СТОРІНКА МОЛОДОГО ВЧЕНОГО

Ribogospod. nauka Ukr., 2017; 2(40): 99-113
DOI: https://doi.org/10.15407/fsu2017.02.099
UDC 621.59:[597-146.31:639.371.2]

DEVELOPMENT OF CRYOPROTECTIVE MEDIA FOR LOW-TEMPERATURE FREEZING OF STERLET (*ACIPENSER RUTHENUS*) SPERM

I. Kononenko, kononenko_irina88@ukr.net, National University of Life and Environmental Sciences of Ukraine, Kyiv

Purpose. *To develop the optimum composition of a cryoprotective solution for freezing sterlet sperm with the use of new and standard constituents and compare the results of the fertilization of eggs with sperm frozen by different ways and in different media. To analyze the results of keeping sterlet males in simulated conditions of natural spawning and to assess the quality of sexual products obtained during early spawning periods in comparison with natural ones.*

Methodology. *The tasks set for the optimization of cryoprotective media for freezing sterlet sperm were solved according to generally accepted cryobiology methods and in accordance with Kopyeyka Ye. F. recommendations. The works with brood sterlet were conducted in accordance with the generally accepted methods of sturgeon breeding and recommendations for natural spawning modeling.*

Findings. *The studies demonstrated a positive effect of creatine, the introduction of which into the solution allowed increasing the cryoprotection of sperm from damaging factors of cryopreservation. During low-temperature freezing of sperm by different ways, the best results were obtained when freezing sperm in samples of smallest volume — granules that was evidenced by the number of live thawed spermatozoa, fertilization rates of eggs and the number of developing embryos. The simulation of the conditions of natural spawning allowed obtaining high-quality sexual products from sterlet males earlier in comparison with natural spawning periods, freezing them and fertilizing eggs after several weeks of storage.*

Originality. *Among a variety of substances used in cryobiology for the optimization of the composition of protective solutions, positive results were tested and obtained with the use of creatine. Optimization of the cryopreservation solution composition was carried out using sterlet sperm obtained at early spawning terms compared to the natural ones.*

Practical value. *The obtained results can be used in the conditions of hatcheries for obtaining sterlet offspring both for market and for the restoration of their populations in natural water bodies. In addition, the obtained results are the basis for further studies on the development of optimum composition of cryoprotective media for freezing sterlet sperm.*

Keywords: *sterlet (Acipenser ruthenus), simulated conditions of natural spawning, pre-spawning group, spermatozoa, methanol, creatine.*

PROBLEM STATEMENT AND ANALYSIS OF LAST ACHIEVEMENTS AND PUBLICATIONS

Since a long time, sturgeon species have been an object of intensive industrial fishery and poaching. With time this and a number of hydroecological factors made impossible their natural reproduction, and consequently, led to a sharp reduction

© I. Kononenko, 2017

in their population size in natural waters. However, a reduction in the number of sturgeons and an increase in the value of their products did not cause a decline in demand, but on the contrary, led to its increase with further annual growth. Nowadays, the reproduction and development of these species are possible only in the conditions of fish farms. If the production of sturgeon products in Ukraine takes place at several farms, the rearing of fish seeds for stocking natural water takes place only at the Dnieper Production and Experimental Fish Hatchery named after academician S. Artyuschik [1]. However, both trends of sturgeon culture face a problem of the lack of high quality sexually mature brood fish [2]. Therefore, for solving this and several other problems in sturgeon culture, both traditional approaches and advanced technology and biotechnology are used, which are the basis for rational environmental management and biodiversity conservation [3].

Currently, one of the current biotechnological methods of the preservation and reproduction of sturgeon is the cryopreservation of male sexual products, followed by the possibility of using this material for producing offspring or for freezing sperm samples in banks as a reserve [4, 5]. The success of the cryopreservation process depends on many factors, among which the most important is the composition of the cryogenic solution, which is used for sperm dilution. Therefore, most studies, which are described in the available literature, are aimed at optimizing the composition of cryoprotective media and searching for new compounds, which can protect the sperm structure from damage during freezing and thawing [6–9].

Regarding the works with the sperm of sturgeon species, the cryopreservation of sexual products requires the development of species-specific freezing technologies, taking into account a number of factors due to a range of specific features of their sperm [9–11]. Perhaps, because of particularly difficult operations with the sperm of sturgeons, despite a large amount of experiments, no successful results on attaining the high quality of the frozen sperm comparable to that of native one were obtained. This fact is a reason for new research and developments in the field of the cryopreservation of sturgeon sperm.

Moreover, sturgeon breeding in controlled conditions, which allows stabilizing the temperature regime, shortening the duration of sturgeon keeping until complete gametogenesis and obtaining sexual products repeatedly, thus intensifying the process of fish cultivation, has been actively used last time [12–14]. This process is important when working with sturgeon species, particularly during the cryopreservation of their sexual products, enabling doing a larger amount of work and getting the desired results.

HIGHLIGHT OF THE EARLIER UNRESOLVED PARTS OF THE GENERAL PROBLEM. AIM OF THE STUDY

Cryopreservation of fish sexual products is a quite complicated biotechnological process, where the desired result is achieved by performing many experiments, and if we take into account the seasonality of spawning works, then it can take more than one year. In the case of sturgeon species, work with low freezing of their sperm is somewhat complicated due to a low quantity of sexual products that may be insufficient to complete the experiment. Simulating natural spawning conditions and controlling the maturation processes of breeding stocks are of great interest for both market sturgeon culture and for work on the reproduction of sturgeon for the restoration of their natural

stocks. This process attracts an evident interest also in the works on the cryopreservation of sturgeon sperm. The efficiency of cryopreservation products increases due to a larger quantity of available sexual products. Therefore, the aim of our research was the development of an optimum composition of a cryoprotective medium for freezing sturgeon sperm with the use of sperm obtained from males at early terms compared to natural ones.

MATERIALS AND METHODS

The work is based on the results of studies, which were performed at the base of educational, scientific and industrial laboratory of the Aquaculture Department of the National University of Life and Environmental Sciences of Ukraine. Eight males of sterlet (prespawning group) were used for the study. They were kept in a special thermocontainer with adjustable system of water heating and maintenance of the necessary water flow regime (at 0.2–0.5 m/s). Simulation of natural spawning conditions by gradual rise of water temperature at 0.5°C daily during 21 day was performed for the maturation of sterlet males and development of their reproductive cells [15, 16]. All manipulations with brood fish of the prespawning group (stimulated maturation, obtaining sexual products, etc.) were conducted according to generally accepted methods used for sturgeon culture [17, 18].

Works on the cryopreservation of sterlet sperm were conducted according to the recommendations of E. Kopyeyka and other cryobiologists [10, 19]. A search for the optimal composition of cryoprotective medium was performed by testing the effect of different substances on sperm quality after thawing based on the results of fertilizing capacity of the thawed sperm (fertilization rates of eggs and number of developing embryos).

STUDY RESULTS AND THEIR DISCUSSION

Sterlet brood stock was kept in a recirculating aquaculture system with water temperature of about 7°C. To trigger the mechanisms, which promote spawning condition in the brood stock in convenient terms, the selected groups of males (prespawning group) were kept in simulated natural spawning conditions with the rise of daily water temperature from 0.5°C to 14.3°C. Monitoring of the physiological state of sterlet males showed that during the study period, which lasted for 21 days, brood fish were active and have good feed intake. The survival rate of sterlet males during this period was 100%, which proved favorable course of the simulation process of natural spawning conditions.

In order to stimulate the maturation of sexual products, the prespawning group of males was divided into two groups of four individuals each (tab. 1).

Quality assessment of males, performed based on major morphometric features, showed relative homogeneity of selected brood fish in both subgroups for all the studied features. The males in the first subgroup had an average age of 8.5 years and body weight from 0.9 kg to 1.2 kg. Their average body length was 52.73±2.04 cm, with a range from 50.20 cm to 57.8 cm. The males in the second prespawning subgroup had an average age of 9 years. Their average body length and weight were virtually equal to these parameters in the first subgroup of males (1.06±0.18 kg and 47.98±2.57 cm respectively).

Table 1. **Some results of works with the prespawning sterlet group, M±m**

Group, № ♂	Index			Stimulanting preparation	Response to stimulation
	age, years	weight (M), kg	length (L), cm		
Subgroups 1					
1	8	0.9	50.2	*Reproginol*	-
2	9	1.1	52.5		+
3	9	1.2	57.8		-
4	8	0.9	50.4		+
M±m	8.50±0.33	1.03±0.09	52.73±2.04		
σ	0.58	0.15	3.54		
Cv, %	6.79	14.6	6.71		
Subgroups 2					
5	9	1.05	55.0	*Vadilen-2*	+
6	10	1.45	61.6		-
7	8	0.7	54.7		-
8	9	1.05	57.6		+
M±m	9.00±0.47	1.06±0.18	57.23±1.84		
σ	0.82	0.30	3.19		
Cv. %	9.07	28.90	5.58		

Stimulation of male maturation in the prespawning group using stimulation preparations *Reproginol* and *Vadilen-2* resulted in the maturation of 50% brood fish: two in the first and two in the second subgroup (tab. 1).

It should be noted that the fish farm has a long positive experience with the use of both stimulation preparations; therefore the low level of maturation rate cannot be fully attributed to poor quality of the preparations used. Presumably, the lack of response in both male subgroups to stimulants may be associated with impaired functioning of neurohumoral systems and, consequently, insufficient preparedness of brood fish for spawning [20]. In addition, males were assessed regarding the quality of their sexual products, which is important when rearing brood fish in controlled conditions and is a determinant factor when deciding the suitability for sperm for cryopreservation (tab. 2).

In total, the first collection of sperm yielded to 11–18 ml; the second one (in 3 hours) — to 9–15 ml. The total amount of two portions of sperm yielded to 27 ml from the male number 2 (first subgroup), 21 ml and 30 ml from the males №№ 5 and 8, respectively, in the second subgroup. The results of rapid evaluation of the sperm quality of in the prespawning group showed the best vital features for the male № 2 of the first subgroup. The sperm of this brood fish had appropriate milky-white color, was characterized by required consistency and high motility of spermatozoa: 95–100% after their activation by pond water. The analysis of the quality of male sexual products in the second subgroups showed that the male № 5 had sexual products with normal consistency, but they had whitish-transparent color and the share of spermatozoa with straight-forward movement was slightly lower (90–95%). Sexual products of the male № 8 had whitish-transparent

color and normal consistency, but the motility of spermatozoa was 70–75% and the G. Persova score was 4. When performing the works on the cryopreservation of the mentioned males under the same conditions, the sperm of males № 2 and № 5 was found to be suitable for low-temperature freezing because the motility of spermatozoa after the process of freezing and thawing remained the highest. The sexual products of the male № 8 from the second subgroup were characterized by the abrupt loss of spermatozoa motor activity even after using different compositions of cryoprotective solutions.

Table 2. **The results of obtaining sexual products of sterlet males at prespawning terms**

Group ♂, №	Ejaculate volume, ml			Sperm color	Consistence	Presence of admixtures*	Score
	Portion I	Portion II	∑				
Subgroup 1							
1	–	–	–	–	–	–	–
2	18	9	27	milk-white	liquid sour cream	–	5
3	–	–	–	–	–	–	–
4	15	–	15	transparent	watery	+	3
	∑, ml		42				
Subgroup 2							
5	11	10	21	whitish-transparent	liquid sour cream	-	5
6	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–
8	15	15	30	whitish-transparent	liquid sour cream	–	4
	∑, ml		51				

Note. * — high content of ovarian fluid, presence of blood, urine, mucus, and others foreign substances.

Testing cryoprotective properties of standard media showed poor results on sterlet sperm. Therefore, for improving the efficiency of the cryopreservation of sterlet sexual products, a decision was taken to optimize the composition of cryoprotective solutions. Thus, four cryoprotective solutions were selected after testing a number of them. They are based on methanol and dimethyl sulfoxide (DMSO) (tab. 3).

Quality assessment of the defrosted sperm diluted by these solutions demonstrated different effects of cryoprotective substances of various substances. Moreover, the quality of sperm was affected by the volume of the frozen sample. In particular, the dilution of sperm with the cryoprotective solution № 1 and freezing it in 1.5 ml test-tubes caused a decrease in spermatozoa motility in the thawed sperm from 35 to 45% compared to the native one. In the sperm frozen in 0.5 ml test-tubes, approximately 50–60% of spermatozoa were able to move after thawing and activation by pond water. The highest spermatozoa motility was observed in the thawed sperm, the samples of which were frozen in the form of granules — 75–80% (fig. 1a).

Table 3. **Blend composition of cryoprotective solutions**

№ solutions	Composition
№ 1	DMSO — 2,115 M, KCl — 13,4 мM, sucrose — 14,6мM, glycine — 74,9 мM
№ 2	methanol — 3,73 M, KCl — 13,4 мM, sucrose — 14,6 мM, glycine — 20,5 мM
№ 3	DMSO — 1,057 M, methanol — 1,86 M, KCl — 13,4 мM, sucrose — 14,6 мM, glycine — 66,6 мM
№ 4	methanol — 3,73 M, $KHCO_3$ — 8,9 мM, creatine — 3,8 мM, sucrose – 14,6 мM

A tendency of the quality reduction of the thawed samples with an increase in their volumes was noted during cryopreservation when using the sperm diluted by the cryoprotective solution № 2. Spermatozoa motility of the thawed sperm in granules remained at the level of 45–55%, whereas when using the sperm frozen in test-tubes (0.5 ml) resulted in a decline to 25–30% (fig. 1b). Therefore, the studies confirmed the earlier results obtained by other scientists who noted the reduction of sperm quality with an increase in the volume of frozen samples [21–23].

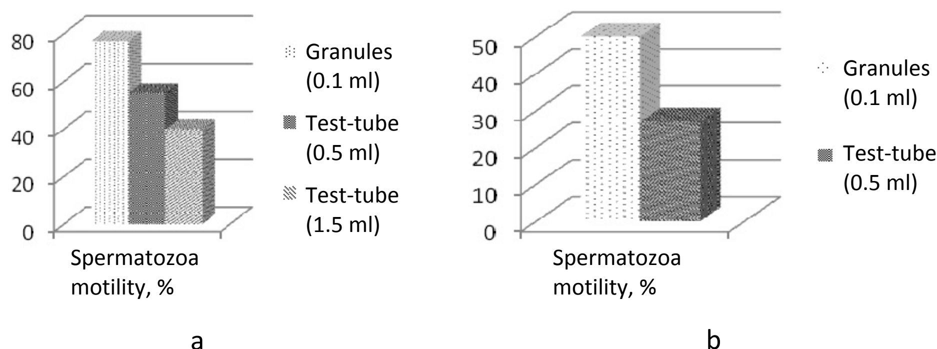


Fig. 1. **Spermatozoa motility (%) after thawing the sperm in different cryoprotective solutions: a — cryoprotective solution № 1, b — cryoprotective solution № 2**

The obtained parameters of spermatozoa motility of the thawed sperm are associated not only with the cryopreservation method but also with the components of cryoprotective media, the components of which ensure the cryoprotection of cells from the extreme factors of cryopreservation and preservation of the functional usefulness of spermatozoa.

When comparing the effects of cryoprotective media on spermatozoa properties after thawing sperm, the highest activity was observed for the sperm diluted by the cryoprotective solution №1 based on DMSO and frozen in the form of granules: 75–80%. The obtained value significantly exceeded the result of freezing the sperm diluted by the cryoprotective solution № 2 based on methanol, when it was possible to produce only 45–55% of active spermatozoa (fig. 2).

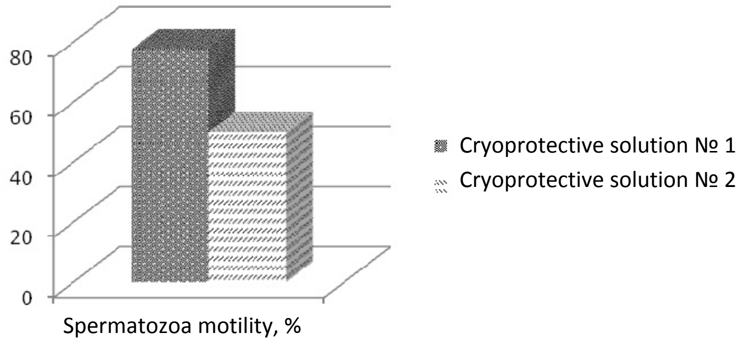


Fig. 2. **Spermatozoa motility after thawing the sperm frozen in different cryoprotective solutions: № 1 (DMSO) and № 2 (methanol)**

The obtained results confirm the data of other scientists who observed a lower quality of the thawed sperm of sturgeons and salmonids, which was diluted by the cryoprotective solution based on methanol [11, 24, 25].

Freezing of the sperm diluted by the cryoprotective solution № 3 (based on DMSO and methanol), has caused a significant decline in the quality of the thawed samples. Spermatozoa motility decreased by more than 55% compared to cases when native sperm was used resulting in 35–45% (fig. 3).

However, despite the better results obtained using the cryoprotective media based on DMSO, due to its observed negative action and toxic effect on spermatozoa [26–29], it was replaced by methanol in further studies. In addition, KCl in the composition of the cryoprotective solution was replaced by $KHCO_3$, the positive experience of working with which was described in the available literature [30, 31]. In addition, we used creatine as an osmolyte. As a result, the spermatozoa survival after sperm preservation increased up to 60–65% that was significantly higher than the results obtained earlier with the use of the cryoprotective solution based on methanol (fig. 3). We can say that the use of these components in the cryoprotective solution contributed to an increase in protective properties and improvement of the thawed sperm quality.

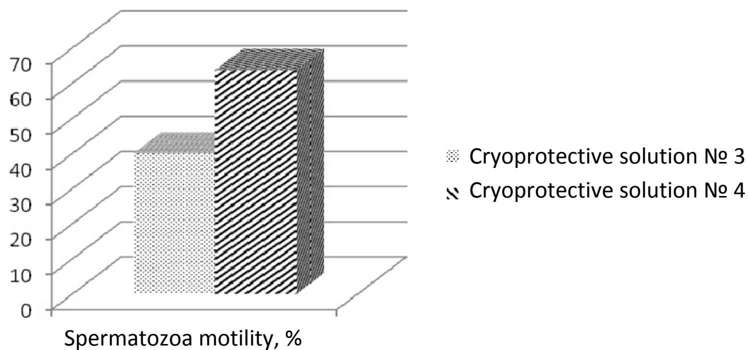


Fig. 3. **The quality of sperm diluted by the cryoprotective solutions №№ 3 and 4**

In general, the highest spermatozoa motility was observed in the sperm, which was cryopreserved in the cryoprotective solution based on DMSO (№ 1). A combination of

DMSO and methanol did not bring the expected result and caused a decrease in spermatozoa activity to 35–45% (cryoprotective solution № 3) compared to the use of the native sperm. At the same time, satisfactory results were obtained when using cryoprotective solution based on methanol with new components (№ 4) — 60–65%, which is promising for further studies.

The next stage of our works was the use of the thawed sperm to fertilize sterlet eggs for testeing their fertilizing ability. For this purpose, small portions of eggs were used (about 2 g), the fertilization and development of which were performed in Petri dishes. Using small portions of eggs is very important for the study because it allows performing fertilization and incubation of embryos without the removal of mucilage [32, 33]. The frozen samples diluted by the cryoprotective solution №№ 1–4 were used for eggs fertilization. The frozen samples were stored for 17 days. Quality assessment of the thawed sperm did not found any deterioration of spermatozoa functional characteristics.

The results of sterlet egg fertilization performed in Petri dishes demonstrated a satisfactory performance (tab. 4).

Table 4. **The results of sterlet egg fertilization by cryopreserved and native sperm in Petri dishes**

Cryoprotective solution, №	Cryopreservation method	Spermatozoa motility, %		№ ♀	Petri dish, №	Fertilized eggs, %	Number of developing embryos, %
		after dilution	after thawing				
1	Test-tube 1.5	80–85	35–45	20	1a	39.0	32.4
					1 b	35.0	29.8
					M	37.0	31.1
				18	2a	38.5	30.5
					2	38.0	28.7
					M	38.3	29.6
	Test-tube 0.5		50–60	20	3	45.0	41.9
	Granules 0.1		75–80		4	55.0	50.7
2	Test-tube 0.5	75–80	25–30	10	5	33.0	28.1
	Granules 0.1		45–55		6a	64.0	55.0
					6 b	66.0	55.9
					M	65.0	55.4
3	Granules 0.1	80–85	35–45	20	7a	53.0	48.4
					7 b	57.0	53.2
					M	55.0	50.8
4	Granules 0.1	75–80	60–65	14	8a	40.0	31.8
					8 b	42.0	34.0
					M	41.0	32.9

Continued Tab. 4

Cryopro-tective solution, №	Cryopre-servation method	Spermatozoa motility, %		№ ♀	Petri dish, №	Fertilized eggs, %	Number of developing embryos, %
		after dilution	after thawing				
Control (♂ № 17)				18	9	79.4	69.1
				14	10	80.7	73.7
Control (♂ № 19)				20	11a	84.8	75.2
					11b	85.2	76.5
					M	85.0	75.8
				10	12	83.5	74.5

The percent of egg fertilization with the thawed sperm fluctuated from 33 to 66% that depended on the cryopreservation method and composition of the cryoprotective solution. For eggs fertilized by sperm granules, the obtained results were higher compared to eggs fertilized by sperm from the tubes of different volumes.

The study of the embryonal development of sterlet eggs on 4 blastomeres and gastrula stages confirmed earlier findings. The highest percentage of egg fertilization was observed in portions fertilized by the smallest samples — granules, and with an increase in the volume of the frozen sample, the egg fertilization rate decreased.

In addition, the studies showed that spermatozoa motility remained high after the cryopreservation of sperm in the cryoprotective solution with DMSO, but the fertilizing ability of the thawed sperm decreased, while the results obtained for the cryoprotective solution based on methanol were consistent with the earlier results obtained for the sperm of sturgeons and salmonids [34–36].

CONCLUSION AND PERSPECTIVES OF FURTHER DEVELOPMENT

Thus, the study on the optimization of the cryoprotective solutions for freezing sterlet sperm demonstrated positive results when using creatine and replacing KCl by $KHCO_3$ that was confirmed by egg fertilization and development data. The use of these components contributed to an increased cryoprotection of sperm from the negative effects of extreme factors of cryopreservation. The obtained result can be important for future studies.

BIBLIOGRAPHY

1. Алхімов Є. М., Шевченко В. Ю. Сучасний стан вирощування ремонтних цьоголіток осетроподібних риб (*Acipenseriformes*) в умовах півдня України//Рибогосподарська наука України. 2017. № 1 (39). С. 52—63.
2. Чебанов М. С., Галич Е. В. Руководство по искусственному воспроизводству осетровых рыб. Технический доклад ФАО по рыбному хозяйству. Анкара, 2013. 325 с.
3. Результаты разработки методов формирования маточных стад стерляди в условиях замкнутого водообеспечения/Пономарева Е. Н. и др.//Вестник АГТУ. 2010. № 1. С. 86—91. (Серия: Рыбное хозяйство).

4. Вепринцев Б. Н., Пилиев С. А. Сохранить генофонд рыб и водных беспозвоночных//Рыбное хозяйство. 1989. № 6. С. 29—32.
5. Карнаухов В. Н. Проблемы и перспективы создания генетических криобанков для целей сохранения биоразнообразия//Биофизика живой клетки. 1994. Т. 6. С. 1—6.
6. Криоконсервация репродуктивного материалы рыб: разработки Южного научного центра Российской академии наук/Пономарева Е. Н. и др.//Современные рыбохозяйственные и экологические проблемы Азово-Черноморского региона: VII Междунар. конф., 20–23 июня 2012 г.: матер. Керчь: ЮгНИРО, 2012. Т. 2. С. 55—58.
7. Концепция сохранения и устойчивого использования биоразнообразия с применением методов криоконсервации геномов гидробионтов/Ананьев В. И. и др.//Избранные труды ВНИИПРХ. Дмитров: Север Подмосковья, 2002. Кн. 1, т. I–II. С. 385—399.
8. Белоус А. М., Гордиенко Е. А., Розанов Л. Ф. Биохимия мембран. Замораживане и криопротекция. Москва: Высшая школа, 1987. 80 с.
9. Использование новых технологий в аквакультуре/Войнова Н. В. и др.//Холодноводная аквакультура: старт в XXI век: Междунар. симп., 8–13 сент. 2003 г.: матер. Санкт-Петербург, 2003. С. 78—80.
10. Технология криоконсервации и хранения в низкотемпературном банке спермы рыб/Цветкова Л. И. и др.//Сборник научно-технической и методической документации по аквакультуре. Москва: ВНИРО, 2001. С. 152—158.
11. Fertilization rate of Siberian sturgeon (*Acipenser baeri* Brandt) milt cryopreserved with methanol/Glogowski J. et al.//Aquaculture. 2002. № 211. P. 367—373.
12. Особенности развития репродуктивной системы донской стерляди в зарегулированных условиях водной среды/Пономарева Е. Н. и др.//Известия Самарского научного центра РАН. 2012. Т. 14, № 5. С. 191—194.
13. Особенности гаметогенеза стерляди в зарегулированных условиях водной среды/Пономарева Е. Н. и др.//Вестник АГТУ. 2011. №2. С. 112—117. (Cepbz: Рыбное хозяйство).
14. Справочник рыбовода. Инновационные технологии аквакультуры Юга России/Матишов Г. Г. и др.; ред. Пономарев С. В. Ростов-на-Дону: ЮНЦ РАН. 2013. С. 109—120.
15. Корчунов А. А. Методы ускоренного формирования маточного стада стерляди в условиях УЗВ//Актуальные проблемы обеспечения продовольственной безопасности Юга России: инновационные технологии для сохранения биоресурсов, плодородия почв, мелиорации и водообеспечения: Междунар. науч. конф., 27–30 сент. 2011 г.: матер. Ростов-на-Дону, 2011. С. 24—25.
16. Результаты разработки методов формирования маточных стад стерляди в условиях замкнутого водоснабжения/Пономарева Е. Н. и др.//Вестник АГТУ. 2010. № 1. С. 86—91. (Серия: Рыбное хозяйство).
17. Судакова Н. В. Технологии и нормативы по товарному осетроводству в VI рыбоводной зоне. Москва: ВНИРО, 2006. 100 с.
18. Чебанов М. С., Галич Е. В., Чмырь Ю. М. Руководство по разведению и выращиванию осетровых рыб. Москва: Росинформагротех, 2004. 135 с.
19. Инструкция по низкотемпературной консервации спермы карпа/ Копейка Е. Ф. Москва: ВНИИПРХ, 1986. 11 с.

20. Залепухин В. В. Индивидуальная рабочая плодовитость как характеристика эндогенной разнокачественности рыб//Вестник АГТУ. 2009. № 1. С. 73—78. (Серия: Рыбное хозяйство).
21. Stein H. Zum Einflus von Schutzsubstanz und Konfektionierung auf das Befruchtungsergebnis mit tiefgefrorenem Bachforellensperma (*Salmo trutta* morpha *fario* L.) // Berl. Munch. Tierarztliche Wochenschrift. 1979. Vol. 92. P. 420—421.
22. О некоторых факторах, влияющих на криоустойчивость спермы карпа *Cyprinus carpio*/Цветкова Л. И. и др.//Избранные труды ВНИИПРХ. Дмитров: Север Подмосковья, 2002. Кн. 1, т. I–II. С. 377—384.
23. Красильникова А. А., Тихомиров А. М. Объем замораживаемого образца как один из факторов выживаемости сперматозоидов осетровых видов рыб при криоконсервации//Естественные науки. Биотехнология. 2014. № 2 (47). С. 62—69.
24. Effects of cryoprotectants and male on motility parameters and fertilizabilization rate in paddlefish *(Polyodon spathula)* frozen-thawed spermatozoa/Linhart O. et al.//J. Appl. Ichthyol. 2006. Vol. 22, suppl. I. P. 384—388.
25. Аквакультура осетровых рыб: достижения и перспективы развития/Цветкова Л. И. и др. Астрахань, 2004. С. 213—214.
26. Acrosome staining and motility characteristics of sterlet spermatozoa after cryopreservation with use of methanol and DMSO/Psenicka M. et al.//Cryobiology. 2008. № 56. P. 251—253.
27. The relationship of the cryoprotectants methanol and dimethylsulfoxide and hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species/Horvath A. et al.//Aquaculture. 2005. № 247. P. 243—251.
28. Chao N.-H., Liao I. C. Cryopreservation of finfish and shellfish gametes end embryos//Aquaculture. 2001. № 197. P. 161—189.
29. Sperm cryopreservation of the Indian major carp, *Labeo calbasu*: Effects of cryoprotectants, cooling rates and thawing rates on egg fertilization/Nahiduzzaman Md. et al.//Animal Reproduction Science. 2012. № 136. P. 133—138.
30. Mounib M. S. Cryogenic preservation of fish and mammalian spermatozoa//J. Reprod. Fert. 1978. Vol. 53. № 1. P. 13—18.
31. Mounib M. S., Hwang P. C., Idler R. R. Cryogenic preservation of Atlantic Cod (*Gadus morhua*) sperm//J. Fish. Res. Board. Can. 1968. Vol. 25, № 12. P. 2623—2632.
32. Черномашенцев А. И., Мильштейн В. В. Рыбоводство. Москва: Легкая промышленность, 1983. 272 с.
33. Иванов А. П. Рыбоводство в естественных водоемах. Москва: Агропромиздат, 1988. 367 с.
34. The relationship of the cryoprotectants methanol and dimethylsulfoxide and hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species/Horváth Á. et al.//Aquaculture. 2005. Vol. 247, № 1–4. P. 243—251.
35. Studies on the sperm biology and sperm cryopreservation in the sterlet, *Acipenser ruthenus* L./Lahnsteiner F. et al.//Aquaculture Research. 2004. Vol. 35. P. 519—528.
36. Lahnsteiner F., Weismann T., Patzner R. A. Methanol as cryoprotectant and the suitability of 1.2 ml and 5 ml straws for cryopreservation of sperm from salmonid fishes//Aquaculture Research. 1997. Vol. 28. P. 471—479.

REFERENCES

1. Alkhimov, Ie. M., & Shevchenko, V. Iu. (2017). Suchasnyi stan vyroshchuvannia remontnykh tsoholitok osetropodibnykh ryb (*Acipenseriformes*) v umovakh pivdnia Ukrainy. *Rybohospodarska nauka Ukrainy, 1(39),* 52-63.
2. Chebanov, M. S., & Galich, E. V. (2013). *Rukovodstvo po iskusstvennomu vosproizvodstvu osetrovyh ryb: Tehnicheskij doklad FAO po rybnomu hozjajstvu*. Ankara.
3. Ponomareva, E. N., Sorokina, M. N., Grigor'ev, V. A., Kovaleva, A. V., & Korchunov, A. A. (2010). Rezul'taty razrabotki metodov formirovanija matochnyh stad sterljadi v uslovijah zamknutogo vodoobespechenija. *Vestnik AGTU, 1,* 86-91.
4. Veprincev, B. N., & Piliev, S. A. (1989). Sohranit' genofond ryb i vodnyh bespozvonochnyh. *Rybnoe hozjajstvo, 6*, 29-32.
5. Karnauhov, V. N. (1994). Problemy i perspektivy sozdanija geneticheskih kriobankov dlja celej sohranenija bioraznoobrazija. *Biofizika zhivoj kletki, 6*, 1-6.
6. Ponomareva, E. N., Tihomirov, A. M., Bogatyreva, M. M., & Krasil'nikova, A. A. (2012). Kriokonservacija reproduktivnogo materialy ryb: razrabotki Juzhnogo nauchnogo centra Rossijskoj akademii nauk. *Sovremennye rybohozjajstvennye i jekologicheskie problemy Azovo-Chernomorskogo regiona: VII Mezhdunarodnaja konferencija*. Kerch', 55-58.
7. Anan'ev, V. I., Gahova, Je. N., Katasonov, V. Ja., Rott, N. N., & Cvetkova, L. I. (2002). Koncepcija sohranenija i ustojchivogo ispol'zovanija bioraznoobrazija s primineniem metodov kriokonservacii genomov gidrobiontov. *Izbrannye trudy VNIIPRH*. Dmitrov: Sever Podmoskov'ja, 385-399.
8. Belous, A. M., Gordienko, E. A., & Rozanov, L. F. (1987). *Biohimija membran. Zamorazhivane i krioprotekcija.* Moskva: Vysshaja shkola.
9. Vojnova, N. V., Mirzojan, A. V., Ivanov, G. Ju., & Fedchenko, V. M. (2003). Ispol'zovanie novyh tehnologij v akvakul'ture. *Holodnovodnaja akvakul'tura: start v HHI vek: mezhdunarodnyj simpozium*. Sankt-Peterburg, 78-80.
10. Cvetkova, L. I., Dokina, O. B., Pronina, N. D., & Milenko, V. A. (2001). Tehnologija kriokonservacii i hranenija v nizkotemperaturnom banke spermy ryb. *Sbornik nauchno-tehnicheskoj i metodicheskoj dokumentacii po akvakul'ture.* Moskva: VNIRO.
11. Glogowski, J., Kolman, R., Szczepkowski, M., Horvath, A., Urbanyi, B., Sieczynski, P., Rzemieniecki, A., Domagala, J., Demianowicz, W., Kowalski, R., & Ciereszko, A. (2002). Fertilization rate of Siberian sturgeon (*Acipenser baeri Brandt*) milt cryopreserved with methanol. *Aquaculture, 211,* 367-373.
12. Ponomareva, E. N., Metallov, G. F., Korchunov, A. A., & Kovaleva, A. V. (2012). Osobennosti razvitija reproduktivnoj sistemy donskoj sterljadi v zaregulirovannyh uslovijah vodnoj sredy. *Izvestija Samarskogo nauchnogo centra RAN, 5*, 191-194.
13. Ponomareva, E. N., Grigor'ev, V. A., Sorokina, M. N., Kovaleva, A. V., & Korchunov, A. A. (2011). Osobennosti gametogeneza sterljadi v zaregulirovannyh uslovijah vodnoj sredy. *Vestnik AGTU, 2,* 112-117.
14. Matishov, G. G., Ponomarev, S. V., & Bakaneva, Ju. M. et. al. (2013). *Spravochnik rybovoda. Innovacionnye tehnologii akvakul'tury Juga Rossii*. Rostov-na-Donu: JuNC RAN.
15. Korchunov, A. A. (2011). Metody uskorennogo formirovanija matochnogo stada sterljadi v uslovijah UZV. *Aktual'nye problemy obespechenija prodovol'stvennoj*

bezopasnosti Juga Rossii: innovacionnye tehnologii dlja sohranenija bioresursov, plodorodija pochv, melioracii i vodoobespechenija: Mezhdunarodnaja nauchnaja konf. Rostov-na-Donu, 24-25.

16. Ponomareva, E. N., Sorokina, M. N., Grigor'ev, V. A., Kovaleva, A. V., & Korchunov, A. A. (2010). Rezul'taty razrabotki metodov formirovanija matochnyh stad sterljadi v uslovijah zamknutogo vodosnabzhenija. *Vestnik AGTU, 1,* 86-91.
17. Sudakova, N. V. (2006). *Tehnologii i normativy po tovarnomu osetrovodstvu v VI rybovodnoj zone*. Moskva: VNIRO.
18. Chebanov, M. S., Galich, E. V., & Chmyr', Ju. M. (2004). *Rukovodstvo po razvedeniju i vyrashhivaniju osetrovyh ryb*. Moskva: Rosinformagrotekh.
19. Kopejka, E. F. (1986). *Instrukcija po nizkotemperaturnoj konservacii spermy karpa*. Moskva: VNIIPRH.
20. Zalepuhin, V. V. (2009). Individual'naja rabochaja plodovitost' kak harakteristika jendogennoj raznokachestvennosti ryb. *Vestnik AGTU. Rybnoe hozjajstvo, 1,* 73-78.
21. Stein, H. (1979). Zum Einflus von Schutzsubstanz und Konfektionierung auf das Befruchtungsergebnis mit tiefgefrorenem Bachforellensperma (*Salmo trutta* morpha *fario* L.). *Berl. Munch. Tierarztliche Wochenschrift, 92*, 420–421.
22. Cvetkova, L. I., Titareva, L. N., Kochetov, A. A. Voronina, V. P., Milenko, V. A., & Pronina, N. D. (2002). O nekotoryh faktorah, vlijajushhih na krioustojchivost' spermy karpa *Cyprinus carpio*. *Izbrannye trudy VNIIPRH.* Dmitrov: Sever Podmoskov'ja, 377-384.
23. Krasil'nikova, A. A., & Tihomirov, A. M. (2014). Ob'em zamorazhivaemogo obraztsa kak odin iz faktorov vyzhivaemosti spermatozoidov osetrovyh vidov ryb pri kriokonservacii. *Estestvennye nauki. Biotehnologija, 2 (47),* 62-69.
24. Linhart, O., Mims, S. D., Gomelsky, B., Cvetkova, L. I., Cosson, J., Rodina, M., Horvath, A., & Urbanyi, B. (2006). Effects of cryoprotectants and male on motility parameters and fertilizabilization rate in paddlefish *(Polyodon spathula)* frozen-thawed spermatozoa. *J. Appl. Ichthyol., 22,* 384-388.
25. Cvetkova, L. I., Dokina, O. B., Pronina, N. D., Milenko, V. A., & Tansykbaev, A. N. (2004). *Akvakul'tura osetrovyh ryb: dostizhenija i perspektivy razvitija.* Astrahan'.
26. Psenicka, M., Dietrich, G. J., Wojtczak, M., Nynca, J., Rodina, M., Linhart, O., Cosson, J., & Ciereszko, A. (2008). Acrosome staining and motility characteristics of sterlet spermatozoa after cryopreservation with use of methanol and DMSO. *Cryobiology, 56,* 251-253.
27. Horvath, A. Wayman, W. R., Urbanyi, B., Ware, K. M., Dean, J. C., & Tiersch T. R. (2005). The relationship of the cryoprotectants methanol and dimethylsulfoxide and hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species. *Aquaculture, 247,* 243-251.
28. Chao, N.-H., & Liao, I. C. (2001). Cryopreservation of finfish and shellfish gametes end embryos. *Aquaculture, 197,* 161-189.
29. Nahiduzzaman, Md., Hassan, Md. M., Roy, P. K., Hossain, Md. A., Hossain, M. Ali R., & Tierach, T. R. (2012). Sperm cryopreservation of the Indian major carp, *Labeo calbasu*: Effects of cryoprotectants, cooling rates and thawing rates on egg fertilization. *Animal Reproduction Science, 136,* 133-138.
30. Mounib, M. S. (1978). Cryogenic preservation of fish and mammalian spermatozoa. *J. Reprod. Fert., 1 (53),* 13-18.
31. Mounib, M. S., Hwang, P. C., & Idler, R. R. (1968). Cryogenic preservation of Atlantic Cod (*Gadus morhua*) sperm. *J. Fish. Res. Board. Can., 12(25),* 2623-2632.

32. Chernomashencev, A. I., & Mil'shtejn, V. V. (1983) *Rybovodstvo*. Moskva: Legkaja promyshlennost'.
33. Ivanov, A. P. (1988). *Rybovodstvo v estestvennyh vodoemah.* Moskva: Agropromizdat.
34. Horváth, Á., Wayman, W. R., & Urbányi B. (2005). The relationship of the cryoprotectants methanol and dimethylsulfoxide and hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species. *Aquaculture, 247,* 243-251.
35. Lahnsteiner, F., Berger, B., Horvath, A., & Urbanyi, B. (2004). Studies on the sperm biology and sperm cryopreservation in the sterlet, *Acipenser ruthenus* L. *Aquaculture Research, 35,* 519-528.
36. Lahnsteiner, F., Weismann, T., & Patzner R. A. (1997). Methanol as cryoprotectant and the suitability of 1.2 ml and 5 ml straws for cryopreservation of sperm from salmonid fishes. *Aquaculture Research, 28,* 471-479.

РОЗРОБЛЕННЯ КРІОЗАХИСНИХ СЕРЕДОВИЩ ДЛЯ НИЗЬКОТЕМПЕРАТУРНОГО ЗАМОРОЖУВАННЯ СПЕРМИ СТЕРЛЯДІ (*ACIPENSER RUTHENUS*)

І. С. Кононенко, kononenko_irina88@ukr.net, Національний університет біоресурсів і природокористування України, м. Київ

Мета. *Розробити оптимальний склад кріозахисного розчину для заморожування сперми стерляді з використанням нових та стандартних складових компонентів і порівняти результати запліднення ікри спермою, замороженою різними способами та у різних середовищах. Проаналізувати результати утримання самців стерляді у модельованих умовах природного нересту та оцінити якість статевих продуктів, отриманих у ранні, порівняно із природними, строки нересту.*

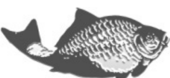
Методика. *Поставлені завдання з оптимізації кріозахисних середовищ для заморожування сперми стерляді вирішувалися згідно з загальноприйнятими методиками робіт у кріобіології та відповідно до рекомендацій Копєйки Є. Ф. Роботи з плідниками стерляді проводилися відповідно до загальноприйнятих методів в осетрівництві та моделювання умов природного нересту.*

Результати. *Дослідженнями встановлено позитивну дію креатину, введення якого до складу розчину дозволило підвищити кріозахист сперми від пошкоджувальних чинників кріоконсервування. В результаті низькотемпературного заморожування сперми різними способами найкращі результати отримані за заморожуваня сперми у зразках найменшого об'єму — гранулах, про що свідчать кількість живих розморожених спермїів, показники запліднення ікри та кількості ембріонів, що розвиваються. Моделювання умов природного нересту дозволило отримати від самців стерляді якісні статеві продукти у ранні, порівняно із природніми, строками нересту, здійснити їх заморожування та запліднити ними через кілька тижнів зберігання ікру.*

Наукова новизна. *Серед різноманіття речовин, що використовуються в кріобіології для оптимізації складу захисних розчинів, було випробувано та отримано позитивні результаті від введення креатину. Оптимізацію складу кріорозчину проведено із використанням сперми стерляді, отриманої у ранні, порівняно із природними, строки нересту.*

Практична значимість. *Отримані результати можуть бути використані в умовах рибницьких заводів з метою отримання потомства стерляді як для товарних потреб, так і для відновлення її популяцій у природних водоймах. Крім того, отримані результати є підґрунтям для подальших досліджень з розроблення оптимального складу кріозахисного середовища для заморожування сперми стерляді.*

Ключові слова: *стерлядь (Acipenser ruthenus), модельовані умови природного нересту, донерестова група, сперматозоїди, метанол, креатин.*



РАЗРАБОТКА КРИОЗАЩИТНЫХ СРЕД ДЛЯ НИЗКОТЕМПЕРАТУРНОГО ЗАМОРАЖИВАНИЯ СПЕРМЫ СТЕРЛЯДИ (*ACIPENSER RUTHENUS*)

И. С. Кононенко, *kononenko_irina88@ukr.net*, Национальный университет биоресурсов и природопользования Украины, г. Киев

Цель. *Разработать оптимальный состав криозащитной среды для замораживания спермы стерляди с использованием новых и стандартных составных компонентов и сравнить результаты оплодотворения икры спермой, замороженной разными способами и в разных средах. Проанализировать результаты содержания самцов стерляди в моделированных условиях естественного нереста и оценить качество половых продуктов, полученных в ранние, по сравнению с естественными, сроки нереста.*

Методика. *Поставленные задачи по оптимизации криозащитных сред для замораживания спермы стерляди решались согласно общепринятым методикам работ в криобиологии. Работы с производителями стерляди проводились в соответствии с общепринятыми методами в осетроводстве и моделированию условий естественного нереста.*

Результаты. *Исследованиями установлено позитивное действие креатина, введение которого в состав раствора позволило повысить криозащиту спермы от повреждающих факторов криоконсервирования. В результате низкотемпературного замораживания спермы разными способами лучшие результаты получены в образцах с наименьшим объёмом — гранулах, о чем свидетельствуют количество живых размороженных спермиев, показатели оплодотворения икры и количества развивающихся эмбрионов. Моделирование условий естественного нереста позволило получить от самцов стерляди качественные половые продукты в ранние, по сравнению с естественными, строки нереста, провести их замораживание и оплодотворить ими через несколько недель хранения икру.*

Научная новизна. *Среди разнообразия веществ, используемых в криобиологии для оптимизации состава защитных растворов, было апробировано и получено позитивные результаты от введения креатина. Оптимизацию состава криораствора проведено с использованием спермы стерляди, полученной в ранние, по сравнению с естественными, строки нереста.*

Практическая значимость. *Полученные результаты могут быть использованы в условиях рыбоводных заводов с целью получения потомства стерляди как для товарных потребностей, так и для восстановления ее популяции в естественных водоемах. Кроме того, полученные результаты являются основой для дальнейших разработок оптимального состава криозащитной среды для замораживания спермы стерляди.*

Ключевые слова: *стерлядь (Acipenser ruthenus), моделированные условия естественного нереста, донерестовая партия, сперматозоиды, метанол, креатин.*