Article
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ASSESSMENT OF SOME MICROSCOPIC PARAMETERS OF RAM SEMEN CORRELATED WITH THE AGE OF THE ANIMALS

Anamaria BLAGA PETREAN[1], Sidonia GOG-BOGDAN[1], Daniel BEREAN [1], Silvana POPESCU[1]

Corresponding author: anamaria.petrean@usamvcluj.ro

Abstract

The study was carried out to evaluate some microscopic parameters (mobility, concentration, viability), to assess the metabolic intensity of spermatozoa (Redox test), and sperm resistance test related with ram fertility and the quality of ram semen in different age groups. The study was conducted in a farm located in Cluj County, on 34 rams of Turcana Alba breed, grouped according to age into 4 batches. Weekly an ejaculate and the mentioned variables were measured. Semen samples were collected from each animal using the artificial vagina (AV). For sperm mobility, the best values were observed for 3 years old rams (X ± S = 88.4 ± 3.02). Variations in sperm viability showed some changes, but for all age groups were obtained values above those indicated in the literature. Assessment of sperm concentration revealed that rams in B6 (X ± S = 2.75 ±0.31) and B5 (X ± S = 2.7 ± 0.38) had the best values. Higher metabolic intensity rate in B6, B5, B3 groups was correlated with higher values of concentration and mobility in these age groups. Thus, the best values regarding sperm resistance were recorded for the rams aged 6 and 5 years, in which the average values were equal to 7022.22. With increase in age, ram showed increase percentages of motility and viability of sperm in all studied batches.

Key words: age, ram, semen, parameters

Many studies in domestic animals have studied the influence of male age on sperm characteristics (Hallap T. *et al*, 2006; Rijsselaere T. *et al*, 2007; Long J.A. *et al*, 2010; Carreira J.T. *et al*, 2017). It was showed that the impact of age on ram semen quality appears to be consistent. Most studies suggest that ram sperm reaches optimum quality at three years of age but begin to decline afterward (Mandiki S.N.M. *et al*, 1998; David I. *et al*, 2007; Hassan M.R. *et al*, 2007; Chella L. *et al*, 2017; Abah, K.O. *et al*, 2023). It was observed that older rams can have better sperm quality than younger ones (≤ 1 year) (Martí J.I. *et al*, 2011).

Other studies have reported a positive correlation between male age and sperm concentration in the ram (Salhab S.A. *et al*, 2003; Hassan *et al*, 2007; Martí J.I. *et al*, 2011; Ntemka A. *et al*, 2019), with one study reporting a 36.6% increase in sperm concentration in rams 3 years of age compared to yearling Yankasa rams (Osinowo O.A. *et al*, 1988).

Several studies have reported an increase in sperm motility with increasing male age in rams (Martí J.I. *et al*, 2011; Chella L. *et al*, 2017; Benia A.R. *et al*, 2018; Andreeva M., Stefanov R., 2020). It was suggested that the hypothalamo-pituitary–gonadal axis of old rams, even up to 13 years of age, is still functioning efficiently,which allows them to maintain good spermatogenesis (Ntemka A. *et al*, 2019). It has been consistently found that sperm membrane integrity in rams increases with age (Chella L. *et al*, 2017; Ntemka A. *et al*, 2019).

In one study, Martí J.I. *et al*, 2011 found that rams aged 8 years and above had a significantly higher sperm viability than those aged 1 year and below, with a viability rate of 64.6 ± 1.08 compared to 57.1 ± 0.82, respectively. This finding was proposed to be as a result of larger sperm head area of the young, which may affect its structural and functional competence (Martí J.I. *et al*, 2011). Our study was designed to determine if the age of the animals influenced the microscopic parameters of ram semen.

MATERIAL AND METHOD

The study was carried out on 34 Turcana Alba rams of different ages, grouped into four batches: batch B2 with 6 rams of 2 years; batch B3 with 10 rams of 3 years; batch B5 with 9 rams of 5 years; batch B6 with 9 rams of 6 years.

[1]Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania

Preliminary stages of ram semen collection

Given the importance of this stage in the collection of semen and its evaluation, measurements were made of the length and circumference of the testicle. The research was carried out during the rams' accommodation period, prior to the semen collection, for 4 weeks. The measurements took place once a week.

We considered it necessary to investigate the way sexual reflexes are manifested in order to select the rams used for semen collection. This is vital in order to obtain quality ejaculates, with a large volume that allows their further processing. This study was necessary because within the breed there is great individual variability regarding sexual behavior. For this purpose, a certain number of rams were randomly selected from each experimental batch. The research focused on the ram's adaptation to the collection site and the harvester due to the fact that it is absolutely necessary to establish a good relationship human – animal.

The experiment lasted a month and a half. The artificial vagina (VA) method was used to collect the semen samples (Groza I.S., 2006). All obtained ejaculates were subjected to an initial macroscopic evaluation and those outside the standard requirements were discarded.

The examination of the semen covered the following aspects: sperm viability, mobility and concentration, assessment of the metabolic intensity of spermatozoa (Redox test), and sperm resistance test according to criteria proposed by Groza I.S., 2006.

Weekly an ejaculate and the mentioned variables were measured. Rams were kept under the same breeding conditions and nutrition.

RESULTS AND DISCUSSIONS

The results obtained in the present study do not show significant changes in testicular length and circumference. Thus, the lowest value of testicular length was recorded in 2-year-old rams (10.65±0.05), while in the other age categories the average values obtained were over 12 cm for both testicles. Regarding the testicular circumference, it can be observed that the lowest values were recorded in 2-year-old rams (32.37±0.05) in comparison with the data obtained in the other experimental groups, where the average values were over 34 cm (*table 1*). The results obtained regarding the determination of the testicular circumference are in accordance with the data reported by other authors. In a study published by Olah *et al*, 2013, the testicular circumference of rams was determined according to breed and season. The highest values were recorded in autumn for Awassi rams (35.5 cm), in spring for Suffolk rams (35.8 cm) and in winter for Merino rams (32 cm).

Table 1

Dispersal of the average values for testicular length and circumference

Batch	Testicular length (cm)		Testicular circumference (cm)
	Right testicle	Left testicle	
B2	10.65±0.05	10.65±0.05	32.37±0.05
B3	12.2±0.07	12.23±0.05	34.11±0.05
B5	12.33±0.07	12.34±0.08	34.22±0.08
B6	12.15±0.07	12.16±0.08	34.44±0.13

The results showed that there are differences in the number of ejaculates obtained from a ram, depending on the age of the animals. Thus, in the case of 3-, 5- and 6-year-old rams, the number of ejaculates obtained was lower compared to those collected from 2 years old rams. The recorded results show differences in volume depending on the age of the animals, the best values being recorded in mature rams, over 3 years old (*table 2*).

Table 2

Dispersal of the average values for the sperm volume according to the age of the rams

Batch	B2			B3			B5			B6		
Parameter	J	E	V	J	E	V	J	E	V	J	E	V
Average	5.5	3.75	1.6	4.83	2.16	2.42	4.2	2.2	2.4	3.8	1.8	2.58
STDEV	±1.29	±0.95	±0.26	±1.16	±0.75	±0.25	±1.30	±0.83	±0.39	±1.30	±0.83	±0.39
J - No. of jumps; E- No. of ejaculates; V – volume (ml)												

Results regarding the average of sperm parameters analysis are presented in Table 3.

In the case of motility, there is a significant increase in rams aged 3 years (X±S=88.4±3.02) compared to those aged 2 years (X±S=82±1.78). For the other age categories, close values were recorded (5 years X±S=88.22±3.41, 6 years X±S= 88.33±3.93). The research carried out by Mandiki S.N.M. *et al*, 1998, regarding the influence of season and age on sperm parameters in Texel, Suffolk and Ile-de-France rams supports the increase in sperm motility and the decrease in the percentage of anomalies with the age of the rams. Mandiki S.N.M. *et al*, 1998 found that mass and progressive motility were similar at ages 2 and 3 but our study showed that ram spermatozoa motility were different between the 2-and 3- year-olds. Same results were found by Chella L. *et al*, 2017. In a study done with Garole rams in a semi-arid tropical environment, Joshi A. *et al*, 2003 found that the age of the ram did not have a significant effect on spermatozoa motility.

The variation of sperm viability shows some changes, but for all age categories, mean values were above those indicated by other studies (Hassan M.R. *et al*, 2007) (*table 3*).

Table 3

Dispersal of the average values of microscopic parameters according to the age of rams

Parameter	B2	B3	B5	B6
Motility (%)	82±1.78	88.4±3.02	88.22±3.41	88.33±3.93
Concentration ($x10^9$ spz/ml)	1.78±0.14	2.6±0.30	2.7±0.38	2.75±0.31
Viability (%)	91.5±2.25	94.1±2.68	92.44±2.78	93.88±2.26
Redox (min.)	7.33±0.36	6.82±0.49	6.6±0.53	6±0.25
Resistance test	6583.33±278.68	6970±188.85	7022.22±148.13	7022.22±192.20

The same authors concluded that normal and live spermatozoa were comparatively better during the 3rd year than the 1st and 2nd year of age while the values were almost similar at 3rd and 4th year of age. With the increasing of age, the semen quality improved and stabilized up to at the age of 3 years.

The assessment of sperm concentration shows the same upward trajectory starting with rams aged 3 years (X±S=2.6±0.30), but also for batch B5 (X±S=2.7±0.38) and B6 (2.75±0.31). We observed that 3-year-old rams exhibited the highest level of spermatozoa concentration than older or younger animals. Similarly, David I. *et al*, 2007, found that in Manech-tete-Rousse and Lacaune rams, spermatozoa concentration was higher between the ages of 2 and 3, thereafter decreasing with age.

The higher intensity of metabolism in the rams from group B6 (X±S=6±0.25), B5 (X±S=6.6±0.53) and B3 (X±S=6.82±0.49) is also correlated with the higher concentration values and mobility in these age categories. The observations made regarding the resistance of the spermatozoa confirm the data presented previously. Thus, the best values were recorded in B6 and B5 where equal average values of 7022.22 were obtained. These data confirm previous studies according to which the increase in resistance is directly proportional to the fertilizing value of the semen (Drugociu D.G, Runceanu L.G., 2004).

As age increased, semen quality improved and stabilized at the age of 3rd to 4th years.

CONCLUSIONS

During this study, there were individual variations in the concentration, motility, viability of the semen depending on the age of the animals. Analyzing the evolution of the average values of the microscopic parameters there is an increase with age of the rams, which could be explained by sexual maturation. Applying the artificial vagina method, the best values of volume semen were recorded in mature rams over 3 years. In our study, as age increase all semen parameters studied improved and stabilized at the age of 3 to 5 years.

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