This study was conducted on 10 crossbred rams consisted of 5 ArkharMerino×Moghani (AM×MG) and 5 Baluchi×Moghani (BL×MG), to analyze correlation between semen characteristics and libido activity. The semen samples were evaluated for semen volume, total sperm per ejaculate (TSE), spermatozoa concentration (SC), color, wave motion, spermatozoa progressive motility, percentage of live and abnormal spermatozoa, pH, rate of metabolic activity of spermatozoa and semen index. The libido of rams was measured by two indices including reaction time (RT) and refractory period (RP). While RT only had a negative and significant correlation with semen volume (r=-0.15, P=0.04), but RP had a significant correlation with seminal traits except for TSE. Moreover, libido test scores between two genetic groups did not have a significant difference (P<0.05). Considering the observed significant correlation between RP and semen characteristics, we suggest that a reliable monitoring with the aim of detecting rams of poor semen quality and libido seems worth making an effort for the future of the herd. In summary, the reported correlation between libido and seminal traits will confirm the importance of simultaneous selection for both libido and semen quality in the herd. Finally, the importance of performing an individual evaluation of semen and libido for each male and for using a ram in breeding programs is highlighted, due to the existence of differences among rams as well as a low correlation between semen characteristics and libido indices.
Assessment of libido

According to Youngquist and ThriftFall (2007), the physical examination of the rams was conducted before the beginning of the study. The libido test was assessed in five-day intervals for five months and it was synchronized with semen collection. The rams were maintained under similar conditions from birth to the examination period. The testing of sexual urge was based on the time taken by a particular ram to react to a sexual stimulus ewe. A camera was used to record time to the libido indicators. Each ram that did not mount the stimulus ewe within 5 min was considered inactive. The reactions are considered by two criterions:

i) Reaction time; measured as the amount of time between the first contact with the teaser ewe and the first false mount with the penis erected (Hoflack et al., 2006).

ii) Refractory period; measured as the time taken between first ejaculate till the second false mount for second serving (Prado et al. 2002).

Each ram was allowed to mount with the stimulus ewe and following the time was recorded for the reaction time and then the refractory period.

Semen collection and evaluation

Semen collection was performed according to Karagiannidis et al. (2000). Briefly, we used an ovariectomized female teaser with quiet temperament for mounting by rams. Semen collection was according to Evans and Maxwell (1987). Concurrent with time recording for test of libido, semen was collected from rams. Ejaculate intervals for each ram was five days and it was constant throughout the study. The short form artificial vagina (internal temperature was 40°C to 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. Immediately after ejaculation, the fresh semen samples were transferred to the lab (maintained at 37°C and was kept out of direct sun light) and then were evaluated. Semen volume was recorded using a graduated collecting glass (0.1 cc accuracy). Semen pH was measured by Pen form pH-meter (with 0.1 grades, model 8685, AZ Instrument, Taiwan). Spermatozoa concentration was determined by the 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. The total number of spermatozoa per 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 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 (400×). For spermatozoa morphology and spermatozoa live/dead ratio, semen was stained with eosin-nigrosin stain and examined microscopically (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 (semen volume×sperm concentration/mL ×live sperm %×progressive motility %) was calculated, as an indicator for estimating semen quality.

Statistical analysis

All statistical analysis was performed using the Statistical Analysis System (SAS, 1996). The MIXED procedure of SAS was used for analysis of the repeated measurement data. The global significance level for all statistical analysis was 0.05. Means were compared to Turkey Test. Pearson correlation coefficient was calculated to evaluate the relationship between quality and quantity of semen characteristics with reaction time and refractory period.

Results and discussion

Means±SE and range of seminal measurements and libido score of ArkharMerino×Moghani and Baluchi×Moghani rams have been presented in Table 2 and Table 3, respectively. The large range for all traits indicated the wide variation between individual rams. Although, RT of BL×MG rams and RP of AM×MG rams showed relatively better values, these values did not differ significantly (P>0.05) between two genetic groups (Table 3). These results are in agreement with the reports of Shackell et al. (1977) that comparing different breeds of ram (Romney and Cheviot) did not find significant differences in the libido characteristics. Galal et al. (1978) reported that libido and semen quality of the rams were not affected by breed except for spermatozoa abnormality and time to first mount. Reaction time only had a negative and significant correlation with semen volume (r=-0.15, P=0.04). Unlike the results of Galina et al. (2007) that promulgated, libido is not related to semen quality, in our study refractory

Table 1. Climate data of the Khalatpoushan Research Station (October 2010 until September 2011). The research was performed from November to March.

|                | Minimum | Maximum | Minimum | Maximum | Average |
|----------------|---------|---------|---------|---------|---------|
|                |          |         |         |         |         |
| Air temperature, °C |         |         |         |         |         |
| October        | 7.6     | 25.1    | 26.9    | 77.5    | 11.3    |
| November       | 0.23    | 16.7    | 32.5    | 71.1    | 10.2    |
| December       | -4.08   | 12.4    | 34.7    | 67.9    | 9.6     |
| January        | -7.93   | 3.65    | 54.26   | 84.06   | 9.9     |
| February       | -7.85   | 4.2     | 51.33   | 85.1    | 10.9    |
| March          | -2.32   | 8.51    | 48.5    | 81.75   | 12      |
| April          | 2.64    | 16.06   | 25.03   | 67      | 13.3    |
| May            | 6.83    | 19.45   | 36.16   | 80.93   | 14.3    |
| June           | 11.51   | 28.03   | 23      | 78.54   | 14.8    |
| July           | 15.75   | 32.61   | 22.8    | 57.87   | 14.6    |
| August         | 16.83   | 33.61   | 15.29   | 56.51   | 13.7    |
| September      | 12.16   | 28.22   | 16.74   | 74.16   | 12.5    |
period (as one of the libido indices) showed a significant correlation with all seminal traits [except for total sperm per ejaculate (TSE)].

The results of this study, the significant and weak correlation ($r=0.15-0.32$) appeared between refractory period with semen characteristics and between reaction time and ejaculate volume ($r=-0.15$, $P=0.039$). Therefore in this study libido was found to be useful in assessing the ram’s semen quality. These findings also coincide with the results of Quirino et al. (2004) that used of scoring system from 0 (no sexual interest) to 10 (two services followed by sexual interest, including mounts, mounting attempts or further services) for assaying sexual activity. Deen (2008) stated that there is a high correlation between, copulation time and semen volume in camels ($r=0.957$). In the study by Wiggins et al. (1953) it was showed that among fertility parameters that were measured (percentage of ewes lambing, percentage of live lambs, percentage of lambs weaned) a significant correlation was only observed between libido criteria (number of ejaculates per trial, ejaculate time for first, second and third mating) and percentage of ewes lambing.

Wiggins et al. (1953) also shown a significant correlation between semen volume, estimated motility count, percentage of normal sperm and percentage of abnormal heads with percentage of ewes lambing ($r=0.062$, $r=0.077$, $r=0.432$, $r=0.35$; $P<0.05$). These findings imply that the libido indices are correlated with fertility and also the fertility parameters have a relative correlation with some of semen characteristics. Studies show that in Bos indicus and Bos taurus, crossbreed bulls generally exhibited higher libido scores in pen-tests than did their parental purebreds, providing further evidence for genetic influence on libido (Chenoweth and Osborne, 1965).

### Table 2. Means ±SE and range of seminal measurements of ArkharMerino×Moghani and Baluchi×Moghani rams.

| Semen parameters | ArkharMerino×Moghani | Baluchi×Moghani |
|------------------|----------------------|-----------------|
|                  | n        | Mean ±SE | Min | Max | Mean ±SE | Min | Max |
| Semen volume, mL | 45       | 1.26±0.20 | 0.67 | 1.90 | 0.97±0.20 | 0.52 | 1.40 |
| Wave motion, range 0-5 | 45        | 4.09±0.18 | 2.00 | 5.00 | 3.82±0.20 | 2.00 | 5.00 |
| Progressive motility, % | 45       | 73.59±3.88 | 50.00 | 90.00 | 67.72±3.78 | 50.00 | 85.00 |
| Semen color, range 0-5 | 45       | 3.58±0.46 | 1.00 | 5.00 | 3.77±0.45 | 2.00 | 5.00 |
| Total sperm output, ×10^9 | 45       | 4.565±0.80 | 1.785 | 30.55 | 3.55±0.81 | 1.616 | 18.99 |
| Sperm concentration, ×10^9 | 45       | 3.55±0.35 | 1.960 | 5.81 | 3.80±0.34 | 2.55 | 5.88 |
| Live spermatozoa, % | 45       | 74.57±3.57 | 56.00 | 94.00 | 68.91±3.59 | 54.00 | 90.00 |
| Abnormal spermatozoa, % | 45       | 10.55±1.71 | 3.00 | 21.00 | 13.02±1.72 | 4.00 | 25.00 |
| Semen pH | 45       | 6.57±0.25 | 5.90 | 7.20 | 6.54±0.25 | 5.90 | 7.30 |
| MBRT, sec | 45       | 111.70±7.07 | 45.00 | 210.00 | 119.39±7.0 | 65.00 | 200.00 |

**Note**: MBRT, methylene blue reduction time. *a* Means in the row of each parameter with different superscripts differ significantly ($P<0.05$). Means within each row within each factor without letters did not differ significantly from each other ($P>0.05$).

### Table 3. Means ±SE and range of libido score of ArkharMerino×Moghani and Baluchi×Moghani rams.

| Libido parameters | ArkharMerino×Moghani | Baluchi×Moghani |
|-------------------|----------------------|-----------------|
|                   | n        | Mean ±SE | Min | Max | Mean ±SE | Min | Max |
| Reaction time, sec | 143       | 23.75±7.63 | 3  | 150 | 18.18±7.46 | 3  | 70 |
| Refractory period, sec | 142       | 233.34±110.6 | 20 | 975 | 258.02±110.9 | 53 | 865 |

*Means within each factor with the same letters did not differ significantly from each other; otherwise, they differ significantly at the level of 0.05.*

### Table 4. Correlation coefficient ($r$) between libido scores and seminal traits of ArkharMerino×Moghani and Baluchi×Moghani rams.

| r     | SV   | WM   | PM   | SL   | SAB  | MBRT | pH   | TSE  | Conc | Color | SI   | RT   |
|-------|------|------|------|------|------|------|------|------|------|-------|------|------|
| RP    | 0.042| 0.001| 0.049| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001 | 0.001| 0.001|
| P value | 0.04 | 0.049| 0.011| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001 | 0.001| 0.001|
| RT    | 0.040| 0.055| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001 | 0.001| 0.001|
| P value | 0.00 | 0.001| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001| 0.001 | 0.001| 0.001|

SV, semen volume; WM, wave motion; PM, spermatozoa progressive motility; SL, percentage of live spermatozoa; SAB, percentage of abnormal spermatozoa; MBRT, methylene blue reduction time; TSE, total spermatozoa per ejaculate; Conc, concentration; SI, semen index; RT, reaction time; RP, refractory period.
Differences in libido scores were also observed between breeding lines and sires-within-lines in young bulls of British breeds (Perry, 1990), and sire strongly influenced serving capacity in young Angus bulls (Ologun et al., 1981). Anzar et al. (1993), in a study on forty-four buffalo bulls determined that semen production was correlated with sexual behavior in only the fair and poor categories of buffalo bulls (r=0.84, P<0.005). In the study that was performed for libido examination of Belgian Blue and Holstein Friesian bulls by Hoflack et al. (2006), it was expressed that regarding the breeding soundness evaluations (BSE) trait semen evaluation, significantly more Belgian Blue bulls failed the BSE as a result of poor sperm quality compared to Holstein Friesian bulls, and the average breed scores for progressive motility and for sperm morphology both indicated a relevant difference in the advantage of the Holstein Friesian breed. Whilst Galal et al. (1978) with seasonal studying on Merino, Ossimi and their crosses stated that relationship between semen quality and libido is not clear across breeding groups. This incoherence in results of different probing may be due to various methods of testing libido, such as the latency 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 specified period of time (Landaeta-Hernandez et al., 2001; Bertram et al., 2002), and scores assigned according to various combinations of these measures (Chenoweth, 1981; Landaeta-Hernandez et al. 2001). On the other hand, sexual activity of males is influenced by the test conditions and the methods applied in tests can vary considerably, even within the same experiment. Therefore, there is a need for the development of a predictive standardized test for estimating sex drive. Yet, many different methods and indices have been introduced for the evaluation of sexual urge of animals. Overall, the interpretation of the results is very difficult. Unlike males, the intensity of female libido is generally assessed through the expression of oestrous and associated with behavioral patterns such as soliciting, mounting, and standing to be mounted, together with the appearance of the vulva and vaginal mucus (Galina and Arthur, 1999; Galina et al. 1996; Landaeta-Hernandez et al. 2002). Significant correlation between semen characteristics and refractory period (time between the first ejaculate to the second false mount for second serving) confirm the fact that probably this parameter of libido is an adequate index for testing sexual activity. Notwithstanding, the ambiguities and inconsistency of these results made a commitment for numerous investigations in these fields.

Conclusions

The results of semen characteristics and libido scores of ArkharMerino×Moghani and Baluchi×Moghani rams showed a remarkable correlation between seminal traits and refractory period. Likewise, the genetic groups did not vary in the expression of libido levels.

In our study it was concluded that the relationship between the libido traits (predominantly refractory period) and seminal characteristics would be a useful index for the selection of the males for genetic improvement in ram breeding schemes.

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