

## Stereological study of Arabian ram testis during different seasons

Dorostghoal, M.<sup>1\*</sup>; Erfani Majd, N.<sup>2</sup> and Goorani Nejad, S.<sup>3</sup>

<sup>1</sup>Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran; <sup>2</sup>Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; <sup>3</sup>Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

\*Correspondence: M. Dorostghoal, Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran. E-mail: mdorostghoal@cua.ac.ir

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### Summary

Quantitative microscopic structure of testis in adult Arabian rams, raised under closed grazing system in Khuzestan province of Iran, was studied in four seasons over a one year period. For this purpose, thirty adult rams were randomly selected and the live weight and scrotal circumference of rams were recorded. At the beginning of each season, among the study group, four rams were randomly selected, slaughtered and their left testes were removed and weighed. For microscopic studies, tissue samples were excised from the left testes, fixed in Bouin's solution and embedded in paraffin. Cross-sections (5 µm thickness) were stained with haematoxylin-eosin and evaluated with quantitative techniques. The results showed that scrotal circumferences, weight and volume of testis varied significantly throughout the year, with the lowest values in early summer ( $P < 0.05$ ). Seminiferous tubule diameter was highest in early winter ( $220.97 \pm 12.15$  µm). Also, relative and total volumes of seminiferous tubules and germinal epithelium gradually increased during the summer and autumn, with highest values in early winter. Higher relative volume of seminiferous tubuli lumina ( $P < 0.05$ ) was found in early summer ( $12.15 \pm 0.35$ ). Lower relative volume of interstitial tissue ( $P < 0.05$ ) was found in early winter ( $15.70 \pm 0.49$ ). Scrotal circumference was significantly correlated with the seminiferous tubule diameter and the total volume of seminiferous tubules ( $r = 0.70$ ,  $P < 0.01$ ). The results indicated that the stereological structure of testis in Arabian rams raised under closed grazing system in the Khuzestan province of Iran is highly affected by season.

**Key words:** Season, Testis, Stereology, Ram reproduction

### Introduction

Reproductive organs are the most dynamic organs in all animals. There are many factors affecting reproduction and fertility such as environmental factors, the most important of which is the photoperiod. However, reproductive activity is not a direct function of day length, but is affected by the photoperiodic history of the animal, the direction of photoperiodic changes and the stage of the annual rhythm at which the photoperiodic signal is received (Robinson and Karsch, 1987). Moreover, the effects of photoperiod on reproduction can be modified to a certain extent by temperature, nutrition, body condition or age of animal (Gerlach and Aurich, 2000). Seasonal

variations of reproductive activity, found in both sexes of many species, are dictated by changes in the secretion of the gonadotropin releasing hormone from the hypothalamus (Edqvist and Stabenfeldt, 1993).

In animals with seasonal reproduction such as the ram, testicular size, testosterone secretion, sperm production and reproductive behavior are decreased during the non-breeding season (Gerlach and Aurich, 2000).

There are marked differences in circannual variations in plasma concentrations of testosterone, FSH and inhibin among different breeds of sheep (Islam and Land, 1977; Dacheux *et al.*, 1981; Lincoln *et al.*, 1990). Moreover, seasonal variations of ram reproductive

activity appear to be influenced by geographical locations (Lincoln *et al.*, 1990; Bielli *et al.*, 1999).

Arabian ram is one of the most important native breeds in the Khuzestan province of Iran, but its reproductive performance has been poorly investigated. Therefore, the present study was performed to determine the basic quantitative morphology and to evaluate the degree of seasonal variations in stereological structure of the testis in Arabian rams raised under closed grazing conditions in Khuzestan province.

## Materials and Methods

### Location

The experiment was carried out on a farm in Ahvaz city in Khuzestan province in Iran. Ahvaz city is located at 31 N and 48 S in a tropical to subtropical zone. In this area, mean temperature varies between 14.5 (December) to 50°C (June) and mean annual rainfall is 226 mm. Daylight length varies from about 173.3 h in December to about 386.5 h in June.

### Animals

Thirty 3–5-year-old adult Arabian rams were randomly selected. All rams were raised under closed grazing condition with similar management practices. The rams were clinically examined and their scrotal circumferences (SC) measured with a flexible tape at the widest scrotal diameter. At the first of each season, four rams were randomly selected for microscopic studies. Then, the rams were slaughtered, the left testes removed and separated from the epididymis and testicular weights were recorded.

### Stereological study

The volume of testis was measured according to Cavellieri's method (Howard and Reed, 1998). For this purpose, the testis was fixed in Bouin's solution for 24 h and its length measured by coulisse. Then, each testis was inserted in 7% agar, divided serially into 9-11 slices of equal thickness and the outline of each slice was traced on a paper. A test grid with 2035 points copied

on a transparent paper was laid on each tracing and the number of points overlying it was counted. The absolute volume of each testis was calculated using the following equation (Howard and Reed, 1998):

$$V = T \times \frac{a}{p} \times \sum_{i=1}^m p_i$$

Where  $p_i$  is the number of points landing within each tracing,  $T$  is thickness of each slice and  $\frac{a}{p}$  is the area associated with each point of the test grid.

Tissue samples from three regions (proximal, middle and distal) of the left testes were excised, and processed for paraffin embedding sections. Serial sections with 5  $\mu$ m thickness were stained with haematoxylin-eosin and used for stereological studies. To measure the seminiferous tubule diameter, 90 round or nearly round cross-sections of seminiferous tubules were randomly chosen in each ram (30 cross-sections in each region of the left testes). Then, using an ocular micrometer of light microscopy (Olympus E.H), at a magnification of  $\times 40$ , two perpendicular diameters of each cross-section of seminiferous tubules were measured.

The volume densities ( $V_v$ ) of the seminiferous tubule, germinal epithelium, seminiferous tubuli, lumina and testicular interstitium were determined by point counting method (Weibel, 1979). Briefly, an ocular square grid of 20 intersecting lines (with 100 test points) was superimposed on 90 microscopic fields per ram. The number of intersections on the grid overlying the tissue component of interest was counted, and the ratio of these points to the total number of the grid was considered to be the volume density of that component, as in the following equation (Bielli *et al.*, 2001):

$$V_v = \frac{P_n}{P_t}$$

Where  $V_v$  is the volume density of the tissue component of interest,  $P_n$  is the number of intersections on the grid overlying the tissue component of interest and  $P_t$  is the total number of points on the grid. The absolute volume of the testicular parameters was calculated by multiplying the corresponding volume density by the testis volume. A correction factor for shrinkage, induced by

processing steps, was applied as described by Howard and Reed (1998).

### Statistical analysis

All data were analyzed using SPSS version 10.0 for windows. Scrotal circumference, testicular weight and volume, seminiferous tubule diameter, total and relative volume of seminiferous tubules, germinal epithelium, seminiferous tubuli, lumina and testicular interstitium in different seasons were compared by one-way ANOVA, and Tukey's test was used as a post-hoc test. The means of testicular parameters during different seasons of the year were compared by analysis of covariance (ANCOVA) using testis weight as a covariate in the model. The Pearson's correlation coefficient was calculated between the scrotal circumference and total volume of seminiferous tubules. A  $P < 0.05$  was considered statistically significant.

## Results

### Clinical findings

The mean live weights of rams were nearly similar (between 49.50 to 51.50 Kg) during all seasons. The means ( $\pm$ SEM) of the scrotal circumferences of the rams in different seasons of the year are shown in Table 1. The means of scrotal circumferences significantly varied by season ( $P < 0.05$ ), with the lowest value in the early summer (June,  $271.7 \pm 1.18$  mm), and

highest value in the early winter (December,  $305.8 \pm 1.11$  mm).

### Stereological findings

The means of weights and volumes of testes differed significantly during different seasons of the year ( $P < 0.05$ ). The lowest values of testicular weight and volume were seen in early summer (June), and the highest values in early winter (December; Table 1).

The mean of seminiferous tubule diameters of Arabian rams varied significantly during different seasons ( $P < 0.05$ ), with the lowest value in the early summer (June,  $186.16 \pm 12.16$   $\mu$ m) and the highest value in early winter (December,  $220.97 \pm 12.15$   $\mu$ m; Table 1). The means of seminiferous tubule diameters did not vary significantly between the three testicular regions ( $P > 0.05$ ). A significant correlation was found between scrotal circumference and the seminiferous tubule diameter of Arabian rams in all seasons ( $r = 0.78$ ;  $P < 0.01$ ).

The means of relative and total volumes of seminiferous tubules and germinal epithelium differed significantly during different seasons ( $P < 0.05$ ). The lowest values of relative and total volumes of seminiferous tubules and germinal epithelium were seen in early summer (June), and the highest values in early winter (December; Table 1). A significant correlation was found between the scrotal circumference and the total volume of

**Table 1: Mean ( $\pm$ SEM) of testicular weight and volume, scrotal circumference, seminiferous tubule diameter, relative and total volumes of parenchyma in Arabian rams at different seasons**

Parameter	March	June	September	December
Testis weight (g)	$242.25 \pm 5.16^a$	$226.37 \pm 5.70^b$	$247.20 \pm 1.44^c$	$265.25 \pm 5.37^d$
Testis volume (ml)	$238.5 \pm 5.07^a$	$223.67 \pm 5.64^b$	$246.45 \pm 4.33^c$	$263.5 \pm 5.26^d$
Scrot. Circum. (mm)	$285.6 \pm 1.14^a$	$271.7 \pm 1.18^b$	$296.8 \pm 1.44^c$	$305.8 \pm 1.11^d$
STD ( $\mu$ m)	$202.18 \pm 8.49^a$	$186.16 \pm 12.16^b$	$210.65 \pm 8.65^c$	$220.97 \pm 12.15^d$
Semini. Tub. $V_v$	$79.35 \pm 0.22^a$	$76.65 \pm 0.37^b$	$80.05 \pm 0.24^c$	$84.30 \pm 0.31^d$
Germi. Epith. $V_v$	$68.65 \pm 0.29^a$	$64.50 \pm 0.25^b$	$69.60 \pm 0.22^c$	$75.45 \pm 0.27^d$
Interstitial $V_v$	$20.65 \pm 0.50^a$	$23.35 \pm 0.36^b$	$19.95 \pm 0.50^a$	$15.70 \pm 0.49^c$
Lumina $V_v$	$10.70 \pm 0.35^a$	$12.15 \pm 0.35^b$	$10.45 \pm 0.33^a$	$9.25 \pm 0.43^c$
Semini. Tub. (ml)	$189.24 \pm 4.16^a$	$171.40 \pm 4.47^b$	$197.38 \pm 3.55^c$	$222.12 \pm 4.55^d$
Germi. Epith. (ml)	$163.72 \pm 3.52^a$	$144.23 \pm 3.73^b$	$171.61 \pm 3.06^c$	$197.75 \pm 3.89^d$
Interstitial (ml)	$49.19 \pm 1.02^a$	$52.20 \pm 1.22^b$	$49.18 \pm 0.86^a$	$41.36 \pm 0.75^c$
Lumina (ml)	$25.51 \pm 0.66^a$	$27.16 \pm 0.73^b$	$25.76 \pm 0.50^a$	$24.37 \pm 0.76^c$

For each parameter, means followed by different superscripts differ significantly ( $P < 0.05$ ). cm: Centimeter; g: Gram; ml: Milliliter;  $\mu$ m: Micrometer;  $V_v$ : Relative volume (%); Scrot. Circum.: Scrotal circumferences; STD: Semineferous tubule diameter; Semini. Tub.: Seminiferous tubule and Germi. Epith.: Germinal epithelium

seminiferous tubule in all seasons ( $r = 0.70$ ;  $P < 0.01$ ).

The means of relative and total volume seminiferous tubuli lumina and testicular interstitium differed significantly between breeding and non-breeding seasons ( $P < 0.05$ ). The lowest values of relative and total volumes of seminiferous tubuli lumina and testicular interstitium were seen in early winter (December), and the highest values in early summer (June; Table 1). Analysis of covariance showed that testicular parameters were significantly related to testis weight during different seasons (Table 2).

**Table 2: Comparisons of testicular parameters during different seasons**

Variable	S.S.	df	F-value	P
STD	274.50	1	12.15	0.002
Semini. Tub	182.16	1	15.69	<0.001
Germi. Epith.	128.26	1	15.152	0.001
Lumina	3.80	1	11.39	0.002
Interstitium	7.57	1	10.51	0.003

A  $p < 0.05$  was considered statistically significant. S.S.: Sum of squares; df: degrees of freedom; P: P-values; STD: Semineferous tubule diameter; Semini. Tub.: Seminiferous tubule and Germi. Epith.: Germinal epithelium

## Discussion

This quantitative microscopic study indicated basic stereological characteristics of Arabian ram testis and their changes in different seasons. The present study showed that there were marked variations in the degree of spermatogenic activity of Arabian ram testis, appearing in the decrease of scrotal circumference, weight and volume of testis, seminiferous tubules diameter, total and relative volumes of seminiferous tubules and germinal epithelium during spring in Khuzestan province, Iran. Zamiri and Khodaei (2005) showed that the time of year significantly affected the volume of semen, scrotal circumferences, scrotal width and serum testosterone levels in Ghezel and Mehraban breeds of Iran. Also, Deldar Tajangookeh *et al.* (2007) showed that season of the year significantly affected the volume of semen, scrotal circumferences, relative testis volume and serum testosterone levels in the Shall, Afshari and Zandi breeds of Iran.

The stereological data showed that the

highest and lowest performance of spermatogenic activity in Khuzestan Arabian rams was seen during autumn and spring, respectively. This study confirms previous reports on the seasonality of spermatogenic function in the ram, with autumn being the normal mating season (Ortavant, 1959; Mickelsen *et al.*, 1981; Gastel *et al.*, 1995). Gundogan (2006) showed that Akkaraman and Awassi rams show seasonality for some reproductive parameters and seminal plasma constituents and could be bred successfully in the autumn months. It is interesting to note that the nadir in the ram sexual cycle is considered to occur during spring in northern breeds (Gastel *et al.*, 1995), which is consistent with our results in Khuzestan Arabian rams. Tabatabaei *et al.* (2007) showed that there is some difference between seasons in Mehraban rams. Total sperm counts during autumn and summer are lower than other seasons. Also, there is a reduction in the concentration of semen and vital sperm during autumn. But, they concluded that there was no impact of season on the reproductive performance of rams in Hamedan province, Iran. However, the greatest volume of semen was seen during autumn in Mehraban rams of the Aminabad region in Tehran (Schahidi *et al.*, 1996) and in local and crossbred rams raised in the United Arab Emirates (Ibrahim, 1997). Also, Mandiki *et al.* (1998) showed that semen concentration increases during winter and spring and decreases during autumn in Suffolk rams, but in Texel rams it increases during autumn and decreases during summer in Western Europe. Karagiannidis *et al.* (2000) indicated that semen concentration increase during autumn and winter in Chios and Friesian rams in Greece.

Stereological data showed that tubular tissue constitutes the main portion of testicular tissue. The present study indicated that germinal epithelium volume in Arabian rams testis is in the range 64.50 to 75.45% during different seasons. Interstitial tissue and lumen formed 15.70 to 23.35% and 9.25 to 12.15% of testis volume depending on the season, respectively. Our results correspond with previous quantitative studies. Massanyi *et al.* (1999) indicated that the testis of fallow-deer was constituted from 63.61 to

76.18% germinal epithelium, 11.47 to 20.05% interstitium and 12.35 to 16.34% lumen depending on the season of study. Moreover, Zibrin (1990) showed that testis of rams at the age of 450 days was formed of 64.75% germinal epithelium, 18.52% interstitium and 12.82% lumen. Massanyi *et al.* (1997) showed that testis of the fox was constituted of 52.7% germinal epithelium, 11.3% interstitium and 36.0% lumen. Also, Toman and Massanyi (1997) showed that germinal epithelium constituted 77.6%, interstitium 12.3% and lumen 10.0% in rabbit testis.

Furthermore, our results showed that there are a few changes in the total amount of interstitial tissue between the breeding and non-breeding season throughout a year.

Our results showed that the seminiferous tubules diameter changed during seasons which is consistent with previous reports in (Gastel *et al.*, 1995) goats (Delgadillo *et al.*, 1995) and fallow-deer (Massanyi *et al.*, 1999).

The present study showed that mean seminiferous tubule diameter decreases during spring. The magnitude of this decrease between breeding and non-breeding seasons (15.75%) is comparable to that of Ile-de-France (12%, Hochereau-de Reviers *et al.*, 1976) rams and lower than that shown by Soay (34%, Mortimer and Lincoln, 1982; 25%, Hochereau-de Reviers *et al.*, 1985) and Corriedale (34%, Gastel *et al.*, 1995) rams. Ile-de-France rams were considered as a domestic breed that shows a highly seasonal pattern of spermatogenic activity (Hochereau-de Reviers *et al.*, 1976). So, Arabian rams are considered a domestic highly seasonal breed.

The correlation found between the total volume of seminiferous tubules and the scrotal circumference can be explained by the fact that tubular tissue forms 70 to 83% of all testicular tissue and that there are few changes in the absolute amount of interstitial tissue throughout a year (Wrobel *et al.*, 1993; 1995).

Since seasonal changes in nutrition decrease under closed grazing conditions, quantitative variations seen in the present study could be due to seasonal changes in photoperiodic stimuli. Kheradmand *et al.* (2006) indicated that improved dietary

intake with higher energy and protein supplementation in Bakhtiary rams can improve their reproductive performance during the breeding season. Martin *et al.* (2002) indicated that Merino and Suffolk rams have similar endogenous rhythms that are similarly modified by photoperiod but, with respect to seasonal changes in nutrition, they differ in both the nature of their reproductive response and the physiological mechanisms that mediate those responses.

In conclusion, our quantitative microscopic data describe basic stereological characteristics of Arabian rams testis and indicate that the structure and function of Arabian ram testis is highly affected by season.

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