Storage and Shipment of semen

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Expected outcome

- Short term storage and long term storage of semen.
- Handling during and after thawing of frozen semen.
- Transport of liquid and frozen semen.
- Care and maintenance of LN2 containers.

STORAGE OF SEMEN

The aim of storage of semen is to prolong the fertilizing capacity of spermatozoa by reducing or arresting their motility and metabolic activity

SHORT-TERM LIQUID STORAGE OF SEMEN

- Semen → stored in **liquid media** which is maintained either at ambient temperature or in a refrigerator at **4-5˚C**.
- **Daily temp. fluctuations** → serious problems in maintaining fertile spermatozoa.
- **Ambient temperature storage** → requires prevention of microbial contamination and proliferation without impairment of sperm viability.

- Ambient temperature storage medium → carbon dioxide → reduced the rate of sperm metabolism.
- **Ilini Variable Temperature IVT)** extender, was saturated with carbon dioxide by bubbling the gas through an egg yolk –citrate extender.
- **CUE (Cornell University Extenders)**, utilize similar metabolic inhibition
- Caprogen extender was extensively use at a storage temperature of 18-24 C.
- The Caprogen extenders is a modified citrate buffered egg yolk medium containing Caproic acid, Glucose, Glycine, sulfacetamide, penicillin, and streptomycin and is saturated with **nitrogen gas.**
- **Fertility**→ maintained for about 3 days using the common ambient-temperature media.

LONG TERM STORAGE (CRYOPRESERVATION TECHNIQUE)

- The limitations of fluid storage of semen, have been over-come by cryopreservation.
- Polge *et al.,* 1949 → cryoprotectant properties of glycerol → preserving semen at ultra-low temperatures.
- *Spermatozoa of many species can be stored at liquid nitrogen temperatures (-196˚C) for indefinite periods.*

STORAGE OF FROZEN SEMEN

- Kept submerged in liquid nitrogen in liquid nitrogen container.
- *Liquid nitrogen should be maintained >15 cm and above.*
- Should be periodically topped with liquid nitrogen.
- Straws should be kept in a plastic goblet which in turn should be kept in canister.
- Same breed from different bulls should be stored with proper partition.
- Goblets must be slightly shorter than straws to enable quick removal of straws.
- The commonly used goblets are 12 cm in height.

- The straws should always be removed with pre-cooled stainless steel forceps.
- **Temperature increase** → is determined by length of time exposed, ambient temperature, air circulation, solar radiation, level of liquid nitrogen in container and height to which the canister is raised above the neck.
- If semen is to be transferred from one canister to other, keep both the canisters submerged in liquid nitrogen kept in a thermocool box and carry out the transfer quickly.

HANDLING DURING AND AFTER THAWING OF FROZEN SEMEN

- The straw should be given a jerk to remove all the liquid nitrogen attached over the surface.
- *Thaw the semen straw at 37 ^{o} C water bath either horizontally or vertically for 30 seconds.*
- After thawing the semen should be used immediately.
- The straw should be wiped thoroughly to remove all the water.
- The semen straw should be cut at laboratory seal end.
- The AI gun should be loaded correctly after pulling the plunger down.
- The sheath should be applied over the AI gun and the O-ring should be placed on the sheath.

SHIPMENT OF SEMEN

- Role of artificial insemination is much more valued when semen of bulls is transported to distant and wide spread areas.
- Semen collection stations are opened at important places from where semen is dispatched to all insemination centres.
- Fertility of bull semen transported to long distance was not deteriorated on first day of use.

SHIPMENT OF LIQUID SEMEN

- Transport should takes less time to reach the insemination centre and gives least jerks and jolts during transport.
- When semen is sent through rail or bus, proper instructions should be issued not to expose the container to sun.
- The container may better be marked **" Living Biological Product**" and "**Gentle Handling Not To Be Exposed To Sun**."
- Transport of semen with cycle is not advisable for more than 10 km during hot season.

Some of the precautions observed to minimize transport shocks, are as follows

- Semen vials should be maintained in jackets (glass or plastic) which are properly secured and capped.
- Sufficient crushed ice should be filled in thermos flask to maintain a temperature below 10˚C during transport. Thermos flasks of 4 to 8 pints capacity should be used for transport.
- In the case of semen preserved at room temperature (20˚C to 25˚C), transport is very convenient
- The transport of semen preserved at room temperature cannot be materialized without some cooling device.
- Iron or wooden crates may be used to protect thermos flasks and shippers from damage during rail or road transport.

SHIPMENT OF FROZEN SEMEN

- The two principal refrigerants used in experimental and routine shipping of frozen semen in recent years dry ice (Co2) and liquid nitrogen.
- Dry ice functions at -110˚ F, **(-79˚C.)** and liquid nitrogen at -320˚F, **(-196˚C),** and hence the latter has a longer safety factor.

LIQUID NITROGEN

- The major advantage of frozen semen is its capacity to remain uninfluenced over long distance transport.
- Another most important advantage is its storage potential extending over several years and the semen may be available even after the death of the bull.
- For storage and transportation of frozen semen, various sizes of liquid nitrogen containers are available.
- The frozen semen can be transported through road, rail or air.

- While transporting frozen semen the temperature should be maintained -196 ^{o} C.
- The liquid nitrogen holding capacity and evaporation rate depends on the size and type of container.
- The liquid nitrogen should be topped up during the transport of straws.
- The wooden crate or similar protective device like hard board or card board may be used during transport of semen to avoid shock to the container.

CARE AND MAINTENANCE OF LN2 CONTAINERS

- The cryogenic containers are double walled vessels with annular space evacuated and sealed. In addition several types of insulation
  - Vacuum alone
  - Expanded foam
  - Gas filled powder and fibrous materials
  - Evacuated powder
  - Evacuated superinsulation is used as thermal insulators.
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- The outer walls of the containers are made up of stainless steel, carbon steel or aluminum alloys.
- Welding or piercing the container wall is dangerous.
- Keep the container in upright position.
- Protect the container against shock and rough handling. During transport support the container with soft padding.
- Protect against direct sunlight or hot blowing winds.
- Avoid frequent cooling and warming of the containers. Thermal stress may cause so much strain within it, that the inner wall of the container may crack.

- *Appearance of moisture on the outer wall of the container is a sign of damaged container.*
- Do not dry the container meant for regular use.
- Evaporation rate → topping
- When vacuum disappears, the insulating capacity is lost.
- *Hence, containers should be handled very gently particularly during transport.*

ASSESSMENT OF LIQUID NITROGEN LEVEL

- The evaporation rate of liquid nitrogen varies
  - From container to container
  - Temperature of the room in which stored
  - Number of times the container is opened.
- ***Hence periodic checking of level of liquid nitrogen is essential. The minimum level of liquid nitrogen should keep the straws completely submerged in the liquid nitrogen.***

- To measure the level of liquid nitrogen, a dipstick (slender stick made of wood or metal) should be used.
- After 5-10 seconds take it out and wave in air.
- The atmospheric air condenses as a frost on the dipstick to the level of liquid nitrogen.
- Read the level one c.m below the end of frost line giving allowance for boiling of liquid nitrogen when dipstick is inserted.
- By using calibration chart the volume of nitrogen can be estimated.
- Frequent measuring leads to unnecessary evaporation of liquid nitrogen.

Weighment method

- Specific gravity of liquid nitrogen is 0.82. (one litre of LN_2 = 0.82kg or one kg of LN_2 = 1.22 litres by volume).
- Based on known weight of empty container and weight after filling liquid nitrogen, the assessment can be made.

THANK YOU