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# Introductory Chapter: Cryopreservation Biotechnology in Aquatic Science

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## 1. Introduction

Cryopreservation is the process of freezing the biological materials at temperature of liquid nitrogen (LN<sub>2</sub>) (−196°C). This means it is possible storing of the biological materials as unchanged for centuries with the capability of recovering the cell functionality following the thawing process [1].

This situation has been increasing the importance of cryobiology as a science that examines the effect of ultra-low temperatures on cell, tissue, organ, and organisms and also freezability of these structures maintaining their viability. In addition, a better understanding of functional properties of thawed cells following the freezing process has been accelerating the development of cryobiology [2].

The cryopreservation method basically includes temperature reduction, cellular dehydration, freezing, and thawing. The lowering of normal temperature to 4°C reduces the cellular metabolic activity and increases the life span of sperm cells. Following thawing process, normal functions of the cells restart [3]. These events at ultra-low temperatures provide basic mechanisms for long-term preservation of biological material in genetically stable form. In practice, no significant change of biological importance occurs below −150°C, and therefore, the material can be conveniently stored in liquid nitrogen at −196°C [2].

Gamete, embryo, and embryonic cell cryopreservation have become of tremendous value in aquatic biotechnologies, which provide an important tool for the propagation of economically important species, and also in the protection of endangered species and genetic diversity in aquatic species [1].

Following successful cryopreservation of avian spermatozoa using glycerol as cryoprotectant by Polge et al. [4], cryopreservation of male gametes became possible in this research area. For the first time, Blaxter [5] applied a similar approach for fish gametes and reported achieving approximately 80% cellular motility following thawing of Atlantic herring spermatozoa in the field of aquaculture. Since then, cryopreservation of fish sperm has been studied and succeeded in more than 200 species [6, 7]. Today, sperm management techniques have been established for freshwater and marine fish species [8–12].

Cold or frozen state preservation of gametes is an important biotechnological tool for aquatic species conservation and has a great concern for aquaculture. Growing in concern to this biotechnology has led to an increase in the number of studies in this research area [13]. Nowadays, it is possible to use preserved semen in routine reproduction applications in aquaculture practices [14].

## 2. Cryopreservation of sperm cells in aquatic species

Sperm cryopreservation is a very valuable tool for the conservation of aquatic species [15]. It is considered as a reliable method for the *ex situ* preservation of biodiversity since it provides opportunity to preserve desired cell samples. In addition, it is possible to reconstruct the original strain, population, or variety following required environmental restoration via this biotechnology [2].

The progressive interest in the application of cryopreservation to aquaculture has revealed how useful this method could be in the management of fish reproduction especially when it is combined with other reproductive technologies such as androgenesis or sex reversal [2].

Two methods can be used for gamete cryopreservation: slow freezing and vitrification. Slow freezing uses low concentrations of cryoprotectants, which are associated with chemical toxicity and osmotic shock. Semen refrigeration with slow cooling rates (0.5–1°C/min) and temperature reduction induces stress on cell membranes and causes modifications in the functional state of membranes [16]. The stress caused by ice crystal formation is associated with the osmotic pressure changes in the unfrozen solution [17].

Cold shock reduces membrane permeability to water and solutes resulting in membrane injury. The main changes occurring during the freezing process are ultrastructural, biochemical, and functional. These changes reduce fertilization of eggs. In frozen/thawed semen, motility of sperm cells is better protected than its morphological integrity. Membrane permeability is increased following cooling process, and this may be a result of increased membrane leakiness and specific protein channels [18].

## 3. Cryopreservation of eggs and embryos in aquatic species

Cryopreservation of egg is more complicated than that of sperm. It is possible to indicate that the large size and the presence of three different membrane layers with different water permeabilities are the main obstacles related to the removal of intracellular water from fish eggs [2].

When the eggs are stripped, the eggs are permeable to ice-reducing cryoprotectants due to opening of channel in the shell-like chorion. According to Harvey and Ashwood-Smith [19], penetration of cryoprotectants such as glycerol, DMSO, and methanol is rather slow in unactivated ova. However, once fertilization or activation occurs, the channel closes and the chorion hardens [20]. At this point, the eggs are impermeable to cryoprotectants and will significantly increase in size due to a brief inflow of the water [21].

Cryopreservation of embryos has become an integral part of assisted reproduction. Successful cryopreservation of embryos is important because the biodiversity of both paternal and maternal genomes will be preserved. Fish embryos are better candidates than eggs for the cryopreservation process due to their higher membrane permeability, less chilling sensitivity, and less complex membrane system.

Studies carried out so far associated to fish egg cryopreservation have been mainly focused on model species such as zebrafish (*Danio rerio*) [22], although other marine and freshwater species have also been studied, for example, gilthead seabream (*Sparus aurata*) [20] and some South American freshwater species [23]. On the other hand, there are factors limiting fish egg cryopreservation including their multicompartamental biological systems, high chilling sensitivity, low membrane permeability, and larger size [20].

#### **4. Cryobanking in aquatic science**

One of the important fields using cryopreservation technology is the cryobanks or sperm banks. Cryobanks are currently more developed for rare domestic animals such as cattle, sheep, and goats than for non-domestic animals. In addition, use of cryobanking to facilitate the management and conservation of endangered species is becoming widespread [24].

The creation of cryobanks for the selected stock to prevent outbreaks or genetic drift is also essential to develop genetic selection programs in commercial aquaculture. On the other hand, conservation of aquatic species which in danger of extinction, is also necessary until the environmental conditions recovered.

In aquatic science, cryobanking has considerable advantages on cultured aquatic species in captivity, in terms of cost, labor, and security since thousands of samples from different generations can be maintained in a minimum space without the risk of loss caused by disease [25]. Moreover, transportation and management of frozen samples are relatively simple, allowing greater flexibility for designing recovery programs. In addition, development of reproductive technologies in aquatic species allows recovery of population from semen samples through cross-breeding programs or application of androgenesis procedures [26].

Research on fish germplasm cryobanking has been carried out on different cell types such as sperm cells, somatic cells, spermatogonia and primordial germ cells, as well as oocytes and embryos. On the other hand, it is well known that sperm cells present advantages compared to other cell types because of their small size and high resistance to chilling [27].

#### **5. Opportunities and new strategies**

The cryopreservation process provides many benefits. Some of the cryobiological applications in the field of aquaculture have been summarized as follows ([6, 8, 28]):

- Storing the sperm for routine fertilization process
- Cross-breeding programming independently the maturation period or availability of breeders
- Utilizing all the sperms from species with a large production
- Increasing the fertile life of the individuals
- Transporting the gametes or embryos between farms instead of breeders
- Marketing the well-characterized and standard quality sperm
- Hybridizing between species with different maturation periods
- Reducing the synchronization treatments
- Year-round supplying the broodstock gametes

Recently, new technologies have been developed to conserve paternal and maternal genetic information. From this point of view, latest studies have focused on cryopreservation of primordial germ cells as an alternative for the cryopreservation

of both paternal and maternal genomes. On the other hand, there is little data about reprogramming of somatic cells into primordial germ cells in fish. In addition, cryopreservation of fish tissues can be considered for cryobanking. However, the regeneration methods should be well studied and established in aquatic species [2].

## **6. Conclusion**

Gamete, embryo, and embryonic cell cryopreservation have become of tremendous value in aquatic biotechnologies, which provide an important tool for the propagation of economically important species, and also in the protection of the endangered species and genetic diversity in aquatic species [1]. This situation has been increasing the importance of cryobiology as a science, examining the effect of ultra-low temperatures on cells, tissues, organs, and organisms and also the freezability of these structures maintaining their viability [2].

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