Artificial Insemination
(A technique with precise handling)

By

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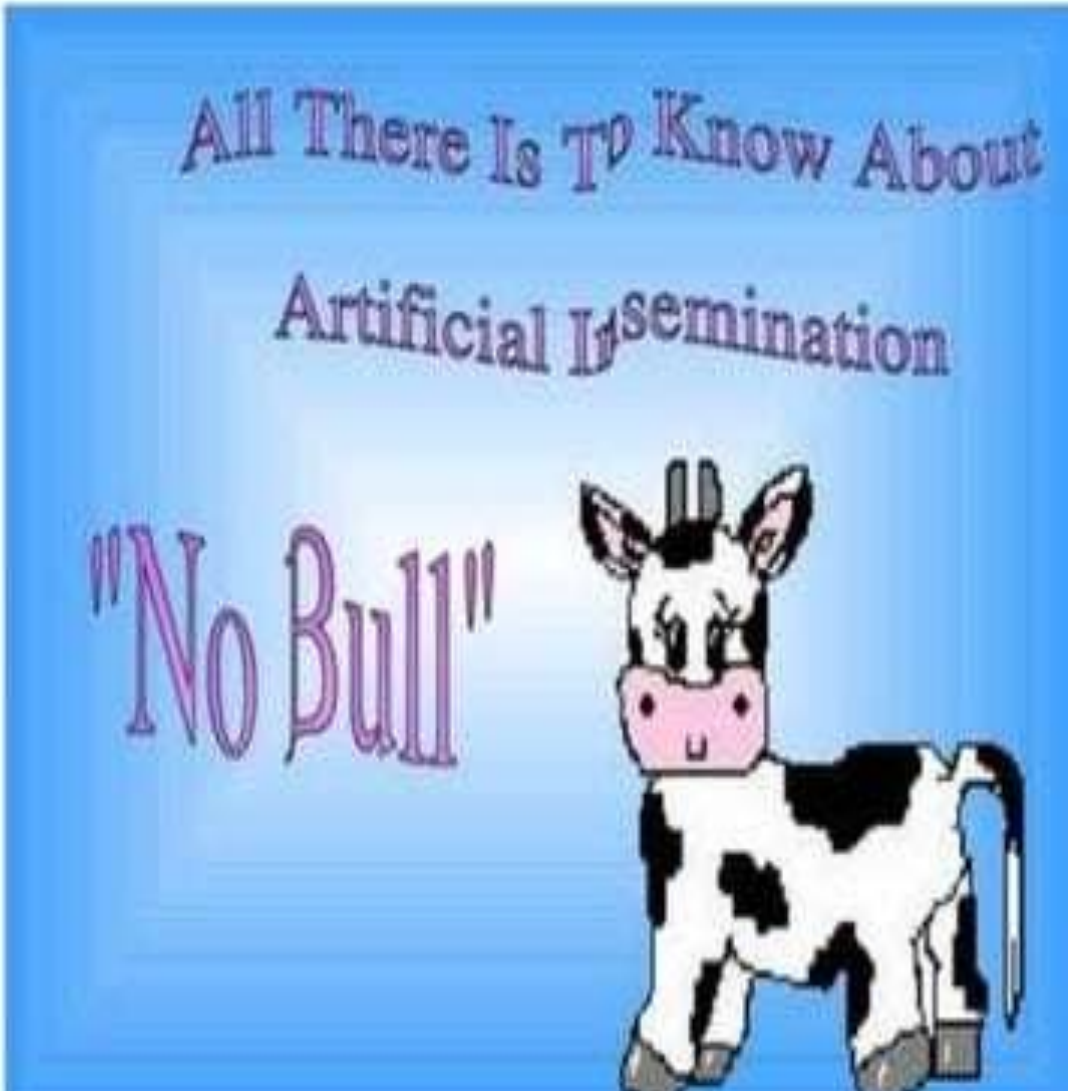
Subject : Analysis of development

Introduction

- Artificial deposition of semen into female genital tract is Artificial insemination.
- Artificial insemination is priority because desirable characteristics of a bull or other male livestock animal can be passed on more quickly.
- Also more progeny can result as compared if an animal mate with females in a natural fashion.



History

- First documented report of successful use of artificial insemination was by **Italian Physiologist L.Spallangini in 1780** when he impregnated a **bitch.**
- In India **Samuath Kumaran** used Artificial insemination for the first time in **1939** at Palace Dairy Farm in Mysore.
- **Degree of success**
- Uptill now it is successful in Ungulates,cat,dog and poultry farms.

Nature of semen and its production

- Semen is the complete discharge of male during ejaculation.
- It has **two components**

❑Cellular elements (Spermatozoa)

❑Seminal Plasma or liquid portion

- **Nature**
- It is creamy or milky in appearance and yellowish grey in color.
- **Consistency**
- It has low viscosity in **dogs and rabbit.**Whereas highly viscous in **cats**.
- **Gel formation** is observed in rodents and **primates.**
- Variation in the **volume** of semen is **due to secretions of accessory**

Steps

- Collection of semen from male
- Evaluation of semen quality
- Semen dilution for preservation
- Deposition of semen in the female reproductive tract

1.Collection of semen from male

Management of male

- Young males are preferred with good nutritional and health status.As they produce good quality semen.

❑12 months in bull

❑7-8 months in goat,boars and ram

❑24 months in stallion(male horse)

Physical status

- Mechanical exercise by bull studs for fit male farm animal.
- Nutritional status has profound effect as sexual development of testis has direct correlation with good sperm production potential.

- **Housing of male**
- It should be convenient and comfortable both for animal and safe for handlers.
- **Preparation of bull**
- It includes pre-collection stimulation of bull(sluggish male) to get increased volume and good %age of motile sperm.
- For this purpose change of teaser and false mounting are effective.
- **Time**
- Early morning before feeding as bull are alert and fresh during morning hours.
- **Frequeny of semen collection**
- Usual practice is to collect semen once a week in bull.However two or three successive collections can be obtained from the same bull.
- Young bulls can be used twice a week for semen collection.

Methods of semen collection

✓Electro-ejaculation method

- In this method **weak alternating current** is provided to **sacral and pelvic nerves** through electrodes placed in rectum.
- Accesory gland secretion takes place at **lower voltage** and **ejaculation at higher voltage.**
- Although semen has less contamination in this method but it **cause discomfort to animal and variable reaction**.It is only prefered when male cannot be trained for artificial collection of semen.

✓Massage method(outdated method)

- Massaging of rectum of vesicular gland and ampullae of vas deferns can induce semen flow.
- But it has more chances of contamination with urine and get imbalance in its

✓Collection on dummy cow or manikin

- A dummy cow is prepared by **heavy steel frame** covered with **hard leather** to give appearance of normal cow.
- An artificial vagina is prepared and fixed at suitable height at rear of dummy cow.
- Although it has advantage over teaser cow **being disease resistance** but male male do not show interest in such dummy cows.

✓Live mount and teasing procedure

- Live mounts using a teaser is most successful procedure.
- Estrogen treated females are selected for semen collection.
- Cow should be of same breed and color.
- Cow should be of medium height as bulls feels difficulty on mounting.
- It is better to select cow which has done 2-3 calving.

Factors affecting semen production

After sexual maturity reproductive capacity of cattles are usually 4-7 years.

Following factors can affect semen production.

- 1.Continental breeds reach early sexual maturity then indigenous
- 2.Long distance travelling bulls have some effect on semen volume
- 3.Seasonal affect on spermatogenesis
- 4.Disease
- 5.Nutritional quality
- 6.Genetic inheritance
- 7.Frequency of ejaculation(more ejaculation less sperm count)

2.Evaluation of semen quality

- Assessment for fertility potential of semen sample before insemination is crucial step.
- Semen is **evaluated for color,volume,density and activity.**
- If **opaque in appearance** it has **high sperm concentration**.But if **translucent** then **few sperms** in semen sample.
- **Curdy appearance indicate infection of reproductive tract** from which semen collected.
- Motility of sperms can be examined under microscope.
- Proportion of live to dead sperms is estimated by **nigrosin-eosin stain.(5% w/v nigrosin+0.6% w/v eosin+3% w/v sodium citrate)**

3.Preservation of semen

- Ejaculated sperms do not servive for long period and high temprature storage is also destructive to spermatozoa.
- So in order to preserve fertilization capacity of sperms.Many diluents are added for different purposes
- **What should be the Qualities of diluents?**

❑1.Proper balance of mineral elements essential for live sperms.

❑2.Diluents should have nutrients for aerobic and anaerobic metabolic processes for survival of sperm.

❑3.Lipoproteins and **lecithins(phospholipids)** to protect **against cold shocks.**

❑4.Diluents should have **buffering capacity** to negate **shift in pH** due to **metabolism and lactic acid production.**

❑5.Inhibit bacterial growth

Some diluting agents.....

- ✓Egg yolk → Against cold shocks
- ✓Sodium citrate → To preserve viability and fertility
- ✓Carbohydrates(glucose+fructose) → As a source of energy.It increase motility and survival of sperm.
- ✓Pencillin,Streptomycin → To inhibit bacterial growth
- Cooling of semen is done to prolong viability and fertilization capacity by reducing metabolic activity.
- Therefore,diluted semen vials are wrapped with cotton,placed in beaker containing water and transferred to refrigerator for further cooling to 5C.

Deep freezing or cryopreservation (Long-term Preservation)

- 1 of 5 cows become pregnant by deep freezing semen.
- For deep freezing **glycerol** is used as it is protective against lethal effects of freezing.

✓**Slow freezing (Ampoule):**

- Slow freezing is carried out in alcohol-co2 ice bath and then transferred in liquid nitrogen container for long preservation.

✓**Instant freezing:**

- 5-5.5 % sugar solution containing glycerol kept for 5-10 hours.After that small drops of 0.5-0.2 ml by micro syrings for 10 min on dry ice.
- Then semen is transferred to liquid nitrogen(-196) for long term storage.

Thawing

- Thawing of frozen semen is essential before use.
- Melting of semen should be as quickly as possible to prevent recrystallization of water into bigger crystals.
- Frozen semen is thawed at 34C for 15 second in water kept in thermos flask.
- **4.Depostion into female reproductive tract**
- Female should be healthy and at heat period.
- Deposition with the help of inseminating gun is done in cervix of female.

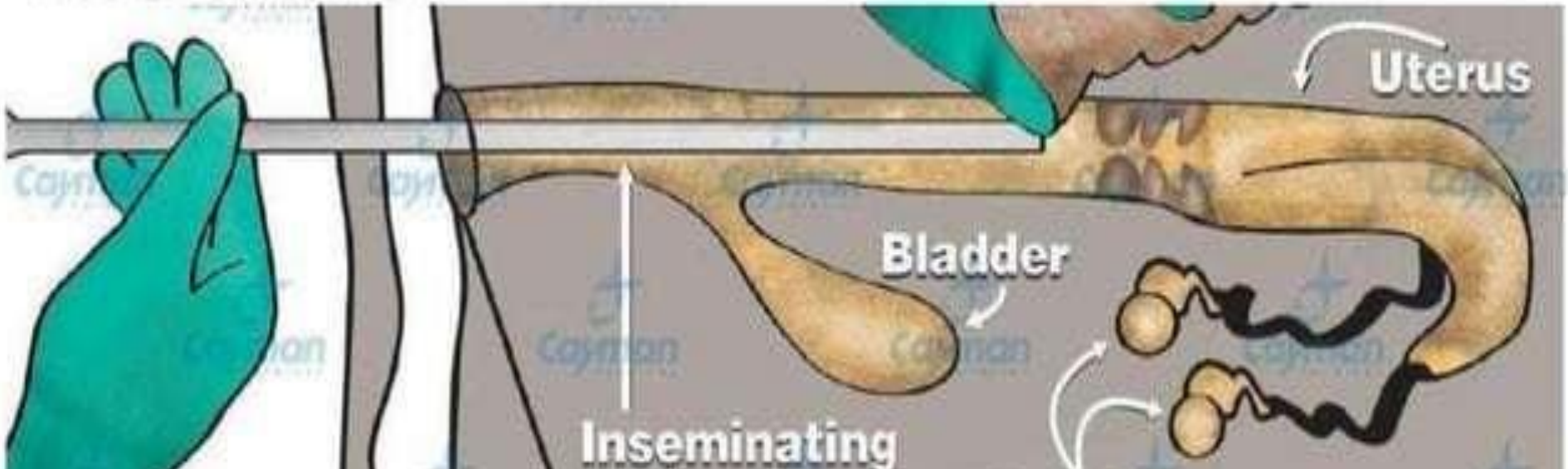
✓**Inseminating equipment**

- 2 ml plastic syring having inside diameter of 1mm
- Pipette is sterilized,dried wrapped with paper and again sterilized with hot

- Diluted semen 0.8-1.2ml is sucked into pipette.Vulva area of cow is cleaned,dilated and pippete is inserted into vagina and semen is lead to mid cervix.

✓**Method of insemination**

- Rectovaginal (cervical fixation) method is used for large animals as it is possible to perform intrauterine insemination by this method.
- Mid cervix is ideal place for deposition as it activate the spermatozoa and increase capacitation.



✓ Detection of heat

- Crystalization pattern of cervical mucous under microscope helpful to detect heat.
- If venation is like fern-tree leaf then female is in heat period.

✓**Timing ideal insemination**

- Cow has wide range of heat period of about 3-28 hours,18 hours being average period.Mostly mid heat is preffered as ideal.
- Cow should not be inseminated before 50 days after calving.
- **Viability of gamete** in female reproductive tract is different for different species.eg 30 hours in cows.

✓**Adrenaline and excitement**

Advantages

- Superior sire animals can be raised
- Small quantity of semen can inseminate many females.
- Proper method help in disease control
- More female at heat can be inseminated simultaneously.
- Golden tool for live stock economic trait.

Demerits

- If veterinarians and professionals are less trained then it is just waste of time,energy and resources.
- Danger of contamination at various stages.
- It is costly in case of materials required in laboratory and transportation.

THANKYOU