**Cooling semen**

Cooling is accomplished by placing the prediluted warm semen container in a container of water at the same temperature 37c. the quantity of water in the container should be sufficient to require a minimum of 2 hours to cool to 5c˚.

**Note**:-

 Optimum cooling rate: - from body temperature to 5c˚for 1.25 to 2 hours ((fast cooling)) or for 2 to 4 hours ((slow cooling)).

**Freezing**

1-nitrogen vapor freezing semen is the nitrogen vapor method. With this method the straws or ampoules of semen to be frozen are simply placed in a rack and positioned in the upper portion (vapor phase) of a wide-mouthed liquid nitrogen semen storage unit or one of the types used for storing cattle semen.

**Note**:- the semen is frozen by the vapor. It should not be placed directly in the liquid nitrogen during the freezing process.

2-dry ice and alcohol freezing method

The dry ice and alcohol method consists of adding dry ice gradually to an alcohol bath and checking temperature so that freezing is at the following rate.

One step: 5c˚ to 0c˚ 30 minutes

Two step: 0c˚ to -5c˚ 10 minute

Third step: -5c˚ to -13c˚ 5 minutes

Forth step: -13c˚ to -17c˚ 3.5 minutes

Fifth step: -17c˚ to -79c˚ 15.5 minutes

**Glycerolation and Equilibration**

Glycerol must be added to semen to protect it during freezing and thawing .damage results from the selective freezing of free H2O both inside and outside the cells. Glycerol binds water and decrease the freezing point of solution.

The level of glycerol varies somewhat with the diluter ingredients

-yolk-citrate

-whole milk

-tris buffered-yolk diluter

All these above diluter must be contains to 7% of glycerol.

-skim milk diluter must be contains 10% glycerol.

Procedure for adding glycerol to semen involves:-

Dividing the diluter into two equal parts

1. One part will not contain glycerol
2. Two part glycerol is added at twice the level desired in the final mixture. This would be 14% for yolk-citrate, whole milk, and tris buffered-yolk diluters and 20% for skim milk diluters.

Freshly collected semen is prediluted with one part diluter for cooling. After the semen is cooled, it is diluted to half the desired final volume with one part diluter.

Two part diluter is added slowly into the one part portion over period of one hours, divided into four volume containing 10% , 20%, 30% and 40% each with one volume added to the one part diluter at 15 minute intervals. After that allowed to sperm remain in contact with the glycerol long enough for the glycerol to reach equilibrium on both sides of the cell membranes. Four hours seems to be a minimum equilibration time before freezing