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SEASONAL VARIATION IN SEMEN QUANTITY AND QUALITY TRAITS OF IRANIAN CROSSBRED RAMS

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ABSTRACT

Evaluation of the seasonal changes in seminal traits of 10 crossbred rams (2-6 year old) was the main purpose of the present study. The crossbred rams consisted of 5 Ghezel×Baluchi (GH×BL) and 5 ArkharMerino×Ghezel (AM×GH). Semen was evaluated for volume, total spermatozoa per ejaculate (TSE), spermatozoa concentration (SC), semen colour, wave motion, sperm progressive motility, percentage of live and abnormal spermatozoa, semen pH, methylene blue reduction time (MBRT) and semen index. Semen index of GH×BL and AM×GH rams in autumn was greater than in spring. Best semen quality was observed in late summer and first autumn month. In AM×GH rams the highest values of TSE, SC and semen colour were recorded during winter. However, there were no significant differences between autumn and summer months except for the semen colour ($P<0.05$). In both the genetic groups the highest and the lowest values of wave motion, progressive motility and live spermatozoa were observed during autumn and spring, respectively. The data showed that the optimal performance of the crossbred rams has been obtained in late summer and the beginning autumn. Seasonal variations of semen characteristics were observed for all of the seminal traits except for the wave motion and MBRT. In conclusion, there were seasonal variations between the crosses in seminal traits and the semen has the capability and quality to be used for AI in breeding programmes throughout the year.

Key words: ram sperm quality; photoperiod; crossbred rams

INTRODUCTION

Unlike most domestic livestock species, sheep are widely known as animals with marked seasonality of breeding activity (Rosa and Bryant, 2003). The most obvious point during the study about reproductive physiology of seasonally polyestrous animals such as sheep is their fertility quality in non-breeding season, because, it is one of the limiting factors for development of sheep breeding flocks. 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 (Dacheux *et al*., 1981; Aurich *et al*. 1996). Also, physiological and behavioural variations of rams are less pronounced than the ewes (Rosa and Bryant, 2003). Therefore, yearlong comparative studies between breeding and non-breeding seasons in rams will be useful for completing the findings and reducing the reproductive challenges of these species. As a result of the revolution in assisted reproductive technologies (ART) in domestic animals in Iran, there has been a growing interest and necessity to have more information concerning the reproductive physiology of farm animals (Talebi *et al.,* 2009). Based on our knowledge there is no published information on the reproductive characteristics of the Iranian crossbred rams and especially Ghezel×Baluchi and Arkharmerino×Ghezel genetic groups. Among many affecting factors on semen characteristics including nutrition, social environment, presence of the female, geographical location, age, testicle traits, body conformation, libido and management system that are perused in many studies (Al-Ghalban *et al*., 2004; Zamiri and Khodaei, 2005; Zarazaga *et al*., 2005) photoperiod

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and breed are the primary treatments that are proximately related to the seminal traits of these species. Therefore, photoperiod and breed became preferable for many researchers (Ibrahim, 1997; Karagiannidis *et al*., 2000; Kafi *et al*., 2004; Barkawi *et al*., 2006; Talebi *et al*., 2009; Zamiri *et al*., 2010). In some of the reports a significant number of ewes were inseminated in non-breeding season (Colas *et al*., 1990), therefore detection of semen characteristics of the crossbred rams in non-breeding season is necessary for reaching the similar goals. Although information is available on the level of ram semen fertility (Mohamed, 1978; Ibrahim, 1997) but this study is the first report from reproductive characteristics of the crossbred rams. The target of producing these crossbred rams were genetic improvement of local breeds (Baluchi and Ghezel) for wool and meat traits (Rafat and Shodja, 2010; Esfandyari *et al*., 2011). Moreover, evaluation of photoperiod and its effect on semen characteristics was the other objective of this study to pursue the effect of genetic group on semen traits, in order to detect the best genetic group for breeding aims in non-breeding seasons.

MATERIAL AND METHODS

Geographical location

This study was carried out from October 2010 to September 2011 at the Khalatposhan Agricultural Research Station, University of Tabriz, Iran. The Research centre is located in the suburb of Tabriz (38º02‘ N, 46º27‘ E and an altitude of 1567 m above sea level).

Animals and management

Ten (n = 10) crossbred rams of the two genetic groups including 5 Ghezel×Baluchi (GH×BL) and 5 Arkharmerino×Ghezel (AM×GH) were used in the study. During 15 days the rams (2-6 years old) were trained (in breeding season) to semen collection by artificial vagina (AV) by the presence of the operator and in the mating pen. Training and sampling were performed via an anoestrous and quiet temperament ewe. The separated rams of the herd were housed in a large cover shelter with an open precinct in order to walk freely. 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. 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 managements were checked during the study.

Semen collection and evaluation

The ten rams were divided into two groups, and each group included 5 rams. Semen collection was followed for 2 days and every day from 5 rams. Ejaculate intervals for each ram was 5 days and it was maintained throughout the study. The short form artificial vagina (40 to 42 ºC) was used for semen collection. A ewe with quiet temperament was used for mounting by the rams. The fresh semen samples taken from the rams were transferred to the laboratory (maintained at 37 ºC) and were surveyed. Seminal traits of the fresh semen were evaluated according to Evans and Maxwell (1987). Semen volume (SV) was recorded as soon as possible after collection using a graduated collecting glass (0.1^{CC} accuracy). Semen pH was evaluated 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 using 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. The total number of spermatozoa per ejaculate (TSE) was calculated (volume × density). Wave motion of fresh semen was evaluated (100 × magnification) according to Evans and Maxwell, (1987), as it was made on the basis of a scale from 0 to 5 (0 = all spermatozoa are motionless, 5 = 90 % or more of the spermatozoa are rapidly moving waves). The assessment of the spermatozoa progressive motility visually scaled from 0 – 100 % on the basis of suspended droplet slide and on a heated (37 ºC) stage using phase-contrast optics (× 400). It has been evaluated in increments of 5 or 10 percentage points. This slide showed individual spermatozoa with more lucidity, as it will be more comfortable for estimating spermatozoa progressive motility. For spermatozoa morphology and spermatozoa live/dead ratio, semen was stained with eosin-nigrosin stain followed by microscopic examination (× 400). Spermatozoa with red head were counted as dead cells and the colourless ones as live spermatozoa. From several parts of the slide about 300 spermatozoa were counted. Metabolic activity of spermatozoa were measured using MBRT method based on colour change from blue to colourless at 37 ºC. In a thin and transparent tube (1mm diameter), 0.2 ml semen was added to 0.2 ml of methylene blue, the time was recorded immediately (per second) in order to get colourless mixture. Semen index (semen volume × spermatozoa concentration/ml × live spermatozoa % × progressive motility %) was calculated, as an indicator of semen quality.

Statistical analysis

Data were expressed as means (x ± S.E.) and all statistical analyses were performed using the Statistical Analysis System (SAS 1996). The analysis was done by using Proc Mixed of SAS (Zamiri *et al*., 2010). For volume, SC, abnormality and MBRT traits the outlier data were deleted. Values were considered to be statistically

significant at P<0.05. Means were compared with the Tukey test. Pearson correlation coefficient was calculated to evaluate the relationship between the quality and quantity of semen traits.

RESULTS AND DISCUSSION

Mean values, standard error, minimum and maximum of semen characteristics in both genetic groups are shown in table 1. The results of seasonal variations of semen characteristics have been shown in table 2 for quantity traits and table 3 about quality semen traits. Effect of photoperiod was observed on semen characteristics and clearly it affected the semen quantity traits (table 4). Semen quantity traits included semen volume, SC, TSE and semen colour (as the index for density estimate), which were significantly influenced by the season of the year (P<0.01). In AM×GH and GH×BL genetic groups, minimum and maximum values of the semen volume were recorded in spring and autumn respectively (P<0.01). Frequently, semen volume increased since the end of June and reached the highest mean values in October, and again decreased gradually at the end of October. This reducing trend followed during autumn and winter. In the AM×GH genetic group differences between the spring (non-breeding season) and the other seasons were significant (P<0.01). But in GH×BL the significant differences between spring and autumn (P<0.01) and between winter and autumn (P<0.01) were noted. This pattern of seasonal variation in semen volume was similarly repeated in SC, TSE and in semen colour too, so that mean values in autumn and/or summer were the peak of amount, and in spring and/or winter were the lowest mean values. However, in winter the amount of TSE in both genetic groups, and values of SC in AM×GH rams were higher than in summer and autumn (P<0.05). Predominantly, the lowest mean values of the quantity traits were recorded in spring, except for the SC in GH×BL, as it was the lowest in winter. In both genetic groups mean value of semen pH increased in the spring (peak value was in May) (P<0.01), while in summer, autumn and winter we did not observe any significant differences. The values of this trait decreased in breeding season (summer and autumn). The highest value of semen index was observed during autumn (in both genetic groups in October) and the lowest value was in spring (June) (P<0.01). In AM×GH rams there were no significant differences in wave motion for all the months. In GH×BL rams only significant difference between spring and autumn was recorded (P<0.05). The results in the AM×GH and GH×BL rams demonstrated that individual progressive motility of spermatozoa was higher during the breeding seasons (autumn and then summer). In AM×GH the highest value was noted in October (75.50 ± 1.65) and the lowest in June (69.90 ± 1.67). On the other hand, in GH×BL rams the highest levels were recorded in September (72.5 ± 1.60) and the lowest amount in June (65.50 ± 1.62). In GH×BL genetic group the highest and the lowest percentages of live

Table 1: Basic statistical characteristics of semen in Ghezel × Baluchi and Arkharmerino × Ghezel 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 (sec)
GH × BL	N	334	334	334	334	334	334	334	330	334	334	331
	Mean	1.16	3.80	68.97	3.47	4.62	3.47	71.45	11.37	19871	6.65	115.80
	S.E.	0.08	0.09	1.70	0.16	0.32	0.18	1.60	0.78	1823.45	0.09	3.10
	Min	0.55	2.00	40.00	2.00	0.885	1.07	40.00	3.00	1140	5.90	65.00
	Max	1.85	5.00	85.00	5.00	23.40	5.80	93.00	28.00	50733.28	8.20	230.00
AM × GH	N	334	334	334	334	334	334	334	330	334	334	331
	Mean	1.03	4.00	71.68	3.41	4.952	3.461	73.51	10.76	18925.31	6.45	111.18
	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	0.50	2.00	45.00	2.00	1.287	1.49	43.00	4.00	2088.5	5.60	55.00
	Max	1.70	5.00	90.00	5.00	17.57	5.44	90.00	28.00	44001.2	7.70	195.00

SV = semen volume, WM = wave motion, PM = progressive motility, SC = semen colour, 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

spermatozoa were recorded in September (72.50 ± 1.60) and in June (65.5 ± 1.61), respectively and in case of the AM×GH rams in October (75.5 ± 1.65) and in June (69.90 ± 1.67), respectively. Among the types of the spermatozoa abnormality, mostly tail abnormalities were observed. In spite of these facts, semen quality from the viewpoint of spermatozoa normality was significantly improved during autumn (in AM×GH) and summer (in GH×BL). Season and genetic group in AM×GH and GH×BL did not have a significant influence on the rate of metabolic activity and wave motion of spermatozoa (P>0.05). However, an inconsiderable seasonal fluctuation was observed in the genetic groups about these traits. The lowest values of MBRT were in autumn for GH×BL and in summer for AM×GH. The correlation between various semen characteristics are presented in table 5. Percentage of live spermatozoa correlated with motility parameters (P<0.01), spermatozoa density (r = 0.14, P<0.01) and semen colour (r = 0.11, P<0.05). Semen volume had a relationship with SC, colour and TSE as showed by the positive correlation of 0.16, 0.13 and 0.42, respectively. The results showed that MBRT decreased over time. MBRT significantly correlated with all of the semen traits (P<0.01) except for the semen volume. Percentage of abnormal spermatozoa was not in correlation with quantity traits, however it significantly correlated with wave motion (r = -0.69, P<0.01), progressive motility (r = - 0.89, P<0.01), percentage of live spermatozoa (r=-0.94, P<0.01). Wave motion and individual progressive motility of spermatozoa showed a significant correlation with semen density (r = 0.19 and r = 0.14 respectively) and semen pH (r = - 0.15, r = - 0.11 respectively). Moreover, semen pH showed a high negative correlation with SC (r = - 0.4, P<0.01).

In this study an obvious influence of photoperiod on semen characteristics was observed and this effect was more prominent in quantitative traits (see table 2). This study is the first report on the seasonal variations in seminal characteristics of the two crossbred rams Ghezel×Baluchi and Arkharmerino×Ghezel in Iran. The effect of season and/or photoperiod on semen quality

Table 2: Seasonal variations in semen quantity (mean±S.E.) of Ghezel × Baluchi and Arkharmerino × Ghezel rams

Semen quantity	Season	GH×BL	AM×GH
Total spermatozoa/ejaculate (×10^9)	Spring	3.537±0.426^{b}	2.852±0.473^{b}
	Summer	4.771±0.310^{ab}	3.912±0.290^{ab}
	Autumn	5.080±0.288^{a}	4.327±0.285^{a}
	Winter	5.113±0.306^{a}	4.718±0.306^{a}
	Mean	4.623±0.326	3.952±0.327
Spermatozoa concentration (×10^9)	Spring	3.408±0.195^{ab}	3.125±0.203^{b}
	Summer	3.510±0.177^{a}	3.394±0.185^{a}
	Autumn	3.682±0.176^{a}	3.630±0.176^{a}
	Winter	3.328±0.179^{b}	3.681±0.179^{a}
	Mean	3.472±0.183	3.461±0.182
Semen volume (ml)	Spring	1.07±0.09^{b}	0.89±0.09^{b}
	Summer	1.14±0.08^{ab}	1.01±0.08^{a}
	Autumn	1.35±0.09^{a}	1.16±0.09^{a}
	Winter	1.08±0.09^{b}	1.07±0.08^{a}
	Mean	1.16±0.08	1.03±0.08
Semen colour (0-5)	Spring	3.375±0.171^{c}	3.125±0.177^{c}
	Summer	3.462±0.166^{b}	3.243±0.173^{bc}
	Autumn	3.659±0.159^{a}	3.583±0.158^{ab}
	Winter	3.390±0.161^{c}	3.687±0.161^{a}
	Mean	3.477±0.164	3.419±0.169

$^{a, b, c}$ Means in the column of each parameter with different superscripts differ significantly (P < 0.05).

Table 3: Seasonal variations in semen quality (mean±S.E.) of Ghezel × Baluchi and Arkharmerino × Ghezel rams

Semen quality	Season	GH×BL	AM×GH
Wave motion (0-5)	Spring	3.70±0.10^{b}	3.93±0.09^{a}
	Summer	3.83±0.09^{ab}	4.00±0.09^{a}
	Autumn	3.95±0.09^{a}	4.09±0.11^{a}
	Winter	3.75±0.11^{ab}	3.99±0.09^{a}
	Mean	3.80±0.09	4.00±0.09
Progressive motility (%)	Spring	66.24±1.61^{b}	70.06±1.75^{b}
	Summer	69.78±1.54^{a}	71.31±1.61^{ab}
	Autumn	71.82±1.50^{a}	74.64±1.50^{a}
	Winter	68.15±1.73^{ab}	70.79±1.54^{b}
	Mean	68.97±1.70	71.68±1.59
Live spermatozoa (%)	Spring	69.61±1.68^{b}	71.73±1.67^{ab}
	Summer	71.09±1.66^{ab}	74.75±1.66^{ab}
	Autumn	75.02±1.51^{a}	76.02±1.51^{a}
	Winter	70.10±1.55^{b}	71.56±1.51^{b}
	Mean	71.45±1.60	73.51±1.57
Abnormal spermatozoa (%)	Spring	12.61±0.79^{a}	11.47±0.71^{a}
	Summer	9.91±0.79^{b}	10.01±0.60^{a}
	Autumn	11.03±0.77^{ab}	9.02±0.77^{b}
	Winter	11.94±0.80^{ab}	12.54±0.79^{a}
	Mean	11.37±0.78	10.76±0.71
Semen index ($\times 10^{9}$)	Spring	16747±1960.25^{c}	13428±1813.59^{b}
	Summer	20316±1843.22^{b}	20817±1756.40^{a}
	Autumn	25121±1733.68^{a}	22211±1728.49^{a}
	Winter	17243±1773.36^{c}	19251±1773.36^{a}
	Mean	19871±1823.45	18925±1767.96
Semen pH	Spring	6.92±0.10^{a}	6.67±0.10^{a}
	Summer	6.52±0.09^{b}	6.40±0.09^{b}
	Autumn	6.56±0.09^{b}	6.39±0.09^{b}
	Winter	6.66±0.09^{b}	6.36±0.09^{b}
	Mean	6.65±0.09	6.45±0.09
MBRT (sec)	Spring	117.68±4.10^{a}	116.99±4.39^{a}
	Summer	114.36±3.07^{a}	107.81±3.09^{a}
	Autumn	111.85±3.07^{a}	109.36±3.06^{a}
	Winter	119.42±3.14^{a}	110.56±3.19^{a}
	Mean	115.80±3.10	111.18±3.17

Means in the column of each variable with different superscripts differ significantly (P<0.05).
Means within each column within each factor having the same letter did not differ significantly from each other (P<0.05).

and quantity has been previously studied in different breeds of rams (Amir *et al.,* 1986; Karagiannidis *et al*., 2000; Kafi *et al*., 2004; Zamiri and Khodaei, 2005; Deldar Tajangookeh *et al*., 2007) and other seasonal breeding animals such as buck (Barkawi *et al*., 2006; Karagiannidis *et al*., 1999) and stallion (Janett *et al*., 2003). Gerlach and Aurich (2000) illustrated that in seasonally breeding male, sperm production is lower in the non-breeding

season. Season significantly affected some of the seminal characteristics. Among the quality characteristics of sperm, a significant effect of season was recorded on spermatozoa progressive motility, percentage of live spermatozoa and abnormal spermatozoa, semen pH and semen index. Moreover, the effect of photoperiod was also observed in semen quantity characteristics. These seasonal variations in both semen quality and quantity are mainly due to the changes in daylight length throughout the year (Chemineau *et al*., 1992). No significant differences were found among 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. It is consistent with the results of Karagiannidis *et al.*, (2000). The results of mean value of semen characteristics in our study were in agreement with other researchers (Al-Ghalban *et al*., 2004; Gundogan, 2007; Zamiri *et al*., 2010). The mean values of their reports included semen volume (0.60 –1.6 ml) SC (2.6 –5.5 × 10^9), percentage of abnormal spermatozoa (4 - 29 %) and percentage of live or motile spermatozoa (60 – 90 %) (Karagiannidis *et al*., 2000; Kafi *et al*., 2004). Therefore, it could be accepted that there is a wide range of this value in several breeds of ram. In the current study mean values for the abnormal spermatozoa of the crosses were generally higher than those reported by Karagiannidis *et al*., (2000) for Chios and Friesian in Northern Greece, Gundogan, (2007) for Akkarman and Awassi in Turkey. In the study of Zamiri *et al.*, (2010) in Moghani breed, minimum value of spermatozoa abnormality was observed in September (7.9 %), which was less than the minimum value in our study. In other words, spermatozoa abnormality in the GH×BL and AM×GH rams was 9.31 % and 9.16 % respectively in the present study. Percentage of live spermatozoa in each of two genetic groups was lower than the values of Kafi *et al*., (2004) in southern Iran (29º 25‘ N, 52º 46‘ E). The mean value of semen volume in the GH×BL

Table 4: Effect of season and ram on semen characteristics of ejaculates obtained during 1 year

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

* P < 0.05, ** P < 0.01, NS= not significant different.
SV= semen volume, WM= wave motion, PM= sperm progressive motility, TSE= total spermatozoa per ejaculate, Conc= concentration, SL= Percentage of live spermatozoa, SAB= Percentage of abnormal spermatozoa, SI= semen index, MBRT= methylene blue reduction time.

Table 5: Correlation coefficients between various semen traits of the rams

	WM	PM	Color	TSE	Conc	SL	SAB	SI	pH	MBRT
SV	-0.04	0.05	0.13**	0.42**	0.16**	0.06	-0.05	0.73**	-0.003	-0.07
WM		0.74**	0.15**	0.001	0.19**	0.70**	-0.69**	0.34**	-0.15**	-0.68**
PM			0.11*	0.01	0.14**	0.91**	-0.89**	0.53**	-0.11*	-0.77**
Color				0.29**	0.93**	0.11*	-0.08	0.47**	-0.34**	-0.54**
TSE					0.30**	0.03	-0.05	0.42**	-0.09	-0.19**
Conc						0.14**	-0.18	0.52**	-0.40**	-0.57**
SL							-0.94**	0.54**	-0.09	-0.78**
SAB								-0.52**	0.03	0.70**
SI									-0.14**	-0.64**
pH										0.17**

* P < 0.05, ** P < 0.01
SV= semen volume, WM= wave motion, PM= sperm progressive motility, TSE= total spermatozoa per ejaculate, Conc= concentration, SL= Percentage of live spermatozoa, SAB= Percentage of abnormal spermatozoa, SI= semen index, MBRT= methylene blue reduction time.

(1.16 ± 0.08) was in agreement with the results of Kafi *et al*., (2004) but it was lower for the AM×GH rams (1.03 ± 0.08). So the comparison of reported data regarding seminal traits is often difficult, it is therefore not surprising that wide amplitude of values have been reported in the literature regarding the seminal characteristics of rams (Gundogan, 2007; Zamiri and Khodaei, 2005; Kafi *et al*., 2004; Karagiannidis *et al*., 2000). In GH×BL during autumn (3.682 ± 0.176), the spermatozoa concentration was high, and lower concentrations were recorded in winter (3.328 ± 0.179), spring (3.408 ± 0.195) and summer (3.51 ± 0.177), respectively. This trend is comparable with the results of Karagiannidis *et al*., (2000) and Talebi *et al*., (2009). These findings confirmed our records of seasonal variations in spermatozoa concentration in GH×BL rams at 38º N latitude. In both of the crosses, reasons of seasonal fluctuations of semen colour and spermatozoa density is very similar. In our study, most of the mean values of the semen characteristics of GH×BL and AM×GH rams (38º 02‘ N, 46º 27‘ E) were almost similar to those reported by other authors in similar temperate regions (Zamiri *et al*., 2010; Gundogan, 2007; Barkawi *et al*., 2006). Therefore, the difference between breeding seasons (late of summer to middle of autumn) and non-breeding seasons, in quantity and quality traits showed that there is significant seasonal trend (P<0.01) in the crossbred rams. In this study, SC followed a similar process like that of the ejaculate volume but it is not in accordance with the results of Talebi *et al*., (2009). Mean amounts of MBRT in our study are rather higher than values reported by Galal *et al*., (1978). But in Egypt (Galal *et al.*, 1978), while studying Merino, Ossimi and their crosses, the best metabolic activity was recorded in spring (76.8 ± 1.04 sec) and autumn (77.2 ± 1.04 sec), and their highest mean values were reported in summer (102.2 ± 1.04 sec). Unlike the findings of Galal et al. (1978) we did not note any significant difference between several seasons of the year in terms of MBRT. Semen characteristics were generally better at the end of summer and the two first months of autumn, than during the winter and spring. In both the genetic groups the lowest mean values of spermatozoa progressive motility were recorded in spring than in winter, although it was in contrast to Karagiannidis *et al*., (2000) at 40º N. As reported previously, the photoperiodic effects on seasonal breeds are determined by the latitude at which they are kept. At latitudes above 40º N, the variations in seminal characteristics are very marked and sperm production increases significantly as daylight length decreases. Seasonal variations, although less marked, were observed between 30º N and 40º N latitude, with higher sperm production during the summer and autumn (Corteel, 1977). Although, the crossbred rams were capable of ejaculation throughout the year, however a few of the rams failed to mount or to ejaculate in non-breeding season. Seasonal breeding animals are a phenomenon occurring in the middle latitudes. Based on the high significant correlation between motility characteristics and percentage of live spermatozoa, it could be concluded that, following the increase in wave motion, the amount of live spermatozoa will be increased. These findings coincided with those of Kafi *et al.*, (2004). However, unlike the reports of Kafi *et al*., (2004) no significant correlation was found between motility and total spermatozoa per ejaculate. MBRT, the method used for metabolic status evaluation of the semen (Salisbury *et al*., 1978) showed high negative correlation with motility traits (P<0.01), which is not consistent with the results of Kishk (2008), while it is in agreement with the findings of Chandler *et al.*, (2000). Methylene blue is a redox dye that changes colour on the basis of the reduction after the addition of hydrogen. Thus respiration rate of spermatozoa at the dense semen leads to rapid reduction of methylene blue. It was agreed with the present study and the study of Kishk (2008), who observed there is the high negative correlation between SC and MBRT (r = - 0.57, P<0.01). High negative correlation between MBRT and the percentage of live spermatozoa (r = - 0.78, P<0.01) showed that following increase in live spermatozoa and progressive motility in semen sample, the rate of hydrogen releasing and thereupon the fructose utilization by sperm cells increased. Then these samples will become acidic in a short time, so long-term storage of these samples is not reliable. The strongest relationship among semen traits with semen pH was observed between motility parameters (r = - 0.15 and – 0.11) and SC (r = - 0.40, P<0.01) and semen index (r = - 0.14, P<0.01). Among the quantity traits, only a significant correlation between MBRT with TSE and sperm density was observed. Thus, it showed that there is no significant correlation between quality and quantity traits of semen. However, Karagiannidis *et al*., (2000) reported a significant correlation between SC and percentage of abnormal spermatozoa. To survey the reproductive activity of the seasonal breeding animals e.g., rams, we should study circannual reproductive rhythm (endogenous mechanisms) and exogenous factors (Gomez-Brunet *et al*., 2012). The most important exogenous factor is photoperiod. It is responsible for the synchronization of reproductive activity with the environment but not for the generation of a circannual reproductive rhythm. In fact an endogenous rhythm exists in the absence of any photic stimulus and therefore the role of photoperiod is to synchronise, but not to create this rhythm. The pineal hormone melatonin is the common link between photoperiod and reproduction (Gerlach and Aurich, 2000). Reproductive activity is not a direct function of day length, but it 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 (Gorman and Zucker, 1995a). Among species that use photoperiod to synchronise breeding activity, sheep is detailed as a short-day breed. But the important point is to know the level of photoperiod influence on the reproductive activity of various breeds. The animals live under the influence of seasonal fluctuations of environmental conditions, with variable amplitudes, which are more marked in the higher latitudes and altitudes (Rosa and Bryant, 2003). Also, supported by the works of Hafez, (1952) and Robinson, (1981) it is argued that breeds whose origins are located between 35º N and 35º S have the tendency to breed at all times of the year. Evans and Maxell (1987) reported the same for breeds whose origins are located between 30º N and 40º N. While at latitudes above 35º N (Hafez, 1952; Goot, 1969) or higher than 40º N (Talebi *et al*., 2009; Zamiri *et al*., 2010) considerable variations were recorded in the seminal characteristics. However in some of the studies for example in Jordan (at 31.5º N latitude and altitude of 350 m, in Damascus bucks) and Iran (34º 18‘ N, 47º 3‘ E, in Markhoz bucks) a significant effect of photoperiod has been found. Dacheux *et al.*, (1981) stated that in temperate latitudes (40º N to 50º N) ram sperm production is a continuous process, but the total number of spermatozoa produced per testis is usually higher in autumn than in spring (Dacheux *et al*., 1981). In the study of Arrebola *et al*., (2010) on the Mediterranean bucks (maintained at 38º N) using photoperiod treatment it was illustrated that no significant difference exists in the annual mean number of sperm per ejaculate between the photoperiod intervals. The results showed that photoperiod treatment allowed adequate sperm production in winter.

CONCLUSIONS

The semen characteristics of GH×BL and AM×GH rams in Northwest of Iran showed a significant seasonal variation in semen quality and quantity. The best semen is produced during late summer up to the second month of autumn. Nonetheless, the magnitude of these seasonal effects should not prevent the animals from breeding, or semen collecting for AI throughout the year. In general, it is concluded that a high reproductive performance can be increased when the crosses are introduced to oestrous ewes in late August till the end of October (breeding season). However, the existence of differences among rams in semen characteristics makes it necessary to perform a semen evaluation on an individual basis for every ram used for artificial insemination or breeding. Therefore it will provide an optimum breeding selection of males in herd.

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