

## Attachment 01

### **Guidelines for Artificial Insemination Activity**

#### 1. Storage of Liquid Nitrogen

- i. Store the liquid nitrogen (LN<sub>2</sub>) storage tank in a well - ventilated cool and dry place and in a clearly visible manner.
- ii. Storage area should be free from dirt such as dust and should be away from direct sunlight and moisture.
- iii. Use clean water and a mild detergent to clean the surface of the tank. (avoid using harsh acidic or alkaline detergents)
- iv. Use a trolley or a wheeled support to move the tank from one place to another.

#### 2. Storage of frozen cattle semen doses

- i. In order to maintain the temperature of the liquid nitrogen tank at 196<sup>0</sup> C, liquid nitrogen should be maintained at the level not less than 10 inches ( 25 cm) from the bottom.
- ii. Liquid Nitrogen level should be measured weekly. For this purpose the special measuring stick should be used.
- iii. In order to maintain liquid nitrogen level of the container where deep frozen semen are stored, it should be filled with liquid nitrogen at least once in every two days.
- iv. Deep frozen semen should always be stored in goblets with the factory end (the cotton plugged end) down wards.
- v. For easy identification of the breed, a proper practice like having a mark on canisters should be adopted. Through this it can be reduced the temperature of deep frozen semen getting changed when lifting up for identification.
- vi. The canister should be kept 2-3 inches below the open of the container when the canister is lifted up to take cattle semen out. ( care should be taken to keep immediately below the freezing point of the tank neck)
- vii. Before taking out the deep frozen semen dose, it should be kept a while near the opening of the liquid nitrogen tank until tweezer end reaches the relevant temperature.
- viii. Having lifted the canister which contains relevant semen straw, only the semen straws doses in the relevant goblet should be taken out as soon as possible using the tweezer

#### 3. Usage of Field Liquid Nitrogen Containers (21/1.5/11)

- i. Containers should be kept in a secured cover during transport
- ii. Should be filled with liquid nitrogen daily.
- iii. Always liquid nitrogen level should be maintained at the optimum level
- iv. Always the deep frozen semen should be stored in goblets keeping in canisters.
- v. A proper practice should be adopted to identify canisters

- vi. Always the canister should be kept below the freezing point when semen doses straws are taken out for thawing.

#### 4. Thawing of frozen cattle semen doses:

- i. It is compulsory to use a tweezer to take semen straws dose out from the liquid nitrogen tank and before using the tweezer it should be kept a while near the opening of the liquid nitrogen tank.
- ii. The canister should be repositioned in the liquid nitrogen tank immediately after removing the semen straws.
- iii. Water in the thawing vessel should be kept at the level to cover semen straw completely and temperature should be measured after stirring the water well.
- iv. Before thawing semen straws which is taken out using the tweezer, hold from the laboratory end shake off liquid nitrogen drops.
- v. When thawing, semen straw should be kept at the temperature of 35-37°C for 30 seconds. For this, it is compulsory to use the digital thermometer/thaw monitor.
- vi. After thawing, semen straw at the laboratory end should be held with the tweezer and shake off drops of water and wipe the pipe with a paper towel.
- vii. Thawed semen should be used within 10-15 minutes after thawing and until it is used care should be taken to maintain temperature nearly at 95 F<sup>0</sup> / (35-37° C)

#### 5. Usage of AI tool kit :

- i. Following tools are included in an AI tool kit and it is compulsory to use the whole AI tool kit.
  - Field Can
  - Canister
  - Goblets
  - Thawing Unit (Flask, Thermometer)
  - Tweezers
  - Semen Straw Cutter
  - AI Gun
  - AI Sheath
  - Sanitary Sleeves
  - AI Gloves
  - Paper tissue
  - Lubricant
- ii. The place where the artificial insemination equipment is prepared: This place should be a shaded place with minimum change in the temperature.
- iii. Preparing of Artificial Insemination Sheaths: One Artificial Insemination Sheath should be partially taken out from the pack. The end of this sheath that is entered into the womb should be free from any damage.
- iv. Hand washing: Wash hands thoroughly before using insemination equipment.
- v. Warming of the AI Gun: Warm the barrel of the AI Gun rubbing it briskly with a paper towel.
- vi. Pulling the Plunger: Draw the plunger about six inches backwards to allow the frozen cattle semen doses to be inserted.

- vii. Drying and testing of frozen cattle semen doses: The frozen cattle semen doses should be thoroughly wiped with a paper towel. Then check if there are air bubbles in the laboratory end of the cattle semen doses. If not, shake the cattle semen dose holding from the end of the laboratory end. Then the air bubble will come to the laboratory end.
- viii. Depositing of semen: The semen straw should be inserted into the AI gun holding from the cotton plugged end (factory end).
- ix. Cutting of Frozen cattle semen straw: With a sterilized straw cutter, cut about  $\frac{1}{4}$  inch below the laboratory end of the cattle semen dose.
- x. Inserting of the AI Sheath: Insert the artificial insemination gun into the sheath to cover the artificial insemination gun. Press the inserted Sheath sufficiently with the help of the AI Gun Ring.
- xi. Prevention of disease using sanitary sleeve: The prepared artificial insemination gun should be covered with a sanitary sleeve. After this is inserted into vagina, before inserting it into the cervix, pierce the end of the sanitary sheath and insert only the artificial insemination gun with the sheath into the cervix. Spreading of disease can be reduced using this sanitary sleeve.
- xii. Temperature Maintenance: Care should be taken to maintain the temperature of the prepared artificial insemination device at  $35^{\circ}\text{C} - 37^{\circ}\text{C}$ .

## 6. How to perform artificial insemination.

### 1) Preparation:

- i. Restrain the cow properly: Care should be taken to restrain the cow without any harm before starting artificial insemination activity.
- ii. Hand hygiene: Wash hands thoroughly with a disinfectant solution before putting on gloves.
- iii. Wearing Gloves: Wash your hand and wear gloves. (AI Gloves)

### 2) Process of Artificial Insemination:

- i. Inserting the hand into the anus and cleaning: Gently insert the hand through the anus to remove dung and clean.
- ii. Hygiene around the vaginal opening: Before inserting the artificial insemination gun, vaginal opening should be cleaned with a paper towel.
- iii. Identifying of the cervix opening.
- iv. The artificial insemination gun should be inserted through the cervical opening by controlling the cervix. This process should be done in a very delicate manner so as not to damage the cervix.
- v. As the artificial insemination gun passes through the cervix, the uterus should be gently pressed by the index finger, and the finger feels sensation as the end of the artificial

- insemination gun enters into the uterus from the cervix. Semen should be released at this point. Releasing of semen should be done slowly (about 5 seconds) by pushing the plunger.
- vi. After releasing semen, the artificial insemination gun should be removed slowly so as not to damage the uterus. Then check whether there is abnormal fluid in the tip of the artificial insemination gun. If there is abnormal fluid, inform the Veterinary Surgeon to take necessary action
  - vii. Then fill in the of artificial insemination receipt book and give a copy of the same to the farmer.

3) Additional facts:

- i. Behavior should be calm throughout the above entire process so as to minimize stress of the cow.
- ii. Body condition Score of the cow. - It is desirable to have a minimum body condition score of 2.5 for a cow and 3.0 for a heifer to undergo artificial insemination.
- iii. Optimum time for insemination - Fertility can be increased by performing the artificial insemination for 12 - 18 hours after standing heat.
- iv. At the veterinary surgeon's office deep frozen semen, liquid nitrogen handling should be done by Veterinary Surgeon/Livestock Development Instructor whenever possible.
- v. All the equipment required for artificial insemination performed with conventional deep frozen semen is applicable in handling, thawing and artificial insemination carried out with sexed semen as well.
- vi. Usage of sexed semen
  - Sexed semen can be used for the heifers in the appropriate weight as per the breed, age and body condition and also for first parity cows free from post-partum disorders.
  - Optimum time for insemination

When using sexed semen, fertility can be increased by performing artificial insemination within 14-20 hours after standing heat. That is, artificial insemination should be done when ovulation is about to occur.

vii. Waste disposal and disinfection of equipment:

- The used AI Sheath, semen straw and the glove should be disposed properly so that they can be recycled.
- Strict adherence to these procedures will optimize the effectiveness of your artificial insemination program.



Dr. B.M.M. Ekanayake  
Director (Animal Breeding)  
Department of Animal Production and Health.

Dr. B.M.M. Ekanayake  
Director (Animal Breeding)  
Division of Animal Breeding  
Dept. of Animal Production & Health  
P.O. Box 13, Getambe  
Peradeniya