

(VCI/CVE/SBT-I)

VETERINARY COUNCIL OF INDIA

(Statutory Body of Government of India established under Indian Veterinary Council Act, 1984)



Continuing Veterinary Education (CVE) Programmes

Training Module on Handling of Frozen Semen

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Published and printed by: The Secretary, Veterinary Council of India, A-Wing, 2nd Floor, August Kranti Bhavan, Bhikaji Cama Place, New Delhi-110066.

Printed at: Creative Graphics, 88 DSIDC Complex, Okhla Phase-I
New Delhi - 110020. Phone: 26816824.

Preface

Consequent upon the decision of the Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India to implement the Continuing Veterinary Education (CVE) programmes, an activity of Professional Efficiency Development Scheme, through Veterinary Council of India as its nodal agency in the country, the Council has started implementing skill based training programmes for the registered Veterinary practitioners. Having conducted trainings on diagnosis of livestock and poultry diseases having zoonotic importance at the first instance, it has now been decided to impart training on Handling of Frozen Semen with the objective to improve upon the breeding efficiency in animals.

The role of Artificial Insemination (AI) in animals and use of frozen semen in achieving rapid genetic gain and in minimizing the risk of sexually transmitted diseases needs no emphasis. Presently, use of frozen semen for AI in animals is a common practice in majority of the field level Veterinary institutions. However, proper handling of frozen semen is known to be the single most important factor determining success rate of AI as the quality of the semen deteriorates rapidly due to inefficient handling during storage, transfer, retrieval, thawing and AI. In order to harvest the optimal benefits of the frozen semen technology, the persons engaged in its practice should have adequate skills and proper training in handling and use of frozen semen. This Module for a three-day training programme developed and finalized by the experts in the subject emphasizes on the procedures for handling and use of frozen semen towards improving breeding efficiency.

The contents of this Module are also available on the website www.vci-india.in.

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Training Module on Handling of Frozen Semen

1. Introduction

Artificial insemination (AI) in animals was the first applied reproduction technology developed in the early decades of last century. This novel technology still remains the only way to achieve rapid genetic gain on a wider scale in addition to minimizing the risk of sexually transmitted diseases. In the beginning, fresh chilled liquid semen, which had short life span was used for AI and was required to be used within 1-2 days of its collection and processing to obtain acceptable fertility rate.

Successful freezing of bull semen in 1950s using glycerol as a cryoprotectant had a revolutionary effect on the spread of AI as a means of breeding. The subsequent availability of liquid nitrogen (LN) as a cheap cryo-agent, prepared by fractional distillation of air, further boosted adoption of AI in cattle all over the world. Presently, frozen semen can be stored indefinitely if appropriate temperature is maintained at all the times as calves have been born from frozen semen stored in liquid nitrogen (-196°C) for two decades. This technology has allowed maximum use of genetically superior bulls and had facilitated international semen trade and exchange of germplasm for introduction of new desirable genes. It has also provided an option of using semen at any time and place and has almost eliminated the wastage of germplasm encountered with liquid semen.

It is reported that inspite of heavy initial investments on equipment and establishment of frozen semen laboratory, the actual cost in terms of each calf born is somewhat lower with frozen semen as compared to liquid semen. While it is possible to maintain frozen semen viable for indefinite period in a cryo-can at ultra-low temperature, the same is easily destroyed in a couple of seconds due to careless-/mis-handling. Other than initial quality of semen and an efficient heat detection system, proper handling of frozen semen is known

to be a major factor determining success rate of this time-tested technology. The good quality semen produced at a renowned semen production centre after being rightly deposited in the females, may not result in conception if the semen is mishandled at any stage. Being living cells, sperm are very delicate and highly prone to mishandling. During storage, transfer, retrieval, thawing and AI, sperm must retain their functional and developmental capabilities, if the objective of birth of a live, viable calf is to be achieved. In any AI unit, proper handling begins with locating the semen in the cryo-container and must continue until it is deposited in the female genitalia at the proper site and time.

Though the mistakes made in handling of frozen semen and AI equipment appear to be small, they have additive effects and conception rate may be more adversely affected than perceived. The most common error during handling of frozen semen is its exposure to temperature fluctuations or to high ambient temperature, which cause thermal injury to the sperms. This injury is irreversible and cannot be rectified by restoring semen back into the liquid nitrogen. In order to harvest the optimal benefits of this technology, the persons engaged in its practice should have adequate skills and proper training in handling and use of frozen semen in addition to access to the essential infrastructure for the purpose.

2. Critical changes in semen during freezing and thawing

At the time of freezing, besides other changes, the water component of the diluter freezes first which gradually makes the solution around sperm hypertonic. This helps in the movement of intracellular water to surrounding environment and consequently exposes the sperm to the state of hypertonicity which may cause damage to sperm membrane. The addition of cryoprotectant, egg yolk and proper rate of cooling minimizes this damage. The process is reversed during thawing when the water liquefies first and leaves the sperm in the hypotonic solution, which is more detrimental to sperm than hypertonicity. Proper thawing, however, exposes sperm to the

state of hypotonocyt for the minimum period of time and ensures higher survival of sperm after this process.

3. Pre-requisites for a successful AI programme using frozen semen

- a) Availability of specialised equipment including cryo-containers, AI guns, pre-sterilized AI sheaths, thawing units etc.
- b) Regular availability of liquid nitrogen (LN) at a reasonable price.
- c) Efficient heat detection system.
- d) Adequately skilled persons who are properly trained for handling frozen semen and AI.
- e) Perfect co-ordination between semen production centres, semen banks, AI centres/units and animal owners.

4. Semen Packaging

French medium (0.5 ml) and mini (0.25 ml) straws are commonly used as packing units for frozen semen. Temperature changes inside the straw depend upon its surface to volume ratio which is more favourable in case of mini straws with an additional advantage of lower requirement for storage space (Table 1). Therefore, the French medium straws are being increasingly replaced by mini straws for semen cryo-preservation all over the world. A standard practice in India is to have a minimum of 10 million progressive motile sperms per insemination dose of frozen- thawed semen. Assuming a survival rate of 50% during the freeze-thaw process, each straw prior to freezing contains at least 20 million motile sperms. Frozen semen is stored at ultra-low temperature (-196°) under LN in highly specialized, vacuum sealed, aluminium containers with an extremely efficient insulation system. The quality of frozen semen including its fertile life can be adversely affected if there is improper handling at any stage till AI, including the management of cryo-containers,

Table 1. Commonly used semen packaging systems

Packaging system	Surface area (~mm ²)	Surface to volume ratio	Remarks
0.5 ml French Straw	1152	2304	0.5 ml French medium straw and 0.25 ml continental straw have approximately similar surface to volume ratio but 0.25 ml French mini straw has higher surface to volume ratio. Accordingly, the freezing of semen is best in the French mini straws. Additionally, a frozen semen container can store nearly double the quantity of mini straws compared to medium straws. Conversely, the impact of mishandling of semen (temperature fluctuation) is more on French mini straws compared to other straws and one has to be more careful in handling French mini straws.
0.25 ml Continental medium straw	555	2220	
0.25 ml French mini straw	823	3292	

5. Frozen semen handling

Sperms are prone to damage due to temperature variations or exposure to high ambient temperature during transfer of straws among cryo-jars from sperm production centre to semen bank or focal points and field AI units.

5.1 Handling of frozen semen during storage

For proper identification and record of use of frozen semen of bull for AI, following details should be legibly printed on the semen packaging system viz.

- Centre of origin
- Breed
- Bull's name/number

- Date of collection or batch number

In the semen banks, the frozen semen is stored in the goblets of different sizes and capacities (Fig. 1). However, if required, the semen may be repacked as per the requirements of different AI centres where it is to be delivered. The direct transfer of goblets to field containers will ensure minimum handling of semen during transfer.

After the transfer of semen straws from storage container to another container, semen inventory in the container should be updated (Fig. 2).

Following tips may help in minimizing the damage to semen during transfer.

- Cryo- jars should be filled with liquid nitrogen prior to transfer.
- Keep the containers side-by-side and as close as possible.
- Remove the canister from its normal slot and have it in the centre of the cryo-jar.
- Transfer of semen among containers should only be done by goblet filled with LN as quickly as possible but not taking more than 5 seconds in any case.
- Handling of individual straws for counting/ transfer must be avoided.

5.2 Handling of frozen semen during shipment/transfer

During repeated transfer of semen straws (goblets) at different AI stations, the LN level in the container will go down. It is, therefore, essential that LN level in shipment container be appropriately maintained. Similarly, the container, which is receiving the semen straws, should also have proper level of LN.

If the frozen semen is packed in appropriate goblet as per the requirement of the field centre, the required semen packed in the goblet can be transferred directly to the field container with minimum handling. Frozen semen container at the AI centre must have following details on it.

- Complete identification of semen in each canister and goblet.

- b) Complete schedule of refilling of LN along with the signature of the person who has filled it. The proper level of LN in the container must be ensured by periodical checking of the level of LN (Fig. 3).

5.3 Handling of Frozen Semen during Thawing

The process of warming the frozen semen to bring it to the liquid state (from solid) is commonly referred to as thawing. Thawing is one of the most critical steps involved in handling of frozen semen. If performed improperly or without due care, the process can adversely affect survival of sperm similar to the freezing process. If the thawing is too slow, many sperm cells may possibly be damaged due to harmful growth of small intracellular ice crystals by re-crystallisation. Thawing at high temperatures may completely damage the semen if there is delay of a few seconds. The semen thawed at high temperature has to be used immediately after thawing to achieve acceptable rate of success. Following are the important tips for thawing of frozen semen:

- a) Always thaw one straw at a time.
- b) Use fresh clean water for thawing.
- c) Thawing can be done in an insulated thawing vessel preferably a wide mouth plastic thermos flask or an electric thawing device (Fig. 4).
- d) Time and temperature are important factors in proper thawing of semen. Generally, higher the temperature of thawing bath shorter should be the time of thawing.
- e) Water at a temperature of $35 \pm 1^{\circ}\text{C}$ for 50-60 seconds is most widely used for thawing of semen and is more practical under field conditions. After thawing, semen should be used quickly as the quality of semen deteriorates gradually after 15 minutes.
- f) Keep thawing vessel near the frozen semen container.
- g) Use an accurate thermometer of a standard make to measure the temperature of thawing bath. Don't guess the temperature. Similarly, the duration of thawing should be timed with a watch to avoid guessing.

- h) The quantity of water should be enough to cover the straw. If there is a doubt regarding intactness of the laboratory seal of the straw, it should be thawed in a vertical vessel with water level up to 4/5th height of the straw to prevent entry of water from the open end (Fig. 4).
- i) Once thawed, semen straw should never be returned into the cryo-jar as it cannot be re-frozen.
- j) Never use pocket, AI gun, air, ice cubes, rolling between palms/ fingers for thawing of semen.
- k) For performing door step AI, carry semen straw either in a small LN jar (and perform thawing at the spot) or in water bath at 35°C in a thermos but never on ice or in chilled water. Once thawed, use straw within 15 mts for optimum results.
- l) Semen must be protected from direct sunlight, heat, water, dust, wind, chemicals, acid, alkali and lubricants etc. during thawing, loading into gun and insemination.

Following are the critical steps during thawing of frozen semen:

- a) Remove the lid of the container.
- b) Remove the identified canister containing the desired semen from its storage position to the centre of the tank.
- c) Lift the canister just high enough to a position where straws are clearly visible. It should preferably be raised up to the lower end of neck tube but in any case not above the frost line, located 5-6 cm from the top of the neck (Fig. 5).
- d) Never raise the canister up to the top of the neck to prevent exposure of straws to the high temperature in this area commonly referred as 'danger-zone'.
- e) Select the desired straw and remove it by grasping with a tweezers (pre-cooled in LN vapours). Do not use fingers to pick straw to prevent frost bite and to avoid warming of straw.

- f) After giving a small flick to the straw to dislodge LN, if any, place it in the thawing water as soon as possible.
- g) Straw should be removed very quickly but not taking more than 5-10 seconds from the time the canister is raised into the neck tube. If the goblet is full of LN, a few extra seconds (45-50 seconds) may not pose any problem.
- h) If the straw can not be retrieved within 10 seconds, the canister should be lowered back into the container for at least one minute to cool it before bringing it up again.
- i) Immediately after the straw is placed in thawing water, lower the canister to the bottom of the container and adjust the canister to its storage position.
- j) Replace the lid of the cryo-container.
- k) Never put a straw once removed from canister back into it as once thawed, straw cannot be re-frozen.

5.4. Deposition of thawed semen into the female genital tract

Prior to retrieving semen, conduct rectal palpation of the female brought for insemination to ascertain the stage of oestrus and whether the animal is fit for insemination. Decide the sire to be used keeping in view the species, breed, breeding policy, production performance and pedigree of the animal. Identify location of the desired semen (canister/goblet) by referring to the semen inventory. This would prevent unnecessary searching and minimize handling of semen.

Once thawed, temperature of the semen straw should be maintained as near to 35°C as possible. Proper handling and correct placement of the thawed semen is the main source of variation among inseminators. All handlings of thawed semen should be done under shade or in a room. The right site for placement of semen is the uterine body, an area 2-2.5 cm just in front of the cervix. The entire process of thawing of semen and loading of straw into the gun followed by insemination of the cow should not take more

than a few minutes. The right time of insemination lies between mid and end of oestrus but breeding a few hours before the end of oestrus may be preferred. The AM/ PM rule is also followed by many practitioners. The animals coming in heat in the morning are inseminated in the evening on the same day. The animals detected in heat in the evening are inseminated next morning. Many buffaloes particularly during off-season have very short oestrus period (a few hours). Such animals should be inseminated as soon as reported or after ascertaining the stage of oestrus by rectal palpation. The essential precautions during AI include:

- a) AI equipment should always be kept clean, properly covered, away from dust and wind. Being living cells, sperms are sensitive to damage by foreign materials and temperature variations.
- b) Insemination gun and disposable plastic sheath should be compatible/ matching with the type of semen straw (French mini or medium) or be of universal type.
- c) Insemination gun should neither be too hot nor too cold.
- d) Lift the straw from the thawing vessel, dry it by gently wiping with paper towel, tissue paper, blotting paper or a clean piece of cloth. Water is spermicidal. Vigorous rubbing should be avoided as it may generate heat.
- e) Quickly re-check identification on the straw.
- f) Take out AI gun from its container, pull plunger of the gun backwards (downwards) by 12-14 cms.
- g) Move the air bubble in the straw to the laboratory end (if not already) by holding the straw from one end and giving it a slight jerk.
- h) Slide the factory end (double seal) of the straw into barrel of the gun as far as it goes. There is an in- built stopper to check it from going too down.
- i) Holding the gun loaded with straw vertically at eye level, cut the straw horizontally (at right angle) through the middle of the air bubble. The straw must be cut at 90° angle to ensure a good seal with the sheath to prevent loss of semen due to back flow.

- j) A pair of sharp scissors or a specially designed straw cutter may be used for this purpose.
- k) Take out sheath from its protective cover holding from the inseminator (split) end. Non split sheath is used with the spiral gun. Tighten the sheath over the barrel of the gun through the central hole of the locking ring and twist it down on the conical end. Tighten 'O' ring by twisting. If the sheath is loose or not fitting properly, semen may flow back.
- l) Sheath is one of the most important components of AI equipment. It holds the straw in place during expulsion of semen. It prevents contamination of gun and introduction of infection into the genital organs. Sheath should be of good quality, non toxic to mucous membrane, pre-sterilized and preferably individually packed. Sheath should never be reused.
- m) Press the plunger of AI gun until it just pushes the factory seal of the straw without any semen being oozed out.
- n) Once the AI gun is ready, it should preferably be covered with vaginal sheath in an aseptic manner.
- o) The cow-end of the vaginal sheath/ sheath / loaded AI gun should not be handled, touched or come in contact with any material. It must remain clean.
- p) Animal must be properly restrained for personal safety. But it should not be excited, frightened or made nervous as release of adrenaline may adversely affect transport of sperm.
- q) Taking normal precautions of rectal palpation, locate genital organs in the pelvic cavity.
- r) Gently remove as much dung as essential without causing the animal to strain or balloon i.e. sucking in of air. The ballooning or straining may make the process difficult and tiring.
- s) Ask the assistant to clean vulvar lips of dirt, dung and lubricants etc. using water and or dry wiping.
- t) The loaded AI gun is inserted into the vagina at angle of 30° between the vulvar lips, opened apart, until it reaches the cervix.

- u) The insemination gun should pass along the top of the vagina to avoid accidental entry into the urethral opening on its floor.
- v) Take the gun to the mouth of the cervix (firm and hard tubular structure), tear the vaginal sheath (if used) and glide the cervix over the gun or alternately hold the cervix firmly and guide the gun through the spiral folds of cervix maintaining a light forward pressure.
- w) The semen should be placed in the body of uterus just in front of the cervix. The site is easily recognized by change in consistency of tissue from hard and firm (cervix) to soft and spongy (uterine body).
- x) Once the gun is in the right place, slowly push the plunger to expel semen drop-wise taking at least 5-6 seconds. The opening of the gun should not be blocked by finger tip or uterine wall to prevent back flow of semen. If the semen is expelled too fast, it may form a stream with pressure, pushing the semen directly into one or the other horn. The number of sperms going into the opposite horn in such cases may be negligible. The chances of conception will be significantly reduced if ovulation is from the contra lateral side. The fate will be the same, if the semen is deposited too deep into one or the other horn. In addition, gun may cause injury to thin and fragile uterine wall during deep insemination.
- y) Withdraw the gun and check it for presence of blood, pus, infection, discharge or back flow of semen.
- z) Give a little massage to the genitalia and let the animal rest for a few minutes before sending it home. Complete the insemination record.
- aa) Dispose off sheath, straw and gloves sensibly into a closed bin. If littered around, these may serve as a source of infection for other animals visiting the premises.
- bb) In certain cases, it may be difficult to penetrate the cervix through its spiral folds/rings or constriction due to scar from previous injury. Sometimes gun may be lodged in the blind pouch of vagina around the posterior end of cervix giving a false impression of penetration into the cervix.

- cc) Heifers have small genitalia, it may be difficult to penetrate the cervix in heifers by inexperienced inseminators.
- dd) Always avoid excessive manipulation and forceful penetration. Injury and bruises may badly affect fertility.
- ee) It is better to deposit semen at the site reached easily, rather than excessive manipulation.
- ff) One should be gentle, careful and patient while performing insemination. There is no match to proper training, experience and dedication.
- gg) Immediately after the AI, necessary details must be filled in the AI card/AI register.

5.5 Common Errors in handling of frozen semen

- a) Exposing semen to elevated temperatures while removing straw from the container or during handling.
- b) Transfer of semen among containers without goblets filled with LN.
- c) Removing straws by bare hands without tweezers.
- d) Incorrect time and temperature of thawing.
- e) Using pocket or air thawing.
- f) Straw not dried completely after thawing.
- g) Improper loading of gun- non matching gun/ sheath/ straw.
- h) Too hot or too cold AI gun.
- i) Straw not cut at right angle and right place (middle of air bubble)
- j) Use of contaminated AI sheath or handling cow end of sheath or loaded gun...
- k) Back flow of semen into the sheath or barrel of AI gun.
- l) Excessive jerky movements to storage jars.
- m) Level of liquid nitrogen going down to critical level or complete evaporation of liquid nitrogen.
- n) Fast expulsion of semen while depositing into the female genital tract...
- o) Depositing semen too deep into the uterine horn.
- p) Not taking proper precautions for hygiene and sanitation.

- q) Lack of proper training and skill in handling of frozen semen
- r) Improper identification and record keeping

6. Management of cryo- containers/ tanks

A typical cryo-jar consists of two separate chambers: an inner holding chamber and an outer chamber.

A non-metallic (fibre glass) neck tube connects the two chambers. The inter-chamber space is filled with foil and special insulating material. To increase the insulation effect, a vacuum is created in the space between the two chambers. The jars have a special loose fitting lid to avoid build up of excessive pressure and prevent entry of air or moisture from the environment into the tank. There are two major types of cryo- containers being used in the semen processing (Fig. 6).

a) Pressurized cryo- jars

They are highly insulated, vacuum jacketed vessels generally with large capacities. They are pressurized and have valves (vents) for filling and dispensing. These jars have pressure gauge and safety valves to protect from excessive pressure build up. Such jars are mainly used as bulk LN storage tanks as a centralized facility at focal points or the production centres with heavy demand for LN. This type of cryo-containers is also used with computerized biological freezers.

b) Non pressurized cryo- containers

They are non- pressurized, double walled, vacuum jacketed vessels more like a thermos flask varying in capacity from small jars of 1.5 litres to wide mouth containers with a capacity of several hundred litres. The wide mouth containers are used for bulk storage of semen and for vapour freezing of semen straws. The large containers with narrow opening having rotatory

columns are employed for long term bulk storage of frozen semen with deferred use such as semen doses of bulls awaiting evaluation. The other category includes the most widely used cryo- jars for semen storage & transportation. They have narrow neck with grooves at the top to hold canisters. The number and size of canisters may vary according to the capacity of the jar. The canisters have small perforations at the bottom or on the sides for entry of LN. Semen straws are generally stored in plastic goblets of different sizes and capacity. The goblets filled with straws are stored in canister either as single or multiple levels. In field units, single level is used as it may be difficult to retrieve semen from goblet lying at the bottom when they are stored one over the other. Small jars of 1.5 to 2.0 litre capacity are more useful for doing AI at farmer's door step. They are easy to carry but have a short holding time. The other common category of jars includes those used for transportation and storage of LN. These jars have a very narrow neck without grooves for holding canisters. They have comparatively long holding time.

Cryo-jars appear to be strong and well built. But actually, they are delicate and highly prone to damage by mishandling. The fragile (non-metallic) neck bears the full weight of the inner vessel and its contents such as liquid nitrogen, canisters, goblets and stored material. A strong mechanical shock, jerk or swinging motion (for example at speed breakers) may act as a pendulum force that may cause crack, wrinkle or break in the neck tube leading to failure of the containers. With proper care and handling useful life of jars can be greatly increased.

6.1 Important precautions for handling cryo- containers

- a) Cryo- containers should be stored in clean, dry, cool and well ventilated room or area away from direct sunlight, heat, wind, dust, doorway etc. The jar should be properly identified. Each canister/ goblet should be labelled to help prepare the semen inventory.

- b) Jar should always be kept in upright position in a quite corner but clearly visible for regular monitoring of its performance and LN level.
- c) Excessive movement of the jar should be avoided. In any case it should never be carried or lifted on its side to avoid damage to the neck tube.
- d) Avoid mechanical shock, jerky movements, abrupt collisions or bumping of jars with corners, doors and other objects. Similarly, jars should never be pulled, rolled or skidded on the floor of a room or vehicle while loading, unloading or shifting places. Rough and careless handling may cause permanent damage to the jar.
- e) Do not store containers on bare concrete floor to prevent corrosion. It may be stored elevated on wooden planks, boards or thick rubber mats. Roller bases with wheels may be a better choice for storage and easy movement of large storage containers.
- f) The original lid of the jar should always be in place. Plugging the jar opening too tight may prove dangerous due to build up of excessive pressure inside.
- g) Appearance of frost or sweat on the container indicates a defective jar. It needs immediate attention. Level of LN should be measured with a marked dip stick. If the level is too low or if there is any doubt, the quality of semen should be evaluated by an experienced person using a phase-contrast microscope prior to transfer of the semen to another good container. If the container has gone empty (dry) the semen stored in it can not be saved.
- h) The cryo- jars used for transportation should have rubber mats at the bottom and circular rubber rings (belts) around to avoid collision and frictional damage. Transportation vehicle should have well- padded floor and roof cover. The vehicle used for transportation should have a good suspension (to absorb shock) and be driven slowly with extra care at speed breakers.
- i) To further prolong life, jars may be transported in well padded wooden boxes (with a small hole for escape of nitrogen).

- j) Welding or piercing wall of the container may cause permanent damage to it.
- k) Warm jars should be charged slowly by pouring in a small quantity of LN at a time to avoid splashing (boiling) of nitrogen and injury.
- l) Frequent cooling and warming of cryo-containers should be avoided. If possible, a jar should be cleaned and sterilized with formalin vapours once in a year.
- m) Holding time and evaporation rate of a liquid nitrogen container vary with its make and design, ambient temperature, wind, storage place, intensity and area of work and handling technique.
- n) Temperature pattern in the neck tube of a cryo-jar depends upon its make and design, level of liquid nitrogen in the jar and ambient temperature.
- o) The level of liquid nitrogen in semen storage containers should be monitored at least weekly. A container should preferably be refilled (topped off) with LN when the tank is half filled.
- p) Ideally, LN should always cover the straws stored in any cryo-container (16 cm if stored in a single level). However, semen may maintain fertility in LN vapours when the level of nitrogen goes down to less than 10 cms. In such cases, temperature increases rapidly towards the neck of the jar and the straws may be highly susceptible to thermal damage if retrieved for use.
- q) Although, one need not panic if the level of LN in the jar has gone low, steps should be taken for its immediate refilling prior to retrieving any straw for use. In all such cases of doubt, a sample of semen should be evaluated under a microscope for progressive motility which is fairly reliable. A non-motile semen is definitely damaged and should be discarded.

7. Safety Concerns while Handling Liquid Nitrogen

LN used for cryopreservation of semen is inert, colourless, odourless, non-corrosive and non-inflammable. But it is extremely cold (-196 ° C). The

vapours and gas released from LN are also extremely cold. Direct contact with any body part can cause frost bite/ cold burns. One litre of LN can release approximately 700 litres of nitrogen gas when warmed to ambient temperature. In a room or an enclosed area, this large volume of nitrogen can displace oxygen in the air to dangerous levels causing threat to the life of workers/ handlers due to asphyxiation. One or two breaths of nitrogen vapours or gas can cause unconsciousness and death if not attended immediately. Following precautions should be taken while working with LN:

- a) As far as possible, handle LN in well ventilated room/ area. Exposure to an oxygen deficient atmosphere (<19.5%) may cause dizziness, drowsiness, nausea, vomiting, excess salivation, diminished mental alertness, loss of consciousness and death.
- b) The habit of bowing head into wide mouth jar or over the semen containers to locate straws must be avoided.
- c) Avoid direct contact of body with LN for more than 1-2 seconds. Keep gas jar out of reach of children and untrained persons.
- d) If there is a frost bite, immediately flush with water and consult doctor.
- e) Wear protective clothing including special cryo- gloves while handling LN.
- f) Even a brief exposure of delicate tissues/ organs like eyes to LN vapours can cause damage. Eyes should be properly protected.
- g) If there is emergency, take the affected person into open area or a well ventilated room and give artificial breathing if necessary. Do not panic and call for medical help.
- h) If LN spills from a container by accident, mishandling or otherwise, leave the room/ area immediately keeping doors open and take stock of the damage only after a few minutes when it has completely vapourized.
- i) Secure the jar properly when being transported, particularly for door-step AI.
- j) Don't carry LN container in a vehicle if the windows are shut.

8. Procurement of quality semen

For optimum results following AI, it is strongly recommended that the frozen semen used must be procured from the laboratories which are following minimum standards for production of frozen semen (MSP) laid down by the Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India.

9. Diseases transmitted through the semen

Presence of any infection in the genital tract is important from the point of it being transmitted through semen. Semen could be infected by micro-organisms reaching it from the testes or any of the accessory organs, and it could be contaminated by micro-organisms present in urine, preputial cavity or the preputial orifice. Semen could also be contaminated by blood or tissue fluid extravasated into the urogenital system. Micro-organisms in the atmosphere, on the teaser animal, on unsterilised equipment, in the semen extender, and in the case of frozen semen, from LN container could also reach semen. There is a possibility of transmission of a number of pathogenic bacteria, mycoplasma or viruses through semen. According to International standards, territory/herd/donor animal/semen should be free from many such diseases.

As per the OIE, the diseases which can be transmitted through semen include Brucellosis, Vibriosis, Trichomoniasis, Infectious Bovine Rhinotracheitis (IBR)/Infectious Pustular Valvovaginitis(IPR), Tuberculosis Leptospirosis, Bovine Viral diarrhea (BVD) and Blue Tongue. In view of this one should be careful in handling of semen in respect of transmission of the above diseases.

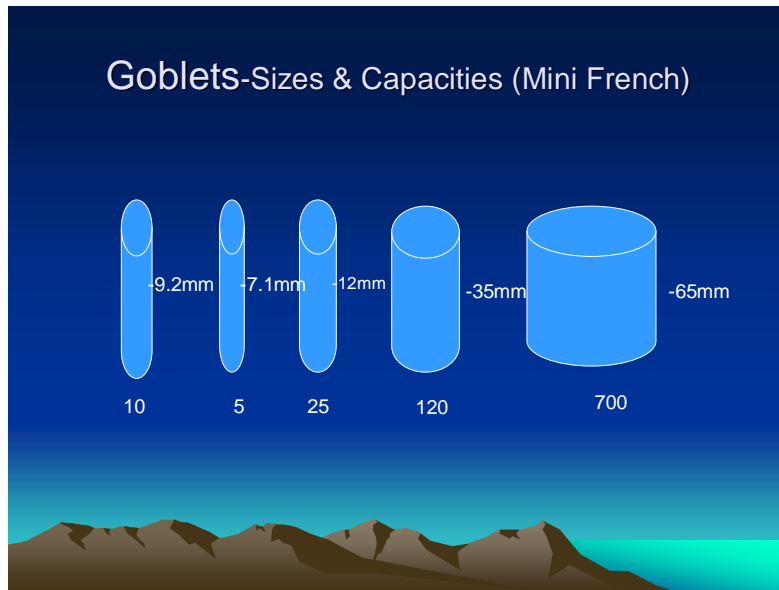


Figure 1. Different types of goblets used for storage of frozen semen

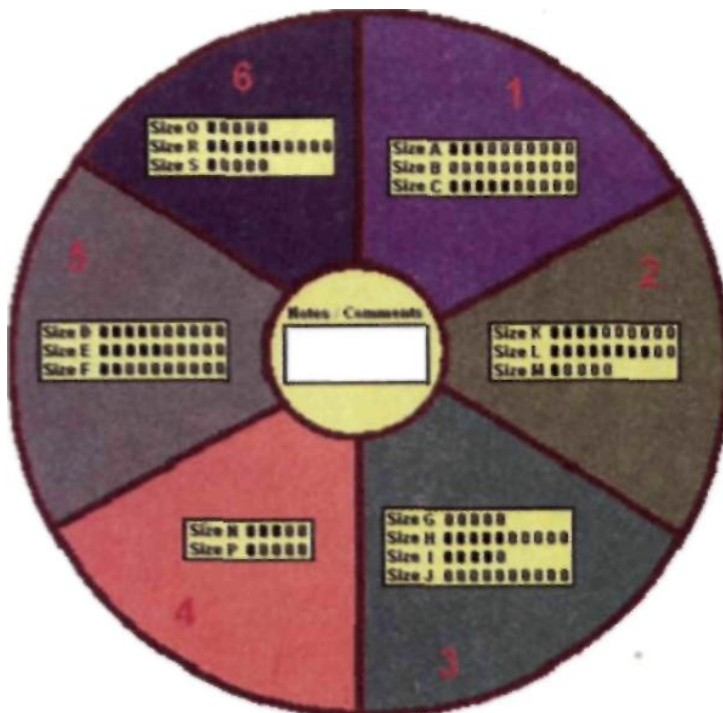


Figure 2. Semen inventory wheel (Sire number can be written on adhesive labels and attached to the wheel or written directly on the wheel with erasable markers. Circles represent straws of semen and can be darkened after each straw is used)

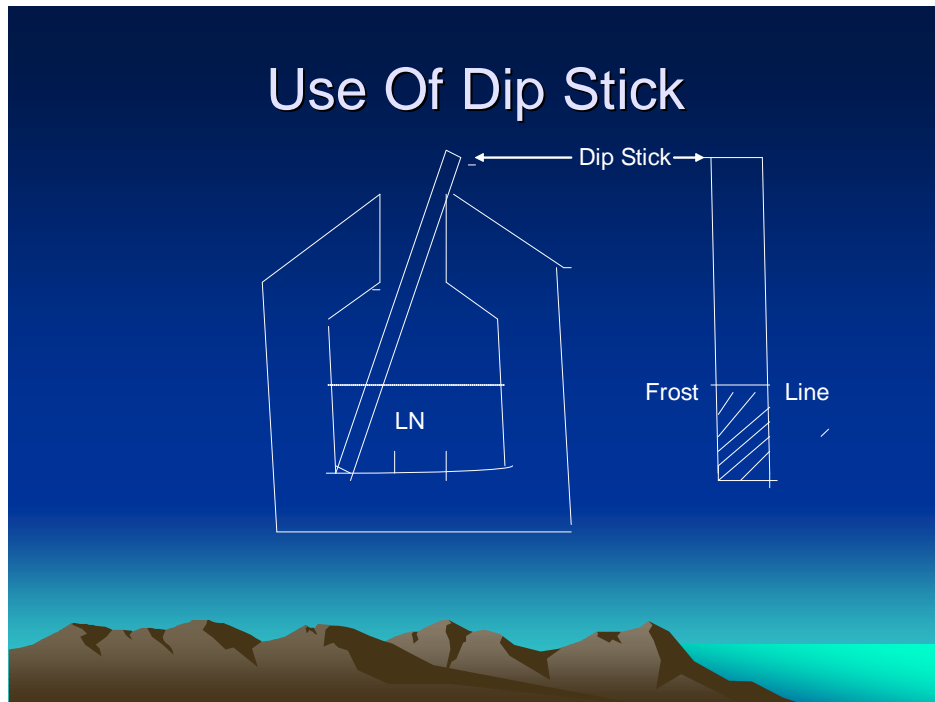


Figure 3. Use of dip stick to measure the level of LN in the container.

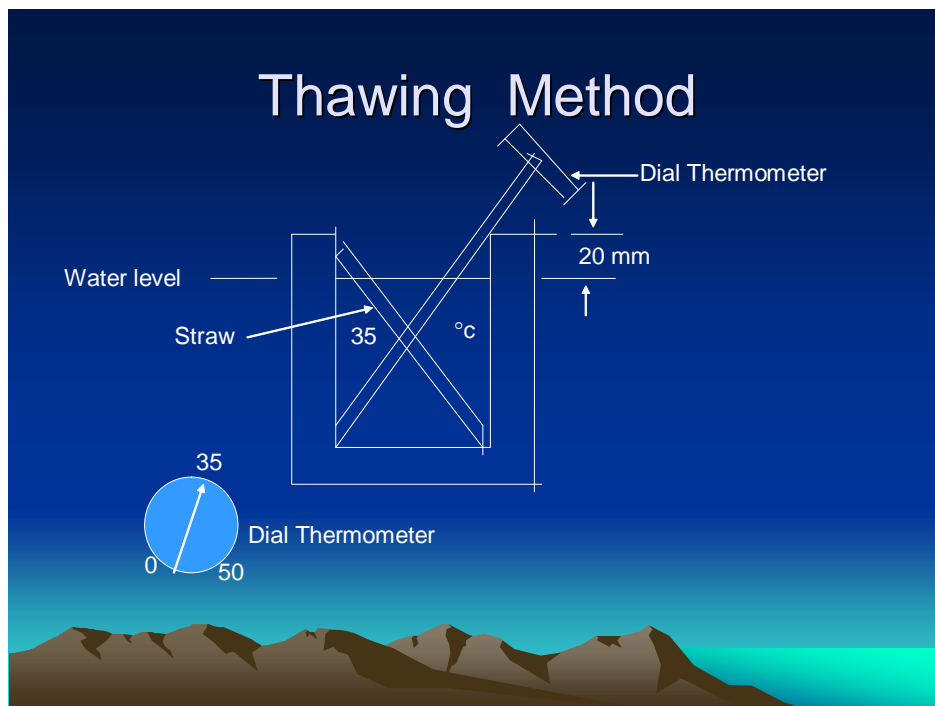


Figure 4. Correct method of thawing of frozen semen

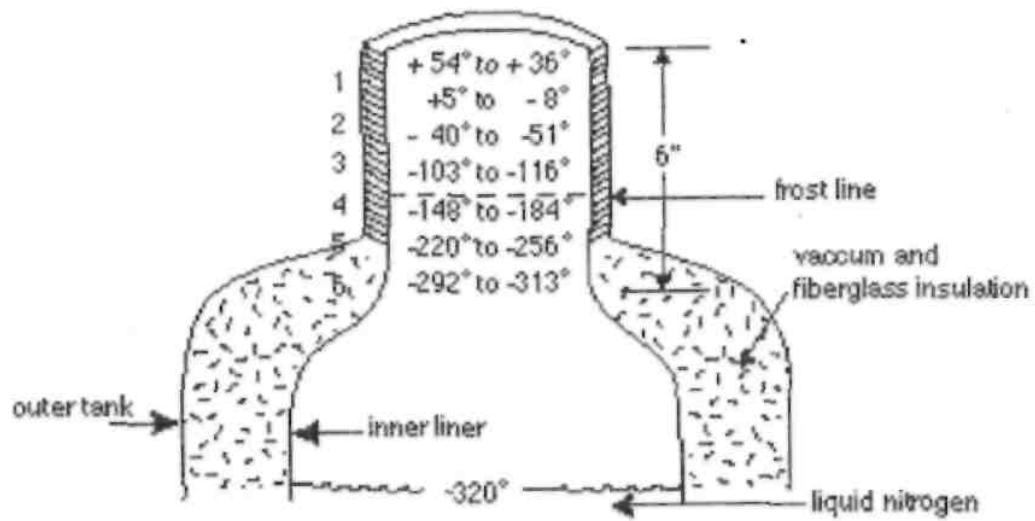


Figure 5. The typical range of Temperatures found in the neck area of a LN container (in °F)

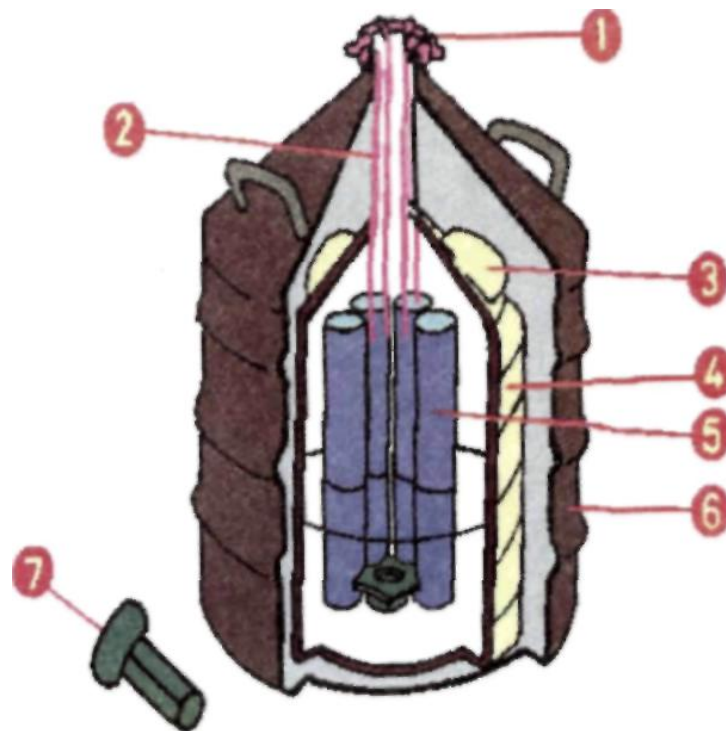


Figure 6. Vertical section of a frozen semen container. Index ring to hold canisters in position (1), Neck tube (2), Molecular sieve adsorbents (3), Inner vessels (4), Canister (5). Outer vessels (6) and Neck plug (7)