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EFFECT OF BREED AND SEASON ON THE QUALITY OF FRESH AND CRYOPRESERVED RAM SEMEN

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Abstract

The study was conducted at the Artificial Insemination Station of the Executive Agency of Selection and Reproduction in Animal Breeding in Sofia, covering the period from June 2018 to September 2020. Ejaculates were collected from four rams of the Pleven Blackhead breed (47 ejaculates), three rams of the Assaf breed (55 ejaculates), and three rams of the Kotel sheep breed (52 ejaculates). The breed significantly influenced ejaculate volume, percentage of progressive motility, and total sperm concentration. The season of collection, on the other hand, significantly affected progressive motility in both fresh ejaculates and semen after cryopreservation. The highest mean ejaculate volume was observed in Pleven Blackhead rams, with an average of 1.108 ± 0.053 ml, while the lowest was recorded in Kotel rams, with an average of 0.718 ± 0.057 ml. The total sperm concentration in Pleven Blackhead rams averaged 2.865 ± 0.146 × 10^9/ml, which was significantly higher compared to the Assaf rams at 2.067 ± 0.124 × 10^9/ml and Kotel rams at 1.919 ± 0.157 × 10^9/ml (p<0.001).

Keywords: rams' semen, semen cryopreservation, Assaf ram, local sheep breeds

INTRODUCTION

Preserving farm animal genetic resources is crucial for maintaining the biodiversity of domestic animals and their adaptation to climate change, shifts in breeding objectives, or epidemic outbreaks (Joost & Bruford, 2015; Mara et al., 2013). Several factors influence semen quality, including breed, season of collection, ram age, and diet. Talebi et al. (2009) found a significant impact of season on serum testosterone levels in rams. They reported a peak in the testosterone levels during summer, which corresponded with observed in summer and autumn the highest semen quality.

Seasonal changes significantly affect sex hormone levels, which in turn influence physiological changes during the estrous cycle (Abecia et al., 2012). Testosterone levels positively and significantly correlated with sperm motility and progressive motility (Swelum et al., 2017).

The cryopreservation of ram semen has a lower success rate compared to that of other animals, such as bulls and stallions (Malinova, 2016; Gáspárdy et al., 2020). This is due to the higher sensitivity of ram semen to freezing and thawing procedures, as well as the low cryotolerance of spermatozoa. Additionally, individual variation in frozen semen quality among small ruminants has been observed, indicating specific differences in the success rates of semen freezing methods (Barbas & Mascarenhas, 2009).

During the various stages of cryopreservation, spermatozoa may experience thermal shock, leading to loss of selective permeability and integrity of the plasma membrane, as well as the release of intracellular enzymes and lipids. The redistribution of ions causes permanent changes in the acrosome and mitochondria membranes, resulting in loss of

motility and impaired metabolic state (Colás et al., 2009). The formation of ice crystals is one of the main biophysical factors leading to cell death during cryopreservation (Arav & Saragusty, 2016). Furthermore, the freezing process induces lipid peroxidation, which causes structural damage to spermatozoa, accompanied by a reduction in motility, membrane integrity, and fertilization capacity (Dorado et al., 2007; Memon et al., 2012).

Semen evaluation is important for determining the reproductive capacity of rams. Assessing motility, concentration, total concentration, and other microscopic parameters of semen are key factors in determining ejaculate quality and the number of doses intended for artificial insemination.

The objective of this study was to examine sperm production and semen quality in rams of different breeds used for semen collection at the Artificial Insemination Station (AIS) in Sofia.

MATERIALS AND METHODS

The study was conducted at the AIS-Sofia under the Executive Agency on Selection and Reproduction in Animals in Sofia, and covers the period from June 2018 to September 2020. The examined ejaculates were obtained from four Pleven Blackhead rams (47 ejaculates – 43 first and 4 second collection), three Assaf rams (55 ejaculates – 49 first and 6 second collection), and three Kotel sheep breed rams (52 ejaculates – 47 first and 5 second collection). Semen samples obtained during December, January, February, and March were considered out of the breeding season, while semen samples collected from June to November were considered to be within the breeding season. During April and May, the rams were not used for semen collection. At the time of obtaining the seminal fluid, the rams were 15 to 18 months old. The rams in the AIS were reared under the same conditions and were fed with a special compound ram feed.

The semen was obtained twice a week, with one or two ejaculates collected using the artificial vagina method. The semen volume was measured using graduated pipettes with an accuracy of 0.1 ml. The progressive sperm motility (%) was determined on a smear slide prewarmed to 38ºC by recording the percentage of progressively motile spermatozoa. The samples were evaluated immediately after collection using a light microscope at a magnification of 200x (Nikolov et al., 2012). The sperm concentration was calculated using a Burker counting chamber. The total concentration (sperm count for the entire ejaculate) was calculated by multiplying the concentration per milliliter by the ejaculate volume (ml).

After examination, the fresh semen was diluted and tempered for four hours at a temperature of 4ºC. The dilution was performed using the colloid egg yolk-based diluent BullXcell® and after the samples have been tempered the semen was packaged in 0.25 ml plastic straws and cryopreserved at a temperature of -80ºC in liquid nitrogen vapors for 10 minutes, then transferred to liquid nitrogen at -196ºC. After thawing, the semen was reevaluated to determine the percentage of progressive motility. All data were analyzed statistically using SPSS Statistics 21 (IBM). The data were processed via multivariate analysis with following statistical model:

$$\mathbf{Y}ijk=\mu+\mathbf{S}i+\mathbf{B}j+\mathbf{e}ijk$$

Where: $Yij\kappa$ - observation vector, μ- total average constant; S*i* and B*j* are the fixed effects of the *i*-th season (1 for anestrous and 2 for estrous) and the *j*-th breed (Pleven Blackhead (1), Assaf (2), and Kotel sheep (3)), respectively; e*ijk*- residual variance.

RESULTS AND DISCUSSION

The breed had the greatest influence on ejaculate volume, progressive motility percentage, and total sperm concentrations (Table 1).

Table 1. Effect of the breed and season of obtaining of the ejaculate on the reproductive performance in rams

Factor	Parameters	F-criteria and Level of significance
Breed	Volume, ml	18.68***
	Progressive motility, %	9.264***
	Concentration, 1.10^9/ml	1.801
	Total concentration, 1.10^9	15.71***
	Post thaw progressive motility, %	1.310
Season	Volume, ml	0.024
	Progressive motility, %	28.78***
	Concentration, 1.10^9/ml	1.371
	Total concentration, 1.10^9	0.157
	Post thaw progressive motility, %	6.447*

Legend: Significance at *p<0.05; **p<0.01; ***p<0.001

There was no statistically significant influence of the breed on the concentration per milliliter and the percentage of progressively motile sperm. The season of obtaining (anestrous or estrous) of sperm significantly influences the percentage of progressively motile sperm in both the fresh ejaculates and those after cryopreservation. Ptáček et al. (2019) and Zaher et al. (2020) also observed the influence of the season on cryopreserved ram spermatozoa.

The average ejaculate volume of the rams from the three breeds examined was 0.88 ± 0.03 ml. The highest average volume was reported for the rams of the Pleven Blackhead (PlB) at 1.108 ± 0.053 ml, and the lowest for the rams of the Kotel breed (KoB) at 0.718 ± 0.057 ml (Fig. 1), and the difference was statistically significant (p<0.001). Similar results were reported for local breeds in Portugal, averaging 0.77 ml across all ten studied breeds, with values of 0.53 ml in the Churro do Campo breed and 0.92 ml in the Churro Algarvio breed (Barbas et al., 2023). In Wera breed rams, the semen volume ranged between 0.2 to 0.9 ml (Jha et al., 2018).

The percentage of progressively motile sperm was lowest in the Kotel breed and highest in Pleven Blackhead and Assaf, with insignificant differences observed between the latter two breeds. In terms of sperm concentration across breeds, Kotel rams exhibited the highest concentration, despite recording the lowest ejaculate volume. Conversely, Assaf rams had the lowest concentration.

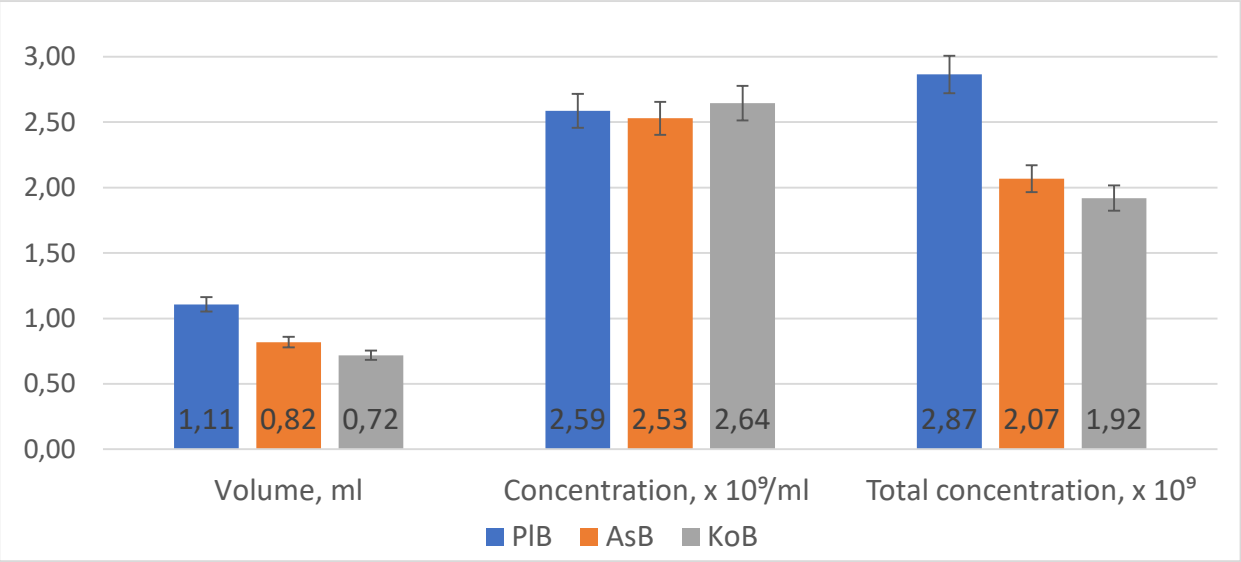

Figure. 1 Volume, concentration and progressive motility of the semen of Pleven Blackhead (PlB), Assaf (AsB) and Kotel (KoB) rams.

Total concentration, which reflects the overall sperm count in the entire ejaculate, was predominantly influenced by ejaculate volume and to a lesser extent by sperm concentration, with more pronounced differences observed across breeds in terms of volume. This parameter is crucial in practice as it indicates the sperm production capacity of rams and consequently, the number of doses per ejaculate. The total concentration of Pleven Blackhead rams averaged 2.865 ± 0.146 x 10^9/ml, whereas significantly lower values were recorded for Assaf (2.067 ± 0.124 x 10^9/ml), and Kotel rams (1.919 ± 0.157 x 10^9/ml) (p<0.001). Similar values for this parameter were reported for certain local Portuguese breeds, such as 2881 x 10^6 per ejaculate in Bordaleiro Entre Douro e Minho rams and 1764 x 10^6 per ejaculate in Churro do Campo rams (Barbas et al., 2023).

Figure 2 illustrates that Assaf rams sperm exhibited a higher cryoresistance compared to Pleven Blackhead and Kotel rams. Even after cryopreservation and thawing, the percentage of progressive motility of spermatozoa of Assaf rams remained relatively high – 42%, compared to 75.6% immediately after collection. The observed loss of motility due to cryopreservation reached 44.4%, 48.2%, and 47.6% for Assaf, Kotel, and Pleven Blackhead rams' spermatozoa, respectively. Nevertheless, the study clearly demonstrates that the local breeds exhibited sufficient sperm cryoresistance, with more than 30% progressive motile sperm, which is adequate for maintaining quality genetic material. This finding could contribute to more effective breed preservation through *in vitro* preservation of their genetic material.

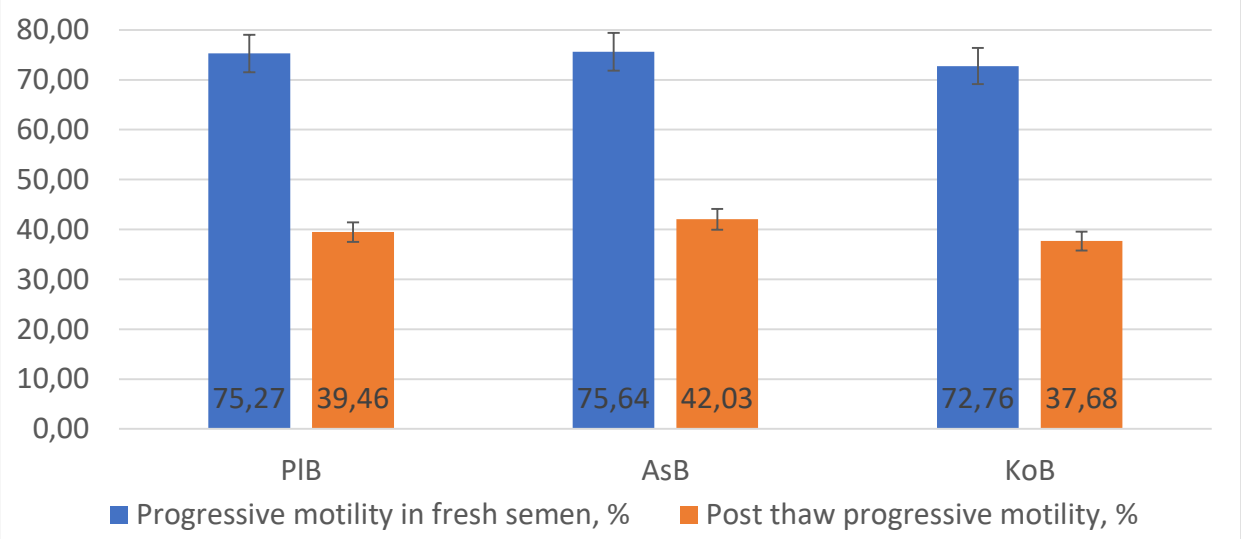

Figure. 2 Average percentage of progressively motile sperm in the ejaculates of Pleven Blackhead (PLB), Assaf (ASB) and Kotel (KOB) rams reported upon obtaining and post thawing of cryopreserved semen.

Table 1 indicates that, consistent with the seasonal reproductive patterns of sheep, the season significantly influences progressively motile spermatozoa both before and after cryopreservation. Ejaculates obtained during the anestrous season exhibited 5.5% lower motility upon collection compared to those obtained during the estrous season (Table 2). Furthermore, a significant loss of motility due to cryopreservation was observed: 50.3% during the anestrous season and 43.3% during the estrous season (p<0.001). Hristev et al. (2007) also reported lower sperm parameter values in SPBM rams during the anestrous season, with statistically significant differences. Similar significant seasonal effects on ejaculate quality have been documented in rams of the Assaf and Awassi breeds (Zaher et al., 2020).

Table 2. Influence of the season on some main sperm parameters of rams

Parameter	Season	N	Mean	± SE	min	max
Ejaculate volume, ml	Anestrous	28	0.887	0.065	0.40	1.50
	Estrous	126	0.876	0.029	0.20	1.90
Progressive motility, %	Anestrous	28	72.45	0.076	70.0	76.0
	Estrous	126	76.67	0.317	60.0	85.0
Concentration, x 10^9/ml	Anestrous	28	2.625	0.059	1.670	2.900
	Estrous	126	2.549	0.026	1.500	2.970
Total concentration, x 10^9	Anestrous	28	2.324	0.180	1.084	3.892
	Estrous	126	2.245	0.081	0.374	5.472
Post thaw progressive motility, %	Anestrous	28	36.01	2.627	21.0	69.0
	Estrous	126	43.44	1.180	7.00	66.0

CONCLUSION

The breed had significant influence on the ejaculate volume, the progressive motility, and the total concentration of the studied ram breeds. The season of sampling, on the other hand, significantly influenced the progressive motility in fresh ejaculates and the ejaculates post cryopreservation.

The highest average volume was reported for the Pleven Blackhead rams- 1.08 ± 0.053ml, and the lowest for the Kotel breed – 0.718 ± 0.057 ml. The total concentration in the ejaculates of the Pleven Blackhead rams was 2.865 ± 0.146 x 10^9 ml on average. The other two studied breeds exhibited significantly lower values of 2.067 ± 0.124 x 10^9/ml and 1.919 ±0.157 x 10^9/ml (p<0.001) for the Assaf and the Kotel rams, respectively. The ejaculates obtained during the anestrous season had a 5.5% lower motility upon obtaining when compared with those in the estrous season. The loss of motility of ram semen as a result of cryogenic damage during the process of cryopreservation was 50.3% during the anestrous and 43.3% during the estrous period (p<0.001).

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