Artificial Insemination

Dr. MA. Nadheer - BVSc,MSc, MBA Deputy Director DAPH Ampara

Definition of Al

- 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 form into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions.

Symptoms of Heat

► The animal will be in excited condition. The animal will be in restlessness and nervousness.

- ► The animal will bellow frequently.
- ► The animal will reduce the intake of feed.
- ▶ Peculiar movement of limbo sacral region will be observed.
- ► The animals which are in heat will lick other animals and smelling other animals.
- ► The animals will try to mount other animals
- ► The animals will standstill when another animal try to mount. This period is known as standing heat. This extends for 14-16 hours.
- ▶ Frequent maturation (urination) will be observed.
- Clear mucous discharge will be seen from the vulva, sometimes it will be string like the mucous will be seen stick to the near the pasts of valva.
- Swelling of the valva will be seen. Congestion and hyperemia of membrane.
- ► The tail will be in raised position.
- ► Milk production will be slightly decreased
- ► On Palpation, uterus will be turgid and the cervix will be opened.

Advantages of Al

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': contagious abortion, vibriosis.

• By regular examination of semen after collection and frequent checking on fertility make, early detection of interior males and better breeding efficiency is ensured.

Advantages of Al con..

- ► The progeny testing can be done at an early age.
- The semen of a desired sire 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 sire without injury to either of the animal.
- It is helpful to inseminate the animals that refuse to stand or accept the male at the time of oestrum.
- It helps in maintaining the accurate breeding and cawing records.
- It increases the rate of conception
- Old, heavy and injured sires can be used.

Disadvantages of Al

- Requires well-trained operations and special equipment.
- Requires more time than natural services.
- 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.
- Necessitates the knowledge of the structure and function of reproduction on the part of operator.

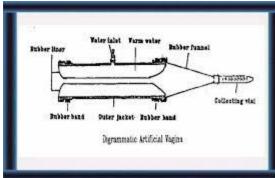
Semen collection methods and evaluation

- Various methods of collection of semen have been devised from time to time. The older unsatisfactory methods have been gradually replaced by the new modern techniques. There are three common methods.
- Use of artificial vagina
- By Electro-stimulation method.
- By massaging the ampulae of the ductus differences through rectal wall.
- The ideal method of semen collection is use of artificial vagina which is safe for sire and the collector also.

Artificial Vagina Method

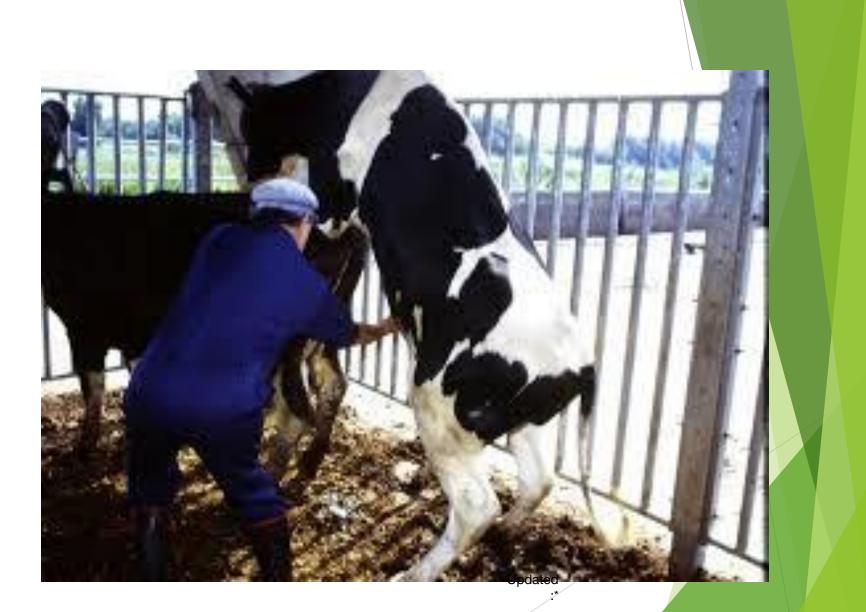
► The artificial vagina has the following parts:

- A heavy hard rubber 2" lose, open at both ends with a nostle for air and water in and outlet.
- Inner sleeve of rubber or rubber liner.
- ► The semen receiving cone or rubber cone.
- Semen collection tube made of glass or plastic graduated in CC and its fraction correct to 0.1 CC









Process

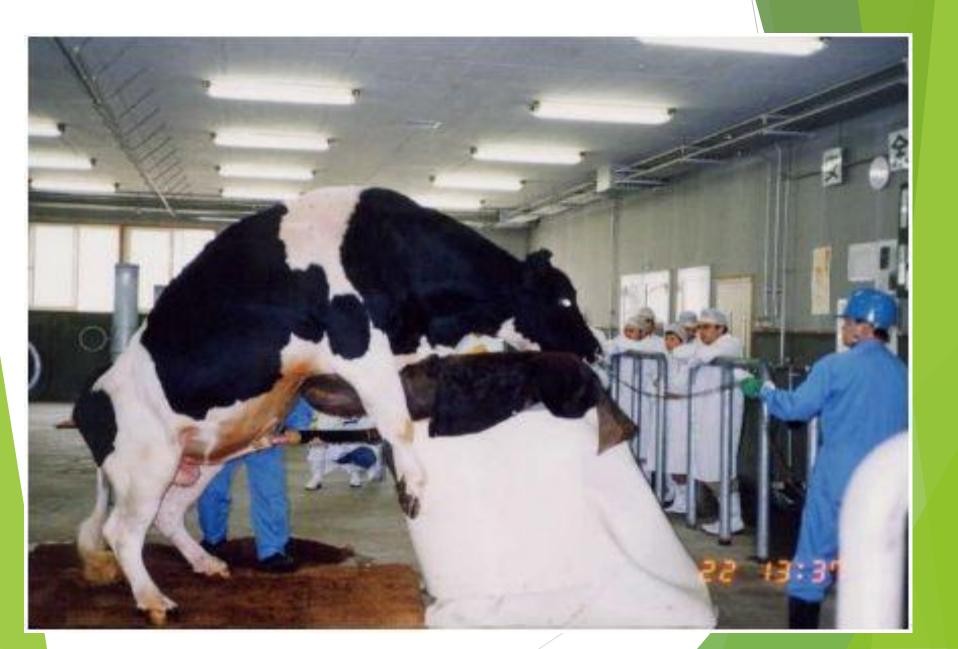
- Insulating bag : Before using for semen collection all the parts are washed thoroughly and sterilized properly, and assembled as artificial vagina, the rubber liner is inserted into the hose; inverting both ends back by folding back from either side opening, and fastening with rubber bands. Now the space between the hard rubber hose and inner rubber liner forms a water tight compartment. The nostle at one end of the hose can be fixed.
- The water jacket of the Artificial vagina is filled with hot water at a temperature of 45°C (113°F) by opening the nostle.
- The graduated semen collection tube is fixed to the narrow end of the artificial vagina hose, and fastened by a rubber band.

Cont..

- The inner side of the rubber liner on the anterior side of the artificial vagina is lubricated with sterile jelly to a length of 3 to 4 inches.
- Air is blown through the nostle into the water jacket, to create pressure in it, and the same is exerted to the rubber linear, to simulate natural vagina.
- The temperature of the artificial vagina is to be checked, at each collection, and it should simulate natural vagina at mounting time.
- If it is too cold ejaculate may not be there after a thrust, or even if ejaculate is there; it may be contaminated with urine, and becomes unfit for use.

Semen collection method

The cow or dummy is secured in service create. The artificial vagina assembled is held at 45° angle from the direction of penis, and the thrust is at that angle. The artificial vagina is held with the left hand by a right-handed person; and when the bull mounts the cow, the sheath of the bull will be graphed by the operator, directing the gland penis into the artificial vagina, and then the bull gives a thrust to ejaculate.



Semen collection and production of frozen semen

 1st step of frozen semen production is collect semen using AV.

If semen is low motatilty the sperm cannot survive after the treatment

Dilution, freezing and thawing.

efficiency of semen producing-reduced.

To collect sperm with good motility-

ejaculate comfortably.

under Natural condition.

So all procedure of semen collection - carefully

Equipment and facilities of semen collection

1.Semen collection.

Varios methods

methods improved

Most common -intercepting method -natully collected from bulls.

- 2. Device and apparatus for semen collection.
 - a. An AV and its composition
 - 2 types of AV

Triple layer

Double layer

used without dummy

both are used putting warm water between inner layer and the outer casine

- Triple layer
- 3 major layers, an outer casing-plastic, a rectangular inner sleeve A,
 - made of rubber and inner sleeve B -rubber.
 - Inner sleeve A fitted to the outer casing.
 - Inner sleeve B is fitted to inner sleeve A,
 - At the end of inner sleeve B -collection tube attached.
 - When semen collected it flows in to collection tube.
 - collection tube is inside the AV-protection from temperature shock.
 - However handling is not easy.- longer and heavier than double layer type.

- Double layer
- Shorter, lighter and easy to handle during semen collection.
- Collection tube is located out side of the casing.
- Even the temperature of the poured water is elevated, no effect to the semen.
- Widely used due to easy to handle.
- Radiator hose of a car can be used as shorter casing which is cheaper and very soft bcos made up of rubber thus injury of penis doesn't occur, even dropped no damage.

Dummy cow

- Fixed dummy is lesser dangerous than than a teaser female bcos stable so bull does not move during collection,
- Possible to adjust the hight of the dummy -easy collection.
- Bull should be trained and accustomed.
- Using mobile dummy (a dummy car) -natural ejaculation-natural position of the penis.
- A collector can avoid a bull from falling down and being stamped down by bull.
- Movable dummy-can be used outside the roompossible to accelerate the sexual excitement.

Teaser cow

- A teaser cow a female, a castrated bull or small bull with warm temper.
- Some times moves furiously-bull can stamp the collector, some times the bull looses the balance and leans on the collector.
- Therefore necessary to restrain the teaser using chute.

Semen collection Room.

- Room should be opened, clean and quite so bull is comfortable and easy collection.
- Full broad space- bull move freely.
- Enough space for dummy, chute and stand for cleaning of prepuce., waiting space for bull, cleaner of prepuce etc.

Techniques for collection of semen

- Preparation for semen collection
- Cleaning of the bull dust, hair and feces
- Cut shorter the hair of scrotum and prepuce if longer.
- Cleaning of bull is so important in term of hygiene.
- Manage temperature of the collection room.
- Then adjust the height of the dummy and observe for any demage or defects.
- ► When female teaser is used, cleaning is necessary.
- Teaser should be restrained in the restraining chute.
- Put palm tree mat or rubber mat to prevent accident by slippery of hind legs when stand to mount.

Preparation of AV

- Use casing and sleeves which not broken.
- Inner sleeves should be cleaned, disinfected and dried.
- Should be stored in hygienic condition keeping box equipped with a sterilization lamp,
- When the AV is composed take not to twist the inner sleeve.
- Put some physical saline solution in to the inner sleeve in which the penis is inserted - smooth flow of ejaculated semen.
- Putting the warm water to AV in a proper temperature Young bull - 40*C, Aged bull 45*C, generally 42 -43 *C
- Bulls don't ejaculate when the temperature is too high or too low.
- Too much water may damage the sleeve by pressure when penis is inserted
- One AV per one bull..

- Cleaning of prepuce
- Necessary to clean inside the prepuce the bacteria increase easily.
- Utilize clean warm water or physical saline solution using electronic vacuum cleaner,
- Clean water or boiled water or 1% invert soap solution.
- ▶ If bull urinates clean up just before collection.
- Collection of semen
- Intercepting method using AV collection could be either left or right of the bull.
- Collector should wear a light costume, boots and helmet for safety for sanitation wear rubber glows to hold the preputial orifice.
- Lubricant smeared to entrance area of the inner sleeve Vaseline and muciliaginous jelly- not too much-contaminate semen.

- Restriction of live mounting
- The controller leads the bull to teaser or dummy and conducts restraint of mounting - an effective method resulting semen high quality.
- When bull mount controller forces the bull to get down before ejaculates.
- When a bull mount collector hold the prepuce to direct the penis to avoid touching the dummy or teaser,
- When restraint the mounting is conducted congested and accessory gland fluid discharge which confirms the satisfiable restrain conducted and the bull is let to mount.
- Not enough restraint-semen contain lot of fluid ph is high and quality is not good.

- Collection of semen.
- After confirming that a bull mounts the collector rapidly approach and collect semen.
- Take care not to be stepped by bull.
- After the bull mount the collector introduce the penis in to the AV.
- Once introduced bull ejacute very fast in amoment of thrust.
- A well trained collector insert the penis at the good timing.
- Immediately after ejaculation hold the entrance of the AV upwards.

The operator should take 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.

The water from the jacket is also drained by opening the nostle. This allows the ejaculate to flow from the cone to the semen collection tube. The semen collection tube is detached from the cone, plugged with cotton wool, and taken to the laboratory for examination. The rubber cone and the semen collection tube can be protected from external contamination or heat or higher, by covering with an insulation bag with zip.

C) SEMEN DILUTION / EXTENSION

- The main reason for extending (diluting) semen is to increase the number of females serviced from one ejaculation.
- A normal ejaculate from a dairy bull contains 5 10 billion sperm which can be used to inseminate 300 to 1000 cows if fully extended (15 to 20 million spermatozoa per straw for deep frozen semen).
- Dilution rate depends on quality of the ejaculate, number of sperm cells, percent alive and mobility.

- Evaluation of semen and sperm
- The points taken account...

a.Do not confuse the semen with the one from another bull.

b.Avoid the temperature shock on the semen.

c.Handle the semen perfectly and hygienically.

d.Examine the semen shortly and rapidly.

Macroscopic examination

- Characteristics of semen.
 - Volume, color, odor, pH and contaminants.
- ▶ To prevent temp. shock- water bath at 34*c at the lab.
- ▶ To avoid mixture of water use quaze to wipe out the tube.
- Measure counting of spermatozoa using a sample.
- Remaining volume measure the pH.

- Volume- spermatozoa and seminal plasma(accessory genital gland secretions). Measured using the graded scale of the tube.
- Changes according to -Individual, age, season, frequency and technique of semen collection.
- Average volume 5 ml (2-10 ml).
- Concentration 1000 million /ml.
- Total number of spermatozoa-5000 million (3000 7000).
- Colour
- Milky white
- More concentration whiter the colour.
- Odor.
- Fresh -Usually a little smell.
- Some times odor of the bull- if not clean well the prepuce.
- Specific odor comes from the protein and phospholipids in the fluid of prostate

- Hydrogen -ion Exponent (pH)
- Use an electric pH meter- accurate.
- Filter paper-generally used, a simple mtd.
- Normal bovine semen ranges from 6.2 6.8.
- As tme passes acidic due to lactic acid by motile spermatozoa.
- Reduction in pH is not good so important to dilute rapidly(pre-dilutin).
- Microscopic examination.
- Concentration, motility, percentage of motile spermatozoa, percentage of abnormal spermatozoa and mixture of different materials.
- Examination of morphology.

Semen Storage

- The discovery that bull semen could be successfully frozen and stored for indefinite periods has revolutionized AI in cattle.
- In 1949, British scientists discovered that addition of glycerol to the semen extender improved resistance of sperm to freezing. Glycerol acts to remove water from the sperm cell prior to freezing and prevents the formation of cellular ice crystals which would damage the sperm.
- There are two methods of freezing and storing semen: dry ice and alcohol (-100 degrees F) and liquid nitrogen (-196 degrees F). Liquid nitrogen is preferred because there is no evidence of fertility deterioration with age. Fertility gradually declines in semen stored in dry icealcohol.

- Semen is usually stored in glass ampoules. Other method that appears promising is the French-straw.
- Artificial coloring is frequently added to semen extenders in order to distinguish one breed from another. Complete identification of the bull is required on each individual semen container.
- Freezing of semen is done with a special diluent, which has the following composition.
- Composition of Diluent
- Major components are Saccharides, buffers and egg yolk.
- Egg yolk-appropriate concentration is 15-20%.-protect spermatozoa from harmful effects by freezing.
- Saccharides-adjustment of osmotic pressure of diluent, protect from harmful effects from freezing and source of energy for spermatozoa. Most commonly glucose is used.

- Buffers effectively affect the maintenance of cell membrane of spermatozoa during the process of freezing and thawing.
- Some buffers-phosphate citrate and citrate sodium solution.
- Amphoteric ionized buffers tris-hydroxy-methyl-animomethane(Tris) and N-tris-hydroxy-methyl-animo-methane (TES) are more superior.
- Concentration of glycerin-7% in the in the egg yolk diluent,10% in milk diluent.which protects spermatozoa from harm effect when freezing.
- The basic principle for freezing semen is to preserve permanently by reversibly inhibiting metabolism of spermatozoa resulting in their little energy consumption but not losing the fertilizability.
- To preserve the cells there is a limit for upper temperature, but not a limit to lower temperature.

- The addition of glycerol to the dilulent makes the cells more resistant to the rigours of freezing and icy crystals, which form are smaller and smoother thus creating less damage to the spermatozoa. The addition of fructose to the diluent luprores sperm resistance to glycerol; and also provides nutrition.
- Frozen semen is packed in single dose glass vials or plastic straws at +5°C.
- The final level of glycerol should be 7.0 to 7.6% during the freezing process.
- The antibiotics are added to inhibit bacteria and to kill pathogenic organisms.
- The semen to be diluted in such a way that one ml. of extended semen will contain 20 million motile spermatozoa.
- The semen must be cooled carefully for spermatozoa to remain with life. The final temperature is lowered to -79°C or still lower.

- Diluents containing egg yolk or milk.
- Diluent NOT containing glycerol called 1st diluent.and with diluent called 2nd diluent bothe are added with antibiotics.

Penicillin	500 -	1000 IU/ml.
Streptomycin	500 -	1000 mg/ml.

- Preparation of straw
- Print the code and the name of the bull, and sterilization using ultra violet rays for 10 minutes.
- Filling and sealing of semen into straw
- Using straw machine semen is filled and sealed into straws. This is an automatic machine.

- Freezing
- Cool the straw rack for freezing at 4*c.
- Put the straw into straw rack.
- Supply the liquid nitrogen into the freezing machine up to 20 cm below the tray.
- Confirm the heater of the freezing machine works well.
- And freez it by monitoring the time and temperature.
- Tranportation of frozen semen
- A container called liquid can or semen storage can 30 -40 l of volume which is transported in a wooden box.
- Check the volume of LN2 insert a gage or weigh the container may be disappearing or leakage of LN2.

Insemination Methods

There-are different methods of insemination in different species of animals i.e. speculum method, vaginal method and recto vaginal method.

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 Al gun covered with plastic sheath.

Cont..

- 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 through the vulva to 'vagina and cervix and observed with the hand in rectum that the A. I gum reaches the cervix, then the semen is deposited by injecting the gun, and after depositing the semen the gun is removed, the empty straw and sheath are disordered.

Evaluation Volume (ml) Color pН Consistency Concentration $(10^6/\text{ml})$ Mass Movement Individual Movement (%) Viability (%) Intact of Plasma Membrane (%) MDA Level (μM) DNA Damage (%)

Average 6.2 ± 0.8 Milky white 6.5 ± 0.1 Medium $1,387.8 \pm 286.6$ 2 + 73.1 ± 2.6 87.8 ± 2.4 85.2 ± 1.8 5.4 ± 0.2 9.5 ± 4.4



Recto vaginal method

Spectrum 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.

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.

Timing of Insemination for Maximum Conception

- A frequent question concerning AI is:
- What time during estrus should cows be bred for greatest chance of conception?
- Since estrus may last from 10 to 25 hours there is considerable latitude in possible time of insemination. Much research work has been conducted on this subject.
- The studies show that conception rate is lower when cows are bred prior to mid estrus or later than 6 hours after cessation of estrus (standing heat in this case).

- Maximal conception is obtained when cows are inseminated between mid-estrus and the end of standing estrus, with good results up to 6 hours after estrus.
- Success in insemination timing is dependent upon a good heat detection program. In large herds, this means assigning individual responsibility for heat detection and a continued education program for labor.
- A successful heat detection program and subsequent proper timing of insemination will pay dividends in increasing reproductive efficiency.

Objectives of Artificial Insemination

- Genetic improvement of livestock
- Disease control mechanism
- Possible to increase fertility
- Decrease breeding expense

Advantages of Al

Genetic Improvement

- Wide spread use and availability of genetically superior sires
- 1 bull can breed 500,000 cows in a lifetime
- After death, semen can be used
 - Oldest frozen semen 40 45 years old
- Rapid proof of sire
 - Progeny testing examines offspring for desired traits
 - With natural mating would only have 100's of offspring

Advantages of Al (cont.)

Availability of sires

Sires anywhere in world







Advantages of AI (cont.)

Availability of sires

- Sires anywhere in world
- Danger of bull (male) removed
- Disease reduction
- Crossbreeding
 - Can try without buying sire
 - Designer animals

Crossbreeding









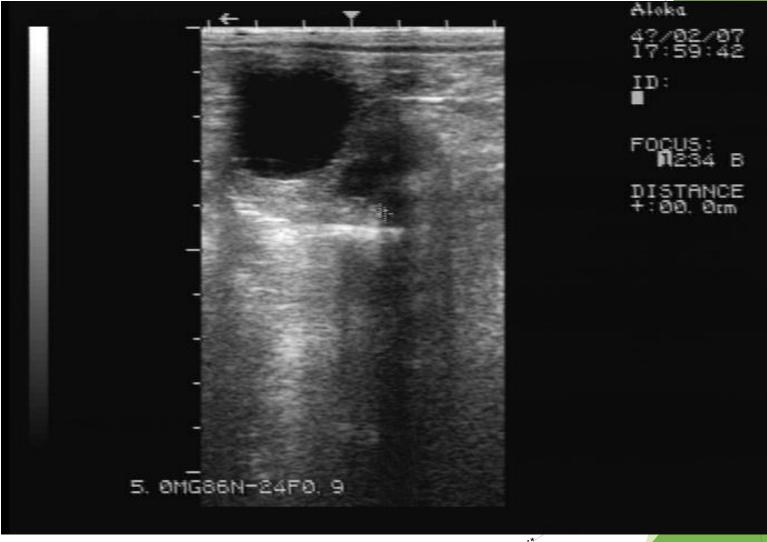


Disadvantages

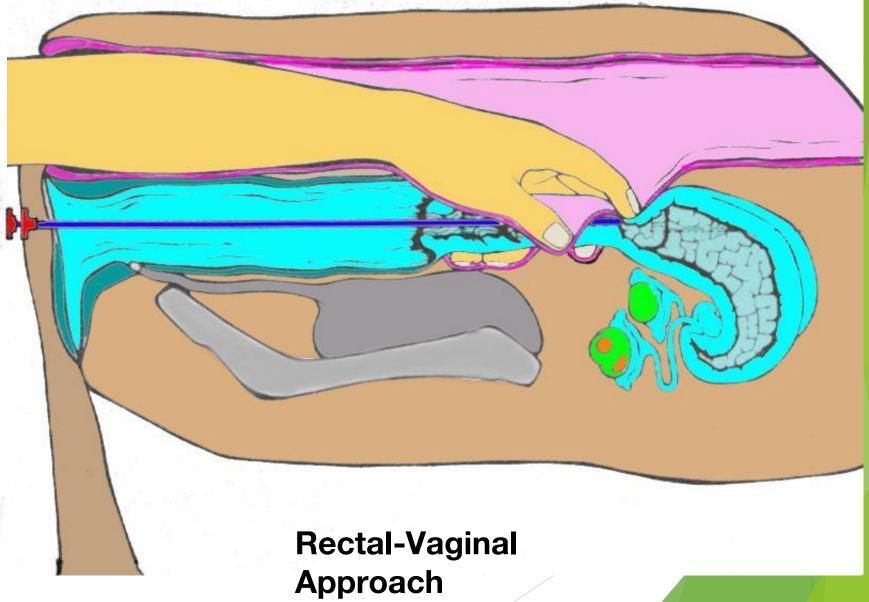
- Estrus detection must be good
- Trained inseminator
- Bull semen the best, other species not as good
- Use of poor male may increase if not tested well
- Technology to store cooled or frozen semen
 - Difficult to maintain



Follicle Size Determination







Factors Effecting Conception Rate

- Time of insemination
 - If after ovulation then get aging of oocytes
 - Exception is the dog
- # of sperm inseminated
- Fertility of males
- Skill of inseminator

Use and Success of Al

Species Liquid		Frozen	Preg. R	ate Maj	or Problems
Dairy Cattle (Heifer/Dry) (Lact. Cow)	OK OK		OK Fair	60-70 Logi 20-35 Do r	istics of timed Al not show heat
Beef Cattle	OK		OK	55-65 Ran logistics of t	ge area larg <mark>e;</mark> imed Al
Sheep OK		Fair	50-65	Large range; of ewe	low value
Swine OK		Fair	65-90	Estrus detec Al not practi	tion, timed
Horses OK		Fair	30-60	Timing insemination, breed restrictions	
Turkey OK		Poor	90	None	
Humans OK		Fair	5-30	Donors; infe	rtility; time
Dogs OK		Fair-Go	od	30-90 Froz	zen must be IUI

Optimal Insemination time

- Its appropriate that the insemination is conducted from 7 -8 hours before the start of heat(from the start of standing heat) to the end of heat.
- ► To inseminate before this is too early.
- Even after the end of heat, a successful insemination is possible for up to 4 hrs but after tis time conception rate is low by the death of embryo due to aging of oocyst.
- In practical AI opt.time decided according to the interval from the time of detection of heat(the time of start of heat) and the condition of follicular growth observed by rectal palpation.

- By rectal palpation- wall of the follicles have elastic hardness and wall of follicles have some thickness in the middle of heat.
- At the end of heat and near the ovulation follicles are 1.5 - 2 cm in size. Walls become thin, hardness disappear and become soft and fluctuant by rectal palpation.
- When follicles show these conditions-most appropriate time for insemination.

Thanks

Embryo Transfer Technology

Introduction

- Embryo transfer is one step in the process of removing one or more embryos from the reproductive tract of a donor female and transferring them to one or more recipient females.
- Embryos also can be produced in the laboratory via techniques such as in vitro fertilization or somatic cell cloning. But the actual transfer of an embryo is only one step in a series of processes that may include some or all of the following

Process includes

- superovulation and insemination of donors.
- collection of embryos.
- Isolation.
- Evaluation.
- short term storage of embryos.
- micromanipulation.
- Genetic testing of embryos.
- Freezing of embryos.
- Embryo transfer.

Steps for Embryo Transfer in Cattle

- Virtually all commercial embryo transfers use nonsurgical recovery of the embryos rather than surgical techniques. The process involves several steps and considerable time as well as variable expense.
- 1) Selection of the Donor Cow
 - The first step is selecting a donor.Beef producers will differ in their opinions regarding the criteria for selecting a genetically outstanding cow.
- 2) Superovulation of the Donor Cow

Superovulation of the donor cow is the next step in the embryo transfer process. Superovulation is the release of multiple eggs at a single estrus.

Cows or heifers properly treated can release as many as ten or more viable eggs at one estrus.

Approximately 85 percent of all normal fertile donors will respond to superovulation treatment with an average of five transferable embryos.

Some cows that are repeatedly superovulated at 60day intervals may produce fewer number of eggs over time.

- 3) Insemination of the Cow Because of the release of many ova from multiple follicles,
- there is a greater need for viable sperm cells to reach the oviducts of the superovulated females.
- Therefore, many embryo transfer technicians will choose to inseminate the cow several times during and after estrus.
- One scheme is to inseminate the superovulated cow at 12, 24 and 36 hours after the onset of standing estrus.

Using highquality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The correct site for semen placement is in the body of the uterus. This is a small target (1/2 to 1 inch) just in front of the cervix. • 4) Flushing the Embryos

To collect the embryos nonsurgically, a small synthetic rubber catheter is in serted through the cervix of the donor cow, and a special medium is flushed into and out of the uterus to collect the embryos seven days after estrus (Figure 1).

▶ 5) Evaluation of the Embryos

As the individual embryos are located using a microscope,

They are evaluated for their quality and

Classified numerically as to the potential likelihood of success if transferred to a recipient female.

6) Selection and Preparation of Recipient Females

Proper recipient herd management is critical to embryo transfer successfully.

Cows that are reproductively sound, that exhibit calving ease and that have good milking and mothering ability are recipient prospect

They must be on a proper plane of nutrition (body condition score 6 for beef cows and dairy body condition score 3 to 4).

Updated

► These cows also must be on a sound herd health program.

7) Transfer of the Embryos

The transfer of the embryo into the recipient cow first requires "loading" the embryo into a 1/4mL insemination straw. This is done under microscopi c viewing with the aid of a 1mL

syringe and requires

considerable practice, patience and dexterity. Deg enerated embryos or embryos of very low grade ne ed not

be loaded and can be discarded. Just prior to emb ryo

transfer, the ovaries of the recipient are palpated rectally to determine which ovary has ovulated.

8) Expected Embryo Transplant Res ults

 Embryo production varies greatly from do nor to donor and flush to flush. Average producti on is approximately six freezable (excellent an d good) and eight transferable (excellent, good, fair a nd poor) embryos per superovulation.

Thanks