ORIGINAL ARTICLE

Developmental changes in testicular interstitium in the Najdi Ram Lambs

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Abstract This experiment was designed to study the relationship between growth rate and sexual development in Najdi ram lambs. Forty-five Najdi ram lambs were used in nine age groups (1–9th month age), keeping 5 lambs in each group. Results show that mean testis size and scrotal circumference increased at a constant rate, reaching a maximum value of 392.00 ± 2.00 g and 25.00 ± 0.32 cm respectively, at the age of 9 months. A similar trend of growth was observed for the mean body weight, reaching a maximum value of 40.2 ± 0.20 kg at the age of 9 months. There was a positive correlation between the increasing rates of body weight and size of the testis. There were significant differences ($P < 0.05$) in testis size, scrotal circumference and body weight of lambs between all ages (1–9 months). The histological section of testis showed that a small number of mature sperms appeared in the eighth month of age and their numbers increased in the ninth month. In conclusion, the current findings indicated that puberty in Najdi rams occurs between the 8th and 9th months of age.

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1. Introduction

With the increasing interest in the application of artificial insemination in sheep, the semen quality is the main parameter limiting male reproductive efficiency which is influenced by testicular size (Zamiri and Khodaei, 2005). Testis size is a standard of fertility and reproductive ability in the ram, as the amount of testicular mass is correlated with the sperm production ability (Salhab et al., 2001), and also it may be useful as a selection criterion for improving reproductive capacity in both the sexes (Toe et al., 2000; Matos et al., 1992). Furthermore, the rate of testis development in the Najdi ram may be a clear evidence of its sexual performance, and has been correlated

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with several reproductive characteristics of female siblings and future female offspring of the ram, such as age at puberty, fertility, prolificacy, and duration of the breeding season (Land and Cart, 1975; Toe et al., 2000). Previous report shows a gradual and linear increase in the testicular volume from 3 to 17 months of age in sheep (Salhab et al., 2001). Scrotal circumference has been described as the best criterion of male sexual development (Notter et al., 1981) but, there is very little evidence correlating age of the ram with scrotal circumference (Notter et al., 1985). In another study, it was reported that various testicular measurements are more correlated with body weight of growing rams than the age (Salhab et al., 2001). A few other reports showed a gradual and rapid increase in both scrotal circumference and testicular volume from 3 to 6 months of age and lambs were in a stage of rapid sexual development as determined by increase in testicular size (Salhab et al., 2001; Ebru, 2005; Ulker et al., 2005).

Detailed information about the onset of puberty and sexual maturation are very important and needed for good reproductive management of domestic animals (Ahmad and Noakes, 1996; Bilaspuri and Singh, 1992). Measurements of ram sexual development may be useful for predicting reproductive capacity in rams sires (Katz et al., 1988; Price et al., 2000). Sexual behavior differs between breeds and herd management plays an important role in the speed of the start of puberty (Godfrey et al., 1988; Maina and Katz, 1997; Price et al., 2000, 2001). Although spermatogenesis is an organized process throughout the life of the male (Cheng et al., 2010; Michelle et al., 2009; Leblond and Clermont, 1952), it does not occur simultaneously in all seminiferous tubules but rather in wave-like sequences of maturation, referred to as cycles of the semniferous epithelium. The objectives of this study were to evaluate the testicular development during postnatal period (age from 1–9 months), and to establish an easy and simple technique for assessing the male fertility that could be useful as a tool in breeding programs in Najdi ram lambs.

2. Materials and methods

2.1. Location, animals and diets

The experiments were carried out at Al-Watania livestock project near Al-Jouf, 150 km from Domat Al-Jandal, Kingdom of Saudi Arabia (29° 48′N, 38° 23′E). Forty-five Najdi lambs were used aged 1–9 months (5 rams in each age group). Rams were weaned at approximately 8 weeks of age and maintained on standard growing ration throughout the experiment, with water and mineral blocks available *ad libitum*.

2.2. Testicular biopsy and histology

Prior to biopsy, scrotal circumference was measured by using vernier caliper and testicular volume was estimated using displacement of water from a cup. All rams were weighed to determine the relationship between testis and body weight, and a gonadosomatic index (GSI) was calculated ($100 \times$ testis weight/body weight). Then, five rams from each age group were assigned to receive a single testicular biopsy at age 1–9 months. Testicular biopsy was performed unilaterally on rams under local anesthesia (Lunstra and Echternkamp, 1988).

After performing biopsy and taking testicular measurement, the tissues were placed in Bouin's fixative for 48 h, then immersed in 70% ethanol for another 48 h. After fixation, samples were washed in Dulbecco's PBS Phosphate Buffered Saline (2×1 h), dehydrated through graded percentages of ethanol (50%, 70%, 80%, 95% and 100%; 2×1 h each), cleared in xylene (2×1 h), pre-infiltrated in a saturated solution of paraffin wax in xylene overnight, infiltrated and were embedded in paraffin wax. Serial sections (5 μm) were made from each sample, mounted onto gelatin-coated glass slides and dried overnight at 37 °C. Tissue sections were stained with hematoxylin-eosin and stored at room temperature until the morphometric analysis. The histological preparations were observed using a compound microscope fitted with photographic attachment (Olympus BX41, Japan). All the observations were made at 400× magnification. For the registration of spermatogenesis, the testicular biopsy score count proposed by Johnsen (1970) was followed.

2.3. Statistical analysis

Least squares analysis of variance was done using the general linear model (GLM) procedure of the statistical analysis system. Differences between groups were assessed to discriminate among the means (Fisher's least significant difference: DNKN procedure). In all cases, the level of statistical significance was considered at $P < 0.05$.

3. Results and discussion

This work presents the first description of the age related changes in body growth and sexual development in Najdi ram lambs. The mean of body weight, scrotal circumference, testicular size and relationship between body weight and testicular size are presented in Table 1. These results appear to be in agreement with corresponding data on other breeds of sheep with few differences that appear to be due to effects of the different environment in which rams were raised (Cui et al., 2003).

The mean body weight increased at a constant rate, from 1st month of age (7.9 ± 0.46 kg) and reached a maximum value of 40.2 ± 0.20 kg at the age of 9 months. There were significant differences ($P < 0.05$) between body weights and lambs aged 1–9 months. The relationships between chronological age and body weight are shown in Fig. 1.

Obvious differences were observed while comparing the body weight of different sheep breeds at seven months of age. Body weight average score for Najdi rams was 34 kg which is less than the other breeds like Serres (44 kg), Friesland (54 kg), Chios (54 kg) and Karagouniki (50 kg) as reported previously (Belibasaki and Kouimtzis, 2000). Variations in the body weight of different breeds could be attributed to the environment and nutrition.

The mean scrotal circumference increased at a constant rate in all lambs, the mean value increased from 1st month of age (6.4 ± 0.24 cm) reaching a maximum value of 25.00 ± 0.32 cm at the age of 9 months. There was a significant difference ($P < 0.05$) in scrotal circumference values (SC) between all ages (1–7 months) except at 8 and 9 months of ages. The difference in SC was insignificant between these two age groups as shown in