INFLUENCE OF SOME EXTERNAL FACTORS ON SPECIFIC CHARACTERISTICS OF SEMINAL MATERIAL FOR KARAKUL DE BOTOȘANI BREED RAMS

Alexandru Marian FLOREA[1], Ionică NECHIFOR[1], Andre CRÎȘMARU[1], Constantin PASCAL[1, 2*]

[1]Research and Development Station for Sheep and Goat Breeding Popăuţi - Botoşani, Romania
[2]University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" from Iaşi, Romania

*Corresponding author email: pascalc61@yahoo.com

Abstract

In order to objectively assess the influence of external factors, the observations were made both in normal breeding seasons and in the off-season, the aim being to obtain conclusive data necessary for a real understanding of specific aspects of reproductive physiology in adult rams. The biological material was represented by breeding rams belonging to the Karakul de Botoşani breed located at the Research and Development Unit for Breeding Sheep and Goats Popăuți - Botoșani. In order to evaluate the mode of influence and the differences due to the experimental factor, the applied protocol aimed to expose the rams to the same effect of photoperiodicity for two consecutive seasons placed in atypical periods. The volume of ejaculate was a basic objective in the applied research, the final results indicating that from rams exposed to a photoperiod placed in the off-season has a lower average value by only 5.04% compared to the volume harvested from rams active in normal season and with over 75% more than the level harvested from those at sexual rest. Differences were also reported in the case of the analysis performed for the determination of the quality indicators of the harvested semen, respectively acidity, density and mobility. The obtained results highlight the existence of multiple factors which, due to the intensity of their influence, can induce changes in the basic characteristics of the semen.

Key words*: external factors, photoperiod, ram, reproduction function.*

INTRODUCTION

The role and influence of different natural factors on the way heat is manifested in females was a more frequently approached topic, while the effect of the same factors on the reproductive function in rams is a subject with a lesser approach.

For a more complex approach to the research objectives, the observations were made in normal breeding seasons but also in the off-season, the aim being to obtain conclusive data necessary for a real understanding of the physiology of reproduction in adult rams.

The obtained results highlight the existence of multiple factors which, due to the intensity of their influence, can induce changes in the basic characteristics of the semen. Of all these, the most important are: nutrition, maintenance conditions, presence of females, geographical location, age, testicular characteristics, libido and management system, an aspect highlighted in some scientific articles in the literature (Nowakowski et al., 1994; Madani et al., 2009; Kridli et al., 2004; Zamiri et al., 2005), but photoperiodicity and race seem to be the main factors influencing the activity of seasonal reproduction.

In this context, conducting research on the influence of natural factors on the main characteristics of semen aims to highlight the ways that can be used to optimize them and other aspects that could be generated by climate change.

MATERIALS AND METHODS

The biological material was represented by breeding rams belonging to the Karakul de Botoșani breed located at the Research and Development Unit for Breeding Sheep and Goats Popăuți - Botoșani.

In order to meet the research objectives, the experimental protocol applied a program to

induce the manifestation of sexual activity in rams in the off-season, based on the following steps:

a. long days simulation, in this case the long day was considered the one with more than 12 hours of light, corresponding to the months of late spring and the beginning of summer;

b. short days simulation, in which case a short day was considered the period in which the rams were kept in the light for less than 12 hours of a day, corresponding to the autumn-winter months.

In order to be able to establish the differences due to the experimental factor, the applied protocol aimed to expose the rams to the same effect of photoperiodicity for two consecutive seasons placed in atypical periods in an area located at northern latitude of 47.76 and longitude of 26.69, respectively in the interval March 10 - May 20, when the ratio between light and darkness is close to or greater than 1/1, and the possibility of late, semi-late and semi-early breeds to manifest sexual cycles is reduced.

At the beginning of each experimental period in the two consecutive years, for 15 days the rams were subjected to a special light program, because it is known that during the 24 hours of a day there is a period in which the animals are more sensitive to light. This period is called the photosensitive phase and is naturally placed 16-17 hours after sunrise, which is considered a landmark of circadian rhythm. Through this mode of action, a progressive exposure of the rams to a total light duration of about 16 hours was achieved, corresponding to 22nd of June.

From that moment on, in order to have conclusive results that could argue the practical possibility of intensifying the reproductive activity in rams, they were subjected to a program of gradual control of both the temperature and the duration of daylight hours of a day.

In the first 17 days there was a gradual increase in the duration of temperature and light until reaching daily values between 26 and 28°C and 15 hours of light, representing the average multiannual values corresponding to 22nd of June, after which we gradually reduced the two parameters, so that after another 18 days to ensure the exposure of batches of rams at an average temperature of 18°C and a duration of 10.45 hours of light, representing the multiannual average values for 15th of October.

From the moment that both parameters were reached, the second stage of the research was passed, namely the collection of semen, and the performance of qualitative and quantitative analyzes of semen.

Parameters that characterize the quality and quantity of semen were evaluated by specific laboratory methods and data processing was performed by methods accepted by the experimental technique.

The non-parametric Mann-Whitney U Test was used to highlight the significance of the differences between the characters analyzed at different times of the year in rams in different conditions in terms of sexual activity.

RESULTS AND DISCUSSIONS

In this species, as in goats, reproduction is determined by the genotype/environment interaction, being largely influenced by the photoperiod, meaning the duration of daylight, to which is added the influence of other factors such as: diet, temperature, humidity, social factors, etc. (Pascal et al., 2008).

Regarding the ratio between light and dark, the effect is received by sheep in the eyes through the retina and is transmitted by nervous system to the pineal gland (epiphysis) which secretes the hormone melatonin (Pascal et al., 2009).

The activity of the hypothalamus and pituitary gland, but also the duration and amount of secreted melatonin, corroborated with the actual dark period, exerts a major influence in the development of sex hormones, meaning follicular stimulation (FSH) and lieutenant hormone (LH) (Andersson et al., 2005; Barid et al., 1981; Cahil et al., 1980; Pascal et al., 2009; Thwaites, 1982).

In sheep and goats, the influence of this phenomenon is major in both sexes from herds in areas located in the northern hemisphere. In this area, reproduction takes place in autumn, and the intensity of the onset sexual cycles is mainly due to the decrease in the intensity of daily light, thus being considered animals that have a negative photoperiod (Delgadillo et al., 2014). The explanation for this behavior would be that shortening the duration of daily light causes the pineal gland to synthesize and

secrete melatonin, which stimulates the hypothalamus, pituitary gland, ovaries and testicles, with positive effects on reproductive activity.

The assessment of the main physical and biological properties of the semen harvested from rams subjected to photoperiodicity, sexually active during the normal breeding season and respectively at sexual rest, was performed for each period considered, the average values being presented in Table 1 and Figure 1.

Table 1. The main biophysical properties of ram semen according to the natural breeding season

Season/ Condition of rams	n	Biophysical behaviors of seminal liquid			
		Volume (ml)	Sperm reaction pH	Density	Mobility (%)
Exposed to photoperiod	8	1.93±0.02	6.71±0.11	1.09±0.01	83.47±0.05
Sexual rest	8	1.08±0.01	5.82±0.09	1.03±0.10	78.99±0.09
Normal season	8	2.04±0.05	6.95±0.20	1.11±0.07	82.48±0.07

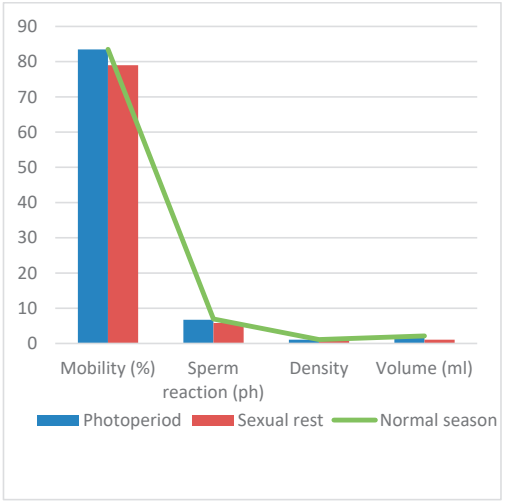

Figure 1. The dynamics of ram's semen main biophysical properties in relation to the natural breeding season

The ejaculate volume was an important objective through which we wanted to verify whether from a quantitative point of view there are differences in the volume of the ejaculate under the influence due to the conditions of the two calendar periods.

In the literature it is specified that in breeding males in the normal season of activity the volume of ejaculate had average values between 1 and 1.5 ml (Luca, 1972) and in males in sexual rest the volume of sperm is lower. Regarding the volume of ejaculate, Kuznetsov quoted by Luca (1972) shows that in rams there were situations in which the level of ejaculate exceeded 6 ml.

Research has shown that there are differences in the volume of semen harvested from rams depending on certain times of the year. Based on the determinations performed, it was observed that there are differences in the volume of ejaculate. The level of ejaculation was higher in the case of harvests performed from rams during the breeding period (2.04 ± 0.02 ml) and who had sexual activity during the autumn season and only 1.08 ± 0.01 ml in those during the sexual rest corresponding to January and February.

In the case of males exposed to the influence of light, the level of ejaculated collected had an intermediate volume of the first two situations, the average level being 1.93 ± 0.02 ml. In the case of harvests from breeding rams during the breeding season, but carried out in the normal season, meaning September-October, the volume of ejaculate was 2.04 ± 0.051 ml, and it was 47.05% higher compared to the volumetric level recorded in the case of harvests performed from rams at sexual rest and by only 5.39% compared to the volume of semen harvested from rams that were exposed to the influence of temperature and duration of light during the off-season (May-June). This difference was statistically significant for $P<0.01$.

However, other studies carried out in areas with a different climate than in the North-eastern part of Romania show that the ejaculation volume was 1.23 ± 0.31 ml being more influenced ($P<0.0001$) of the season and in very small measure of age ($P<0.84$) of rams (Barid et al., 1981).

All these values confirm that in rams subjected to the influence of photoperiodicity, the testicular secretory activity takes place in almost normal conditions and in the situation when the reproductive activity is placed in the months considered to be in the off-season.

This aspect is very important when in some farms is applied an advanced management of sheep breeding. In this case, the application of a controlled program of exposure of breeders to

the influence of light and temperature can change the general condition of rams because through photoperiodicity can stimulate the intensification of sexual activity and their use in off-season breeding.

The economic importance of the data obtained for this indicator is also ensured by the fact that the volume of ejaculate obtained from rams stimulated by external factors reaches the level obtained during normal activity. At the same time, the practical importance is obvious because when a reproductive technology based on artificial insemination is applied, the number of sown females increases with the resulting doses per ejaculate, even when the rams exposed to the influence of temperature and light have a lower ejaculate volume. This aspect does not affect the fertilizing capacity of the semen even if by reducing the volume of ejaculate there is a reduction in the total number of sperm, and the final results of insemination do not decrease significantly, an aspect reported in other specialized bibliographic sources (Nacu et al., 2011; Davis et al., 2001; David et al. 2007).

In a similar study performed on the breeders of the Merino de Palas undergoing an experimental treatment based on the influence due to photoperiodism on the reproductive characters, an average ejaculate level of 1.91 ± 0.021 ml was obtained, and from those at rest and in natural season the volume was 0.98 ± 0.06 ml and 1.94 ± 0.03 ml, respectively (Moise et al., 2015).

Regarding the volume of ejaculate from rams in the natural breeding season Kuznetov quoted by Pascal (2015) states that there are situations in which the ejaculate level can exceed 2 ml, sometimes even higher than 6 ml.

Sperm acidity or reaction is assessed by determining the pH value and may be influenced by the concentration of semen fluid in the sperm. The higher the sperm density, the more the pH tends towards acidity, an aspect determined by more intense metabolism but also by the greater accumulation of lactic acid in the seminal fluid.

Regarding the values of this character but specific to ram sperm, in the literature it is specified that in males in the normal breeding season the pH has average values of 6.8 (Luca, 1972; Nadolu et al., 2007; Pascal et al., 2008; Thwaites, 1982; Zarazaga et al., 1997).

The evaluation of this indicator is important because the duration of sperm motility proved to be pH dependent. Relatively longer periods of motility occur mainly at a range of pH levels between 5.8 and 6.4 with a maximum placed at an average value of 6.26.

In the determinations made, differences were found regarding the reaction of the semen harvested from the rams at different times of the year and under different conditions in terms of sexual activity.

It should be noted that the determined average values are close only to the groups of ram's subject to the influence of the photoperiod and those in the normal season of reproductive activity. Thus, it was found that in the case of semen harvested from rams subjected to a photoperiod, the acidity records an average value of 6.71 ± 0.11. Samples collected at the time when the breeding activity took place in the normal season from the same ram, the average acidity was 6.95 ± 0.20. This aspect allows us to conclude that there is an almost constant relationship between the epidermal contents and the secretions of the secondary glands, a sign that the spermatogenesis process evolves normally in rams under the influence of natural factors and their optimization at other times of the year.

The statistical processing of the data does not show that the difference between the average values obtained in the two periods is not significant for the statistical thresholds taken into account.

In contrast, when determining the acidity of semen harvested from rams during sexual rest, the average pH was 5.82 ± 0.09. Placing average values well below these limits is undesirable because at low pH sperm motility is reduced and has a negative effect on their viability.

Density and mobility are important indicators of assessing the quality of semen because the act of fertilization depends on mobility and the number of viable sperm.

Analyzing the specific weight of sperm in relation to the interrelationships between the physical properties and the biological value of semen, it is found that a specific weight of

semen corresponds to a particular density and mobility of sperm (Andersen et al., 2005).
After Lindhal and Kihlstrom, quote Pascal et al. (2008) the specific weight of sperm also depends on the ratio between mature (heavier) and immature (lighter) sperm in the sperm fluid. Other studies state that sperm-specific weight is directly influenced by osmotic pressure and is directly related to the cryoscopy point of sperm (Mann, 1960).
The sperm density assessed under a microscope is quite subjective and greatly influenced by the experience of the examiner. Due to this fact, in laboratory practice determinations are made by counting the spermatozoa in the ejaculate to be analyzed or other indirect procedures by which the number of sperm in a sperm volume can be established as correctly as possible. The number of sperm can be reported in millions per microliter, or in billions per ml of sperm. At the same time, it is mandatory to establish the correct degree of semen dilution (Pascal et al., 2008).
When assessing dignity, the results obtained are placed in a narrower range in rams during periods of ongoing sexual activity, the differences not being statistically significant. Based on the average values determined from semen samples collected from rams under the direct influence of temperature and light exposure time, the main density value was 1.09 ± 0.01 and for those with sexual activity during the normal season the density increases to 1.11 ± 0.07.
The research carried out wanted a comparison of the average values specific to this character in rams in conditions of intense sexual activity carried out at different times of the year. In the case of analysing this character on the semen harvested from rams during sexual rest, the average value was 1.03 ± 0.10.
Mobility is one of the quality indicators that are used to analyze the basic characteristics of semen specific to any animal species. Normal sperm are able to move quickly, in a straight line, by undulating movements of the tail. Only sperm with active linear motion participate in fertilization.
Simultaneously with the assessment of mobility and concentration, observations are made regarding the energy of movement of sperm. On the samples analyzed from the three groups of rams that were in sexual periods placed at certain intervals of the year or in those at sexual rest, the sperm mobility registered some differences of the average values. In the group of rams during sexual activity, the average value was 83.47 ± 0.05 in those who went through an off-season stimulation program and 82.48 ± 0.07 in rams who had sexual activity during the normal breeding season. In contrast, in the rams that were in the period of sexual rest, the mobility had average values of 78.99 ± 0.09. Between this average value and those determined for the two groups of rams that had sexual activity in the normal season and in the off-season, it is found after applying the non-parametric Mann-Whitney U Test that the differences are significant for $P<0.05$.
The existence of differences between the average values of rams in different conditions of use, but also the approximation of data for density and mobility to samples collected from rams in the natural breeding season and those subjected to photoperiodicity allows us to say that by optimizing light and temperature in atypical seasons the quality of semen has characteristics close to the values determined in those in full season of reproductive activity.
Based on the data obtained from the evaluation of the semen quality from breeding rams, it can be stated that the exposure of breeders to a program to simulate temperature and light duration during off-season has a positive influence on the main characteristics of sperm fluid.

CONCLUSIONS

In case of harvests from breeding rams during the normal breeding season, i.e. September-October, the ejaculate volume was 2.04 ± 0.051 ml, being 47.05% higher than the volumetric level recorded in the case of harvests from rams at sexual rest and only 5.39% compared to the volume of semen collected from rams that were exposed to the influence of temperature and light duration during off-season periods (May-June).
When analyzing the acidity of the semen, differences were found regarding the reaction of the semen harvested from the rams at different times of the year and activity.

The determined average values are close only to the groups of rams subjected to the influence of the photoperiod (pH = 6.71) and those in the normal season of reproductive activity (pH = 9.65)

Regarding the differences between the sperm density of semen, it is found that in the samples collected from rams after photoperiod treatment and from those in the normal breeding season the average values of specific gravity are very close, meaning 1.039 in those subjected to photo-periodism and 1.038 for those who work in the natural season.

Based on the data obtained from the evaluation of the quality of semen from breeding rams, it can be stated that the exposure of breeders to a program to simulate temperature and light duration during off-season has a positive influence on the main characteristics specific to semen quality.

REFERENCES

Andersson, H., Johnston, J.D., Messager, S., & Hazlerigg, D.G. (2005). Photoperiod regulates clock gene rhythms in the ovine liver. *General and Comparative Endocrinology* 142. 357-363. Scotland, UK.

Barid, D.T., & Mac Nelly, A.S. (1981). Gonadotripic control of follicular development and function during the oestrus cycle of the ewe. *J. Reprod. Fert*. Suppl.

Cahill, L.P., & Mauleon, P. (1980). Season and photoperiod effects on follicles and atresia in the sheep ovary. *Aust. J. Biol. Sci*., 37.

David, I., Bodin, L., Lagriffoul, G., Manfredi, E., & Christèle, R.,G. (2007). Character process model for semen volume in AI rams: Evaluation of correlation structures for long and short-term environmental effects. *Genetics Selection Evolution, 39*(1), 55-71

Davis, G.H., Dodds, K.G., Wheeler, R., & Jay, N.P. (2001). Evidence that an imprinted gene on the X chromosome increases ovulation rate in sheep. *Biology of Reproduction*, 64, 216-221.

Delgadillo, J.A., Flores, J.A., Duarte, G., Vielma, J., Hernández, H., Bedos, M., Fitz-Rodríguez, G., Fernández, I.G., López-Sebastián, A., Gómez-Brunet, A., Santiago-Moreno, J., Zarazaga, L.A., Keller, M., & Chemineau, P. (2014). Out-of-season control of reproduction in subtropical goats without exogenous hormonal treatments. *Small Ruminant Research*., 121, 7-14.

Khidri, R.T., Taobaa, M.J., Sawalha R. M., & Amashe M.G. (2004). Comparative study of scrotal circumference and semen characteristics of Mountain Black goat and its crossbred with Damascus goat as affected by different factors. *Jordan Journal of Agricultural Sciences*, Volume 1, No. 1.

Luca, N. (1972). *Reproduction of domestic animals*. Bucharest, RO: Agro-Silvica Publishing House.

Mann, T. (1960). *Evolution of sperm by chemical analysis. The artificial insemination of farm animal*. E.J. Perry (edit) New Brunswick N.J. Rutgers, University, Press.

Madani, T., Chouia, F., & Abbas, K. (2009). Effect of oestrus synchronisation and body condition on reproduction of anoestrous ouled djellal ewes. *Asian J. Anim. Vet. Adv*., 4, 34-40.

Nacu, G., Pascal, C., & Hoha, G. (2011). Research on fertility and prolificacy of the sows artificially inseminated with different amounts spermatozoids. *Lucr. şt. Seria Zootehnie,* 55, 278-280.

Nadolu, D., & Anghel, A.H. (2007). The influence of the photo-periodic variations upon the rams sexual activity. *Lucrari Stiintifice, Seria Zootehnie*, 277-280.

Nowakowski, P., & Cwikkla, A. (1994). Seasonal variation in testes size in Polish Merino rams and its relationship to reproductive performance in spring. *Theriogenology*, *42*(4), 613-22, doi: 10.1016/0093-691x (94).

Pascal, C. (2015). *Tratat de creşterea ovinelor şi caprinelor / Treated for raising sheep and goats.* Iaşi, RO: Ion Ionescu de la Brad Publishing House.

Pascal, C., Ivancia, M., & Gherasim, N. (2008). The influence of some factors on the reproductive function of Romanian local sheep. *Reproduction in Domestic Animals*, 43, 99.

Pascal, C., Ivancia M., Gîlcă, I., & Nacu, Gh. (2009). Research regarding the influence of natural factors on activity reproduction behavior at rams, *Reproduction in Domestic Animal Reproduction*, 44, 119.

Moise, L., Moise, V., & Şonea, C. (2015). Management of Assisted Reproduction in the Palas Merino sheep breed. *Scientific Papers: Animal Science and Biotechnologies*, *45*(1).

Thwaites, C.J. (1982). Development of mating behaviour in the prepubertal ram. *Anim. Behav*. 30, 1053–1059.

Zamiri, M.J., & Khodaei, H.R. (2005). Seasonal thyroidal activity and reproductive characteristics of Iranian fat-tailed rams. *Animal Reproduction Science,* 88, 245-255.

Zarazaga, L.A., Malpaux B., & Chemineau, P. (1997). The characteristics of the melatonin secretory rhythm are not modified by the stage of pregnancy in ewes. *Reproduction, Nutrition, Development*, 37, 105-112.