

## STIMULATION OF SPERMATOGENESIS IN ARIES IN THE SECONDARY SEASON

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### Abstract

The studies were performed on ram semen, harvested in the secondary breeding season. Aries studied in the secondary breeding season were given the preparation ZooBioR<sup>1</sup>-1 (BioR 0.5%, Zn 2.5 mg / g, Se 25 mcg / g) and ZooBioR<sup>1</sup>-2 (BioR 0.5%, spirulina extract 10 mg / g, Zn 2.5 mg / g, Se 25 mg / g) of 5 g head / day for a period of 50 days. The following parameters were analyzed: testosterone dynamics, ejaculate volume, sperm concentration and number in the ejaculate, sperm mobility. Investigations into the influence of ZooBioR-1 and ZooBioR-2 showed a statistically significant influence on the increase in testosterone levels at the end of the experiment in the experimental groups compared to the control group ( $P \leq 0.1$ ). Increased testosterone levels led to increased sexual activity in rams in the secondary season. The preparations had a positive influence on the spermogram indices in rams in the experimental groups at the end of the experiment in the secondary season, compared to the control group. Ejaculate volume increased statistically significantly in the experimental groups ( $P \leq 0.001$ ), sperm concentration in the ejaculate increased insignificantly and mobility increased statistically significantly ( $P \leq 0.1$ ) in the first experimental group at the end of the experiment the other sperm indexes had oscillations towards the minimum limit and the maximum limit of the followed indices, the data being statistically insignificant.

**Key words:** ram, ejaculate, mobility, secondary season

### INTRODUCTION

Male breeders of zootechnical interest used in artificial insemination are an important means by which valuable characters are spread in the population.

Starting from this truth for the realization of the sperm bank which involves the preservation by freezing of the sperm material from the rams, it is necessary for the rams to be very well known not only in terms of morpho-productive characters but also in terms of reproductive skills. (Milovanov VC, 1962).

When choosing rams used for semen harvesting in the off-season, studying how to manifest sexual reflexes is essential to obtain high-volume quality ejaculates that allow their processing for preservation.

This research is necessary because there is great individual variability in sexual

behavior induced by the type of nervous system and temperament of males (Miclea V., 2003).

The normal sexual activity of male breeders is conditioned by the morpho-functional integrity of the genital tract. Knowledge of the theoretical and practical aspects of the biological reproduction of rams has a special importance in the organization and development of reproductive technology in this species and especially for the practice of artificial insemination. Sperm formation and quality is associated with increased testosterone secretion in the off-season and the appearance of the specific sexual compartment. In sheep breeding, the biological efficiency is influenced by the way it performs the reproductive activity at the level of each breed.

Therefore, the reproductive function must be directed towards the intensity of sheep breeding but bent in relation to the particularities of the breed (Nadolu D., 2007, Pascal C., 2009).

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One of the ways to intensify production is to use factors to stimulate spermatogenesis in the off-season in rams and sheep (Pascal C. et al. 2009)

The quality of sperm in rams is influenced by the season, maintenance conditions, diet and regime of use during reproduction.

The breeding season is determined by the level and dynamics of light and temperature, respectively. Although spermatogenesis is not completely stopped during the year and the best quality ejaculates are obtained in autumn when the light of day gradually decreases.

It is known that there are animals in which ejaculate, especially in the off-season, cannot be cryopreserved because the percentage of sperm that retain their fertilizing capacity after thawing is too small. When making the sperm bank in order to preserve the genetic potential, it is important that the chosen rams are used for reproduction throughout the year.

## MATERIAL AND METHOD

The research was conducted in May 2019 on a number of nine rams of different ages belonging to the Moldovan Karakul breed within the Technological Experimental Station belonging to the Scientific-Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine of the Republic of Moldova.

In order to compose the batches, the rams were subjected to an examination of the male genital tract.

Rams used in the season in the research were grouped into two experimental groups and one control:

- lot 1 witness-3 rams
- experimental lot 2 - 3 rams who were given 5 g head / day of ZooBioR-1 for 50 days.
- lot 3 experimental- 3 rams who were given 5g head / day ZooBioR-2 for 50 days.

The semen was harvested using the artificial vagina method. All phases of the research complied with the requirements in force regarding the ethics of the use of

animals for scientific research purposes. After harvesting, the analysis of the qualitative and quantitative parameters of the sperm was performed. Macroscopic analyzes of sperm appearance and volume were followed by microscopic analyzes of mobility, sperm count and ejaculate rate, sperm counts, and testosterone levels analyzed at the beginning and end of the experiment. The experimental plan used to determine the various changes between the parameters of raw semen was the one in repeated measurement blocks. This plan allowed a small number of individuals to analyze the factors and the interaction between factors that determine the quality of semen in rams.

Each individual who constituted the experimental unit was considered as a block of data and the repeated measurements were constituted in treatments.

In order to detect the possible differences in size and their significance according to the quality of the variance, we proceeded according to the known methodology and the statistical parameters were calculated after Mercurieva (1964). Microscopic indices on the quality of raw semen taken from rams from experimental groups or studied by the CEROS program.

Tracking sperm velocity assessed three characteristics: VAP velocity that measures the distance traveled by sperm in a general direction and in a given observation period; VSL velocity that measures the distance in a straight line traveled by sperm from one point to another in an observation period; VCL velocity that measures the total distance traveled by the sperm head in an observation period. Hematological indices of the blood were studied using the method of Menisicov V. (1986) and Poloziuc O. (2019) and testosterone levels using the method of Tietz N.W. (1986)

## RESULTS AND DISCUSSIONS

Experimental data on hematological indices of blood collected from rams studied in the secondary season are presented in Table 1.

Table 1 Hematological indices of the blood

Specification	witness		Experimental 1 ZooBioR -1		Experimental 2 ZooBioR 2	
	The beginning of the experience	The end of the experience	The beginning of the experience	The end of the experience	The beginning of the experience	The end of the experience
Erythrocytes (x) $10^{12}/L$	$10.7 \pm 1.1$	$6.3 \pm 0.6$	$10.1 \pm 0.7$	$7.9 \pm 0.4$	$9.9 \pm 1.1$	$8.7 \pm 0.8$
Leukocytes (x) $10^{12}/L$	$6.4 \pm 1.1$	$8.0 \pm 1.0$	$8.6 \pm 1.1$	$10.1 \pm 0.9$	$12.4 \pm 0.5$	$6.8 \pm 0.5$
Hb g/L	$91.7 \pm 6.0$	$118.3 \pm 4.4$	$108.0 \pm 4.2$	$101.7 \pm 4.4$	$108.3 \pm 1.7$	$106.7 \pm 3.3$
VSE mm/oră	$1.7 \pm 0.3$	$3.3 \pm 0.3$	$4.0 \pm 1.4$	$2.3 \pm 0.3$	$3.7 \pm 1.2$	$5.7 \pm 0.3$
E, %	$13.0 \pm 4.2$	$11.3 \pm 1.8$	$12.0 \pm 0.6$	$13.7 \pm 1.8$	$15.0 \pm 4.6$	$11.3 \pm 1.5$
Segmented, %	$10.7 \pm 3.2$	$11.0 \pm 2.5$	$10.3 \pm 2.6$	$7.7 \pm 2.0$	$8.3 \pm 0.9$	$14.0 \pm 2.0$
Unsegmented, %	$3.7 \pm 1.8$	$16.3 \pm 4.2$	$6.3 \pm 2.3$	$13.7 \pm 5.4$	$6.7 \pm 2.9$	$10.7 \pm 3.9$
Lymphocytes, %	$72.7 \pm 8.3$	$61.3 \pm 5.5$	$71.3 \pm 4.7$	$65.0 \pm 3.8$	$70.0 \pm 4.9$	$64.0 \pm 3.8$

The data obtained showed oscillations of hematological constants in all experimental groups of rams studied determined by the influence of the administered preparations. The values of the erythrocyte series in rams from the experimental groups or located in the limits of physiological variations with oscillations between the lower level of the values (number of erythrocytes), the average level of hemoglobin and the upper level of the reference values at the beginning and end of the experiment.

The total level of leukocytes was within the physiological limits in the experimental groups being increased in the first experimental group (insignificant) at the end of the experiment and increased at the end of the experiment in the second group.

The leukocyte formula showed different values of leukocyte fractions in the experimental groups in the sense of

insignificant decrease of eosinophils at the end of the experiment in the second experimental group.

The other indices studied recorded average values in the physiological limits of the studied groups.

More and more research has shown that testosterone is essential in expressing sexual manifestation in males but also allows the development and maintenance of sexual behavior in males. Sexual activity increases with increasing testosterone levels during puberty. Moreover, the secretion of this hormone is activated by external stimuli such as the behavior and smell of sheep, the secretion of androgens being directly correlated with the production of pheromones.

Experimental data on blood testosterone levels in rams studied in the secondary period are presented in Table 2.

Table 2 Dynamics of testosterone in rams

Specification	witness		Experimental 1 ZooBioR -1		Experimental 2 ZooBioR 2	
	The beginning of the experience	The end of the experience	The beginning of the experience	The end of the experience	The beginning of the experience	The end of the experience
Testosterone (ng / ml)	$4.2 \pm 0.2$	$4.2 \pm 0.3$	$4.0 \pm 0.1$	$5.2 \pm 0.4^*$	$4.3 \pm 0.4$	$6.0 \pm 0.1^*$

\* $P \leq 0.01$

Investigations into the influence of ZooBioR-1 and ZooBioR-2 have shown a statistically significant influence on the increase in testosterone levels at the end of the experiment in the experimental groups compared to the control group. Increased

testosterone levels led to increased sexual activity in rams in the secondary season. The experimental data on the ram spermogram studied in the secondary season are presented in Table 3.

Table 3 Spermogram in rams

Specification	n	Sperm concentration, billion / ml	Volume, ml	Mobility, %	Sperm with rectilinear movements, %
At the beginning of the experience					
witness	20	2.21 ± 0.01	0.5 ± 0.05	75.0 ± 2.3	33.7 ± 1.6
Experimental 1 ZooBioR -1	20	2.30 ± 0.1	0.5 ± 0.04	74.4 ± 1.7	28.5 ± 2.0
Experimental 2 ZooBioR 2	20	2.24 ± 0.1	0.5 ± 0.05	71.7 ± 3.0	28.7 ± 2.3
At the end of the experience					
witness	20	2.18 ± 0.1	0.6 ± 0.04	74.4 ± 3.7	29.1 ± 2.0
Experimental 1 ZooBioR -1	20	2.34 ± 0.09	0.8 ± 0.04***	80.0 ± 1.6*	32.9 ± 2.0
Experimental 2 ZooBioR 2	20	2.48 ± 0.09	0.9 ± 0.04***	79.9 ± 2.7	33.7 ± 2.4

The experimental data presented in the table demonstrate that the preparations ZooBioR-1 and ZooBioR-2 had a positive influence on the spermogram indices in rams in the experimental groups at the end of the experiment in the second season compared to the control group. Ejaculate volume increased statistically significantly in the experimental groups ( $P \leq 0.001$ ) sperm concentration in the ejaculate increased statistically insignificantly and mobility increased statistically significantly ( $* P \leq 0.1$ ) in the first experimental group at the end of the experiment.

Computer-assisted sperm analysis CEROS has become useful for its ability to evaluate calculations at a much faster rate with a larger data storage space used in measurements made to determine the quality of semen. Experimental data on computer-assisted sperm velocity are presented in Table 4.

The study of VAP velocity characteristics found in all experimental groups at the beginning of the experiment was  $92 \mu\text{m} / \text{sec}$ . At the end of the experiment this index increased but the differences are not statistically significant compared to the control group.

 Table 4 Sperm advance rate,  $\mu\text{m} / \text{sec}$ 

Specification	n	Calculated statistical parameters, $\mu\text{m} / \text{sec}$		
		VAP	VSL	VCL
At the beginning of the experience				
witness	20	92.5 ± 3.7	71.2 ± 5.3	136.7 ± 6.6
Experimental 1 ZooBioR -1	20	92.1 ± 6.0	74.8 ± 4.8	143.5 ± 8.7
Experimental 2 ZooBioR 2	20	93.3 ± 8.3	70.7 ± 8.6	145.2 ± 9.2
At the end of the experience				
witness	20	111.8 ± 8.6	90.5 ± 7.4	153.0 ± 13.3
Experimental 1 ZooBioR -1	20	111.2 ± 10.2	91.8 ± 9.3	167.5 ± 12.2
Experimental 2 ZooBioR 2	20	104.4 ± 3.3	85.3 ± 2.9	158.0 ± 6.2

Measuring the velocity of VSL sperm, we found that this index was on average  $70 \mu\text{m} / \text{sec}$  and at the end of the experiment it increased to  $91.8 \pm 9.3$  in the first experimental group but compared to the control group the data are statistically insignificant. Assessing the speed of VCL sperm, it was established that this index increased in ejaculates taken from rams and reached mean values of  $167.5 \pm 12.2$  in the first experimental group and  $158.0 \pm 6.2$  in the second experimental group compared to  $153.0 \pm 13.3$  obtained in the control group at the end of the experiment the data being statistically insignificant.

## CONCLUSIONS

1. The preparations ZooBioR-1 and ZooBioR-2 had a positive influence on the modification of sexual reflexes and spermatogenesis in Moldovan Karakul rams in the off-season. The volume of ejaculate increased significantly ( $P \leq 0.001$ ), and the level of testosterone in the experimental groups compared to the control group increased significantly.

2. The studied preparations showed average values of the oscillation of hematological constants in the experimental groups of rams studied

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