Acoustic-mechanical effect on the sperm of sturgeon fish using piezoactuators

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Abstract. To improve the quality of the frozen material during cryopreservation, scientists apply various effects on cells: mechanical, chemical or physical. In this work we use acoustic-mechanical effects on cells before cryopreservation. As a result of the studies, the optimal parameters of the impact of the piezoactuator were selected to improve the quality of defrosted reproductive cells of male sturgeons. The object of research was the sperm of the Russian sturgeon. The progressive motility time of native spermatozoa posure time (0.5 min; 1 min, 1.5 min) and frequency (300 Hz, 500 Hz, 550 Hz) were used. Analysis of the motility of thawed sperm showed that the best result in terms of the percentage of sperm motility was obtained when using a frequency of 500 Hz for 1 minute (27%). At the same time, the best indicator of sperm motility time was given by using a frequency of 300 Hz for 1 minute (390 s).

1 Introduction

Cryopreservation of sperm is the most effective method of long-term storage of sperm from domestic animals [1]. Sperm cryobanking is a valuable tool for preserving the genetic resources of various species, providing an opportunity to restore populations and maintain diversity.

Currently, despite the development of innovative methods of artificial reproduction of fish, sperm cryopreservation is still the most effective method of storing reproductive cells for managing genetic diversity [2-3]. The use of frozen sperm from animals and fish minimizes the spread of diseases, eliminates geographical barriers and preserves the genetic material of the animal for an unlimited time [4].

The main problem in creating a cryobank of animal sperm is the development of a successful freezing protocol [5]. The freezing-thawing process leads to a sharp decrease in the quality of sperm and, consequently, fertility. Cryopreservation reduces the viability and motility of spermatozoa subjected to cryopreservation [6].

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Thus, improving sperm survival after cryopreservation and increasing fertility during artificial insemination with frozen-thawed sperm continues to be the focus of attention when cryobanking fish sperm [2, 6].

Over the past decade, significant progress has been made in the process of cryopreservation of fish sperm, and, as a result, fertilization. Progress was associated with the use of new cryopreservation methods, including the search for new tread compositions [7-8] and high-speed freezing modes [9], which lead to better cryo-survival of spermatozoa.

The success of cryopreservation depends on the quality of the sperm, and, therefore, its quality should be assessed based on the state of the sperm before cryopreservation [10]. Sperm quality assessment is very important because it provides the necessary information for optimal protocols for processing and storing sperm used in artificial insemination. The quality of sperm is species-specific and may depend on the feeding regime, feed quality, growing temperature and spawning season of males [11].

To improve the quality of the frozen material during cryopreservation, scientists apply various effects on cells: mechanical, chemical or physical [12]. Thus, the use of magnetized water as a base solution of freezing media can protect plasma, acrosomal and mitochondrial membranes of sperm cells against damage caused by cryopreservation [13]. Low-level laser irradiation is also a new biophysical approach to improving the quality of sperm after thawing. Low-level laser irradiation affects the mitochondrial respiratory chain of spermatozoa, which leads to a possible increase in sperm survival during freezing [14]. The use of sublethal stress in spermatozoa before freezing is a new strategy for cryopreservation of sperm. Stress exposure can cause general adaptation and increased resistance to various future stresses. Thus, the use of hydrostatic pressure (40 MPa, 80 min) before cryopreservation of sperm increases sperm motility without adversely affecting the health of the resulting offspring [15]. Mild oxidative stress caused by 200 mmol/l of hydrogen peroxide (H_2O_2) increases the rate of fertilization and penetration of bull sperm after thawing [16]. In other studies, the quality of frozen-thawed spermatozoa significantly improved when very small doses of stressors (e.g., ethanol and nitric oxide) were added to the cryopreservation medium before freezing [17].

In this work, to improve the quality of sperm, we use acoustic-mechanical effects on cells before cryopreservation. Previously carried out works it has been shown that this contributes to a better penetration of protectors into cells in a shorter period of time, thereby reducing the period of equilibrium, that is, the time of the toxic effect of the protector components on the cells. As a result, the percentage of live sperm after deep freezing increases.

Piezoelectric actuator (piezoactuator) is a device that uses the ability of piezoceramics to expand under the influence of an electrostatic field to generate force and displacement in the micrometer range. The piezoactuator is designed to activate mechanisms, systems or controls based on the piezoelectric effect, converting electrical signals into mechanical motion or force [18]. They convert the electrical voltage into a small, but extremely precisely controlled linear displacement with a high developed force. The principle of their operation is based on the reverse piezoelectric effect, that is, the mechanical deformation of the crystal when exposed to an electric field. In this case, reciprocating motion or other types of it is carried out.

2 Material and research methods

The object of research was the sperm of the Russian sturgeon. Motility of spermatozoa with in native sperm was 90%. The progressive motility time of native spermatozoa posure time (0.5 min; 1 min, 1.5 min) and frequency (300 Hz, 500 Hz, 550 Hz) were used. The experimental options were: A - 300 Hz, 1min; B - 500 Hz, 1min; C - 550 Hz, 1min; D - 500

Hz, 0.5 min; E - 500 Hz, 1.5 min. Sperm quality was evaluated before and after cryopreservation, while the time of translational movement of spermatozoa and the percentage of motile spermatozoa were determined.

Before low-temperature preservation, cryoprotectant was added to the sperm of the Russian sturgeon, the solution was treated with a piezoactuator and kept for 40 minutes at 4 ° C. After balancing, the samples were frozen by a stepwise method in a programmable Planer freezer (Figure 1) to a temperature of -70 °C with further immersion of the samples in liquid nitrogen (-196 ° C).

Fig. 1. Program freezer Planer.

3 Results and discussion

The results of the assessment of sperm motility before freezing are presented in Figures 2, 3.

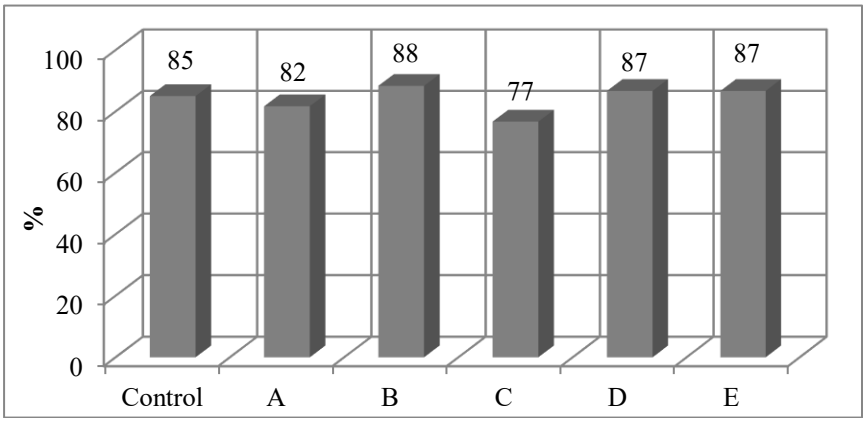

Fig. 2. Motility, %.

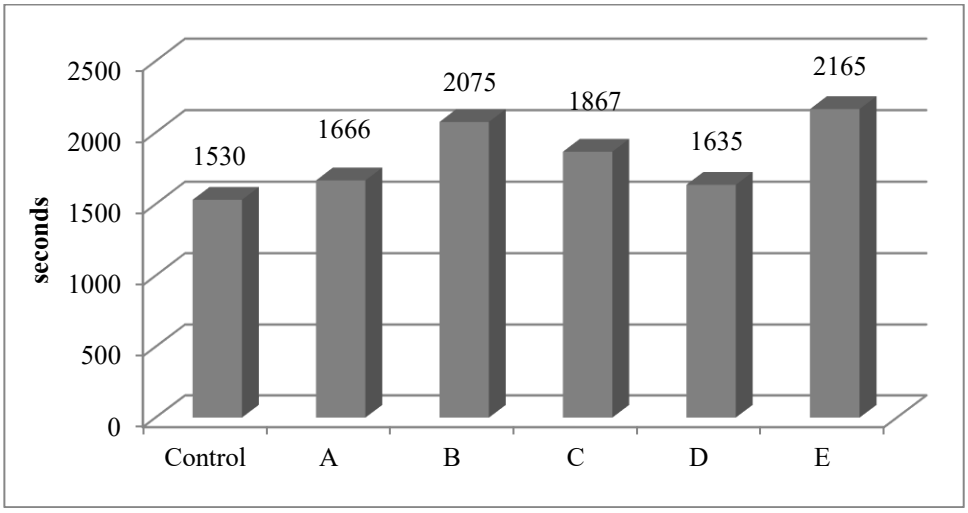

Fig. 3. Time of motility.

As can be seen from Figures 1 and 2, the acoustic-mechanical effect has an effect on spermatozoa even before freezing. When conducting acoustic-mechanical effects on the sperm of the Russian sturgeon, the frequency of irradiation plays an important role. The lowest result in the percentage of sperm motility was obtained using a frequency of 550 Hz. When using a frequency of 300 Hz, the percentage of motility was at a fairly high level (82%), but the time of translational motility of spermatozoa decreased by 25% relative to native sperm. The best result was obtained when using a frequency of 500 Hz (variants B, D, E).

Exposure time also had a significant impact. Thus, when exposed for 0.5 minutes, the time of translational motility of spermatozoa was significantly reduced (option D), while the percentage of motility remained at a high level (87%). The best results on sperm activity were obtained when exposed for 1 and 1.5 minutes. At the same time, the percentage of sperm motility was higher (88 and 87%, respectively) than in the control sample without acoustic-mechanical action (85%).

Thus, when analyzing sperm before freezing, the best results were obtained when using a frequency of 500 Hz for 1 and 1.5 minutes.

The results of assessing the mobility of thawed sperm are presented in Figures 4 and 5.

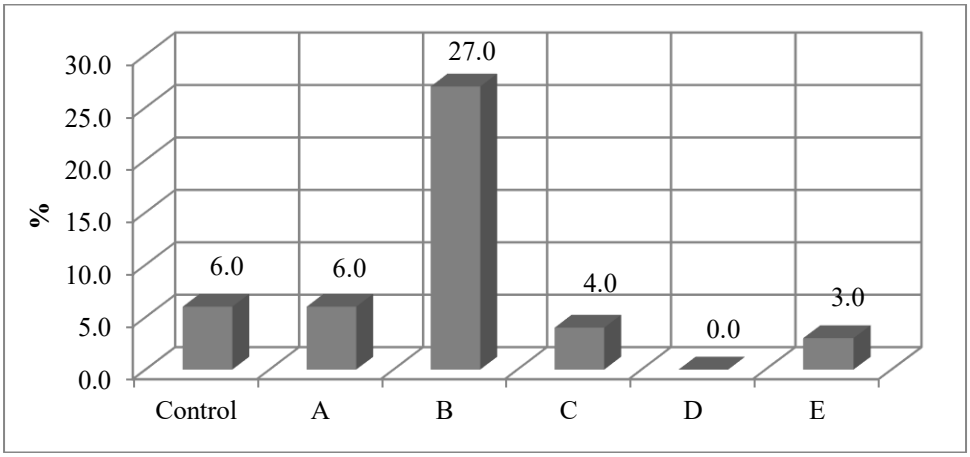

Fig. 4. Motility, %.

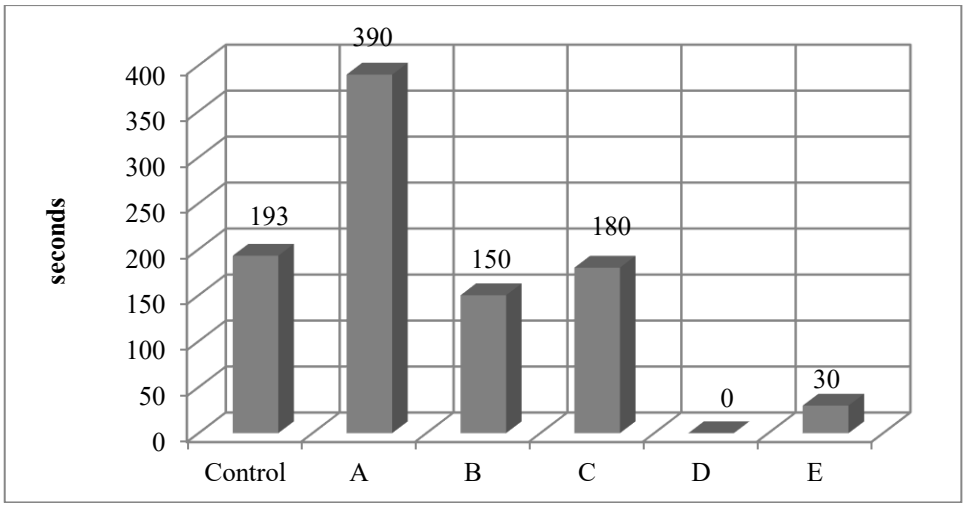

Fig. 5. Time of motility, sec.

Analysis of the motility of thawed sperm showed that the best result in terms of the percentage of sperm motility was obtained when using a frequency of 500 Hz for 1 minute (27%). At the same time, the best indicator of sperm motility time was given by using a frequency of 300 Hz for 1 minute (390 s).

Due to the fact that artificial insemination of eggs gives preference to sperm with a higher percentage of sperm motility, it is recommended to use acoustic-mechanical action with a frequency of 500 Hz for 1 minute for cryopreservation of sperm.

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