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Seasonal variation in seminal characteristics, testicular measurements and plasma testosterone concentration in Iranian Khalkhali bucks

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Abstract The aim of the present study was to determine seasonal variations in semen characteristics, testicular size, and plasma testosterone concentration in Iranian Khalkhali goats. An artificial vagina was used to collect semen from 10 bucks (2–4 years of age), from November 2013 to October 2014. Testicular circumference was higher ($P<0.05$) from September to October. Ejaculate volume, sperm concentration, and percentage of normal sperm were higher ($P<0.05$) during September and October and lower during December and January ($P<0.05$). Plasma testosterone concentration was higher during October ($P<0.05$) than other months. Testicular circumference was positively correlated with serum testosterone concentration, percentage of live and normal sperm and semen volume. The lowest level of lactate dehydrogenase (LDH) in the seminal fluid (1.92 U per mL) was recorded during early October (autumn) and the highest level in early spring (2.80 U per mL). The lowest levels of K^+ and Na^+ in the seminal fluid were recorded in spring (59 and 72.3 mg per dL, respectively). The correlation coefficients of live and normal sperm percentage and sperm motility with seminal fluid K^+ level (r = 0.60 and 0.46, respectively) and Na^+ level (r = 0.48 and 0.35, respectively) were positive, and with LDH (r = -0.50 and -0.42, respectively) were negative ($P<0.01$). Despite monthly variations, semen quality was within the range regarded as satisfactory for normal fertility; however, the highest quality semen was collected during September and October.

Keywords: Khalkhali goats, semen, testicular circumference, testosterone

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Introduction

Bucks are generally classified as seasonal breeders (Hafez and Hafez, 2000). Compared with other farm animals, less attention has been paid to the reproductive physiology of male goats (Zamiri and Heidari, 2006). Sexual behavior, semen quality and quantity limit male reproductive efficiency, and sexual characteristics are affected by environmental and physiological conditions such as nutrition, latitude, breed, testicular size and circulating gonadotropins (Hammoudi et al., 2010; Karagiannidis et al., 2000; Zamiri and Heidari, 2006). The seasonality of reproduction is mainly controlled by neuro-endocrine mechanisms that are modified by external factors such as photoperiods and changes in the daylight length throughout the year (Hafez and Hafez, 2000). Factors affecting semen quality and quantity are important especially in artificial insemination (AI). Scrotal circumference and semen characteristics vary with time among different breeds and among individual bucks of the same breed (Pandey et al., 1985; Langford et al., 1999; Noran et al., 1998) and scarce information available for bucks may not be applicable to all goat breeds.

Seminal plasma contains substances that support the sperm cells and maintain a favorable micro-environment for sperm movement and storage (Zamiri et al., 2010). Information on the seasonal variation in seminal plasma composition can thus be used for preparing a suitable media as a diluent for sperm storage. The Na^+ and K^+ cations in the seminal plasma establish the osmotic balance, while the essential trace elements are the

components of many important enzymes (Zamiri and Khodaei, 2005; Jafaroghli et al., 2014; Zargari et al., 2016). Biochemical evaluation of seminal plasma may thus be important for the assessment of sperm quality. Lactate dehydrogenase (LDH) plays an important role in the sperm metabolism, sperm capacitation and fertilization (Duan and Goldberg, 2003). In addition, seminal LDH has been proposed to be a good indicator of sperm viability (Stamatiadis et al., 1984).

The Khalkhali goat is one of the most important breeds in Iran, reared mainly for milk production. Approximately 250000 heads are in the southern regions of Ardabil province. This breed is also regarded as an endangered species, and some herds have been established to conserve this breed. There is no published information on the reproductive characteristics of these goats. The Khalkhali goat is a long-haired and small-sized goat and commonly reared in extensive mixed farming systems, together with sheep and cattle, or in semi-intensive oasis systems. Currently, national projects for the development of the small ruminant sector and biodiversity conservation have been implemented in Iran. Therefore, knowledge of reproductive traits of Khalkhali bucks is important in herd management, and for determining the reproductive and productive potential of this breed. There are no published reports on the reproductive characteristics of this breed; therefore, this study was carried out to determine selected semen and testicular characteristics, and serum testosterone levels in Khalkhali bucks throughout the year.

Materials and methods

Animals and location

This experiment was performed at the Khalkhal Goat Station, located in north-west of Iran (37°, 37', 08" N latitude and 48°, 31', 33" E longitude), a semi-arid region with cold winter and moderately warm summer. Temperatures vary between - 4 and 31°C, and the annual rainfall ranges between 250 and 500 mm. Ten fertile and healthy bucks (2–4 years old; 38-45 kg live weight) were randomly selected, housed separately from the does, and received a daily diet consisting of (as fed) 670 g alfalfa hay and 780 g of a commercial concentrate mix. The diet contained (dry matter basis) 2.15 Mcal metabolizable energy per kg, 14.0% crude protein, 1.04% calcium and 0.5% phosphorus. The bucks had free access to mineral blocks and fresh water.

Semen collection and evaluation

Semen was collected from November 2013 to October 2014, at 15-day intervals from the bucks using an artificial vagina. Semen was collected in the morning, transferred to the laboratory at 35 to 37 °C within 15 to 20 minutes of collection, and kept in a water bath at 35 °C. The ejaculates were evaluated for volume (mL), sperm concentration ($\times 10^9$ sperm/mL), percentage of live and normal sperm (eosin-nigrosin staining) and pH. For eosin-nigrosin staining, a mixture of 10 µL of diluted spermatozoa and 10 µL eosin-nigrosin stains was smeared on a slide and allowed to air dry in a dust-free environment. The staining solution contained1.67 g eosin-Y (Merck, CAS N. 115935), 10 g nigrosin (Merck, CAS N. 115928) and 2.9 g sodium citrate (Merck, CAS N. 567446) dissolved in 100 mL distilled water (Evans and Maxwell, 1987).

Percentage of live sperm and sperm morphology were determined using 200 sperm per semen sample at 400 × magnification. Semen volume was estimated directly in calibrated semen collection tubes, and sperm concentration was determined with the aid of a Neubaur hemocytometer, after dilution (1:200) of the semen sample with 2% eosin solution. A sample of the diluted semen was placed under a cover slip on a pre-warmed (37°C) microscopic slide and the sperm progressive motility was subjectively assessed using a phase contrast microscope (× 400 magnification). Seminal pH was measured using a digital pH meter (pH 211 Microprocessor, Hanna, Italy). At monthly intervals, scrotal circumference was measured using a tape measure.

Seminal fluid chemistry

After quality evaluation, the ejaculate was centrifuged (8944 g for 30 min) and the seminal fluid aspirated. Lactate dehydrogenase (LDH) activity in the seminal fluid was determined immediately after centrifugation, using a commercial kit (Pars-Azmoon Co., Tehran). A portion of seminal fluid was frozen (-20°C) until analyzed for Na^+ and K^+ concentrations (Flame photometer, Seac-FP20, Italy).

Plasma testosterone measurement

Blood samples were collected monthly via the jugular vein into heparinized tubes, placed on ice, transported to the laboratory, and centrifuged at 1096 g for 10 min. Plasma was harvested and stored at −20°C, until analyzed for plasma testosterone concentration using a commercial RIA kit (Radim Co., Italy). The minimum detectable plasma testosterone concentration for the assay was 0.35 ng/mL and the intra- and inter-assay coefficients of variation were 5.5 and 7.7%, respectively.

Statistical analysis

The percentage data were arcsine-transformed and subjected to repeated measure analysis using Proc Mixed (SAS 9.1.3, 2003). The autoregressive (TYPE=**AR(1)**) covariance structure was selected based on the Schwarz Bayesian criterion. Live weight was included in the model as the covariate. Least squares means and standard errors of the back-transformed data are reported in the text. The level of significance was set at $P<0.05$.

Results

Testicular circumference was greater during late summer (September) to early autumn (October and November) and the lowest values were recorded during the winter months ($P<0.05$), Table 1). Semen volume, and sperm concentration, were higher during autumn (late September to late October) and the lowest values were found during the winter months ($P<0.05$), Table 1).The highest (1.6 mL) and lowest volumes of semen were collected in October and December, respectively. Percentage of abnormal sperm in the semen varied between 6.5 to 13.1% and was higher from mid-winter to early spring, and reached its lowest values in late summer and early autumn (Table 1). Seminal fluid pH (6.1 to 6.5) was slightly acidic, and generally lower during winter and higher in autumn (monthly data not tabulated).The lowest level of LDH in the seminal fluid was recorded during early autumn (1.92 U per mL) and the highest level (2.80 U per mL) in early spring (Table 2). The lowest concentrations of K^+ and Na^+ in the seminal fluid were recorded in spring (59 and 72.3 mg per dL, respectively). Maximal concentration of testosterone (Figure 1) was recorded in autumn (4.2 ± 0.52 ng/mL), but it decreased progressively during winter (3.1 ± 0.52 ng/mL) and reached its minimal level in spring (2.64 ± 0.52 ng/mL).Testosterone concentration increased again from late summer (3.60 ± 0.52 ng/mL).

Correlation coefficients of live and normal sperm percentage and sperm motility with seminal fluid K^+ (r=0.60 and 0.46, respectively), Na^+ (r=0.48 and 0.35, respectively) and LDH (r=-0.50 and -0.42, respectively) were significant ($P<0.01$).

Discussion

This study is the first to report variations in seminal and scrotal characteristics of Iranian Khalkhali goat. The largest value for testicular measurements of Khalkhali bucks was observed during late summer to early autumn and the lowest values during winter. Testicular size (e.g. scrotal circumference) is a highly heritable trait, and considered to be a good index of sperm production potential. Temperate, day length, feed quality and average daily weight gain (ADG) are the main factors that affect testicular size (Hafez and Hafez, 2000).

A wide range of values has been reported for goat semen characteristics including the volume (0.5 to 1.5 mL), sperm concentration (1.5 to 5.0 x 10^9 mL^{-1}) and percentage of abnormal sperm (5 to 29%) in the semen (Karagiannidis et al., 2000; Shamsuddin et al., 2000; Talebi et al., 2009; Zamiri and Heidari, 2006). The mean ejaculate volume of Angora goats in Australia for two consecutive years were 0.80 to 0.98 mL and the sperm concentrations were 2.94 to 3.33 ×10^9 mL^{-1} (Mendoza

Table 1. Least squares means of monthly seminal characteristics and testicular (scrotal) circumference in Khalkhali bucks

Month	Semen volume (mL)	Sperm density (10^9/mL)	Abnormal sperm (%)	Circumference (cm)
Nov	1.04^{ef}	3.57^{cd}	9.2^{c}	29.2^{bc}
Dec	0.88^{g}	3.6^{cd}	9.3^{c}	28.3^{cd}
Jan	0.86^{g}	3.18^{f}	12.6^{a}	26.2^{fg}
Feb	0.97^{fg}	2.74^{g}	12.5^{a}	25.3^{h}
Mar	0.93^{fg}	2.65^{g}	13.1^{a}	25.9^{gh}
Apr	$1.09d^{e}$	3.31^{ef}	10.6^{b}	26.2^{fgh}
May	1.19^{cd}	3.43^{de}	12.1^{a}	27.1^{ef}
Jun	1.24^{c}	3.52^{cde}	12.2^{a}	27.8^{de}
Jul	1.20^{cd}	3.68^{bc}	10.9^{b}	28.2^{cd}
Aug	1.38^{b}	3.76^{b}	8.1^{d}	28.8^{bc}
Sep	1.50^{ab}	3.81^{b}	6.5^{e}	29.5^{ab}
Oct	1.60^{a}	4.05^{a}	8.2^{d}	30.4^{a}
S.E.M.	0.06	0.05	0.42	0.40
P value	<0.001	<0.001	<0.001	<0.001

^{a,b,c}: Within columns, means with common superscript (s) are not different (P>0.05).

Table 2. Least squares means of monthly concentrations of seminal Na^+ and K^+, lactate dehydrogenase (LDH) and pH in Khalkhali bucks

Month	Na^+ (mg/dL)	K^+ (mg/dL)	LDH (U/mL)	pH
Nov	73.6^{bc}	75.8^{ab}	2.25^{defg}	6.43^{a}
Dec	72.7^{bc}	75.4^{ab}	2.52^{bcd}	6.32^{b}
Jan	72.6^{bc}	71.8^{bc}	2.57^{abc}	6.25^{b}
Feb	73.0^{abc}	61.6^{fg}	2.74^{ab}	6.13^{d}
Mar	72.3^{c}	59.0^{g}	2.80^{a}	5.95^{e}
Apr	72.8^{bc}	60.7^{fg}	2.40^{cde}	5.84^{f}
May	73.1^{ab}	$63.1e^{fg}$	2.35^{cdef}	5.83^{f}
Jun	73.2^{abc}	65.2^{def}	2.10^{fgh}	5.73^{g}
Jul	73.3^{abc}	68.1^{cde}	2.11^{fgh}	5.95^{e}
Aug	73.8^{ab}	70.1^{cd}	2.30^{efg}	6.09^{d}
Sep	73.9^{a}	77.6^{ab}	2.00^{gh}	6.25
Oct	73.9^{a}	79.6^{a}	1.92^{h}	6.34
S.E.M.	0.54	1.76	0.14	0.03
P value	<0.001	<0.001	<0.001	<0.001

^{a,b,c}: Within columns, means with common superscript (s) are not different (P>0.05).

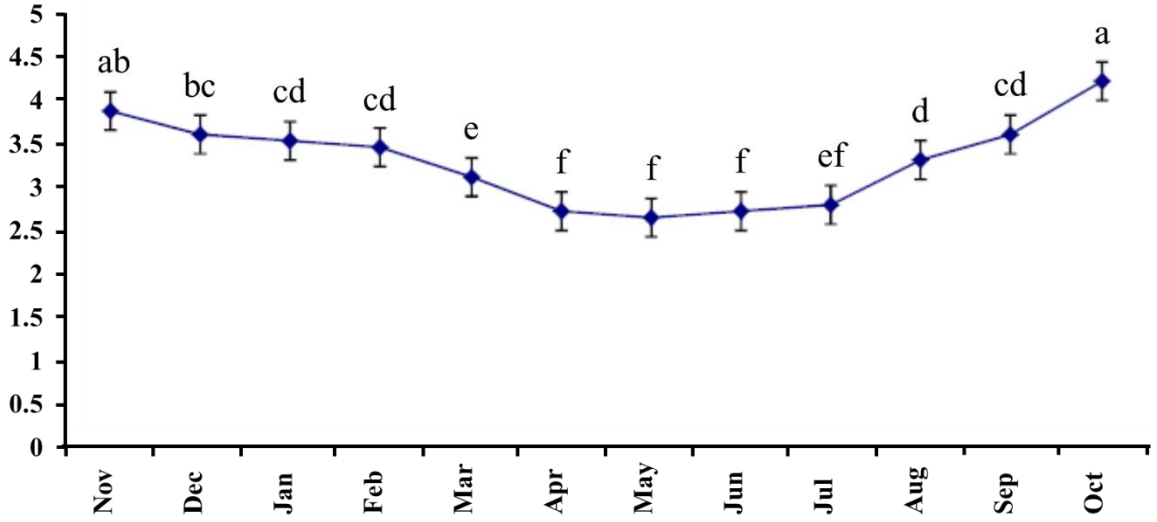

Figure 1. Monthly variation of serum testosterone concentration (ng/mL) in Khalkhali bucks (least squares means and error bars).
^{a,b,c}: Means with common superscript (s) are not different (P>0.05).

et al., 1989). Zamiri and Heidari (2006) reported that average semen volume, percent of abnormal sperm and sperm concentration in Rayini goats varied between 1 to 1.4 mL, 7 to 13% and 1.8 to 2.6 × 10^9 mL^{-1}, respectively.In Black Bengal bucks, sperm concentration varied between 4.2 to 5.1 × 10^9 mL^{-1}, total sperm number per ejaculated between 1.14 to 1.75 × 10^9 mL^{-1}, and motility between 77 to 79% (Shamsuddin et al., 2000).

Reproductive activity in the goat breeds is highly seasonal and closely related to changes in the photoperiod (Leboeuf et al., 2008). Seasonal variation in quality and quantity of goat semen has been associated with environmental factors such as changes in photoperiod and temperature (Roca et al., 1992; Hammoudi et al, 2010). The effect of season on the reproductive characteristics of several buck breeds such as British (Ahmad and Noakes, 1995), Damascus (Al-Ghalban et al., 2004), Markhoz (Talebi et al., 2009) and Rayini (Zamiri and Heidari, 2006) has been previously reported. Breed and individual differences in scrotal circumference have been found in bucks (Noran et al., 1998; Pandey et al., 1985).

Total sperm abnormality in Khalkhali goats (6.5 to 13%) was almost identical to that of Black Bengal (6 to 10%) in Bangladesh (Shamsuddin et al., 2000), Rayini (6.8 to 12.8%) in Iran (Zamiri and Heidari, 2006) and Alpine and Saanen goats in Greece (Karagiannidis et al., 2000) while it was lower for Damascus goats (29%) in

Jordan (Al-Ghalban et al. 2004) and Nubian (22%) goats in Sudan (Ali and Mustafa, 1986). It is noteworthy that Damascus goats in Greece (Karagiannidis et al., 2000) had a lower percentage of abnormal sperm (about 8%) than Damascus goats in Jordan (Al-Ghalban et al., 2004). Although spermatogenesis is active throughout the year in the buck, considerable seasonal variations have been reported for different regions. Sperm concentration was higher during autumn (late September to late October) and the lowest values were observed during the winter months. Sperm concentration in Khalkhali bucks was about 50 % higher than for Rayini (Zamiri and Heidari, 2006) or Alpine (Karagiannidis et al, 2000) goats. Al-Ghalban et al. (2004) found higher sperm concentration during summer and autumn, but Roca et al. (1992) reported that sperm concentration was lower during autumn and higher during spring and summer. This is a reflection of various factors that influence sperm production, including genetics, environment and nutrition. Seminal fluid pH values (monthly data not tabulated) were not affected by time in Khalkhali goats (6.1 to 6.5), similar to the findings of Barkawi et al. (2006) and Zamiri and Heidari (2006). Seminal Na^+, K^+ and LDH concentrations were within the range recorded for other goat breeds (Zamiri and Heidari, 2006). The correlation coefficient of LDH level with the percentage of live sperm (-0.50) was smaller than the value (-0.65) reported for Rayini bucks (Zamiri and Heidari, 2006). Each increase in the percentage of live and normal sperm corresponded to a decrease in LDH activity in the seminal fluid. LDH is an intracellular enzyme and increased levels in seminal fluid may be an indication of the integrity of the sperm plasma membrane and it has been proposed that LDH can be used as a good indicator of sperm viability (Dube et al., 1982; Stamatiadis et al., 1984). In Khalkhali goats, K^+ ion levels gradually decreased from September to December, accompanied by a decrease in live cell percentage and an increase in the percentage of abnormal sperm. In the present study, low levels of Na^+ and K^+ ions were associated with low percentages of motile sperm, and such semen was considered to be of lower quality. The Na^+ and K^+ cations generally establish the osmotic balance, and seminal plasma osmolality ultimately plays an important role in the activation of the sperm cell. The K^+ ion has a role to play in keeping sperm in a quiescent state (Zamiri and Khodaei, 2005). Khalkhali bucks kept under a natural photoperiod showed marked seasonal variations in testosterone secretion. In early October, the scrotal circumference attained its maximum size, corresponding to the highest plasma testosterone level during the experiment. In seasonal breeders, variations in gonadotropins secretion are responsible for seasonal variations in sexual activity with alternate active and inactive sexual periods. This effect is attributed to the photoperiodic variations which affect the central nervous system through the modification of the duration of nocturnal secretion of melatonin (Delgadillo and Chemineau, 1992). In the present study, seasonal plasma testosterone pattern was similar to that reported by Todini et al. (2007) who found that mean plasma testosterone concentration in four Mediterranean breeds were affected by season, being higher during summer and autumn.

The effect of photoperiod on seasonal breeders depends mostly on the latitude, and in latitudes above 40º N, considerable variations are found in the seminal attributes (Corteel, 1977). Increased sperm production is found during the decreasing day length. At latitudes between 30º N and 40º N, seminal characteristics show less marked seasonal variations with higher sperm production during summer and autumn. Khalkhali goats of the present study live at 37º N latitude and fall within this range.

Conclusion

There were seasonal fluctuations in semen quality and quantity of Khalkhali bucks. Semen with better quality and quantity was produced during the breeding season (late summer and autumn). In spite of monthly variations in sperm quality, semen quality out of breeding season was within the range regarded as satisfactory for normal fertility.

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تغییرات فصلی در ویژگی‌های منی، اندازه بیضه و غلظت تستوسترون پلاسمایی در بزهای نر خلخالی ایران

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چکیده هدف از مطالعه حاضر تعیین تغییرات فصلی در خصوصیات منی، اندازه بیضه و غلظت تستوسترون پلاسمایی بزهای نر خلخالی بود. بدین منظور، جمع آوری منی از ۱۰ بز نر بالغ خلخالی (۲-۴ سال) با استفاده از مهبل مصنوعی از نوامبر ۲۰۱۳ تا اکتبر ۲۰۱۴ انجام گرفت. محیط دور بیضه در فاصله سپتامبر تا اکتبر بیشتر بود ($P < ۰/۰۵$). حجم منی، غلظت اسپرم و درصد اسپرم طبیعی از سپتامبر تا اکتبر بالاتر و از دسامبر تا ژانویه پایین تر بود ($P < ۰/۰۵$). غلظت پلاسمایی تستوسترون در طی اکتبر پایین تر از ماه های دیگر بود ($P < ۰/۰۵$). محیط دور بیضه همبستگی مثبتی با غلظت تستوسترون سرم، درصد اسپرم های زنده و طبیعی و حجم منی داشت. پایین ترین سطح لاکتات دهیدروژناز در مایع منی (۱/۹۲ واحد در میلی لیتر) در طی اوایل اکتبر (پاییز) و بالاترین سطح در اوایل بهار ثبت گردید. پایین ترین سطوح سدیم و پتاسیم در مایع منی در بهار (بترتیب ۵۹ و ۷۲/۳ میلی گرم در دسی لیتر) ثبت گردید. ضرایب همبستگی درصد اسپرم های زنده و طبیعی و تحرک اسپرم با سطح پتاسیم (بترتیب r=۰/۶۰ و r=۰/۶۰) و سطح سدیم (بترتیب r=۰/۴۸ و r=۰/۳۵) مثبت و با فعالیت لاکتات دهیدروژناز (بترتیب r= -۰/۵۰ و r= -۰/۴۲) منفی بود ($P < ۰/۰۱$). علیرغم تغییرات فصلی، کیفیت اسپرم در دامنه قابل قبول برای باروری قرار داشت اما منی با بالاترین کیفیت در طی سپتامبر تا اکتبر جمع آوری شد.