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CRYOPRESERVATION OF REPRODUCTIVE CELLS OF MALE RUSSIAN STURGEON

Currently, cryopreservation of sperm is recognized as one of the promising ways to preserve the genetic diversity of fish, not only rare and endangered species, but also aquaculture objects. Scientific research was carried out in the scientific research center "Fisheries" of the S. Seifullin Kazakh Agro Technical University and the JSC "Republican Center for livestock breeding "Asyl Tulik" in 2022. The purpose of the research was to study methods of cryopreservation of Russian sturgeon sperm using cryoprotectors using dimethyl sulfide oxide with a concentration of 5% and 10% and methanol with a concentration of 3% and 8%. In the course of the research, generally accepted methods for assessing and freezing the sperm of the studied object were used. During the experimental work, the method of freezing the reproductive cells of the Russian sturgeon with a 15-minute exposure to -21°C in a box with subsequent immersion in a Dewar vessel was studied. The survival and lifetime of spermatozoa after defrosting were also studied. According to the results of the assessment of the quality of defrosted sperm of the Russian sturgeon, it was found that the most effective cryoprotective medium for cryopreservation is based on methanol with a concentration of 8%. The number of spermatozoa with translational movements in the three groups ranged from 23.8% to 31.2%. At the same time, the lifetime of spermatozoa in this cryoprotective medium was 118s. Methanol with a concentration of 8% provides the best resistance to oxidative stress experienced by cells during freezing. The results of the research make it possible to create a cryobank of the Russian sturgeon gene pool at fish hatcheries to preserve genetic diversity.

Key words: cryopreservation, cryoprotector, reproductive cells, sperm, Russian sturgeon.

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Орыс бекіресі аталықтарының репродуктивті жасушаларының криоконсервациясы

Қазіргі уақытта сирек кездесетін және жойылып бара жатқан түрлердің ғана емес, сонымен қатар аквакультура объектілерінің де балықтардың генетикалық әртүрлілігін сақтаудың перспективті жолдарының бірі шәуетті криоконсервациялау болып табылады. Ғылыми зерттеулер 2022 жылы «С. Сейфуллин атындағы Қазақ агротехникалық университетінің» «Балық шаруашылығы» ғылыми-зерттеу орталығында КеАҚ және «Асыл түлік» республикалық мал шаруашылығын асылдандыру орталығы» АҚ жүргізілді. Ғылыми зерттеулердің мақсаты 5% пен 10% концентрациясы бар диметилсульфидоксид және 3% пен 8% концентрациясы бар метанол негізіндегі криопротекторларды қолдана отырып, орыс бекіресінің шәуетін криоконсервациялау әдістерін зерттеу болып табылды. Зерттеу барысында зерттелетін объектінің шәуетін бағалау және мұздату бойынша жалпы қабылданған әдістер қолданылды. Эксперименттік жұмыстарды жүргізу кезінде орыс бекіресінің репродуктивті жасушаларын 15 минут -21^{0}С дейін ұстап, кейіннен Дьюар ыдысына батырып қатыру әдісі зерттелді. Сондай-ақ, дефростациядан кейін сперматозоидтардың өмір сүру деңгейі мен өмір сүру уақыты зерттелді. Орыс бекіресінің дефростирленген шәуетінің сапасын бағалау нәтижелері бойынша криоконсервациялау үшін 8% концентрациясы бар метанол негізіндегі криоқорғау ортасы неғұрлым тиімді болып табылатыны

анықталды. Үш топ бойынша ілгерілемелі қозғалысы бар сперматозоидтардың саны 23,8%-дан 31,2%-ға дейін ауытқыды. Сонымен қатар, осы криопротекторлық ортаны қолдана отырып, сперматозоидтардың өмір сүру уақыты 118 с құрады, 8% концентрациясы бар метанол мұздату кезінде жасушалар бастан кешіретін тотығу стрессіне жақсы қарсылықты қамтамасыз етеді. Зерттеу нәтижелері генетикалық әртүрлілікті сақтау үшін балық зауыттарында орыс бекіресінің гендік қорының криобанкін құруға мүмкіндік береді.

Түйін сөздер: криоконсервация, криопротектор, репродуктивті жасушалар, шәует, орыс бекіресі.

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Криоконсервация репродуктивных клеток самцов русского осетра

В настоящее время одним из перспективных путей сохранения генетического разнообразия рыб не только редких и исчезающих видов, но и объектов аквакультуры признана криоконсервация спермы. Научные исследования проводились в научно-исследовательском центре «Рыбное хозяйство» НАО «Казахский агротехнический университет им. С. Сейфуллина» и в АО «Республиканский центр по племенному делу в животноводстве «Асыл түлік» в 2022 году. Целью научных исследований являлось изучить методы криоконсервации спермы русского осетра с использованием криопротекторов на основе диметилсульфидоксида с концентрацией 5% и 10% и метанола с концентрацией 3% и 8%. В ходе исследований были использованы общепринятые методики по оценке и заморозке спермы исследуемого объекта. При проведении экспериментальных работ изучен метод замораживания репродуктивных клеток русского осетра с 15-минутной выдержкой в ящике до -21^{0}С с последующим погружением в сосуд Дьюара. Также были изучены выживаемость и время жизни сперматозоидов после дефростации. По результатам оценки качества дефростированной спермы русского осетра было установлено, что наиболее эффективным для криоконсервации является криозащитная среда на основе метанола с концентрацией 8%. Количество сперматозоидов с поступательными движениями по трем группам колебалось в пределах от 23,8% до 31,2%. При этом время жизни спермиев с использованием данной криозащитной среды составило 118 с. Метанол с концентрацией 8% обеспечивает наилучшую устойчивость к оксидативному стрессу, испытываемому клетками во время заморозки. Результаты исследований дают возможность создать криобанк генофонда русского осетра на рыбоводных заводах для сохранения генетического разнообразия.

Ключевые слова: криоконсервация, криопротектор, репродуктивные клетки, сперма, русский осетр.

Introduction

The Caspian Sea is the richest reservoir in the world in terms of the abundance and number of sturgeon species. Of the 26 known species of sturgeon, 6 live here – beluga (*Huso huso*), Russian sturgeon (*Acipenser güldenstädtii*), Persian sturgeon (*Acipenser persicus*), stellate sturgeon (*Acipenser stellatus*), sterlet sturgeon (*Acipenser ruthenus*), barbel sturgeon (*Acipenser nudiventris*). The Russian sturgeon, stellate sturgeon and beluga are of the greatest commercial importance. Throughout the history of the Caspian fishery, sturgeon catches have varied significantly depending on the reproduction and intensity of fishing. The current catastrophic decline in the number of sturgeon in the Caspian Sea, due to irrational fishing, reduction of migration routes and natural reproduction, determines the need to take effective measures to preserve them. These measures should take into account the species-specific adaptation complexes and migration mechanisms of sturgeon. The discovery of the patterns of the formation of sturgeon populations living in the Caspian Sea is crucial not only for the preservation of natural reproduction, but also for the improvement of the biotechnics of industrial sturgeon breeding[1-5].

Currently, the increasing anthropogenic impact on aquatic ecosystems not only affects the physiological state of aquatic organisms, but also leads to a decrease in the number of species. This is especially noticeable on such species as sturgeon, whitefish (endemic to the Caspian Sea), walleye, etc. If earlier Russian sturgeon, stellate sturgeon and beluga,

as well as barbel stugeon in the Caspian and Azov basins had commercial significance, nowadays their capture is prohibited. Beluga and stellate sturgeon in these reservoirs have become so rare that they have become endangered species, and the populations of Russian sturgeon have sharply decreased. At present, the barbel sturgeon and the native species of the Caspian Sea, the inconnu, can be attributed to rare species, the number of which has decreased to a critically low level [6-8].

Preservation and increase of stocks of rare and endangered species of fish is possible only with the development of factory breeding. However, the efficiency of population recruitment based on artificial reproduction is reduced due to a sharp decrease in the number of producers [9]. There is a need to develop various approaches to the use and preservation of the population gene pool of natural generation producers for the purposes of artificial reproduction. Cryobiotechnology methods for hydrobionts have been actively used for the last 10-15 years and the effectiveness of their implementation with a reduction in the number of natural populations and a shortage of producers can be quite high [10-11].

Cryopreservation remains one of the most effective and rapidly developing areas for preserving the genetic diversity of fish, not only rare and endangered species, but also aquaculture objects. The presence of genetically representative collections of fish genomes from breeding herds at sturgeon hatcheries and from natural populations in the cryobank makes it possible to preserve the genetic diversity of these valuable commercial objects with maximum effect [12-13]. Cryopreservation of biological objects involves a certain composition of the preserving solution as well as freezing and defrosting conditions. In the experiments of foreign scientists on the cryopreservation of fish sperm, the preserving solution is a cryoprotective medium, which is a solution of a cryoprotector in a diluent. Cryoprotectors are understood to be cryoprotectors penetrating through the cell membrane, such as glycerin, ethylene glycol, dimethyl sulfoxide (DMSO), methanol, dimethylacetamide, etc., while non-penetrating cryoprotectors (most often sucrose) are part of the diluent [14-22].

The aim of the research is to study methods of cryopreservation of Russian sturgeon sperm using a cryoprotective medium based on DMSO and methanol with different concentrations.

Materials and methods of research

The research was conducted in 2021-2022 at the scientific research center “Fisheries” of the Department of Hunting and Fisheries, S.Seifullin Kazakh Agro Technical University. The research material was the sperm of a Russian sturgeon (*Acipensergüldenstädtii)*, harvested at the Ural-Atyrau sturgeon hatchery during the spawning campaign. Also, the biomaterial after freezing was studied for quality in the laboratory of JSC “Republican Center for livestock breeding “Asyl Tulik””.

When studying the quality of sperm, a visual assessment was carried out by color and consistency directly during roe straining. The quality of sperm was determined by the number of sperm with translational movement and the lifetime of sperm after its activation. A drop of sperm was applied to the microscopic slide, then diluted with water in a ratio of 1:300, thereby activating sperm cells. For cryopreservation, sperm with a mobility of 90-100% was used.

Low-temperature preservation of reproductive cells of male Russian sturgeon was carried out according to the generally accepted method [13]. However, in the cryoprotective solution, the content of cryoprotectors and the introduction of the base solution were adjusted due to the fact that the Russian sturgeon was chosen as the object of the study. During the freezing process, several cryosolutions containing a multicomponent base solution of 0.1% sucrose, 0.08% potassium chloride, 5-10% dimethyl sulfoxide (DMSO) or 3-8% methanol were used. The cryosolution was prepared in a cool room 16-18°C. The seminal fluid was mixed with a 1:1 cryomedium, and then frozen.

Defrosting of cryovials with cryopreserved sperm was carried out in a water bath at a temperature of 38-40°C for 1 minute. The mobility of defrosted sperm was recorded on a personal computer monitor using a video setup under a microscope at magnification from 180 to 400 times when activated with distilled water.

The following materials were used for the experimental work: a Dewar vessel for storing biological material in nitrogen, a trinocular microscope with a camera and CEROS software of CASA computer technology (IMV-technologies, France) for assessing the quality of defrosted fish sperm, a microscope at x40 magnification for

assessing the quality of native sperm, cryovials, a water bath, a stopwatch, a micropipette. During freezing, Eppendorf cryovials with a volume of 0.2ml and 0.9ml and polypropylene straws with a volume of 0.25ml were used.

Statistical processing was carried out according to the guidance of G.F. Lakin [23] and on a PC using the Excel program [24].

Research results and their discussions

To conduct research after assessing the quality of freshly obtained sperm, groups of fish were formed, which included 2-3 males. Before freezing, semen cooled to 10-12°C was diluted with a cryoprotective medium cooled to the same temperature in a volume ratio of 1:1. The medium was added slowly, drop by drop, with continuous stirring. The resulting suspension of sperm – cryoprotective medium was poured into cryovials with a volume of 0.2-0.9 ml for 5-10 minutes. The cryovials were placed on a raft of foam with a size of 14.5 x 14.3 cm with a thickness of 4 cm. Next, the raft was immersed in a foam box measuring 33.5 x 21cm, 26cm high from the outside and 20cm inside filled with liquid nitrogen. The raft with cryovials was kept for 15 minutes until the temperature dropped to -21 ^{0} C, then all cryovials were transferred to a Dewar vessel for long-term storage at a temperature of -196°C .

The speed and methods of freezing are of great importance, using a programmable freezer for cryopreservation of Persian sturgeon sperm (*Acipenserpersicus*) in the cryoprotective medium MT (modified Flower diluent) + 10% methanol, the authors achieved the best results at a maximum freezing rate of – 40°C/min [25]. We used the field method of freezing on a raft proposed by Russian scientists.

Sperm defrosting was carried out by removing test tubes with frozen sperm from liquid nitrogen and placing them in a water bath with a temperature of 38-40°C, then activated with distilled water. The number of motile spermatozoa and the lifetime were determined in defrosted samples (Table 1).

Table 1 – Results of cryopreservation of Russian sturgeon sperm

Group of fish	Sperm volume, ml	Mobility of defrosted sperm, %			
		DMSO 10%	DMSO 5%	Methanol 3%	Methanol 8%
1 (semen mixture of 3 males)	20 ml	10,2±0,3	24,3±0,24	22,1±0,18	31,2±0,12
2 (semen mixture of 2 males)	14 ml	7,4±0,11	14,3±0,21	12,6±0,16	23,8±0,17
3 (a mixture of sperm from 3 males)	17 ml	8,6±0,14	18,6±0,2	19,3±0,13	27,2±0,26

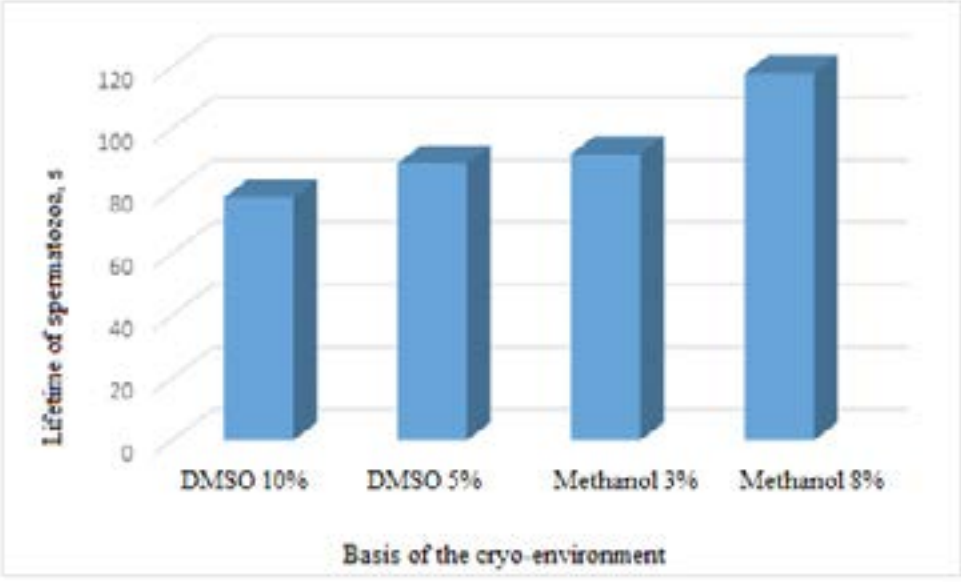

Figure 1 – The time of activity of Russian sturgeon sperm, s

Special attention during the main research was paid to optimizing the qualitative and quantitative composition of the protective medium as the most important factor in ensuring the protection of spermatozoa from the damaging effects of low temperatures. The authors, based on their results, found that methanol is the most suitable cryoprotector for sperm of sturgeon fish (sterlet), providing the best resistance to oxidative stress [26].

In the experiments on cryopreservation of Russian sturgeon sperm, 4 types of cryoprotective medium were used: DMSO with a concentration of 5%, 10%, methanol with a concentration of 3%, 8%. The motility of sperms after defrosting was studied. High results were obtained when using DMSO with a concentration of 5% and methanol with a concentration of 8%, where sperm motility was in the range of 14.3-24.3% and 23.8-31.2%, respectively. In this regard, when comparing the use of DMSO, a high effect was obtained when using DMSO with a concentration of 5%, and when using methanol with a concentration of 8%.

According to the average values of the cryoprotective medium, graphs of the lifetime of sperms after defrosting were constructed. Figure 1 shows that different compositions of the cryoprotective medium act on spermatozoa in different ways. The greatest activity of sperm was shown under the action of methanol at a concentration of 8% and amounted to 118s. When conducting experiments using DMSO, the highest result was obtained when used at a concentration of 5%, which is 12% higher than using DMSO with a concentration of 10%. When using a cryomedium based on methanol with a concentration of 8%, the result was 22% higher than methanol with a concentration of 3%. According to the conducted experiments, the optimal cryoprotective medium is methanol 8%.

Conclusion

In the conducted studies, a field method of freezing the sperm of Russian sturgeon was used with 15 minutes in a box on rafts up to -21°C with subsequent immersion in a Dewar vessel. This raft method showed a good result, where the survival rate of motile spermatozoa ranged from 7.4 to 31.2%.

When assessing the quality of the defrosted sperm of the Russian sturgeon, it was found that the cryoprotective medium based on methanol with a concentration of 8% is the most effective for cryopreservation. The number of sperm with translational movements in the three groups ranged from 23.8% to 31.2%. At the same time, the lifetime of spermatozoa in this cryoprotective medium was 118s.

Conflict of interest

All authors have read and are familiar with the content of the article and have no conflict of interest.

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