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Photoperiod as a Factor for Studying Fluctuations of Seminal Traits during Breeding and Non-Breeding Season

Research Article

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ABSTRACT

The main purpose of this study was to evaluate the influence of the photoperiod on the seminal traits of crossbreed wool-producing rams throughout on year period. For the effect of photoperiod two periods were considered: decreasing daylight length (summer and autumn) and increasing daylight length (winter and spring). For the study 5 Baluchi × Moghani (BL×MG) and 5 Arkharmerino × Moghani (AM×MG) rams were used. Semen collection started from first of October 2010 to end of September 2011. After a training period of 2 weeks semen ejaculates were evaluated for volume, total sperm count per ejaculate (TSE), spermatozoa concentration, semen color, wave motion, percentage of progressive motility, percentage of live and abnormal spermatozoa, semen pH, methylene blue reduction time (MBRT) and semen index (semen volume × spermatozoa concentration/mL × live spermatozoa % × progressive motility %). Analysis of the yearlong data showed that semen samples with the best quality were collected in September to November ($P<0.05$). Significant seasonal variations of semen traits were observed for all of seminal traits except for progressive motility, percentage of live spermatozoa and MBRT. Yet, no statistical differences were found between the two genetic groups ($P>0.05$). Although there were significant seasonal changes in semen characteristics of the crosses, the fresh semen showed adequate quality to be used for artificial or natural insemination all around the year. Photoperiod was found to influence semen production in two genetic groups at 38 ˚02' N, 46 ˚27' E at an altitude of 1567 m above sea level of Iran. However, these effects should are not detrimental to the use of rams for breeding purposes throughout the year.

KEY WORDS photoperiod, ram, seasonal variation, sheep crossbred, spermatozoa.

INTRODUCTION

Sheep production is a traditional economic activity that is used for meat and milk production in Iran. One of the most important factors for economical development of sheep industry is lambing in throughout year. Seasonal breeding is a limiting factor in this species. Therefore, it was decided to evaluate the quality of semen in breeding and non-breeding seasons before using artificial insemination in sheep.

The Arkharmerino is a breed of sheep obtained by cross-breeding between wild Arkhar rams with ewes of the Novocaucasian Merino, Précoce and Rambouillet breeds (Ernst and Dmitriev, 2007). These two genetic groups were developed through targeting the improvement of local breeds (Baluchi and Moghani) for wool traits. There is no published information on the reproductive traits of Baluchi × Moghani or on Arkharmerino × Moghani genetic groups. In addition to gathering information on reference values for semen characteristics, evaluation of the effects of photoperiod on ram semen characteristics at this latitude was another pursuit for this study, along with the evaluation of putative differences on semen traits between the two ge-

netic groups, to identify the most suitable line for breeding purposes. Unlike most domestic livestock species, sheep are widely known for their marked seasonality of breeding activity linked to annual cycle of daily photoperiod (Rosa and Bryant, 2003). The annual cycle of daily photoperiod has been identified as the major determinant for this phenomenon in sheep (Rosa and Bryant, 2003). Understanding of their fertility quality in the non-breeding season will be helpful for developing sheep industry. In contrast to ewes and most horse mares, that become anovulatory outside the breeding season, stallions and rams are not azoospermic during the non-breeding season despite a significant reduction in sperm production or quality (Aurich *et al.* 1996). Also, overall physiological and behavioral sexual variations are also less pronounced in rams than ewe (Rosa and Bryant, 2003). Therefore, yearlong comparative studies comprising breeding and non-breeding seasons in rams will be useful for understanding their reproductive physiology. As a result of the revolution in assisted reproductive technologies in domestic animals in Iran, a growing interest and necessity demands more information concerning the reproductive physiology of farm animals (Talebi *et al.* 2009).

The breeding season starts in most ovine breeds during summer or early autumn (Chemineau *et al.* 1992) and its length varies largely among breeds but in general it ends during the winter (Hafez, 1952). Many other factors affect the semen characteristics, including nutrition, social environment, the presence of females, geographical location, age, testicle and body conformation, libido and management system, as reported in many studies (Nowakowski and Cwikla, 1994; Mandiki *et al.* 1998; Al-Ghalban *et al.* 2004; Zamiri and Khodaei, 2005; Zarazaga *et al.* 2005), but the photoperiod and the breed are primary factors regulating the seasonal reproduction. Therefore, they became the focus for many researchers (Simplicio *et al.* 1982; Ibrahim, 1997; Karagiannidis *et al.* 2000; Kafi *et al.* 2004; Al-Ghalban *et al.* 2004; Barkawi *et al.* 2006; Talebi *et al.* 2009; Zamiri *et al.* 2010). In intensive management systems, a significant number of ewes are inseminated in non-breeding season (Colas *et al.* 1988; Colas *et al.* 1990). Therefore a detection of semen characteristic of the crosses in non-breeding season is necessary. Although information is available on the level semen fertility of ram (Mohamed, 1978; Haynes and Schanbacher, 1983; Nowakowski and Cwikla, 1994; Ibrahim, 1997) the yearlong research is first report of the semen characteristics of the crossbreed rams reared at the northwest of Iran.

MATERIALS AND METHODS

Location

This trial was performed at the Sheep Breeding Research Center, in Tabriz (38° 02' N, 46° 27' E atan altitude of 1567 m above sea level), East Azerbaijan, Iran. This experiment was carried out from October 2010 to September 2011, with the training period performed during early September 2010.

Animals

Ten crossbreed and fertile rams consisting of 5 Baluchi × Moghani (BL×MG) and 5 Arkharmerino × Moghani (AM×MG) aged of 3-6 years old and with a live weight of 74-88 kg were used in this study. The animals were maintained under natural photoperiod conditions and at equal levels of nutrition per day -20% concentrate (75% barley, 25% corn, soya, bran) and 80% alfalfa hay. The rams were initially trained (beginning of September) for 15 days in September to ejaculate semen by using artificial vagina (AV) in the mating pen (210 cm length, 60 cm width, and 120 cm height). Training and semen sampling was performed via an anoestrous teaser with quiet temperament. The rams were separated from the herd and housed in a large cover shelter with an open precinct for walking freely. Levels of nutrition remained equal and all rams had free access to salty stones and were sent for drinking fresh water twice or three times a day. Hoof trimming, shearing, crutching, dipping, disease prevention and another general management were checked up during the study. The temperature, relative humidity and photoperiod were recorded during the experiment (Table 1).

Semen collection

Randomly all the rams were divided into two groups. Each group included 5 rams of different genetic make-up. Semen collection was done for 2 days and every day from 5 rams. Ejaculation intervals of each ram were five days throughout the study. A short form artificial vagina (AV) was used for semen collection. Collecting glass of AV was warmed at 37 °C before the operation and was maintained at this temperature until processed. An anoestrous ewe with quiet temperament was used for mounting the rams. Immediately after collection the fresh semen samples were transferred to the lab (avoiding sunlight) and were surveyed.

Semen appraising

Seminal traits of the fresh semen were evaluated according to the procedure adopted by Evans and Maxwell (1987). Volume of ejaculates was measured in a conical tube graduated at 0.1 mL intervals. Semen pH was surveyed with two methods, Pen form pH-meter (with 0.1 grades, model 8685, made in Malaysia) and indicator pH-meter strips (Merck, made in Germany, with 1.0 grade). Spermatozoa concentration was determined by use of a Thoma slide (haemocytometer method).

Table 1 Climatic data during the experiment (October 2010 until September 2011) at Khalat Poshan Research Center, University of Tabriz

Month	Air temperature (ºC)		Relative humidity (%)		Average
	Minimum	Maximum	Minimum	Maximum	Day length (h)
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

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 under a microscope. The overall number of sperm per ejaculate was then calculated (volume×density). Wave motion of fresh semen was evaluated (at 100×magnification) (Evans and Maxwell, 1987).

The assessment of the spermatozoa progressive motility was via a visual scale from 0-100% on basis of suspended droplet slide and on a heated (37 ˚C) stage using phase-contrast optics (×400). The evaluation was done in increments of 5 or 10 percentage points for viewing individual spermatozoa with more lucidity and estimating spermatozoa progressive motility. For sperm morphology and sperm live / dead ratio, semen was stained with eosin-nigrosin stain and examined microscopically (×400). From several parts of the slide, about 300 spermatozoa were evaluated for mortality 200 for abnormality percentages. Metabolic activity of spermatozoa was measured by methylene blue reduction time (MBRT) method based on color change from blue to colorless at 37 ˚C.

In a thin and transparent tube (1 mm diameter), 0.2 mL semen was added to 0.2 mL of methylene blue and time for color change recorded. Semen index (semen volume×spermatozoa concentration/mL×live spermatozoa %×progressive motility %) was calculated, as an indicator for appraising semen quality.

Statistical analysis

All statistical analysis was performed using the MIXED Procedure of Statistical Analysis System (SAS 1996) and outliers deleted for volume, concentration, abnormality and MBRT traits.

Values were considered to be statistically significant at $P \leq 0.05$. For volume, SC, abnormality and MBRT traits the outlier data has been deleted. Means values were compared with Tukey test. Pearson correlation coefficient was calculated to evaluate the relationship between quality and quantity of semen attributes.

RESULTS AND DISCUSSION

Descriptive statistics for seminal parameters are shown in Table 2. Results of seasonal fluctuations of semen characteristics were surveyed for eleven traits and presented in Table 3 and Table 4. Photoperiod influence on semen characteristics has been shown in Table 5. In AM × MG and BL × MG genetic groups, minimum and maximum values of semen volume were recorded at spring and autumn respectively and reported significantly difference volumes ($P<0.01$). Frequently, semen volume increased from the end of June and the highest mean values were reported at the beginning of October, and then decreased gradually at the end of October. This falling process followed during autumn and winter except for BL × MG and in April were there occurred a sudden increase (Figure 1). The highest mean vales of percentage of live spermatozoa were recorded in December (for BL×MG) and September (for MR×MG). Monthly variations of percentage of live spermatozoa are shown in Figure 2. In AM × MG rams difference between the spring (the part of non-breeding season) and other season was significant ($P<0.01$). But in the BL × MG a significant difference was found between non breeding season (spring and winter) with breeding season ($P<0.01$). In both genetic groups sperm concentration, TSE and semen color were highest in winter and lowest in spring. In the both genetic groups, spring was the peak for mean values of semen pH. In BL × MG rams mean values of semen pH increased concurrent with the spring. The most semen index was observed during autumn (in both genetic group observed at October) and the lowest mean values in spring (June). In AM × MG rams there was significant difference in wave motion between breeding season (summer and autumn) and non breeding season (Table 4). But in BL×MG rams most mean values were recorded in summer and fewest in winter, and these were found to significantly differ from each other ($P<0.01$).

Table 2 Semen characteristics in BL × MG and AM × MG genetic group over the year

Genetic group		SV (mL)	WM (0-5)	PM (%)	SC (0-5)	TSE ($\times10^9$)	Conc ($\times10^9$)	SL (%)	SAB (%)	SI ($\times10^9$)	pH	MBRT (sec)
BL × MG	n	334	334	334	334	334	334	334	332	334	333	331
	Mean	0.86	3.72	67.42	3.73	3.55	3.77	68.91	13.02	14769	6.54	119.39
	SE	0.08	0.09	1.70	0.16	0.33	0.17	1.60	0.76	1823.50	0.09	3.10
	Min	0.45	2.00	40.00	2.00	0.916	1.95	45.00	4.00	722.40	5.70	65.00
	Max	1.40	5.00	85.00	5.00	18.90	5.68	90.00	26.00	43834.50	8.20	230.00
AM × MG	n	334	334	334	334	334	334	334	330	334	334	331
	Mean	1.02	3.93	72.79	3.55	4.495	3.516	74.57	10.95	19472.29	6.57	111.70
	SE	0.08	0.09	1.59	0.16	0.327	0.182	1.57	0.71	1767.06	0.09	3.17
	Min	0.45	2.00	45.00	1.00	0.985	0.960	50.00	3.00	1395.2	5.90	45.00
	Max	1.90	5.00	90.00	5.00	31.55	5.81	94.00	21.00	104058	7.80	220.00

SV: semen volume; WM: wave motion; PM: progressive motility; SC: semen color; TSE: total spermatozoa per ejaculate; Conc: spermatozoa concentration; SL: percentage of live spermatozoa; SAB: percentage of abnormal spermatozoa; SI: semen index; MBRT: methylene blue reduction time.

Table 3 Seasonal variations in semen quantity (Mean±SE) of Baluchi × Moghani (BL×MG) and Arkharmerino × Moghani (AM×MG) rams

Semen quantity	Season	BL × MG (Mean±SE)	AM × MG (Mean±SE)
Total spermatozoa / ejaculate ($\times10^9$)	Spring	2.430±0.430^{b}	2.326±0.463^{c}
	Summer	3.210±0.298^{a}	3.730±0.290^{b}
	Autumn	3.636±0.316^{a}	4.336±0.285^{b}
	Winter	4.105±0.306^{a}	5.853±0.306^{a}
	Mean	3.345±0.337	4.061±0.336
Spermatozoa concentration ($\times10^9$)	Spring	3.443±0.195^{b}	3.315±0.199^{b}
	Summer	3.625±0.177^{ab}	3.555±0.185^{ab}
	Autumn	3.796±0.176^{a}	3.482±0.176^{ab}
	Winter	3.952±0.180^{a}	3.676±0.179^{a}
	Mean	3.704±0.182	3.507±0.184
Semen volume (mL)	Spring	0.69±0.09^{c}	0.70±0.09^{b}
	Summer	0.90±0.08^{a}	1.02±0.08^{a}
	Autumn	0.96±0.09^{a}	1.15±0.09^{a}
	Winter	0.84±0.09^{b}	1.06±0.08^{a}
	Mean	0.84±0.09	0.98±0.08
Semen color (0-5)	Spring	3.351±0.171^{c}	3.326±0.177^{b}
	Summer	3.512±0.156^{bc}	3.419±0.173^{ab}
	Autumn	3.712±0.159^{ab}	3.566±0.158^{ab}
	Winter	3.979±0.161^{a}	3.671±0.161^{a}
	Mean	3.638±0.162	3.495±0.167

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

The results in the AM × MG and BL × MG rams demonstrated that individual progressive motility of spermatozoa was higher in breeding seasons (autumn and summer). In the AM × MG genetic group, greatest value synchronized occurred in October (75.09±1.53) and lowest in January (69.78±1.58).

In GH × BL genetic group the highest levels were recorded in September (71.1±1.59) and the lowest were found in June (62.43±1.70).

The highest and the lowest percentage of live spermatozoa were recorded in September (71.56±1.56) and June (64.66±1.65), respectively in BL × MG genetic group; but in October (75.59±1.54) and February (71.43±1.51) in AM × MG rams. The spermatozoa abnormality occurred mostly as tail abnormalities. In spite of these facts, semen quality from the viewpoint of sperm normality improved significantly during autumn in AM × MG and summer in BL × MG groups.

Season and genetic group did not influence the rate of metabolic activity and wave motion of the spermatozoa. Seasonal fluctuation was observed with respect to methylene blue reduction time (MBRT) in the two genetic groups. The BL × MG group showed lowest MBRT in autumn and AM × MG group in summer. Correlation coefficients between various semen characteristics (Table 6) exhibited good correlation of live spermatozoa with motility (r=0.90, P<0.01) and sperm density and semen color (r=0.30, P<0.01). Semen volume could be correlated with sperm concentration, color and TSE as shown by the positive correlation of 0.21, 0.24 and 0.39, respectively. The MBRT decreased over time and correlated with all of semen traits (P<0.01). Percentage of abnormal spermatozoa was correlated with all the semen quantity traits except for TSE. The percentage of abnormal spermatozoa was significantly correlated with wave motion (r=-0.69, P< 0.01), progressive motility (r=-0.88, P<0.01) and percentage of live spermato

zoa (r=-0.92, P<0.01). Wave motion and individually progressive motility of semen samples showed a significant correlation with semen density (r=0.19 and r=0.33, respectively) and semen pH (r=-0.38 and r=-0.39, respectively). Moreover, semen pH showed the high negative correlation with semen concentration (r=-0.6, P<0.01). At the University of Tabriz the interest arose to find a suitable sheep breed which could produce more uniform wool, from the Arkhar Merino breed that was imported from Kazakhstan and to assess the influence of photoperiod on seminal characteristics.

As expected, the summer and autumn with decreasing daylight length (breeding season) and winter and spring are seasons with increasing daylight length (non-breeding season), affected the seminal indices of the crossbreed rams. This study is the first to report on the seasonal variations in seminal indices of the two crossbreed genetic groups of rams (Baluchi×Moghani and Arkharmerino×Moghani) in Iran. The effect of season and / or photoperiod on semen quality and quantity has been previously reported in different breeds of rams (Amir *et al*. 1986; Ibrahim, 1997; Karagiannidis *et al*. 2000; Kafi *et al*. 2004; Zamiri and Kh-

Table 4 Seasonal variations in semen quality variables (Mean±SE) of Baluchi × Moghani (BL×MG) and Arkharmerino × Moghani (AM×MG) rams

Semen quantity	Season	BL × MG (Mean±SE)	AM × MG (Mean±SE)
Wave motion (0-5)	Spring	3.60±0.10^{bc}	3.81±0.09^{b}
	Summer	3.92±0.09^{a}	4.00±0.09^{a}
	Autumn	3.72±0.09^{ab}	4.05±0.11^{a}
	Winter	3.51±0.11^{c}	3.75±0.09^{b}
	Mean	3.68±0.09	3.90±0.09
Progressive motility (%)	Spring	65.37±1.61^{b}	72.00±1.75^{a}
	Summer	70.51±1.54^{a}	74.67±1.61^{a}
	Autumn	69.89±1.50^{a}	74.89±1.50^{a}
	Winter	64.40±1.73^{b}	71.00±1.54^{a}
	Mean	67.54±1.70	73.14±1.59
Live spermatozoa (%)	Spring	65.91±1.68^{c}	73.33±1.67^{a}
	Summer	70.37±1.66^{ab}	75.73±1.66^{a}
	Autumn	70.96±1.51^{a}	75.26±1.51^{a}
	Winter	66.54±1.54^{bc}	72.28±1.51^{a}
	Mean	68.44±1.60	74.16±1.57
Abnormal spermatozoa (%)	Spring	12.71±0.76^{ab}	11.47±0.75^{a}
	Summer	11.86±0.70^{b}	9.67±0.67^{b}
	Autumn	12.65±0.74^{ab}	9.11±0.73^{b}
	Winter	14.04±0.75^{a}	10.84±0.77^{a}
	Mean	12.81±0.74	10.27±0.72
Semen index (×10^{9})	Spring	11346±1859.61^{b}	12123±1854.65^{b}
	Summer	16511±1881.39^{a}	19852±1769.43^{a}
	Autumn	17249±1813.56^{a}	22645±1827.49^{a}
	Winter	14023±1797.32^{ab}	20071±1769.46^{a}
	Mean	14782±1837.97	18672±1805.25
Semen pH	Spring	6.88±0.10^{a}	6.69±0.11^{a}
	Summer	6.42±0.09^{b}	6.60±0.10^{a}
	Autumn	6.37±0.11^{b}	6.57±0.09^{ab}
	Winter	6.51±0.10^{b}	6.51±0.09^{b}
	Mean	6.54±0.10	6.59±0.09
MBRT (sec)	Spring	120.22±3.61^{a}	111.18±3.70^{a}
	Summer	110.74±3.77^{a}	106.27±3.11^{a}
	Autumn	116.69±3.81^{a}	108.33±3.20^{a}
	Winter	123.47±3.17^{a}	116.08±3.25^{a}
	Mean	117.78±3.59	110.46±3.31

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 Effect of genetic group, season and ram on semen characteristics of ejaculates obtained during one year

Effect	Semen characteristics										
	SV	WM	PM	Color	TSE	Conc	SL	SAB	SI	pH	MBRT
Genotype	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Season	**	*	NS	**	**	**	NS	*	**	*	NS
Ram	*	*	*	*	NS	*	*	*	*	*	*

NS: non significant; * P<0.05 and ** P<0.01.
SV: semen volume; WM: wave motion; PM: progressive motility; SC: semen color; TSE: total spermatozoa per ejaculate; Conc: spermatozoa concentration; SL: percentage of live spermatozoa; SAB: percentage of abnormal spermatozoa; SI: semen index; MBRT: methylene blue reduction time.

odaei, 2005; Deldar Tajangookeh *et al*. 2007; Zamiri *et al*. 2010) and also in other seasonal breeding animals such as bucks (Barkawi *et al*. 2006; Karagiannidis *et al*. 1999) and stallions (Janett *et al*. 2003) with sperm production down regulated in the non-breeding season (Gerlach and Aurich, 2000).

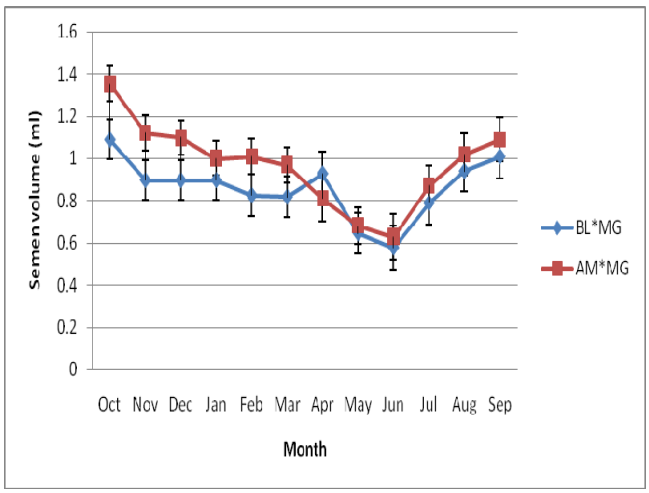

Figure 1 Monthly variations of semen volume in two genetic groups throughout the year

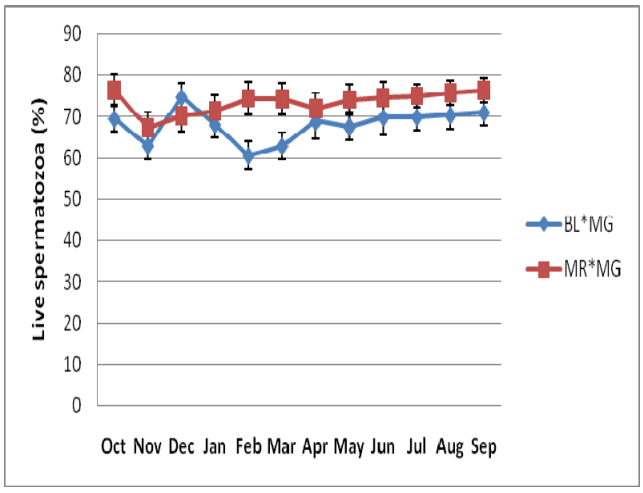

Figure 2 Monthly variations of live spermatozoa in two genetic groups throughout the year

In the present study, the photoperiod significantly affected some of the seminal characteristics. Among quality characteristics of sperm, a significant effect of season was recorded on percentage of abnormal spermatozoa, semen pH ($P<0.05$) and semen index ($P<0.01$). Sperm progressive motility, percent of live spermatozoa and MBRT did not have significantly seasonal variations. Moreover, photoperiodic effect also was observed clearly on semen quantity characteristics ($P<0.01$). These seasonal variations in both semen quality and quantity were attributive mainly to changes in daylight length throughout the year (Chemineau *et al*. 1992). Any significant differences were not found on all traits between the two genetic groups. Significant differences among rams within each genetic group ($P<0.05$) were found in some of seminal traits, but, non significant differences were found between the two genetic groups in any other traits in agreement with the previous reports of Karagiannidis *et al*. (2000).

The results of mean values of semen characteristics observed in our study were in agreement with those of other researchers (Zamiri *et al*. 2010; Gundogan, 2007; Kafi *et al*. 2004, Al Ghalban *et al*. 2004; Karagiannidis *et al*. 2000).

The semen volume of 0.60-1.6 mL, spermatozoa concentration of 2.6-5.5 × 109, abnormal spermatozoa of 4-29% and live or motile spermatozoa of 60-90% is on record (Karagiannidis *et al*. 2000; Kafi *et al*. 2004; Gundogan, 2007). Therefore, it could be accepted that there is wide amplitude of semen characteristics in several breeds of ram. In the current study mean values for the abnormal spermatozoa of the crosses (9-14 %) were generally higher than the other researchers (Karagiannidis *et al*. 2000) for Chios and Friesian rams in Northern Greece and by Gundogan (2007) for Akkarman and Awassi in Turkey. Zamiri *et al*. (2010) reported in Moghani breed a minimum spermatozoa abnormality of 7.9% in September much lesser than the value observed in our study 11.42% in BL × GH rams in September and 8.91% in the AM × MG genetic group in November. Percentage of live spermatozoa in the two genetic groups was lower than the values recorded by Kafi *et al*. (2004) in south of Iran (29˚ 25' N, 52˚ 46' E). The semen volume in the BL × MG (0.84±0.09) did not coincide with results of Kafi *et al*. (2004) and was lower (1.03±0.08) in AM × GH group than the reported value (Kafi *et al*. 2004) making the comparison of seminal attributes often difficult. Thus, it is not surprising that wide variations have been reported in the seminal attributes of rams (Gundogan, 2007; Zamiri and Khodaei, 2005; Kafi *et al*. 2004; Karagiannidis *et al*. 2000; Ibrahim, 1997). In BL × GH genetic group, the sperm concentration remained high (3.952±0.180) during winter and low in spring (3.443±0.195), summer (3.625±0.177) and autumn (3.796 ±0.176) a trend comparable to that reported by Karagiannidis *et al*. (2000) and Talebi *et al*. (2009). These findings confirmed the previous records of seasonal variations of sperm concentration in BL × MG rams at 38˚ N latitude. In both crosses, circumstance of seasonal fluctuations of semen color and sperm density was similar. In our study, most of the mean values for the semen characteristics of BL × MG and AM × MG rams, born and raised in northwest of Iran (38˚ 02' N, 46˚ 27' E), were almost similar to those reported by other authors (Barkawi *et al*. 2006; Zamiri *et al*. 2010; Gundogan, 2007), in similar temperate regions.

Table 6 Correlation coefficients between various seminal traits of the rams

	MBRT	pH	SI	SAB	SL	Conc	TSE	Color	PM	WM
SV	-0.21**	-0.20**	0.71**	-0.10	0.13*	0.21**	0.39*	0.24**	0.15**	0.21**
WM	-0.76**	-0.38**	0.50**	-0.69**	0.74**	0.19**	0.19*	0.37**	0.78**	-
PM	-0.84**	-0.39**	0.59**	-0.88**	0.90**	0.33**	0.11*	0.34**	-	-
Color	-0.67**	-0.56**	0.57**	-0.31*	0.30**	0.92**	0.34**	-	-	-
TSE	-0.22**	-0.17*	0.41**	-0.05	0.09	0.29**	-	-	-	-
Conc	-0.67**	-0.60**	0.61**	-0.28*	0.30**	-	-	-	-	-
SL	-0.81**	-0.35*	0.57**	-0.92**	-	-	-	-	-	-
SAB	0.80**	0.29**	-0.54**	-	-	-	-	-	-	-
SI	-0.67**	-0.30**	-	-	-	-	-	-	-	-
pH	0.52**	-	-	-	-	-	-	-	-	-

* P<0.05 and ** P<0.01.
SV: semen volume; WM: wave motion; PM: progressive motility; SC: semen color; TSE: total spermatozoa per ejaculate; Conc: spermatozoa concentration; SL: percentage of live spermatozoa; SAB: percentage of abnormal spermatozoa; SI: semen index; MBRT: methylene blue reduction time.

The quantity and quality attributes of seminal characteristics in the crossbreed rams differed in breeding (late of summer to middle of autumn) and non-breeding seasons.

Sperm concentration did not follow a similar trend to that of the ejaculate volume in this study and was comparable with the results obtained by Talebi *et al*. (2009). Mean values of MBRT in our study were quite different from those found by Galal *et al*. (1978). Also the seasonal variations were different. BL × MG and AM × MG rams performed best in breeding season. In Egypt, Galal *et al*. (1978) recorded in their study on Merino, Ossimi and their crosses the best metabolic activity in spring (76.8±1.04 sec) and autumn (77.2±1.04 sec). While summer (102.2±1.04 sec) was greatest mean values in these breeds. On the contrary, Galal *et al*. (1978) did not observe significant difference in MBRT traits between several seasons of the year. In the present study, the semen characteristics were generally better towards the end of summer (onset of improvement) and in the two first months of autumn, than during the winter (onset of decrease in quality) and spring (usually was lowest quality and quantity). In both genetic groups the progressive motility of sperm was lowest in winter and spring in contrast to the findings of Karagiannidis *et al*. (2000) at 40˚ N. The data suggest that summer and autumn with decreasing daylight length (breeding season) and winter and spring with increasing daylight length (non-breeding season) influenced the seminal characteristics of BL × MG and AM × MG rams. This indicated that the two test groups of rams were sensitive to photoperiod in respect of their reproductive behaviour. Photoperiodic effects on seasonal breeders have been reported to be dictated by the latitude at which they are kept. At latitudes above 40˚ N, marked variations in seminal characteristics and increased sperm production with decreasing daylight length have been observed (Zamiri *et al*. 2010). Seasonal variations, although less marked, were observed between 30˚ N and 40˚ N latitude, with higher sperm production during the summer and autumn.

Although, the crossbreed rams were capable of ejaculating throughout the year. Seasonal breeding animals have previously been shown to occur in middle latitudes (Rosa and Bryant, 2003) such as here. A high correlation between wave motion and sperm progressive motility with live sperm (r=0.74, r=0.90, P<0.01, respectively) demonstrated that, concurrent with improved sperm motility (one of most important of semen quality indicators) increased the percent of live sperm which, resembled the findings of Kafi *et al*. (2004). A significant correlation was found between motility indices and TSE again in agreement with the results of Kafi *et al*. (2004). A high negative correlation of MBRT with motility traits (r=-0.76 to r=-0.84, P<0.01) similar to the findings of Chandler *et al*. (2000) but in contrast to the results of Kishk, (2008). MBRT is an evaluator of the metabolic status of the spermatozoa (Salisbury *et al*. 1978). Methylene blue is redox dye that changes color upon reduction by addition of hydrogen. Thus, respiration rate of spermatozoa at the dense semen lead to rapid reduction of methylene blue. The observed high negative correlation between sperm concentration and MBRT (r=-0.67, P<0.01) and between MBRT and percent of live sperm (r=-0.81, P<0.01) were similar to the findings of Kishk, (2008). This can be attributed to the rate of release of hydrogen upon fructose utilization the by sperm cells. Thus, these samples might become acidic and not reliable for long-term storage. Most relationship among semen traits with Semen pH could be well correlated with concentration and color (r=-0.60 and -0.56, P<0.01) watery sample trending alkaline. Among the quantitative traits, a high correlation between MBRT with semen color and sperm concentration was observed. Karagiannidis *et al*. (2000) also reported a significant correlation between sperm concentration and abnormal sperm percentage (r=-0.19, P<0.05).

The results of the present investigations suggest that ewes exhibiting estrus could be artificially inseminated by fresh semen throughout the year and consequently, reproductive performance of herd increased considerably.

Seasonal fluctuations of environmental conditions markedly influenced reproduction of animals at higher latitudes and altitudes (Rosa and Bryant, 2003). Robinson (1981) argued that breeds located between 35˚ N and have the tendency to breed at all times of the year. Evans and Maxell, (1987) reported 30˚ N and 40˚ N latitudes for the breeds to follow this tendency. Latitudes above 35˚ N (Hafez, 1952; Goot, 1969) or higher than 40˚ N (Talebi *et al*. 2009; Zamiri *et al*. 2010) considerably influenced the seminal attributes.

However in the some studies, for example in Jordan (at 31.5˚ N latitude and altitude of 350 m, in Damascus bucks) and Iran (34˚ 18' N, 47˚ 3' E Kermanshah, Iran, in Markhoz bucks) the photoperiod was found to have significant effect on breeding behaviour of sheep. In temperate latitudes (40-50˚ N) sperm production of rams is a continuous process, although the total number of spermatozoa produced per testis is usually higher in autumn than in spring (Dacheux *et al*. 1981).

The present study showed that the reproductive activity of the seasonal breeding animals e.g., rams, may be improved by exploitation of photoperiod synchronized circannual reproductive rhythm (endogenous mechanisms) and exogenous factors.

The pineal hormone melatonin has been established as the common link between photoperiod and reproduction (Gerlach and Aurich, 2000). Reproductive activity is not a direct function of day length, but is affected by the photoperiodic history of the animal, the direction of photoperiodic changes and the stage of the circannual rhythm at which a photoperiodic signal is received (Robinson and Karsch, 1987; Gorman and Zucker, 1995a).

Our study clearly showed linkage between photoperiod and reproduction of Baluchi × Moghani (BL×MG) and 5 Arkharmerino × Moghani (AM×MG) breeds located at 38˚ 02' N, 46˚ 27' E and an altitude of 1567 m above sea level in Iran.

CONCLUSION

Semen evaluation does have an important role in artificial insemination programs or in flocks where single sire joining groups are used. This will be useful for identifying rams with poor performers. Thus, it will provide optimum breeding selection of males in herd. The semen characteristics of BL × MG and AM × MG rams in Northwest of Iran showed a significant seasonal variation in semen characteristics. The best semen is produced during late summer to second month of autumn (breeding season). Nonetheless, the magnitude of these seasonal effects should not prevent the animals from serving or semen collecting for artificial insemination throughout the year but it is necessary to perform semen evaluation on an individual basis for every ram used for artificial insemination or breeding.

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