C) Semen processing:

The purpose of semen processing is: to keep the sperm fertile for as long as possible. Semen processing involves the following steps: Dilution, Stabilization (Preservation) at 5°, filling in straws and freezing.

I. Dilution :

Once the sperm concentration is determined, the amount of diluents or extender that can be added to the semen and number of straws that can made are calculated, based on the following formulas :

Vol. Dil = NP × 0.25 – V

NP = V × MI × C (ml) \ N° of spermatozoa per straw.

Vol.Dil : Volume of diluents

NP: Number of straws

0.25: Straw volume in ml

V: semen volume in ml

MI: percentage individual motility

C: spermatozoa concentration per ml

The number of spermatozoa per straw (dose) is usually 30 million.

II. Stabilization (Preservation) at 5°:

After calculating the dilution and number of straws, the diluted semen is cooled from 25c° to 5c° (at rate of 0.2 – 0.3c° per minute) then stabilized at 5c° for least 2 hours before filling the straws.

III. Filling the straws:

The straws are filled at 4c° manually or automatically (filling machines). The filling can be done at laboratory temperature of 24c° before cooling and stabilizing at 4c°. Once completed, the straws are sealed with polyvinyl material and frozen.

IV. Freezing:

After filling and sealing the straws and allowing stabilizing, the sperm is frozen. This process can perform using (Liquid Nitrogen or Biofreezer). The freezing temperature is typically (-120c°).

The typical freezing ramp:

1. From [5 to ⁻ 6 c°], 4c°\minute.
2. From [⁻6 to ⁻25c°], 50c°\minute.
3. From [⁻25 to ⁻ 10 c°], 50c°\minute.
4. From [⁻10 to ⁻ 120 c°], 80c°\minute.

The straws are then immediately placed into liquid nitrogen to reach a final temperature of [⁻196 c°].

D) Evaluation of frozen semen:

Post thawing evaluation is done to ascertain whether the semen is able to overcome the freeze-thawing process. Factors that may affect the evaluation are due to the sperm itself or its handling (lack of liquid N_2 in the storage container, handling of straws, etc).

The thawing of straws is performed in water at 37c° for 30-50 second. After this, one end of the straw is cut and put in a tube inside a water bath; the other end is then cut to empty the contents. The evaluation of semen does consist of the following steps:

1.Motility examination:

The percentage progressive motility and vigor are determined immediately after thawing the semen and after 2 hours of incubation. Various methods can be used to determine the motility. Automated system such as CASA (Computer Aided Semen Analysis) is the most objective. With experience, bright field microscopic examination without counting cells provides quick estimates. A drop of semen is spread thinly and evenly between a warm slide and cover slip at a magnification of 1000 to evaluate the percentage of spermatozoa with progressive motility, then the rate of progression (vigor) is assessed on the following scale:

0: No movement.

1: Slight ripple or vibration at the tail, without progression.

2: Slow progression, including stop and start movements.

3: Continuous progressive movement at a moderate speed.

4: Progressive and rapid movement.

5: Very fast progressive movement, with the cells being difficult to follow visually.

Semen of good quality, which has been recently thawed, usually has: 40-50 % of spermatozoa with progressive motility with vigor of 3-4.

After 2 hours of incubation, these values usually decrease by 10-15 % and 1 degree, respectively.

The minimum standards required for motility are:

- 0 hours = 25 % of spermatozoa with progressive motility vigor 3.
- 2 hours = 15% of spermatozoa with progressive motility vigor 2.

2. Percentage of intact acrosome:

Determining the percentage of intact acrosome is a morphological method for measuring the viability after thawing, and is related to fertility. Determining the viability and potential fertility of frozen semen is a valuable addition to motility.