Artificial Insemination

For

Veterinary Practitioner

By

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Semen of bull and Artificial Insemination

Introduction:

Although we can be confident that semen from artificial insemination organization is of high quality (as bull might be scientifically selected) semen may be damaged while in processing, during storage, during transportation or by mishandling in the field.

Whenever the quality of frozen semen is in question or fertility is depressed, a portion from semen lot should be evaluated by experienced person.

In most semen processing organizations, each ejaculate is evaluated immediately after collection for volume, spermatozoal concentration, the percentage of progressively motile spermatozoa and the speed of movement. Spermatozoal morphology is also examined at regular interval.

It is enough for semen in the field to evaluate from time to time the percentage of progressively motile spermatozoa, rate of motility and number of motile spermatozoa per inseminate. Additional tests may be used under especial circumstances.

Semen Compositions:

1- Sperm cell ⟹ testes
2-Seminal plasma ⟹ Accessory glands

1-Structure of Sperm:

A. *Head*:

a- Nucleus: contain genetic code.

b- Post nuclear cap : cover posterior portion of nucleus

c- Acrosome: cover anterior portion of nucleus and contain enzymes: hyalouronidase, corona penetrating enzymes and acrosin

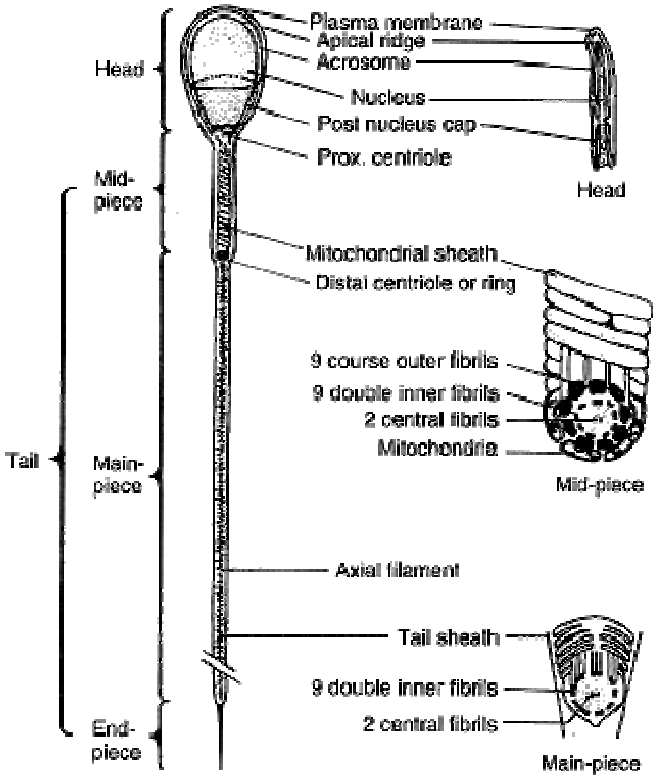
B .*Neck*:

Proximal centriole join head and tail which separate during fertilization and in heat damaged semen.

C. *Tail:*

a- Mid-piece: Mitochondrial sheath

b- Main- piece: Axial filament, inner fibrils and outer fibrils.



2- Seminal plasma:

a- Inorganic compounds: Na, CL, K, Ca and Mg

b- Buffering agent: Bicarbonate

c- Energy substrates: fructose, sorbitol, glycerylphosphorylcholine and citric acid

d- Antimicrobial constituent as seminal plasmin and EgA

e- Hormonal substances: androgen, estrogens prostaglandins, FSH, LH and other hormones.

Sperm Capacitation:

Physiological changes that make sperm able to penetrate the zona pellucida and fuse with the ovum.

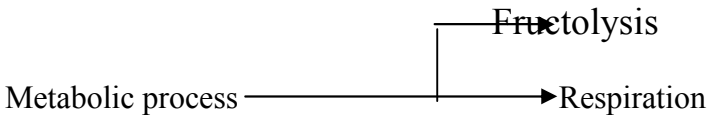
Semen Evaluation:

A-Macroscopic examination

B- Microscopic examination:

- Motility
- Concentration
- live/died
- Sperm morphology
- Maturity
- Acrosome morphology

Sperm Metabolism:



Factors affect the rate of metabolism:

1- Temperature :

a) Increase temperature leads to increase the rate of metabolism and decrease sperm life span

*if temperature reach 50 C this leads to irreversible loss of motility

*causes of death are due to exhaustion of substrate and decrease pH.

b) Decrease temperature leads to slow metabolic rate and extend fertile life if cold shock avoided

2-pH: optimum activity of sperm enzymes at pH 7.0.

3- Osmotic pressure

Storage of Semen

-Semen extended to increase its volume and extend itf fertility life
- Properties of good semen extender
- Constituent of extender
- Extension of semen in liquid form

Freezing of Semen:

A. Processing steps of frozen bull semen: Dilution، cooling، glycerolation & equilibration، packaging، freezing

B. Control of semen temperature: With placing semen in warm water bath

1-Semen dilution

A. Predilution: Warm semen with 3 to 4 volumes of diluter tempered in the same water bath ، Lecithin and lipoprotein from yolk of diluter: Protect sperm from cold shock and prevent changes in cell wall permeability during cooling process

B. Dilution: After cooled to 5°، the prediluted semen is diluted to final volume with diluter cooled to 5°

2- Dilution rate

A. Optimum number of motile sperm per breeding unit at the time of insemination: 10 million sperm (7-8 million sperm for high fertility bulls and 15 million for low fertility bulls), and % sperm with post-thaw intact acrosome may be involved in determining optimum sperm number per breeding unit

B. Post-thaw sperm motility of an individual bull: can be determined by post-thaw evaluations on several ejaculates of the bull

C. Breeding units = semen volume x sperm concentration x post-thaw sperm motility / 10 million

D. Dilution rate = Total volume of diluted semen (total volume of diluter added + semen volume) = Breeding units x diluted semen volume/ breeding unit (0.5 ml for 0.5 ml straw packaging)

3- Cooling semen

Optimum cooling rate: From body temperature to 5° for 1.25 to 2 hours (fast cooling) or for 2 to 4 hours (slow cooling)

4- Glycerolation and equlilibration

A. Optimum final level of glycerol added:

a. Yolk-citrate and tris buffered-yolk diluters: 7%
b. Milk diluters: 10 - 13%

B. Temperature at glycerolation: 5℃ in yolk-citrate and milk diluters; Glycerol damage when added at 35℃; No temperature problem in glycerolation for tris buffered-yolk diluter

C. Procedure for glycerolation:

a. Divide diluter into Part I and Part II of equal volume
b. Part I: no glycerol and semen will be prediluted with Part I
c. Part II: glycerol added at twice the level desired in the final mixture = 14% glycerol for yolk-citrate or tris buffered-yolk diluter and 20-25% for milk diluter
d. Prediluted semen will be diluted with Part I remainder and then further diluted by dripping slowly with Part II: 10%, 20%, 30% and 40% in the interval of 20 min.

D. Equilibration:

a. Equilibration time: needed for the sperm and diluter to completely mix: at least 1 hour
b. Temperature during equilibration: 5℃
c. Additional research is needed for optimum equilibration time

-5- Semen packaging: See Figure 16-3

Plastic straws:

a. Size: 0.5 ml with 113 mm long and 2.8 mm in diameter (In Europe 0.25 or 0.3 ml straws are used)

b. Straw preparation: One end of straw contains 3-parts plug: Cotton-Polyvinyl alcohol powder- Cotton; This PVA powder allow air to pass through until aqueous material (the semen) comes in contact with the powder and vacuum is used to siphon the diluted semen into the straw and when the fluid reaches the powder, it forms a gel and seals. Heat may be used to fuse the plastic open end.

c. Storage of straws in liquid nitrogen container: Placed in goblets within a cane after labeled to identify the donor bull and the semen producing business

6- Freezing

A. Freezing procedure: A single layer of straws are placed on a tray at 5.5 cm above the liquid nitrogen level; Straws will reach the temperature of liquid nitrogen vapor in about 2 min.

B. Optimum freezing rate:: -126°c/min to -7°c/ min.: satisfactory results: influenced by the type of package, glycerol level, thawing rate and diluter composition

7- Storage and handling of bull semen

Semen storage in liquid nitrogen container:

a. Double wall stainless steel or aluminum container with a vacuum between the walls

b. Field unit:
Liquid nitrogen capacity: 20 liters
Holding time: 90 days
Storage capacity: 1,200 straws of 0.5 ml capacity
Recharging interval: 60 days
Canister, cane and goblets: goblets should always be filled with liquid nitrogen

Evaluation of frozen semen

ASSESSMENT OF QUALITY OF FROZEN SEMEN:

1-Motility:

It is adopted 20% progressively motile spermatozoa as the minimum acceptable motility for frozen semen. The spermatozoa must show a reasonable rate of forward movement.

2-Number of motile spermatozoa per inseminate:

The number of motile spermatozoa per inseminate must be determined to know if too few or too many sperm are present for maximum reproductive efficiency. The optimal number of spermatozoa depends upon the breed and the fertility level of each bull.

As an average for all bulls the non return rate (NR) was lower for inseminates containing 5 million motile sperm compared with 10 or 15 million motile sperm after thawing. Fertility of bulls increased when the number of spermatozoa increased from 5-10 million.

Final conclusion that for most bulls each inseminate should contain at least 10 million motile sperm after thawing.

3-Acrosomal Integrity :

Since the acrosome is involved in the fertilization process, it has been postulated that spermatozoa with deteriorated or damaged acrosome are unlikely to be capable of fertilization.

A relatively high positive correlation has been reported for acrosomal integrity and fertility of Holstein bulls**.**

METHODS USED TO EVALUATE FROZEN SEMEN:

A minimum number of about five straws is required for this evaluation.

1-Motility:

The thawing bath**,** slide warmer, hot stage, phase contrast microscope and all glassware that will be in contact with semen are checked for cleanliness. All material in contact with the semen should be at 38°C. Two straws are removed from the liquid nitrogen, thawed, thoroughly dried and opened. Because of higher spermatozoal concentration, semen from each straw is diluted to final volume of 1 ml. with worm extender and gently mixed. A small drop of

semen is then placed on each end of slide, maintained on a warming plate and is covered with worm cover slide.

Semen at both ends of slide is viewed under phase contrast microscopy at approximately 480 magnifications. Motility is estimated to the nearest 5 percent.

2- <u>Number of motile spermatozoa per inseminate</u> :

if the sample meets minimal standards for spermatozoal motility, the number of motile spermatozoa per inseminate must be then determined.

For this. Three straws are removed from LN and their contents are thawed. The volume per inseminate is determined by aspirating the contents of the straws in a graduated syringe. Spermatozoal concentration is determined by hemocytometry.

The number of motile spermatozoa per inseminate is calculated as follows:

Number of motile sperm per inseminate =

Number of sperm/ml X inseminate volume X motility %

3- <u>Acrosomal Integrity</u> :

Acrosomal integrity is evaluated when fertility of the semen is low but the sample meets minimum standards for motility and number of motile spermatozoa per inseminate.

Acrosomal integrity can be estimated in wet seminal smear under dark field microscopy. One hundred spermatozoa is examined and the percentage of spermatozoa with intact acrosome is determined.

Handling Frozen Bovine Semen in the Field

Most of fertility problems associated with the use of frozen semen are due to improper handling or deposition of semen by the technician. Herewith, we will discus how to minimize the reduction in fertility resulting from improper handling techniques for frozen semen packaged in straws.

Maintaining temperature of frozen semen:

For maximum reproductive efficiency, the temperature of semen should be maintained at -130 C or lower at all times. There are numerous opportunities for exposure of frozen semen to elevated temperatures from the time of freezing at the central artificial insemination organization to the time of thawing for deposition in the cow.

The unit in which the semen is received must contain LN. If the temperature has risen above -80 C the semen should not be used to breed cows because the fertility is greatly reduced.

Transfer of semen:

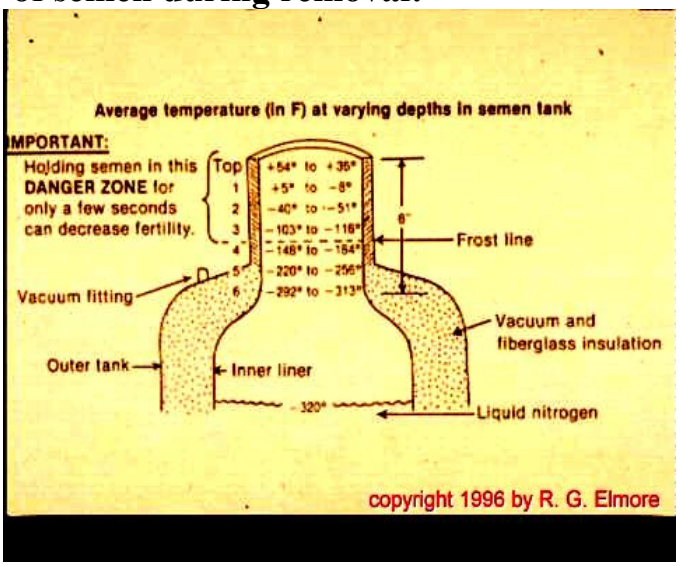
The first opportunity for improper handling of frozen semen comes during delivery or picking up. Semen can be exposed to elevated temperatures when it transferred from one tank to another. Seminal temperature rose more quickly within long straw with small diameter than the short one with greater diameter.

In the same time, semen expose to rise in temperature and immediately plunged back into LN, produce a reduction in post thaw motility which is a reliable measurement of fertility and that occurs more rapidly in small diameter straws.

So, straws must never exposed to ambient temperature, such as during transfer to storage tank. Plastic goblets are effective in reducing temperature changes within straws during exposure to ambient temperature.

Finally, We concluded that, straws should be transferred while in goblets containing LN, and the transfers should be completed as quickly as possible, away from wind and sunlight.

Exposure of semen during removal:



Semen may also be exposed to damaging temperature each time it is raised into the neck of tank to remove an individual straw for insemination. The temperature within the neck of tank is much higher toward the top of the tank. Since spermatozoa will be damaged at these temperatures, the canister containing the straws should be raised no higher than absolutely necessary, and the individual straw should be removed for thawing as quickly as possible. The damage for the rest of straws from exposure to elevated temperature is additive.

So, seminal temperature reached during exposure to the neck of the tank depends upon the height to which semen is raised, length of exposure, level of LN in the tank and the interval between exposures.

Therefore, , semen should be raised no higher than necessary to facilitate removal of straw for thawing and removal should be quickly as possible.

Thawing straws:

1- **Prepare the thawing bath containing water at 35 C**
2- **Remove the lid from the LN tank, identify the canister holding the straws that are to be used, lift the canister no higher than the frost line in the neck of the LN tank**
3- **With the thumb and forefinger, quickly grasp the tip of the goblet holding the straws and lower the canister back into the tank**
4- **With a long forceps grasp an individual straw from the goblet and lower the goblet to the bottom of the canister within the tank.**
5- **Immediately transfer the straw from the storage tank to the thawing bath. Where French straws should be held in the water for 6 seconds.**
6- **After removing the straw from the thawing bath, carefully dry the straw with taolit paper and inspect it for any cracks or defective seals.**
7- **Carefully clip one end of the straw with especial sharp cutter or scissors. The clip line must be straight**
8- **Place the straws into the funneled end of the plastic insemination sheath**
9- **Complete the parts of inseminating device**
10- **Inseminate the cow as will mentioned after**

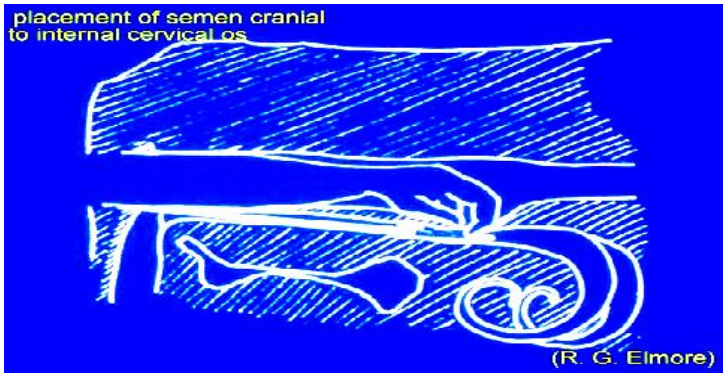
The temperature of the semen must be prevented from reaching excessive levels and the thawing time must be controlled. In the same time be careful and avoid condition that will reduce the temperature of the thawed semen.

Insemination by Recto-vaginal techniques:

Although it is somewhat difficult for the biginner but it is the method of choice for its high conception rate. The technique is done as follow:

1- introduce the left hand through the rectum and make pack racking, after that make dry cleaning of the vulva with taollit paper.
2- Grasp the cervix with lift hand
3- Insert inseminating instrument through vagina.
4- Hold cervix by its posterior end with index and middle finger and thumb, leaving the other two fingers free to help guide the inseminating instrument.
5- Guide the instrument into the opening of cervix and manipulate the cervix in all directions to pass the instrument through the cervix.

6- Move the fingers and thumb forward so that the manipulation is taking place just forward to the end of instrument.
7- Stop the instrument as soon as it reaches the anterior end of the cervix, and do not with draw the instrument, especially when the cow urinates.

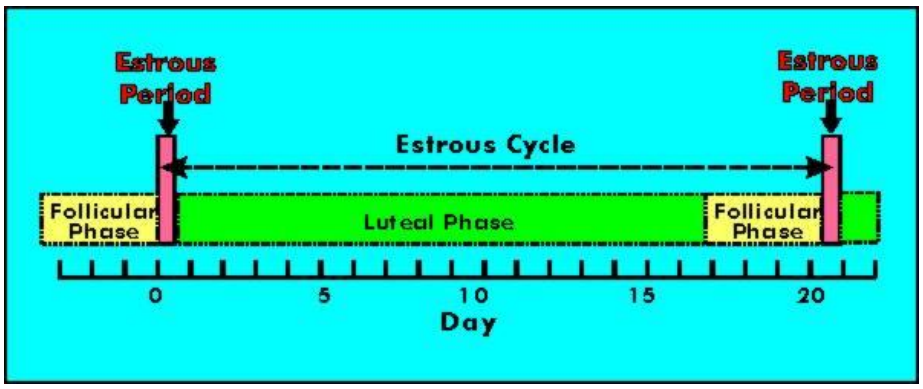


Some Problems during insemination:

1- Insert the instrument into vagina not to inter sub-urethral diverticulum or external urethral orffice
2- When muscular contraction force the reproductive tract toward anus and cause vagina to become folded, grasp the cervix with left hand and push it forward to straighten the vagina.
3- When the cow attempt to expel left hand from rectum with peristaltic muscular contractions, wait until the rectum will be relaxed.
4- When the cervix can not be grasped or manipulated through contractions of rectal muscles, cup the fingers over the handed rectal wall and pull the hand toward the anus to cause the contracted rectal muscle to be relaxed and softened.
5- When the vagina fills with air, dispel the air by firm pressure with the hand toward the vulva.
6- When the bladder is ex tremely full, manipulate the clitoris to cause urination.

Estrus Detection and AI of Cattle

Infertility occurs in all herds but reproductive problems may become more obvious with artificial insemination since mating related events are recorded and summarized. Animal attendants often blame breeding failures on reproductive abnormalities such as acyclicity, silent estrus, follicular defects or insemination deficiencies. However, the inability to detect estrus so that females can be mated at the opportune time is, although often overlooked, the most frequent cause whenever artificial insemination is the principal mating procedure. Those people responsible for executing insemination procedures need a comprehensive understanding of reproductive function and sexual behavior to appreciate how important their estrous detection activities are in contributing toward the success of the entire program.



The bovine estrous cycle usually covers 17 to 23 days so normal, nonpregnant cows should show signs of estrous behavior (heat) every three weeks.

A number of physical and behavioral signs may indicate that a cow or heifer is in or near estrus. Some of the things to watch for are:

Sexual Behavior

- standing for monting by a bull (heterosexual behavior)
- standing for mounting by another female (homosexual behavior)
- being mounted by another female but moving away rather than standing (homosexual behavior)
- attempting to mount another female (homosexual behavior)

Associated Behavior

- restless
- persistent licking or sniffing other animals
- chin pressing on rump or back of another animal
- When on pasture, animals coming into estrus may bellow persistently. Confined cows, however, rarely exhibit this vocalization behavior.

Other (Physical) Signs

- mucus discharges from the vulva
- swelling of a vulva
- reddening of vaginal mucosa
- soiling or roughening of hair covering rump and/or flanks

The best indicator of estrus is when any cow or heifer repeatedly stands and accepts mounting by one of her herdmates.