12. REPRODUCTIVE TECHNOLOGIES

Can you recall ?

- **The terms sperm, ovum, estrous, fertilization and parturition.**
- **How the reproduction takes place in animals?**

12.1 BASICS OF REPRODUCTION

12.1.1 Estrous Cycle

Estrous cycle is the chain of physiological events that begins at one estrous period and ends at the next**.**

The estrous cycle consist of a long luteal phase (days, 1-17) where the cycle is under the influence of progesterone and shorter follicular phase (days, 18-21) where the cycle is under the influence of estrogen and can be completed in four stages.

1. **Follicular Phase (Days, 18-21)**
   i) Proestrus stage. ii) Estrus stage.
2. **Luteal Phase (Days, 1-17)**
   i) Metestrus stage. ii) Diestrus stage.

The details of various stages of estrous cycle are as follows.

1. **Proestrus :** It is the initial stage of estrous cycle. It usually lasts for 2 to 3 days in cow. It is characterized by follicular growth in ovaries and production of estrogen.

2. **Estrus :** It is the period of sexual desire. The duration of this stage in cows is around 24 hours. The ovary presents mature *graffian follicle.* There is high level of *estrogen* in blood. The animal exhibits signs of heat during this stage.

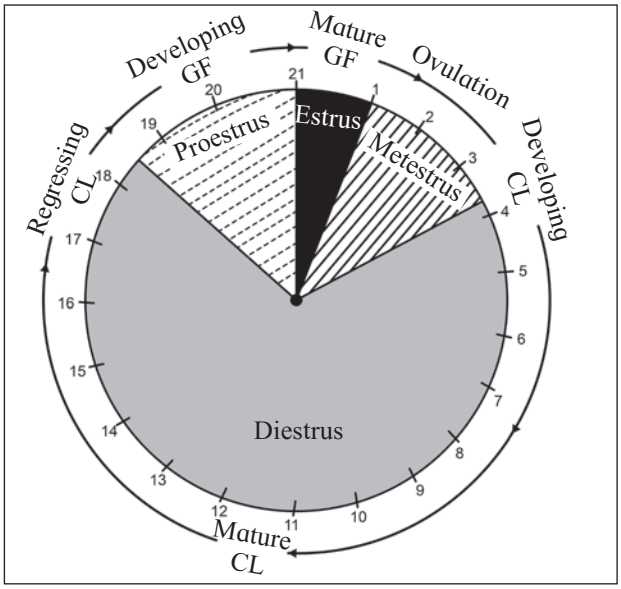


Fig 12.1 Estrous Cycle

3. **Metestrus :** It is the period after estrus. It lasts for 2-3 days in cows. It is characterised by cessation of visible signs of heat. The Graffian follicle ruptures and ovulation takes place during this period in cows and buffaloes. The ruptured follicle in the ovary is replaced by small, soft, developing corpus luteum which secretes a hormone called as ***progesterone.***

4. **Diestrus :** It is the longest stage of estrous cycle. It usually lasts for 15 days in cow. The corpus luteum fully develops. This stage is followed by either pregnancy or next estrous cycle or anestrous in animals.

Remember...

Heat/estrus is the period of intense sexual desire or sexual excitement experienced by females.

Table 12.1 : Reproductive cycle of domestic animals

Particulars	*Cow*	*Buffalo*	*Ewe*	*Doe*
Age of Puberty	24-30 months	36-42 months	6-12 months	6-12 months
Length of estrous cycle	21 days	21 days	17 days	20 days
Duration of heat	12-24 hrs	12-24 hrs	24-36 hrs	24-36 hrs
Time of ovulation	10-14 hrs after end of estrous	10-14 hrs after end of estrous	Towards end of estrous	Towards end of estrous
Period of Maximum fertility	Last 8 hrs of estrous	Last 8 hrs of estrous	2nd half of estrous	2nd half of estrous
Gestation period	280 days	310 days	150 days	150 days
Breeding Season	Throughout Year	Sept. - Feb.	June-Aug. Jan - Feb.	June-Aug Jan.-Feb.

Symptoms of heat/estrus

The cows in heat exhibit signs of excitement, restlessness, reduced appetite and rumination, frequent bellowing, drop in milk production, swollen and congested vulval lips, licking other animals, frequent urination with raised tail, peculiar movement of back and clear, shiny, stringy vaginal mucous discharge. Cows in heat attempts to mount other cows and also stands to be mounted when other cow/animal mounts.

Observe and record...

The behaviour of cow and buffalo during heat/estrus.

12.1.2 Fertilization

Fertilization is the union of male and female gametes.

Ovum : Ovum is a female gamete. It is produced in the ovary by oogenesis. It is roughly spherical, non-motile structure. It is covered by vitelline membrane, zona pellucida and cells of *cumulus oophorus* / *corona radiata*.

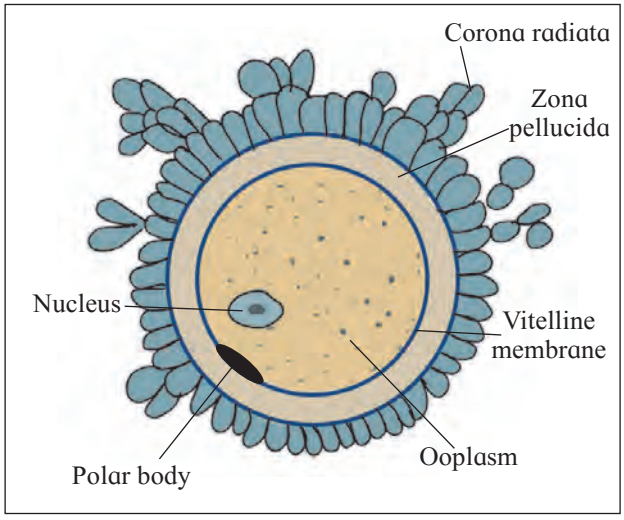


Fig. 12.2 Structure of ovum

Sperm : Sperm is a male gamete. It is produced in the testis by spermatogenesis. It is microscopic, motile structure. It consist of head, neck, middle piece and elongated tail.

Process of fertilization

The process of fertilization involves following steps.

1. **Transport of sperm :** Sperms deposited in the female genital organ either by natural service or artificial insemination are transported to the site of fertilization (ampulla) by both sperm motility and contractions of female genital tract.

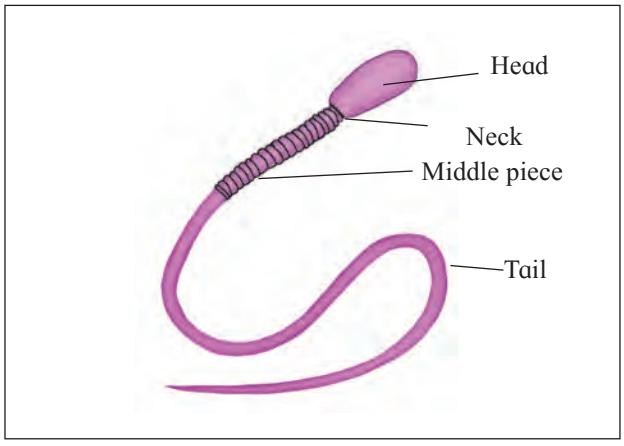


Fig. 12.3 Structure of sperm

2. **Capacitation :** When the sperms are exposed to female reproductive tract for 4-6 hours, they attain the capacity to penetrate and fertilize the ovum. This process of preparing the sperms for fertilization is known as ***capacitation***.

3. **Ovulation :** Under the effect of LH, the matured Graffian follicle from ovary ruptures and the ovum is released. This process of release of ova is called as ***ovulation***. Ovulation usually takes place about 8 hours after the end of estrus in cows. The released ovum is immediately picked up by infundibulum and reaches the ampulla in another 6 hours.

Do you know ?

The sperm remains viable for 24 hours in female genital tract whereas, ovum remains fertile for only 10 hours after ovulation.

4. **Penetration of the ovum by a sperm :** The sperm head contains ***hyaluronidase*** enzyme that breaks the *cumulus oophorus* of ovum and the sperm reaches the zona pellucida. The sperm also carries an enzyme called ***zonalysin*** **or** ***acrosin*** which breaks the zona pellucida, and allows sperm to reach perivitelline space. After entry of first sperm, zona pellucida becomes unresponsive to other sperm, called as ***zona reaction.*** The sperm head then passes through vitellus and loses its tail. When sperm contacts with vitelline membrane, there is reaction in the membrane which makes it unresponsive to other sperm, called as ***vitelline block***.

5. **Formation of pronuclei :** The penetration of the ovum by sperm activates the egg/ova to complete its second reduction division. This results in formation of second polar body and female pronucleus. The sperm head consisting of nucleus is considered as male pronucleus.

6. **Syngamy :** The male and the female pronuclei fuse together to form one cell. This process of fusion of male and female pronuclei is called as **syngamy.**

12.1.3 Pregnancy

Can you recall ?

The terms pregnancy, gestation, embryo and foetus.

Pregnancy is the condition of female characterized by presence of developing young in the uterus.

Remember...

Gestation period is the period from fertilization/conception to parturition.

Stages of pregnancy

The gestation period is divided into following three parts based on the developmental stages of the conceptus in cattle.

1. **The period of ovum/zygote :** It is the period up to 10-12 days after fertilization. The zygote reaches uterus on 3rd - 4th day of fertilization where the zygote is nourished by the secretions of uterine glands i.e. uterine milk.

2. **The period of embryo :** It extends from (implantation) 13th to 45th day of pregnancy in cow. It is characterized by formation of germ layers which gives rise to different body parts/organs. The digestive system, lungs, liver and circulatory system develops. The nervous, musculoskeletal and urogenital systems begins to develop. The extra embryonic membranes (placenta) usually formed in 20-30 days.

Remember...

The heart of embryo starts beating by 21st to 22nd day.

3. **The period of foetus :** It extends from 46th day of pregnancy till the day of parturition. It is characterised by progressive increase in size and weight of foetus. There is development of bones and cartilages in different body parts. The different body systems progressively develop to the fully functional form. There is growth of placenta and accumulation of fluids.

Do you know ?

Hairs appear around the muzzle and eyes at 150 days and hair coat covers the body at about 230 days of pregnancy.

Symptoms / Signs of pregnancy

Cessation of estrous cycle after artificial insemination or natural service, gradual drop in milk production, sluggish, docile behavior, glossy hair coat, fattening tendency, gradual increase in the size of the abdomen and increase in the size of mammary glands/udder.

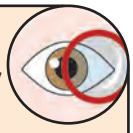
12.1.4 Parturition

Parturition is the act of giving birth to fully developed and viable young one.

Signs of Approaching Parturition

The animals about to parturiate exhibit reduced appetite, irregular rumination, sluggish movements, hollow flanks with pendulous abdomen, elevated tail base and sunken rump, enlargement/distension of udder, engorgement of teats with colostrum, loose, soft and swollen vulva, strings of thick mucus hangs from vulva, rapid pulse and respiratory rate and decrease in body temperature by 1 to 2^0 F.

Observe and record :



The signs of parturition in cow/ buffalo/goat.

Causes of initiation of parturition

The exact cause of initiation of parturition is still not known. However, the following factors may be responsible for initiation of parturition.

1. Increase in the irritability of the uterus due to increase in the size of foetus at the end of pregnancy.
2. Excessive distension of uterus gives equal and opposite reaction by means of straining/contraction.
3. Degenerative changes in placenta towards the end of gestation resulting into separation of foetus from maternal tissues.

4. Decreased blood supply to foetus due to compression of arteries by distended uterus.

5. Accumulation of waste products and increased CO_2 tension in blood enhances foetal movements which in turn stimulates uterine contractions.

6. Increased secretion of corticosteroids by foetal adrenals.

7. Gradual fall in progesterone level removes/decreases inhibitory action on uterine musculature.

8. Rise in estrogen level towards the end of gestation sensitizes the uterus for the action of oxytocin and prostaglandins ($PGF_2\alpha$).

9. Release of oxytocin at the time of parturition produces strong uterine contractions leading to expulsion of foetus.

10. Relaxin hormone secreted at term causes relaxation of cervix, vagina and pelvic ligaments and thereby helps in expulsion of foetus.

Stages of parturition

The process of parturition is divided into three stages -

1. **First stage :** It is also called as ***stage of dilatation of cervix.*** It is the period between onset of uterine contractions and dilatation of cervix. It lasts for 2 to 6 hours in cow. It is characterized by restlessness, mild labour pain, rapid pulse, rapid and distressed breathing, appearance and rupture of allantoic bag (first water bag).

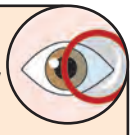
2. **Second stage :** It is also called as ***stage of expulsion of foetus.*** It lasts from complete dilatation of cervix to expulsion of foetus. It usually lasts for ½ to 1 hour in cow. This stage is characterized by recumbency, severe straining, entry of foetus in birth canal, appearance and rupture of amnion (second water bag) and expulsion of foetus.

3. **Third stage :** It is also called as ***stage of expulsion of placenta.*** It consists of the period between the birth of calf and expulsion of placenta. It lasts for ½ to 8 hours in cow. It is characterized by separation of placenta from cotyledons and its expulsion from vagina.

Remember...

- **Allantoic bag is also called first or false water bag.**
- **Amnion bag is also known true water bag.**

Observe and record...



The act of parturition in cow/ buffalo

Table 12.2 : Common names for the sex, young one and parturition of Animals

Species	*Male*	*Female*	*Young one*	*Act of parturition*
Cattle	Bull	Cow	Calf	Calving
Buffalo	Buffalo bull	She Buffalo	Buffalo Calf	Calving
Goat	Buck	Doe	Kid	Kidding
Sheep	Ram	Ewe	Lamb	Lambing

12.2 ESTROUS SYNCHRONIZATION

Estrus synchronization (ES) programs are available for the past 25 years and used as a tool to make artificial insemination more practical. At present number of products and protocols are available and all have its advantages and disadvantages. All synchronization programs require good management, cows having regular estrous cycles, and in good body condition

Estrus synchronization involves the use of one or more hormones to bring cows into heat within a short time period (36 to 96 hours).

Remember...

Estrous synchronization is the manipulation of the reproductive process so that females can be bred with normal fertility during a short predefined interval.

Hormones used in estrus synchronization

The following two hormones are usually used for estrus synchronization

1. **Prostaglandins (PG)** are produced naturally in the animal's body and function, in part, to affect the estrous cycle. In cyclic females, estrus occurs within 2 to 6 days after they are given intramuscular injections of prostaglandin $F_2 \alpha$
2. **GnRH or gonadotropin-releasing hormone** is a naturally occurring hormone that causes the release of other required hormones.

Protocols used in estrus synchronization

The "Synch" protocols viz. Select Synch, Ov Synch, Co Synch, and Modified Select Synch are currently used to synchronize estrus and ovulation in postpartum cow using GnRH – Prostaglandin $F_2\alpha$ (PG) protocol. Among all these synch protocols Ovsynch protocol is commonly used (Fig. 12.4).

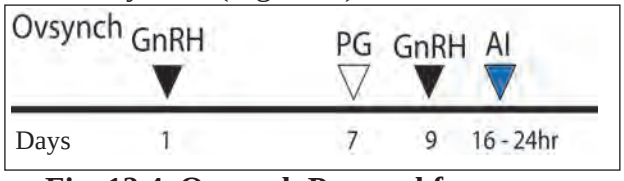


Fig. 12.4. Ovsynch Protocol for estrous synchronization

Advantages

1. Maintain the milk production round the year in organized dairy farm
2. The time required for detection of estrus is reduced
3. Uniformity of calves at weaning is possible
4. Management of calves and cows during gestation and calving period becomes easier
5. Calf crop will be more uniform in age and size which can lead to an advantage in the market.
6. Cow nutrition can be improved by grouping cows according to stage of gestation and feeding each group accordingly.

Disadvantages

1. Requires skilled management
2. Cows will respond poorly if not fed properly or if body condition is poor/weak.
3. More expenses on hormones/drugs and labour
4. High level of management is required

12.3 ARTIFICIAL INSEMINATION

Artificial Insemination (AI) is the most popular technique used in veterinary practice for genetic improvement of the animals. This technique is now days widely used in cattle and buffaloes and to some extent in other species of animals.

Artificial insemination (AI) is defined as the process in which semen is collected from genetically superior male animal and introduced into the female genital tract by an artificial means.

Advantages of AI

1. It increases the use of superior / outstanding sires for genetic improvement.

Table 12.3 : Comparison between AI and Natural service

No.	Parameter	AI	Natural Service
1.	Bulls required for breeding	Very less	More
2.	Use of proven sire	More / Extensive	Less / limited
3.	Progeny testing of sire	Easy	Difficult
4.	Intensity of spread of venereal diseases	Very less	More
5.	Special equipments	Required	Not required
6.	Trained/skilled persons	Required	Not required
7.	Expenditure on maintaining breeding bulls by farmer	Nil	Heavy
8.	Periodical change of breeding bull	Not needed	Essential
9.	Mating of animals having physical disparity/ disability	Possible	Not possible
10	Semen evaluation and examination of female in estrous	Strictly carried out	Not carried out

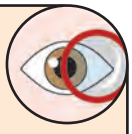
2. It reduces the number of bulls required for breeding purpose.
3. It solves the problem of searching and purchasing a new bull every two years to avoid inbreeding.
4. It helps in early and more rapid progeny testing of bulls.
5. It reduces the chances of spread of venereal diseases like brucellosis, trichomoniasis.
6. It saves labour and expenditure on maintaining breeding bulls on farm.
7. It encourages farmers to keep records and to adopt improved animal husbandry practices.
8. It eliminates difficulties in mating of animals having physical disparity due to differences in size and weight.
9. It enables use of injured or old but superior sires for breeding purpose.
10. It permits crossbreeding to improve the production performance.
11. It improves the breeding efficiency and leads to improved performance.

Disadvantages of AI

1. It requires well trained persons for operations like semen collection, semen evaluation, insemination etc.
2. It needs special facilities like laboratory and equipments.
3. It reduces the market value of bulls.
4. Its use is limited in rural areas due to illiteracy and religious taboos.
5. It involves very strict hygienic precautions at every step.

Observe and record :

The process of artifical insemination in cow/buffaloes.



12.3.1 Semen Collection

The objective of semen collection is to collect the adequate quantity of semen with maximum number of viable spermatozoa per ejaculation.

The different methods of semen collection are artificial vagina method, electro-ejaculation method and massage method.

The details of each method are as given below.

1. Artificial vagina method :

- It is the most commonly used method of semen collection in which Artificial Vagina (AV) is used for collection of semen.
- The different kinds of AV are used for various types and species of animals.

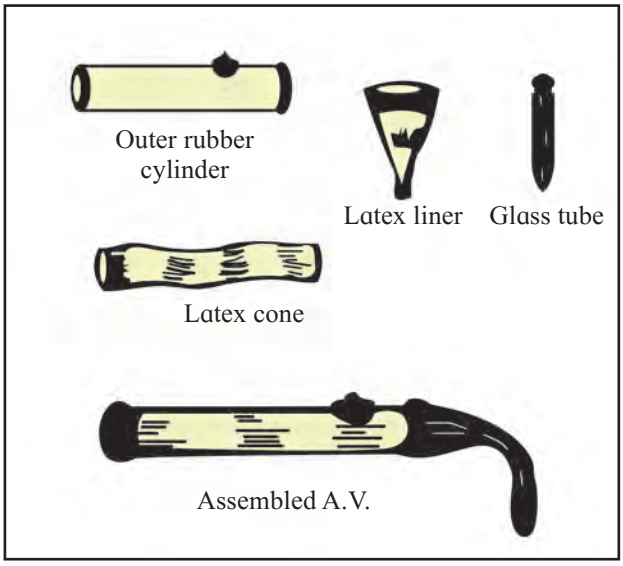


Fig. 12.5. Different parts of artificial vagina

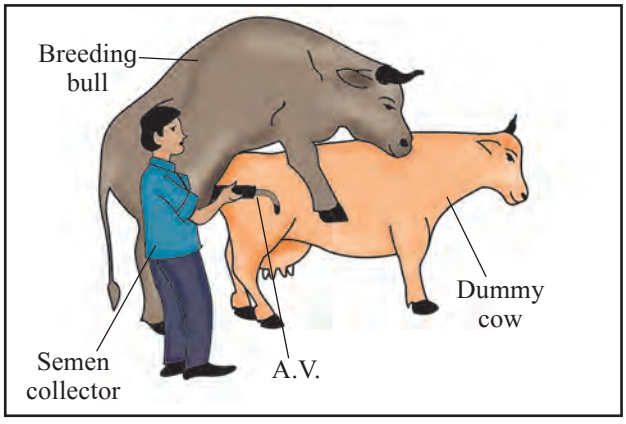


Fig. 12.6. Semen collection by AV method

Advantages

1. Semen is collected in uncontaminated and natural state.
2. Semen collected is usually with better sperm concentration .
3. The semen is clear and free from other secretions viz. urine after collection of semen.
4. The volume, color and consistency of the semen is readily seen in the graduated tube.
5. Semen can be collected successfully by using a dummy / teaser cow.

Disadvantages

1. The use of AV requires technical skill.
2. Semen collection is difficult from injured and old males.
3. Some males may refuse to serve the AV due to variation in temperature, pressure and lubrication.

Do you know ?

The best time of semen collection is early morning before feeding.

2. Electro - ejaculation method

This technique is rarely used for semen collection in bulls.

This method involves electrical stimulation to the ampulla and seminal vesicles of bulls through rectum.

The different types of electro ejaculators with rectal probes specially designed for individual species are used.

Advantages

1. Semen can be collected from bulls having low sex libido or leg injury or paralysis.
2. Semen can be collected from young and untrained / ferocious bulls.
3. Teaser or dummy cow is not required.

Disadvantages

1. Semen gets contaminated with urine.
2. Semen collected by this method is usually with poor sperm concentration.
3. Few males may refuse to donate semen and sometimes exhibits muscle tremors.
4. The sperms are more susceptible to cold shock and less likely to survive during freezing and thawing.

3. Massage method

This method involves semen collection by massaging the seminal vesicles and ampullae of breeding bull per-rectally.

Advantages

1. This method is useful for semen collection in bulls having low sex libido and leg injuries.
2. Semen can be collected from bulls untrained for AV.

Disadvantages

1. The semen collected is usually with poor sperm concentration and poor keeping quality.
2. The chances of contamination with urine are more.

Can you tell ?

Which is the best method of semen collection ?

12.3.2 Semen composition and properties

Semen is a suspension of spermatozoa in seminal fluid.

Semen composition

- Semen is composed of sperms and seminal plasma.
- Sperms are produced in testes and stored in epididymis.
- Seminal plasma comprises secretory fluids of accessory organs like seminal vesicles, prostate gland, Cowper's gland, epididymis and vas deferens
- Seminal plasma contains fructose, citric acid, minerals, proteins and vitamins.

Properties of semen

1. The normal colour of bull semen is milky white to creamy.
2. The consistency of bull semen varies from thick milky to thick creamy.
3. The normal volume of semen per ejaculate is 5-8 ml in cow bull and 3-4 ml in buffalo bull.
4. The specific gravity of semen is 1.035 in bull.
5. The normal semen pH is 6.4 – 6.8.
6. The sperm concentration ranges between 800 to 2000 million per ml of semen in bull.

Internet my friend

Normal quantity of semen ejaculated by different adult livestock species.

12.3.3 Semen preservation

Purpose of semen preservation

1. It helps to store semen for long period and transport of semen for long distance.
2. It helps to retain fertility potential of spermatozoa for long period. It helps to inhibit growth of bacteria and prevents death of sperms in semen.
3. It helps to use maximum semen from proven sire

Methods of semen preservation

1. Storage at room/ambient temperature

1. Semen can be preserved for 4 to 7 days at 25 - 30 ^{0}C by this method.
2. Illini Variable Temperature (IVT), Millovanov's dilutors and Coconut Milk Extenders (CME) are commonly used semen extenders for preservation of semen at room temperature.

Remember...

Semen dilutor or extender is an agent or a medium which increases the volume and keeping quality of collected semen and retains fertility potential of sperm cells in semen.

2. Storage at refrigeration temperature

1. Semen preserved at 4 to 5^{o}C in refrigerator is called as **liquid semen.**
2. Semen can be preserved for 4 days at 4 to 5^{o}C.
3. Egg Yolk Citrate (EYC) and Egg Yolk Phosphate (EYP) diluters are commonly used diluents for preservation of semen at refrigeration temperature.

3. Storage at sub-zero temperature (cryopreservation)

1. Semen preserved at -196 ^{0}C in liquid nitrogen is called as **frozen semen.**
2. Semen is preserved for years together at -196 ^{0}C.
3. The commonly used extenders for frozen semen are Glycerol-Egg-Yolk-Citrate dilutor, Milk-Glycerol diluter and Glycerolated Egg Yolk Tris dilutor.
4. This method is commonly used because of its advantages over other methods.

Do you know ?

The frozen semen is the best method of semen preservation.

Advantages of frozen semen

1. Semen can be stored/preserved for very long time i.e. for years together.
2. Semen retains its viability and fertility as good as fresh semen.
3. Semen from proven but injured sires can be used efficiently.
4. Semen can be transported any time and any where in the world.
5. This technique enhances efficiency and accuracy of progeny testing programmes.

Disadvantages of frozen semen

1. Freezing, packaging and storage equipments are very costly.
2. Frozen semen increases chances of spread of contagious diseases if bulls used are infected or strict hygienic measures are not followed during processing and storage of semen.
3. Semen of some bulls may not withstand freezing.
4. The more number of spermatozoa per insemination are required because freezing process kills about 50% spermatozoa.

Internet my friend

Collect information on storage of semen in livestock species.

12.3.4 Insemination

Insemination means introduction of semen into female reproductive tract by natural or artificial means.

Methods of insemination

1. **Vaginal speculum method :** This method is used in case of sheep and goat.

Method

1. Take clean and sterilized vaginal speculum and lubricate it with liquid paraffin.
2. Introduce the speculum into vagina and view the cervix.
3. Take the semen straw into insemination gun.
4. Introduce the insemination gun into vagina and place it in cervix and push the semen.
5. Remove the insemination gun and vaginal speculum.

Disadvantages

1. Introduction of speculum into vagina is painful.
2. Requires frequent cleaning and disinfection of speculum.
3. Abnormalities of uterus and ovary can not be ascertained.

2. **Recto-vaginal method :** This method is commonly used in cows and buffaloes

Method

1. Secure the cow/buffalo in heat in service crate.
2. Put the polythene gloves/ rubber sleeves in left hand and lubricate it with soap water.

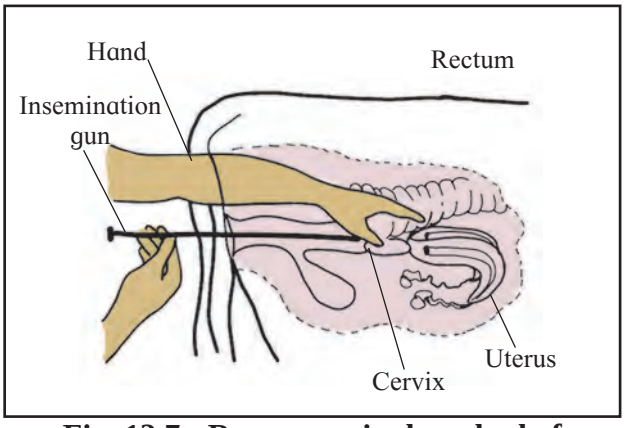


Fig. 12.7 : Recto - vaginal method of insemination

3. Insert the left hand into rectum, remove the dung and grasp the cervix.
4. Wash the vulva with clean water and then wipe it dry with the help of clean absorbent cotton or towel.
5. While grasping the cervix with palm and fingers inserted in rectum, pull apart vulval lips and pass the AI gun at an angle of 45^0 through vagina with other hand.
6. Pass the AI gun by manipulation up to mid cervix and deposit the semen.
7. Remove the AI gun as well as left hand from the rectum.

Advantages

1. Abnormalities of genital organs can be ascertained.
2. Chances of spread of infection are very less.

Remember...

- **The proper time of insemination in cattle and buffaloes is mid to late estrus.**
- **Mid-cervix is the most ideal site for semen deposition.**
- **Dose of frozen semen is 0.25 ml diluted semen**
- **Number of sperms required is 12 million motile sperms per insemination for frozen semen.**

12.4 : EMBRYO TRANSFER TECHNOLOGY

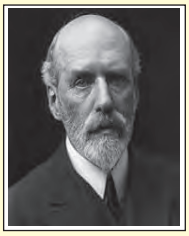
Embryo transfer (ET) is a technique by which embryos are collected from donor female and transferred to recipient female which serve as surrogate mother for remaining period of pregnancy.

Remember...

Artificial insemination technique allows greater use of superior sires; however ET technique allows the best use of superior / elite females for genetic improvement.

Embryo transfer (ET) was first performed and recorded in rabbits by Walter Heap in 1891. Embryo transfer in animals began in the 1949 with sheep and goats. In our country first embryo transfer cattle calf was born in 1986 in Andhra Pradesh Agricultural University, Tirupati, whereas first embryo transfer buffalo calf was born in 1987 at Sabarmati Ashram, Gaushala, Bidaj. In recent years the technology has become fairly well established in cattle and many institutions have adopted ET technique successfully in cattle resulting in several pregnancies and birth of genetically superior calves.

Introduction to scientist



Walter Heape (1855 - 1929) was a british zoologist and embryologist famous for the first successful mammallian embryo transfer.

Advantages

1. The technique makes it possible to produce many off springs / calves from genetically superior / elite cows.
2. It is the quickest and most efficient method of increasing, the rate of genetic improvement within the herd.
3. It is economic to import and export embryos with desirable genetic traits as compared to export and import of pride animals.
4. It helps in conservation of endangered animals species and breeds.

Limitations

1. The cost of embryo transfer technology is high
2. It requires highly sophisticated and well equipped laboratory.
3. The success rate is low.

Technique

1. The highly fertile cow having desirable traits is selected as donor cow for retrieval of embryo.
2. The Follicle Stimulating Hormone (FSH) is injected to donor cow twice daily for 4 days from day 11 of estrous / heat for induction of super ovulation.
3. Following prostaglandin therapy on day 14 of estrous, the donor cow comes in heat in 48 hours and releases more number of ova (10-15) i.e. superovulation.
4. The donor cow in heat is then inseminated with high quality semen of the outstanding bull 3 times at an interval of 12 hours.
5. The ova are naturally fertilized within the donor's womb and then zygote reaches uterus for implantation.

Remember...

It is necessary that both donor and recipient cow should be in heat on the same day.

6. About 7 days after insemination the embryos which are freely floating within the uterus are flushed out carefully with Foley's catheter in suitable biological medium in graduated cylinder.

7. The flushed out embryos are then isolated, classified and good embryos are transferred within 4 to 5 hours of collection to the recipient cows or they may be frozen for later use.

8. The embryo develops to foetus in the womb of recipient cow i.e. surrogate mother. However the offspring born derives its genes from donor cow and from male to which the donor was bred.

Do you know ?

The embryo's can be stored for indefinite period in liquid nitrogen

12.5 CLONING TECHNIQUE

Cloning : It is the process of making identical genomic copies of an original animal.

Clone : It can be defined as a group of two or more individuals with identical genetic make up, derived by asexual reproduction, from a single common parent or ancestor.

Introduction to scientist

J. B. S. Haldane (1892 – 1964) was a British-Indian scientist. He is remembered for coining the words "clone" and "cloning" in human biology and "ectogenesis". He willed his body for medical studies, as he wanted to remain useful even after death.

The word clone refers to creation of a precise copy of a gene (gene cloning), production of therapeutically relevant embryonic stem cells (therapeutic cloning) and production of genetically alike animals (reproductive cloning). In 1963, J.B.S. Haldane coined the term clone. Nuclear transfer technique was used in 1980's to clone cattle and sheep from cells taken directly from early embryos of respective species. In 1995, live limbs namely Megan and Morag were born from embryo derived cells which were cultured in the laboratory for several weeks. For the first time in 1996 Ian Willmut at the Roslin Institute, Scotland, cloned a sheep Dolly from mature mammary gland cell of six year old Dorset sheep.

Remember...

- **Dolly was the first cloned mammal.**
- **Pratham is the world's first IVF buffalo calf born through ET technique at NDRI Karnal in 1990.**
- **Garima is the first live cloned buffalo calf born on June 6, 2009 at NDRI, Karnal.**

Advantages

1. Animal cloning could enable the rapid dissemination of superior genotypes from nucleus breeding farm and herds directly to commercial farmers. Thus technique could provide genotypes / animals suited for high milk yield, disease resistance or extreme environmental conditions.

2. Cloning can be used for conservation of indigenous livestock breeds especially threatened or endangered animals. Somatic cells obtained from endangered animals can be propagated and maintained in vitro

for use as nuclear donor to produce animal clones.

3. Animal models can be developed to study the genetic basis of several human diseases.
4. The cloned animals that are capable of producing recombinant proteins can provide an economic and safe system for production of pharmaceutical proteins for prevention and treatment of animal diseases.
5. Stem cell therapy can be used for treatment of various diseases.
6. Cloning can create organ transplants that would never be rejected by a recipient.

Limitations

1. The current cloning technique is not efficient and is more expensive.
2. Development abnormalities, illness and premature deaths are common in cloned animals.
3. The ethical issues related to cloning are unresolved.
4. The safety of cloned animals as food source has not been well established.

12.6 TRANSGENIC ANIMALS

Transgenic animals are defined as animals whose genetic make up has been improved by introducing DNA from other source than parental germplasm by using reconstruction DNA technology.

Technique : Transgenic animals can be produced as follows :

1. The desired gene is identified, isolated and prepared for insertion into a fertilized egg.
2. The host embryo is obtained for insertion of gene.
3. The desired gene is then transferred to host cell.
4. The recombinant embryo is duplicated and then embryo is transferred to recipient cow that has earlier been synchronized.

Advantages

1. It is possible to produce animal that possess trait of economic importance viz. growth, reproduction and production.
2. It provides base for human organ transplant (xenotransplantation).

12.7 SEXED SEMEN/EMBRYO

Sexed semen : Semen having X or Y bearing sperm to produce progenies of a desired sex either female or male with 80-90% accuracy is known as sexed semen.

Sex sorting technology was developed by the USDA (United States Department of Agriculture) researchers in Livermore, California, and Beltsville, Maryland. The technology was patented as "Beltsville Sperm sexing technology". The commercialization of sexed semen started in United States in the year 2001. At present, commercially sex sorted semen is available in many countries of Europe, USA, Canada, Mexico, Brazil, India, China, Japan etc.

Do you know ?

Sexed semen gives 80-90% accuracy to get female progenies as compared to conventional semen straws where male : female ratio is about 50:50.

How sexed semen is produced?

1. Sperms are sorted by identifying differences between the X- and Y-bearing sperm.

2. The X-chromosome (female) contains 3.8 per cent more DNA than the Y-chromosome (male) in cattle. This difference in DNA content is used to sort the X from the Y bearing sperm.

3. The flow cytometry based sorting method is mainly used as a most efficient technique with more than 90 per cent purity.

Can you imagine ?

The sperm concentration of sexed semen ranges between 2 and 4 million/dose whereas, it is 20 million/ dose in conventional semen.

Advantages

1. It produces maximum female calves which help the farmers to save resources shared with unwanted males.
2. It speeds up genetic improvement by increasing efficiency of Progeny Testing (PT), Embryo Transfer (ET) and In-vitro Fertilization (IVF) programme.
3. It is an economic way to increase herd strength with no risk of introducing diseases by purchasing heifers from outside.
4. It is more successful even at a low sperm concentration in semen than conventional semen as dead, dying or damaged sperm cells are removed during the sorting process.

Disadvantages/Limitations

1. It requires highly skilled person to operate sex sorting machines.
2. The cost of sexed semen is high.
3. The efficiency and speed of sex sorting machine is generally low.
4. Approximately 50 per cent sperms are lost during sorting and hence having less conception rate.

EXERCISES

Q.1 Fill in the blanks.

1. The best site for semen deposition is ……
2. Embryo can be preserved in liquid nitrogen at …….^{0}C.
3. Artificial insemination technique makes better use of …….. for genetic improvement.
4. …… is the most commonly used method of semen collection.
5. The sperm concentration of bull semen is ……. millions/ml.
6. The process of preparing the sperms for fertilization is known as ……
7. The young one in goat is termed as…….
8. The conception rate with sex sorted semen is ……..percent less than the conventional semen.

Q.2 Make the pairs

Group A	Group B
1. Goat	a. Process of preparation the sperms for fertilization
2. Sheep	b. Kidding
3. Capacitation	c. Lambing
4. Ovulation	d. Process of fusion of male and female pronuclei
5. Syngamy	e. Process of release of ova
	f. Calving

Q.3 State true or false

1. Diestrus is the longest stage of estrous cycle.
2. Gestation period in ewe is 165 days.
3. The best time of semen collection is early morning before feeding.
4. Embryo transfer was first performed and recorded in sheep.
5. Act of giving birth is called as parturition.
6. Estrous in cattle usually occurs every 60 days.
7. Dolly the famous animal was a cloned sheep.
8. Fertilization result into formation of eggs.
9. The normal semen pH ranges from 6.4 – 6.8.

Q.4 Answer in brief

1. Define the term artificial insemination.
2. Mention the different parts of AV.
3. Enlist the methods of insemination.
4. What do you mean by cloning?
5. Name the hormone used for induction of super ovulation.
6. Enlist the steps involved in the process of fertilization.
7. Give the signs/symptoms of pregnancy.
8. Enlist the signs of heat in cattle.
9. What are the different methods of pregnancy diagnosis?

Q.5 Answer the following questions

1. Write advantages of AV method of semen collection.
2. Define semen, give its properties.
3. Write the advantages of frozen semen over liquid semen.
4. Enlist the benefits of embryo transfer technology.
5. What are the limitations of cloning technique?
6. Give the advantages and disadvantages of estrous synchronization.
7. Compare the AI and Natural service.

Q.6 Answer the following questions in detail

1. Define artificial insemination and give its advantages and disadvantages.
2. Enlist different methods of semen collection and describe in brief artificial vagina method.
3. Elaborate the concepts of sexed semen along with its advantages and disadvantages.
4. Define estrous synchronization along with its procedure.
5. Describe the estrous cycle in detail.

Practicals (XI)

1. Study of external body parts of livestock and poultry.
2. Demonstration of morphological features of various breeds of cattle.
3. Demonstration of morphological features of various breeds of buffalo.
4. Demonstration of morphological features of various breeds of sheep and goat.
5. Demonstration of morphological features of various breeds of poultry.
6. Identification of commonly used feeds and fodder crops.
7. Study of unconventional feed and fodder resources.
8. Study of azolla fodder Production
9. Demonstration of chaffing of fodder and hay making
10. Different methods of silage making
11. Study of hydroponic fodder production system
12. Study of various body systems of ruminants.
13. Study of various body systems of poultry and structure of egg.
14. Study of different breeding systems adopted in cattle improvement programme.
15. Signs and symptoms for heat detection in livestock.
16. Study of methods of pregnancy diagnosis in livestock.
17. Signs and stages of parturition.
18. Study of equipments required for collection of semen.
19. Visit to local veterinary dispensary to demonstrate A.I. technique.

Project Work (XI)

1. Collect the information and photographs of cattle breeds from your locality.
2. Collect the information and photographs of buffalo breeds from your locality.
3. Collect the information and photographs of sheep breeds from your locality.
4. Collect the information and photograph of goat breeds from your locality.
5. Collect the information of and photographs of poultry breeds from your locality.
6. Collect the specimens of common fodder crops cultivated in your area.
7. Collect the samples of different feeds/concentrates fed to livestock in your locality
8. Visit the different livestock improvement projects and record their objectives and breeding programme.
9. Visit and collect the information on modern dairy farm.
10. Visit to feed manufacturing company and record different types of feed manufactured.

Note : Teachers can allot any one above project work.

Specimen question paper (Practical) — 30 Marks

Q. 1 Identification — **6 Marks**

A. Identify

B. Sub question

Note : Total 6 specimens should be kept, each carrying 1 Marks (½ mark for identification and ½ mark for sub question)

Q. 2 Solve any two of the followings (Each 3 marks). — **6 Marks**

A. Draw a neat Diagram/sketch and label it correctly.

e.g. Draw a neat sketch of cow and buffalo and label the body parts.

B. Calculation from practical syllabus.

e.g. Calculate feed and fodder requirements of lactating crossbred cow weighing 500 kg as per thumb rule.

C. Theoretical question from practical syllabus.

e.g. What is the importance of pregnancy diagnosis ? What are the external signs of pregnancy in the farm animals.

Q. 3 Practical exercise (any one) — **6 Marks**

1. Hay making
2. Silage making
3. Chaffing of fodder
4. Azolla production
5. Urea molasses treatment

Q.4 A. Vivavoce — **2 Marks**

b. Journal — **4 Marks**

Q.5 Project or visit report — **6 Marks**

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