What is artificial insemination (AI)?

Artificial insemination (AI) is the manual placement of semen in the reproductive tract of the female by a method other than natural mating.

It is one of a group of technologies commonly known as "assisted reproduction technologies" (ART), whereby offspring are generated by facilitating the meeting of gametes (spermatozoa and oocytes).

Advantages and disadvantages of artificial insemination

AI in animals was originally developed to control the spread of disease, by avoiding the transport of animals with potential pathogens to other animal units for mating and by avoiding physical contact between individuals. The use of semen extenders containing antibiotics also helped to prevent the transmission of bacterial diseases.

The advantages and disadvantages of AI are as follows:

Advantages:

- AI helps prevents the spread of infectious or contagious diseases, that can be passed on when animals are in close contact or share the same environment;
- The rate of genetic development and production gain can be increased, by using semen from males of high genetic merit for superior females;
- It enables breeding between animals in different geographic locations, or at different times (even after the male´s death);
- Breeding can occur in the event of physical, physiological or behavioural abnormalities;
- AI is a powerful tool when linked to other reproductive biotechnologies such as sperm cryopreservation, sperm sexing;

 AI can be used in conservation of rare breeds or endangered species.

Disadvantages:

1- Some males shed virus in semen without clinical signs of disease ("shedders").
2- Some bacterial pathogens are resistant to the antibiotics in semen extenders or can avoid their effects by forming bio-films;
3- Estrus detection must be good .
4- Trained inseminator .
5- Bull semen the best, other species not as good .
6- may increase number of inherited or lethal defect transmitted by carrier sire.
7- it need Technology to store cooled or frozen semen .

Steps of A.I.

1- collection of semen.
2- semen evaluation.
3- semen dilution.
4- semen storage.
5- inseminating cow

SEMEN COLLECTION

Various methods of collection of semen have been devised from time to time.

The older unsatisfactory methods have gradually been replaced by the newer modern techniques. The three most common methods are:

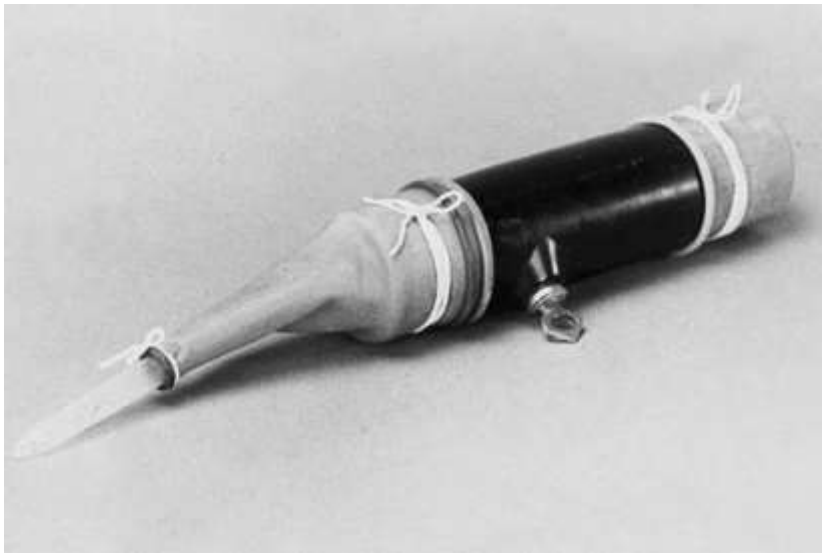
(1) Use of an Artificial Vagina (A.V.),

(2) -by Electro-stimulation and

(3) by massaging the ampula of the ductus

1- The Artificial Vagina

Artificial vagina is used to collect semen from many species, most prominently cattle and horses, but also sheep, goats, rabbits and even cats.



The AV consists of a strong outer rubber cylinder containing a latex liner. At one end of the AV a latex extension cone carrying a graduated collecting tube is attached. The length of the AV should be adjusted so that the bull ejaculates into the extension cone, thereby producing semen that is as free as possible of particulate or bacterial contamination. The space between the outer cylinder and latex liner is filled with warm water, so that the temperature in the lumen of the AV is between 45°C and 48°C. If this temperature falls below about 4Y C ejaculation is unlikely.

The main stimulus to ejaculation is the temperature of the AV but its pressure upon the bull's penis is relatively less important. A little inert lubricant (liquid paraffin or soft paraffin) is placed in the lumen of the AV just prior to use.

An AV uses thermal and mechanical stimulation to stimulate ejaculation.

ADVANTAGES OF USING THE A.V.:

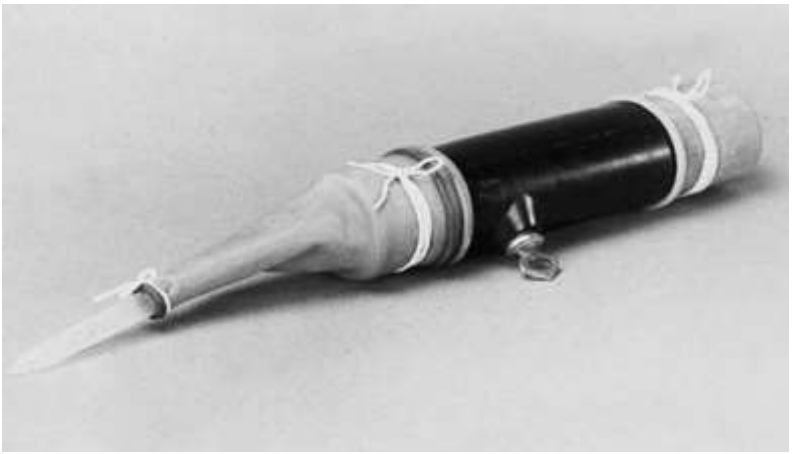
1. Basically a "natural way" to collect semen
2. Clean
3. Collects good volumes

DISADVANTAGES OF USING THE A.V.:

1. Takes a lot of time, especially in breeds with decreased libidos
2. Bulls need to be trained
3. Increase in labor
4. Cannot be used with structurally unsound bulls
5. Hazardous to the collector

Requirements of AV:

a. Pressure: By filling water and a little air into the space between casing and inner liner.
b. Temperature: By warm water of 45 C at collection (temperature of water at filling: 55 to 60 C).
c. Lubrication: Around front area of liner with sterile and harmless lubricating jelly



Sexual stimulation prior to collection with the artifical vagina

A. Reasons for sexual stimulation:

1) to insure that bull will mount and ejacualte normally.

2) to insure the collection of maximum number of sperm with highest possible quality per ejaculate.

B. Method of sexual stimulation:

1- By exposing bulls to teaser for several min.

2- 2 to 3 false mounting

2- Electro-ejaculation

Principle:

Electrically stimulating nerves to male reproductive system.

Electro-ejaculation involves applying a series of short, low-voltage pulses of current to the pelvic nerves which are involved in the ejaculatory response.

Equipment:

A bipolar electrode and a variable source of alternating current: voltage: 0 - 30 V, amperage: 0.5 - 1.0 A.

ADVANTAGES OF USING THE E.E.:

1. Can be used with a wide range of bulls and rams

2. Can be used on untrained bulls and rams

3. Usually good collection volumes

4. Can be used on bulls unable to mount

5. Can be used for initial inspection of bull's penis and sheath, to check for any growths or extension problems

6. Can be used on bulls with decreased libido

7. electro-ejaculation is the only technique useful for collecting semen from wild animals, in which case the male is anesthetized prior to the procedure.

DISADVANTAGES OF THE E.E.:

1. Possible injury of the bull due to confinement

2. Increased incidence of spermless ejaculates

3. Possibility of muscle tetany

4. Semen quality as compared with AV ejaculates: larger volume, lower in sperm concentration, sometimes semen contamination by urine

Evaluation of semen

- Semen must be evaluated immediately after collection.
- After collection of semen, the ejaculate evaluate by two main types of examinations, visual, microscopic and chemical examination.

Visual examination or Appearance:

Semen evaluation should be as rapid as possible so that the semen sample collected is processed further for preservation in shortest possible time.

1. Volume: a graduated test tube used for semen collection, therefore the volume of ejaculate can be determined immediately after semen collection. The average of volume is about 2,5-4,5 ml and sometime 8-10 ml.
2. Color : Normal and fresh semen appears as a thick whitish to slightly yellowish fluid. The thickness of the semen sample is a reflection of the number of sperm present.
3. Odor : There should be no odor associated with the semen sample. Presence of odors indicate of an infection or the presence of urine.

- Some abnormality can be detected in the color of the semen as blood, urine and feces can cause the semen to be pink to brownish.
- White clumps or flakes indicate pus and the presence of an infection in the reproductive tract of the male.

4- Density or consistency of semen should normally be thick opaque but variations are also noted ranging from the watery to thick creamy and opaque. This again depends upon the frequency of collection. The volume, and consistency changes according to frequency of collection body size and age, nutrition, exercise, etc.

Microscopic examination of semen:

1. **Motility:**

- Motility is very important parameter and it highly correlated with semen fertility.
- Motility estimated by light microscopy is by now the criterion mostly used for the assessment of semen quality, especially for routine semen evaluation in AI laboratories.
- There are two type of motility examination:

A- Mass Motility:

- This motility is a result of movement of many sperm in one direction. The motility can be observed in the form of recurrent swirling waves.
- This examination can be done by placing a drop of semen on the slide and examined under low power.

B- Individual Motility:

- The ideal motility of sperm is progressive, forward motility with characteristic swing of head and tail. But during the examination other forms of motility are seen.
- Moderate damage sperm may swim around in circles or backwards.
- More severely damaged and dying sperm roll from side to side.
- The test can be done by placing a small drop of semen on slide and diluted with normal saline and covered by slide cover and observed at high power.
  - https://www.youtube.com/watch?v=MOJVyBC0Fvc

2. Live-dead sperm:

The principle of these techniques is depending on using vital stains. The stains cannot penetrate through the intact membrane, but if the plasma membrane is damaged the stains enter the sperm and bind to the nucleus.

-The combination stain of eosin – nigrosine is most commonly used. This stain is used to evaluate both sperm dead & live and sperm morphology.

-Fluorescence microscopy can be used to evaluate the sperm viability by using fluorescence supravitally staining techniques such as SYBR-14 and Propidium iodide.

3. **Acrosomal integrity** :

Acrosomal integrity can be estimated using fast green fast. This stain is color the intact acrosome and can be tested using the light microscope.

Fluorescence microscopy can be used to detect the acrosome integrity using some fluorescence stain such as Lyso Tracker Green.

4. **Sperm concentration:**

The concentration of sperm in the semen is needed to determine how to dilute semen and provide adequate sperm in each breeding dose. The number of sperm ejaculated varies greatly among males but is dependent on age, size of the testes. The sperm production on any day is also dependent on the collection frequency and the intensity of the sexual stimulation.

While young bulls produce from 2 to 5 ml of semen, older mature bulls might produce 6 to 15 ml. The concentration of sperm in semen is usually between 500 to 2,000 million sperm/ml Holstein bulls.

The concentration of sperm can be estimated manually using hemocytometer or can be quantitative evaluated by measurement of the differentiation in the optical density of the semen sample using the spectrophotometer.

To calculate the number of sperm per ml of original sample:

1. Calculate the mean number of sperm counted for each chamber (i.e. for each of the central counting areas of each chamber).
2. Multiply the mean obtained in (1) by 10,000 to obtain the number of cells per ml of diluted sample.
3. Multiply the count obtained in (2) by the dilution factor.

Example: Assume that you dilute the original semen sample by adding 0.1 ml of semen to 9.9 ml of diluent (1:100 dilution factor). You then count the number of sperm in 5 of the 25 large squares within the central counting area of two chambers, (obtaining counts of 132 and 128 cells).

1. The mean number of sperm per chamber is thus 130 x 5 or 650 cells per counting area (650 cells per 0.1 microliter).
2. Multiply the 650 cells per counting area by 10,000 to obtain the number of cells per ml of diluted sample (answer = 6,500,000)

Multiply 6,500,000 cells per ml of diluted sample by 100 (the dilution factor) to obtain 650,000,000 per ml of original sample.

Morphology:

There is a high correlation between the fertility of semen and the percentage of abnormal sperm. The morphology of sperm can be studied using light and electron microscope.

The abnormalities in sperm morphology can cause decreased ability to reach the site of fertilization, fertilization.

There are two type of abnormalities:

-Primary abnormalities: These abnormalities occur due to disorders the germinal epithelium. This mean, it occur due to an effect during spermatogenesis.

-Secondary abnormalities: These abnormalities occur during ejaculation or in manipulation of the ejaculate including over heating, too rapid cooling or due to presence of water, urine or antiseptic in the semen.

-Primary abnormalities have more effect on fertility than secondary abnormalities.

The primary abnormalities include:

Microcephalic sperm, Macrocephalic sperm, Double head, Elongated or narrow head, Pyriform head, Round head, Double mid piece or tail, swelling of mid piece, Abaxial attachment, Abnormal acrosome, filiform neck.

The secondary abnormalities include:

Free head, Proximal or distal protoplasmic droplets, coiled tail, broken tail. Spermatozoa for morphological test are tested under the oil immersion.

Chemical methods of evaluation:

* pH :

pH of the semen can be tested approximately by taking a drop of semen in watch glass and adding a drop of bromothymol blue indicator and mixing it well by a bulb and capillary tube. The mixed content is drawn in capillary tube with the help of bulb and the colour is watched against a standard colour capillaries for different pH. The tube where exactly the colour matches is the approximate pH of semen.

The other method is with the help of pH meter. The single electrode pH meter is most suitable for this purpose and most accurate estimations can be done with the help of pH meter.

The normal pH of bull semen is 6.2 to 6.8 while that of buffalo is 6.8 to 7.2 . Excellent quality semen gives pH of 6.4 to 6.5 while poor quality semen shows pH towards neutrality. The pH of semen also decreases on time lapse between collection and measurement of pH since the fructose in the semen is broken down by spermatozoa under anaerobic condition converting it to lactic acid. The anaerobic conditions are usually expected to exist in narrow collection tube. The pH is likely to decrease .

Methylene blue reduction test: *is a very simple test used to measure the metabolic activity of the spermatozoa.*

- This test is based on the principle that the hydrogen ions are liberated during sperm metabolism which will reduce the blue colored methylene blue into colorless leucomethylene blue.
- The hydrogen ions are released due to the *dehydrogenase enzyme* present in active sperm.
- The time taken to change the color is directly related with the motility and concentration of the spermatozoa.
- More the motility and concentration will lead to more the hydrogen ion release which will cause less time taken by the methylene blue to colourless leucomethylene blue.

Other test for semen quality: Resistance to temp. shock:

1. Resistance to hypertonic salt solutions.
2. Osmotic pressure.
3. Catalas test.
4. Fructolysis rate test.
5. Respiratory activity.