INTRODUCTION

Detailed information about the onset of puberty and sexual maturity is needed for the good reproductive management of domestic animals (Al-Kawmani et al. 2014; Ake-Lopez et al. 2016). In sheep, testis size may be useful as a selection criterion for improving reproductive capacity in male lambs (Toe et al. 2000; Al-Kawmani et al. 2014; Ake-Lopez et al. 2016; Hassan et al. 2013), and the measurement of ram sexual development parameters may be useful for predicting reproductive capacity in rams (Kridli and Said, 1999; Price et al. 2000). Furthermore, the characterization of puberty and early sexual development are important criteria used in the selection of ram-lambs within a breed (Kridli and Al-Yacoub, 2006; Hassan, 2013). In this context, it is significant that while most breeds of sheep are seasonal breeders, the age of puberty in males differs from one breed to another (Hassanin, 2013). The age of 4–5 months, however, appears to be a critical period for pubertal changes, and testosterone plays an important role in the appearance of sexual desire among ram lambs, as determined by the first existence of spermatozoa in ejaculated semen (Cui et al. 2003; Salhab et al. 2001). The postnatal development and function of the testes are controlled by a complex interaction of circulating gonadotropins with cytokines and growth factors, as well as the establishment of the hypothalamo-hypophyseal gonadal axis (Cardenas-Gallegos et al. 2015; Sarlos et al. 2013). This work was therefore designed to determine the specific changes to the testes and to testosterone concentrations occurring during the postnatal period until puberty is attained.

Sheep are one of the most important livestock species in Saudi Arabia and play a vital role in the livelihood of a large proportion of small, marginal farmers and nomadic peoples. The Najdi and Naemi breeds are among the most important in the Kingdom of Saudi Arabia, but understanding the reproductive processes and selection of breeding males is challenging in the harsh desert conditions. The declining numbers of local sheep and increased amount of imported meat have prompted this study, which aims to provide scientific information that might help efforts to increase the domestic production of sheep in the Kingdom.

It is important to know whether Najdi and Naemi breeds reach sexual maturity early or late since a lot of farm animal breeders find it difficult to determine the males with the best chance of exhibiting superior reproductive
performance, even though it is crucial to take care of such males at an early age to prepare them efficiently for the reproductive process. From a scientific perspective, a particular issue is that the testosterone concentration in blood plasma has not been fully studied in these breeds to date, especially in terms of its relationship with testicular parameters and postnatal body weight, which is useful in determining the commencement of spermatogenesis (Swelum et al. 2016).

The objectives of this study was therefore to evaluate testicular development during the postnatal period in the Najdi and Naemi ram lambs, and to investigate the occurrence of spermatogenesis in these breeds in relation to age and body weight. In addition, the exact age at which testosterone levels are elevated and the relationship of these levels with the histological appearance of the testes will be determined.

MATERIALS AND METHODS

The experiments were carried out at the Al-Watania Livestock Project in the Bosaitta area near to Al-Jouf, Kingdom of Saudi Arabia (29°.00′ – 48.00′N, 38°.00′ – 23.00′E). A total of 90 male lambs (45 Najdi and 45 Naemi) were used, with five lambs at each month of age between one and nine months for each of the two breeds. The rams were maintained on a standard growing ration throughout the experiment, with water and mineral blocks available ad libitum. All the samples were collected between March to July, i.e. 90 samples in total covering all animals in all age groups according to the plan of maintaining five samples in each age group.

Testicular biopsies were collected from each male lamb (from 2-9 months) by the operative removal of a piece of tissue from the testis, small enough as to have no deleterious effect on the testis yet large enough to include a representative group of tubules. Prior to biopsy, scrotal circumference was measured using a Vernier calliper and testicular size was estimated by displacement of water (from 1-9 months). The body weight (BW) of each lamb was recorded at the commencement of the experiment.

Plasma Testosterone test: Blood samples were collected from the jugular vein of each ram in 10 ml heparinized vacutainer tubes. These were then centrifuged directly at 3500 x g for 20 min. The harvested plasma was stored at -20°C until the hormonal assay. The testosterone plasma concentration (ng/ml) was determined in all the samples according to Kicklighter and Norman (1989), using simple solid phase competitive ELISA commercial kits (Human Diagnostics Worldwide, Wiesbaden, Germany), following the manufacturers protocol. The intra and inter-test coefficients of differences were 3.4 and 4.1%, respectively for concentrations between 0.2 and 16 ng/ml.

Histological preparations: Upon completion of the testicle measurements and collection of biopsy samples, the samples were prepared for histology by fixing them in Bouin’s fixative for 48 hours. This was followed by washing with 70% alcohol and Dulbecco’s Phosphate Buffered Saline (PBS) respectively. Samples were then dehydrated overnight with increasing concentrations of alcohol series, cleared with three changes of xylene and impregnated with melted paraffin wax using an automatic tissue processor (VIP tissue, Tek 5 Jr, Sakura, Japan). The processed tissue samples were embedded in paraffin blocks and serial sections with a thickness of four microns were obtained using a rotary microtome (RM2245, Leica, Germany). The sections were stained with routine haematoxylin and eosin stains (H&E) and mounted with DPX using an autostainer machine (Leica ST 5020, Leica Company, Germany) before being stored at room temperature until quantitative morphometric analysis and microscopic examination and photography, (DeVento et al. 1992).

Statistical analysis: Least square analysis of variance (ANOVA) was done using the general linear model (GLM) procedure in the SAS statistical analysis system (1996). Differences among groups were assessed by Fisher’s least significant difference (LSD) and Duncan procedures (1955) to check the significance among the means. In all cases, the level of statistical significance was set at P < 0.05.

RESULTS AND DISCUSSION

Body weight: The mean body weight of Najdi and Naemi rams increased at a constant rate, reaching a maximum value of 42.3±0.33 kg and 32.8±0.20 kg, respectively, at the age of nine months. There were significant differences (P < 0.05) in body weight with increasing age in both breeds. The relationship between chronological age and body weight is shown in (Fig. 1). The mean body weight of Najdi rams was significantly greater than that of Naemi rams from first to ninth month (Table 1).

Scrotal circumference: The mean scrotal circumference in Najdi and Naemi rams increased at a constant rate, reaching a maximum value of 27.19±0.22 cm and 22.6±0.24 cm, respectively, at the age of nine months. There were significant differences (P < 0.05) in scrotal circumference with age in both breeds at every month compared to the preceding month. The relationship between chronological age and scrotal circumference is shown in (Fig. 2). The mean scrotal circumference of Najdi rams was significantly greater than that of Naemi rams from the first to the seventh month (P < 0.05).

Testis Size: The mean testis size in Najdi and Naemi rams increased at a constant rate, reaching a maximum value of 395.00±3.00 g and 316.00±2.45 g, respectively, at the age of nine months. There were significant differences (P < 0.05) between testis sizes with age in both breeds at every month compared to the preceding month. The relationship between chronological age and testis size is shown in (Fig. 3). The mean testis size of Najdi rams was significantly greater than that of Naemi rams from the first to the tenth month with as shown in Table 1 (P < 0.05). The statistical analysis revealed significant (P < 0.05) differences for breed (B) effect as well.
Effect of age on body weight, scrotal circumference, testis size and testosterone concentration in Najdi and Naemi male lambs. (Mean ± SE, n = 5 for each Najdi and Naemi lamb).

<table>
<thead>
<tr>
<th>Age (Month)</th>
<th>BW(kg)*</th>
<th>SC(cm)*</th>
<th>TS(g)*</th>
<th>TC(ng/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Najdi</td>
<td>Naemi</td>
<td>Najdi</td>
<td>Naemi</td>
</tr>
<tr>
<td>1</td>
<td>8.2±0.56k</td>
<td>6.50±0.27m</td>
<td>6.34±0.22l</td>
<td>4.1±0.10a</td>
</tr>
<tr>
<td>2</td>
<td>16.1±0.44k</td>
<td>12.10±0.29l</td>
<td>9.54±0.31k</td>
<td>9.4±0.40b</td>
</tr>
<tr>
<td>3</td>
<td>18.8±0.46j</td>
<td>15.50±0.50k</td>
<td>13.11±0.34i</td>
<td>10.6±0.24c</td>
</tr>
<tr>
<td>4</td>
<td>24.3±0.43i</td>
<td>17.60±0.24j</td>
<td>14.59±0.33gh</td>
<td>12.8±0.37d</td>
</tr>
<tr>
<td>5</td>
<td>26.5±0.32h</td>
<td>23.60±0.24i</td>
<td>17.31±0.23f</td>
<td>14.6±0.24e</td>
</tr>
<tr>
<td>6</td>
<td>33.4±0.34e</td>
<td>27.60±0.24g</td>
<td>21.59±0.34d</td>
<td>17.4±0.75f</td>
</tr>
<tr>
<td>7</td>
<td>36.4±0.53c</td>
<td>30.00±0.45f</td>
<td>23.39±0.23bc</td>
<td>21.2±0.37g</td>
</tr>
<tr>
<td>8</td>
<td>39.5±0.31b</td>
<td>30.80±0.20ef</td>
<td>24.39±0.42b</td>
<td>21.4±0.40g</td>
</tr>
<tr>
<td>9</td>
<td>42.3±0.33a</td>
<td>32.80±0.20d</td>
<td>27.19±0.22a</td>
<td>22.6±0.24h</td>
</tr>
</tbody>
</table>

* For each character, means with different superscripts within each month differ significantly (P < 0.05).

Table 2: Source of variability and probability for studied parameters in Najdi and Naemi ram lambs.

<table>
<thead>
<tr>
<th>Character</th>
<th>Variability</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>A</td>
<td>603.41</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1641.58</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>A×B</td>
<td>17.52</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>SC</td>
<td>A</td>
<td>103.19</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>624.64</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>A×B</td>
<td>4.04</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>TS</td>
<td>A</td>
<td>463.68</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3004.66</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>A×B</td>
<td>29.94</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>


Testosterone concentration: The mean testosterone concentration in Najdi and Naemi rams increased at a constant rate, reaching a maximum value of 7.58±0.13 and 7.23±0.12 ng/ml, respectively, at the age of nine months. There were significant differences (P < 0.05) in testosterone concentration with age in both breeds at every month compared to the preceding month. The relationship between age and testosterone concentration is shown in (Fig. 4). The mean testosterone concentration was significantly higher in Najdi rams than in Naemi ones between the first and the sixth month, as shown in Table 1 (P < 0.05).

Histological examination: Histological sections of the testes of Najdi rams showed no cellular divisions for spermatogonia within the seminiferous tubules during the second, third, and fourth months of age, except for an increase in the diameter of the seminiferous tubules diameter at the expense of cavity with increasing age. Interstitial tissue containing Leydig cells was clearly observed. In Naemi ram lambs, the histological sections showed no divisions for spermatogonia inside the seminiferous tubules during the second, third, fourth and fifth months of age, with a small expansion in the diameter of the seminiferous tubules; Sertoli cells and Leydig cells were increasingly observed, as shown in (Fig. 5).

In five month old Najdi ram lambs there were few large diameter spermatocytes in the sections as a result of the growing phase of spermatocytogenesis. At the sixth month of age, although no spermatozoa were present, several spermatocytes and a few spermatids existed as a result of initiation of meiotic division, as shown in (Fig. 5). At the seventh month of age there were still no spermatozoa but all types of spermatogenic cells, especially spermatids could be seen. At the eighth month of age many spermatozoa were present but the germinal epithelium was disorganized with marked sloughing or obliteration of the lumen, as shown in (Fig. 5). At the ninth month of age, however, complete spermatogenesis with many spermatozoa were clearly observed. The germinal epithelium was organized in a regular thickness leaving a relatively small open lumen, as shown in (Fig. 5).
The results of the present work showed the existence of significant monthly differences in the body weight (BW) of both Najdi and Naemi ram lambs at every month from the first to the ninth, but these differences were greater with Najdi rams than with Naemi ones. These results are in broad agreement with data from other studies (Al-Kawmani et al. 2014; Cui et al. 2003), with just a few differences that appear to be due to the effects of breed and the environment in which the rams were raised. The body weight also increased at a constant rate in both breeds as the animal aged up until the point of sexual maturity and thus a fully developed body (Emsen, 2005; Belibasaki and Kouimtzis, 2000). The exact rate of body weight increase, however, is mainly dependent on the breed of sheep (Hassanin et al. 2013).

A sudden and rapid increase in testosterone concentration was observed in both Najdi and Naemi ram lambs between five and nine months of age. While scrotal circumference increased between three and six months of age. These results are similar to those obtained by several researchers. Emsen (2005) and Hassanin (2013), for example, found an increase in both scrotal circumference and testicular volume between three and six months of age in Redkaraman and Awassi sheep. In another study of Awassi ram lambs Salhab et al. (2002) reported a sharp increase in testicular dimensions starting around seven months of age and at an average body weight of 34.6 kg. From all these data, it could be suggested that lambs experience rapid sexual development between three and six months of age.
nutritional and breed effects. Indeed, in these other four breeds there was a significant difference in SC: at puberty the SC of Friesland rams was 33.9 cm, which was 4 - 6 cm greater than in the three remaining breeds. Indeed, Friesland ram lambs had a greater SC throughout the entire period studied.

In all Najdi ram lambs, a gradual and rapid increase was observed in the mean testis size between three and seven months of age, while in Naemi ram lambs this increase occurred between four and seven months of age. These data suggest that from three to seven months of age, lambs are in a stage of rapid sexual development as determined by an increase in testicular size. These results are similar to those obtained by Emsen (2005) and Ulker et al. (2005). The increase in testicular size, length, width and circumference between three and seven months of age was in accordance with the development of body weight, while the increase of testicular volume was three times greater than the growth of body weight or the three other testicular parameters in a study conducted by Salhab et al. (2002).

There was a positive relationship between body weight and testicular size in both Najdi and Naemi ram lambs. Salhab et al. (2002) found that testes’ measurements were progressively correlated with body weight more than with age. A clear difference in testis size for different sheep breeds at different months of age has been demonstrated also in other studies (Koyuncu et al. 2005; Emsen, 2005). Thus, early pubertal development is associated with increased body weight which is desirable in terms of improved reproductive performance.

As shown in Fig 5, there was no clear difference in spermatogonia during the second, third and fourth months of age in Najdi ram lambs, while in Naemi ram lambs, observation was found in spermatogonia during the second to the fifth months of age. There was no appearance of cell division within the seminiferous tubules at all except for an increase in the diameter of the seminiferous tubules at the
Fig 5: Cross section of testis showing the process of spermatogenesis in Najdi left and Naemi right ram lamb at different postnatal ages (2–9 months), (A) two months of age, showing immature spermatogonia and sertoli cells, (B) three months of age, showing basal spermatogonia, (C) four months of age, showing spermatogonia, (D) five months of age, few spermatocytes were observed, (E) six months of age, more spermatocytes were present, (F) seven months of age, showing spermatids, (G) eight months of age, showing few spermatozoa, (H) nine months of age, showing uniformly distributed Spermatozoa. Abbreviations, (St) seminiferous tubules, (Sg) spermatogonia, (Sc) spermatocytes, (Rs) spermatids, (Sp) spermatozoa, (Le) leydig cells, (Sc) sertoli cells. (400. X, H & E stain)
expense of cavity and the interstitial space. The first spermatogonial division began at the fifth month in Najdi ram lambs, during the sixth month in Naemi ram lambs, and spermatogenesis was completed with many spermatozoa present during the eighth month in Najdi and the ninth month in Naemi rams.

Overall, this, this showed that Najdi and Naemi ram lambs reached puberty around the eighth and ninth month, respectively. Sexual development and the appearance of spermatozoa in ejaculates are more correlated with body weight than chronological age of ram lambs (Al-Kawmani et al. 2014; Toe et al. 2000; Salhab et al. 2002; Lunstra and Cundiff, 2003). The Tanyang breed reached puberty between the eighth and ninth months of age (Cui et al. 2003), while other breeds, such as Chios, Karagouniki, Serres and Friesland, reached puberty between sixth and seventh months of age. (Belibasaki and Koumitzis, 2000; Cui et al. 2003). It appears, however, that Najdi and Naemi breeds may be among the breeds that are intrinsically of late puberty and this could be the subject of further study. The current findings indicate that puberty in Najdi and Naemi rams occurs in the eighth and ninth months of age, respectively.

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REFERENCES


