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Experience in obtaining and cryopreserving semen of Mid-Caucasian Tur, *Capra Caucasica* Güldenstaedt et Pallas, 1783

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ABSTRACT

The study was primarily aimed at determining the possibility of preserving the quality parameters and functional value of the semen of Caucasian tur, *Capra caucasica* after cryopreservation. For this purpose, the following objectives were set: to find out the possibility of obtaining the semen of Caucasian tur by electroejaculation and to study the parameters of its fresh undiluted semen; to study the cryoprotective properties of the experimental synthetic medium for dilution of its semen; to study the quality parameters and functional value of the frozen semen; and to evaluate the fertilizing capacity of the frozen and thawed semen. In the field study, semen was obtained during the breeding season (late November) from three mature turs by electroejaculation. A total of 19 ejaculates were obtained and evaluated. At each retrieval, ejaculates were pooled to exclude the influence of individual male characteristics and separated into two equal aliquots. One aliquot was then diluted with TRIS-based synthetic medium (control medium) and the other was diluted with the same medium supplemented with 10% platelet-rich plasma (PRP) from the same animal from which the biomaterial had been collected. Subsequently, all samples were cryopreserved in liquid nitrogen. The volume, total motility, velocity of sperm, acrosome, and plasma membrane integrity of sperm at different stages of cryopreservation (after dilution, equilibration, and freezing and thawing) were investigated. The results have shown that the semen obtained by electroejaculation had an average volume of 0.8 mL (range 0.3 to 1.4 mL), satisfactory quality parameters (sperm velocity 76% (7.6 points on a 10-point scale), average concentration 2.85×10^8 sperms mL^{-1}). Semen diluted with the experimental medium supplemented with 10% PRP had significantly higher total motility ($p < 0.05$) compared to the control medium after equilibration and freezing, and thawing. In the experimental diluent after freezing and thawing, the percentage of sperm with high motility has significantly increased ($p < 0.05$) compared to the control medium. The addition of 10% PRP resulted in improved progressive motility and viability of sperm after cryopreservation ($p < 0.05$), as well as increased acrosome preservation and plasma membrane integrity compared to the control medium. The fertilizing ability of cryopreserved semen frozen in the experimental medium was 19.1% higher compared to the control medium.

Keywords: Caucasian tur, Semen quality, PRP, Dilution, Cryopreservation, Field conditions.
Article type: Research Article.

INTRODUCTION

One form of rational animal husbandry is the involvement of wild fauna resources in agricultural production, including the employment of interspecific hybridization. Distant hybridization is one method available to enrich the gene pool of domestic animals by introducing unique allelic combinations in various genes inherent in wild animals. It is used both in the development of new livestock breeds combining valuable properties of the original breeds and for the reconstruction and restoration of endangered species (Nasibov 2010b). Russia is home to many

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wild species of small ruminants in the pronghorn family. These include snow sheep, argali, mouflon, Siberian ibex, Caucasian tur, saiga antelope, chamois, and other species (Bagirov *et al.* 2014). Some of them are considered ancestors of domesticated sheep and goats, while others belong to more distant taxonomic groups. All these species are characterized by exceptional adaptability to harsh environmental conditions, strong constitution, strong skeleton, well-developed cardiovascular system, endurance, unpretentiousness to feed, and high vitality. In addition, they are characterized by certain productive parameters that make them attractive for breeding, such as increased fat yield (up to 8.4%), and some of them are very large (up to 120-150 kg; Aybazov *et al.* 2014b). One such wild resource species is Caucasian tur, *Capra caucasica*, which is one of the relict endemic species and has a wide distribution throughout the northern slope of the Caucasus Range. The phylogeny of turs reflects the complex history of local fauna formation. Caucasian tur is subdivided into three species that differ in their distribution range and some morphological features. Most researchers (Bobyr 2008; Aybazov *et al.* 2014a; Ivanina 2018) tend to consider Caucasian turs as a single polymorphic species with three subspecies: (i) West Caucasian tur (*C. caucasica* severtzovov. *caucasica severtzovi* Menzbier, 1887); (ii) Mid-Caucasian tur (*C. caucasica* Guldenstaedt et Pallas, 1783); and (iii) East Caucasian tur (*C. cylindricornis* Blyth, 1841). *C. caucasica* was assessed for The IUCN Red List of Threatened Species in 2019. It is listed as Endangered under criteria B1ab (i,iii,v; Weinberg 2020). These subspecies have fairly clear habitat boundaries, although their ranges may overlap and result in the appearance of hybrid specimens (Semyonov 2010). On the other hand, E.Yu. Zvychaynaya (2008) believes that there is no clear internal differentiation or geographical boundary between different forms. The author explains the existing morphological cline of Caucasian turs by secondary hybridization of originally different forms. It is concluded that despite the fact that genetic distances between mitochondrial and nuclear lines of *C. caucasica* and *C. cylindricornis* are quite significant, relatively recent evolutionary events such as hybridization of basal groups of the Caucasian tur genus, formation of several mitochondrial lineages of *C. aeorgrus* due to introgression of mtDNA, and appearance of alien mtDNA haplotypes played a significant role in the formation of genetic diversity of Capras. Therefore, in limited populations of Caucasian turs, genetic isolation, and inbreeding may have deleterious effects on some components of female adaptability and male reproductive ability (Kazanskaya 2007). The continuing decline in the number of mature specimens requires the establishment of a cryopreserved sperm bank to guarantee the conservation of its different subspecies. Mid-Caucasian tur was first described by I.A. Güldenstaedt in 1772, and after a while P.S. Pallas included it into the zoological classification. So, its full name is *Capra caucasica* Güldenstaedt et Pallas, 1783 (Pallas 1779). This subspecies of Caucasian tur inhabits rocky areas of the highlands of the Main Caucasian Range and Lateral Ranges, and their offspurs from N 43020′45″, E 42°26′55″ in the west to N 42^{0}30′00″, E 44027′11″ in the east in the zone from 1000 to 4200 m above sea level and is a common species of ungulates. Scientific interest in the study of some biological and reproductive parameters of Mid-Caucasian tur, the possibility of obtaining its semen, the possibility of crossbreeding turs with Karachaev goats, obtaining hybrid young has been determined by several scientific premises. From a fundamental point of view, on the basis of such crossing, it is possible to establish some regularities of inheritance of exterior and interior traits, as well as to clarify the phylogenetic origin and the existing zoological classification of animals. As for the practical purposes of crossbreeding turs with domestic goats, the potential use of Caucasian turs to increase the live weight and meat productivity parameters of native Karachaev goats is of the greatest interest. In particular, in wild mountain goats, the weight of musculoskeletal tissue amounts to 75% of body weight, while in autochthonous Karachaev goats, it reaches 45-50%. Furthermore, hybridization with Caucasian turs can be considered a method of breeding new highly productive breeds of small horned livestock, developing new forms of farm animals well adapted to harsh environmental conditions, and rationally using vast territories unsuitable for breeding other animal species. Thus, Caucasian turs can serve as a model object for a variety of biological studies. When working with them, researchers have obtained interesting results in population dynamics, special and general ecology, ethology, and physiology. We have found some data on the breeding biology of Caucasian tur in the work by Y.L. Morozov (2009). At the same time, there are no systematic data on the parameters of their reproduction. Accordingly, there is currently no scientific data on the technology of obtaining, quality assessment, and cryopreservation of their semen. Existing technologies for animal semen cryopreservation include semen dilution with special synthetic media (diluents). Their primary function is to protect sperm from destruction during freezing at ultra-low temperatures (Nasibov 2010a). Over the past few decades, different protocols for semen cryopreservation have been developed, taking into account the type of animal, the degree of semen dilution, the rate of cooling and freezing (slow freezing and vitrification), the form

of cryogenically frozen material (pellets, straws, cryo-loops) and thawing techniques, which have generally improved the cryo-survival rate of sperm and the preservation of its functional integrity (Peris-Frau 2020). Without questioning the fact that all factors involved in cryopreservation must be considered to ensure sperm survival and viability, it seems worth emphasizing that the composition of the medium used for dilution prior to cryopreservation plays a crucial role in maintaining sperm integrity (Toker *et al.* 2016; Aybazov *et al.* 2021). The best synthetic media ensure that sperm motility after freezing and thawing is within 50% of the quality of the original sperm, and the fertility of cryogenically frozen sperm varies from 35 to 65% (Aybazov *et al.* 2022). Lipid peroxidation, which increases the production of reactive oxygen species (ROS), has been identified as the main cause of sperm damage during preparation for freezing, cryopreservation and subsequent defrosting (Kargar *et al.* 2017). Increased production of ROS during cryopreservation leads to disruption of the sperm plasma membrane, significantly reduces sperm motility and viability after thawing (Peris-Frau *et al.* 2020), and increases DNA damage (Mustofa *et al.* 2021). At the same time, such susceptibility to cryogenic destruction may be attributed to the features of sperm of a certain animal species. In particular, goat semen has been reported to have a high concentration of poly-unsaturated fatty acids, which may serve as a trigger for ROS formation and subsequent sperm damage (Wahjuningsih *et al.* 2021; Salama *et al.* 2024). The Caucasian tur is taxonomically close to domesticated goats. Therefore, it is logical to assume that the semen of the tur may have similar characteristics to goat semen, including by cryopreservation. Electroejaculation has been successfully used in some wild ruminants (Casinello *et al.* 1998), including Iberian ibex (*Capra pyrenaica*; Santiago-Moreno *et al.* 2009). However, no information on its use in Caucasian turs has been found. Early studies on domestic goats showed that the quality of semen collected using electrical stimulation was lower than that of semen collected using artificial vaginas (Greyling *et al.* 1983). In addition, goat semen collected by electroejaculation has a higher pH and contains more seminal plasma than semen collected with artificial vaginas (Santiago-Moreno *et al.* 2006). Therefore, the response to cryopreservation of samples collected in this way may differ. Due to the difficulties in collecting and cryopreserving reproductive cells, the establishment of germplasm collections of wild species such as the Caucasian tur is not yet widespread. Therefore, the development of optimal collection techniques and successful field freezing protocols could be a breakthrough for these species.

MATERIALS AND METHODS

Research site and animals

The studies were conducted in Khurzuk village of the Republic of Karachay-Cherkessia (N 43°26′00″, E 42°08′48″). The altitude above sea level is 1850 m, the average temperature in November is +3.5 ℃, and in April - 4.3 ℃. The average precipitation is about 450-500 mm. As semen donors, three mature (6-year-old) turs, *Capra caucasica* with an average annual live weight of 125.0, 128.0, and 137.0 kg were used. The animals were caught at the age of 2-4 weeks by a gamekeeper and raised in cages. In order to create conditions that maximally simulate the natural habitat of the turs, the enclosures were surrounded by extensive fenced paddocks with an area of more than 200,000 m^2 with preserved natural relief and mountain landscape. Taking into account the high secrecy of turs, shelters were built to minimize stress for them. The animals' daily diet consisted of 3.0 kg of alpine hay and 1.5 kg of crushed barley, oats, peas, and corn. The animals had free access to clean drinking water and blocks with vitamins, minerals and salt.

Semen collection

Semen from turs was collected during the breeding period (end of November). Despite the fact that turs were raised in captivity and kept together with a group of Karachaev goats for a long time, they retained their wild habits, which were characterized by fearfulness and aggression towards humans. Due to the impossibility of using an artificial vagina, the method of electroejaculation (EE) was used to obtain semen from them. EE procedures were performed in the morning hours for all semen collections. Before electroejaculation, the eyes of the animal were covered with a mask to minimize stress from manipulations. General anesthesia was provided by intravenous injection of Telasol solution (Telasol, Zoetis, USA) at a dose of 0.3-0.4 mL as a sedative. After completion of all manipulations, the effect of the anesthetic was eliminated by intramuscular injection of atipamezole at a dose of 0.30 mg kg^{-1} (Antisedan®, Pfizer Inc., Amboise Cedex, France). A probe 30 cm long and 2.5 cm in diameter was used for electroejaculation. The procedure of electroejaculation was as follow: the animal was fixed in the prone position. The probe was coated with carboxymethylcellulose gel for ultrasound to improve electrical conductivity.

The operator inserted the probe with ventrally located electrodes into the rectum to a 15-20 cm depth. Then, the second operator manually protruded the penis of the animal, after which the first operator created electrical pulses of 4-5 s duration, alternating with periods of no stimulation of 2-3 s duration. The current was 0.1...1.0 mA, and the voltage was 5 V at the beginning and increased by 1 V until ejaculation occurred. This protocol was used because it was previously tested on Karachaev goats and showed high efficiency. The released semen was collected in a sterile double-walled semen collection tube. Since the ambient temperature during manipulations was below 18 ℃, to prevent the cold shock of semen, the inter-wall space of the semen collection tube was filled with water heated to 30-35 ℃. A total of 19 ejaculates were obtained, of which 18 were evaluated (one ejaculate was disposed of due to urine contamination).

Evaluation of fresh semen

Since the study was conducted in the field, the quality of fresh semen was evaluated in a simplified procedure. The volume of ejaculation was measured with a graduated micropipette (MiniMedProm, Russia). Total sperm concentration was determined using a Goryaev chamber (MiniMed, Russia). The total number of sperm cells in the ejaculate was calculated as the product of the volume and concentration of sperm in each ejaculate. Sperm motility was evaluated subjectively using a MB-2 microscope (Russia) at ×100 magnification on a ten-point scale (0 - lowest, 10 - highest score). Only ejaculates with total sperm motility of more than 60% were cryopreserved.

Semen cryopreservation

The pooled ejaculates were reassessed for quality and divided into two equal aliquots. The basal medium was a synthetic medium based on TRIS: 78 mM citric acid, 254 mM TRIS, 70 mM fructose, 5% glycerol (by volume) and egg yolk (15% by volume), pH 6.8, which was the control medium. The experimental medium was characterized by the addition of PRP (medium supplemented with 10% platelet-rich blood plasma) to the basal medium at a concentration of 10%. The procedure for obtaining autologous platelet-rich plasma (aPRP) included collection of venous blood into a 15-mL tube with 1.5 mL anticoagulant (3.1% sodium citrate). Then, within 10 min, centrifugation at room temperature (+18 to +24 °C) was carried out at a speed of 3500 rpm for 5 min with a step of 500 rpm (centrifuge "ELMI SM-6MT" (Latvia) with an angular rotor and rotor inclination angle of 45°). A timer to set centrifugation time was used to control the operation. Then a syringe (10.0-20.0 mL) with a 27-G blunt-tipped thin-walled cannula was used to withdraw the supernatant containing platelet-rich autoplasm from upper part of the tube. The concentration of platelets in the autoplasm was at least 1000×10 cells mL^{-1}. The final concentration of sperm cells in each dilution was 500×10^6 cells mL^{-1}. After dilution, semen samples were packed into disposable labeled tubes and equilibrated at 2...3 ℃ in a thermos with melting ice for 120 minutes. After equilibration, sperm motility was assessed in the field. Then, the semen was frozen on a fluoroplastic plate with wells cooled in liquid nitrogen to – 100 ℃. The plate, during freezing, was in nitrogen vapor 5 cm from the upper edge of the foam box and 2-3 cm from the nitrogen surface. The semen was pipetted into wells so as to obtain pellets of 0.25 cm^3 (approximately 120×10^6 sperm cells). The pellets were transferred to a liquid nitrogen tank and stored 10 to 30 days before evaluation.

Thawing and evaluation of thawed semen

Frozen semen was evaluated under stationary laboratory conditions. Semen pellets were thawed in a test tube at 37 ℃ until fully defrosted. After thawing, total and progressive sperm motility as well as fast, medium and slow sperm motility were assessed using computerized sperm movement analysis equipment (CASA; Semen Vision™). Sperm motility was calculated using speed standards set as fast (VCL > 75 μm s^{-1}), medium (VCL 45-75 μm s^{-1}), and slow (VCL < 45 μm s^{-1}). For this purpose, thawed samples were diluted in a proportion of 1:10 (by volume) with 0.9% NaCl solution. Then, 10 μL diluted semen was placed into a Makler counting chamber (10 μm depth) preheated to 37 ℃ on a thermoplate. Each sample was evaluated in five microscope fields. *In vitro* survival of thawed sperm was performed by placing it in a thermostat at 36.6 ℃ and recording the time (in min) until complete cessation of sperm motility.

Insemination

Insemination of Karachaev goats with frozen and thawed semen of Caucasian tur was carried out artificially. Goats in heat were selected using vasectomized test bucks. Insemination was performed twice: after detection of

the signs of heat and 10 hours later—each time, a dose of semen containing 120 × 10^6 motile sperm cells was injected intracervical.

Statistical analysis

Descriptive statistics of experimental data were performed in the Microsoft Excel program. Mean values (M) for sperm motility, acrosome, plasma membrane integrity, live weight of hybrid kids, and standard errors (± m) were calculated. Detected differences were considered statistically significant at $p \leq 0.05$. Experiments on animals were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/index.htm). The research protocol was approved by the Bioethics Commission of the Institute of Zootechnics and Biology of the Russian State Agrarian University - Moscow Agricultural Academy named after K.A. Timiryazev (Protocol No. 15, dated October 20, 2023).

RESULTS

Although the same technique was used in the experiment and the same operators performed all manipulations, the variables studied showed significant discrepancies. An average current of 0.28 mA (variation from 0.1 to 0.4 mA) and 6.5 pulses (variation from 4 pulses to 18) were required to achieve ejaculation. The time of sperm discharge also varied widely (from 30 to 135 sec). The volume of ejaculate varied considerably, with the average being 0.8 mL (range 0.3 to 1.4 mL). Total sperm motility in freshly obtained ejaculate averaged 76.0% (or 7.6 points, range 50.0% to 80.0% or 5.0 to 8.0 points). The sperm concentration averaged 2.2 billion mL^{-1} while also showing great variability, from 1.0 to 3.2 billion mL^{-1}. As can be seen, the main quantitative and qualitative indicators of the fresh tur semen obtained by electroejaculation were low. However, the main conclusion is that the electroejaculation method is effective and can be used if necessary. After dilution, sperm motility indices in the control medium and the experimental medium did not differ significantly, amounting to 7.4 and 7.5 points, respectively. After equilibrating the diluted semen in a thermos with melting ice at a temperature of 2...3 ℃ for 120 minutes, sperm motility decreased and amounted to 6.3 points in the control medium and 6.9 points in the experimental medium. The cryopreservation process had the greatest impact on semen quality parameters. Since the thawed semen was evaluated in stationary conditions, we had the opportunity to analyze the sperm motility (fast, medium, and slow) in more detail. It has been found that the rate (%) of motile sperm in the experimental diluent after freezing and thawing was significantly ($p < 0.05$) higher than in the control medium (46.0 ± 0.36 vs. 36.0 ± 0.53). In this group, the best motility consisted of an increase in the number of sperm with fast and medium speed and a significant decrease in the number of sperm with slow speed (Table 1). From the data obtained, it can be concluded that adding 10% PRP to the control diluent allows a significant increase in the percentage of sperm that retained motility after cryopreservation. With almost equal initial motility after dilution in the control and experimental medium, which can be taken as 100.0%, after thawing the percentage of sperm that retained total motility was 48.7% and 61.3%, respectively, indicating a higher cryoprotective potency of the experimental medium. Sperm motility is one of the most important quality characteristics of freshly obtained, diluted, frozen, and thawed semen. It is believed that the higher the percentage of sperm with rectilinear-progressive movement, the higher the sperm fertility and fertilizing ability. At the same time, the sperm functional value, i.e., the ability to fertilize the egg, depends to a greater extent on the integrity of the acrosome and plasma membrane. The results of the study about the effect of adding PRP to sperm on the integrity of the sperm acrosome and plasma membrane are presented in Table 2.

Table 1. Effect of adding PRP to TRIS-based basal medium on Capra caucasica sperm motility after thawing.

Synthetic medium	Sperm motility after thawing (%)			
	Total	of which by speed		
		fast (>75 μm s^{-1})	medium (45-75 μm s^{-1})	slow (<45 μm s^{-1})
Control medium (TRIS-based)	36.0±0.53	48.4 ± 0.44	23.7 ± 0.54	27.9 ± 0.90
Experimental medium (TRIS-based + 10% PRP)	46.0±0.36	57.4 ± 0.57	28.1 ± 0.39	14.1 ± 0.81

As Table 2 shows, the number of sperm with an intact acrosome significantly depended on the diluent. The highest integrity of the acrosomal apparatus and plasma membrane of sperm was found in thawed sperm diluted before

cryopreservation with experimental diluent (56.1% and 60.6%), while the sperm head and its membrane were significantly more damaged in the control medium (45.8% and 45.4%).

Table 2. Effect of adding PRP to TRIS-based basal medium on integrity of the acrosome and plasma membrane of *Capra caucasica* sperm.

Synthetic medium	Integrity preservation after thawing (%)	
	of acrosome	of plasma membrane
control medium (TRIS-based)	45.8 ± 0.87	45.4 ± 1.17
experimental medium (TRIS-based + 10% PRP)	56.1 ± 0.87	60.6 ± 1.14

Sperm survival, i.e., the time from thawing to complete cessation of sperm motility, was 20.2% higher in the experimental medium than in the control medium (548 min vs. 456 min). Out of 26 goats inseminated with frozen and thawed semen of Caucasian turs, seven animals gave birth, from which eight viable hybrids were obtained, including five males and three females. In the group of goats (n = 14) inseminated with semen frozen in the experimental medium, the number of goats that gave birth was 2.5 times higher than in the group where the standard medium TRIS-based was used. Accordingly, the fertilization rate in the experimental group was 35.7%, and one double was obtained, while in the control group, the fertilization rate was 16.7%, and all kids were born as singletons. The average gestation period of goats did not differ significantly between groups and amounted to 156.6 ± 2.14 days. The first group produced only female kids, while the second group produced both females and males. The average live weight of hybrids was 4.70 ± 0.25 kg for males and 4.10 ± 0.15 kg for females. There was no significant difference in the live weight of females between the groups (Table 3).

Table 3. Results of kidding of Karachaev goats under artificial insemination with frozen and thawed semen of *Capra caucasica* using different media.

Synthetic medium	Number of goats		Conception rate (%)	Number of offspring	Live weight (kg)
	Inseminated	Kidding			
control medium (TRIS-based)	12	2	16.7	2	4.15 ± 0.35 (females)
experimental medium (TRIS-based + 10% PRP)	14	5	35.7	6	4.05 ± 0.05 (females) 4.70 ± 0.25 (males)

The offspring are monitored, and their growth, development, and some biological characteristics are studied.

DISCUSSION

The Caucasian tur (Sarga caucasica) is a mountain goat endemic to the Western Caucasus. Its numbers are stable, but it has been assigned Endangered status, which requires the establishment of a cryobank of sperm to ensure the conservation of its various subspecies. The results of our experiments have shown that the electroejaculation method can be successfully applied to sperm production. The sperm production of the Caucasian tur has an average volume of 0.8 mL (range from 0.3 to 1.4 mL), satisfactory qualitative parameters (total motility 76% (7.6 points on a 10-point scale), average concentration 2.85×10^8 sperm cells mL^{-1}). The low concentration of sperm in the ejaculate appears to be because the tur's reproductive glands are stimulated to a greater extent during the electroejaculation, causing the semen samples to have a normal volume but a low concentration of sperm compared to the samples obtained with an artificial vagina, although sperm motility remains normal. Data on the quality of semen of small ruminants obtained by electroejaculation are contradictory. Studies by Greyling & Grobbelaar (1983) on domesticated goats have shown that the quality of semen collected by electrostimulation is significantly lower than that with an artificial vagina. Others have noted that semen collection by electrostimulation reduces the volume to some extent. However, the quality remains acceptable for further use (Casinello *et al.* 1998), e.g., in Iberian ibex, *Capra pyrenaica* (Santiago-Moreno *et al.* 2009). In our studies, it was

not possible to compare the two irradiation methods, but the main conclusion is the acceptability of the electroejaculation method for obtaining Caucasian tur semen. The freezing and thawing process significantly decreased the values of the main sperm variables ($p < 0.001$). At the same time, cryopreservation of sperm in TRIS-based diluent with the addition of 10% PRP has been shown to have significant advantages over the control synthetic medium (without PRP), improving sperm quality, acrosome and plasma membrane integrity during and after sperm cryopreservation (Aybazov & Selionova 2024). The different PRP proteins provide buffering capacity of the diluent, which in turn leads to osmotic stability and reduces the formation of ice macrocrystals during cryopreservation and defrosting, protecting the integrity of sperm membranes. This was confirmed by the studies of Taher-Mofrad *et al.* (2020) conducted on human sperm. Better preservation of acrosome integrity during cryopreservation may also be attributed to a component of platelet-rich plasma, such as insulin-like growth factor-1 (IGF-1), which is known to be a successful protector of proteins associated with the acrosomal membrane (Selvaraju *et al.* 2016). The improvement in the integrity of the sperm plasma membrane may also be due to the presence of nerve growth factor (NGF) in plasma, which interacts with receptors in the sperm head, activates kinases, and preserves membrane integrity. It is more difficult to explain the fact revealed in our studies that PRP can improve sperm motility, the percentage of sperm with rapid movement, and their survival rate after cryopreservation. We do not have a scientifically substantiated explanation. However, we have found evidence in the literature that some scientists believe this phenomenon to be also related to various growth factors rich in PRP. Some (Castellini *et al.* 2019) argue that NGF plays a key role in improving sperm viability and motility by modulating its receptor in the middle part of the sperm, while others (Ferraguti *et al.* 2022) believe that NGF reduces apoptosis in healthy sperm and reduces oxidative damage caused by cryopreservation. Saucedo *et al.* (2018) also found improved sperm kinematics and motility but attribute this to another important growth factor in PRP, fibroblast growth factors (FGF), which they believe increases phosphorylation of the FGF receptor in the flagella and acrosome of sperm, activating protein kinase signaling pathways. The formation of reactive oxygen species leads to lipid peroxidation (Kargar *et al.* 2017). It is the main cause of sperm damage in all animal species during preparation for freezing, cryopreservation, and subsequent defrosting of sperm. Lipid oxidation significantly reduces sperm motility and viability after thawing (Peris-Frau *et al.* 2020) and leads to sperm plasma membrane disruption (Mustofa *et al.* 2021). We assume that adding PRP to the Caucasian tur semen diluent significantly increases the antioxidant parameters of the synthetic medium. Bader *et al.* (2020) indicated the good antioxidant properties of PRP and showed that PRP at a concentration of 2% can counteract the deleterious effects of H_2O_2-induced oxidative stress in human sperm. It is known that the trigger of ROS formation and subsequent damage to tur sperm may be a feature of the sperm of this species, i.e., a high concentration of polyunsaturated fatty acids (Wahjuningsih *et al.* 2021; Salama *et al.* 2024). The Caucasian tur is phylogenetically and taxonomically close to domesticated goats, so it is logical to assume that tur sperm may have features similar to those of goat sperm, including the formation of ROSs during preparation and cryopreservation. The effect of preservation of motility and membrane integrity of tur sperm in diluent with PRP in our experiments has shown the validity of this assumption.

CONCLUSION

The present results show that it is possible to obtain sperm of male tur by electroejaculation. That platelet-rich plasma can be used effectively as a diluent for cryopreservation of tur sperm, which has led to a significant improvement in the progressive motility and viability of sperm after cryopreservation, as well as an increase in the preservation of the acrosome and the integrity of the plasma membrane compared to the control medium.

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