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Photoperiod as a factor for studying fluctuations of seminal traits during breeding and non-breeding seasons

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The main purpose of this study was to evaluate the influence of photoperiod on the seminal traits of crossbreed wool-producing rams throughout one year period. For the effect of photoperiod (PTP), two periods were considered: Decreasing daylight length (summer and autumn) and the other, increasing daylight length. For this 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/ejaculate (TSE), concentration (SC), color, wave motion (WM), percentage of progressive motility (PM), percentage of live sperm (LS) and abnormal sperm (SAB), pH, methylene blue reduction time (MBRT) and semen index (SI). Analysis of the year long data showed that semen with the best quality was collected in September to November ($P < 0.05$). Significant seasonal variations of semen traits were observed for all seminal traits except for PM, LS and MBRT. Yet, no statistical differences were found between the crosses ($P > 0.05$). Although, there were significant seasonal changes in seminal traits of the crosses, the fresh semen showed adequate quality to be used for artificial or natural insemination throughout the year. PTP was found to influence semen production in two genetic groups at 38°02' N, 46°27' E and an altitude of 1567 m above sea level of Iran. However, these effects were not detrimental to the use of rams for breeding purposes throughout the year.

Key words: Genetic group, crossbreed ram, photoperiod, seasonal variation, spermatozoa.

INTRODUCTION

Sheep production is a traditional economic activity that is mostly used for meat and milk production in Iran. One of the most important factors for economical development of sheep industry is lambing throughout the year. Seasonal breeding is a limiting factor in this species. Therefore, it was determined to evaluate the quality of semen in breeding and non-breeding seasons before using artificial insemination (AI) in sheep. Unlike most domestic livestock species, sheep are widely known for their marked seasonality of breeding activity linked to annual cycle of daily photoperiod (PTP) (Rosa and Bryant,

2003). The annual cycle of daily PTP has been identified as the major determinant for this phenomenon in sheep (Rosa and Bryant, 2003). Understanding the fertility quality in non-breeding season will be helpful in 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

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

Month	Air temperature (°C)		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

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 (Mandiki et al., 1998; Al-Ghalban et al., 2004; Zamiri and Khodaei, 2005; Zarazaga et al., 2005), but the PTP and the breed are primary factors regulating the seasonal reproduction. Therefore, they became preference for many researchers (Simplicio et al., 1982; Ibrahim, 1997; Kafi et al., 2004; Barkawi et al., 2006; Talebi et al., 2009; Zamiri et al., 2010).

The Arkhar-Merino is a breed of sheep obtained by crossbreeding between wild Arkhar rams with ewes of the Novocaucasian Merino, Précoce and Rambouillet breeds (Ernst and Dmitriev, 2007). These two genetic groups are developed targeting the improvement of local breeds (Baluchi and Moghani) for wool traits. There is no published information on the seasonal variation of seminal traits of Baluchi × Moghani and Arkharmerino × Moghani genetic groups. In addition to gathering information on reference values for semen characteristics, evaluation of the effects of PTP 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 genetic groups, to identify the most suitable line for breeding purposes. In intensive management systems, a significant number of ewes are inseminated in non-breeding season (Colas et al., 1988, 1990).

Therefore, detection of semen characteristic of the

crosses in non-breeding season is necessary. Although, information is available on the physicochemical parameters of semen of the crosses and on the part of their reproductive traits (Moghaddam et al., 2012; Asadpour et al., 2012; 2012a), however, this yearlong research is the first report on the seasonal variation of semen characteristics of these crossbreed rams reared at the northwest of Iran.

MATERIALS AND METHODS

Location

This trial was performed at the Sheep Breeding Research Center, Tabriz (38°02' N, 46°27' E and an altitude of 1567 m above sea level), Iran. This experiment was carried out from October 2010 to September 2011.

Animals

Ten crossbreed and fertile rams consisting of 5 BL × MG and 5 AM × MG aged 3 to 6 years and with a live weight of 74 to 88 kg were used in this study. The animals were maintained under natural PTP and 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 in length, 60 cm in width and 120 cm in height). Training and semen sampling was performed via an anoestrous teaser with quiet temperament. The rams were separated of the herd and housed in a large cover shelter with an open precinct for walking freely. All rams were sent to drink fresh water twice or three times a day. The other general management was checked during the study. The climate conditions of the research center were recorded during the experiment (Table 1).

Semen collection

Randomly, all the rams were divided into the two groups. Each group included 5 rams of different genetic groups. Semen collection

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 ($\times 10^9$)	Conc ($\times 10^9$)	SL (%)	SAB (%)	SI ($\times 10^9$)	pH	MBRT (s)
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
	S.E.	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
	S.E.	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.

was done for every 2 days from 5 rams. Ejaculation intervals of each ram were five days throughout the study. Short form 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. Immediately, the fresh samples were transferred to the laboratory (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, a) pen form pH-meter (with 0.1 grades, model 8685, AZ Instrument, Taiwan), b) indicator pH-meter strips (Merck, made in Germany). SC was determined by use of a Thoma slide (haemocytometer 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 under a microscope. TSE was then calculated (volume × density). WM was evaluated according to the study of Evans and Maxwell (1987). The assessment of the PM was a visual

scale from 0 to 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 to 10 percentage points for viewing individual spermatozoa with more lucidity and estimating PM. For SAB and LS, semen was stained with eosin-nigrosin stain and examined microscopically (400×). From several parts of the slide, about 300 spermatozoa were evaluated for mortality and 200 for SAB percentages. Metabolic activity of spermatozoa was measured by 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 was recorded. SI (volume × SC/ml × LS% × PM%) was calculated, as an indicator for appraising semen quality.

Statistical analysis

All statistical analysis were 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 was 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

The quantity traits of semen (semen volume, color, SC and TSE; Table 2) were significantly influenced by season of the year. In AM × MG and BL × MG genetic groups, minimum and maximum values of semen volume were recorded at spring and autumn, respectively ($P < 0.01$). Frequently, semen volume increased from end of June and received the highest values at the October, and again decreased gradually at the end of October. This falling process continued during autumn and winter except for BL × MG that had a sudden increase in April (Figure 1). The highest mean values of LS were recorded in December (in BL × MG) and September (in MR × MG). In AM × MG

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

rams the significant differences was observed between the spring (the part of non-breeding season) and the other seasons ($P < 0.01$). But BL × MG rams had a significant difference between non-breeding season (spring and winter) and breeding season ($P < 0.01$). In the two genetic groups SC, TSE and semen color were highest in winter and the lowest was in spring. In the crosses, spring was the peak for semen pH. In BL × MG rams, mean values of semen pH increased concurrent with the spring. The most SI was observed during autumn (in both genetic group observed at October) and the lowest mean values was in spring (especially June). In AM × MG rams, there was significant difference in WM between breeding season (summer and autumn) and non-breeding season (Table 3). But most mean values in BL × MG rams were recorded in summer and the fewest were in winter ($P < 0.01$).

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 AM × MG genetic group, greatest value synchronized with the October (75.09 ± 1.53) and the lowest was in January (69.78 ± 1.58). In GH × BL genetic group, the highest levels were recorded in September (71.1 ± 1.59) and lowest in June (62.43 ± 1.70). The highest and lowest percentage of live spermatozoa in BL × MG genetic group were recorded in September (71.56 ± 1.56) and June (64.66 ± 1.65), respectively. But the highest and lowest LS in AM × MG rams were recorded in October (75.59 ± 1.54) and February (71.43 ± 1.51), respectively (Figure 2). In our study, the tail abnormalities were the most spermatozoa abnormality that occurred. 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 genetic groups. The season and genetic group did not influence the rate of

metabolic activity and WM ($P > 0.05$). The BL × MG genetic group showed the lowest MBRT in autumn and AM × MG group in summer. Correlation coefficients between various semen characteristics (Table 4) exhibited a near relationship between LS and motility traits ($r = 0.90$, $P < 0.01$) and sperm density and semen color ($r = 0.30$, $P < 0.01$). Semen volume showed a positive correlation with SC, color and TSE ($r = 0.21$, 0.24 and 0.39 , respectively). The MBRT decreased over time and correlated with all semen traits ($P < 0.01$). SAB was correlated with all the semen quantity traits, except for TSE. The percentage of abnormal spermatozoa was significantly correlated with WM ($r = -0.69$, $P < 0.01$), PM ($r = -0.88$, $P < 0.01$) and LS ($r = -0.92$, $P < 0.01$). WM 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 high negative correlation with SC ($r = -0.6$, $P < 0.01$).

DISCUSSION

As expected, the summer and autumn with decreasing daylight length (breeding season) and winter and spring seasons with increasing daylight length (non-breeding season), affected the seminal indices of the crossbreed rams. The effect of season and/or PTP on seminal traits has been previously reported in different breeds of rams (Karagiannidis et al., 2000; Kafi et al., 2004; Zamiri and Khodaei, 2005; Deldar Tajangookeh et al., 2007; Zamiri et al., 2010) and also in other seasonal breeding animals such as buck (Barkawi et al., 2006; Karagiannidis et al., 1999) and stallion (Janett et al., 2003). Among quality traits, a significant effect of season was recorded on SAB, semen pH ($P < 0.05$) and SI ($P < 0.01$). PM, LS and

Table 3. Seasonal variations in semen characteristics (mean $\pm$ S.E.) of Baluchi $\times$ Moghani (BL $\times$ MG) and Arkharmerino $\times$ Moghani (AM $\times$ MG) rams.

Semen characteristics	Season	BL $\times$ MG (Mean $\pm$ S.E.)	AM $\times$ MG (Mean $\pm$ S.E.)
Total sperm/ejaculate ($\times 10^9$)	Spring	2.430 $\pm$ 0.430 ^b	2.326 $\pm$ 0.463 ^c
	Summer	3.210 $\pm$ 0.298 ^a	3.730 $\pm$ 0.290 ^b
	Autumn	3.636 $\pm$ 0.316 ^a	4.336 $\pm$ 0.285 ^b
	Winter	4.105 $\pm$ 0.306 ^a	5.853 $\pm$ 0.306 ^a
	Mean	3.345 $\pm$ 0.337	4.061 $\pm$ 0.336
Sperm concentration ($\times 10^9$)	Spring	3.443 $\pm$ 0.195 ^b	3.315 $\pm$ 0.199 ^b
	Summer	3.625 $\pm$ 0.177 ^{ab}	3.555 $\pm$ 0.185 ^{ab}
	Autumn	3.796 $\pm$ 0.176 ^a	3.482 $\pm$ 0.176 ^{ab}
	Winter	3.952 $\pm$ 0.180 ^a	3.676 $\pm$ 0.179 ^a
	Mean	3.704 $\pm$ 0.182	3.507 $\pm$ 0.184
Semen volume (ml)	Spring	0.69 $\pm$ 0.09 ^c	0.70 $\pm$ 0.09 ^b
	Summer	0.90 $\pm$ 0.08 ^a	1.02 $\pm$ 0.08 ^a
	Autumn	0.96 $\pm$ 0.09 ^a	1.15 $\pm$ 0.09 ^a
	Winter	0.84 $\pm$ 0.09 ^b	1.06 $\pm$ 0.08 ^a
	Mean	0.84 $\pm$ 0.09	0.98 $\pm$ 0.08
Semen color (0-5)	Spring	3.351 $\pm$ 0.171 ^c	3.326 $\pm$ 0.177 ^b
	Summer	3.512 $\pm$ 0.156 ^{bc}	3.419 $\pm$ 0.173 ^{ab}
	Autumn	3.712 $\pm$ 0.159 ^{ab}	3.566 $\pm$ 0.158 ^{ab}
	Winter	3.979 $\pm$ 0.161 ^a	3.671 $\pm$ 0.161 ^a
	Mean	3.638 $\pm$ 0.162	3.495 $\pm$ 0.167
Wave motion (0-5)	Spring	3.60 $\pm$ 0.10 ^{bc}	3.81 $\pm$ 0.09 ^b
	Summer	3.92 $\pm$ 0.09 ^a	4.00 $\pm$ 0.09 ^a
	Autumn	3.72 $\pm$ 0.09 ^{ab}	4.05 $\pm$ 0.11 ^a
	Winter	3.51 $\pm$ 0.11 ^c	3.75 $\pm$ 0.09 ^b
	Mean	3.68 $\pm$ 0.09	3.90 $\pm$ 0.09
Progressive motility (%)	Spring	65.37 $\pm$ 1.61 ^b	72.00 $\pm$ 1.75 ^a
	Summer	70.51 $\pm$ 1.54 ^a	74.67 $\pm$ 1.61 ^a
	Autumn	69.89 $\pm$ 1.50 ^a	74.89 $\pm$ 1.50 ^a
	Winter	64.40 $\pm$ 1.73 ^b	71.00 $\pm$ 1.54 ^a
	Mean	67.54 $\pm$ 1.70	73.14 $\pm$ 1.59
Live spermatozoa (%)	Spring	65.91 $\pm$ 1.68 ^c	73.33 $\pm$ 1.67 ^a
	Summer	70.37 $\pm$ 1.66 ^{ab}	75.73 $\pm$ 1.66 ^a
	Autumn	70.96 $\pm$ 1.51 ^a	75.26 $\pm$ 1.51 ^a
	Winter	66.54 $\pm$ 1.54 ^{bc}	72.28 $\pm$ 1.51 ^a
	Mean	68.44 $\pm$ 1.60	74.16 $\pm$ 1.57
Abnormal sperm (%)	Spring	12.71 $\pm$ 0.76 ^{ab}	11.47 $\pm$ 0.75 ^a
	Summer	11.86 $\pm$ 0.70 ^b	9.67 $\pm$ 0.67 ^b
	Autumn	12.65 $\pm$ 0.74 ^{ab}	9.11 $\pm$ 0.73 ^b
	Winter	14.04 $\pm$ 0.75 ^a	10.84 $\pm$ 0.77 ^a
	Mean	12.81 $\pm$ 0.74	10.27 $\pm$ 0.72
Semen index ($\times 109$)	Spring	11346 $\pm$ 1859.61b	12123 $\pm$ 1854.65b
	Summer	16511 $\pm$ 1881.39a	19852 $\pm$ 1769.43a
	Autumn	17249 $\pm$ 1813.56a	22645 $\pm$ 1827.49a
	Winter	14023 $\pm$ 1797.32ab	20071 $\pm$ 1769.46a
	Mean	14782 $\pm$ 1837.97	18672 $\pm$ 1805.25

Table 3. Continued.

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

^{a, b, c} Means in the column of each parameter with different superscripts differ significantly ($P < 0.05$). Means within each column within each factor having the same letter does not differ significantly from each other ($P < 0.05$).

Figure 2. Monthly variations of live spermatozoa in two the genetic groups throughout the year.**Table 4.** Correlation coefficients between various seminal traits of the rams.

Parameter	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**									

*Significant at $P < 0.05$, **Significant at $P < 0.01$, coefficients without symbol (* or **) are not significant. SV: semen volume, WM: wave motion, PM: progressive motility, 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.

MBRT did not have significantly seasonal variations. Moreover, effect of PTP was also observed clearly on semen quantity characteristics ($P < 0.01$). These seasonal variations in semen quality and quantity were attributive mainly to changes in daylight length throughout the year (Chemineau et al., 1992). No significant difference was found on all traits between the two genetic groups ($P > 0.05$). Significant differences among the rams within each genetic group ($P < 0.05$) were found in some of the seminal traits, but, non-significant differences were found between the two genetic groups in any of the other traits which is consistent with the previous reports (Karagiannidis et al., 2000). Our results on mean values of semen characteristics are in agreement with those of other researchers (Zamiri et al., 2010; Gundogan, 2007; Kafi et al., 2004; Ghalban et al., 2004; Karagiannidis et al., 2000). The semen volume of 0.60 to 1.6 ml, SC of 2.6 to 5.5×10^9 , SAB of 4 to 29% and live or motile spermatozoa of 60 to 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 traits in several breeds of ram. In the current study, mean values for the SAB (9 to 14%) were generally higher than that of other researchers (Karagiannidis et al., 2000) for Chios and Friesian rams and for Akkarman and Awassi rams (Gundogan, 2007). Zamiri et al. (2010) reported in Moghani breed a minimum SAB of 7.9% in September much which is lesser than the value observed in our study, 11.42% in BL $\times$ GH rams in September and 8.91% in the AM $\times$ MG genetic group in November. LS in the two genetic groups was lower than the values recorded by Kafi et al. (2004) in Iran (29°25'N, 52°46'E). The semen volume in the BL $\times$ MG (0.84 ± 0.09) did not coincide with the results of Kafi et al. (2004) and was lower (1.03 ± 0.08) in AM $\times$ GH rams than 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). In BL $\times$ GH genetic group, the SC 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 SC in BL $\times$ MG rams at 38°N latitude. In both crosses, circumstance of seasonal fluctuations of semen color and SC was similar. In our study, most of the mean values for the semen characteristics of BL $\times$ MG and AM $\times$ MG rams, were almost similar to those reported by other authors (Barkawi et al., 2006; Zamiri et al., 2010; Gundogan, 2007), in similar temperate regions. The semen quantity and quality attributes in the crossbreed rams differed in breeding (late summer to middle of autumn) and non-breeding seasons. SC did not follow a quite similar trend

with 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 with the reports of Galal et al. (1978) in Egypt. BL $\times$ MG and AM $\times$ MG rams had best performance in breeding seasons. Galal et al. (1978) recorded in their study on Merino, Ossimi and their crosses the best metabolic activity in spring (76.8 ± 1.04 s) and autumn (77.2 ± 1.04 s). While summer (102.2 ± 1.04 s) had the 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 with lowest quality and quantity). In both genetic groups, PM 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 and winter and spring with increasing daylight length influenced the seminal traits of BL $\times$ MG and AM $\times$ MG rams. PTP effects on seasonal breeders have been reported to be determined by the latitude at which they are kept. At latitudes above 40°N, marked variations in seminal traits and increased sperm production with decreasing daylight length have been observed (Zamiri et al., 2010). Seasonal variations, although less marked, were observed between 30 and 40°N latitude, with higher sperm production during the summer and autumn (Corteel, 1977). Although, the crossbreed rams were capable of ejaculating throughout the year, Rosa and Bryant (2003) illustrated that seasonal breeding animals occurred in middle latitudes. High correlation between WM and sperm progressive motility with live sperm ($r = 0.74$, $r = 0.90$, $P < 0.01$, respectively) demonstrated that concurrent with improved PM (as one of the most important semen quality indicators) there is increased LS which resembled the findings of Kafi et al. (2004). The significant correlation found between motility and TSE is in agreement with the results of Kafi et al. (2004). A high negative correlation of MBRT with motility traits ($r = -0.76$ to $r = -0.84$, $P < 0.01$) was similar to the findings of Chandler et al. (2000) but was inconsistent with the results of Kishk (2008). MBRT is an evaluator of the metabolic status of the spermatozoa (Salisbury et al., 1978).

The observed high negative correlation between SC and MBRT ($r = -0.67$, $P < 0.01$) and between MBRT and LS ($r = -0.81$, $P < 0.01$) were similar to the findings of Kishk (2008). This can be attributed to the rate of release of hydrogen upon fructose utilization 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 SC and color ($r = -0.60$ and -0.56 , $P < 0.01$), and watery sample that is alkaline. Among the quantitative traits, a

high correlation between MBRT with color and SC was observed. Karagiannidis et al. (2000) also reported a significant correlation between SC and SAB ($r = -0.19$, $P < 0.05$). 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 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 traits.

However, in some studies, for example in Jordan (at 31.5°N in Damascus bucks) and Iran (34°18'N, 47°3'E in Markhoz bucks) the PTP was found to have significant effect on breeding behaviour of sheep. In temperate latitudes (40 to 50°N) sperm production of rams is a continuous process, although the total number of sperm 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 example, rams, may be improved by exploitation of PTP synchronized circannual reproductive rhythm (endogenous mechanisms) and exogenous factors. 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 PTP and reproduction of BL × MG and AM × MG genetic groups located at 38°02'N, 46°27'E in Iran.

Conclusion

Semen evaluation does have an important role in AI 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 seminal traits 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 November. Nonetheless, the magnitude of these seasonal effects should not prevent the animals from been used for semen collection for AI throughout the year. But it is necessary to perform semen evaluation on an individual basis for every ram used for AI or breeding.

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