

SEASONAL VARIATIONS IN TESTICULAR MEASUREMENTS, FRESH SPERM QUALITY AND POST-THAW SPERM MOTILITY IN GURCU GOAT BUCKS

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ABSTRACT

This study was aimed to determine seasonal variations in testicular measurements and semen characteristics of four Gurcu goat bucks. Data were collected over a whole year. Body weight and morphometric testicular parameters were measured once a month. Semen samples were collected weekly through an artificial vagina, then diluted in a skim milk extender and frozen. Semen volume, sperm concentration, the total number of spermatozoa and the mass activity were evaluated in each extended semen sample. In contrast, sperm progressive motility was assessed prior to the cryopreservation process. In autumn, the testis was larger than in other seasons ($P < 0.01$). Ejaculate volume increased in winter ($P < 0.01$), while the total number of spermatozoa increased during the winter even the seasonal effect was not significant ($P = 0.24$). Post-thaw motility was highest for the semen collected in autumn. In conclusion, although seasonal variation in the characteristics of fresh semen was limited, it may be appropriate to collect semen in autumn, when sperm doses should be cryopreserved for the genetic preservation of this breed.

Key words: Gurcu goats; freezability; seasonal variation; semen characteristics; testis

INTRODUCTION

Gurcu goats are locally raised in the North-Eastern Anatolia region especially around Ardahan-Çıldır City, Turkey. These goats are critical Turkish genetic resources that are threatened by an extinction. They are a dairy-type local breed producing approximately 200-250 liters of milk for a lactation period of 150-180 days (Yalcin, 1990). Recently, the Gurcu breed has been officially recognized. However, the number of goats of this breed is in the hundreds and decreasing over time. There is currently only one herd that breeds Gurcu goats. Generally, preservation of the Gurcu

goat is essential for local adaptability and genetic diversity of Turkish goats. Cryopreservation of sperm has been a preferred method for the genetic preservation of goats in Turkey (Şezgin *et al.*, 2010; Kulaksız *et al.*, 2016; Kuru *et al.*, 2017; 2018).

Because fewer males than females are kept, therefore, an individual male makes a more significant contribution to the next generation. Hence, it is crucial to assess their reproductive fitness. Characterizing both testis morphology and semen traits are essential for determining fertility rates and ensuring the continuity of breeds (Tekin, 1994). Gordon (1999) reported that goat buck semen has a relatively small amount of seminal

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fluid (0.5–1.5 mL), a high density of spermatozoa ($\sim 4 \times 10^9$ cells per mL) with a high rate of motility (70%–90%). Semen quality and quantity, as indicators of reproductive efficiency, are influenced by factors such as breed, age, season, frequency of ejaculation, techniques of breeding and even by variation among individual goats within the same herd (Greyling and Grobbelaar, 1983; Webb *et al.*, 2004). Among these factors, the season has important influences on semen quality. Previous studies have focused on seasonal variation in testicular measurements and the characteristics of fresh semen of goats (Ahmad and Noakes, 1996; Kamal *et al.*, 2005; Zamiri and Heidari, 2006; Talebi *et al.*, 2009).

The varieties of goat breeds are another critical factor affecting the success of cryopreservation. For instance, semen from native or cultured goat breeds may have different sensitivity against cryopreservation (Kulaksız *et al.*, 2013). However, there are several studies that investigated the effect of a season on frozen-thawed goat semen (Tuli and Holtz, 1995; Wang *et al.*, 2015). Therefore, this study was aimed at examining the effect of a season on testicular measurements and characteristics of fresh and frozen-thawed semen of Gurcu bucks.

MATERIAL AND METHODS

Four male bucks of the Gurcu goat breed were used in this study. They were 12 months old, when the study commenced, and weighed 40–45 kg. The bucks were housed at the Education Research and Practice Farm, Faculty of Veterinary Medicine, Kafkas University, Turkey at 40° 34' 33" N, 43° 02' 35" E at an altitude of 1751 m. The bucks were kept under natural photoperiod. They were fed 1 kg of hay and 0.9 kg concentrate mix, containing 12 % protein per animal daily, and had free access to vitamin/mineral block and freshwater all the time. A general management schedule for de-worming, disease prevention, and hoof trimming was followed throughout.

The seasons were classified as autumn (September–October–November), winter (December–January–February), spring (March–April–May), and summer (June–July–August). These seasons were determined by considering the descriptions

of the mating period that Kuru *et al.* (2017) reported in their study conducted under the natural conditions of Kars province, Turkey.

Body weight (BW) and testicular measurements (TM) were recorded once a month throughout the study. The scrotal circumference was measured with a flexible metric tape (A Neogen Company, MI, USA). Testes width, testes length and scrotal thickness were determined by using a digital caliper (Insize Co., Ltd., GA, USA). The volume of each testis was measured volumetrically using the Archimedes principles of water displacement in a measuring cylinder (Demirci, 2002). Before the start of the research, all bucks were trained for semen collection using an artificial vagina. Each time semen was collected, the same female goat (in heat or not) was used as a phantom. Semen was always collected at the same time (09:00–10:00) and by the same person. Semen was obtained from each buck once a week for an entire one-year period.

A total of 113 ejaculates were evaluated. The volume of ejaculated semen was recorded immediately after collection into a graduated collection vial. Sperm progressive motility was subjectively evaluated under a phase contrast-supplied microscope equipped with a heating stage at 37 °C and magnification of 400× after dilution (1:10) with skimmed milk extender. Sperm concentration was determined using a hemocytometer after diluting semen (1:400) with Hayem solution (Tekin, 1994). Before freezing, the semen was diluted with a skim milk-based egg yolk extender (Kulaksız and Daşkın, 2010). The composition of the skim milk-based solution was 10 g of skim milk powder and 0.9 g of glucose dissolved in a water to a final volume of 100 mL, to which 10 % (v/v) egg yolk, 5 % (v/v) glycerol, 500 IU of penicillin and 500 µg of streptomycin sulphate per mL were added. The extended semen samples were stored at 5 °C. Diluted semen was loaded into 0.25 mL straws (IMV. Technologies; L'Aigle, France) in a concentration of approximately 10^8 spermatozoa/straw. Plastic straws were sealed with a polyvinyl alcohol powder. The straws containing the semen were then placed into a refrigerator at 5 °C and allowed to equilibrate for 2 h before being frozen. After equilibration, the straws were frozen horizontally on a rack about 4 cm above a liquid nitrogen (LN₂) level in an insulated container. The nitrogen vapour reduced the temperature within

the straws to $-120\text{ }^{\circ}\text{C}$ in approximately 15 min. Then, the frozen straws were plunged into LN_2 and were stored for a month before thawing.

Two straws from each buck were thawed in a water bath at ($37\text{ }^{\circ}\text{C}$) for 1 min and sperm progressive motility was subjectively examined for each frozen-thawed semen sample. Precisely, a $3\text{-}\mu\text{L}$ aliquot of each sample was placed on a warm ($37\text{ }^{\circ}\text{C}$) slide and covered with a coverslip. Four or five different fields were recorded using a phase-contrast microscope (Nikon Eclipse E400, Nikon Corp., Japan) at $400\times$ magnification and results were expressed as the percentage of progressive motile sperm for each sample. Throughout the experiment, two trained technicians (as a double-blind manner) evaluated all the samples and results were expressed in percentages as the mean value of their observations (Kulaksız and Daşkın, 2010).

Statistical analysis

The SPSS software program (SPSS 20.0, Chicago, IL, USA) was used to analyze the data. Distributions of the data were evaluated by the Shapiro–Wilk test. Repeated measured analysis of variance (ANOVA) was used to determine the significance of effects and the Bonferroni test was used for comparing means of live weight, scrotum and testicular measurements, as well as pre- and post-thaw semen characteristics (semen volume, mass activity, progressive sperm motility, sperm concentration, total spermatozoa and post-thaw progressive sperm motility) by seasons. If "n" was

not equal in comparison variables, one-way ANOVA and Dunnett T3 (if n equals, Tukey's (honestly significant difference (HSD) test) tests were used for multiple comparisons of data. The results were presented as mean \pm standard error. $P < 0.05$ was considered as statistically significant in evaluating the results.

RESULTS

The bucks ranged in weight between 47 and 55 kg. Bodyweight increased in autumn and winter seasons compared to spring and summer season ($P < 0.01$; Table 1). Except for scrotal skin thickness, the testicular characteristics also changed seasonally ($P < 0.01$). Scrotal circumference, right and left testicular length, right and left testicular width and testicular volume were generally highest in autumn and least in spring ($P < 0.01$; Table 1).

The quantity and intensity of the native spermatological characteristics of the Gurcu bucks were significantly affected by a season ($P < 0.001$) (Table 2). The ejaculate volume was higher during the winter season than in other seasons ($P < 0.001$). The effect of a season, on the concentration of spermatozoa was found to be statistically significant ($P < 0.001$). Semen with the highest spermatozoa concentration was obtained in the spring and summer seasons ($P < 0.001$). Progressive spermatozoa motility was significantly affected by a season ($P < 0.001$). The rates of progressive

Table 1. Seasonal variation in body weight and testicular measurements of Gurcu goats

Parameters	Spring	Summer	Autumn	Winter	P-value
Body weight (kg)	47.07 ± 1.66^a	50.27 ± 0.81^a	55.20 ± 0.87^b	54.60 ± 1.03^b	< 0.001
Scrotal circumference (cm)	23.10 ± 0.52^a	25.40 ± 0.35^b	26.77 ± 0.25^c	25.73 ± 0.24^b	< 0.001
Testes length (cm) right	9.37 ± 0.21^a	10.64 ± 0.25^b	11.68 ± 0.26^c	10.21 ± 0.25^b	< 0.001
Testes length (cm) left	9.67 ± 0.20^a	10.97 ± 0.25^b	11.94 ± 0.22^c	10.51 ± 0.20^b	< 0.001
Testes width (cm) right	5.02 ± 0.10^a	5.53 ± 0.13^b	6.11 ± 0.09^c	5.47 ± 0.09^b	< 0.001
Testes width (cm) left	5.03 ± 0.12^a	5.61 ± 0.15^b	6.11 ± 0.10^c	5.45 ± 0.11^{ab}	< 0.001
Testicular volume (mL)	280.33 ± 6.41^a	342.67 ± 8.89^b	380.33 ± 9.12^c	317.33 ± 7.64^b	< 0.001
Scrotal skin thickness (cm)	0.66 ± 0.04	0.66 ± 0.03	0.65 ± 0.03	0.71 ± 0.03	0.269

Spring: March–April–May, Summer: June–July–August, Autumn: September–October–November, Winter: December–January–February. Different subscripts in the same row (a-c) indicate significance difference ($P < 0.001$). The Data are presented as mean \pm standard error (SE).

Table 2. Seasonal variation in semen characteristics of Gurcu goats

Season	Semen volume (mL)	Mass activity	Progressive sperm motility (%)	Sperm concentration (x 10 ⁹ mL)	Total Spermatozoa (x 10 ⁹ mL)	Post-thaw progressive sperm motility (%)
Autumn	1.07 ± 0.07 ^a	4.83 ± 0.08	78.04 ± 1.32 ^a	2.50 ± 0.17 ^a	2.49 ± 0.27	36.50 ± 3.05 ^a
Winter	1.39 ± 0.06 ^b	4.68 ± 0.08	81.70 ± 1.35 ^a	2.48 ± 0.80 ^a	3.34 ± 0.28	23.89 ± 3.11 ^b
Spring	0.84 ± 0.06 ^{ac}	4.83 ± 0.09	81.25 ± 1.45 ^a	3.94 ± 0.22 ^b	3.11 ± 0.37	20.96 ± 2.77 ^b
Summer	0.66 ± 0.06 ^c	4.45 ± 0.26	66.25 ± 4.00 ^b	3.92 ± 0.29 ^b	2.70 ± 0.37	15.45 ± 5.58 ^b
P-value	<0.001	0.176	<0.001	<0.001	0.244	0.021

Autumn (n = 23 for fresh semen): September – October – November, Winter (n = 41 for fresh semen): December – January – February, Spring (n = 29 for fresh semen): March – April – May, Summer (n = 20 for fresh semen): June – July – August. Different subscripts in the same row (a-c) indicate significance difference ($P < 0.05$). The Data are presented as mean ± standard error (SE).

spermatozoa motility decreased in summer but did not change dramatically in other seasons. The examination of post-thawing sperm progressive motility revealed that the best motility was reached in the autumn season ($P = 0.021$).

DISCUSSION

No information or scientific study is available about the reproductive characteristics of Gurcu goat bucks in Turkey, particularly regarding the seasonal variation in semen quality and quantity. The present study demonstrates novel and relevant data regarding the reproductive aspects of Gurcu bucks.

This study showed that the testicular measurement was significantly affected by the season and testicular measurement values (scrotum circumference, testis length, and testis width) of the Gurcu bucks were higher in autumn than in spring, summer or winter. These results are consistent with the findings of Webb *et al.* (2004), Kamal *et al.* (2005), Barkawi *et al.* (2006) and Chentouf *et al.* (2011), who reported seasonal variations in the testis measurements. Souri and Mirmahmoudi (2014) found the highest values of the Markhoz bucks' scrotum circumference (35.2 cm), testis length (14.7 cm) and testis width (6.1 cm) in autumn. These values were higher than the values in the breed used in the present study. Thus, the results obtained in the present study were lower than those shown in the literature, and the factors attributing to this fact

were: the breed, age, weight, care and nutrition of the goats used in the study, the measurements periods, the person making the measurements and the measurement technique.

This study showed that the ejaculate volume was significantly affected by the season. The ejaculate volume of the Gurcu bucks was higher in winter than in spring, summer or autumn. These results were consistent with the findings of Greyling and Grobbelar (1983), Ahmad and Noakes (1996) and Zamiri and Heidari (2006), who reported monthly and seasonal variations in the amount of semen. Chentouf *et al.* (2011) reported that Morocco domestic bucks had the highest (0.92 mL) and lowest (0.44 mL) semen volumes during the summer and winter months, respectively, indicating that seasonal differences were significant. Roca *et al.* (1992) and Barkawi *et al.* (2006) reported the seasonal variation in the amounts of semen in the Murciana-Granadina and Zaraibi bucks, respectively. They have also indicated that the highest sperm count was obtained in the autumn season. In this regard, the findings of the present study were different from those of Chentouf *et al.* (2011), Roca *et al.* (1992) and Barkawi *et al.* (2006). This difference might be due to different goat breeds used, geographical location, climatic conditions and care-feeding conditions of the region where the study was conducted.

Spermatozoa motility of the Gurcu bucks was significantly influenced by seasons. Spermatozoa motility was highest in winter (81.70 %) and lowest in summer (66.25 %). Some researchers reported

that changes in semen motility were significantly affected by months and seasons (Barkawi *et al.*, 2006; Kridli *et al.*, 2007; Talebi *et al.*, 2009; Wang *et al.*, 2015), while other researchers did not observe this (Chentouf *et al.*, 2011; Dorado *et al.*, 2010). Besides, the fresh spermatozoa motility in this study was higher than the year-round motility values detected by some researchers (Kamal *et al.*, 2005; Talebi *et al.*, 2009; Chentouf *et al.*, 2011; Qureshi *et al.*, 2013; Wang *et al.*, 2015). The reasons for the differences between the motility values obtained in this study and those obtained in other published studies included the breed, age, evaluator and methods of the evaluation. However, the motility values in this study were similar to those reported by Roca *et al.* (1992), Dorada *et al.* (2010) and Farshad *et al.* (2012).

In the present study, ejaculates with the higher spermatozoa concentration were collected in the spring season. In Korean domestic bucks (Choe *et al.*, 2006) and in Saanen and Nubian bucks (Kamal *et al.* 2004), no difference was found between the seasons in terms of spermatozoa concentration. In this regard, the findings of the present study differed from those of Choe *et al.* (2006) and Kamal *et al.* (2004). Furthermore, the spermatozoa concentration of the Gurcu buck semen observed in the present study was found to be either higher (Talebi *et al.*, 2009; Souri and Mirmahmoudi, 2014; Qureshi *et al.*, 2013; Wang *et al.*, 2015), lower (Ahmad and Noakes, 1996; Karagiannidis *et al.* 2000; Barkawi *et al.*, 2006; Al-Ghalban *et al.*, 2004), or similar (Roca *et al.*, 1992; Kamal *et al.*, 2005; Kridli *et al.*, 2007; Chenteouf *et al.*, 2011) to the values reported in the literature. The reasons for the difference in the spermatozoa concentrations in the present study and other studies included the breed, age, care method, nutritional conditions, geographical location of the region where the research was conducted, climatic conditions, the evaluator and the methods used.

Studies examining the seasonal variation in freezability of the spermatozoa are limited. Tuli and Holtz (1995) found that the freezability of Boer buck semen was influenced by a season. They detected the best post-thawing spermatozoa motility and viability percentages during winter. In this regard, the present study differs from the study conducted by Tuli and Holtz (1995). Wang *et al.* (2015) have been reported that the best seasons for the freezing of Xinong Saanen buck semen were the summer

and autumn seasons. The results of this study are consistent with the findings of Wang *et al.* (2015).

CONCLUSION

Although many studies have been conducted to examine the semen characteristics in different goat breeds, studies on the semen characteristics of regional/domestic genotypes are limited. This novel study determined the sperm characteristics of Gurcu goat bucks and paved the way to establish an infrastructure for future studies about artificial insemination in Gurcu goats. Although the Gurcu bucks have continued sperm production throughout the year, getting regular semen ejaculate from the some Gurcu bucks throughout the year is not possible; also the sustainability of such studies is challenging. It would be more appropriate in Gurcu bucks to carry out the sperm intake process in a short time interval during the breeding and nonbreeding seasons rather than spreading this process all year round. Although seasonal variations in fresh spermatological characteristics were limited, it may be appropriate to prefer the autumn season in this breed, when the semen usually is cryopreserved. Taking into account the native and post-thawing spermatological characteristics of the Gurcu bucks, determined in this study, the success rate in future artificial insemination studies on the Gurcu bucks might be increased.

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