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Seasonal Changes in Serum Testosterone, LDH Concentration and Semen Characteristics in Markhoz Goats

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ABSTRACT : This experiment was conducted to study variations of serum testosterone and seminal characteristics of Markhoz male goats. Blood samples were obtained via jugular vein, and semen was collected by using an artificial vagina from 14 fertile male goats (2-3 years of age), at 15-day intervals starting on 15 July and ending on 30 October 2010 (during breeding and non-breeding season). Semen volume, total sperm (volume×concentration), live sperm (%), abnormal sperm (%) and semen pH were significantly superior during the late summer and early autumn (breeding season). Variation of sperm density, motility and progressive motility was not significant during the sampling period. The results presented show that the lowest and highest levels of lactate dehydrogenase in the seminal plasma were recorded in late October (2.82 U/ml) and in late August (4.81 U/ml), respectively. Moreover, the study indicated that the serum testosterone concentration was higher during late summer and early autumn ($p<0.05$) than at any other of sampling period. There were negative correlations between volume and sperm density (-0.135, $p<0.05$), and positive correlations between volume and percentage live sperm (0.224) and percentage progressive motility (0.194, $p<0.01$). Sperm density was correlated with live sperm (0.200, $p<0.05$) and progressive motility (0.202, $p<0.01$). The correlation between live sperm and progressive motility was 0.554 ($p<0.01$). Furthermore, the results in this study indicated a significant positive correlation between live sperm and LDH (0.450) and a negative correlation between sperm density and LDH concentration (-0.272) ($p<0.01$). Significant, but positive correlations were found between sperm motility and LDH (0.542) and testosterone concentration (0.522), respectively ($p<0.05$). In conclusion, this study demonstrated that the best obtained semen was collected in late summer (during decreasing photoperiod) and early autumn (September and October). This also coincides with the natural breeding season of Markhoz goats in Iran. (**Key Words :** Markhoz Goat, Semen Characteristics, Testosterone, Lactate Dehydrogenase)

INTRODUCTION

Male goats of temperate latitudes are seasonal breeders. It is well known that the sexual behavior, semen quality and quantity are the main factors that limit male reproductive efficiency during the year. These factors could vary according to different environmental and physiological factors such as climate (Ibrahim, 1997; Zarei et al., 2009); latitude, breed season of the year (Roca et al., 1992a; Karagiannidis et al., 2000), testicular size (Ahmad and Noakes, 1995) and circulating gonadotropins (Perez and Mateos, 1996; Hammaudi, 2010). Seasons however, seem to be the principle cue affecting semen quality in goats (Barkawi et al., 2006). Seasonal variations of fertility in goats are mainly due to the change of day length throughout the year (Chemineau et al., 1992; Talebi et al., 2009). Short days stimulate secretion of luteinizing hormone (LH), which in turn, induces testicular growth, release of testosterone, sperm production, mating activity and fertility (La Falci et al., 2002). Testosterone is the hormone responsible for spermatogenesis and sexual behavior, thus the seasonal pattern of testosterone secretion could limit the male reproductive efficiency during some periods of the year (Todini et al., 2007). Lactate dehydrogenase (LDH) is essential for metabolic processes which provide energy for survival, motility, capacitation and fertility of spermatozoa (Sirat et al., 1996). It has been proposed that the seminal fluid LDH can be used as good indicator of sperm viability (Stamatiads et al., 1984). Markhoz goat, known as Angora

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goat in other places, is one of the most important goat breeds in Iran. This breed presents a seasonality in reproductive activity and the breeding season commences in the September-October with the maximum reproductive activity in November (Farshad et al., 2008; Talebi et al., 2009. However it is currently considered a breed at risk of extinction. In this context, knowledge of the factors affecting variation in semen quality is an essential parameter that can be used to improve this breed of Iran.

MATERIAL AND METHODS

Geography and climate

This study was performed at the testing station, located in Sanandaj, Iran (35°20′ N latitude, 47° E longitude and 1,373 m above sea) during the breeding (September to October) and non-breeding (July to August) season. Climatologically information for this location during the experimental year is summarized in Table 1.

Animals

Fourteen fertile Markhoz male goats were chosen randomly from among the animals available at the testing station. They were 2 to 3 years old and weighed from 35 to 45 kg at the start of the experiment. The animals were kept under natural photoperiod, and nutritional levels were adjusted to meet maintenance requirements (NRC, 1981). Each buck was fed daily with 2 kg alfalfa hay and 0.5 kg commercial concentrate. As calculated, the daily feed supplied 1.9 Mcal metabolizable energy and 300 g crude protein (DM basis). All bucks had free access to water and mineral blocks.

Semen collection and quality evaluation

Semen was collected at 15-day intervals by an artificial vagina (42 to 43°C) using a female goat as a mount. Within 15 min after collection, the semen was taken to the laboratory and kept in a water bath at 37°C. Fresh ejaculates were evaluated for volume, motility (diluted with normal saline), progressive motility, live spermatozoa (eosin nigrosin stain), sperm concentration or density (sperm cells were counted in four squares of a hemocytometer after 1:200 dilution of semen with 0.5% eosin solutions) and semen pH and then the number of spermatozoa per ejaculate was calculated (volume×density). To evaluate progressive motility, a sample of the diluted spermatozoa was placed under a cover slip in the center of pre-warmed (37°C) slide, transferred to a heated microscope stage set at 37°C and subjectively assessed by phase contrast microscopy (×100 magnification). The percentage of live sperm was determined using a modification of eosin-nigrosin stain procedure described by Chauhan and Anand (1990). A mixture of 10 μl of diluted spermatozoa and 10 μl Eosin- nigrosin stain were smeared on slide and allowed to air dry in a dust-free environment. Two hundred spermatozoa from different microscopic fields were examined under a bright-field microscope using a 400× objective, and the number of non-stained (viable) spermatozoa was counted. The ejaculated semen volume was recorded immediately after collection from a graduated collection vial. The seminal pH was also directly measured using a digital pH meter (microprocessor 211- Hanna, Italy). After examination of the spermatozoa the remaining part of the ejaculates were centrifuged for 30 min at 10,000 rpm and the seminal fluid aspirated. Lactate dehydrogenase (LDH) activity in seminal fluid was measured (Moss and Handerson, 1994) immediately after centrifugation, using a commercially available kit (Pars-Azmoon co, Tehran; LDH analyzer, Kone co., Finland). Blood samples were collected at 8-9 am, using syringes without anticoagulant. Blood samples were centrifuged within 1 h of collection at 3,500 rpm for 15 min and serum stored at -20°C for later testosterone assay using a commercial RIA kit (Demeditec Diagnostic, GmbH, Germany). The intra- and inter assay coefficients of variation were 5.1 and 7.9 respectively.

Statistical analyses

All percentage data were transformed to arcsine, and subjected to repeated measures ANOVA, using Proc mixed of SAS (SAS, 1999). The AR (1) covariance structure was selected, based on Schwarz Bayesian criterion (Littell et al., 1998). Least squares means and standard errors are reported in the text.

RESULTS

Table 2 presents the semen characteristics of Markhoz

Table 1. Climatological data for the experimental year

Month	Air temperature (°C)			Relative humidity (%)		Average day length (h)
	Mean	Minimum	Maximum	Minimum	Maximum	
July	26.1	16.4	30.3	8	35	12
August	27.8	17.8	37.0	11	47	11
September	18.7	11.2	28.2	16	61	10
October	14	7.6	23.3	23	69	9

Table 2. Least squares means (±SEM) of semen characteristics of Markhoz goats during the sampling period

Period	Semen characteristics									
	Semen volume (ml)	Spem density (10^9ml^{-1})^{NS}	Total sperm (10^9)	Live sperm (%)	Abnormal sperm (%)	Motility (%)^{NS}	Progressive motility (%)^{NS}	pH	Testosterone (ng/ml)	LDH (U/ml)
15 Jul	0.94±0.04 ^{cd}*	3.78±0.05	3.53±0.22^{a}	89.78±0.24^{b}	9.57±0.27^{a}	85±0.74	78.78±0.74	6.97±0.02^{a}	3.08±0.25^{a}	4.53±0.08^{a}
30 Jul	0.97±0.03 ^{cd}	3.75±0.06	3.61±0.23^{a}	90±0.23^{b}	9.60±0.26^{a}	85.14±0.75	78.43±0.75	6.95±0.02^{a}	2.85±0.26^{a}	4.49±0.08^{a}
15 Aug	0.98±0.03 ^{cd}	3.78±0.06	3.64±0.2^{a}	90±0.26^{b}	9.74±0.26^{a}	85.14±0.72	78.43±0.72	6.91±0.05^{a}	3.02±0.51^{a}	4.32±0.07^{a}
30 Aug	1.01±0.03 ^{bc}	3.78±0.06	3.77±0.22^{ab}	89.93±0.29^{b}	9.52±0.27^{a}	85.07±0.71	79.14±0.71	7±0.02^{a}	2.93±0.38^{a}	4.81±0.09^{a}
15 Sep	1.10±0.03 ^{ab}	3.87±0.05	4.22±0.20^{b}	90.85±0.25^{a}	8.7±0.18^{b}	86.57±0.78	80.35±1.17	6.74±0.08^{a}	5.16±0.43^{b}	3.47±0.36^{b}
30 Sep	1.12±0.04 ^{a}	3.94±0.05	4.37±0.20^{b}	90.78±0.22^{a}	8.72±0.19^{b}	86.86±0.81	80.35±1.22	6.85±0.05^{a}	4.85±0.51^{b}	3.30±0.11^{b}
15 Oct	1.14±0.03 ^{a}	3.92±0.05	4.44±0.23^{b}	90.85±0.23^{a}	8.68±0.18^{b}	86.71±0.79	80.50±1.26	6.82±0.05^{a}	4.57±0.4^{b}	3.12±0.1^{bc}
30 Oct	1.15±0.03 ^{a}	3.92±0.05	4.45±0.20^{b}	91.21±0.3^{a}	8.69±0.17^{b}	86.64±0.79	80.35±1.25	6.83±0.05^{a}	4.69±0.42^{b}	2.82±0.14^{c}

* Means with the same superscript (s) are not significantly different. NS = Not significant (p>0.05).

bucks collected during the breeding and non-breeding season. Significant differences (p<0.05) were observed for the various measurements, except for sperm density, motility and progressive motility, during the recording period. Within each season, there was no significant differences in semen characteristics, LDH and serum testosterone concentration. As indicated in Table 2, semen volume, sperm density, motility, progressive motility, total sperm, live sperm serum testosterone showed a uniform pattern with low scores during non-breeding season and high scores in breeding season. The percentage abnormal sperm, semen pH value and seminal LDH levels increased during the non-breeding season. Mean serum testosterone concentrations and seminal LDH levels (±SEM) and photoperiod changes during the recording period are set out Table 1 and 2.

There were negative correlations between volume and sperm density (-0.135, p<0.05), and positive correlations between volume and percentage live sperm (0.224) and percentage progressive motility (0.194, both p<0.01). Sperm density was correlated with live sperm (0.200, p<0.05) and progressive motility (0.202, p<0.01). The correlation between live sperm and progressive motility was 0.554 (p<0.01).There was a significant positive correlation between live sperm and LDH (0.450) and a negative correlation between sperm density and LDH concentration (-0.272) (p<0.01). Significant, but positive correlations were found between sperm motility and LDH (0.542) and testosterone concentration (0.522), respectively (p<0.05).

DISCUSSION

Reproductive activity in the Markhoz goat is highly seasonal and closely related to changes in the photoperiod (Talebi et al., 2009). Seasonal variation in quality and quantity of goat semen has been associated with environmental factors such as sinusoidal changes of photoperiod or/and air temperature variations (Roca et al., 1992a; Hammoudi et al., 2010). Photoperiodic influences on seasonal breeders depend mostly on the latitude at which they are kept. In latitudes above 40°N, the variation in seminal characteristics is very marked (Corteel, 1977) and sperm production increases significantly when day length is decreased. Seasonal variations, although less marked, are observed between 30°N and 40° latitudes, with higher sperm production during summer and autumn (Evans and Maxwell, 1987). Markhoz goats live at latitude of 35°N, and can be included in this category of seasonal breeders. The results of this study show that Markhoz bucks have seasonal reproductive tendencies as indicated by the variation in semen characteristics (Table 3). These results are in agreement with the finding of Roca et al. (1992a) with Murciano-Granadina goats, Barkawi et al. (2006) with Zaraibi goats and Talebi et al. (2009) with Markhoz goats.

Table 3. Semen characteristics of Markhoz goats during the breeding and the non-breeding season

	Semen characteristics									
	Semen volume (ml)	Spem density (10^9ml^{-1})	Total sperm (10^9)	Live sperm (%)	Abnormal sperm (%)	Motility (%)	Progressive motility (%)	pH	LDH (U/ml)	Testosterone (ng/ml)
Breeding season	1.14±0.04	3.91±0.03	4.37±0.22	90.93±0.12	8.7±0.09	86.7±0.39	80.39±0.61	6.81±0.03	3.19±0.10	4.82±0.22
Non-breeding season	0.97±0.02	3.77±0.03	3.64±0.22	89.93±0.14	9.61±0.14	85.09±0.37	78.69±0.47	6.96±0.02	4.38±0.04	2.97±0.19
	*	**	*	**	*	**	*	*	*	**

* p<0.05. ** p<0.01.

The goats of temperate zones express an important variation in their sexual activity. There is a period of minimal sexual activity which last from February to September. As with other breeds (Zamiri and Khodaei, 2005) the highest values for most parameters were found from the end of summer to autumn and the lowest values were recorded for many parameters in early summer. These findings were in agrrement with the report of Talebi et al. (2009) and the results in this Study. The significant seasonal differences in semen volume observed in the present study have also been reported for the other breeds of goats under different environmental conditions (Loubser and Von Niekerk, 1983; Delgadillo and Chemineau, 1992; Tuli and Holtz, 1992). However, Grayling and Grobbeloar (1983) observed no significant seasonal variation in the semen volume of Boer goat bucks in South Africa. They reported slightly higher volumes in July and the lowest volumes in September, which correspond with our findings. Their observations were made in the Southern Hemisphere. Age and weight of animals, sexual stimulation prior to semen collection, method of semen collection and the geographical location may be responsible for the differences. In Saanan and Alpine goats (Delgadillo and Chemineau, 1992), sperm production and percent live normal cells were higher during August and September. Changes in the percentage of abnormal sperm in Markhoz goats are in agreement with the data from Rayini (Zamiri and Haidari, 2006), Murciano-Granadina (Roca et al., 1992b) and Verata (Perez and Mateos, 1996) bucks. In Alpine, Saanen and Damascus bucks from Greece the highest percentage of abnormal sperm was observed in spring and summer and the lowest in autumn, while the winter season was a transitional period for this parameter (Karagiannidis et al., 2000). Evans and Maxwell (1987), reporting on small ruminants, considered semen with 15 to 20% sperm abnormalities to be normal and of good fertilizing quality. If this is true also for the Markhoz breeds of goats, then the males in our study were well within the range of normal fertility. Furthermore, the results in this study were in agreement with the findings of Talebi et al. (2009). Motility, progressive motility and sperm density were greater during late summer and early autumn (breeding season) in this breed, but the difference was not significant ($p>0.05$). These results are in agreement with results from Saanen bucks reported by Ahmed et al. (1997) and reports of Talebi et al. (2009) for Markhoz goats. Seasonal variation in the pH value of goat semen was slighly lower (6.74 to 7.0) than the values of 7.01 and 7.02 reported for Angora goats in Australia (Mendoza et al., 1989) and the values of 7.1 and 7.3 reported by Talebi et al. (2009) for Markhoz goats. LDH is an intracellular enzyme and increased levels in the seminal fluid may be an indication of the integrity of the sperm plasma membrane. It has thus been proposed that increased LDH levels can be used as a good indicator of less of integrity of the plasma membranes (Dube et al., 1982). LDH values were higher than the range reported for Rayini goats in Iran (2.82-4.81 vs. 2.12-2.44; respectively). The testosterone profiles of bucks in present study displayed a well-defined seasonal pattern. During the experimental period two main period resulted, characterized, respectively, by low (from July to August) and high (from September to October) serum testosterone concentrations. The increase of testosterone secretion as the result of sexual stimulus has been established for bucks (Perez Liono and Mateos Rex, 1994).

CONCLUSION

The seasonal pattern of reproductive activity in the Markhoz buck is associated with changes in the day length. The best semen is obtained during the late summer and early autumn (breeding season). Seasonal variation in serum testosterone concentration and LDH seminal fluid suggest that seasonality occurs in reproductive performance and seminal quality of Markhoz goats that are maintained at 35°N latitude in the Northern Hemisphere. The results indicate that at the geographic location of Iran, seasonality is to be reckoned with when collecting semen from Markhoz goats.

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