 cm. The size of AV for zebu bulls is almost similar to buffalo bulls.

All the components of the AV should be thoroughly washed and sterilized before assembling for semen collection. Hot water (45 – 50^0C) is filled through the ventil on the rubber cylinder in the space between the cylinder and latex liner. The temperature of the AV at the time of collection should be between 43 and 48^0C. Sterilized Vaseline is applied over the inner surface of the latex liner up to proximal two third distance. Air is pumped through the valve in the ventil of the rubber hose to construct the inner passage of AV and also to create folds resembling the vagina of cow. All the rubber parts should be thoroughly scrubbed with soap and hot water, rinse with alcohol and distilled water and then dried and stored in dust free cabinet.

Proper sexual stimulation of bulls just prior to semen collection is a pre-requisite for harvesting optimum quality and quantity of semen. A bull or castrated male is convenient

as a teaser and is as good as cow. The main criteria for selection of a teaser are physical strength, appropriate height and immobility. The bull is brought near the dummy and few false mounts are allowed before collecting semen. This practice increases the volume of semen and concentration of sperms. After mounting the sheath of the erected penis is hold in hand and directed towards the AV. Most of the ruminants are thrust ejaculators and their penis is made up of elastic tissues and hence AV temperature is very much essential rather than the pressure inside the AV. Once the semen is ejaculated and the donor dismounts the collection tube is pulled down and the valve is opened to release the air and water. The collection tube should be covered to protect the semen from intense sunlight and fluctuating ambient temperature.

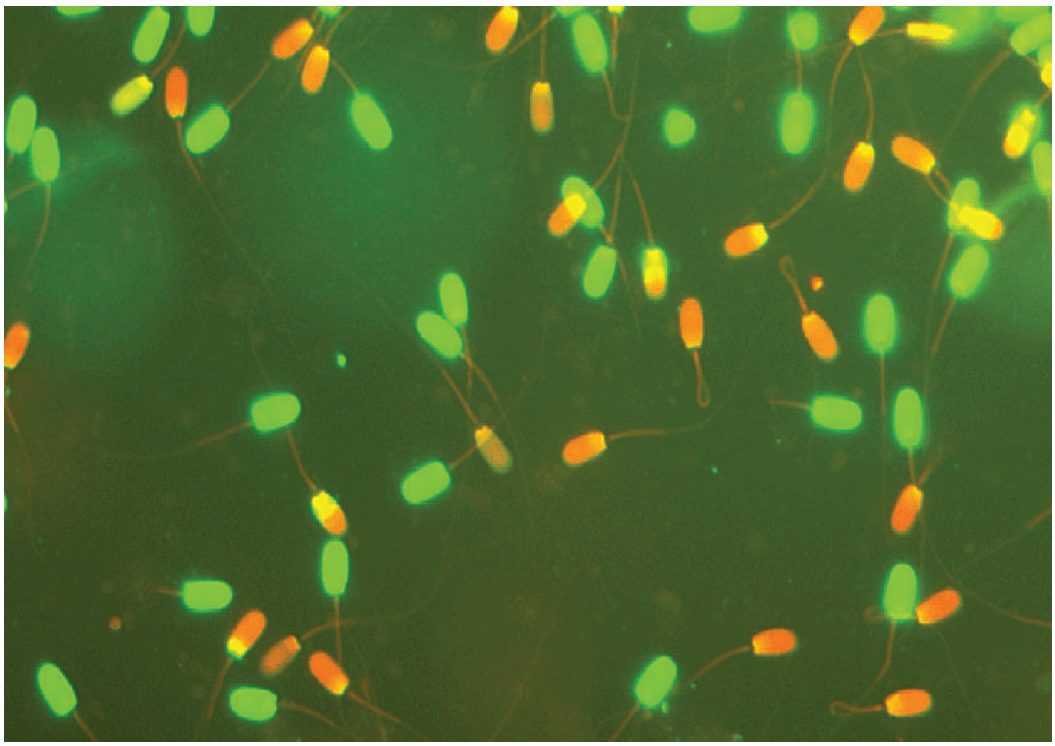
Artificial vagina for semen collection



Semen collection from bulls

Semen evaluation

It is economically and biologically important that only semen with a high fertilizing potential should be used in AI programme. Generally, in most of the semen stations in the country, the ejaculate quality is assessed to find out its suitability for preservation. These tests include the following. Immediately after collection, the ejaculate is examined for colour, volume, mass activity and for contamination if any. Then the sperm motility is estimated using microscope. It is advised to estimate the proportion of live spermatozoa, intact acrosome spermatozoa, membrane intact spermatozoa and sperm abnormality. Nowadays computer assisted semen analyzers (CASA) are commonly used to quantify different motility parameters.



Quality assessment of bull spermatozoa (live spermatozoa – green colour; dead spermatozoa – red)

Semen preservation and storage

Semen is then diluted in an extender which provides an appropriate concentration of spermatozoa, allowing more inseminations from each sample. The extender also nourishes and protects the spermatozoa during storage and distribution. Bull semen can be preserved either at refrigerated temperature (short term; 3-4 days) or at ultra low temperature (-196^{o}C). The extension rate depends upon the purpose, sperm concentration and semen volume. Generally, the extension ratio is 1 part of semen with 10 parts of extender for preservation at refrigeration temperature. For refrigerated storage, semen is extended at minimal part

(semen 1 part: extender 3 parts) and cooled to 5°C over a period of 2 hours. Initially extended semen is further extended with precooled extender (5°C) to the final amount and stored in a refrigerator. In case of bovine, the dilution rate is kept in such a way that each ml of the extended semen contains at least 25 million sperm/ml. The motility and fertility of the semen is good up to 72 hrs in refrigerated temperature.

Ultra low preservation or freezing of semen is of great importance in livestock breeding and farm management. It has made it possible to make available the use of outstanding proven sizes for larger number of cows, covering larger area, frozen semen shipment has become possible to different continents in the globe to any place connected with any service. At present frozen semen is used in most of the states in India. For ultra low temperature, the semen is extended with extender (generally Tris Egg Yolk Citrate extender is used) containing a cryoprotectant (generally glycerol) and extended semen is filled in straws (French straws) and equilibrated at 5°C for 4 hours. Then the straws are exposed to liquid nitrogen vapour for 8 – 10 minutes and plunged into the liquid nitrogen. The semen can be stored for an indefinite period in liquid nitrogen, however care should be taken to maintain proper liquid nitrogen level in the container. Identification of the bull is done on each individual semen container. Each straw contains around 20 million spermatozoa.

Transportation

The frozen semen is transported dipped in liquid nitrogen, the main precautions in the transportation of frozen semen are:

1. It should be ensured that the level of liquid nitrogen does not go down and the semen straws/ampoules remain dipped in liquid nitrogen.
2. Liquid nitrogen containers should be protected from damage during transportation.
3. Undesirable material should not be put in the liquid nitrogen containers.
4. Transportation with public vehicle should be avoided. It may lead to serious consequences.
5. The consignee should be well informed about details of semen, date of dispatch and mode of transportation etc.
6. The container should be labelled as; living biological product, handle with care, rush it etc.

Insemination methods

Insemination can be carried out surgically or non-surgically, the later being most commonly used in farm animals. Non-surgical method of insemination can be carried out using a speculum or recto vaginal method. Recto vaginal method is the most popular technique

of insemination for large animals. An advantage in this method is that it is possible to find out the estrus stage and other pathophysiological conditions of the female genital tract. In this method, gloved, well-lubricated hand is introduced into the rectum and the faeces is removed. Inside the rectum the lower wall is pressed down with the hand to locate the uterus and the cervix is grasped. Before inserting the insemination rod into the vagina, the vulval area is cleaned with towel and while inserting the rod care should be taken to prevent the tip of the rod touching the vulva lips. The rod initially is introduced at 45^0 angle and then made straight line with the animal's body. When the rod reaches the anterior vagina it is guided through the external os of the cervix with the help of gently pressure and manipulation of cervix with the hand in rectum until desired penetration is achieved. Then the semen is deposited by pushing the stillet of the insemination rod.

Site of semen deposition

In natural service the site of semen deposition varies with species. In cattle, buffaloes and ram, the semen is ejaculated in the anterior part of the vagina i.e the semen is sprayed on and around the os uteri. In horse, the semen is partly ejaculated in the cervix and partly into the uterus. In the sow, most of the semen usually is ejaculated into the uterus since the penis of the boar enters in the cervix. In AI with liquid semen, the preferred site of deposition in mid cervix however, uterine insemination is also possible. When frozen semen is used it may be deposited in the mid cervix or in the uterine body. In case of sperm deposition at mid cervix the cervical mucus provides optimum environment for longer survival of sperm, and cervix is less susceptible for trauma by the catheter compared to the uterus. Also, chances of induced abortions especially in early pregnancy and gestational heat are eliminated. However, when sperm are deposited in the mid cervix, a significantly higher degree of retrograde loss of spermatozoa occurs. Hence it is now recommended that frozen semen is to be deposited at the beginning of the uterine body but due care need to be given so that no damage occurs to the uterus.

Time of insemination

Spermatozoa require some period of stay in female reproductive tract to acquire final changes for fertilization to occur. This process is called as "capacitation". On an average a cow is in heat for 12 - 24 hours and ovulation occurs approximately 10 - 12 hours after the end of the estrus. Thus, insemination between mid estrus to end of the estrus gives maximum conception rate. In routine practice cows and buffaloes that are in detected in heat in morning are inseminated in evening and those, which are diagnosed in heat in the evening, are inseminated in the morning. Animals whose cervical mucus shows typical fern pattern are more suitable for successful insemination. Several studies suggest insemination of cattle and buffaloes at 12 - 18 and 18 - 24 h after onset of estrus, respectively results in better conception rate.

Accomplishing high conception rate in bovines using AI

Several steps are to be followed in day-to-day insemination practices to achieve high conception Rate in cattle and buffaloes under field conditions.

Frozen semen quality: In order to achieve high post thaw semen quality, it is essential to obtain high quality fresh semen. From collection of ejaculate to deposition of semen in female reproductive tract, spermatozoa are extremely sensitive to deviations in temperature. Usually, semen stations supply frozen semen straws of ejaculate obtained from highly fertile bulls after they full fills the post thaw requirements. The steps to be followed after receiving the frozen semen straws are discussed in detail.

Semen storage, transfer and retrieval: Once frozen, exposure of straws to temperatures above –130°C and re-cooling results in irreparable sperm cell damage. This results in reduced sperm motility, viability and acrosomal integrity. Progressive motility of sperm is required for transport of spermatozoa from the site of deposition to the site of fertilization, while acrosomal integrity is essential for membrane specific recognition and binding of sperm with ovum. Hence, it should be ensured that the canister containing semen is well below the top of the tank neck.

Handling of straws and thawing: The straws should be removed from LN_2 container using a tweezers/forceps as quickly as possible (with in 3-5 seconds) and shaked to remove excess nitrogen. It is generally advocated to thaw the frozen semen straws at 37°C for 30 seconds. The temperature and time must be followed strictly to achieve high post thaw sperm recovery rate. Hence, it is important to maintain necessary equipments for heating arrangements, thermos flask and thermometer. Frozen semen straws can be thawed vertically as well as horizontally, but, it is important that in both the methods straws should be fully dipped in water. After thawing, the straws should be wiped gently to remove water. It should always be remembered that water is lethal to spermatozoa. If the air bubble is located in the single plug (laboratory seal) side of the straw, then it can be cut with clean scissors. If it is located in the middle of the straw, it should be moved towards the single plug side by shaking gently before cutting. The straw should be gently placed in the gun and slided into the sheath and the sheath should be secured with the gun tightly using 'O' ring. Before loading, the AI gun should be warmed. There should be no gap between the cut end of the straw and the sheath otherwise part of semen may remain in the sheath thus reducing the number of spermatozoa per insemination.

Selection of female for insemination: It is essential that insemination should be done at proper estrus to obtain high CR. Though the owner/herds man of the animal to be inseminated claim that the animal is in estrus, it should be confirmed by clinical/gynecological examination. Efficiency of detection/confirmation of estrus using clinical methods vary with inseminators depending upon their expertise, knowledge level etc. In India, besides

veterinarians, village level workers and technicians are also performing AI. The arborization pattern (fern pattern) of cervical mucus can be used to predict the optimum time of insemination. It is not advocated to palpate ovaries during estrus or before insemination unless one has proper expertise in gentle handling of ovaries. Rough handling may lead to rupture of the Graffian follicle and anatomical disposition of fimbria of oviduct leading to alterations in egg pick up.

Properly timed insemination: The most important primary requisite to obtain high CR is inseminating cows and buffaloes at appropriate time. It is generally advocated that insemination should be done from the middle to end of estrus. Thumb rule under field conditions is if the animal exhibits beginning of estrus signs in late night or early in the morning it should be inseminated evening of the same day. If the estrus signs start at late morning/ afternoon/ evening then the animal should be inseminated next morning.

Proper method of insemination: All efforts to make AI successful using proper collection, handling and processing of semen are worthless if insemination is not properly carried out. The cow or buffalo to be inseminated should be restrained well; otherwise there is every chance to damage the uterus by AI gun and improper deposition of semen leading to poor CR. Before introducing the gun, perineum and vulval area of the animal has to be wiped properly to avoid infection carried through the gun. Insemination gun should be inserted at 30-45^{o} angle after opening vulval lips to avoid urethral opening. In case of frozen semen, the site of deposition is the body of uterus, just next to the internal os of the cervix. After withdrawing the gun, uterus can be massaged gently as it may hasten the sperm transport.

Clitoral stimulation: In normal cyclic buffaloes, ovulation has been reported to occur between 11 and 20 hrs after the end of estrus. But, the time period between end of estrus and ovulation has been found to be longer (25-48h) in higher percentage of sub-estrus buffaloes. It is well known that in buffaloes the incidence of sub-estrus is high. Hence, delayed ovulation has been reported to be a cause for low conception in buffaloes under field conditions. Mechanical stimulation of reproductive tract by massaging clitoris after AI has been shown to improve CR by hastening the surge of luteinizing hormone and ovulation. The clitoris should be massaged gently and immediately after insemination to get favorable results.

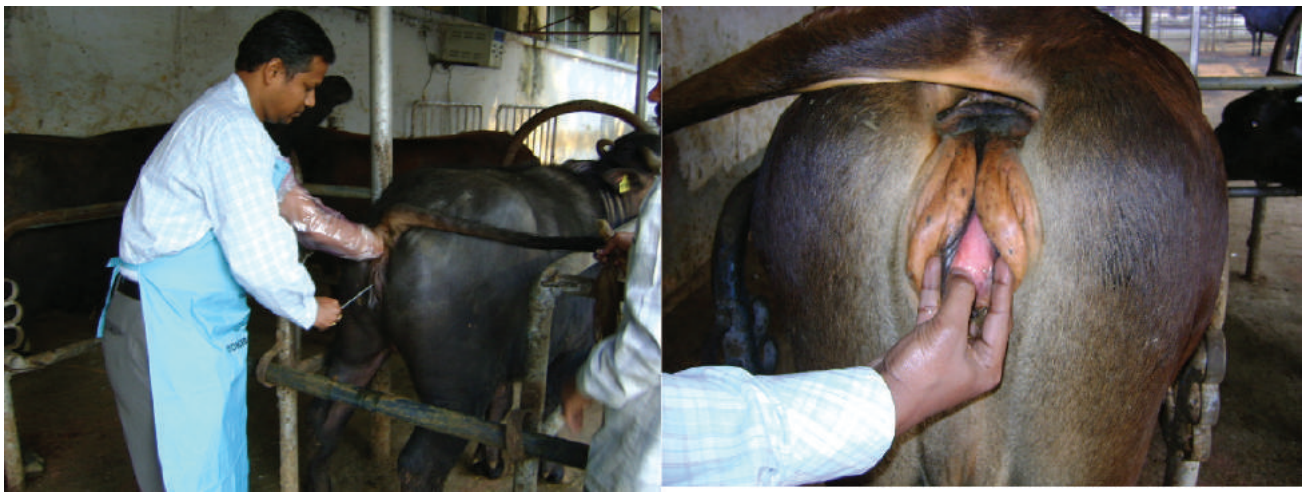


Figure: Proper method of artificial insemination and messaging of clitoris

Activity

1. Go to nearby veterinary hospital and observe how artificial insemination is performed

Review Questions

1. Artificial insemination is comparatively easier in ———————————— than in ————————————

2. Write about AV method of semen collection

3. What is the common extender used for cryopreservation of semen?