

SIMULTANEOUS STUDY OF SOME OF MALE BREEDING SOUNDNESS INDICES AND SEXUAL URGE ON THE CROSSBREED RAMS

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ABSTRACT

The aim of this trial was investigation of the relationship between seminal parameters, sexual urge (SU) and some of body measures (BMs) in five ArkharMerino×Ghezel (AM×GH) and five Ghezel×Baluchi (GH×BL) rams during 5 month. The semen samples were evaluated for semen volume (SV), total sperm/ejaculate (TSE), spermatozoa concentration (SC), color, wave motion, spermatozoa progressive motility, percentage of live and abnormal spermatozoa, pH and metabolic activity of spermatozoa (MBRT). SU of the rams was measured by two indices including reaction time (RT) and refractory period (RP). BMs of the rams consisted of body weight (BWT), body length (BL), hip width (HW) and height at withers (HTW), which were recorded in monthly intervals. No significant differences were found between the two hybrid groups in any traits except for SU indices. RT only showed a significant correlation with SV and pH ($r = -0.14$ and $r = -0.17$, $P < 0.05$ respectively). RP showed a significant correlation with semen traits except for SV, TSE, pH, semen color. A significant correlation was revealed between the all BMs except for BWT with HTW. Semen quantity characteristics had a significant correlation with HTW, HW and BWT. RP showed a negative correlation with BMs. These results suggest that BMs can be used to predict the SU of the rams and also they will confirm the necessity of synchronized selection for the breeding soundness indices in the herd.

Key words: crossbreed ram body measurements; sexual urge; semen characteristics

INTRODUCTION

Reproduction is one of the most important factors for the economics of livestock production (Chenoweth, 1994; Makarechian *et al.*, 1985). Evaluation of reproductive ability of rams is an integral part of management programs of sheep flocks. The objective of a breeding soundness examination (BSE) in rams is to evaluate and classify their breeding ability. Hence, evaluation of male fertility prior to breeding is one of paramount factors to achieve breeding success (Ford *et al.*, 2009). The potential fertility of breeding males can be evaluated in the field by assessment of mating ability, testicular and physical examination and semen quality evaluation (Hoflack *et al.*, 2006). Semen evaluation has been used as an index of ram fertility especially in

those used in AI programs. Strongly sexual urge or libido of rams influences overall flock fertility (Matos and Thomas, 1991).

Differences in sexual behavior among rams have been recognized since long ago (Hafez, 1951) and positive associations between rams with high scores for sexual performance and ewe fertility have been reported (Mattner *et al.*, 1971; Perkins *et al.*, 1992). Study of relevance between fertility and quality of sexual desire can be useful for selection purposes and also for obtain an optimize fertility in the herd. Many studies indicated that sexual urge is an important factor affecting male fertility and there are some evidences that it is strongly influenced by genetic factors e.g. breed or genetic group (Ologun *et al.*, 1981; Chenoweth, 1983). Quirino *et al.* (2004) reported that direct selection for libido would

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be effective and it would lead to desirable correlated response in body weight, physical and morphological characteristics of spermatozoa and undesirable correlated response for scrotal circumference. In contrast, Galina *et al.*, (2007) showed that libido is neither related to semen quality nor to scrotal circumference, so that it is possible to obtain an excellent semen sample in bulls with low libido. This incoherence in results between the probers may be caused by various methods of libido testing (Landaeta-Hernandez *et al.*, 2002; Landaeta-Hernandez *et al.*, 2001; Bertram *et al.*, 2002). Therefore, there is a need for a standard libido testing in all breeding and commercial flocks to remove the rams with poor performance before than serving with female. Seminal physicochemical characteristics of these genetic groups have been well studied previously (Asadpour *et al.*, 2012a; Asadpour *et al.*, 2012b; Moghaddam *et al.*, 2012a; Moghaddam *et al.*, 2012b). However, there is low data of some aspects of the reproductive characteristics of ArkharMerino×Ghezel and Ghezel×Baluchi genetic group. The present study designed to determine the relationship between semen characteristics, sexual urge and body conformation traits. Therefore, the data from the crosses were studied according phenotypic correlation between these traits.

MATERIAL AND METHODS

Animals and management

This project was performed using 5 AM×GH and 5 GH×BL rams (3-5 years old) and via a female teaser (from Oct 2011 to Feb 2012). The males were trained to mounting and serving with anoestrus ewe with quiet temperament. The location for performing this study was in suburb of Tabriz, Iran (38° 02' N, 46° 27' E). During 15 days the rams were trained (in peak of breeding season) to semen collection by artificial vagina (AV) by the presence of the operator and in the mating pen (210 cm length, 60 cm width, 120 cm height). All the examinations were done by the same technician. The rams separated from the herd were housed in a large cover shelter with an open precinct in order to walk freely. All of the rams were kept under natural photoperiod. Any of rams were not able to seeing mounting and serving of other rams. Levels of nutrition remained equal and without changes as each ram's diet daily consisted of 20 % concentrate (75 % barley, 25 % corn, soya, bran, supplement and lime) and 80 % alfalfa hay. Also, all the rams had free access to salty stones and fresh water twice or three times a day. Hoof trimming, shearing, crutching, dipping, disease prevention and other general management were checked during the study.

Assessment of body measurements (BMs)

Height at wither (HTW) was measured vertically from thoracic vertebrae to the ground using a metal ruler. Body length (BL) was measured from the sternum to the aitch bone. Hip width (HW) was measured using a plastic measuring tape. BWT, BL, HW and HTW were recorded in monthly intervals.

Estimation of sexual urge (SU)

Two traits reaction time (RT) and refractory period (RP) were used for assessment of sexual urge (SU) of the rams. Simultaneous with semen collection the SU indices were evaluated at five-day intervals. The rams were reared under similar conditions from birth until the examination period. The testing of SU is based on the time taken by a particular ram to react to a sexual stimulus ewe. A camera was used for recording time to the SU indices. Each ram that did not mount the stimulus ewe within 5 minutes was considered inactive. The reactions are included by two criteria: a) Reaction time; measured as the amount of time between first contact with the teaser ewe and first false mount with the penis erected (Hoflack *et al.*, 2006). b) Refractory period; measured as the time taken between first ejaculate till the second false mount (Prado *et al.*, 2002). Each ram was allowed to mount with the stimulus ewe and following the time was recorded for the RT and then the RP.

Semen evaluation

Concurrent with video recording for the ram's sexual activity, the ram semen samples were collected. Ejaculates of rams were collected in the intervals of five days and it was constant throughout the study. Artificial vagina (AV) with internal temperature maintained at about 40 - 42°C was used for semen collection. Collecting glass was warmed at 37°C before the operation and it was maintained at this temperature until processed. A ewe with quiet temperament was used for mounting by the rams. Immediately after ejaculation the fresh semen samples were transferred to the laboratory (keeping out of direct sun light) and evaluated. SV (semen volume) was recorded using a graduated collecting glass (0.1cc accuracy). Semen pH was measured by the Pen form pH-meter (with 0.1 grades, model 8685, AZ Instrument, Taiwan). SC (spermatozoa concentration) was determined by use of a Thoma chamber following haemocytometer counter method. The fresh semen was diluted using 0.1 M sodium citrate dehydrate 2.9 % (pH = 6.7 - 6.9) plus one drop of formalin (1:400) at 400×magnification. TSE (total sperm/ejaculate) was then calculated (volume×density). Wave motion of fresh semen was evaluated (100 × magnification) according to Evans and Maxwell (1987). The assessment of the spermatozoa progressive motility was done using a visual scale from 0 to 100 % on the basis of suspended droplet slide and on a heated (37°C)

stage using phase-contrast optics ($\times 400$). Suspended droplet slide showed individual spermatozoa with more lucidity. For spermatozoa morphology and spermatozoa live/dead ratio, semen was stained with eosin-nigrosin stain and examined microscopically ($\times 400$). About 300 spermatozoa were counted from several parts of the slide. Metabolic activity of spermatozoa was measured using the Methylene Blue Reduction Time (MBRT). It was estimated by use of the method adopted by Herman and Madden (1953). Semen index was calculated according to Talebi *et al.*, (2009).

Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (SAS, 1996). There were a few outliers on some of the traits (SV, SC, sperm abnormality, MBRT and SU). Therefore, to reduce the effect of sampling error, we have removed the outlier data. The Proc Mixed procedure of SAS was used for analysis of the repeated measurement data. The mean values were compared using a Tukey' test. Pearson correlation coefficient was calculated to evaluate the relationship between the traits. The mean values were considered to be statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The minimum, maximum and mean \pm SE of seminal characteristics of AM \times GH and GH \times BL rams are presented in Table 1. AM \times GH rams showed best semen quality than the other genotype but it was not significant ($P > 0.05$). According to the descriptive statistics, AM \times GH genetic group had a higher time scores (equivalent with the lower libido) than GH \times BL rams in case of the SU traits (Table 2). The large range for all traits indicated the wide variation between individual rams. An inconspicuous and non-significant dominance of BMs ($P > 0.05$) was observed in the mean values of AM \times GH rams than GH \times BL genetic group (Table 2). The rams with high SU presented the highest live spermatozoa, motility, spermatozoa metabolic activity, SC and the fewest spermatozoa abnormalities. However, these relationships were ranged from - 0.13 to 0.24 and were not significant (Table 3). Small and negative correlation was observed between RT with SV and pH (varying from 0.14 - 0.17, $P < 0.05$). RP did not show a significant correlation with TSE, semen pH, color and volume ($P > 0.05$). Correlation coefficient between the SU indices (RT and RP) demonstrated that, the rams with fewer RT had a shorter RP ($r = 0.13$, $P = 0.04$). Thus, reaction time could be a factor for estimating refractory period of the rams. A highly significant correlation was revealed between BMs e.g. BWT and BL ($r = 0.54$), HW and HTW ($r = 0.835$) and HW with BL ($r = 0.49$), indicating high

level of association between these variables (Table 4). As it is shown in table 4 a high and significant correlation coefficients between HTW and HP vs. RP were observed ($r = - 0.47$). Data of semen evaluation as a determining factor for breeding soundness examination did not indicate any high and clear correlation with body sizes except for some of semen quantity traits e.g. TSE with HTW and HP ($r = 0.39$ and 0.31 respectively), SV with HTW and HW ($r = 0.36$ and 0.30 respectively) and also SC with BWT ($r = 0.29$, $P < 0.05$).

Many researchers emphasized that genetics plays an important role in determining sexual urge and it has a clear effect on sexual urge (libido) and inherent fertility differences between individual males (Ologun *et al.*, 1981; Chenoweth, 1997; Petherick, 2005). These studies show that in *Bos indicus* and *Bos taurus*, crossbred bulls generally exhibited higher libido scores in pen-tests than did their parental purebreds, providing further evidence of genetic influence on libido (Chenoweth and Osborne, 1965). Contrary to the results of Ford *et al.*, (2009) who did not observe significant difference between Boer and Kiko bucks in terms of SU indices ($P > 0.05$), in our work it was found that GH \times BL rams were better compared to the other genotype. The non-significant difference between the two genetic groups (in body weight and body length) was in agreement with results of Lavvaf *et al.*, (2012). In our study SU was found to be useful in semen quality estimating. These findings also coincide with the results of Quirino *et al.*, (2004) who used scoring system from 0 (no sexual interest) to 10 (two services followed by sexual interest, including mounts, mounting attempts or further services) for the assessment of sexual urge. Deen (2008) revealed that there is a high correlation between, copulation time and semen volume in camels ($r = 0.957$). The results of Wiggins *et al.*, (1953) showed exists a significant correlation between some of libido criteria (including number of ejaculates per trial, ejaculate time for first, second and third mating) and percentage of ewes lambing.

Wiggins *et al.*, (1953) reported that significant correlation was revealed between semen volume ($r = 0.062$, $P < 0.05$), estimated motility count ($r = 0.077$, $P < 0.01$), percentage of normal sperm ($r = 0.432$, $P < 0.01$), percentage of abnormal heads ($r = - 0.35$, $P < 0.01$) and percentage of ewes lambing. These findings indicated that the sexual urge indices are correlated with fertility and also the fertility parameters have a relatively correlation with some semen characteristics. This simultaneous trend between SU and physical semen characteristics in our study is in agreement with findings of Barkawi *et al.*, (2006). Anzar *et al.*, (1993) after study on 44 buffalo bulls reported that semen production was correlated with sexual behavior urge only in the fair and poor categories of buffalo bulls ($r = 0.84$, $P < 0.005$). Galal

Table 1: Range of seminal measurements of Ghezel×Baluchi and ArkharMerino×Ghezel rams

Genetic groups		AM × GH			GH × BL		
Semen parameters	N	Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max
Semen volume (ml)	145	1.12 ± 0.18	0.45	2.00	1.17 ± 0.25	0.48	2.20
Wave motion (0-5)	143	4.05 ± 0.18	2	5	3.82 ± 0.24	2	5
Progressive motility (%)	145	69.60 ± 4.21	50	90	67.75 ± 3.83	45	85
Semen color (0-5)	145	3.61 ± 0.41	2	5	3.55 ± 0.45	2	5
Total sperm output (×10 ⁹)	144	4.275 ± 0.73	1.654	19.55	4.616 ± 1.21	1.506	21.6
Sperm density (×10 ⁹)	145	3.623 ± 0.39	1.950	5.56	3.45 ± 0.44	1.85	5.42
Live sperm (%)	145	73.52 ± 3.42	50	90	72.91 ± 3.46	54	90
Abnormal sperm (%)	143	10.50 ± 1.53	4	28	11.30 ± 1.72	4	29
Semen index (×10 ⁹)	145	21133 ± 3923	4088	48876	20460 ± 3659	1040	64255
Semen pH	143	6.45 ± 0.27	5.8	7.1	6.69 ± 0.35	5.9	7.7
MBRT (s)	143	107.47 ± 7.07	55	190	119.16 ± 8.4	64	281

Means within each row within each factor without letters did not differ significantly from each other.

Table 2: Range of sexual behavior urge and body feature traits of Ghezel×Baluchi and ArkharMerino×Ghezel rams

Genetic groups		AM × GH			GH × BL		
SU parameters	N	Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max
Reaction Time (s)	144	24.45 ± 7.51 ^a	3	110	11.76 ± 7.02 ^b	2	48
Refractory Period (s)	144	234.47 ± 109.1 ^a	42	983	79.01 ± 108.5 ^b	20	305
Body Measurements	N	Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max
Body weight (kg)	50	69.79 ± 5.74	49.8	90	74.37 ± 5.74	53.2	92
Body length (cm)	50	77.78 ± 3.45	70	83	74.07 ± 3.73	68	80
Height at withers (cm)	50	71.30 ± 4.74	63.5	87	69.64 ± 4.72	64.5	77
Hip width (cm)	50	18.78 ± 1.93	15	25	18.00 ± 1.79	16	21

^{a,b} – significant difference at P<0.05

Table 3: Correlation coefficient (r) between sexual behaviour urge and seminal traits in ArkharMerino×Ghezel and Ghezel×Baluchi rams

r	SV	WM	PM	SL	SAB	MBRT	pH	TSE	Conc	Color	RT	RP
RP	-0.06	-0.24	-0.22	-0.20	0.22	0.19	-0.12	-0.02	-0.13	-0.11	0.13	1
P value	0.76	0.002	0.009	0.001	0.008	0.002	0.07	0.686	0.04	0.10	0.04	1
RT	-0.14	-0.02	-0.01	-0.004	0.01	0.005	-0.17	0.01	-0.05	-0.05	1	0.13
P value	0.03	0.72	0.85	0.94	0.83	0.96	0.01	0.84	0.44	0.47	1	0.04

SV = semen volume, WM = wave motion, PM = progressive motility, TSE = total sperm per ejaculate, Conc = sperm concentration, SL = Percentage of live spermatozoa, SAB = Percentage of abnormal spermatozoa, MBRT = methylene blue reduction time, RP = refractory period, RT = reaction time

et al., (1978) in their seasonal study on Merino, Ossimi and their crosses stated that relationship between semen quality and libido is not clear across breeding groups. It is not surprising that the findings on the relationship between measures of libido and fertility are inconspicuous, with some workers reporting positive correlations (Lunstra, 1984, 1986; Crichton and Lishman, 1988) and others, contradictory or negative (Christensen *et al.*, 1982; Boyd *et al.*, 1989; Bertram *et al.*, 2002; Holroyd *et al.*, 2002). The high and significant correlation among BMs and BWT will provide a valuable data for early selection of the crossbred rams in genetic improvement schemes. Due to the strong correlation between hip width and body length, these criteria (HP and BL) could be used for prediction of the ram body weight. These results are in agreement with results of Keith *et al.*, (2009). Maksimovic *et al.* (2012) in their study reported that body mass of three crossbred rams (Wurtemberg, Il-de-France and Pirot Pramenka) has a significant correlation with their body length ($r = 0.58$, $P < 0.01$). Also they stated that HTW did not have a significant correlation with the ram body mass. In the other study expressed that many Belgian Blue bulls with poor semen quality were failed in breeding soundness evaluations (Hoflack *et al.*, 2006). Hassan *et al.*, (2009) reported there are

a significant correlation between body weight and SV, SC and sperm motility ($r = 0.568$, 0.664 , 0.494 respectively). Fields *et al.*, (1979) reported a non-significant correlation between BWT with SV, sperm motility and SC and these results are in agreement with our work except for SC. Previously was also reported a positive correlation between sperm production and body condition score (Ikhatua and Olayiwole, 1982). Okere *et al.*, (2011) indicated that semen production is fairly independent of most body conformation traits. A positive correlation between hip width and height at withers with semen quantity characteristics ($r = 0.27$ to 0.39), indirectly indicate that the rams with bigger HW and HTW may have more semen output. Overall in the present study the correlations between seminal traits and body measurements were quite low. Unlike the results of Ford *et al.*, (2009), in our research SU scores and especially refractory period were correlated to the body size traits ($P < 0.05$). Refractory period could be defined as a period of time during which testis are incapable of repeating another ejaculation. Among two libido traits, RP showed more correlations with the other traits than RT and probably this trait of sexual urge (RP) could be an appropriate clue for male libido estimating. This discrepancy in the libido results of

Table 4: Correlation coefficient (r) between body conformation traits with seminal and sexual urge traits in the both genetic groups

Traits	r	Body weight	Height at withers	Hip width	Body length
Body conformation traits	Body weight	1	0.21	0.44**	0.54**
	Height at withers	0.21	1	0.835**	0.36**
	Hip width	0.44**	0.835**	1	0.49**
	Body length	0.54**	0.36**	0.49**	1
Sexual urge traits	Reaction time	- 0.40**	- 0.19	- 0.27*	- 0.22
	Refractory period	- 0.22*	- 0.47**	- 0.47**	- 0.37**
Seminal Traits	Semen volume	0.03	0.36**	0.30*	0.02
	Wave motion	0.22	0.20	0.18	0.002
	Progressive motility	0.17	0.21	0.21	0.01
	Live sperm	0.15	0.21	0.21	0.05
	Abnormal sperm	0.17	- 0.22	- 0.22	- 0.05
	MBRT	0.29*	- 0.22	- 0.22	0.05
	pH	0.16	- 0.08	- 0.13	- 0.03
	Total sperm/ejaculate	0.11	0.39**	0.31*	0.03
Sperm concentration	0.22*	0.27*	0.23	- 0.08	
Semen color	0.17	0.22	0.19	0.07	

** $P < 0.01$, * $P < 0.05$, ns Non-significant. MBRT: methylene blue reduction time.

different probers may be caused by various methods used for testing libido such as the latency (refractory period) for males to copulate, or reaction time (Chenoweth, 1981; Landaeta-Hernandez *et al.*, 2001), counts and durations of interest, such as sniffing at the vulva and time spent with females (Bertram *et al.*, 2002), the number of mounts and/or serves during a set period of time (Landaeta-Hernandez *et al.*, 2001; Bertram *et al.*, 2002) and scores assigned according to various combinations of these measures (Blockey, 1981; Chenoweth, 1981; Landaeta-Hernandez *et al.*, 2001). Therefore, there is a need for the development of a predictive standardized test for estimating sexual urge of males. Overall the interpretation and comparison of the results of these researches will be very difficult.

CONCLUSION

There is a paucity of data on breeding soundness evaluations in ArkharMerino×Ghezel and Ghezel×Baluchi rams. Therefore, this trial compared some of breeding soundness indices (BMs, semen evaluations), SU and their relationship with each other. Striking correlation between semen characteristics and RP in the crosses confirms the fact that probably this parameter of SU is an adequate index for libido testing. Nevertheless, ambiguities and inconsistency in results of the researchers made a commitment for numerous investigations in these fields. Generally, our results indicated that measurements of external body dimensions, body weight, sperm output characteristics and sexual urge can accurately guide the assessment of the reproductive performance of the crossbred rams.

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