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Research Article
Testicular Parameters and Scrotal Measurements in Relation to Age and Body Weight in Growing Naemi-Rams

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Abstract

Background and Objective: The attainment of pubertal age in farm animals is a crucial stage in the reproductive life cycle. The objective of this study was to discover the association between the testicular parameters and scrotum and body weight and age and detection of stages of spermatogenesis, during the before and after puberty period. **Materials and Methods:** Eighty Naemi ram lambs in Saudi Arabia were divided into 8 groups corresponding to ages of 2-16 months (10 males in each group). Testis parameters, scrotum circumference (SC) and Testicular volume (TV) and A testicular histological were measured. **Results:** The results showed that the mean body weight (b.wt.), SC and TV of the rams increased at a quick rate to reach the maximum at 16 months. This character was significantly ($p<0.05$) different between all the age groups. A positive relationship was found between b.wt. and Testicular parameters. A testicular histological examination revealed that before 4 months of age seminiferous tubules (ST) contained nearly spermatogonia only, while Sertoli cells were seen for the first time at 6 months of age. At 14 month of age complete spermatozoa filled the ST lumen. **Conclusion:** It could be concluded that all the testicular and scrotal parameters measured in this study were greatly influenced by b.wt. and age of Naemi ram lambs. This research suggests that Naemi rams reach full pubertal development around one year of age and should not to be used for mating before that age.

Key words: Naemi ram lambs, spermatogenesis, testicular parameters, sertoli cells, puberty period, seminiferous tubules, reproductive life cycle

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The animal livestock sector is considered to be one of the most important economic sectors in the Kingdom of Saudi Arabia, with a role in achieving food security in different kinds of meat, dairy and related products. Sheep are one of the most important livestock species in Saudi Arabia and play a crucial role in the livelihood of small and marginal farmers and nomads. Testis size is considered to be an indicator of reproductive capacity and fertility in the ram as the amount of testicular mass is highly correlated with sperm production capacity and extra gonadal sperm reserves[1-3]. Furthermore, the rate of development of the testes in young rams may be an indicative of their sexual performance[3,4].

Scrotum circumference (SC) is often considered to be the best indicator of male sexual development[5] but little study has been undertaken to describe the association between age and scrotum circumference[6]. Salhab *et al.*[1] showed that the various testicular measurements are more correlated with the body weight of growing lambs than with their age, nonetheless, a gradual but ever more rapid increase in both SC and testicular volume (TV) was observed between 90 and 180 days of age. This data suggests that from 4-7 months of age, lambs are in a stage of rapid sexual development as determined by an increase in testicular size[7-9]. The increase of SC (215%) and TV (442%) between 1 and 360 days of age was twice and four times higher than that of body weight[8]. There is an urgent need for detailed information on the onset of puberty and the sexual maturity of good reproductive management of animals[10]. Because of the above findings, the current experience was to highlight the problems that arise during the selection of breeding males at the early reproductive stage and to be cared for by farmers. The objective of this study, therefore, was to evaluate testicular development and histological appearance and SC dimensions during the postnatal period from 2-16 months of age in relation to body weight in Naemi ram lambs of Saudi Arabia and compare them with other regional and international breeds.

MATERIALS AND METHODS

Location: The experiments were carried out at Al-Watania Livestock in Bosaitta area Project near to Al-Jouf, (150 km from Dumat Al-Jandal city) Kingdom of Saudi Arabia (29°.00-48.00′ N, 38°.00-23.00′ E).

Animals: Eighty growing Naemi lambs were divided into equal groups according to eight age categories (2, 4, 6, 8, 10, 12, 14 and 16 months). The rams were weaned at approximately 8 weeks of age and then maintained on a standard growing ration throughout the experiment, with water and mineral blocks being available *ad libitum*. All rams were weighed every 2 weeks regularly until the end of the experiment and the gonadosomatic index was calculated. The experiments were carried out during the summer (June and August), when the average temperature was 44.6±1.68°C.

Testicular and scrotal measurements: Prior to biopsy, testis diameter (TD) and length (TL), scrotum circumference (SC) and length (SL) were measured using a Vernier calliper and testicular volume (TV) was estimated by displacement of water from a cup as described in Gemeda and Workalemahu[11]. All ram-lambs were weighed every 2 weeks to determine the relationship between testis development and body weight (b.wt.) and a gonadosomatic index (GSI) was calculated (testis volume/b.wt., X 100), according to Al-Kawmani *et al.*[3].

Testicular biopsy and examination: Five ram-lambs from each age group were subjected to a single testicular biopsy, which were performed unilaterally under local anaesthesia[12]. The testicular biopsy involved the operative removal of a piece of tissue from the testis, small enough as to have no deleterious effect on the gland yet large enough to include a representative number of tubules. In the present study, testicular development was evaluated using tissues obtained by a biopsy gun from viable animals, since a biopsy gun is considered to be the most cost-effective, simple and reliable means of conducting testicular biopsies and to have negligible deleterious effects on farm animals either on a short or long-term basis[12]. The testicular biopsy tissues were kept in Bouin's fixative for 48 h and then immersed in 70% ethanol for another 48 h. After fixation, samples were washed in Dulbecco's Phosphate Buffered Saline (DPBS, 2×1 h), dehydrated through graded ascending 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 embedded in paraffin wax. Two serial sections (5 μm) were made from each sample, mounted onto gelatine-coated glass slides and dried overnight at 37°C. Tissue sections were stained with haematoxylin-eosin and stored at room temperature until examination[13]. The histological preparations were examined

under a compound microscope fitted with a photographic attachment (Olympus BX41, Japan) at 400 × magnification.

Statistical analysis: Least square analysis of variance was done using the general linear model (GLM and one-way ANOVA) procedure of the statistical analysis system (SPSS Inc., Chicago, IL, USA). Differences among groups were assessed to discriminate between the means using the Duncan procedure[14]. In all cases, the level of statistical significance was considered to be $p<0.05$.

RESULTS

Body weight (b.wt.): The relationship between chronological age and b.wt., is shown in Fig. 1. In all the ram lambs, the mean b.wt., increased at a constant rate, increased at a constant rate, reaching a maximum value of b.wt., at the age of 16 months. As would be expected, body weight differed significantly ($p<0.05$) between all age groups.

Relationship between body weight, testis size and GSI: There was a positive relationship between b.wt. and the volume of the testicle, i.e., as the body weight increased, there was an associated increase in TV (Fig. 2). A sudden increase in testis size occurred at 14 and 16 months of age. The differences in GSI could be divided into three groups of significant difference ($p<0.05$), months 2, 4 grouped together to be lower than the other months, while the 14 and 16 month-age groups (almost similar) comprised a further significantly different group from all other groups. The remaining age groups (6, 8, 10 and 12 months) did not differ significantly among each other (Table 1).

Scrotal circumference (SC) and length (SL): The relationships between chronological age and SC are shown in Fig. 1. In all the ram lambs, the mean SC increased at a constant rate, reaching a maximum value (43.0±0.33 cm) at the age of 16 months. Inevitably, therefore, the growth rates of the scrotum were significantly different ($p<0.05$) at 2, 4, 6, 8, 10, 12, 14 and 16 months of age followed the same trend. It was found that the significant differences of SL followed the same trend as for SC except at 12 and 14 months of age.

Testicular parameters: In all the Naemi lambs, the mean testis volume (length+diameter) increased at a constant rate, reaching a maximum value at the age of 16 months. Testis volume showed a significant ($p<0.05$) difference in all months, except between 14 and 16 months of age. Testicular volume was also positively correlated with ram lamb b.wt., in all the Naemi lambs. The mean testis length increased at a constant rate, reaching a maximum length (6.91±0.19 cm) at the age of 16 months. The differences between months 2 and 4 were not significant ($p<0.05$), but at these months' values were significantly lower from the other months. The remaining months grouped together without significant differences. Therefore, slight differences in this increase were observed between age groups. There was a positive relationship between b.wt. and testis diameter (TD). Also, there was a gradual increase in TD. But Months 2 and 4 did not show a significant difference between them but were significantly different from the other groups shown in Fig. 3.

Table 1: Gonadosomatic index throughout post-natal till sexual maturity periods in Naemi ram-lambs (Mean±SE, n = 80)

Age (month)	GSI*
2	0.48±0.036[a]
4	0.58±0.028[a]
6	0.94±0.016[b]
8	0.96±0.051[b]
10	0.95±0.014[b]
12	0.94±0.067[b]
14	0.87±0.012[e]
16	0.86±0.057[e]

*Different superscripts in the same column are significantly different ($p<0.05$).
*GSI: Gonadosomatic index, No. of ram-lambs in each age group is 10

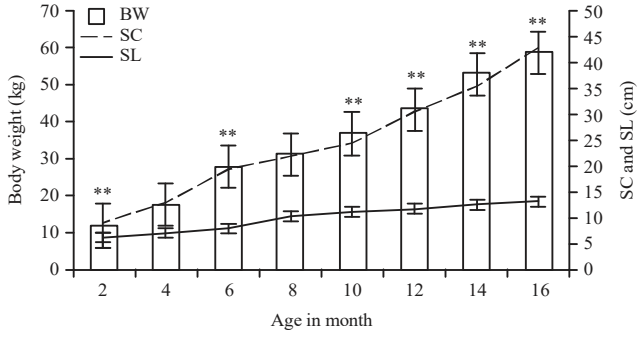

Fig. 1: Body weight and scrotal parameters of the Naemi ram-lambs as affected by the advancement of age and body weight
SC: Scrotal circumference, SL: Scrotal length), The symbol (**) indicate highlight significant values in the figures ($p<0.05$)

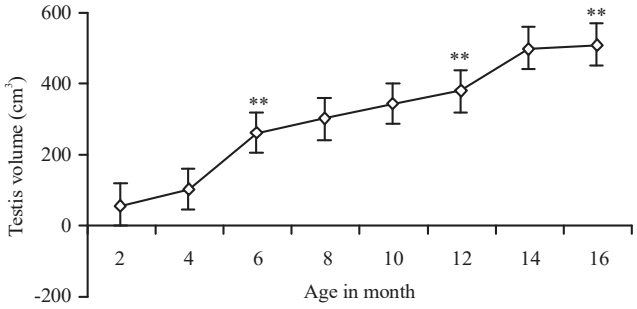

Fig. 2: Testis volume as affected by the advancement of age in Naemi ram-lambs
The symbol (**) indicate highlight significant values in the figures (p<0.05)

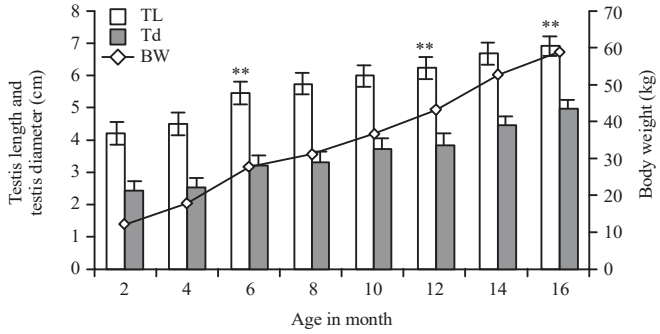

Fig. 3: Relationship between testicular dimensions and body weight as Naemi ram-lambs proceeded from 2-16 months of age
TL: Testis length, Td: Testis diameter. The symbol (**) indicate highlight significant values in the figures (p<0.05)

Histological examination: Microscopically, in Fig. 4a-h each age group had a different histological picture. During the first 4 months of age, there were no cells in the tubular sections except one layer of spermatogonia lying on the basement membrane. Sertoli cells first appeared during the 6th month. At 6 and 8 months of age, only a few spermatocytes were present and at 10 and 12 months of ages, many sperms were present but the germinal epithelium was disorganized with marked sloughing or obliteration of lumen. However, complete spermatogenesis occurred with many spermatozoa were percentage of at 14 and 16 months of age. The germinal epithelium was organized in a regular thickness leaving an open lumen filled with spermatozoa. In Fig. 4 c and d it was observed that the seminiferous tubules exhibited an expanded lumen with a reduced amount of thickening of the spermatogenic epithelium.

DISCUSSION

Few studies have investigated the relationship between age, weight and sexual development parameters in Naemi ram lambs, despite these being one of the most important sheep breeds. This study has begun to bridge this gap in knowledge by showing that testicular and scrotal changes are associated with increased age and weight, starting from two months' post-lambing to 16 months of age. The mean of body weight (b.wt.), testicular volume (TV), scrotal circumference (SC) and scrotal length (SL) were close association with each other, which an agreement with corresponding other breeds of sheep Cui *et al.*[15] in Chinese rams, Emsen[8] in Al-Awassi, Al-Kawmani *et al.*[3] in Najdi, Ake-Lopez *et al.*[10] in Pelibuey rams and Hassanin *et al.*[16] in Najdi and Harriram lambs. A gradual and rapid increase in both SC and TV was observed from 6 months to 12 months of age with an accompanied increase in b.wt., (19.6 and 30.4 kg, respectively) which demonstrates a correlation between the sexual parameters and the b.wt., of the growing lambs with age as ascertained previously by Salhab *et al.*[1] in Awassi ram lambs, Yarney *et al.*[17] in Suffolk rams and Koyuncu *et al.*[18] in Kivircik (Western Thrace) ram lambs. This also may indicate that the age at which ram-lambs reached puberty and at which they may therefore be considered to be sexually mature, is a few

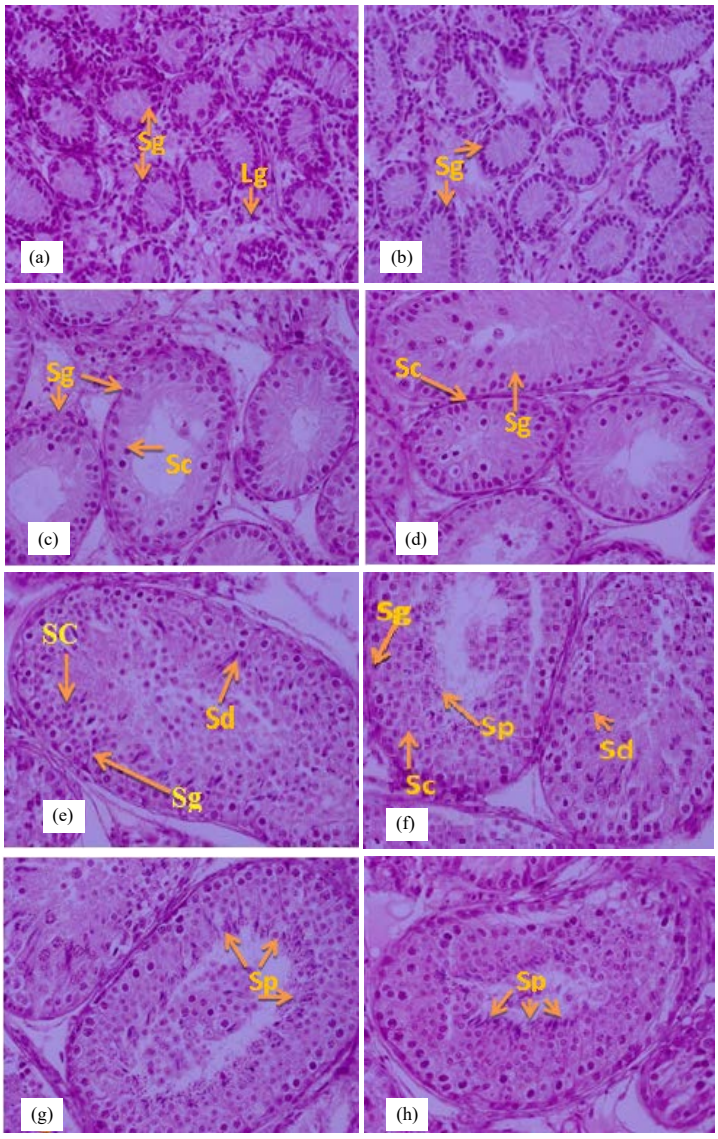

Fig. 4(a-h): Light micrographs of the testis in male Naemi ram-lambs from 2 months of age till 16 months of age (a) 2, (b) 4, (c) 6, (d) 8, (e) 10, (f) 12, (g) 14 and (h) 16 months

Sg: Spermatogonia, Sc: Spermatocytes, Sd: Spermatids, Sp: Spermatozoa, Lg: Leydig cells. Magnification at 400x

months later in the Naemi sheep breed, probably occurring around one year of age when a drastic increase was observed in testicular and scrotal parameters as ascertained previously by Salhab *et al.*[1] in Awassi ram lambs, Yarney *et al.*[17] in Suffolk rams. Statistical analysis revealed significant differences among age groups in b.wt., SC, SL and TV especially at 12 months of age when compared to the previous age groups and this suggests that most of the measured parameters increased around one year of age, corresponding to a sudden increase in b.wt. It could be suggested, therefore, that Naemi rams are better suited to mating duties from 12 months of age onwards as ascertained previously by Al-Kawmani *et al.*[9] in Najdi and Naemi ram lambs and also Mukasa-Mugerwa and Ezaz[19] in Menz ram lambs.

In respect to the histological examination of the testis, the first appearance of spermatocytes occurred at 6 and 8 months of age but a considerable number of spermatozoa were observed at 12 months of age, this result is similar to study of Nazari-Zenouz *et al.*[20]. In general, it could be concluded that the histological appearance of the testis sections by light microscopy of different ages was typical to evaluate testicular development during the postnatal period and to investigate

spermatogenesis in the ram-lambs[9,20]. Anyway, there were relationships between all testicular parameters and body weight, on one hand and also between testis size and spermatogenesis development in testicular interstitium, on another hand[20]. Therefore, this study found that sheep breeds that live in desert environments often develop late sexual maturity and also, this study will help researchers discover the critical areas of reproduction, assessment of sexual performance and stages of spermatogenesis in different ages. Thus, a new theory can be found and easy tool to evaluate testicular development during the postnatal period and to investigate spermatogenesis in most breeds.

SIGNIFICANCE STATEMENT

Because of the above findings, the current experience was to highlight the problems that arise during the selection of breeding males at the early reproductive stage and to be cared for by farmers. By finding a simple and easy tool to evaluate testicular development during the before and after puberty period and to investigate spermatogenesis in Naemi ram lambs. Which live in the harsh desert environment of Saudi Arabia and compare them with other regional and international breeds'.

CONCLUSION

The results showed that the scrotum circumference (SC) is an important selection criterion and an indicator for determining the testicular development and safety of young rams as they are closely related to testicular parameters and sperm production, thereby improving performance sexual in the rams. Maybe the scrotum circumference varies depending on season and body weight but should be at its highest peak during the breeding season.

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