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Significance of Cryopreservation Biotechnology for Protection of Aquatic Species

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Abstract

Cryopreservation is a method of long term storage of living cells at very low temperature mostly at the temperature of liquid nitrogen that is -196^{o}C. These cells are stored in those conditions in which their capabilities of movement, regeneration and reproduction should not disturb. This process is very helpful for the fish farming as preserved sperm, oocytes can be used for the off season fertilization of fish species. Cryopreservation is helpful for conservation of specific genetic traits and to extant endangered species. By cryobanking transportation of gemplasm from one farm to another farm is also become easy. In this process some chemicals are used as cryoprotectant agents like DMSO (dimethyl sulfoxide). In this review we describe both advantages and disadvantages of cryopreservation.

Keywords: Cryopreservation, Conservation, Dimethyl sulfoxide, Fertilization, Germplasm

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INTRODUCTION

Cryopreservation is defined as the long time storage of Individual living cells and biological tissues at very low temperatures, like the temperature of liquid nitrogen, usually at -196ºC (Bakhach, 2009). At this temperature, the cellular activities are temporarily prevented and cells can be genetically stable for a long time until needed. This procedure is very important for biomedical, clinical, species conservation and biotechnology research areas. It is a best method for preserving living tissues for long time because it's a cheap method as compared to other procedures.

Cryoinjury is the most important area of research for checking the response of cell changes according to inner and outer environment (Mazur, 1984). It also considered the properties of freezing and defrosting. Important parameters which involve in these research areas are diffusion, osmosis, Cryoprotectants, cooling and thawing process.

Cryopreservation method comprises conversion of cell maintenance media to culture media which have cryopreservation agent, like dimethyl sulfoxide (DMSO). Then Cells are cooled at temperature of -80°C in specific cooling container. After cooling cells are transferred to very low temperature storage of below -135°C. Liquid nitrogen is commonly used for this extreme low temperature.

Cryopreservation has many applied uses in fisheries and aquaculture. They are:

1. Wider transfer of gametes from one point to another point
2. Male progeny fish numbers reduced
3. Provide more time for progeny availability
4. large number of families should be conserved through Selective propagation
5. genetic resources preservation

Fish population is in alarming condition due to water pollution and overfishing. Endangered species can be preserved by cryopreservation of aquatic germplasm, and by fish farming. By these strategies genetically important characteristics can be conserved and saved from loss occur through diseases and natural disasters.

Many fish species has been preserved completely by cryopreservation of semen for propagation of many wild and domestic species. Researchers did many efforts from more than last three decades for cryopreservation of fish embryos but still they are unsuccessful (Streit et al., 2014). Successful cryopreservation of gametes, eggs, and embryos will provide a new way of completely limitless production of more vigorous and healthy generations of fish species as needed (Godoy, 2013). Genetic biodiversity of aquatic resources can be maintained by saving the Genomes of endangered species (Rana, 1995).

Cryopreservation of sperm from aquatic species

According to IUCN 5,161 aquatic species are in endangered condition and these can be recovering by using the cryopreservation methods in farming of naturally present species (IUCN Red List, 2015). Researchers are focusing on aquatic animals species for the purpose of life maintenance in controlled condition and for checking the effect of environmental pollution for future maintenance. This environmental pollution become a great risk for Killer whales (Orcinus orca) and dangerous for movement, production and strength of sperm. As a result it can create the infertility in Killer whales.

This problem was recovered by directional solidification technology and by using cryprotectant agents and glycerol (Robeck et al., 2011). This method also used for cryobanking of gametes to maintain the population of sea aquariums.

Androgenesis

Cryopreservation also used for the purpose of changing in chromosome set by stopping activity of the oocyte genome through irradiation or stop fertilization by using cold, heat or pressure shock at the first stage of mitotic division. This complete process of inactivation is called Androgenesis (Dunham, 2004; Komen & Thorgaard, 2007). This procedure is helpful for the recovery of specific species which sperms were cryopreserved by fertilizing with eggs of relevant species. This technique was successfully applied on rainbow trout (Babiak et al., 2002; Scheerer et al., 1991), sturgeon species (Grunina et al., 2006), and between fertilization of common carp and goldfish (Bercsényi et al., 1998).

Germplasm Cryobanking of aquatic species

Cryobanking of fish germplasm involve many types of cells, like sperm, eggs, oocytes, embryos, somatic cells, spermatogonia and primordial germ cells. Endangered natural reservoirs of fish species also can be saved by using Germplasm cryopreservation. The first successful cryopreservation process was done on bull semen to save and reproduce the threatened species (Polge et al., 1949). In fish Aquaculture sperm is mostly common for the propagation and administration of related species involving cyprinids, silurids, salmonid (Magyary et al., 1996; Tsvetkova et al., 1996). Cryopreservation of embryos and oocytes in aquatic species is only successful for eastern oyster eggs (*Crassostrea virginica*) (Tervit et al., 2005), and for larvae of sea urchin and eastern oyster (Paniagua-Chavez & Tiersch, 2001; Adams et al., 2006).

Fish genome is small in size, so it is best model for studying the human genetic diseases (Barbazuk et al., 2000). More than 200 fish species sperm was successfully manage and cryopreserved from marine and fresh water (Kopeika et al., 2007; Tsai et al., 2010) including carp, salmonids, catfish, cichlids, medakas, white-fish, pike, milkfish, grouper, cod, and zebrafish (Scott & Baynes, 1980; Harvey & Ashwood-Smith 1982; Stoss & Donaldson 1983; Babiak et al., 1995; Suquet et al., 2000; Van et al., 2006; Bokor et al., 2007; Tsai et al., 2010). Frozen-thawed spermatozoa have more fertility and survival power than freshwater species (Drokin, 1993; Gwo, 2000).

Tissue collection and cryopreservation

Tissue culture is necessary for getting the more tissues before cryopreservation or it is also required for reproduction of fish. It is difficult to manage all samples collectively at the time of tissue collection so these are cryopreserved as soon as possible after harvesting of tissues (Moritz & Labbe, 2008). Fish sperms and somatic cells can be saved in cryobank by collecting them in straws and cryovials. Procedures of tissue collection, culturing them and cryopreservation have been designed for different aquatic species (Lakra et al., 2011), but their response can be varied from specie to specie (Chenais et al., 2014).

Pros and Cons of Cryopreservation in Fisheries Science

Biological material can be preserved for thousands of years without damage.

Total volume of sperm can be used without any wastage.

Off-season fertilization can be done by using preserved sperms.

Transportation of germplasm is easy for farming system as compared to transport of fish.

Conservation of genetic resources of specific required traits (Cabrita et al., 2010).

Conservation of genetic material of threatened species which become very important model specie in biomedical research (Tsai, 2003; Iwai et al., 2009).

Fish gametes can be preserved from both parents for maintenance of genetic biodiversity.

Fish embryo and oocytes cannot be cryopreserved because of damage by very low temperature (Tsai & Lin, C, 2012).

Cryopreservation Quality

For getting the best results of cryopreservation evaluation of every step is necessary. This process has different steps for the quality checking is following:

- Checking the movement of sperm after collection
- After putting in extender solution
- After storage at low temperature
- After addition of cryoprotectant
- After melting of sample
- Fish quality sperm can also be checked by using software "computer-assisted sperm analysis"
- Flow cytometry and comet assay also used for checking cell characteristics and DNA quality (Daly & Tiersch, 2011).

Cryoprotectants

Cryoprotectants used to prevent damage of cells from the crystallization and recrystallization process during storage at freezing temperature. Chemicals which used as cryoprotectants are following (Meryman, 1966).

- Methanol
- Dimethyl sulfoxide (DMSO)
- Sucrose

Evaluation strategies used in cryopreservation:

There are four main stages in the cryopreservation process including condition of fish at the time of collection, preparation, cryostorage and thaw conditions of sperm at the time of usage. All these steps are given in fig 1.

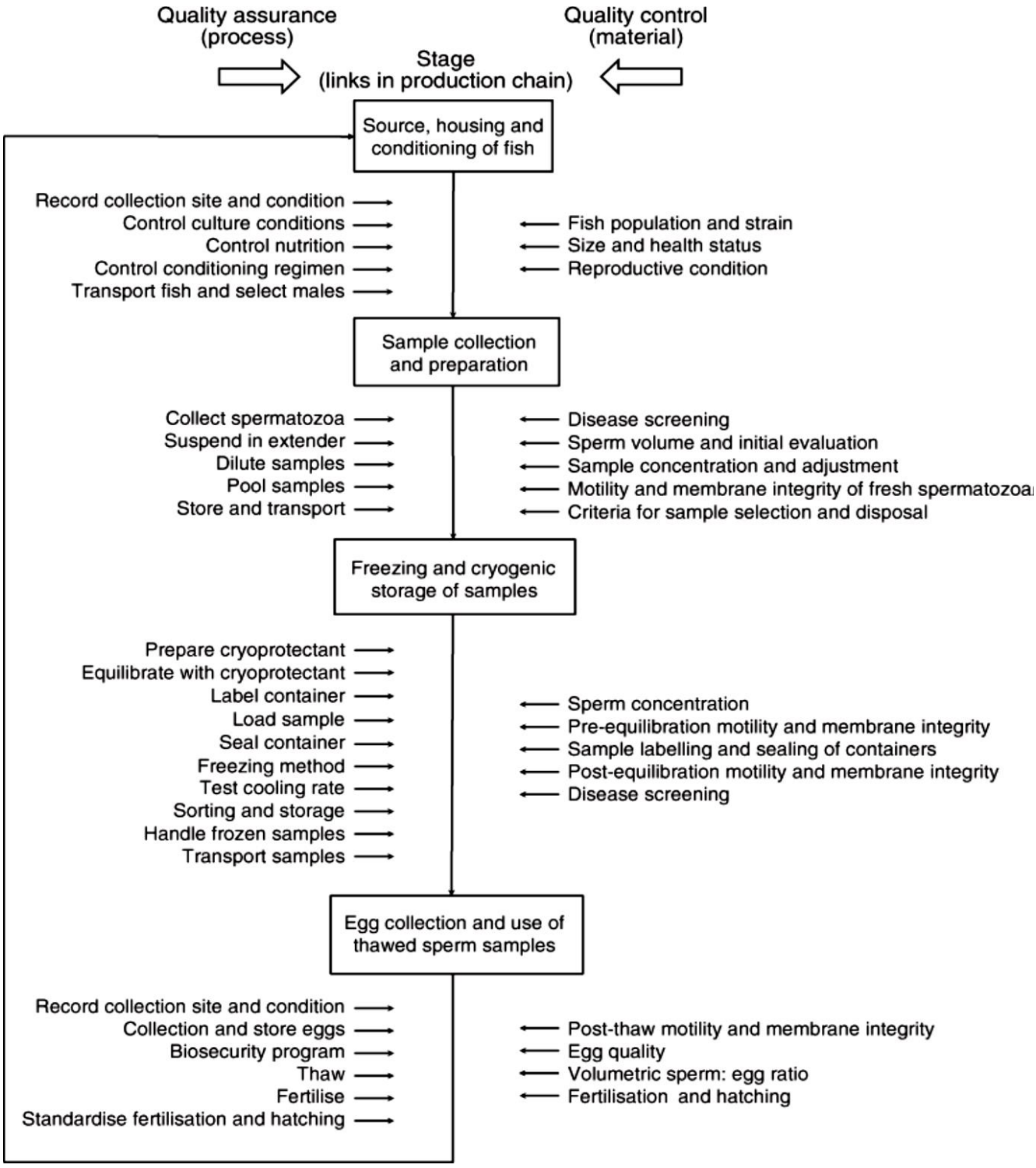

Figure 1. Key quality assurance (left) and quality control (right) activities that take place along the four main stages of the cryopreservation process (Torres et al., 2016).

Difficulties in Cryopreservation

Sometime cells are not able to use after cryopreservation due to damage of cell membrane (Kim et al., 2015; Chaytor et al., 2012). During cryopreservation two methods can create problem are slow-freezing and vitrification (Fahy et al., 1984). These processes can create crystallization, recrystallization and formation of glass solid instead of crystals inside and outside of the cell and causes the injury of cell even cryoprotectants also not enough to solve this problem (Fahy et al., 1984). Anti-freezing proteins are used to solve this problem by preventing the ice recrystallization, so it can improve the process of cryopreservation (Zilli et al., 2014).

New Trends and Future Works in the Area

Researchers are trying to find out solutions for the preservation of fish embryos and ovarian tissues. Genetic and behavioral changes of cells should be checked in Larvae and juveniles stages and even in adult form when they are exposed to cryo-solutions. Scientists are trying to find out new solutions for overcome the problems of cell damage produced by ice crystallization.

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