

# JOB ROLE – ANIMAL HEALTH WORKER

Sector – Agriculture

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Class X



PSS Central Institute of Vocational Education  
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# **UNIT 4: Implementation of Animal Breeding Services in Dairy Animals**

## **Session 7: Handling of Liquid Nitrogen Containers and Frozen Semen**

# Content

Title	Slide No.
Session Objectives	4
Introduction	5
Handling of Liquid Nitrogen Containers and Frozen Semen	6
Safety and Care during Handling Liquid Nitrogen Containers	7-11
Measurement of Volume of Liquid Nitrogen in LN2 Containers	12
Handling and Maintenance of an AI Gun	13
Handling of AI Sheath	14
Handling of Semen Straw	15
Handling of Semen Straw During Retrieval from LN2 Container	16-21
Summary	22

# Session Objectives

The student will be able to :

- Learn about the techniques of AI involving handling of liquid nitrogen containers and frozen semen
- Safety and care during handling liquid nitrogen containers.
- Measurement of volume of liquid nitrogen in LN2 containers
- Handling and maintenance of an AI Gun, AI Sheath and Semen Straw

# Introduction

Handling of semen and liquid nitrogen containers is a very important step in Artificial Insemination .

The metabolic activity of the semen needs to be retained for effective AI therefore it is stored in Liquid Nitrogen at extreme low temperatures (-196 °C) to retain the metabolic activity.

# Handling of Liquid Nitrogen Containers and Frozen Semen

Semen used for artificial insemination (AI) is stored at an ultra-low temperature ( $-196^{\circ}\text{C}$ ) in specialised containers called liquid nitrogen (LN2) containers.

The metabolic activities of semen cease at this temperature, which can be revived at the time of AI.

Liquid nitrogen (LN2) containers are double-walled containers. The walls of these containers are made up of high quality insulation material.

# Safety and Care during Handling Liquid Nitrogen Containers

The following aspects are taken into consideration while handling the liquid nitrogen containers

- (i) Avoid direct contact of LN<sub>2</sub> containers with the hard floor.
- (ii) Always keep LN<sub>2</sub> containers in a dry place, on a rubber or wooden plank.
- (iii) Always keep the containers in a vertical position in a cool and well ventilated room. Liquid nitrogen is non-toxic and noninflammable but continuous evaporation of LN<sub>2</sub> in poorly ventilated rooms leads to suffocation.

Cont...

- (iv) Avoid direct exposure of LN2 containers to sunlight or hot air.
- (v) Frost formation on top of the outer shell of the LN2 container and evaporation of LN2 is an indicator of vacuum loss.
- (vi) Do not tilt or roll the LN2 container as it may lead to spilling.
- (vii) The container should always be kept closed with the neck plug and lid to minimise the LN2 loss. LN2 containers should be opened only to retrieve the semen straw or filling LN2.
- (viii) Do not interchange the lid or canister of liquid nitrogen containers.

Cont...

- (ix) A loosely fitted plug may lead to excessive LN2 loss while tightly fitted one may damage the neck plug.
- (x) Avoid scrapping, welding, drilling or punching on the walls of the container.
- (xi) Use a funnel to transfer LN2 in to the container.
- (xii) Never overfill the container.
- (xiii) Use protective measures (gumboots and gloves) while handling LN2. Direct exposure of liquid nitrogen (-196°C) to body parts may lead to frostbite. In case of spillage, use plenty of water immediately to wash the affected part.
- (xiv) Use tweezer forceps for removing semen straw from LN2 container.

Cont...

- (xv) Regularly check LN2 level in the container with a wooden or solid metal dipstick.
- (xvi) Do not stack LN2 containers one above the other.
- (xvii) Utmost care must be taken while transporting and handling liquid nitrogen in public transport or gathering as sudden evaporation of LN2 may cause chaos leading to accidents.



Keep the container upright



Do not keep the container horizontally on the ground



Do not pour liquid nitrogen directly without using a funnel



Always use a funnel to pour LN2 from one container to the other



Routinely check the level of LN2, preferably, twice in a week



Do not stack anything over the container

# Measurement of Volume of Liquid Nitrogen in LN<sub>2</sub> Containers

Wooden or solid metal graduated dipsticks can be used to measure liquid nitrogen in the container



Dipstick is slowly immersed/inserted vertically in the container till it touches the bottom and left for 5 to 10 seconds

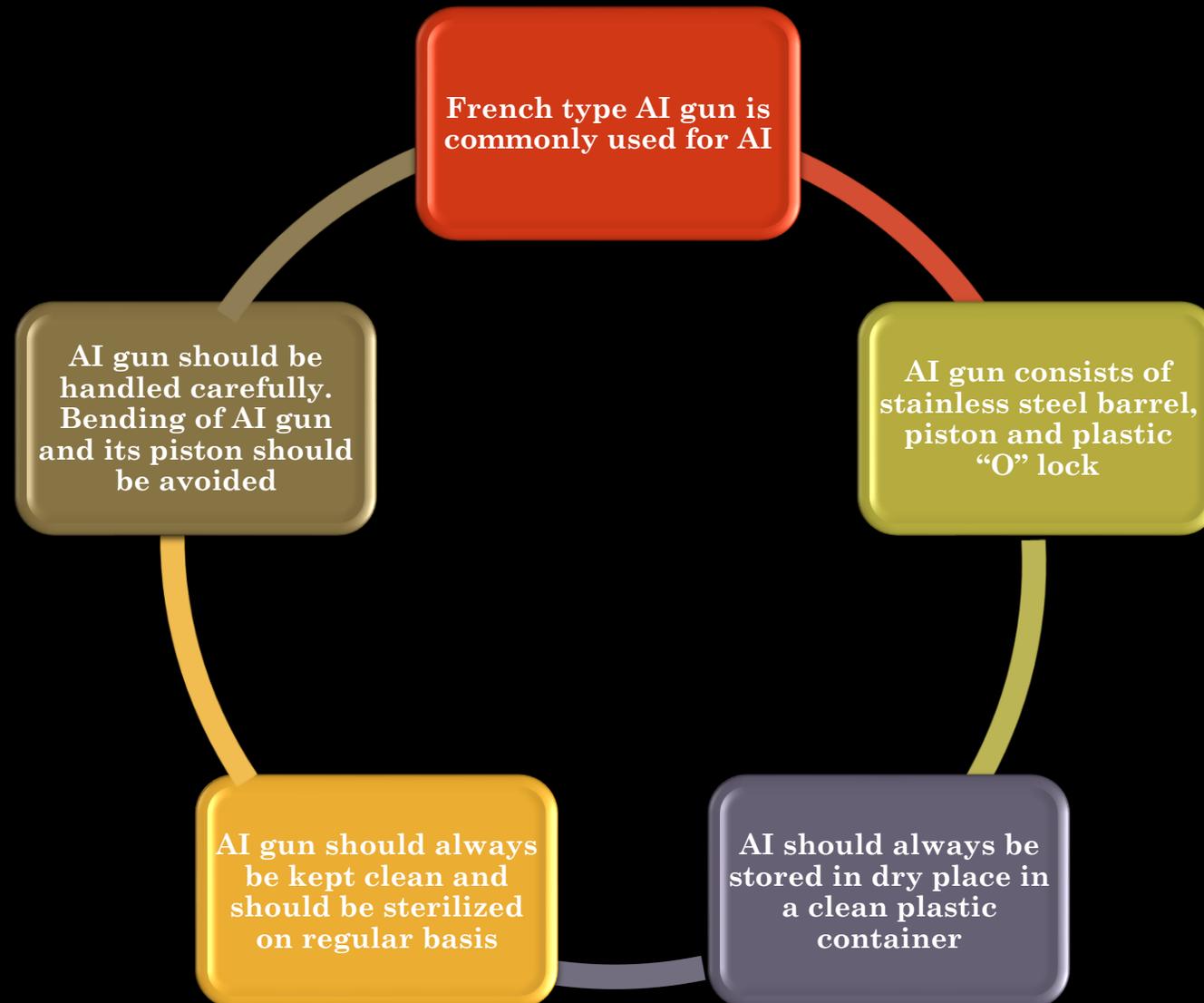


Dipstick is taken out from container and waved in air, lower meniscus at the middle of frost line is noted. Volume of liquid nitrogen is then measured by comparing the frost line reading with the calibration chart of particular model



More frequent measurement of liquid nitrogen should be avoided as it causes evaporation of gas. Dipstick is always kept hanging when not in use so as to prevent damage to the dipstick.

# Handling and Maintenance of an AI Gun



# Handling of AI Sheath

AI sheath are made up of plastic and should always be stored in clean and dry plastic container

AI sheath commercially available in single packing should preferably be used

Packing of sheath should only be opened at the time of AI

Touching of AI sheath should be avoided as it may lead to infection of female genital organs

Discard AI sheath if accidentally touched or contaminated with dirt, filth or dung

Protective sheath can be added over AI sheath to prevent genital infections

# Handling of Semen Straw

Frozen semen straws should be handled very carefully while retrieving from the LN2 container during insemination and also during transfer from one tank to another.

# Handling of Semen Straw During Retrieval from LN<sub>2</sub> Container

- (i) Semen straw should not be exposed to high temperature. The canister should be kept below the frost line of the LN<sub>2</sub> container during retrieval of the straw from the container or transfer of semen dose from one container to another.
- (ii) The canister from which semen straw is to be taken out should be identified before retrieval from the container.

Cont...

- (iii) Pre-cool the tweezer forceps in LN2 vapours before removing the desired semen straw from the canister.
- (iv) Semen straw should be removed within 10 seconds from the canister raised in LN2 container below the frost line. If the task of straw removal from canister is not completed in 10 seconds, then the canister should again be lowered in LN2 and lifted again to complete the task of straw retrieval.

- (v) Once removed, the straw should never be placed back into the container as exposure to room temperature will make it useless.
- (vi) The canister containing semen straws should immediately be lowered to the desired position after the straw retrieval.

# Handling of Semen Straw During Insemination

- (i) After thawing, the semen straw should be maintained at 35°C.
- (ii) Semen straw should be wiped with a tissue paper to prevent its rapid cooling.
- (iii) The straw should be shaken in air to move air space to the laboratory sealed end. This prevents semen loss while cutting the straw at the laboratory sealed end.

- (iv) Semen straw should be cut at a right angle and not obliquely, to prevent semen loss due to backflow while insemination.
- (v) Insemination with the straw should be performed within 15 minutes of thawing of semen.

# Precautions to be Taken While Handling Semen Straw During Transfer from One Tank to Another

- (i) Liquid nitrogen containers should be kept side by side before transfer of semen straw.
- (ii) Containers should be filled with LN2 up to an optimum level before transfer.
- (iii) Use an appropriate and compatible canister.
- (iv) Semen doses should be transferred from one container to the other within five seconds.
- (v) Semen straw should not be touched with bare hands while transferring from one container to the other as it may result in thermal injury to the semen.

# Summary

- In this session you have learnt about techniques of AI involving handling of liquid nitrogen containers and frozen semen
- Safety and care during handling liquid nitrogen containers.
- Measurement of volume of liquid nitrogen in LN2 containers
- Handling and maintenance of an AI Gun, AI Sheath and Semen Straw

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