Application of piezoactuators in the technology of low-temperature preservation of fish reproductive cells

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Abstract. The sperm of Russian sturgeon (*Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833) was the research object. Acoustic-mechanical influence on sturgeon semen improves fish-biological and reproductive indices of the seminal fluid. The most optimal indices for reproductive cells of male Russian sturgeon are 500 Hz, 90 V with the exposure duration of 1 minute. It has been shown that when a piezo-actuator with a frequency of 500 Hz and an amplitude of 90 V is applied to the sperm mixture with the cryoprotectant, ice crystals are formed later, which may influence the survival of cells after defrosting. **Keywords:** cryopreservation, reproductive cells of fish, sturgeon, acoustic-mechanical impact, freezing, cracking ice, microparticles

1 Introduction

The genotypic principle, as an independent method, involves ensuring long-term storage of genotypes - creating genetic banks of rare and endangered species. The use of frozen semen of animals and fish minimizes the spread of diseases, eliminates geographical barriers and preserves the genetic material of an animal for an unlimited time [1].

Cryobanking is a valuable tool for preserving genetic resources. Cryopreservation is widely used in assisted reproductive technologies, agriculture, and endangered species conservation programs, playing an important role in genetic breeding programs, biodiversity conservation, and restocking programs [2-3]. Cryopreservation of gametes in aquatic animals, in contrast to terrestrial vertebrates, especially mammals, has very limited success. Sperm cryopreservation has been successful in a number of commercially important aquatic species, especially some bony fish. Nevertheless, the reproductive success of cryopreserved sperm in general is still low, and the technology used requires further improvement. The development of sperm cryobanks in a number of other aquatic animals is not at the stage of advanced commercial application as in domestic mammals [4].

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Artificial insemination in sturgeon farming implies certain standards of quality of the sperm used to ensure sufficient production yield. The successful result of deep freezing of spermatozoa depends on the initial quality of sperm, and, therefore, its quality must be evaluated before cryopreservation [5]. The most important indicators of sperm quality are activation ability, which gives the required concentration of motile spermatozoa at the moment of fertilization, as well as spermatozoa motility retention time. These indicators provide a more adequate assessment of the fertile potential of the sperm. Nevertheless, sturgeon breeding guidelines do not recommend the use of semen with a concentration of less than 1 bln/ml and motility after activation of less than 75% [6].

The quality indicators of defrosted semen are inherently lower compared to fresh semen, so the technology of semen cryopreservation is not used in production and remains mainly experimental [7].

Cryobiologists around the world are working to improve the survival rate of defrosted sperm of different fish species, as well as their fertilizing ability. For this purpose, optimal compositions of cryoprotective media, freezing and thawing regimes, and various kinds of stimulating effects are being selected [8-11].

To improve the technique of cryopreservation of reproductive cells of sturgeon fish, it is advisable not only to use optimal cryopreservation media [12-13], but also to perform stimulatory effects on the seminal fluid [14].

One of the newest issues being studied in the freezing of sturgeon semen is the use of ultrasonic waves to create optimal conditions for semen preservation at low temperatures. Acoustic-mechanical influence with the set parameters on biological fluids is practically unstudied, so conducting experiments in this field is relevant for today [15]. A new methodological approach to low-temperature preservation of fish reproductive cells using acoustic-mechanical effects opens up great opportunities for creating effective methods of deep freezing.

Previous work [16-17] showed that the use of acoustic-mechanical effects on cells before cryopreservation increases the percentage of live spermatozoa after deep freezing. As a result, after developing a mathematical model of acoustic-mechanical effects on fish sperm, a number of indicators were selected that require testing their effectiveness in practice.

2 Material and research methods

To increase the survival rate of spermatozoa with active forward movement, acoustic-mechanical influence during equilibration was used in our works. The object of the study was the sperm of Russian sturgeon (*Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833), obtained at the fish farm of Rostov region during the spawning campaign in 2022. Before the experiment, the quality of native semen was evaluated. Then, cryoprotector was added to the Russian sturgeon semen, the solution was exposed with a piezoactuator using various parameters, and incubated for 20 minutes at 4°C. After exposure with a piezoactuator the semen quality was evaluated: the time of progressive motion of spermatozoa and the proportion of mobile spermatozoa were determined.

In earlier experiments it was determined that the best result in terms of sperm survival after cryopreservation was obtained with acoustic-mechanical exposure for 1 minute at a frequency of 500 and 300 Hz. These indicators were taken as a basis in the present work. Further investigation consisted in comparing different amplitudes (45 V, 90 V) at frequencies of 500 Hz and 300 Hz. Thus, the following parameters were investigated in a series of experiments with Russian sturgeon sperm: A-control (semen with cryoprotectant without exposure), B - 500 Hz, 45 V; C - 500 Hz, 90 V; D - 300 Hz, 45 V; E - 300 Hz, 90 V. The exposure time was 1 minute.

Then we carried out freezing of the samples to study the process of crystallization. To study the shapes of microparticles and the changes occurring during crystallization, an apparatus consisting of a microscope, a foam bath, a Goryaev chamber, a thermometer, a video camera, and a table lamp was assembled [18]. The sample was applied in a thin layer (10 µl) to the chamber (Fig. 1). The sample was covered with a 0.15 mm coverslip (Deltalab, Spain). The sample was cooled with liquid nitrogen by gradually adding it to the bath and bringing the sample temperature to minus 196oC. The temperature was measured using an ATT-2004 thermometer. The changes were recorded in "Video" mode using a Sony Alpha A57 camera.

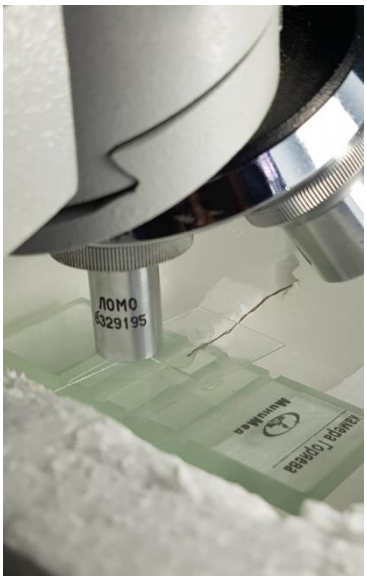

Fig. 1. Sample freezing apparatus - Goryaev chamber with a thermocouple and a sample under the microscope lens.

3 Results and discussion

Assessment of the quality of Russian sturgeon semen showed that the proportion of spermatozoa with progressive motility in the native semen reached 70%. The time of progressive motility of native spermatozoa was 558.7 seconds. After diluting sperm with cryoprotectant in the ratio 1:1 and equilibration for 20 minutes (control version of the experiment), the proportion of live spermatozoa decreased to 53.3%. This is probably due to the poisoning effect of the protector components on the cells. The results of sperm motility estimation in all variants of the experiment are presented in table 1 and figures 2 and 3.

Table 1. Semen quality of Russian sturgeon.

Parameters	Motility. sec.	Survival. %
Fresh sperm	558.7	70.0
A (Control)	594.0	53.3
B (500 Hz. 45V)	525.3	66.7
C (500 Hz. 90V)	695.7	76.7
D (300 Hz. 45V)	399.3	36.7
E (300 Hz. 90V)	447.7	43.3

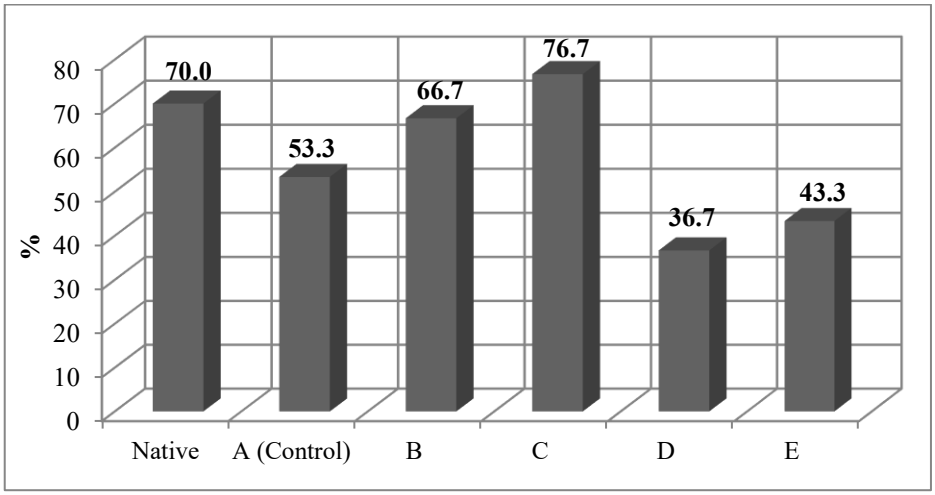

Fig. 2. Motility of spermatozoa, %.

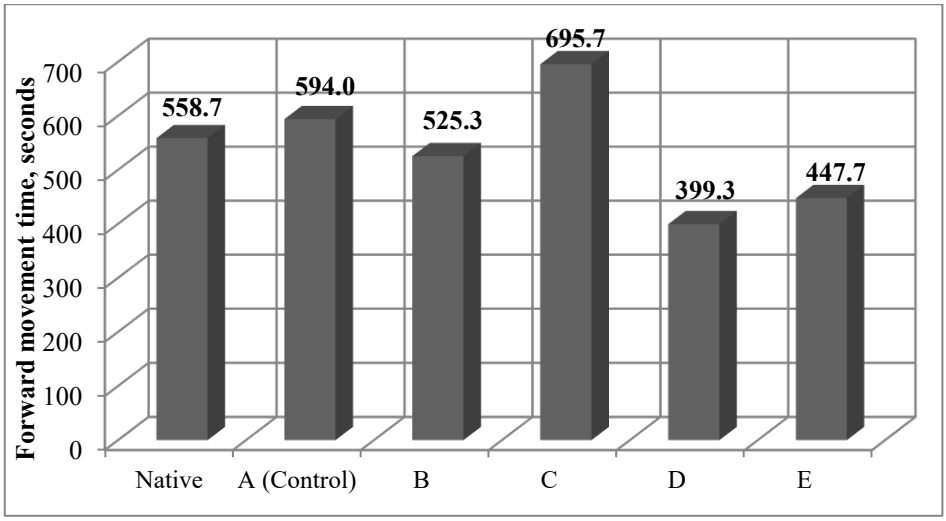

Fig. 3. Time of motility, in seconds.

As can be seen from Table 1 and Figures 2 and 3, acoustic-mechanical influence has a positive effect on the percentage of motility and life time of Russian sturgeon spermatozoa. Variant C (500 Hz, 90 V) showed an increase in the percentage of spermatozoa with translational motion by 23.4% (76.7%) relative to control (53.3%). Also, at these exposure parameters, the sperm lifetime increased relative to control - 695.7 s and 594.0 s, respectively.

When comparing the amplitude of exposure (45 V and 90 V) at the used frequencies of 500 and 300 Hz, in both cases the use of an amplitude of 90 V gave the best result. Thus, at a frequency of 500 Hz and an amplitude of 90 V, the proportion of live spermatozoa was 10% higher than with the 45 V amplitude (66.7% and 76.7%, respectively), and the lifetime was 170.4 seconds longer. At a frequency of 300 Hz, the use of an amplitude of 90 V yielded 6.6% more live spermatozoa and 48.4 seconds longer cell life than with an amplitude of 45 V at the same frequency.

Based on the results obtained, we can conclude that acoustic-mechanical influence on the sperm of sturgeon fish improves fish-biological and reproductive indices of seminal fluid. At

the moment, the most optimal parameters for reproductive cells of male Russian sturgeon are 500 Hz, 90 V with the exposure time of 1 minute.

To study crystallization of the solutions, 3 samples were taken: A - control, C - 500 Hz and 90V, E - 300 Hz and 90V. On the graph (Fig. 4) the critical temperature points, at which the cracking of the sample, recrystallization occurred, are marked.

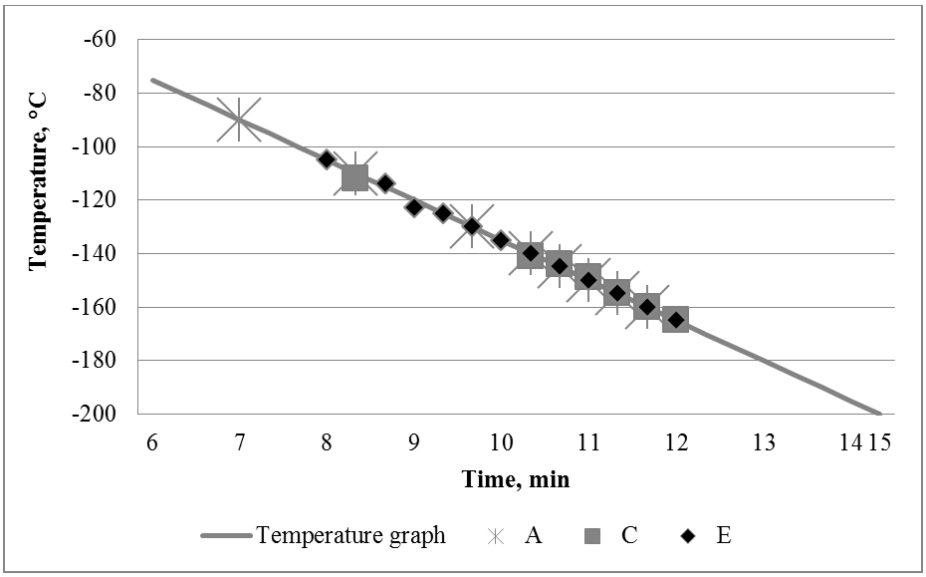

Fig. 4. Main crystallization points of sperm mixture and cryoprotectant medium: A - control, native+protectant; C - 500 Hz, 90V; E - 300 Hz, 90V.

It was shown that when a piezo-actuator with a frequency of 500 Hz and an amplitude of 90 V is applied to the mixture of sperm with cryoprotectant, ice crystals are formed later, which can affect the survival of cells after defrosting.

Thus, a number of parameters of acoustic-mechanical effects on the sperm of sturgeon fish were investigated during experimental works. An increase in reproductive qualities in Russian sturgeon was noted when exposed to the range of 500 Hz, 90 V for 1 minute. Further research is required to optimize the cryopreservation process using acoustic-mechanical effects on the reproductive cells of sturgeon.

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