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CHANGE OF PHYSIOLOGICAL AND MORPHOLOGICAL SPERM QUALITY TRAITS IN REINDEER (*Rangifer tarandus*) DURING CRYOPRESERVATION

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Abstract

Assisted reproductive technologies allow effective preservation and use of endangered animal gene pool and creation of new breeding forms. In reindeer (*Rangifer tarandus*) herding, the technique of sperm cryopreservation is still under development. This is due to the difficulty of collecting reindeer sperm in the Arctic conditions. Besides, the rutting season of reindeer begins in the autumn and lasts about a month. Only during this period is it possible to collect sperm as spermatogenesis in reindeer stops after rutting season. The aim of the work was to study the effects of cooling and freezing-thawing on the physiological and morphological traits of reindeer sperm quality. Reindeer sperm was collected by electric ejaculator or by washing out of the epididymis. After assessing the quality of ejaculated and epididymal semen (volume, total and progressive motility and sperm concentration), the sperm was diluted with Steridyl medium to a final concentration of 100 million/ml, packed in 0.25 ml straws and cooled to 5 °C for 120 min. After cooling and balancing, the straws were kept in liquid nitrogen vapors on a float at –110 °C for 12 min and then lowered into liquid nitrogen. Semen was thawed at 37 °C. The initial assessment of ejaculated sperm showed that the average volume of reindeer ejaculate was 0.5±0.08 ml, with concentration of 0.520±0.069 billion/ml, total motility of 64.3±4.07 %, and progressive motility of 47.9±4.24 %. The epididymal sperm cell concentration was on average 0.260±0.078 billion/ml, total and progressive motility was 43.6±8.49 % and 20.8±5.25 %, respectively. There was a large variability between ejaculates on the extent of changes in sperm motility after cryopreservation. Thus, total motility decreased by 41.9±5.38 % on average with fluctuations from 1 % to 89 %, progressive — by 36.8±5.29 % with fluctuations from 0 % to 75 %. A total of 42 % of ejaculates lost more than 50 % total motility. In some cases, there was a complete loss of motility after freezing, and in some samples the changes were insignificant. Large variability in changing cell motility was observed both in ejaculated and in epididymal semen. Epididymal sperm cells had higher motility after freezing than ejaculated spermatozoa, but showed more pronounced disturbances in the motility character. Sperm morphology analysis showed that there is an increased percentage with wrinkled or missing acrosome as compared to other animal species, i.e. 6.9±0.76 % in both ejaculated and epididymal reindeer sperm cells. There was no significant increase in damages of the sperm tail, neck and acrosome. The number of cells with injuries in tail and neck increased by 4.2±1.05 % with a range from 0.01 % to 15.7 %, and acrosome — by 2.5±0.35 with a range from 0.6 % to 8.3 %. High variability in the increase of plasma membrane damages was observed, i.e. 10.9±5.02 % with fluctuations from 0.13 % to 45 %. Such a large variability is due to the peculiarities of the reindeer sperm cryoresistance and differ-

ences between individual ejaculates. Significant difference in physiological and morphological changes in semen quality after cryopreservation between ejaculated and epididymal sperm were not found. Thus, the greatest changes in the cryopreserved reindeer semen are in motility and membrane integrity. The obtained data on physiological and morphological changes in reindeer semen during freezing should be taken into account when optimizing the composition of diluents and cryopreservation protocol.

Keywords: *Rangifer tarandus,* reindeer, cryopreservation, sperm motility, acrosomes, cell membranes

In the Russian Federation, there are about 2 million wild and domestic reindeer (*Rangifer tarandus*) whose habitat occupies more than half of the country's territory. For the indigenous peoples of the Arctic, reindeer husbandry has become a major industry. Venison is a dietary product with a high content of important microelements and vitamins. Improving breeding methods aimed at improving meat productivity is an urgent problem for the breeding and selection of reindeer in Russia [1]. The existing methods do not provide the required growth rates for the production of reindeer-breeding products [1, 2]. In addition, the active development of the Arctic led to climate change, a reduction in the number of pastures and, as a consequence, a decrease in the number of domestic and wild reindeer [3-5].

Modern reproductive technologies make it possible to preserve and use the gene pool of endangered species indefinitely, to conduct intensive selection and choose the best animal units. However, these technologies have not yet found wide application in reindeer husbandry [6-8]. The high cost and complexity of transporting deer in the conditions of the North, as well as an unfavorable prognosis for male acclimatization, necessitate the development of methods for cryopreservation of reindeer semen and the creation of a sperm cryobank. The use of such sperm will allow the exchange of genetic material between remote regions of the Arctic [1, 5, 6].

There are references in the literature to only a few cases of artificial insemination in reindeer husbandry. Scottish researchers Dott and Utsi presented the data on artificial insemination of reindeer females with fresh sperm [9, 10]. Mkrtchyan and Deryazhentsev in the 1970s published the work on the assessment and cryopreservation of reindeer sperm [11-13]. In the 1990s and 2000s, there were reports of artificial insemination with cryopreserved sperm [14-17]. Mkrtchan et al. [18] were the first people in the world who managed to get reindeer calves after artificial insemination with frozen-thawed sperm. However, the technique of cryopreservation of reindeer sperm has not yet been developed [5, 19].

An important feature of the adaptation of the deer organism to the conditions of the Arctic is the cessation of spermatogenesis outside the rutting season. The harsh Arctic climate and the short breeding season complicate the procedure for obtaining the sperm of these animals [17, 20, 21].

To develop a method for deep freezing of reindeer sperm as part of the program for the preservation of the Arctic biological resources, a comprehensive study of the processes occurring in spermatozoa under the influence of low temperatures is required. However, there is no published data on the changes occurring with the sperm of these animals during cryopreservation.

In the present work, the quality of semen of reindeer before and after freezing was first characterized. It was shown that the largest changes during cryopreservation were undergone by the motor activity of germ cells, as well as membrane integrity. Reindeer have an increased proportion of sperm with a wrinkled or missing acrosome. After freezing, epididymal sperm cells retain

higher motility compared to the ejaculated ones, but more disturbances in the nature of their movement are noted.

The aim of the study was to assess changes in physiological and morphological indicators of reindeer sperm quality during cryopreservation.

Techniques. In the experiments, the sperm of reindeer (*Rangifer tarandus*) aged 1.5 to 7.5 years living on the Taimyr Peninsula was used. From 11 animal units, sperm was obtained by electro-ejaculation (DC100-240V ejaculator, Minitьb GmbH, Germany), at least three samples from each. Moreover, sperm was received from the epididymis from two males aged 2.5 and 3.5 years, who were castrated and left in the herd to work in harness, and post-mortal from six wild animals (age from 1.5 years) after the shoot-off. Sperm was obtained with automatic dispensers after cutting the epididymis and then transferred to 1.5-ml tubes with Steridyl diluent (Minitьb GmbH, Germany). Blood was avoided from getting into the sample.

After assessing the quality of ejaculated and epididymal sperm (volume, total and progressive motility, and sperm concentration), it was diluted in Steridyl medium to a final concentration of 100 million/ml, packed in 0.25-ml straws and cooled to 5 °C within 120 minutes. After cooling and equilibration, the straws were kept in liquid nitrogen vapor on a float at a temperature of –110 °C for 12 min and then lowered into liquid nitrogen. The samples were thawed at 37 °C.

The concentration, general and progressive sperm motility were determined using the CASA method (Computer-Assisted Semen Analysis) using the Argus-CASA program (OOO Argussoft, Russia), and the morphology and condition of acrosomes were evaluated by phase-contrast light microscopy. The damage level of the sperm plasma membrane was studied using a Sperm VitalStain stainer (Nidacon International AB, Sweden). Staining was conducted in Eppendorf tubes (50 μl of sperm was mixed with 50 μl of stain), the smears were prepared on glass slides. The preparations were scanned with ×1000 zoom with oil immersion, counting at least 200 cells in each sample, white cells are intact, red or pink are sperm cells with damaged membranes. For visualization, the Argus-CASA system and a BA410 microscope (Motic China Group Co., Ltd., China) were used.

The data were processed using the SigmaPlot 12.5 program (Systat Software Inc., USA) and Microsoft Excel. The mean values (*M*), standard errors of the mean (±SEM), minimum (min) and maximum (max) values of indicators are given in the article.

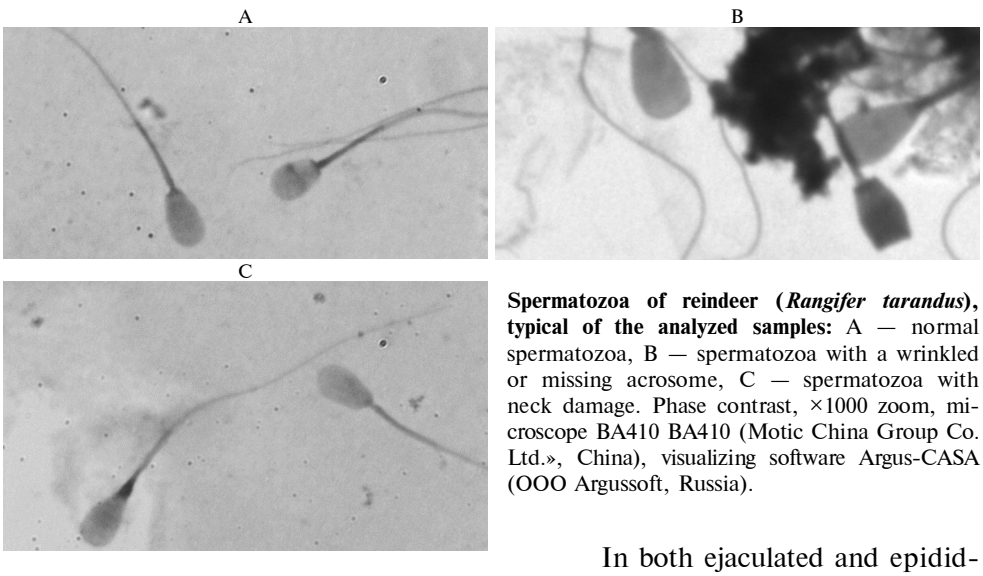
Results. Currently, there is a lot of data on the damage to spermatozoa of different animals during cryopreservation [22, 23]. For the most part, there are changes in the motility and nature of the movement of sperm, as well as damage to cellular structures – tails, acrosomes, and membranes. Cell damage occurs mainly due to the formation of ice crystals and osmotic stress [24]. For example, in bulls' sperm, even under optimal cooling and thawing conditions, 40-50% of cells cannot be frozen [25]. In horse sperm, the greatest damage after cryopreservation was observed in the acrosome area [26]. Boar sperm has the lowest level of cryoresistance [23].

According to the data, the average volume of reindeer ejaculate was 0.5±0.08 ml, the difference between ejaculates was from 0.02 to 2 ml, the concentration was 0.520±0.069 billion/ml (with a difference of 0.195 to 1.2 billion/ml), total motility 64.3±4.07 (10-84%), progressive motility 47.9±4.24% (3-79%). For epididymal sperm, the concentration, total and progressive sperm motility were 0.260±0.078 billion/ml (0.042-0.441 billion/ml), 43.6±8.49% (10-94%) and 20.8±5.25% (3-45%), respectively. These results are consistent with the data of

foreign [16, 17] and USSR researchers [13, 18], as well as with the data, previously obtained by the authors [20]. For cryopreservation, 25 samples of sperm were taken with total motility of at least 65%.

General and progressive sperm motility after thawing on average amounted to 23.2±5.25% (0-64%) and 16.3±3.46% (0-45%) in ejaculated sperm and 40.8±6.92% (21-63%) and 18.0±3.6% (8-32%) in epididymal sperm. For comparison, the total motility of thawed sperm of another *Cervidae* family member, the the Javan rusa (*Rusa timorensis*) was from 7.5 to 39.8%, the progressive one from 2.5 to 14.7% [27]. The authors froze the sperm diluted in a Tris-based extender, similar in composition to that we used (Steridyl). In our tests, the total motility of spermatozoa in thawed ejaculated reindeer sperm was lower than in the epididymal one, the progressive motility was almost the same, and the number of motile cells that had rectilinear-translational motion was 70 and 45%, respectively. Martínez et al. [28] note that although the cell motility of the epididymal and ejaculated deer sperm after cryopreservation does not differ much, the pattern of cell motion is better in ejaculated sperm.

An important criterion in sperm selection is determination of the number of normal (Fig., A) and pathological spermatozoa in the ejaculate [25]. Spermatozoa can be damaged by rapid cooling or low temperature [29]. Spermatozoa with abnormalities in the structure of the head, neck, and tail are considered pathological. There are several types of morphological changes in the acrosome. We noted such abnormalities as partial deformation of the outer membrane of the acrosome, ruptures and wrinkled outer membrane, as well as the absence of the acrosome.



Spermatozoa of reindeer (*Rangifer tarandus*), typical of the analyzed samples: A — normal spermatozoa, B — spermatozoa with a wrinkled or missing acrosome, C — spermatozoa with neck damage. Phase contrast, ×1000 zoom, microscope BA410 BA410 (Motic China Group Co. Ltd.», China), visualizing software Argus-CASA (OOO Argussoft, Russia).

In both ejaculated and epididymal sperm of reindeer, an increased proportion of spermatozoa with a wrinkled or missing acrosome was observed (see Fig., B) compared to other animal species, on average 6.9±0.76% with fluctuations from 1 to 15.2% (Table 2). Perhaps, this is a special feature of reindeer sperm. It should be noted that after freezing, there was no significant increase in the number of cells with acrosome damage, on average 10.1±0.78% with fluctuations from 3.6 to 18.3%. The number of cells with injuries in the tail area (see Fig. C) on average amounted to 11.9±1.31% (1.6-33.2%) before freezing and 15.5±1.15% (5.0-34.4%) after thawing.

Membrane integrity is a prerequisite for the functioning of cells. In fresh (ejaculated and epididymal) reindeer sperm, an average of 15.5±4.09% (1.78-

50.0%) of membrane damage was observed, with 21.8±4.44% (3.5-53.3%) for frozen-thawed sperm.

According to the change in sperm motility after cryopreservation, the ejaculate significantly differed. General motility decreased on average by 41.9±5.38% (the difference between ejaculates was from 1 to 89%), progressive motility by 36.8±5.29% (0 to 75%). Spermatozoa in 42% of ejaculates lost more than 50% of their total motility during cryopreservation, and in 25% of ejaculates, less than 20% of their motility. In some samples, a complete loss of motility after freezing was observed; in some cases, the changes were insignificant. Such great variability is due to both the characteristics of the cryoresistance of deer sperm and the differences between animal units. Significant individual variability in cell motility after thawing is also noted in red deer [30].

We did not observe a noticeable increase in the frequency of damage in the tail and neck of spermatozoa and acrosome. The number of cells with damage to the tail and neck increased on average by 4.2±1.05% (0.01-15.7%), with damage to the acrosome by 2.5±0.35% (0.6-8.3%). In other deer species, for example, in red deer, the number of damages in acrosomes by freezing and thawing increased on average by 20% [31]. In stallion sperm, the number of spermatozoa with acrosome hypoplasia and lack of internal contents after cryopreservation increased by 20.9%, and with acrosome degradation by 10.4% [32]. In the studies on the effect of cryopreservation and thawing on the sperm of red deer, the authors noted individual variability between males for the preservation of membrane integrity and acrosomes [33, 34].

We also did not reveal high variability between the samples in terms of their susceptibility to damage to the plasma membranes of spermatozoa. On average, the damage increased by 10.9±5.02% (0.13 to 45%). This confirms the theory that in reindeer sperm, membranes are most sensitive to low temperatures, which also affects cell motility. There was no significant difference in changes in morphological indicators of reindeer sperm quality after cryopreservation between ejaculated and epididymal sperm. It should be noted that sperm retains its fertilizing ability, despite a sharp decrease in sperm motility after thawing. Thus, as a result of in vitro fertilization of reindeer egg cells that we conducted using thawed epididymal sperm of a male which sperm motility reduced after cryopreservation by more than 50% (from 96 to 40%), embryos at the blastocyst stage were obtained (data are not presented).

Thus, the motor activity of the cells and the integrity of the membranes are subjected to the greatest changes during cryopreservation of reindeer sperm. The concentration of sperm cells in freshly obtained ejaculates is 0.520±0.069 billion/ml, total and progressive motility is 64.3±4.07 and 47.9±4.24%, in epididymal semen samples 0.260±0.078 billion/ml, 43.6±8.49 and 20.8±5.25%, respectively. After cryopreservation, the overall motility decreases on average by 41.9±5.38%, the progressive one by 36.8±5.29%. The frequency of damage to plasma membranes of spermatozoa on average increases by 10.9±5.02%. No significant differences in changes in physiological and morphological indicators of sperm quality during freezing and thawing between ejaculated and epididymal sperm were found. However, after cryopreservation, high individual variability is observed for all indicators of sperm quality. The data obtained on changes in physiological and morphological quality indicators of reindeer sperm after freezing and thawing expand the understanding of the reproductive biology of this species. The identified features should also be taken into account when optimizing the composition of diluents and the cryopreservation protocol to reduce damage in germ cells.

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