

# CRYOPRESERVATION OF FISH GAMETES

- Method by which living cells/tissues/ organs are preserved and are viable for an indefinite period by storing the same at  $-196^{\circ}\text{C}$  .
- Cryopreservation of sperms are successful .
- Large size of egg, complex str., presence of several membranes with variable permeability- obstacles in teleost egg preservation.
- Even fertilization of normal egg with cryopreserved spermatozoa were not successful.
- Knowledge of the composition of milt and sperm quality essential for optimum rate of fertilization and healthy hatchlings.

# PRINCIPLES OF CRYOPRESERVATION

## 3 essential steps In freezing

1. FREEZING
2. STORING
3. THAWING

The rates of freezing and thawing are critical in preserving the vitality and viability of spermatozoa.

- @ if cooling rate – too low, cells get sufficient time to lose water, it can maintain the osmotic equilibrium with the extra cellular solution. Cell death is caused by excessive dehydration.
- @ if cooling rate - very high , the time for water to escape out of the cell is very limited. little or no water escapes out of the cell. Intracellular freezing takes place. Ice crystals are formed, fatal to the cells.
- @ Cooling rate moderate- only part of the freezable water leaves the cell. water in the cell vitrifies/ forms ice crystals which are tolerable if thawing is fast enough to avoid recrystallisation.
- @ **Use of cryoprotectants increase the rate of vitrification and optimise the post thaw survival.**

**CRYOPROTECTANTS**- chemicals that minimise injuries to the cell due to ice formation or it suppresses ice formation.

### CRITERIA FOR CHOOSING A CRYOPROTECTANT

1. Least toxic to cells
2. Should be permeable to cells
3. Should be soluble in water during freezing

### TWO categories

#### **A. Permeating cryoprotectants – permeable to cell memb.**

Function by

1. Reducing the rate of diffusion of water from cell to extracellular ice crystal.
2. Reducing the cell volume change
3. Reducing the rate of ice crystal growth.

#### **Common Permeating cryoprotectants –**

1. **DMSO (Dimethyl Sulfoxide)** ✓
2. Glycerol
3. Methanol
4. Propylene glycol

## B. Non permeating cryoprotectants

1. Not permeable to cell memb.
2. Act by depressing the freezing point and raising the ice formation temperature of extracellular solution.

Commonly used chemicals- sucrose, glucose, dextran, egg yolk serum, skim milk Antifreeze protein .

**Extenders** – Solution of balanced salts.

Used to dilute the milt as undiluted milt is not suitable for freezing.

Also inhibits the activation of spermatozoa and functions as a medium for cryoprotectant.

The **diluent** used for milt - **combination of extender and cryoprotectant.**

Kept in the fridge, as it is an exothermic reaction.

Three categories of diluents-

- I. Extender A 90ml, DMSO- 5 ml, Glycerol- 10ml.
- II. Extender B 90ml, DMSO- 8 ml, Glycerol- 10ml.
- III. Extender C 65ml, DMSO- 15 ml.

Any one of the diluents – mixed with milt in a proportion- 4:1(diluent : milt)

## ADVANTAGES OF CRYOPRESERVATION

1. In seasonal breeders such as carps seed can be produced and supplied to farmers at any time of the year.
2. Helps in selective breeding and in development of other techniques meant for genetic upgradation of cultivable fishes- production of strains which have faster growth rate, disease resistance , adaptability to extreme conditions and better feed conversion efficiency.
3. Help in preventing inbreeding depression.
4. Helpful in preserving genetic diversity and in conserving endangered species by developing gene banks
5. Reduces the cost of brood stock maintenance through the exploitation of a limited stock.
6. Cryopreservation is useful in conditions where male and female mature at different times

## CRYOPRESERVATION OF CARP MILT- PROTOCOL

1. Healthy male breeders are selected during peak breeding season.
2. Subjected to Hypophysation.
3. Within 3 hrs, milt from the males is collected by stripping into an ice cold sterilized tube.
4. Yield of milt- 6-10 ml/kg body weight.  
.5-1 ml/kg body weight(with out hormone)
5. Care is taken to avoid contamination of milt by urine, faecal matter, mucus etc.
6. Milt stored in fridge.
7. Sperm count and sperm motility is assessed using a microscope and through spermatocrit value .
8. If more than 80% of the spermatozoa are motile – sperm count is taken as  $2 \times 10^7$  per cubic mm, and spermatocrit value- 70.
9. The milt is diluted by a mixture of extender and cryoprotectant.
10. Three types of extender solution are used A, B, C.-  
Sodium Cl, Calcium Cl, Potassium Cl, Sodium bicarbonate, Sodium phosphate, and Magnesium sulphate are the chemicals used for extender solution.
11. Extenders can be stored in refrigerator for 1 month.
12. Diluents are prepared by mixing the DMSO or Glycerol with extender.

13. Milt : diluent = 1 :4
14. This mixture kept at 8- 10 deg C for 45 min for equilibration.
15. Mixture is filled in French straw (0.5 ml) or visotubes (5ml vials ) and sealed.
16. In case of French straw – it is fumed over liq. Nitrogen for 10 min and then stored in liquid Nitrogen at  $-196^{\circ}\text{C}$
17. If visotubes are used it is cooled at  $15^{\circ}\text{C}$  per minute in a cooling chamber.
18. Whenever required the reactivation of sperms can be done by thawing.
19. Fast thawing is preferred over slow thawing.
20. French straws are made to slush by swirling them in a water bath at  $38^{\circ}\text{C}$  for 7-9 seconds.
- 21 . Few drops of fresh water are put into the tubes with milt, this reactivates and restores the mobility of the sperm.
22. Visotubes need 65-70 seconds for slush formation.
23. Visotubes with 3 ml thawed dilute milt is sufficient to fertilise 1 to 1.5 lakh eggs.