- Introduce the AI gun first at 45 ° angle, then horizontally and glide along the dorsal side of the vagina. Never use force while introducing the AI gun. This would lead to injuries in the reproductive tract and affect the conception rate. If an animal jumps or falls down, withdraw the AI gun immediately to avoid injury to the cow.
- Insemination of a cow should not be done in hot climate, as pregnancy can never be established.
- Remember that poor fertility with frozen semen is due to improper handling or improper deposition of semen by the inseminator.

Efficiency of inseminator

Artificial insemination is an unnatural act to which cows may react unfavourably. Extreme excitement or irritation at this time may upset the delicate nervous and hormonal relationship. Practical experience shows that careful, skillful technique is a must for successful AI programme. Some trained inseminators have lower than normal conception rates even though they are able to deposit the semen at the right place. These persons usually are too rough in their technique. For the best psychological cooperation from the cow irritation and excitement should be minimized, both before and after manipulations of the genital system.

Safe handling of liquid nitrogen (LN₂)

- LN, should be handled carefully as it can produce "frost bite" on the skin similar to burn, even if it is in contact for more than few seconds.
- Cold vapour of LN, can damage delicate tissues such as eyes if exposed for a short period. One should not look into an open LN, container without eye protection.
- Boiling and splashing always occur when filling up a warm container. Always perform these slowly to minimize boiling and splashing and keep your eyes away from the container.
- Always use stainless steel tongs with long handles to remove any object immersed in LN₂. loose fitting gloves made of woolen or leather can be used for handling. Cold metal may stick to skin and tear flesh when attempts are made to withdraw the cold objects from bare hands or fingers. Specially made cryo-gloves are also available for this purpose. Care should be taken to avoid spilling of LN, into shoes.
- Use containers specially designed to hold LN, All

containers have vent or safety device to allow the escape of nitrogen vapour. Inadequate venting or closing tightly can result in excessive gas pressure, which can damage or burst a container. Use only the supplied by the firm; never plug the containers tightly.

Ventilation



Always handle liquid nitrogen containers in a well ventilated excessive area to prevent concentration of gas. Excessive amounts of nitrogen reduce the concentration of oxygen in the air. When the oxygen level goes below

20.5 % can cause asphyxiation. A person

can become unconscious, without sensing any warning symptoms such as dizziness.

Transferring of liquid nitrogen

Use plastic funnel while pouring liquid nitrogen into another smaller container. The top of the funnel should be partly covered to reduce splashing. When it is not safe to tilt a container, use a discharge tube to remove liquid nitrogen.

Care and maintenance of containers

The cryogenic containers are double walled vessels, with annular space evacuated and sealed. In addition several types of insulation viz., vacuum alone, expanded foam, gas filled powder and fibrous material are used as thermal insulators. The outer walls of containers are made of stainless steel, carbon steel or alluminium alloys.

- Extreme care should be exercised in handling containers.
- The cryogenic container is specific and they are meant to store only the particular liquid for which they are made.
- Welding or piercing the container wall is dangerous.
- Keep the container always in upright position.
- Protect container against shock and rough handling. During transport support the container with soft padding (rubber, thermocole etc.)

- Protect against direct sunlight and hot blowing winds.
- Filling of warm container should be slow.
- Avoid frequent cooling and warming of containers. Thermal stress may cause so much strain within it that the inner wall of container may crack. Appearance of moisture on the outer wall of container is a sign of damaged container. Do not dry the container meant for regular use.
- Find out the evaporation rate for each container, so that the periodicity of topping could be organized.
- When the vacuum disappears, the insulating capacity is lost. Repairing of damaged containers has to be done by the manufacturer only. Hence, containers should be handled very gently particularly during transport in vehicles.

Assessment of liquid nitrogen level

The evaporation rate of liquid nitrogen varies between containers (even among the same make and capacity), temperature of the room in which it is stored and number of times it is opened. Hence, periodic checking of the level of the liquid nitrogen is essential. The minimum level of liquid nitrogen should keep the straws completely submerged in liquid nitrogen.

To measure the level, take a dipstick (slender stick made of wood) and gently lower it into the container and rest it at bottom. Hollow tube should not be used. After 5 - 10seconds, take it out and wave in the air. The atmospheric air condenses as a frost on the stick to the level of liquid nitrogen. Read the level 1 cm below the end of frost.

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Artificial Insemination in Dairy Cows **Procedure and Facts** for **Improving Conception Rate**





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ARTIFICIAL INSEMINATION OF DAIRY COW

rtificial insemination (AI) is a technique in which semen is collected from the male, evaluated, preserved, stored and inseminated to females. The role of man who stands between the cow and bull play an important role. The efficiency of the person handling the semen and depositing in the cow will influence to a great extent on the conception rate in AI programmes.

The efficiency of reproduction in dairy cows is one of the most important factors in determining profitability of the herd. To remain profitable, dairy cows must produce one calf at 12 to 14 months interval. In a healthy herd the average number services per conception is 1.6 to 1.7. If satisfactory fertility rate is to be achieved, many events of the reproductive process have to occur in a well balanced manner.

It is always possible that certain portion of bovine population either fails to conceive with a single or more number of inseminations, looses the conceptus during gestation or produces a dead calf at delivery. The affected cows may have long calving interval which tend to decrease the average daily production of milk.

Factors affecting conception rate

For successful AI, fertile semen must be deposited in the healthy cow's reproductive tract at the right stage of the estrous cycle.

Important factors affecting the conception rate include

a) Fertility of the bull selected for semen collection

- b) Processing of the semen
- Storage procedures of semen c)
- d) Estrous detection and insemination technique
- e) Time of AI
- f) Fertility of cow

a) Fertility of males

- Semen quality of the bulls (sperm motility, concentration, viability and morphology)
- Infections involving the male reproductive organs
- Chromosomal disorders, developmental abnormalities and immunological factors

b) Processing of the semen

- Aseptic and all scientific care is essential while processing semen for preservation.
- c) Storing and handling of semen
 - Maintain cold chain
- d) Heat detection efficiency and inseminator's skill On an average, the duration of estrum in cow is 15 to 20 hours and ovulation occurs approximately 12

hours after the end of estrum. Identification of cows in estrus is very important as it facilitates the breeder to perform AI at appropriate time.

Symptoms of estrus in the cow

- Standing to be mounted
- Attempt to mount other cows •
- Stringy mucous hanging from vulva
- Increased restlessness
- Drop in milk vield
- Reduced feed intake •
- Frequent bellowing
- Frequent urination and tail raising •
- Vulval oedema

Estrus detection aid

Advanced but easily available techniques like "fern pattern" of mucous can help to detect correct time of ovulation.

Procedure: Collect a drop of cervical mucus, place it on a glass slide and spread it evenly. Dry it in air and examine under microscope (10X) for different fern patterns.

Three types of fern patters are,

Typical- clear fern leaf like appearance. The branches are well marked and have bright and thick boundaries. Atypical- mixed type appearance. Fern branches remain discontinuous and are not well cut out into further branching.

Nil type- no fern like appearance.

Interpretation:

Early heat: fern patterns are scattered and are small in size. Branching is thin and fine.

Mid heat: arborization or crystallization is visible through out smear. The branches are well marked and have bright and thick boundaries. Tertiary or quaternary or quinquinial branching is observed. This type of pattern is generally referred as typical.

Late heat: Crystallization is not typical. Fern branches are discontinuous and not well cut into further branching. There is an increased infiltration of lymphocytes in the smear also.





e) Time of AI and conception rate in cows The influence of time of insemination on conception

rate in cows is furnished in the following table.		
Time of AI	Conception rate	
art of estrus	44 %	

Start of estrus	44 %
Middle of estrus	82.5 %
End of estrus	75 %
12 hrs after estrus	32 %
24 hrs after estrus	12 %
36 hrs after estrus	8 %
48 hrs after estrus	0 %

Post- partum breeding

After parturition, adequate sexual rest is a must for cows to the uterine involution. The average time required to complete uterine involution in cow is 45 to 60 days. Therefore, cows should be bred after 60 days postpartum.

General management of females

Nutritional factors such as energy, protein, vitamins and minerals needed for successful reproduction are the same as those needed for maintenance, growth and lactation. Deficiency or excess of any of these components which is serious enough to affect reproduction as well as other physiological functions.

Functional disorders in females

- Anoestrus- may be physiological due to the effect of season, nutrition, lactation stress, ageing, etc. or may be pathological due to inflammatory conditions of ovary.
- Atypical estrus like short estrus, prolonged estrus, split estrus, nymphomania and silent estrus etc.
- Ovulatory failure

Role of inseminator

The present conception rate for AI with frozen semen at the field level is reported to be low. The main reason is improper handling and / or deposition of the semen. Thawing and insemination are the two important phases to be handled by the inseminator with utmost care. With chilled semen, deposition of semen either in cervix or uterus results in same conception. While with frozen semen, if semen is deposited in the vagina conception is very low, if deposited in cervix the conception rate is lowered and optimum conception occurs with deposition in the body of uterus. The service of skilled inseminator is essential in maintaining optimum fertility in the field. It has been observed that inseminators who had been inseminating for many years were only 25 % accurate in semen deposition. Though it is very difficult to motivate experienced inseminators it certainly demonstrates the need for retraining.

Lvkascope

Thawing of semen

Theoretically the faster a sperm is frozen; the more rapidly it should be thawed for optimal survival. To bypass crystallization zone, thawing must be rapid and uniform. The most convenient method is to plunge the straw in warm water. The temperature should be 38- 40 °C for 60 seconds for optimal survival of spermatozoa. During thawing entire straw must be completely submerged in water bath.

Procedure for loading the AI Gun

- Identify the canister from which the desired semen is to be taken. Ascertain the colour of the straw by reading identification tag.
- Remove the lid from the container and lift the proper canister up to the level of the frost line. Never lift the canister above the neck level.
- With a help of forceps, grasp an individual straw and remove it, at the same time lower the canister immediately back into the container. If you are unable to take the straw within 10 seconds, lower the canister back to nitrogen, wait for some time and make next attempt.
- With the wrist movement give one or two jerks to the straw to expel liquid nitrogen trapped at the end of factory seal.
- Dip the straw into a clean water bath at 37-40 °C for 60 seconds.
- Remove the straw from bath and dry the straw with a clean tissue paper or cotton. Inspect the straw carefully and discard straw with cracks or defective seals. Semen must never come in contact with water.
- Place the straw in the chamber of insemination gun. To obtain a perfect fit, it is essential that the laboratory seal be removed by cutting at right angle through middle of the airspace. Make sure that the clipped end of straw has a straight clean cut with no jagged edges. Straws cut at other angles will result in back flow and wastage of semen at the time of insemination.
- The post thaw survival of spermatozoa is poor. For maximum reproductive efficiency, thawed semen should be used immediately. Therefore, do not thaw more than one straw at a time.

Precautions while inseminating a cow

• Work under very hygienic conditions, handle semen and AI equipments correctly and do not try your own methods.