Influence of laterality on testis anatomy and histology in Ghezel rams

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Abstract

Whether paired organs are equivalent has to be analyzed by assessing both structural and functional aspects. The present study compared the paired left and right testis from Ghezel sheep originating from Northwestern Iran (Azerbaijan) and Northeastern Turkey. Twenty-five pairs of testes were collected from mature Ghezel rams from Tabriz slaughterhouse. The two glands were compared for their size, weight and activity rate. Weight, length, width and thickness were measured. Then, paraffinized blocks were prepared by routine histological techniques and sections were stained by the haematoxylin-eosin method. Five 6 μm sections were prepared from each paraffinized block and 50 seminiferous tubules (STs) were analysed in each section for tubular differential index (TDI) and spermiogenesis index (SPI). TDI and SPI were compared between the paired left and right testes. Weight, length and thickness of left testes were significantly higher than in right testes (P < 0.05). Moreover, TDI and SPI were found to be higher in left testes than the right (P < 0.05).

Key words: activity, anatomy, Ghezel ram, histology, laterality, sheep, testes.

Introduction

Ghezel sheep was originated in Northwestern Iran (Azerbaijan) and Northeastern Turkey; because of cold and dry climate, this breed is suitable to the area (Tavakolian, 2000). Testes are endocrine and exocrine glands which produce the male gametes (spermatozoa) and secrete male sex hormones. The shape and size of testis vary in different animals and species and also between breeds within a breed. The shape of sheep testes is oval and its weight is between 80 and 300 g. The weight of testis depends on the breed, feeding and, critically, the mating season (Mukasa-Mugerwa et al. 1986; Galmessa et al. 2000).

The sequence of events in the development of spermatozoa from spermatogonia is referred to as spermatogenesis and is subdivided into three phases: (1) spermatocytogenesis, the process during which spermatogonia develop into spermatocytes; (2) meiosis, the maturation division of spermatocytes that result in spermatids with a reduced (haploid) number of chromosomes; and (3) spermiogenesis, the process of transformation of spermatids into spermatozoa. The duration of spermatogenesis is 40–50 days in rams (Junqueyra et al. 1986; Wrobel 1993). The size of left testis in mammals and poultry is reported greater than right testis (Sisson & Grossmann 1975). But there is no information about the activity rate in left and right testes.

The aim of our study was to compare left and right testicular size and activity to understand if there is any difference or not. Four anatomical factors as weight, length, width and thickness were measured in each testis. In addition, to address testes function, tubular differential index (TDI) and spermiogenesis index (SPI) were assessed by histology.
Materials and method

Twenty-five pairs of testes from mature Ghezel rams were collected from Tabriz slaughterhouse in autumn and winter 2015. Testes were taken immediately to the laboratory on ice; and were removed from scrotum, and the epididymis and other around tissues of the testes were separated (Fig. 1).

Anatomy – Morphology

Weight, length, and thickness and width (Fig. 2, 3) were measured by a scale and a caliper.

Histology

For histological studies, testicular tissue samples 1 x 1 cm were prepared and fixed in 10% formalin for a week. Paraffinized blocks were prepared by routine histological techniques and 6 μm sections were stained (haematoxylin-eosin staining). The activity of testes was evaluated by measuring the tubular differential index (TDI) and the spermiogenesis index (SPI). The average of SPI and TDI was compared between the left and right testes by t-Test (Paired Two Sample for Means).

Five sections (6 μm) were prepared from each paraffinized block and 50 seminiferous tubules (STs) were analyzed in each section for TDI and SPI. To estimate the TDI, 50 STs were counted in each section. STs with less than three layers of differentiated germinal cells were considered negative. STs with more than three layers were considered TDI positive. The proportion of positive and negative seminiferous tubules was counted. To estimate the (SPI, 50 STs were considered and proportion of STs with spermatozooids compared to seminiferous tubules without spermatozoid was counted. STs containing spermatozooids were considered positive and STs without spermatozoid were considered to be negative. (Fig. 4–6).

Fig. 1. Left and right testes of a Ghezel ram after removal of around tissue. (A) epididymis attachment region.

Fig. 2. Method of measuring testes length.

Fig. 3. Method of measuring width and thickness in two planes.

Fig. 4–6.
Results

Morphometry results

Anatomical results

Anatomical results are shown in Table 1. Weight, length, width and thickness of left testis were higher than those of their paired right testes and were significantly different ($P \leq 0.05$) in all measures.

Histological results are shown in Table 2. The TDI and spermiogenesis index (SPI) in right and left testes of Ghezel ram were significantly greater in the left compared to the paired right testes ($P < 0.05$).

Discussion

Testes are paired organs that produce male germ cells. They are enclosed in a diverticulum of the

![Fig. 4. Histological section of a seminiferous tubule.](image)

1. Seminiferous tubule which contains less than three layers of differentiated germinal cells (A).
2. Seminiferous tubule which contains more than three and/or four layers of differentiated germinal cells. (B) seminiferous tubule which contains lower than three and/or four layers of differentiated germinal cells.

![Fig. 5. SP, spermatozoids.](image)

(A) Seminiferous tubules which contain spermatozoids. (B) Seminiferous tubules without spermatozoids. (C). (H&E staining x 400).

![Fig. 6. Histological section of a seminiferous tubule that shows positive SPI and positive TDI.](image)

1. Spermatocyte (A); Sertoli cell (B); Spermatozoids (C). (H&E staining x 1000).

### Table 1. Anatomical results of Ghezel sheep testes ($n = 25$ rams)

| Testes-studied factors | Weight (g) | Length (cm) | Width (left to right) cm | Thickness (Cranial to caudal) cm |
|------------------------|------------|-------------|--------------------------|----------------------------------|
| Left testes            | 164.2 ± 78.5$^a$ | 9.0 ± 1.6$^a$ | 6.4 ± 0.9$^a$ | 5.9 ± 0.8$^a$ |
| Right testes           | 158.9 ± 78.3$^b$ | 8.8 ± 1.5$^d$ | 6.3 ± 0.7$^f$ | 5.7 ± 0.8$^h$ |

Statistically significant differences were noted. Same letter superscripts in each vertical column indicate no significant difference ($P \leq 0.05$).
abdomen termed the scrotum (Sisson & Grossmann 1975). Depending on the species, the testis is typically oval to nearly spherical and varies considerably in size. In the ruminants, right and left testes usually differ in size and position, one being slightly more dorsal than another. The weight of the testes in bull is 250–300 g, those of the ram 200–300 g and that of the goat is 145–150 g (Nickel 1979). They vary greatly in size in different subjects and are commonly of unequal size, the left one more often being larger (Sisson & Grossmann 1975). It is always stated that the left testis is usually larger than the right in birds (Marshall 1961), but Gray (1930) found that in Leghorns, the right testis is usually the larger. In a series of White Leghorns of various ages up to 6 months old, the average weight of the left testis was about 17% greater than that of the right; in older birds, the right testis was about 10% heavier than the left (King 1975).

Table 2. Activity indices of Ghezel sheep testes (n = 25)

| Testes/Activity index | SPI       | TDI        |
|-----------------------|-----------|------------|
| Left testes           | 67.86 ± 21.44393a | 281.02 ± 94.95111c |
| Right testes          | 39.30 ± 10.49839524b | 168.36 ± 41.50305d |

Superscript initials in each vertical column are not same indicating a significant difference.

Testicular weight, a reliable index of semen producing ability has been shown to vary between three breeds of sheep in Nigeria. It was concluded that the testicular biometry of Uda and Balami sheep observed were suggestive of higher spermatozoa per unit mass of the testes and epididymis and subsequently higher fertility (Ibrahim et al. 2012). Yarney & Sanford (1985) studied the relationship between prepubertal reproductive endocrine parameters and postpubescent testicular size together with rams’ function, and they observed a positive correlation between prepubertal male hormone concentrations and subsequent testicular size and mating frequency.

The right testis in mammals being smaller than the left one has been attributed to an enlarged pampiniform plexus on the left side (El-Jack 1987). It is reported that in sheep, the left testis was 10% larger than the right testis on subsequent examinations (Mahmud 2015). Perumal (2014) showed that the scrotal circumference was highly correlated with testicular parameters and body weight compared to age. The males with bigger testis produce more sperm than the males with smaller testis (Kaymakci et al. 1988).

This study has shown that the left testis size (without epididymis and pampiniform plexus) is significantly larger than right testis in Ghezel ram (P < 0.05). Also the weight of the left testis was significantly higher than those of the paired right testes (P < 0.05).

There is no information about different activity rates between left and right testes. However, in the female, there are some reports of differences between right and left reproductive activity. During oestrus, a single oocyte is usually released and there is a slight preponderance of ovulations from the left ovary. Assessing the functional activity of the two ovaries on the basis of post-mortem counts of corpora lutea in 792 equine genitalia, Arthur et al. (1996) recorded an incidence of 52.2% of ovulations occurring from the left ovary. In a series of 1506 uteri of dairy cattle examined in the USA, Erdheim (1942) found the fetus in the right horn in 1015 (67.4%) and in the left in 474 (31.4%) but in a series of 2318 uteri of beef cattle, however, the side incidence of pregnancy was approximately equal: right 1178 (50.8%), and left, 1121 (48.3%). Among all Erdheim’s (1942) specimens, there was one exceptional single pregnancy in which he found the CL in the left ovary, and fetus in the right horn, out of 133 pregnant uteri from Swedish Highland cattle. Settergren & Gallaway (1965) found 59.4% pregnant on the right side and 40.6% on the left. This series also included one specimen in which the CL was in the left ovary and the fetus in the right horn (Arthur et al. 1996). In the aves, during fourth day of incubation, many germ cells transfer from the right gonad to the left and it has also has reported that the left gonad then has up to five times as many germ cells as right (Venzke 1954; Swift 1915; King 1975). In the genetic female, the left ovary soon becomes larger than the right. Even before the end of the indifferent phase of embryonic development, the left gonad is larger of the two, consistent with its far greater endowment of...
germ cells (King 1975). Libman et al. (2010) studied the microanatomy of the left and right spermatic cords and their related arteries and lymphatics at subinguinal microsurgical varicocelectomy. On the left there were more arteries and lymphatics than on the right, with a significant correlation between the number of right and left internal spermatic arteries \((r = 0.42)\). Ali Abdullahi et al. (2012) reported that testicular morphometric characteristics could be used to predict the sperm production capacity in camels, and that reliable selection for breeding based on the testicular morphometry was possible.

Spermatogenesis index (SPI) is an important marker for estimation of testes activity. High rate of SPI showed high rate spermatozoid production and resulted high rate of testis activity. This research histological results (SPI) were showed which, left testes of Ghezel sheep were more active than right testes significantly \((P < 0.05)\). Tubular differential index (TDI) is another important landmark for estimation of testes activity. In TDI, STs that contained over three layer of germinal cell are active in spermatozoid production. This research histological results on TDI were appeared which, left testes TDI were more active that right testes significantly \((P < 0.05)\).

In generally, this research results showed that left testes of Ghezel ram are not only significantly \((P < 0.05)\) larger than right testes but also significantly more active (SPI and TDI) than right testes \((P < 0.05)\). It is not known why there is laterality in testicular anatomy and function. There are a series of factor which could influence them. For example, endocrine, blood vessels branching pattern, gene expression and other factors could influence. Further work is needed to identify different factors and determine their influence in laterality.

Conflicts of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all the named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Ethics statement

This study was performed on Ghezel sheep testes. Testes are eliminated organs in slaughter house and they did not, have nutritional usage for human and animals. We collected Ghezel sheep testes from Tabriz industrial slaughter house.

Contributions

HK designed and carried out this project. MRS and FBK contributed in samples collection, data collection and lab works, in a team. HK supervised the project and checked the writing of this article.

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