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The Influence of Seasonal Factors on the Quantitative and Qualitative Parameters of Ram Semen of the Dzhalginsky Merino Breed

Tatyana Mamontova[1*], Konstantin Katkov[1], Natalia Kizilova[2] and Ali-Magomet Aybazov[1]

[1]North Caucasus Federal Agrarian Research Centre, 355004, Stavropol, Russian Federation
[2]Stavropol State Agrarian University, 355004, Stavropol, Russian Federation

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Key words
Ram, Semen, Seasonal variation, Daylight gradient

ABSTRACT

The present study was aimed at studying the influence of seasonal parameters (ambient temperature, atmospheric pressure, air humidity, duration and gradient of daylight hours) on the quantitative and qualitative parameters of sperm (volume, motility, concentration, survival) of rams bred in mid-latitude. Sperm samples were collected from 5 Dzhalginsky merino rams once a week for twelve calendar months using an artificial vagina. The lowest quality sperm was produced in the spring, high quality indicators were observed in the autumn period (semen volume: 0.96 ± 0.14 ml versus 1.42 ± 0.15 ml, $P < 0.05$; sperm concentration ($\times 10^9$ ml^{-1}): 2.66 ± 0.10 versus 3.61 ± 0.11, $P < 0.001$; total sperm ($\times 10^9$): 2.56 ± 0.06 versus 5.12 ± 0.09, $P < 0.001$; motility (1–10) 7.42 ± 0.55 versus 9.08 ± 0.57, $P < 0.05$; relative survival: 35.85 ± 2.94 h versus 67.4 ± 2.77 h, $P < 0.001$). The results revealed strong positive correlations between semen quality parameters ($P < 0.001$). The most significant negative correlation was found between semen volume and daylight hours ($r = -0.638$; $P < 0.001$). With a 95% probability, a curvilinear correlation was established between the length of daylight and the semen volume (*yv*= 0.31±015). In conclusion, it should be noted that the season of the year influences the quality of the rams sperm, with the value and direction of the daylight gradient having the greatest influence. It is with a negative gradient aimed at reducing the length of daylight that the highest rates of ram sperm are observed.

INTRODUCTION

An objective assessment of the reproductive qualities of male farm animals is based on a direct method based on insemination and the number of pregnancies that have occurred. However, it takes a long time. In some cases, a convenient and quick indirect method is used, based on laboratory assessment of quantitative and qualitative parameters of sperm, such as motility, concentration, morphology and viability of semen and other parameters (Morrell *et al*., 2017).

Long-term studies show that changes in the characteristics of these indicators are influenced by external factors, such as ambient temperature, humidity, pressure, feeding level and housing conditions (Bhakat, 2014; Brown, 1994; Ibrahim, 1997; Mislei *et al*., 2020), as well as internal, such as breed, age, collection frequency and ejaculate number (Folch, 1984; Leahy and Gadella, 2011; Turner, 2019).

The photoperiod affects the production of hormones and, as a result, the quality parameters of semen and the sexual behavior of males, which differ in the breeding season and the period of non-breeding (Leahy *et al*., 2010). The manifestation of these fluctuations is influenced by the genetic factor, i.e. the geographical location of the breed. For example, sheep raised in mid-latitudes > 40° are more seasonal (Ibrahim, 1997) than sheep from tropical and subtropical regions 10–30° (Chemineau *et al*., 1986). In the process of gametogenesis in sheep and rams, there are significant differences, both at the behavioral and endocrine levels. In rams, the seasonal reactions of extinction (inhibition) of reproductive function are less pronounced, while the process of spermatogenesis in rams occurs throughout the year. This can make it possible to obtain semen from rams, accumulate it throughout the year and use it for insemination of sheep with good results (Magomedov, 2007).

Sheep breeding in Russia is a traditional type of

* Corresponding author: mamontova.vniiok@gmail.com
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farming activity that allows you to receive significant profits and natural products for marketing. However, the key problem remains the clearly pronounced seasonality of domestic breeds, in which the reproductive use of sheep occurs only during the breeding season (August-November).

Recently, great interest has been shown in the promising breed of the Dzhalginsky merino, developed in 2013 on the maternal basis of the Stavropol breed and Australian rams from the Uardry plant. Australian merino species are of agricultural importance in many countries and are distinguished by their adaptability and resilience in various climates. They are also believed to be less dependent on seasonal factors (Killeen and Dawe, 1966; Knight *et al*., 1975). One of the important economic indicators of the productivity of sheep is the level of reproduction, which mainly depends on the quality of the semen of the breeding rams and the possibility of their use in the reproductive process during all seasons of the year. Previously, studies of the reproductive qualities of Dzhalginsky merino sheep were carried out only in the breeding season. Due to the use of the Australian merino gene pool in the breeding of the studied breed, it is logical to assume that the reproductive activity of the Dzhalginsky merino will show itself and change depending on the season of the year.

The purpose of this study was to obtain information on the possibility of reproductive use of the Dzhalginsky merino sheep of the Russian breed in different seasons of the year, as well as to determine the set of seasonal parameters that have the greatest impact on seasonal fluctuations in semen quality.

MATERIALS AND METHODS

Experimental design

The Animal Ethics Committee of the North Caucasus Federal Agrarian Research Centre has approved the procedures outlined in this document (No. 137, March 6, 2019).

To achieve the aims of our study, the following sperm parameters were analyzed: volume *V* (ml), sperm concentration *D* (× 10^9 ml^{-1}), total sperm/ejaculate *T* (× 10^9), semen progressive motility *M* (1–10), survival rate *S* (h). A posteriori analysis was carried out that determines the dependence of sperm quality parameters on climatic and seasonal factors (daylight length (min), air temperature (°C), atmospheric pressure (hPa), humidity (%)) registered by the local meteorological office.

Animals and breeding conditions

The studies were carried out in a sheep-breeding farm located in the Ipatovsky district of the Stavropol Territory at 45°43′ N. Climatological information for this area during the experimental year (2019–2020) is summarized in Table I.

Table I. Climatological data for the experimental year[a].

Season	Air temperature, ℃		Daylight length, min	Humidity, %	Atmospheric pressure, hPa
	Min.	Max.			
Autumn	- 10	+ 29	654.69	71.31	1004.84
Winter	- 9	+ 18	567.31	81.23	1002.08
Spring	- 1	+ 29	814.23	63.54	998.79
Summer	+ 12	+ 39	899.62	39.31	996.12

[a]Summer and autumn is a period of decreasing day length, winter and spring is a period of increasing day length.

The experiment involved five rams of the Dzhalginsky merino breed at the age of 2.5–3.5 years. The rams were selected based on the experience of their use in the previous breeding period. All animals were kept in the same conditions with the same ration with daily pasture grazing (Table II). A general plan was drawn up and treatments for deworming, disease prevention and hoof trimming were carried out.

Table II. Composition and nutritional value of rations for rams[1].

Parameter	Breeding period (live weight 99.4 kg)	Non-breeding period (live weight 108.5 kg)
Wild mixed hay	-	2.4 kg
Grass-legume hay	2.2 kg	-
Mixed feed	1.2 kg	0.7 kg
Fodder beets	1.5 kg	1.0 kg
Common salt	18 g	18 g
Premix (P 80-2)	10 g	10 g
The ration contains:		
EFU	2.87	2.43
Crude protein	410 g	4285 g
Digestible protein	285 g	191 g
Lysine	19.8 g	13.5 g
Methionine with cystine	13.4 g	11.5 g

[1]The ration is for one ram per day.

Recording of seasonal condition descriptors

Daylight (minutes) were calculated as the difference between the official sunrise and sunset times for each day of the experiment. Atmospheric pressure and humidity were recorded daily during the experiment by the local meteorological office. Average daily air temperature (°C) was measured daily. The air temperature was also measured at the time of semen collection. For each descriptor, the average values for the month and for the season of the year were calculated.

Semen collection

Semen were collected once a week in the morning using an artificial vagina (AV) (40–42 °C). An ovariectamized sheep, which was fixed in a special machine, was used as a dummy animal when obtaining semen. One sample of ejaculate was collected once daily, from each animal. Occasionally, the rams failed to mount or to ejaculate and so some ejaculates were not available. Therefore, in our calculations, we indicate the number of ejaculates studied. Each single instance of ejaculation was considered as an individual sample. A total of 243 samples were utilized and provide the data for this analysis. The study period consisted of four equal intervals: autumn (September-November), winter (December-February), spring (March-May), summer (June-August).

Semen evaluation

The volume of ejaculated semen was recorded with an accuracy of 0.1 ml immediately after collection using a glass graduated semen collector. The concentration was determined by direct counting of semen in a counting chamber, for which semen was pre-diluted with a 3 % sodium chloride solution (1:200) in an erythrocyte melanger (mixer). The number of cells was counted under a phase-contrast microscope at 400× magnification. Sperm concentration was calculated and expressed as 10^9/ml. Finally, the total sperm count per ejaculate was calculated by multiplying ejaculate volume times the sperm concentration. The wave motion was evaluated by preparing a wet nozzle (2 μl) of spermatozoa and examined under a phase-contrast microscope at 100× magnification equipped with a step warmer, and subjectively evaluated from 0 (no movement) to 10 (numerous fast waves) on a scale with steps equal to 1, according to the method adapted by Evans and Maxwell (1987).

Following evaluation of the freshly collected sample, which was then diluted in a ratio of 1:2 with a commercially available dilutent "AndroMed" (Mintube, Germany). Diluted in accordance with the manufacturers instructions. The diluted sperm was then allowed to acclimatise to room temperature for 30 min. Then the sperm was packed in sterile sealed tubes, which were placed in containers with water and stored in a domestic refrigerator at a temperature of 2-4℃. To determine the survival rate, diluted chilled sperm was evaluated by sperm motility for the first two days at 8 hourly intervals. Thereafter, evaluation was performed hourly until the complete death of all sperm cells.

Statistical analysis

The measured values of sperm parameters were pre-processed using the data analysis package of the MS Excel spreadsheet processor. At this stage, anomalous measurements were excluded and the average values were calculated. Further, the data were processed using the integrated mathematical package MATLAB 7 (version 7.0, The Math Works and Cleve Moler, USA). The analyzed data were subjected to one-way analysis of variance using the anova1 function built into MATLAB. The factor investigated here is the semen sampling season. The gradient function built into MATLAB was also used to calculate gradients. Full correlation analysis, plotting graphs, diagrams, gradient fields was implemented using programs developed by the authors in MATLAB. The characteristic of the correlation was assessed using the Chaddock scale.

RESULTS

Sperm quality

Table III shows the average values of the studied characteristics of sperm for 243 ejaculates. The season of the year influenced both the quantity and quality of sperm. Differences between seasons were found for *S* and *D*. For *V* and *M*, significant differences were found between autumn and spring. The lowest semen volume observed was 0.96ml in the spring compared to 1.41ml in the autumn.

Table III. Quality parameters of rams sperm[1].

Parameters[2]	Autumn (n = 65)[3]	Winter (n = 65)[3]	Spring (n = 53)[3]	Summer (n = 60)[3]
V (ml)	1.42±0.15[a]	1.22±0.11	0.96±0.14[a]	1.04±0.18
D (×10^9 ml^{-1})	3.61±0.11[ac]	3.18±0.08[a]	2.66±0.10[c]	2.85±0.09[c]
T (×10^9)	5.12±0.09[c]	3.86±0.05[c]	2.56±0.06[c]	2.97±0.08[c]
M (1 – 10)	9.08±0.57[a]	8.49±0.49	7.42±0.55[a]	8.13±0.61
S (h)	67.4±2.77[c]	44.06±1.83[c]	35.85±2.94[c]	40.07±3.44[c]

[a,b,c] Different lowercase superscripts indicate differences between seasons (a, $P < 0.05$; b $P < 0.01$; c, $P < 0.001$). [1]Values are means±SD; [2]V, volume; D, sperm concentration; T, total sperm/ejaculate; M, sperm progressive motility; S, relative survival. [3]The number of studied ejaculates

One of the objective indicators of the biological usefulness of sperm is their relative survival. In our studies, this indicator also showed its seasonality with the maximum value in the autumn (67.4 h) and the minimum in the spring (35.9 h). It should be noted that in our studies at 7–9 weeks of summer, one or two rams sometimes refused mating, so we could not get the planned amount of ejaculates, or they were of unsatisfactory quality.

Figure 1 shows the results of analysis of variance in the form of a range diagram. The season of semen sampling has a significant effect on all the studied parameters of semen. A small exception is sperm motility (Fig. 1c), the average values of which were equal in winter and summer.

The maximum values of the parameters are observed in autumn; a decrease in average values in winter; minimum values in spring and an increase in average values in summer. This general trend in sperm parameters allows us to hypothesize that their values are strongly correlated with each other.

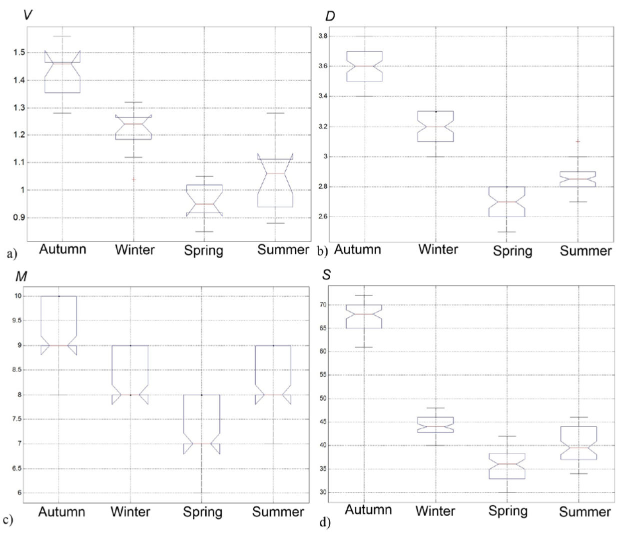

Fig. 1. Span diagram illustrating the results of variance analysis of a) semen volume, b) concentration, c) mobility and d) survival.

Table IV. Correlations between parameters of sperm quality (multivariate analysis).

Parameter	Concentration	Motility	Survival
Volume	0.879±0.03***	0.778±0.05***	0.870±0.03***
Concentration		0.826±0.04***	0.931±0.02***
Motility			0.808±0.05***
Survival			

* P < 0.05; ** P < 0.01; *** P < 0.001.

Correlations within sperm quality parameters

Table IV shows the values of the correlation coefficients, their errors and the characteristics of the correlation. All correlations were strong, especially the correlations between concentration and survival.

Correlations of sperm quality with climate

Table V shows the correlation between the parameters of sperm and the duration of daylight. There is a curvilinear correlation between the duration of daylight hours and the volume of semen. The measure of the linearity of the connection is equal to yv=0.31±0.15. The curvilinear correlation coefficient is equal to the value rn=0.945. The dependence of sperm volume on daylight hours is shown in Figure 2. Other parameters of sperm behave similarly to the volume.

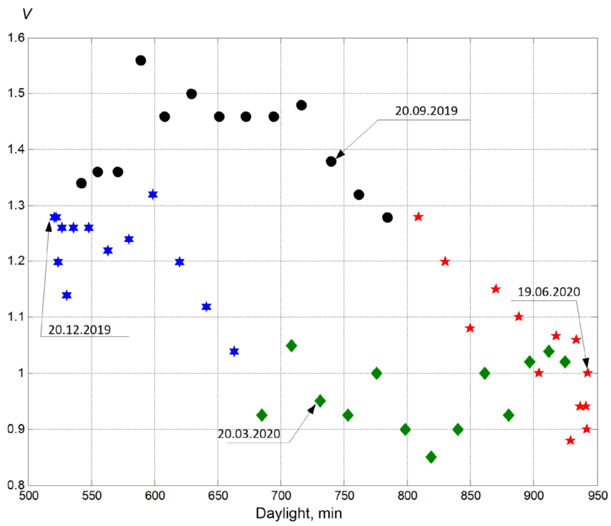

Fig. 2. The dependence of semen volume on the duration of daylight.
Autumn semen values are marked with ●. The values obtained in winter are shown by *. Spring measurements are shown by ♦, summer semen measurements by *. The arrows show measurements taken on days as close as possible to the days of the summer and winter solstices (June 19, 2020 and December 20, 2019), as well as to the days of the autumn and spring equinox (September 20, 2019, and March 20, 2020).

Regression analysis of semen parameters and seasonal factors

The linear regression coefficient of semen volume by daylight hoursours is $Rv=-0.00085 \pm 1.36 \cdot 10^{-8}$. Due to the high correlation of sperm parameters with each other (Table IV), it can be assumed that the data obtained on the basis of semen volume studies can also be extended to other parameters.

Dependence of sperm quality parameters on daylight gradient

The results of the research show that the length of daylight hours in days close to the autumn equinox is equal to the length of the day in days close to the spring equinox. At the same time (Fig. 2) the volume of semen collected in the autumn is significantly higher than the volume of semen collected in the spring. Analysis of the data in Figure 2 suggests that the dynamics of changes in the duration of daylight hours affects the parameters of sperm. This dynamics is described by the gradient of daylight (1)

$$G = \partial F / \partial t \ldots\ldots \quad (1)$$

Where F is the duration of daylight hours in minutes; t – measurement interval, week.

The gradient of daylight characterizes the speed and direction of change in the length of the day. It can have both positive and negative values. Figure 3 shows the relationship between this gradient and ram semen volume. Figure 3a shows change in the semen volume of rams depending on the week of semen collection. As mentioned above, the first sperm collection was carried out in autumn, September 6, 2019. During the first 13 weeks of collection, the autumn period lasted. As can be seen from Figure 3a, the maximum volume of semen obtained is precisely during this period, from the 1st to the 11th week. During this period, a negative gradient of daylight hours is observed. Daylight are decreasing (Fig. 3c), and the value of the gradient is negative and modulo greater than or equal to 20 min / week (Fig. 3b).

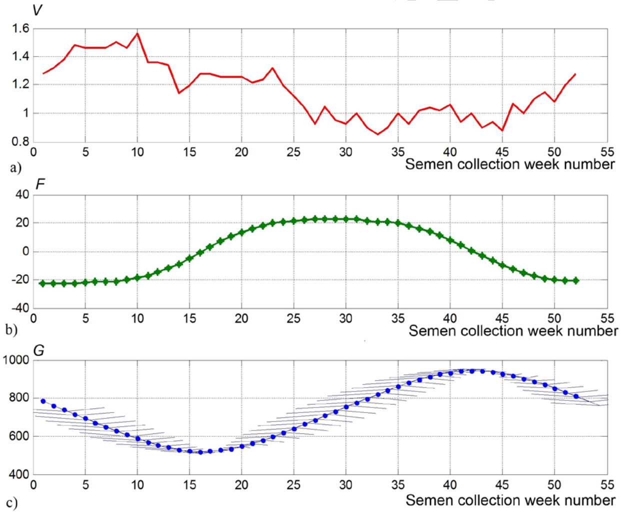

Fig. 3. The ratio of changes in the volume of semen in rams and the duration of daylight: a) semen volume versus collection week number; b) dependence of the gradient of daylight on the week number of sperm collection; c) graphic area gradient of daylight.

Further, with a negative gradient (weeks 11 to 15) and with a slight positive gradient (weeks 16 to 20), the semen volume remains quite high with insignificant variance (Fig. 3a). Then, with an increase in the positive gradient to a value of 20 min / week and higher (weeks 24 to 35), the volume of semen obtained from brood rams decreases sharply and reaches a minimum at the 33rd week of collection.

Semen volume continues to be low with positive values of the gradient of daylight hours. An increase in sperm volume is observed only with the transition of the gradient value through zero to the region of negative numbers. At the same time, a sharp increase in volume is noted when the gradient reaches negative values close to the value of –20 min/week. Figure 4 shows the dependence of the obtained semen volume on the gradient of daylight hours.

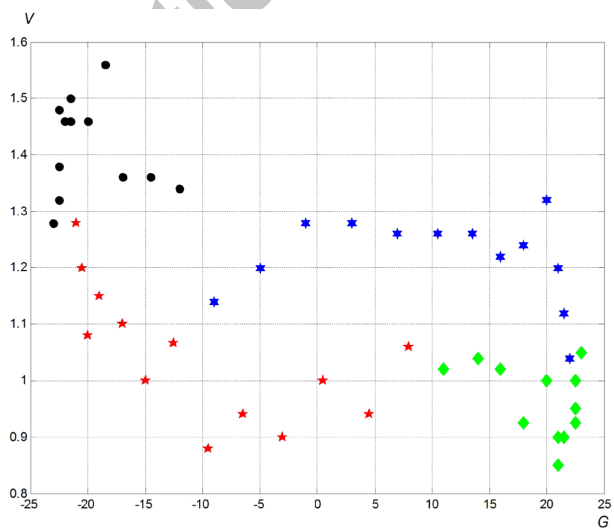

Fig. 4. Fig. 4. Dependence of semen volume on the gradient value of daylight.
For statistical details, see Figure 2.

Thus, it can be stated that both the value of the gradient and its direction influence the semen volume. It is with a negative gradient aimed at reducing the length of daylight hours that the greatest value of the semen volume obtained from brood rams will be observed.

The conclusions about the dependence of the semen volume of rams on the gradient of daylight hours can be extended to other parameters of sperm: concentration, motility, and survival.

Correlation between sperm parameters and gradients of seasonal factors

In the process of the work, similar studies were

Table V. Correlation coefficients between semen parameters and seasonal factors.

Semen parameters	Seasonal factors			
	Daylight length	Average daily temperature	Atmosphere pressure	Humidity
Volume	-0.638±0.08 ***	-0.236±0.13	0.323±0.12 *	0.372±0.12 **
Concentration	-0.615±0.09 ***	-0.278±0.13	0.404±0.12 **	0.357±0.12 **
Motility	-0.487±0.11***	-0.130±0.13	0.308±0.13 *	0.152±0.14
Survival	-0.426±0.11 **	-0.083±0.14	0.346±0.12 *	0.219±0.13

* P < 0.05; ** P < 0.01; *** P < 0.001.

Table VI. Coefficients of correlation between sperm parameters and gradients of seasonal factors.

Sperm parameters	Gradients of seasonal factors			
	Daylight gradient	Average daily temperature gradient	Atmospheric pressure gradient	Air humidity gradient
Volume	-0.608±0.09 ***	-0.260±0.13	0.073±0.14	0.196±0.13
Concentration	-0.598±0.09 ***	-0.277±0.13 *	0.041±0.14	0.095±0.14
Motility	-0.656±0.08 ***	-0.324±0.12 *	-0.051±0.14	0.147±0.14
Survival	-0.714±0.07 ***	-0.291±0.13 *	0.088±0.14	0.124±0.14

* P < 0.05; ** P < 0.01; *** P < 0.001.

carried out on the influence of gradients of average daily temperature, pressure and humidity on the parameters of sperm of stud rams. No clear dependence of sperm parameters on the gradients of these seasonal factors was found. The values presented in Table VI show that significant correlations for all sperm parameters are observed only with a gradient of daylight hours.

DISCUSSION

The seasonality of reproductive functions is a generally accepted fact for sheep raised in middle and high latitudes (Dacheux *et al*., 1986; Lindsay *et al*., 1984; Pelletier *et al*., 1989). In Russia, a study of the fertility of local rams in different seasons of the year has not been conducted before. Perhaps this is due to the lack of the need to use stud rams outside the breeding season, since local sheep have a pronounced sexual season, which once again confirms the theory that sheep breeds bred in temperate climates have a seasonal sexual cycle. The results of our research have shown that it is possible to obtain sperm from the Dzhalginsky merino bred in the middle latitude of the Russian Federation in all seasons of the year. At the same time, the influence of the season of the year on both the quantity and quality of sperm is noted, which is confirmed by the analysis of variance carried out. The highest indicators were observed in autumn, slightly decreased in winter, reached the lowest indicators in spring and gradually increased in summer. Similar studies have also shown an increase in the number and quality of ram sperm during the breeding season (Chella *et al*., 2017; Dacheux *et al*., 1986; Gundogan, 2006; Karagiannidis *et al*., 2000; Mandiki *et al*., 1998). At the same time, almost all average values of sperm parameters were within the normal range indicated by Mann and Lutwac-Mann (1981). Thus, the results obtained confirm the idea that the use of Australian merino in breeding the Russian breed Dzhalginsky merino can have a positive effect on the reproductive qualities of the new breed, namely its aseasonality in reproduction.

The proposed hypothesis about the strong correlation of sperm parameters with each other was confirmed in our studies. Similar studies on Chios and Frisian rams in Greece also showed a significant correlation between basic sperm parameters (Volume × total sperm / ejaculate = 0.855; Motile sperm × abnormal sperm (%) = −0.874) (Karagiannidis *et al*., 2000).

Correlation analysis between sperm parameters and seasonal factors showed the greatest correlation with daylight. This is agreed with research by Boland *et al.* (1985) and Perez C *et al.* (1997) on Merino sheep. Flores-Gil *et al*. (2020) also confirms that the seasonality of reproduction of small ruminants from temperate regions is mainly regulated by changes in daylight.

Another important conclusion of this study was that the parameters of sperm are influenced not by the length of daylight itself, but by its dynamics. It is the negative value of the gradient and its direction in the direction of decreasing daylight hours that has a greater effect on the increase in the values of sperm parameters. The results of the study confirm that, despite the differences in the maturation of germ cells and the expression of sexual behavior between sheep and rams, a decrease in daylight hours also affects the reproductive qualities of rams.

CONCLUSIONS

The present study has shown that five experimental Dzhalginsky merino rams bred in mid-latitude with their sexual exploitation throughout the year, full feeding and appropriate housing conditions can regularly ejaculate in all seasons. At the same time, the season affects the qualitative and quantitative parameters of sperm. The maximum values of the studied parameters of sperm are achieved in the period: the end of summer the beginning of winter, completely covering the autumn period. During the specified period of time, the highest negative values of the gradient of the duration of daylight hours are observed. In this case, the dynamics of changes in the duration of daylight hours plays a key role. From the obtained results, it can be assumed that, if conditions are met, rams of various breeds raised in Russia can be used to obtain and collect sperm of a sufficiently high quality throughout the year. To confirm this hypothesis, we will continue extensive studies of the influence of the season of the year on various parameters of the sperm of merino sheep bred in Russia.

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Statement of conflict of interest

The authors have declared no conflict of interest.

Research data for this article

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