Cryopreservation of Fish Reproductive Cells: Development of Research in Southern Russia

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In the context of current global environmental challenges, the problem of biodiversity conservation has become increasingly important. Maintaining and increasing stocks of fish fauna of the Southern Seas of Russia is possible while maintaining natural reproduction and mandatory development of breeding to the greatest extent possible. However, hatcheries currently take a simplified approach to the formation of female populations due to a lack of broodstock. The use of closely related pairs in mating is fraught with the loss of natural genetic polymorphisms, inbreeding, and consequently, a significant reduction in the adaptive potential of the population.

At the moment, cryotechnologies are of strategic importance, including anti-crisis technologies to solve problems related to conservation of fish genetic biodiversity. The use of cryopreserved sperm in hatcheries that apply artificial reproduction and aquaculture enterprises will allow the creation of a genetically diverse population, and will reduce the area required and the cost of maintaining males, thereby allowing an increase in the production of a population of females. Application is possible at any time, without the risk of delayed maturation of broodfish or obtaining sexual products of inadequate quality.

Since 2004, the staff of the Southern Scientific Center of the Russian Academy of Sciences, jointly with Astrakhan State Technical University, have been conducting research on low-temperature preservation and long-term storage of genetic material of valuable fish species. The aim of the research is development of technology for cryopreservation and storage of fish reproductive cells to ensure their structural and functional integrity and the development of techniques for using cryopreserved sperm in aquaculture.

The two most important issues of low-temperature preservation of gametes are the freezing rate and the composition of cryoprotective mixtures. Laboratory staff has conducted research on optimization of freezing and selection of compounds of cryoprotective mixtures for different fish species, including Russian sturgeon, Beluga sturgeon, stellate sturgeon, sterlet, carp, silver carp, common carp, and whitefish. Earlier studies formed the basis for development of cryopreservation methods for sturgeon sperm. The composition of cryoprotectant, which can prevent destruction and loss of germ cells during low-temperature preservation, is species-specific in relation to the different characteristics of a particular type of sperm. Providing electrical stimulation of germ cells (Tikhomirov and Ponomareva 2008) increases the rate of penetration of the cryoprotectant into reproductive cells of sturgeon.

In 2010, the Southern Scientific Center, Russian Academy of Sciences, jointly with Astrakhan State Technical University, received a patent for a method of increasing the survival of germ cells in sturgeon cryopreservation. Developed with the Institute of Cell Biophysics, a method of reducing the low-temperature jump during crystallization of cryoprotectant solutions was developed, allowing an increase in the integrity of thawed cells after cryopreservation. In the method, freezing the suspension with the biological material and cryoprotectant in liquid nitrogen is carried out with application of ultrasonic waves with a frequency of 0.50-10 MHz. Studies demonstrated the effectiveness of freezing sperm on mesh in a thin film. Reducing the volume of toxic substances in the composition of cryoprotective medium for sturgeon sperm has led to increased lifespan of thawed cells.

These studies demonstrated the effectiveness of freezing sperm on mesh in a thin film (Krasilnikova and Tikhomirov 2014a). Reducing the volume of toxic substances in the composition of cryoprotective medium for sturgeon sperm, which in turn reduced the toxic effect of cryoprotectants, has led to increased lifespan of thawed cells. The results obtained allowed us to recommend adjustment of the concentration of penetrating cryoprotectant solutions as a function of the quantity of intracellular water to improve survival of sperm cells after the double temperature shock (Krasilnikova and Tikhomirov 2014b, Krasilnikova and Tikhomirov 2015).

To increase the level of heterogeneity in the resulting offspring and eliminate the negative consequences of inbreeding, a scheme was proposed for the formation of broodstock sturgeon in industrial conditions with the use of cryopreserved reproductive sperm stored in a low-temperature sperm bank. In the formation and operation of broodfish, it is recommended to use 10 percent of the sperm stored in liquid nitrogen in the cryobank annually. This gives the possibility of using high-quality sperm at any time, eliminating the risk of delayed maturation of broodfish and using a greater number of females in spawning.

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Work was done to produce offspring and determine the physiological usefulness of juveniles obtained with the use of cryopreserved sperm (Bogatyreva 2010, Krasilnikova 2015). Fish obtained using thawed sperm had the best survival rate, growth rate, fertility, physiological and biochemical characteristics, compared with fish produced using traditional methods.

Years of research to develop methods of cryopreservation of fish eggs in southern Russia has led to the development of a cryoprotectant with “enveloping action,” the basic component of which is a mixture of unrefined vegetable and animal oils containing linoleic, linolenic acids and phosphatides, which are glycerol esters of fatty acids and a substituted phosphoric acid. Comparative evaluation of a standard treatment with this new penetrating cryoprotectant showed that, after freezing in liquid nitrogen, oocytes of the sterlet, Russian sturgeon and Beluga sturgeon were capable of fertilization (20-45 percent).

Ongoing collaborative work between the Southern Scientific Center of the Russian Academy of Sciences and Astrakhan State Technical University has led to the creation of a cryobank, whose activities are fundamentally different from existing experimental collections. The basis of these activities has led to the accumulation, conservation and use of genetic material for the shortfall of broodfish and the correction of existing technologies of artificial reproduction of rare and endangered fish species of the Volga-Caspian Sea and Azov-Black Sea basins.

The creation of a low-temperature bank will:
1) Keep genetic information of rare and endangered species at the temperature of liquid nitrogen for decades without loss of genetic information.
2) Transport genetic material into the areas with a disappearance or sharp reduction in fish populations for species restoration or to provide genetic material according to the needs of customers;
3) Provide opportunities for breeding and genetic work on fish farms.
4) Create a fairly complete genetic collection of different species of hydrobionts for the subsequent full restoration of hydrobionts of the basin and of ecosystems;
5) Reduce the area used for holding broodfish, thereby improving the economic efficiency of artificial reproduction and commodity fish cultivation.

The laboratory has all necessary equipment for the cryobank: cryostorage for long-term storage of sperm samples; for experimental works and transportation of gametes and liquid nitrogen; a programmable freezer; a personal computer; a microscope equipped with a video-ocular for visual determination of sperm quality; fish tanks for the works to produce offspring; and an installation to determine the behavioral responses of juvenile fish.

Material will be collected from the hatcheries of the Astrakhan, Volgograd and Rostov regions of Russia, enabling the exchange of genetic material in the southern federal district of Russia. Every year the work of freezing and accumulation of sperm samples of various fish species is carried out. All data about the broodfish from which the sperm is taken is entered into a database, which is used to choose the sample for fertilization of the spawn. Systematic quality control of semen stored in the repository for 1-3 years is implemented. During this time the sperm does not lose its capacity to fertilize eggs.

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Notes
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