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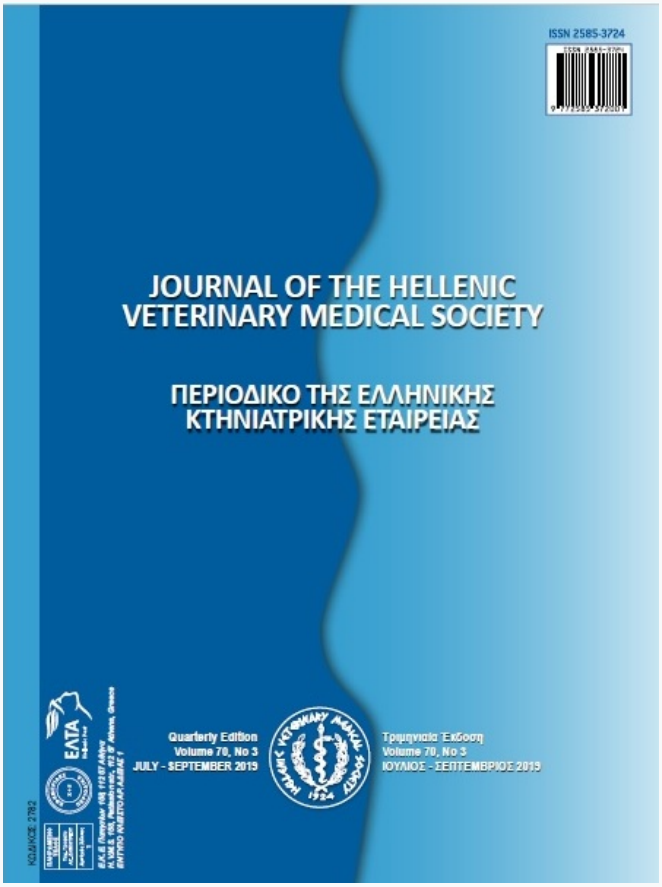

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Research article
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Investigation of the changes observed in scrotal circumference, and native and post-thaw semen characteristics in karayaka rams during the breeding and non-breeding seasons

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ABSTRACT. The aim of the present study was to investigate and compare the impact of the breeding (BS) and non-breeding (NBS) seasons on scrotal circumference, and native and post-thaw semen characteristics in Karayaka rams. Six (6) Karayaka rams were used as semen donors, and ejaculates were collected with the aid of an artificial vagina during the BS (October-November) and NBS (April-May). Interestingly, sperm freezability was found to be higher in the NBS, when compared to the BS, and the sperm motility rate (36.00±2.74%, 55.0±3.24%) and percentage of abnormal sperm (44.00±1.90%, 57.00±2.09%) were found to significantly differ with season ($P<0.05$). In conclusion, the post-freezing sperm motility was better during the non-breeding season. Post-thaw sperm abnormality was higher during non-breading season. However, if ram semen is used fresh for AI, it can be collected and used during both during the breeding and non-breeding season.

Keywords: freezability, karayaka rams, seasonal variation, semen characteristics

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INTRODUCTION

The Karayaka sheep is a native breed of Turkey, which is raised in the Black Sea Region. The Karayaka breed is renowned for its mutton production and high quality meat, while it is also well adapted to the warm, humid and irrigated semi-arid regions of northern Turkey (Kaymakcı et al., 2001). Sperm quality, which is an indicator of fertility in rams, is affected by several factors including season, temperature, humidity and day length. Furthermore, some other factors, such as breed and age of the animal and the production system used, have been reported to influence sperm production and quality (Tajangookeh et al., 2007). Among these factors, season is one of the most important factors that influences variations in semen quality and fertility. The season may show direct or indirect effects on variations in ram reproductive activity (D'Alessandro and Martemucci, 2005; Olah et al., 2013). Rams, that are raised in the northern hemisphere, are particularly affected by seasonal variations, and their testicular activity either increases or decreases, thereby altering sperm characteristics.

There is limited research on seasonal effects on cryopreservation of ram semen in the literature (D'Alessandro and Martemucci, 2005; Makawi et al., 2007). To our knowledge, there has not yet been any study about Karayaka rams concerning these issues and our study is the first to describe these parameters in this breed. Thus, this study aims at the investigation of the impact of seasonality on alterations in scrotal circumference, and native and post-thaw sperm quality in Karayaka rams. This study not only describes sperm characteristics of the Karayaka ram for the first time, but is also expected to help strengthen the preformance of AI in the Karayaka breed.

MATERIALS AND METHODS

Animals, feeding and management: In this study, the breeding season has been determined between October and November, while the non-breeding season has been defined as the months April and May (Olfaz et al., 2010). Six 2-3-year-old Karayaka rams, weighing 65-70 kg, were used in this study. The animals were raised under uniform feeding conditions, natural photoperiod and natural temperature and environmental humidity. All rams were housed in a covered shelter and were provided with 1 kg of hay per animal daily. A supplement containing 12% protein was also offered each morning (0,9 kg for each ram). Clean and safe water was available at all times. A general management schedule for de-worming, disease prevention, and hoof trimming was followed throughout the study.

Scrotal measurements, semen collection and evaluation: Scrotal circumference was assessed twice a month during the breeding and non-breeding season, with a flexible metric tape (Tape, Scrotal Metric, A Neogen Company) (Kulaksiz et al., 2010). Semen was collected in both the breeding season (BS, October-November) and the non-breeding season (NBS, April-May). During both seasons, the first five ejaculates of all rams were discarded to minimize the extragonadal sperm reserve. Semen collection was performed once a week on 8 occasions per each season for each ram. In total, ninety six (96) ejaculates were collected from six rams (sixteen ejaculates from each ram, eight collected in the BS and eight in the NBS) by means of an artificial vagina (Minitube model) after stimulating rams with ewes in estrous.

The ejaculates collected from each ram were evaluated for native sperm characteristics, including semen volume, sperm concentration, motility, and percentage of abnormal sperm. Ejaculate volume was measured immediately after collection using a graduated glass tube. Ejaculates were diluted 1:10 with a skim milk-based extender, and percentage of motile spermatozoa was estimated by subjective microscopic examination at x400 magnification using a phase-contrast microscope equipped with a heated stage at 37°C. Sperm concentration was determined using the haemocytometer method, after diluting the semen samples 1:400 with Hayem's solution. Sperm morphology was evaluated after fixation with Hancock's buffered formol saline solution (Schafer and Holzmann, 2000). Abnormal sperm morphology percentage was determined by counting a total of 200 spermatozoa under a phase-contrast microscope (×1000 magnification; oil immersion).

Semen cryopreservation: A skim milk-based egg yolk extender protocol was used for semen cryopreservation. The extender contained 10 g of skim milk powder and 0.9 g of glucose per 100 ml. Egg yolk and glycerol were added to the solution to final concentrations of 10% and 5% (v/v), respectively. The skim milk-egg yolk-glycerol (SEG) extender contained 500 IU of penicillin and 500 μg of streptomycin sulphate per mL, and was stored at 5°C. Diluted semen was loaded into 0.25 mL French straws were used, so that each straw contained a dose of 100 x 10^6 spermatozoa. Plastic straws were sealed with polyvinyl alcohol powder. Loaded straws were stored at

5°C and were allowed to equilibrate for 2 hours before being frozen. After equilibration, the straws were suspended on a styrofoam rack 4 cm above the liquid nitrogen (vapour) for 15 min. At this stage, the straws were rapidly transferred to LN2 containers at -196°C. Straws were stored in LN2 until evaluation. After being stored for a month, two straws belonging to each ram were thawed in a warm bath (37°C) for 1 min. Next, according to the method described above, the contents of the straw were examined for sperm motility and sperm abnormalities (Evans and Maxwell, 1987).

Statistical analysis

The data obtained in the present study was statistically analysed using the SPSS® (SPSS 18.0, Chicago, IL, USA) software package. Distribution of the data was assessed with the Shapiro-Wilk test. As the data had a normal distribution, the groups were compared with parametric tests. The comparison of all data for the breeding and non-breeding seasons was made with the t-test (independent). Results were expressed in mean±standard error of mean (SEM). Statistical significance was set at $P<0.05$.

RESULTS

Scrotal circumference and native and post-thaw sperm characteristics of the Karayaka rams included in this study are presented in Figures 1, 2, 3 and 4. Season had a statistically significant impact on scrotal circumference ($P<0.05$). Scrotal circumference was greater in the breeding season, when compared to the non-breeding season (Figure 1). While ejaculate volume was also significantly larger in the BS (1.45 ml, $P<0.05$), no statistical difference has been found in sperm concentration, sperm motility, or percentage of normal sperm with respect to season ($4.40x10^9$ ml, 85%, 95%, respectively) (Figures 2 and 3, $P>0.05$). Furthermore, post-thaw sperm motility rates and abnormal sperm percentages were significantly affected by season ($P<0.05$), whereas freezability of semen was better in the NBS, in comparison to the BS (Figure 4).

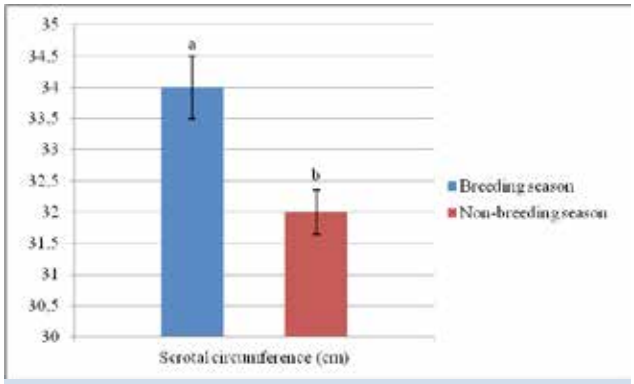

Figure 1. Scrotal circumference of Karayaka rams in the breeding and nonbreeding season

Different letters (a-b) indicate significanct difference ($P<0.05$)

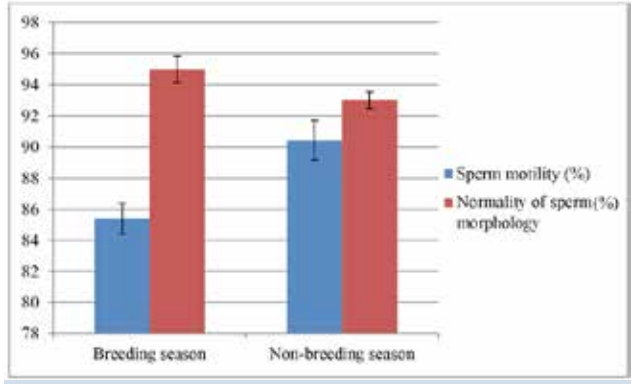

Figure 3. Sperm motility and normality of sperm morphology of Karayaka rams in the breeding and nonbreeding season

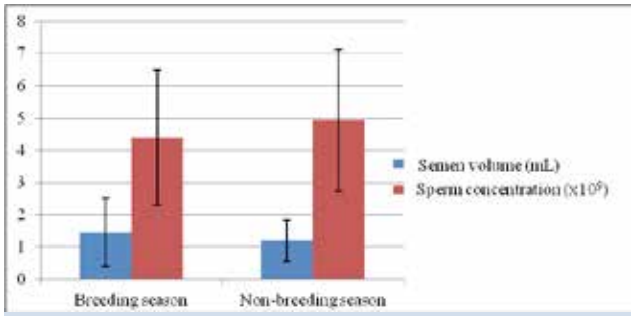

Figure 2. Semen volume and sperm concentration of Karayaka rams in the breeding and nonbreeding season. Different letters (a-b) indicate significanct difference ($P<0.05$)

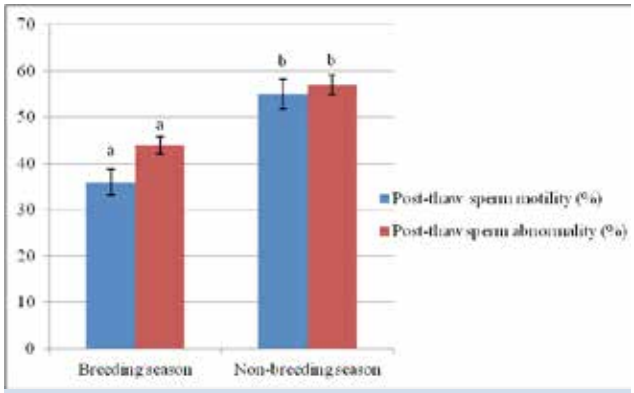

Figure 4. Post-thaw sperm motility and post-thaw sperm abnormality of Karayaka rams in the breeding and nonbreeding season. Different letters (a-b) indicate significanct difference ($P<0.05$)

DISCUSSION

This study is the first report on seasonal changes observed in reproductive indices of Karayaka rams reared in northern Turkey. In the present study, the highest values recorded for scrotal circumferences of the Karayaka rams, were measured in autumn, while the lowest values were recorded in spring. This is in line with the results reported by Zamiri et al. (2010). Similarly, in a study on Dorper rams, Budai (2014) reported the smallest scrotal circumference 30.1 cm in May, and the largest scrotal circumference 36.3 cm in October, and indicated the difference between these months to be statistically significant. Moreover, Oláh et al. (2013) reported the highest values for testicular circumference and scrotal width in winter in Merino rams. Kafi et al. (2004) have recorded the largest scrotal circumference (33.3 cm) in autumn and the smallest scrotal circumference (31.1 cm) in winter. On the other hand, other studies such as that of Jackson et al. (1990) suggest that season has no significant effect on scrotal circumference. Our findings showed that season affects scrotal circumference. These differences observed among the results of various similar studies probably occur due to several uncontrollable factors, including the absorption rate of nutrients.

Several factors including breed, body weight, age, management, weather, nutrition (feed quality and availability), method of semen collection, and degree of sexual stimulation affect the characteristics of ram semen (Folch 1984). In the current study, ejaculate volume of Karayaka rams significantly ($p<0.05$) increased during the BS, in agreement with the findings of Tajangookeh et al. (2007), Karagiannidis et al. (2000), Kafi et al. (2004) and Makawi et al. (2007). Surprisingly, our findings showed that season has no effect on fresh semen quality of Karayaka rams. In contrast to our expectations, the results of the present study showed that, in Karayaka rams, sperm concentration and motility measured in the BS (85.39%) were lower than the respective values measured in the NBS (90.43%). Mean sperm motility determined in the NBS was numerically higher than that determined in the BS, yet this difference was statistically insignificant. In contrast to the present study, Ali and Taha (2012) reported that sperm concentration of Awassi rams was highest during the natural breeding season (from late summer to early autumn), in comparison to the non-breeding season (winter). The increase detected in sperm concentration of Awassi rams was associated with a marked decrease of the photoperiod, which agrees with the findings of Zamiri and Khodaei (2005). Morever, Karagiannidis et al. (2000) reported that during the summer months, semen quality decreased in Chios rams. On the other hand, in a study carried out in crossbred Chios rams in the United Arab Emirates, Ibrahim (1997) found that semen quality did not decline during the hot months of summer. These findings suggest that semen quality of Karayaka rams is not affected by season in northern Turkey. The results of the present study showed that Karayaka rams had continuous and acceptable spermatogenic activity throughout the year. The differences among the results of the present study and previous research might be related to the length of the study period, as well as to age and breed differences of the rams used. These variations could also be attributed to management-related conditions and the laboratory method used to estimate sperm concentration.

Mandiki et al. (1998) recorded higher semen quality (lower percentage of abnormal sperm and higher percentage of live sperm) during the winter in both Suffolk and Ile-de-France rams. Karagiannidis et al. (2000) reported a higher abnormal sperm percentage during spring and summer, and indicated that winter is a transitional period (between the anestrous and breeding season) for the indigenous breeds of Greece. Furthermore, Aller et al. (2012) reported that seasonal changes significantly affected abnormal sperm percentage, that reached its highest level in winter and fall and its lowest in autumn. The low abnormal sperm percentage detected in these studies in autumn is in line with the results of the present study. On the other hand, in the present study the percentage of abnormal sperm was affected by seasonal changes only to a limited extent. Our findings showed that, excluding volume, individual motility, sperm concentration, and percentage of abnormal sperm remained stable throughout the study. Makawi et al. (2007) reported that the most significant ($p<0.05$) increase in seasonal changes occurred in semen volume, sperm concentration, mass motility, sperm individual motility and percentage of live sperm in Awassi rams in the period from late summer to early autumn; this finding is in agreement with that of Tajangookeh et al. (2007). Several factors such as age and breed of the animal, interspecies variations, number of specimens, sperm collection and evaluation methods, nutrition, management practice and environmental conditions may be responsible for the variations in the results of different studies.

One of the most striking findings obtained in the

present study was the fact that freezability of Karayaka ram sperm was significantly higher during the NBS, when compared to the BS, in contrast to the widely accepted view that freezability of ram semen is higher during the BS. Information available in literature reports regarding the freezability of ram sperm collected during the breeding and non-breeding seasons is contradictory. In contrast to the result, it has been reported that ram sperm freezability was higher during the breeding or non-breeding season (D'Alessandro and Martemucci, 2005; Makawi et al*.,* 2007). Olah et al. (2013) reported that freezability of ram sperm during the breeding season or outside may vary depending on the ram breeds.

In the present study, freezability of Karayaka ram semen was better in the non-breeding season. Endocrine status may also alter the resistance of sperm cells to freeze-thawing. It has been suggested that increased plasma testosterone level has a negative effect on the cryosurvival of Capra ibex spermatozoa (Coloma et al., 2010). Although testosterone levels were not measured in the present study, it is well known that, in rams, testosterone levels are much higher during the breeding season, in comparison to the non-breeding season (Ali and Taha 2012; Aller et al., 2012; Zamiri et al., 2010). Furthermore, seasonal alterations in seminal plasma composition may have also influenced sperm freezability *(*Rickard et al., 2014). In fact, in a study carried out in stallions, cryotolerance of sperm frozen in the breeding season was found to be lower than that of sperm frozen in the non-breeding season (Kumar et al., 2014).

CONCLUSION

The season in which semen is collected influences fresh semen quality and freezability of spermatozoa in Karayaka rams. The post-freezing motility of sperm was better during the non-breeding season. Overall, the most favourable fresh and post-thaw semen characteristics of spermatozoa, diluted with a skim milk-egg yolk extender, occur in spring, which corresponds to a sexually hypoactive season for this breed.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

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