The influence of blood, seminal plasma testosterone, growth hormone and cortisol levels on the sperm quality in merino rams

ABSTRACT

The research was conducted on 10 Merino rams to determine the effect of testosterone, growth hormone and cortisol levels on the sperm quality. Beginning in January, blood samples were taken from the jugular veins of rams every month, centrifuged for 20 min at 3000 rpm and obtained plasma. On the days the blood samples were taken, the semen samples which were collected with an artificial vagina, were divided between two eppendorf tubes, one of which was used for the determination of the semen characteristics, while the other was centrifuged to obtain seminal plasma. A significant relationship was detected between blood and seminal plasma levels of testosterone ($P<0.01$). No growth hormone (GH) on a measurable level was detected in the seminal plasma, and it was determined that plasma GH levels, just as testosterone levels began to increase with the approach of the reproductive season, and peaked at the start of the season. Plasma and seminal plasma cortisol levels, on the other hand, while increasing before the season, decreased significantly at the start of the season, and a remarkable correlation between plasma and seminal plasma levels was ascertained ($P<0.01$). An important negative relation which was observed between plasma GH levels and seminal plasma cortisol levels in Merino rams. It was found out that the hormones that were examined did not have a significant influence on sperm qualities, but there was an important negative relation only between the plasma GH levels and sperm volumes in the Merino ($P<0.01$). In conclusion, it may be stated that in rams, especially plasma testosterone and cortisol levels determine the plasma levels; both plasma and seminal plasma testosterone levels increase to a significant degree in the reproductive season. It can be said that there is no significant relationship between semen quality and plasma and seminal plasma hormone levels.

Keywords: Cortisol, Growth Hormone, Sperm, Testosterone.

INTRODUCTION

In all living beings, sexual activity and reproduction are healthy maintained under the effect of hormones and several other factors. Among the domestic livestock, sexual activity in sheep in particular follows a seasonal course, and after the months of August and September when the days are shorter, the hormone profiles of animals change and they enter the sexual cycle. As the days shorten, melatonin production in sheep stimulates the biosynthesis and release of gonadotropins from the anterior lobe of the pituitary gland, gonadotropins activate the ovaries and the sexual cycle begins (Boland et al., 1985; Hamidi et al., 2012). Despite the fact that rams do not display a precise seasonal cycle, producing semen throughout the year, it has been hypothesized that they experience changes in hormone profiles, testicular weight and diameter, and semen characteristics, and this has been proven with subsequent studies (Abecia et al., 2012; Belkadi et al., 2017).

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Research Article

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An important point in animals is how the relationship between plasma levels of hormones such as testosterone, growth hormone, cortisol, and seminal plasma levels, affects qualities of sperma. For example, Graves and Eiler (1979), found that exogenous cortisol administration in bulls increased cortisol concentrations in both the plasma and seminal plasma significantly, and that increasing the cortisol concentrations caused an increase in total sperm counts. The number of studies of rams addressing this issue is limited. In the present study, the plasma and seminal plasma concentrations of growth hormone, testosterone and cortisol were analyzed in rams every month for a period of one year, and annual variations in hormone concentrations, relationships between hormone concentrations and the effects of hormones on semen characteristics were determined.

MATERIAL and METHOD

Selection of animals

The study was carried out with healthy 10 Merino rams. All rams were barned in the Research Farm of Selcuk University, Faculty of Veterinary Medicine throughout the study period, and fed ad libitum.

Collection of blood and semen samples

Beginning in January, blood samples were taken from the jugular veins of rams every month, centrifuged for 20 min at 3000 rpm, and kept in the freezer at - 20 °C until the analyses were carried out. On the days the blood samples were taken, the semen samples which were collected with an artificial vagina, were divided between two eppendorf tubes, one of which was used for the determination of the semen characteristics, while the other was centrifuged to obtain seminal plasma, and kept in the freezer at -20 °C until the analyses were carried out.

Hormone analyses

Testosterone, cortisol and growth hormone concentrations were analyzed in the plasma and seminal plasma. The growth hormone analyses were carried out using the sheep growth hormone enzyme immunoassay (EIA) method developed by the Endocrinology Laboratory of Selçuk University Faculty of Veterinary Medicine, Department of Biochemistry (Serpek and Haliloğlu, 2000) while cortisol and testosterone concentrations were analyzed using DRG Cortisol EIA (EIA – 1887) and DRG Testosterone EIA (EIA – 1559) kits developed by DRG Instruments GmbH, Germany Division of DRG International, Inc.

Determination of semen characteristics

In the semen samples that were taken, semen volume, sperm motility, mass motility in the seminal plasma, semen concentration, host values, sperm counts, percentage of normal spermatozoa, percentage of spermatozoa with head defects and the percentage of spermatozoa with other defects were determined.

Mass Movement examination was carried out with 4× microscope lens in 1 drop fresh sperma and spermatozoon groups scored 0 - 5 intervals. The Mass Movement was evaluated done as follows; 0 (-): No movement. 1 point (+): There is very little mass movement. Ripple motion is not observed. 2 points (++): There is a low degree of mass movement and superficial ripple movement. 3 points (+++): Vivid ripple movement with a moderate mass movement. There is some blackening in places. 4 points (++++): Black areas are formed with a strong mass movement, strong wave movements andswirls are visible. 5 points (+++++): Dense black areas are formed with a very strong mass movement. Very strong wave movements and eddies are seen.

For motility examination, sperma was diluated with tris base extender solution to 100 $\times 10^6$ and 1 drop sample was dripped on slide and invastigated in 5 different zones with 20×

lens phase contrast microscope and rates were calculated in percentage value.

Sperm density was carried out with hemocytometric method by using thoma slide and spermatozoon was counted in 1 / 200 diluated sperm in 10 big square with 40× lens microscope. Hayem solution [sodium sulfate (5 grams) sodium citrate (1 gram) mercury chloride (0.5 grams) bidistilled water (200 ml)] was used to dilute the samples taken from native semen. Finally density was calculated with formula that given belove. [Number of spermatozoon / (big square volume × number of square × dilution rate)] × 1000.

Abnormal sperma exemination was carried out with liquid fixation method by using Hancock solution and 300 spermatozoon were observed in 1 drop sample obtained from 1 / 200 diluated sperm with 100× lens microscope and disorder rates were calculated in percentage value.

For HOST (hypo osmotic swelling test) thawed sperm and HOST solution mixture that incubated for 1 hr at 37 °C was examinated 100× lens microscope and counted 300 spermatozoons. Curl tailed ones were evaluated as live and strong.

Statistical analysis

The statistical analysis of the data was performed using the SPSS 21. Because of the abnormal distribution of data Mann – Whitney U Test was performed to determine the statistical significance. Correlation analyses was performed with Spearmen's method.

RESULTS

Hormone concentrations in blood plasma

The plasma testosterone, cortisol and growth hormone concentrations of rams, and variations in the concentrations by month, are presented in Table 1. The plasma testosterone concentrations, which were low in the spring months, started to increase gradually from August, and concentrations remained high until December. The plasma cortisol concentrations were low in the winter months, but increased in the spring, and it was observed that concentrations decreased significantly as the reproductive season began (Table 1). The mean plasma growth hormone concentrations, which were 7-8 ng/ml in January, started to decrease significantly in the Merino samples in June but bounced back significantly to 12-13 ng/ml in the in July. (Table 1).

Table 1. Plasma hormone levels in merino rams, ng/ml (n=10)

M	1	2	3	4	5	6	7	8	9	10	11	12
T	2.92 ± 0.89	3.19 ± 1.3	2.58 ± 1.42	3.99 ± 0.85	3.22 ± 0.95	2.51 ± 0.82	1.76 ± 0.51	6.35 ± 2.21	9.03 ± 2.34	9.09 ± 2.03	10.36 ± 1.96	6.67 ± 1.97
C	9.04 ± 1.97	16.72 ± 2.05	13.83 ± 3.9	36.27 ± 6.37	16.54 ± 4.25	21.4 ± 4.81	17.88 ± 4.99	26.54 ± 10.8	9.41 ± 2.6	8.13 ± 2.46	14.66 ± 2.52	13.2 ± 5.08
GH	8.01 ± 0.86	7.03 ± 0.94	8.23 ± 1.82	8.92 ± 0.9	6.52 ± 0.54	4.86 ± 0.45	13.43 ± 0.8	13.23 ± 0.81	15.33 ± 1.17	8.67 ± 0.74	5.66 ± 0.97	6.57 ± 0.39

(M: Months, T: Testosterone, C: Cortisol, GH: Growth Hormone)

Hormone concentrations in the seminal plasma

No growth hormone was detected at any measurable concentration in the seminal plasma. In Merino rams, the testosterone concentration in the seminal plasma, which was 8.01 ± 0.86 ng/ml in January, showed fluctuations, but the concentrations were maintained until June, after which they started to increase, reaching a peak in October (15.33 ± 1.17 ng/ml) (Table 2).

Table 2. Seminal plasma hormone levels in merino rams, ng/ml

M	1	2	3	4	5	6	7	8	9	10	11	12
T	8.01 ± 0.86	7.03 ± 0.94	8.23 ± 1.82	8.92 ± 0.9	6.52 ± 0.54	4.86 ± 0.45	13.43 ± 0.8	13.23 ± 0.81	15.33 ± 1.17	8.67 ± 0.74	5.66 ± 0.97	6.57 ± 0.39
C	9.11 ± 2.69	7.76 ± 2.01	7.37 ± 2.62	21.3 ± 9.18	8.91 ± 2.55	15.9 ± 5.49	9.49 ± 3.61	10.8 ± 5.09	1.78 ± 0.61	2.62 ± 0.58	5.27 ± 1.32	3.54 ± 1.16
n	10	10	10	10	9	10	9	7	8	7	5	6

(M: Months, T: Testosterone, C: Cortisol, n: Number of animals)

Despite the fact that the cortisol concentrations in the seminal plasma only showed a statistically significant bounce in April and June. The seminal plasma cortisol levels decreased significantly in September. (Table 2).

Semen characteristics

Among the semen characteristics, semen volume showed high monthly fluctuations in the 1-year period in Merino rams, while the lowest volumes were observed in July and the highest volumes were observed in December. The volumes noted in February, May, June and December were significantly higher ($P < 0.05$) than in the other months. (Table 3).

Table 3. Some semen charasteristics of merino rams.

M	1	2	3	4	5	6	7	8	9	10	11	12
SA (ml)	1.31 ± 0.16	1.72 ± 0.2	1.35 ± 0.15	1.26 ± 0.14	1.66 ± 0.11	1.64 ± 0.11	1.06 ± 0.18	1.2 ± 0.42	1.28 ± 0.13	1.31 ± 0.16	1.4 ± 0.26	1.92 ± 0.25
MM	2.0 ± 0.2	2.1 ± 0.2	2.3 ± 0.3	2.6 ± 0.3	2.8 ± 0.2	2.2 ± 0.3	2.6 ± 0.3	2.0 ± 0.5	2.3 ± 0.3	2.1 ± 0.3	2.6 ± 0.4	2.17 ± 0.4
SD ($\times 10^6$)	1334 ± 223.2	1860 ± 335.5	1505 ± 190.2	2280 ± 283	1850 ± 161.8	1995 ± 230.9	2189 ± 61.1	1600 ± 305.2	2425 ± 302.7	1644 ± 160.4	2030 ± 133.6	2600 ± 216.4
SM (%)	76.5 ± 2.36	77 ± 2.38	76.1 ± 3.05	81.0 ± 2.08	80.0 ± 2.35	75.5 ± 2.41	80.0 ± 1.67	78.3 ± 2.79	77.5 ± 2.99	73.9 ± 2.64	81.0 ± 5.51	79.17 ± 2.66
HV (%)	77.5 ± 1.64	77.5 ± 2.01	78.0 ± 2.26	81.0 ± 2.08	73.3 ± 3.73	67.0 ± 2.94	80.0 ± 1.67	80.0 ± 2.37	78.1 ± 2.83	76.2 ± 2.45	82.0 ± 1.23	79.17 ± 3.0
NSR (%)	77.2 ± 2.19	77.2 ± 1.14	76.0 ± 1.46	76.5 ± 2.25	76.4 ± 2.49	76.1 ± 1.89	78.9 ± 1.87	76.3 ± 2.81	74.88 ± 2.75	76.88 ± 1.68	76.6 ± 2.21	76.3 ± 2.40
HDR (%)	6.20 ± 2.62	7.10 ± 1.66	7.30 ± 1.42	5.20 ± 2.04	6.89 ± 3.26	5.80 ± 2.62	4.67 ± 2.55	8.33 ± 1.97	6.0 ± 2.27	6.88 ± 1.55	7.20 ± 1.64	6.67 ± 0.52
ODR (%)	16.60 ± 2.10	15.70 ± 1.53	16.70 ± 1.09	18.70 ± 1.68	16.67 ± 2.54	17.1 ± 2.04	16.89 ± 1.07	17.0 ± 2.61	19.38 ± 2.21	16.25 ± 1.17	16.2 ± 2.04	17.0 ± 2.27
n	10	10	10	10	9	10	9	7	8	7	5	6

(M: Months, SA: Semen Amount, MM: Mass Movement, SD: Sperma Density, SM: Sperma Motility, HV: Host Value, NSR: Normal Spermatozoon Rate, HDR: Head Disorder Rate, ODR: Other Disorders Rate, n: Number of animals)

The highest mass motility in sperma was observed in April and May (2.60 ± 0.30 and 2.80 ± 0.20), and for one year the values fluctuated between 2.00 ± 0.20 and 2.80 ± 0.20. No differences were detected in terms of the reproductive season or the seasons of the year (Table 3).

Sperm concentrations showed very high fluctuations in Merino rams throughout the year. Concentrations were low in the first

months of the year, but started to increase from April, with the highest concentrations observed in September and December, being significantly higher than the concentrations in January, March, August and October (P < 0.05) (Table 3). The highest sperm motility percentages were observed in April, May, July and November in Merino rams (81 ± 2.08, 80 ± 2,35, 80 ± 1,67 and 81 ± 5.51%, respectively) while the lowest percentage was observed in October (73.88 ± 2.64%) (Table 3). Host values showed a variation similar to sperm motility, with the lowest percentage observed in June (67.00 ± 2.94%) and the highest percentages in April, July, August and November (81 ± 2.08, 80 ± 1.67, 80 ± 2.37 and 82 ± 1.23% respectively), representing a statistically significant difference (P < 0.05) (Table 3). The mean percentage of the normal spermatozoa varied between 74.88 ± 2.75 and 78.89 ± 1.87 in Merino rams, although no significant difference was found between the monthly percentages over the year (Table 3). The mean percentage of spermatozoa with head defects varied between 4.67 ± 2.55 (July) and 8.33 ± 1.97 (August) in Merino rams, with no significant difference noted between the monthly percentages (Table 3). The ratio of spermatozoa with other defects varied between 15.70 ± 1.63 and 19.38 ± 2.21 throughout the year in Merino rams, with no statistically significant difference noted between the monthly percentages (Table 3).

Relationships between analyzed parameters

In Merino rams, a statistically significant negative correlation was found between the plasma concentrations of growth hormone and cortisol (P < 0.01) and, it was found that the same negative correlation continued with seminal plasma cortisol levels. (P<0,05). It has been determined that plasma testosterone levels and plasma cortisol levels significantly affect seminal plasma testosterone and cortisol levels and there is a significant positive relationship between them (P< 0.01). In the examination of the relationship between semen qualities and plasma and seminal plasma hormone levels, a statistically significant difference was determined only between plasma GH levels and semen volume (P<0.05). It was also found that plasma testosterone and cortisol concentrations significantly affected seminal plasma testosterone and cortisol concentrations, with a significant positive correlation (P<0.01). When the semen characteristics and relationships between the plasma and seminal plasma hormone concentrations were analyzed, a statistically significant difference was noted only between plasma growth hormone concentrations and semen volume (P<0.05) (Table 4).

Table 4. Relationships between parameters in merino rams

		Pl.Cor	Pl.GH	SpTes	SpCor	Vol	MM	Mot	Dens.	Host	Normal	Head D.	Other D.
Pl. Tes	r	0.174	0.039	0.325**	0.007	-0.067	-0.1	-0.047	0.085	0.001	-0.01	0.054	0.002
Pl. Cor	r		-.213*	-0.141	0.630**	-0.038	0.04	0.095	0.15	0.064	-0.174	0.07	0.171
Pl. GH	r			0.119	-0.253*	-0.325**	0.07	0.013	0.122	0.124	-0.052	-0.087	0.148
Sp.Tes	r				-0.113	0.021	0	0.029	0.182	-0.03	-0.038	0.035	0.025
Sp.Cor	r					-0,045	0.06	0,16	0.03	0.009	-0.088	0.07	0.053
Vol	r						0.16	0.146	0.004	-0.046	0.128	-0.087	-0.142
MM	r							0.530**	0.291**	0.397**	0.047	0.015	0.001
Mot	r								0.359**	0.316**	0.105	-0.006	-0.075
Dens	r									0.403**	-0.012	-0.068	0.006
Host	r										0.007	0.109	0.025
Normal	r											-0.512**	-0.875**
Head D.	r												0.184
Other D.	r												

(Plasma: Pl, Cor: Cortisol, Tes: Testosterone, GH, Growth Hormone Vol: Volume, MM: Mass Movement, Mot: Motility, Dens: Density, D: Disorder, r: Correlation)

DISCUSSION

In January, being the first month of the study, plasma testosterone concentrations were similar to Zamiri et al. (2010), who indicated a decrease in testosterone concentrations during winter months. As reported by researchers (Zamiri and Khodaei, 2005; Hamidi et al., 2012), testosterone concentrations show fluctuations in rams between the pre-reproductive and reproductive season. Palacios et al. (2016) reported that high plasma cortisol concentrations in sexual activity, when rams kept inside, separate from ewes. Ansari et al. (2017) observed that serum cortisol concentration in antelopes, higher in summer than winter in line with present study findings. The high concentrations of plasma testosterone levels, which were detected until October and November, returned to the concentrations noted in January due to the separation of the rams and ewes (Table 1).

Plasma growth hormone concentrations, which were 8.01 ± 0.86 ng/ml in Merino rams in January, decreased gradually during the spring months, similar to the findings reported in literature with a statistically significant decrease occurring in May (Table 1) and the concentrations started to increase in July with the increase in temperatures, as well as the increase in plasma testosterone concentrations associated with the stress of the beginning of the reproductive season (Bex et al., 1978; Bartlett et al., 1990; King et al., 2005; Kalra et al., 2008). The growth hormone concentrations were statistically significant until September when compared to other months, which was a finding supported by Hamidi et al. (2012) whose findings indicated that growth hormone and testosterone concentrations increased during the reproductive season. In the present study, testosterone concentrations in seminal plasma showed a seasonal variation similar to the figure observed by Casao et al. (2013) and Javed et al. (2000) findings.

Graves and Eiler (1979) reported that plasma and seminal plasma basal cortisol concentrations in bulls (3-4 µ/dl) increased 238 - fold in the plasma and 32 - fold in the seminal plasma 20 min after the administration of 500 mg exogenous cortisol. Ansari et al. (2017) investigated the effect of the summer and winter seasons on epididymal sperm quality in antelopes, and found that heat stress increased serum cortisol concentrations, but no significant variation in T3 and T4 concentrations. Maurya et al. (1990) found that the incorporation of low levels of concentrates (100 g/day) into the forage diets of Malpura rams with low body condition scores (2.5) between March and June caused a decrease in live weights, as well as in seminal plasma cortisol concentrations ($P<0.05$) when compared to rams with medium and high body condition scores, and while semen volume and sperm motility were affected negatively, sperm counts did not change. In the study, the variations in the seminal plasma cortisol concentrations of Merino rams by month were similar to the variations in the plasma cortisol concentrations, and this finding is in line with the findings of Graves and Eiler (1979), who suggested that exogenous cortisol administration in bulls increased cortisol concentrations in both the plasma and seminal plasma, suggesting that blood cortisol passes to the seminal plasma (Table 2).

İleri et al. (1998) reported that the average semen volume, which can vary according to age, season, nutrition, the technical ability of the person taking the sample, the management of and method used for taking the samples, the sampling frequency, the temperament of the ram and the body condition is around 1 ml (0.7 - 3.0 ml). According to the data from the study, the semen volumes taken of the Merino rams were within the normal range, but showed fluctuations, with higher volumes seen in the 2nd and 12th months than in the other months (Table 3). In an analysis of the relationship

between semen volume and hormone concentrations in the plasma and seminal plasma, a significant negative correlation was found only between the plasma growth hormone concentrations ($P<0.05$) (Table 4). Semen with a mass motility score (varying between 0 and 5 in adult rams (Rege et al., 2000; David et al., 2015) higher than 4 can be used for artificial insemination (Alvarez et al., 2012; David et al., 2015), Rege et al. (2000) reported that concentrations in 9 - month old Menz and Horro rams in Ethiopia were 1.92 ± 0.27. In the present study, concentrations were seen to vary between 2.00 ± 0.20 and 2.80 ± 0.20 over one year, with no significant variation noted between months (Table 3). The detected concentrations were lower than those reported by David et al. (2015) and Alvarez et al. (2012), but in line with the figures reported by Rege et al. (2000). This difference was considered to be related to the breed or to regional climatic variances. When the relationships between semen characteristics were analyzed (Table 4), a significant correlation was found between mass motility and sperm motility, semen concentration and host values ($P<0.01$), as could be expected. İleri et al. (1998) reported sperm concentrations of $1 – 4 \times 10^9$/ml in rams, and these concentrations were reported to vary according to the age of the bull (Maurya et al., 2010) and rams, and were affected also by nutritional status, season and melatonin, cortisol, growth hormone and testosterone concentrations in the blood (Bartlett et al., 1990; King et al., 2005; Kalra et al., 2008; Hamidi et al., 2012; Ansari et al., 2017). The lowest concentrations in Merino rams were found in January (1334 ± 223.2) and the highest concentrations in December (2600 ± 216.4 × 10^6/ml), with concentrations were found to be within the normal range throughout the year (Table 3). In Merino rams, sperm motility varied between 75.5% (June) and 81% (May and November) throughout the year, and it was observed that the monthly variations were not significant, with concentrations falling within the normal range determined for healthy animals (Benmoula et al., 2017) (Table 3). Camara et al. (2017) reported host values of 44.6 ± 3.4% shortly after diluting the semen samples of Santa Ines rams. Ömür and Çoyan (2014) found host values of 52 ± 2.6% in semen samples taken from Merino rams during the reproductive season and subjected to freezing and thawing. Akalın et al. (2015) identified host values of 57.0 ± 1.2% after freezing and thawing semen samples from Konya Merino rams. In the present study, the semen samples were not subjected to any processes, and so the host values were lowest in June (67 ± 2.94%), which is outside the reproductive season, and highest in November (82 ± 1.23%), which is within the reproductive season. The percentages were found to be significantly higher during the reproductive season when compared to the months outside the reproductive season (Table 3). Mickelsen et al. (1981) found that the ratio of normal spermatozoa in the semen samples of Suffolk and Lincoln rams, taken during the reproductive season using an electro - ejaculator was 92,8% for both breeds in October, and 77.0% for Suffolk rams and 84.7% for Lincoln rams in November, while Moore (1985) found that the ratio of normal spermatozoa in the semen samples of Romney rams, taken using an artificial vagina, was 79%, and 82% in samples taken using an electro - ejaculator In the present study, the ratio of normal spermatozoa was lowest in September (74.88 ± 2.75%) and highest in July (78.89 ± 1.87%), but the percentages followed a horizontal course. Although these ratios were lower than those reported by Mickelsen et al. (2000) and Moore (1985) in samples taken using an electro - ejaculator, our findings were in line with the ratios in the samples taken using an artificial vagina, as in our study (Table 3).

CONCLUSION

As a result, it could be suggested that testosterone concentrations in blood plasma of Merino rams fed ad libitum for one year increase significantly at the beginning of reproductive season, and are maintained until the end of the year; that testosterone concentrations in the seminal plasma of Merino rams followed a course similar to the testosterone concentrations in the plasma, with a statistically significant correlation ($P < 0.01$) between them; that plasma cortisol concentrations, which were low at the beginning of the year, increased gradually from May, when the weather started to get warmer, due to heat stress, and with the end of heat stress the concentrations started to decrease rapidly and were maintained until the end of the year; that a significant correlation between plasma and seminal plasma concentrations can be seen in Merino rams ($P < 0.01$); that plasma growth hormone concentrations follow a similar course to the reproductive cycle; that the concentrations, which increased significantly as the reproductive season approaches, start to decrease with the beginning of the fall; that no significant correlation exists between hormone concentrations, that there is no significant correlation between plasma and seminal plasma concentrations of hormones and semen characteristics; and that a significant correlation exists among sperm mass motility, semen concentration and host values, as well as a significant correlation between head defects and other defects, as expected. It is considered that the detection of blood testosterone and growth hormone concentrations during the reproductive season could be useful in determining the performance of rams.

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Ethical approval:

The necessary board of ethics approval was obtained from the Board of Ethics of the Selçuk University Faculty of Veterinary Medicine (No: 2009/50).

Conflict of interest: The authors declare that there is no conflict of interest.

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