

## Sperm cryoresistance

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### EFFECT OF CRYOPRESERVATION ON BIOLOGICAL PARAMETERS OF SEMEN IN ROMANOV BREED × ARGALI HYBRID RAMS

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#### Abstract

Cryopreservation of genetic material and artificial insemination is an important element of assisted reproductive technologies. Establishing cryobanks of biomaterial, derived of high-valuable breeding rams, provides the production of the maximal number of offspring. This technology allows more effectively use the genetic material with higher efficiency, save and restore the populations of rare and endangered species and carry out crossbreeding and hybridization between isolated populations. The most common biomaterials are the sperm, which are used in the programs for the conservation and restoration of wild and domestic genetic resources. This is associated with the availability and easiness of producing sperm. In sheep breeding, the use of reproductive technology has the local character in contrast to other livestock industries. One reason for this is the low efficiency of application of frozen-thawed ram semen that is caused by some complexities involved in sperm cryopreservation of this animal species. During the freezing and thawing process, the sperm undergoes significant technological impact. Some stages of this process are the shock to semen and lead to the destruction of a large part of cells or to the damaging their individual organelles or segments. Less is known about the freezing capacity of sperm derived from hybrid rams, which are produced by hybridization of domestic and wild *Ovis* species. Taking this into account the aim of our research was to study the effect of freezing and thawing process on the biological parameters of sperm derived from purebred Romanov ram and hybrid rams carrying argali (*Ovis ammon*) blood. As a material for our research, we used the sperm of hybrid rams of different origin: F<sub>1</sub> ( $n = 1$ , 50 % argali, produced by surgical insemination of Romanov ewe with frozen-thawed sperm of argali ram); BC<sub>1</sub> ( $n = 5$ , 25 % argali blood, produced by backcrossing of Romanov ewes with F<sub>1</sub> ram); BC<sub>2</sub> ( $n = 5$ , 12.5 % of argali blood, produced by backcrossing of Romanov ewes with BC<sub>1</sub> rams). The sperm of purebred Romanov ram (RAM) was used as a control. Qualitative and quantitative parameters of freshly derived and frozen semen of the experimental animals, the percentage of motile and immobile spermatozoa, their cryoresistance, the state of the acrosome and the degree of chromatin condensation were studied. The motility of spermatozoa was decreased significantly in sperm of both of hybrid and purebred rams (by 47 and 50 %, respectively). The cryoresistance of spermatozoa of F<sub>1</sub> hybrid ram was increased by 9 %, the osmotic resistance increased — by 20 % and dehydrogenase activity — by 44.5 % comparing to purebred Romanov ram. With the decrease of blood ratio of wild species, the decrease in differences for above-mentioned parameters was observed: the differences between BC<sub>2</sub> and ROM were 2.2, 4.7 and 10.0 %, respectively.

Keywords: argali hybrids, cryopreservation, sperm, freeze-thaw

Artificial insemination (AI) is one of the main elements of farm animal reproduction. At present, effective animal husbandry necessitates AI [1-5]. The cryopreservation of biological material (generative plasma, embryos, germ and somatic cells) is the most important auxiliary reproductive technology [6-8].

Spermatozoa are the most commonly used in conservation and restoration of genetic resources of wild fauna and domestic animals [9]. This is due to the availability and ease of obtaining sperm (5-6 billion germ cells per ejaculate).

In addition, for many animal species, artificial insemination technology using frozen-thawed semen has been developed.

Cryopreservation of sperm allows to accumulate viable generative material from high-value producers, to use it more efficiently, to obtain the maximum number of offspring, to preserve and restore rare and endangered species, and to cross and hybridize between isolated populations. Cryopreservation of spermatozoa consists of several treatments that lead to changes in individual structural units of spermatozoa, which is often accompanied by a decrease in their biological full-value [10-14]. The effect of the freeze-thaw cycle on the spermatozoa morphology is shown [15-19]. In all agricultural animals, when the cryopreserved semen is used, the yield of the offspring is reduced. According to a number of researchers, this is associated with a decrease in the motility of spermatozoa, since they must have a high speed of rectilinear motion to reach the ovum [20]. Spermatozoa with high activity, but ultrastructural, biochemical, or functional damage also have low probability of ovum fertilization [21, 22].

The use of assisted reproductive technology in sheep breeding is of a local character, due to the low efficiency of cryopreservation of ram semen. It is much more difficult to freeze it than the sperm of other mammalian species. About 10 % of the ejaculate obtained from rams using modern diluents and protocols does not undergo cryopreservation [23], and, therefore, to optimize cryopreservation of spermatozoa in this species is of importance.

Adding blood of wild species is one of the promising ways of genetic improvement of sheep breeds. At the L.K. Ernst All-Russian Research Institute of Animal Husbandry a model population has been created, including hybrids of domestic Romanov ewe (*Ovis aries*) and argali (*O. ammon*) with different ratio of the wild species blood. The change in biological and productive indices in individuals with the blood of wild animals compared to the original domestic species was shown [24, 25]. A semen bank of hybrid animals was created [26].

In the present paper, we first studied the indices of the biological full-value of the semen of hybrid rams with different percent of Romanov breed and argali as compared to their purebred analogues. The effect of cryopreservation on cryoresistance, osmotic resistance and dehydrogenase activity of the semen was shown. An increase in these indices in hybrid animals is noted, indicating a higher cryostability of their semen as compared to the semen of purebred rams.

The purpose of this work was to study the effect of freezing-thawing cycle on the biological parameters of spermatozoa in hybrid (with argali blood) and purebred Romanov rams.

*Technique.* The sperm was collected from the hybrid rams: F<sub>1</sub> (obtained by surgical insemination of the Romanov ewe with the frozen-thawed semen of argali,  $n = 1$ , 50 % argali), BC<sub>1</sub> from backcrossing Romanov ewe with F<sub>1</sub> ram ( $n = 5$ , 25 % argali), BC<sub>2</sub> from backcrossing Romanov ewe with BC<sub>1</sub> rams ( $n = 5$ , 12.5 % argali). The Romanov ram semen (ROM) ( $n = 3$ ) was used as a control. Feeding and housing were the same for all the rams. The semen samples were collected in an artificial vagina. Dilution, deep freezing and thawing were carried out in accordance with the proprietary method of ram semen cryopreservation in granules. Quality indicators of freshly prepared diluted and frozen-thawed semen were studied.

For recognition and evaluation of sperm parameters, the software Zoo-sperm 1.0 (OOO VideoTesT, St. Petersburg, Russia) was used. The program is based on video image processing [27]. The integrity of the acrosome was studied by differential staining with Diakhim-Diff-Quick set (ABRIS+, Russia). The DNA fragmentation index in chromatin was determined by detecting DNA gaps in a test with acridine orange (AO-test) and fluorescence microscopy (Nikon,

Japan).

The data was processed statistically using the Microsoft Office software package. The tables show the mean ( $\bar{X}$ ) and their deviations ( $x$ ).

**Results.** Analysis of the motility of spermatozoa in fresh and frozen semen of hybrid animals and their purebred analogues showed no significant differences. The proportion of spermatozoa with rectilinear motion (class A + B) in both experimental groups was almost the same reaching 90.0 % for freshly collected semen and 43.0 % for cryopreserved semen in hybrids, and 93.0 and 43.0 %, respectively, in purebred individuals. The proportion of spermatozoa of class C (with curvilinear movement) and class D (immobile) in freshly obtained semen did not exceed 4.3 and 5.7 %, respectively, in the hybrids, and 3.6 and 3.4 % in the purebred animals. In freezing-thawing, the number of immobile spermatozoa increased to 48.6 % in hybrid rams and to 51.2 % in their purebred analogues.

The indices of osmotic and hypoosmolar resistance, as well as dehydrogenase activity of spermatozoa in hybrid and purebred animals were within the established requirements (Table 1). At the same time, these in parameters were higher in hybrids. The differences between ROM and F<sub>1</sub> were 9.0 % for cryoresistance, 20.0 % for osmotic resistance, and 44.5 % for dehydrogenase activity. In consequent generations, the differences were leveled out: in the third generation, hybrids exceeded their purebred analogues in cryoresistivity by 2.2 %, in osmotic resistance by 4.7 %, and in dehydrogenase activity by 10.0 %.

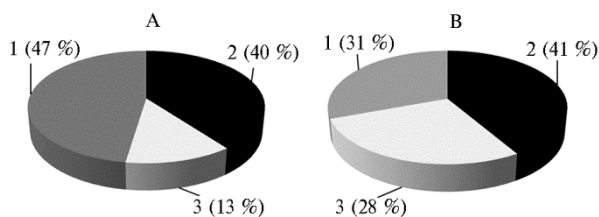
**1. The indices of the cryostability of spermatozoa obtained from hybrid and purebred Romanov rams ( $\bar{X} \pm x$ )**

Groups	Cryoresistance	Osmotic resistance	Dehydrogenase activity, min
ROM	0.44±0.02	0.21±0.06	7.82±0.65
F <sub>1</sub>	0.48±0.03	0.25±0.08	11.31±0.38
BC <sub>1</sub>	0.45±0.02	0.23±0.06	9.09±0.47
BC <sub>2</sub>	0.43±0.03	0.22±0.01	8.61±0.22

Note. For description of the groups see the *Technique* section.

The study of the influence of cryopreservation on the morphometric parameters of spermatozoa revealed some differences between the experimental groups. The portion of spermatozoa with disturbed morphology in fresh ejaculates of hybrid rams was 10.7 %, which was 3.1 %

higher than that for purebred animals. There was a difference between groups in the occurrence of abnormal morphology in segments of spermatozoa. A higher disorder percentage in the structure of spermatozoa in purebred producers was noted in the flagellum region, in hybrid rams — in the middle part (Fig.).



**The frequency of morphological disorders in the segments of spermatozoa of purebred Romanov rams (A) and hybrids with argali (*Ovis ammon*) (B) in frozen-thawed semen (on average in three groups): 1 — abnormal flagella, 2 — abnormal head, 3 — abnormal middle part.**

When freezing and thawing, the number of spermatozoa with morphological disorders increased in both hybrid and purebred animals, however, the percentage of the frequency of abnormalities in spermatozoa segments within the experimental groups remained practically unchanged.

Sturdy of the acrosome and chromatin fragmentation in fresh and frozen semen revealed a decrease in the indices in the hybrid animals compared to the purebred analogues. The difference between the Romanov rams and BC<sub>1</sub> and BC<sub>2</sub> was 2.5-3.2 % for fresh semen and 3.9-5.2 % for frozen-thawed semen in the number of spermatozoa with intact acrosomes, and 25.8-43.9 and 0-21.2 %, respectively, in the chromatin fragmentation (Table 2). At the same time, we did not reveal a correlation between the changes in these indices and the percent of argali blood.

**2. Estimation of spermatozoa in hybrid and purebred Romanov rams according to the state of the acrosome and the degree of chromatin fragmentation before and after cryopreservation ( $X \pm x$ )**

Groups	Proportion of spermatozoa with intact acrosomes, %		Degree of chromatin fragmentation, %	
	FOS	FTS	FOS	FTS
ROM	92.4±5.2	90.3±8.7	11.6±3.9	12.7±1.7
F <sub>1</sub>	96.5±6.7	88.3±4.7	8.6±2.8	12.7±3.5
BC <sub>1</sub>	89.9±8.3	86.4±5.7	6.5±2.7	9.5±2.4
BC <sub>2</sub>	89.2±1.6	85.1±4.6	8.2±3.8	12.3±3.2

Note. FOS — fresh semen, FTS — frozen-thawed semen. For description of the groups see the *Technique* section

In freezing-thawing, the proportion of spermatozoa with intact acrosomes decreased and the DNA fragmentation index increased in all groups. In hybrid animals, the differences were more pronounced and amounted to 4.6-8.4 % and 45.1-47.6 % respectively, in purebred — to 2.2 and 9.5 %, respectively.

Thus, after cryopreservation of the semen of both hybrid and purebred animals, the biological full-value of the spermatozoa decreased. The semen of the hybrids was more cryoresistant than the semen of Romanov rams. After cryopreservation, the motility of spermatozoa decreased by 47.0 % in hybrid animals and by 50.0 % in the purebreds. The spermatozoa cryoresistance indices in the hybrids also increased compared to the domestic parent. With the decrease in the argali blood percent towards the domestic form, differences in cryoresistance, osmotic resistance and dehydrogenase activity were leveled out and were 2.2, 4.7 and 10.0 % for the rams of maternal breed and hybrids BC<sub>2</sub>, respectively. In the F<sub>1</sub> hybrid ejaculates, the content of sperm with intact acrosomes was 4.1% higher. In the fresh ejaculates of hybrids, the chromatin DNA fragmentation was lower than in the Romanov breed.

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