Cryopreservation Of Sperm Antigens And Embryo

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Cryopreservation

- Cryopreservation derives from the Greek word *cryos,* meaning “cold”.
- Thus it refers to the preservation of biological tissues in sub zero temperatures, typically -196 º C.
- At these temperatures, all biological activities of cells and tissues is effectively stopped or ceased.
- Cryogenic storage at very low temperatures is presumed to provide an indefinite, if not near infinite, longevity to cells.
- Pre-implantation embryos, oocytes , spermatozoa ,ovarian tissue can be cryopreseved.

Benefits of Cryopreservation

- Effective means to conserve the germ plasms of endangered species.
- Fertility preservation.
- Methods to reduce multiple pregnancies
- Larger range of stocks available
- Easy disease-free exchange of stocks, nationally and internationally
- Stocks remain viable indefinitely
- Safety from disease, genetic contamination and breeding failure

What You Need For Cryopreservation?

Liquid nitrogen (liquid phase or vapor phase)

- Characteristics of liquid nitrogen:
  - Chemically inert
  - Relatively low cost
  - Non toxic
  - Non flammable
  - Readily available

Cryofreezer

Cryoprotectant: organic or inorganic additive which will protect the cell from freezing injuries during cryopreservation.

- Characteristics of cryoprotectants:
  - Should easily penetrate into cell
  - Non- electrolyte
  - Easily misible with water
  - E.g: Glycerol, DMSO, PVP, PEG etc.

Cryopreservation Of Sperm

- It is commonly known as **sperm banking**.
- Sperm banking is a procedure to preserve sperm cells for future use.
- The first successful cryopreservation of spermatozoa was initiated over 50 years ago.
- For human sperm, the longest reported successful storage is 22 years.
- It can be used for sperm donation where the recipient wants the treatment in a different time or place, or for men undergoing a vasectomy to still have the option to have children.
- It is also useful for men suffering from azoosprmia (Lack of motile sperm) & Gonadial cancer.
- These frozen sperms can be used in association with one of the **Assisted Reproductive Techniques (ART)** to induce pregnancy.

Process of Sperm Cryopreservation

1. **Obtaining semen sample:**

- Semen samples are collected in sterile container.
- The semen so collected is suspended in 10-20% glycerol in egg yolk buffer.

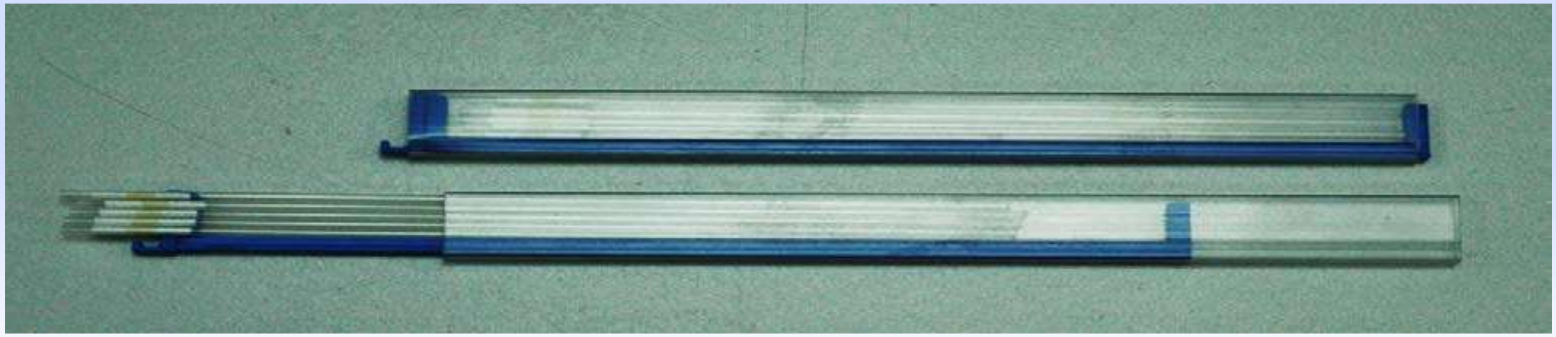
2. **Semen analysis:**

- Semen samples are analyzed for volume, viscosity and pH levels, and microscopically evaluated to determine motility, sperm count and morphology.

3. Freezing:

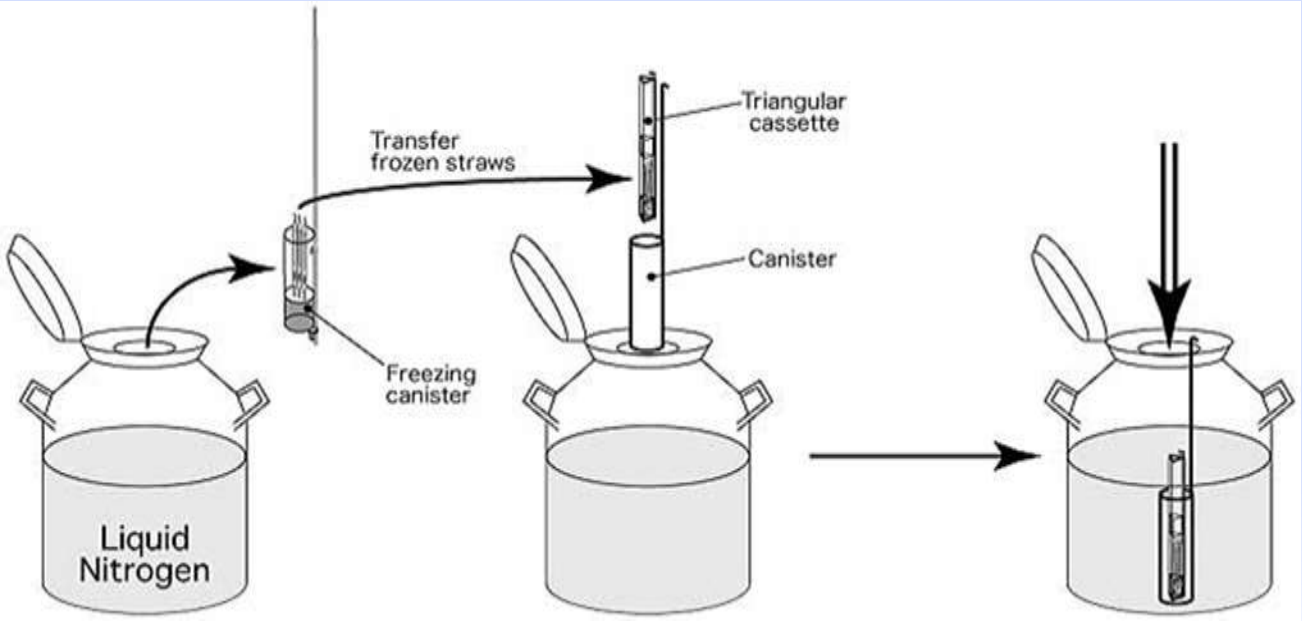
- Freezing of semen is done either in straw or ampoules.
- Freezing can be achieved in three ways:

I. **Slow freezing:** Liquid nitrogen is poured into the tank and cooling rate is obtained from 20°C to −80°C at rate of 1.5°C/min and then at 6°C/min; at completion of the freezing the straws are removed and stored into liquid nitrogen at −196°C. This takes about 40 min.



II. **Rapid freezing:** requires direct contact between the straws and the nitrogen vapors for 8–10 min and immersion in liquid nitrogen at −196°C.

- The sample is initially mixed in dropwise manner with equal volume of cold cryoprotectant; the mixture is loaded into the straws and left to incubate at 4°C for 10 minutes.
- The straws are then placed at a distance of 15–20 cm above the level of liquid nitrogen (−80°C) for 15 min; after this stage, the straws are immersed in liquid nitrogen.



4. Thawing:

- It is done by putting ampoule containing the sample in a warm water bath (35 to 40°c).
- Frozen tips of the sample in tubes or ampoules are plunged into the warm water with a vigorous swirling action just to the point of ice disappearance.
- Just a point of thawing quickly transfer the tubes to a water bath maintained at room temperature and continue the swirling action for 15 sec to cool the warm walls of the tube.

Cryopreservation Of Embryo

- Embryo cryopreservation or embryo freezing is a method used to preserve embryos, generally at embryogenesis stage by cooling and storing them at low temperatures.
- Started in 1984.
- It is one of the most common and well established fertility preservation treatments, with proven successful pregnancy rates.
- In addition, the duration of storage had no significant effect on clinical pregnancy, miscarriage, implant, or live birth rate.

Process Of Embryo Cryopreservation

1. **Embryo collection:**

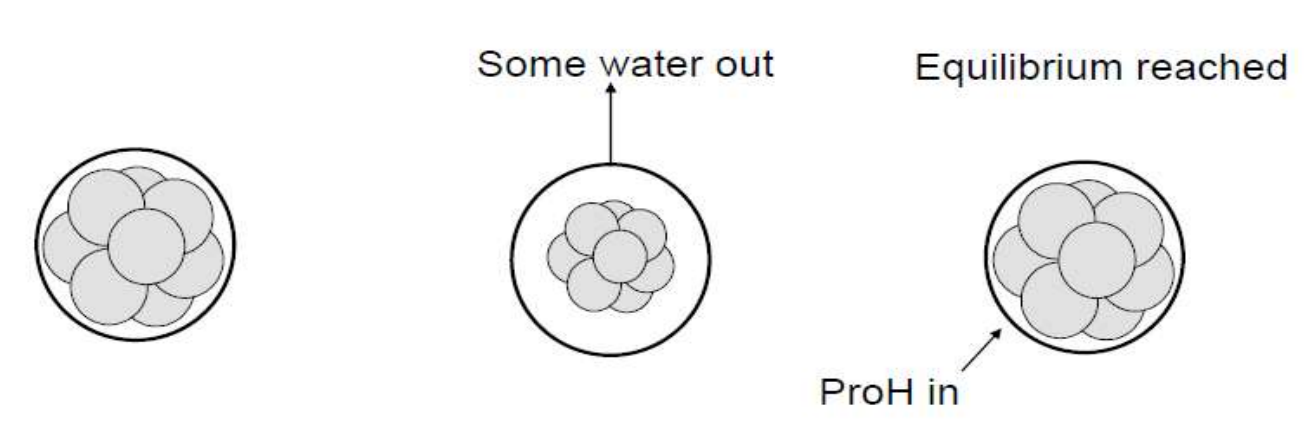
- Egg retrival is done under ultrasound guidance and subsequent fertilization and embryo culture is carried out.
- If there is surplus of embryos, then embryos of sufficient quality are collected for cryostorage.

2. **Embryo selection:**

- While the embryos can be frozen at any pre implantation stage between one cell to the blastocyte stage, generally embryos at blastocyte stage are chosen to cryopreserve.
- In cases where embryo needs to be frozen without a fresh transfer, freeze all the embryos the day after egg collection at one cell-stage.

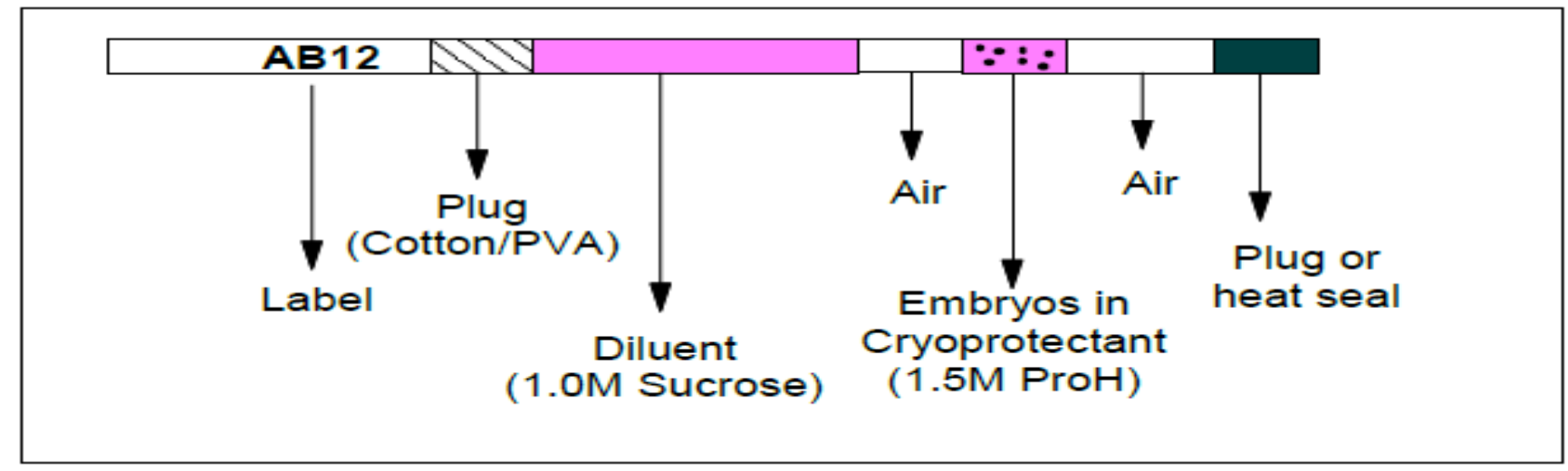
3. Freezing:

- Selected embryos are transferred to freezing medium and kept for 20 mins at 20ºC for equilibration.



- Controlled-rate freezing technique is utilized that slowly cool embryos in cryoprotectant fluid from body temperature down to -196ºC at the rate of 0-4ºC/min.
- The embryos are contained within special labelled embryo straws or vials, that are sealed prior to freezing.

- Once frozen, embryos are placed inside labelled tubes attached to aluminium canes.



- After reaching -35ºC, the straw is removed from freezer and quickly placed in LN2 container and stored in it.

4. Thawing:

- Embryo thawing is the reverse of the freezing process, and involves warming the embryos.
- Embryo thawing takes approximately 2 hours.
- During the thawing process, embryo is placed in water bath.
- When embryos return to room temperature, the embryos are passed through a series of solutions to remove the cryoprotectant that is no longer needed.
- The thawed embryos are kept in the incubator until the embryo transfer, during which time they should resume development and undergo cell division.

Comparison Of Cryopreservation Of Sperm And Embryo

Comparison of Cryopreservation of Mouse Sperm vs Embryos		
	Sperm	Embryos
Advantage	Cheaper to cryopreserve, requires only one day of work for TAF, requires only two males/line	Embryos are frozen post fertilization, recover to live offspring easier
Disadvantage	Only freeze one gamete, revival through IVF (labor extensive and costly), must use wildtype females as egg donors	Requires at least 8 females, PI may need to give ip injections of hormones
# animals needed	2 males/line	At least 8 het or homo stud males and 8-16 weanling females
# days to complete	One day	One to three days
Cost	$360/2 males	$400-$1200 depending on # females used and procedure used


Thank You