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Relationship between Levels of Peripheral Blood Testosterone, Sexual Behavior, Scrotal Circumference and Seminal Parameters in Crossbred Rams

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ABSTRACT

***Background*:** Measurements of testosterone, scrotal circumference and libido have a great value as indicators of onset of puberty, total semen production, semen quality, control of spermatogenesis, testicular state, pathological conditions of testes and the potential sub-fertility or infertility. Therefore, the aim of this study was to determine relationship between levels of peripheral blood testosterone, sexual behavior, scrotal circumference and seminal parameters in crossbred rams.
***Materials, Methods & Results*:** Twelve crossbred and sexually mature rams consisting¬ of three Arkharmerino × Moghani (AM × MG), three Baluchi × Moghani (BL × MG), and three Ghezel × Baluchi (GH × BL) and three Ghezel × Arkharmerino (GH × AM) were used in this study. The scrotal circumference (SC) was measured at monthly intervals. Every two weeks the 12 crossbred rams were evaluated for the degree of libido using three ovariectomized ewes. Sexual behavior of crossbred rams was evaluated in terms of (1) reaction time for the first and second ejaculate (2) time taken for the first and second ejaculate (3) number of mounts for the first and second ejaculation. Ram semen was collected by artificial vagina and blood samples were obtained via jugular vein. Soon after the collection, semen characteristics and testosterone plasma were assayed. For estimation of the relationship between genetic group and other indices, Statistical analyses were performed by One-way ANOVA and Bivariate correlation coefficient was used to calculate the correlations between testosterone by spermatozoa parameters and libido in each genetic group; $P < 0.05$ considered significant. The results showed that there were no significant differences between crossbred rams in terms of semen characteristics except for spermatozoa progressive motility, semen volume and pH. The highest level of plasma testosterone was recorded in GH × BL rams (7.12 ± 1.87 ng/mL) and the lowest was for AM × MG genetic group (2.99 ± 1.90 ng/mL). However, any significant difference wasn't observed among the four genetic groups about plasma testosterone. At the present study the mean values of scrotal circumference of Arkharmerino × Moghani were higher than other genetic groups ($P < 0.05$). Also scrotal circumference showed a positive correlation with reaction time for the second ejaculation, semen pH and spermatozoa concentration ($P < 0.05$).
***Discussion*:** The sexual activity of males is influenced by test conditions and the methods applied in tests can vary considerably, even within the same experiment for example with regard to test duration and number of males and females. Among libido indices, only a significant correlation was observed between reaction time for second ejaculate and scrotal circumference. A non-significant correlation was observed between plasma testosterone, concentration, and semen traits. These differences between various reports could be due to the season of the study, breed, age of rams and many other environmental factors. It could be concluded that testosterone profiles is not a notable factor for estimating the quality of crossbred rams spermatogenesis and sexual activity. In addition to the above points, the males should be examined for a number of different tests have been used to assess the fertility or performance of crossbred rams, including scrotal measurement, semen examination, libido testing, hormonal profile and the other examinations.

Keywords: crossbred ram, libido, scrotal circumference, semen characteristics, testosterone.

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INTRODUCTION

Measurement of scrotal circumference (SC), libido and testosterone are an important aspect of breeding soundness examination of rams. Testosterone, the primary male hormone is responsible for male characteristics. Testosterone plays a central role in control of spermatogenesis, testicular state, ewe estrous manifestation and sexual behavior in rams. [20,27,31].

Libido is a useful measure of the reproductive efficiency which is influenced by breed, geographical location, season of the year and testicular size [32]. Moreover, positive associations between rams with high scores for sexual performance and ewe fertility have been reported [20]. Measurements of scrotal circumference have a great value as indicators of onset of puberty, total semen production, semen quality, pathological conditions of testes and the potential subfertility or infertility [19]. It was illustrated that among all testicular measurements, scrotal circumference has the highest within operator repeatability [24]. They also indicated that scrotal circumference provides a bigger scope for genetic improvement of fertility in ram lambs than do any of the semen characteristics [24].

A lot of reports exist about blood plasma testosterone and scrotal circumference with many semen parameters [28]. However, the correlation between blood plasma testosterone and scrotal circumference with blood testosterone, sexual behavior, scrotal circumference and seminal parameters in crossbred rams. Semen characteristics in crossbred rams with differing fertility has not hitherto been well studied. Therefore, the aim of this study was to determine relationship between levels of peripheral blood testosterone, sexual behavior, scrotal circumference and seminal parameters in crossbred rams.

MATERIALS AND METHODS

Animal and location

This trial was performed at the sheep breeding Station, located in Tabriz, Iran (38º 02' N and 46º 27' E). Twelve crossbred and fertile rams (3 Baluchi × Moghani; 3 Ghezel × Baluchi; 3 Ghezel × Arkharmerino; 3 Arkharmerino × Moghani) 3-6 years old with a live weight of 63-90 kg were used in this study. The animals were maintained under natural photoperiod and were housed separately from the ewes during the trial. Levels of nutrition remained equal and without changes as each ram was daily fed by using 65% hay (1.3 kg) and 35% commercial concentrate (400 g) consisting of 250 g barley, 36 g soya, 60 g corn, 64 g bran, 14 g supplementary. All rams had free access to salt stone and were sent for drinking fresh water twice or three times per day.

Scrotal circumference and live weight

Monthly measurement of scrotal circumference in all crossbred rams were recorded using a tape measure, and the combined testes diameter. The live weight of the rams was recorded at the beginning and end of the study.

Sexual activity test

Every two weeks the 12 crossbred rams were evaluated for the degree of libido using three ovariectomized ewes. Sexual behavior of crossbred rams was evaluated in terms of (1) reaction time for the first and second ejaculate (2) time taken for the first and second ejaculate (3) number of mounts for the first and second ejaculation.

Semen collection and evaluation

The 12 rams were divided into two groups randomly, and each group included six rams. Semen collection was followed for two days and every day from six rams. Ejaculate intervals for each ram was 15 days and it was regarded throughout the study. Therefore, two ejaculates from each ram were collected during a month. Overall 93 semen samples were collected from September 2010 to the December of 2011 (breeding season in this location). Semen collection was performed by an artificial vagina (AV) short form (temperature 42-43ºC) from the 12 fertile crossbred rams. Prior to collection, the prepuce was wiped and cleaned to prevent contamination of the semen. Semen was collected in the mornings, and quickly transported to the laboratory (at 37ºC), and placed in a water bath at 37ºC.

Semen volume was recorded using a graduated collecting glass (0.1^{mL} accuracy). Semen pH was measured by Pen form pH-meter[1] (with 0.1 grades). Spermatozoa concentration was determined using of a Thoma slide following haemocytometer counter method, as fresh semen was diluted by 0.1 M sodium citrate dehydrate 2.9% (pH = 6.7- 6.9) plus one drop of formalin (1: 400) at 400× magnification. Wave motion of fresh semenq was evaluated (at 100× magnification)

according to Evans and Maxwell [7], as it was made on the basis of a scale from 0 to 5 (0 = all spermatozoa are motionless, 5 = 90% or more of the spermatozoa are very rapidly moving waves). The assessment of the spermatozoa progressive motility was a visual scaled from 0 - 100% on the basis of suspended droplet slide and on a heated (37ºC) stage using phase-contrast optics (×400). For spermatozoa live/dead ratio, semen was stained with eosin-nigrosin stain followed by microscopic examination (×400). Spermatozoa with red head were counted as dead cells and the colorless ones as live spermatozoa. From several parts of the slide, about 300 spermatozoa were counted [7].

Measurement of plasma testosterone concentrations

Blood samples were collected from 12 crosses and it was repeated four times for each ram at 30-days intervals. Blood sampling was via jugular vein using heparinised venoject tubes and centrifuged (1500×g for 20 min). All twelve blood samples were collected after the first semen collection in each month. The plasma was decanted and stored at -20ºC until assay. Plasma concentrations of total testosterone were measured by enzyme-linked immunospecific assay (Testosterone Enzyme Immunoassay Test Kit)[2] according to the manufacture procedure. The reagents had been previously validated for sheep plasma using a parallelism test. Specificity of the assay was 100% and sensitivity was 0.2 - 16.0 ng/mL. The intra- and inter assay coefficients of variations were 6.5 and 9.3 %, respectively.

Data analysis

All statistical analyses were performed using SPSS (version 16.0). For estimation of the relationship between genetic group and other indices, One-way ANOVA and LSD post hoc were used. Bivariate correlation coefficient was used to calculate the correlations between testosterone by spermatozoa parameters and libido in each genetic group. The relationship between SCF ≥30 and < 30 with other indices were assumed by independent t-test. The global significance level for all statistical analyses was 0.05.

Table 1. Descriptive statistics of semen characteristics, plasma testosterone and scrotal circumference in Arkharmerino × Moghani (AM × MG), Baluchi × Moghani (BL × MG), Ghezel × Baluchi (GH × BL) and Ghezel × Arkharmerino (GH × AM) crossbred rams.

Genetic group		SV (mL)	WM (0-5)	SPM (%)	pH	Conc ($\times 10^9$)	SL (%)	TST (ng/mL)	SCF (cm)	LW (Kg)
GH × BL	n	24	24	24	24	24	24	12	12	6
	Mean	1.32	3.70	70.0	7.07	3.82	71.25	7.17	31.47	77.37
	Min	0.90	3.3	65.0	6.30	3.21	65.00	4.20	28.70	64.5
	Max	1.60	4.0	80.0	7.60	4.53	80.00	8.70	32.70	90.0
	SEM	0.12	0.15	5.37	0.40	0.44	5.06	1.88	1.57	13.26
AM × GH	n	23	23	23	23	23	23	12	12	6
	Mean	1.15	3.78	70.75	6.45	3.92	70.50	5.37	29.58	70.37
	Min	0.90	3.30	65.00	6.70	2.83	64.00	3.10	28.00	63.0
	Max	1.40	4.10	80.00	6.70	5.38	79.00	9.40	30.50	84.5
	SEM	0.12	0.15	5.37	0.40	0.44	5.06	1.88	1.57	11.34
AM × MG	n	24	24	24	24	24	24	12	12	6
	Mean	1.47	4.23	80.00	6.83	4.67	70.33	2.95	32.87	77.66
	Min	1.30	3.90	70.00	6.70	4.08	63.00	2.38	31.80	73.0
	Max	1.60	4.50	90.00	7.00	5.23	77.00	3.50	35.00	85.5
	SEM	0.12	0.15	5.37	0.40	0.44	5.06	1.88	1.57	6.82
BL × MG	n	22	22	22	22	22	22	12	12	6
	Mean	1.00	3.70	65.00	6.40	3.48	70.67	4.16	31.17	66.83
	Min	0.80	3.37	60.00	6.00	3.20	60.00	2.50	30.00	63.0
	Max	1.20	4.00	75.00	6.80	3.80	76.00	6.20	33.50	73.0
	SEM	0.15	0.22	6.01	0.47	0.50	7.13	1.88	1.57	5.39

SV=semen volume; WM=wave motion; SPM=spermatozoa progressive motility; Conc=spermatozoa concentration; SL=percentage of live spermatozoa; TST=testosterone; SCF=scrotal circumference; LW=live weight.

Table 2. Least square means (LSM) of Semen characteristics, scrotal circumference, plasma testosterone levels and live weight Arkharmerino × Moghani (AM × MG), Baluchi × Moghani (BL × MG), Ghezel × Baluchi (GH × BL) and Ghezel × Arkharmerino (GH × AM) crossbred rams.

Parameters	No.	Genetic group				C.V
		AM × MG	AM × GH	GH × BL	BL × MG	
Semen volume (mL)	93	1.48 ± 0.10[a]	1.14 ± 0.11[b]	1.33 ± 0.10[a]	1.02 ± 0.13[c]	25.85
Progressive motility (%)	93	81.1 ± 5.87[a]	71.85 ± 5.87[b]	71.07 ± 5.88[b]	66.11 ± 6.61[b]	3.40
Spermatozoa concentration ($\times 10^9$)	93	4.66 ± 0.42	3.89 ± 0.42	3.80 ± 0.41	3.49 ± 0.48	16.59
Wave motion (0-5)	93	4.25 ± 0.15	3.79 ± 0.14	3.69 ± 0.14	3.66 ± 0.20	10.29
Live spermatozoa (%)	93	70.33±5.02	70.50 ± 5.02	71.25 ± 5.01	70.67 ± 6.63	3.30
Semen pH	93	6.80±0.43[a]	6.46 ± 0.42[b]	7.03 ± 0.43[a]	6.38 ± 0.40[b]	9.80
Scrotal circumference (cm)	48	32.81±1.49[a]	29.57 ± 1.48[b]	31.47 ± 1.53[b]	31.20 ± 1.51[b]	4.01
Testosterone (ng/mL)	48	2.99±1.90	5.40 ± 1.81	7.12 ± 1.87	4.11 ± 1.87	27.77
Live weight (kg)	24	77.54±6.87	71.02 ± 11.21	78.12 ± 13.54	66.80 ± 5.11	4.30

[a,b,c.]Values in the rows with different superscripts are significantly different between the genetic groups ($P < 0.05$).Values in the rows within each parameter without superscripts did not differ significantly between the genetic groups ($P < 0.05$). C.V=coefficient of variation.

Table 3. Least square means ± SEM for libido parameters in Arkharmerino × Moghani (AM × MG), Baluchi × Moghani (BL × MG), Ghezel × Baluchi (GH × BL) and Ghezel × Arkharmerino (GH × AM) crossbred rams.

Libido Parameters	BL × MG	GH × BL	AM × GH	AM × MG	C.V
Reaction time for first ejaculation (s)	13.0 ± 4.0	18 ± 10.0	18.0 ± 3.0	12.0 ± 6.0	15.71
Time taken for first ejaculation (s)	30.0 ± 9.0	3.2±4.0	16.0 ± 7.0	10.0±5.0	16.90
No. of mount for first ejaculation	6.0±1.01[a]	1.2±0.65[b]	2.5 ± 0.85[b]	1.6±0.39[b]	30.0
Reaction time for second ejaculation (s)	3.63±0.68	1.34±0.51	1.79 ± 0.57	4.1±0.70	28.8
Time taken for second ejaculation (s)	23.33±14.37	84.0±51.0	34.0 ± 17.0	42.0±40.0	12.05
No. of mount for second ejaculation	7.0±1.81	3.66±1.76	4.0 ± 1.9	6.5±4.0	29.0

[a,b]Values in the rows with different superscripts are significantly different between the genetic groups ($P < 0.05$).Values in the rows within each parameter without superscripts did not differ significantly between the genetic groups ($P < 0.05$). C.V=coefficient of variation.

Table 4. Correlation coefficients between semen characteristics and plasma testosterone and scrotal circumference in crossbred rams.

Variables	LS	WM	SPM	Conc	pH	SV	SC
Testosterone (ng/mL)	0.374	0.275	0.118	0.02	- 0.132	0.05	0.09
Scrotal circumference (cm)	- 0.51*	0.083	0.159	0.739*	0.567	0.393	1

LS=Live spermatozoa; MO=wave motion; SPM =spermatozoa progressive motility; Conc=spermatozoa concentration; SV=semen volume; SC=Scrotal circumference. *Significance at $P < 0.05$.

Table 5. Correlation coefficients between libido parameters and plasma testosterone and scrotal circumference in crossbred rams.

Parameter	RT1	RT1E1	NOM1	RT2	RT2E2	NOM2
Scrotal circumference (cm)	0.256	-0.102	-0.181	- 0.622*	0.066	-0.117
Testosterone (ng/mL)	-0.125	-0.496	-0.177	-0.469	0.239	0.142

RT1=reaction time for first ejaculation; RT1E1=Time taken for first ejaculation; NOM1=Number of mount for first ejaculation; NOM2=Number of mount for second ejaculation; RT2=Reaction time for second ejaculation; RT2E2=Time taken for second ejaculation.*Significance at $P < 0.05$.

RESULTS

Means value, standard error, minimum and maximum of semen characteristics, blood testosterone levels, scrotal circumference and live weight in four crossbred rams have been shown in Table 1. Least square means (LSM) and coefficient of variation (CV) of semen characteristics, blood testosterone, scrotal circumference and live weight in four crossbred rams are presented in Table 2. There were no significant differences between crossbred rams in terms of semen traits except for spermatozoa progressive motility, semen volume and pH ($P < 0.05$). Highest and lowest mean values of spermatozoa motility and semen volume were recorded in AM × MG and BL × MG genetic groups, respectively ($P < 0.05$). The highest level of plasma testosterone was recorded in GH × BL rams (7.12 ± 1.87 ng/mL) and the lowest was for AM×MG genetic group (2.99 ± 1.90 ng/mL). However, any significant difference wasn't observed among the four genetic groups about plasma testosterone. At the present study the mean values of scrotal circumference of Arkharmerino × Moghani were higher than other genetic groups ($P < 0.05$).

The mean values (± SEM) of sexual behavior have been shown in Table 3. There were no significant differences among the four crossbred rams in terms of libido parameters except for the number of mount for the first ejaculation ($P < 0.05$). As it was higher in BL × MG rams than other genetic groups (NOM1= 6.0 ± 1.01, $P < 0.05$).

The correlation coefficients for plasma testosterone and scrotal circumference with semen characteristics and each others are presented in Table 4. The results of Table 4 showed that peripheral blood testosterone is not correlated with semen characteristics. It is also noticed that scrotal circumference correlated with spermatozoa concentration (r = 0.739, $P < 0.05$) and percentage of live spermatozoa (r = - 0.51, $P < 0.05$).

The correlation coefficient for plasma testosterone and scrotal circumference (SC) with sexual behavior are presented in Table 5. About SC, a significant correlation was only observed between scrotal circumference and reaction time for the second ejaculation (r = - 0.622, $P < 0.05$).

DISCUSSION

As reported previously, there is a wide range of ram semen characteristics, including ejaculate volume (0.60 - 1.6), sperm concentration (2.6-5.5 × 10^9 sperm/mL), and percentage of live or motile sperm (60 - 90%) [8,30]. In our study, the mean values of semen volume ranged between 0.8 to 1.6 mL. Predominantly, this variety in semen characteristics was observed for the other semen traits. These findings imply that there are many affecting factors in spermatogenesis process including genetics, environmental conditions and nutrition. However, these values are in agreement with data on several sheep breeds in the temperate climates e.g. the Moghani rams [33], Ghezel and Mehraban breeds [32], and Persian Karakul rams in Iran [12]. Testosterone is involved in several male reproductive processes [26]. The plasma testosterone levels in this study are coincides with many authors who also studied testosterone profiles of ram serum [8]. The highest levels of plasma testosterone were recorded in Ghezel × Baluchi rams. In the current study there is no correlation between circulating levels of testosterone and the level of sexual activity. The results obtained in this study showed that libido in the crossbred rams are not directly related to plasma levels of testosterone. This is in agreement with other work, which reported that there is no correlation between plasma testosterone and libido [11]. Moreover, most previous studies in other species have found no differences in circulating testosterone concentration between sexually active and inactive males [9]. While, Perkins and Roselli [21] in accordance with many evidences illustrated that a metabolite of testosterone (Estradiol) is responsible for maintenance of sexual behavior in rams. The reduction in reaction time together with the increase in testosterone concentration observed in high rams during short days, in comparison with low rate animals, suggests that testosterone concentration might be related to the sexual activity of the male [3]. Elevated testosterone concentrations have also been found in high compared with low sexual performance rams in response to female exposure [20]. In our results, a non-significant correlation was observed between plasma testosterone concentration, and semen traits, while they reported a significant correlation between testosterone with semen volume, sperm motility and sperm concentration in rams [15,25]. They also reported that a high correlation between semen volume and testosterone profile in male camels [6]. These differences between various reports could be due to the season of the study, breed, age of rams and many other environmental factors.

Scrotal circumference has been widely used in predicting the reproductive capacity of male domestic animals [4,23]. Also scrotal circumference, a high heritable trait, is considered as an excellent index of sperm production in the ram. In this study no significant different were observed among genetic groups. The variation in scrotal circumference within temperate climates throughout the year seems to be due mainly to changes by photoperiod, level of nutrition and daily body growth [12]. Among semen characteristics, a significant correlation was observed between the percentage of live spermatozoa and spermatozoa concentration with scrotal circumference. These results was confirmed by other experiment [10,24]. In other studies, it has been reported that scrotal circumference has a significant positive relationship with semen volume and spermatozoa concentration in bulls [1,13,17].

Genetics plays an important role in determining libido, but there are many environmental factors affecting its expression. Moreover, the sexual activity of males is influenced by test conditions and the methods applied in tests can vary considerably, even within the same experiment for example with regard to test duration [16] and number of males and females. Consequently, there is a need for the development of a predictive standardized test for estimating libido. Among libido indices, only a significant correlation was observed between reaction time for second ejaculate and scrotal circumference. The results of this study are in agreement with other reports [3,22]. They concluded that there was little evidence of a direct relationship between libido and scrotal circumference. In generally, notwithstanding a significant difference in some of seminal traits and in the scrotal circumference between the genetic groups but it was not pervasive for the other reproductive traits.

CONCLUSIONS

It could be concluded that among these traits, testosterone profiles is not a notable factor for estimating the quality of ram spermatogenesis and sexual activity. In addition to the above points, the males should be examined for a number of different tests have been used to assess the fertility or performance of rams, including scrotal measurement, semen examination, libido testing, hormonal profile and the other examinations.

SOURCES AND MANUFACTURERS

[1]Pen pH-meter, model 8685, AZ Instrument, Taiwan.

[2]Medix Biotech Inc., CA, USA.

***Declaration of interest*.** The authors report no conflicts of interest. The authors are responsible for the content and writing of the paper.

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