

## REPRODUCTIVE EFFICIENCY OF AN INDIGENOUS IRANIAN GOAT (*Capra hircus*)

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### ABSTRACT

*Goats (Capra hircus) are economical animal in livestock production especially in rural areas. It is well known that goat can tolerate harsh conditions; however there are little information about its reproduction functions such as testis histomorphometry and efficiency of sertoli cells. This study aimed to estimate germ cell types and number per sertoli cell of an indigenous Iranian goat (Lori goat). Semen was collected from five Lori goats by means of an artificial vagina. Semen volume, concentration, normality and motility of spermatozoa were determined. After removing both testes, tissue paraffin section (5 $\mu$  thickness) were prepared and stained using Haematoxylin and Eosin method. Mean weight of testis was 114.40  $\pm$  27.00 grams. Means of seminiferous tubule diameter and tubule length per gram testis were 197.20  $\pm$  1.80  $\mu$ m and 9956.00  $\pm$  93.00 m, respectively. The mean number of spermatids per each tubule, spermatogonia and spermatocytes at stage I were 163.00  $\pm$  99.9, 140.30  $\pm$  28.60 and 146.40  $\pm$  19.80, respectively. From this study it can be concluded that the Lori goat has high reproductive potentials probably due to high number of sertoli cell per germ cells.*

**Keywords:** Lori goat, Testis, Sertoli cell, Seminiferous tubule, Spermatocytes, Spermatogonia, Spermatozoa, Semen

### INTRODUCTION

The goat is an economically important species in livestock production in rural areas. Goats are able to tolerate high rainfall areas and other harsh conditions associated with the semi-arid climate. Goats can efficiently ingest low quality feedstuff especially fibrous materials; they are favorite animal in rural area of most sub-tropical countries (Green and Baker, 1996; Adams *et al.*, 1997). Although it is well known that goat can

tolerate harsh conditions, our knowledge is limited about indigenous goat reproduction functions such as testis morphometry and efficiency of sertoli cells. Goats have been used as a model for spermatogonial transplantation and a number of studies have described spermatogenesis in goats (Gordon, 1997). Even though goat fertility has been studied in different environmental conditions (Ahmed *et al.*, 1997; Al-Ghalban *et al.*, 2004; Martemucci *et al.*, 1998), published data on testis function

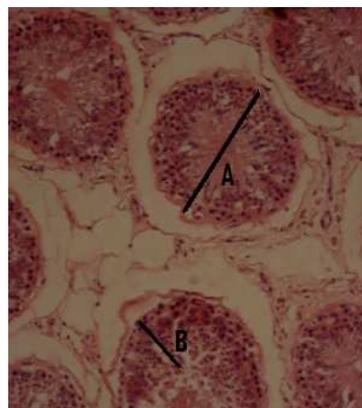
and spermatogenesis (Courtens and Loir, 1981; Bilaspuri and Guraya, 1984; Oke *et al.*, 1984; Onyango *et al.*, 2000) is limited. Efficiency of spermatogenesis (i.e. estimated number of spermatozoa produced per gram of testicular parenchyma) is highly correlated to germ cells supported by single sertoli cell (Russell and Peterson, 1984; Franca and Russell, 1998). Efficiency of reproduction is also highly correlated with density of seminiferous tubules, number of sertoli cell per gram of testis and the duration of the spermatogenic cycle (Russell *et al.*, 1990; Sharpe, 1994; Neves, 2001; Leal, 2004). Thus it has been proposed that daily sperm production can be accurately determined from the total number of sertoli cell (Franca and Russell, 1998; Franca and Godinho, 2003).

In Iran, there are about 25 million goats playing critical and economical roles for their producers in rural area. About 2 millions of the Iranian indigenous goats are Lori goats and are reared in west of Iran mainly in Lorestan province. Lori goat is highly economic for goat producers in terms of meat production. Therefore, governmental strategies such as breeding and estrus synchronization programs have been started to protect these beneficial animals and their producers. However, there is a lack of knowledge about reproduction characteristics of the Iranian indigenous goats in the country. Zamiri and Heidari (2006) studied the reproductive characteristics of Rayani goat in central area in Iran. There are no published information about the reproductive characteristics and testis histomorphometry of the Lori goat. Therefore this study aimed to investigate reproductive characteristics of Iranian indigenous goat (Lori goat) that is mainly reared in west of Iran.

## MATERIAL AND METHODS

Five mature Lori bucks (2 – 3 years old) were selected. An artificial vagina was used to collect semen. Semen volume, concentration of sperm per milliliter, sperm normality and motility were determined using the methods of Sorensen (1979). Goats were slaughtered and testis were trimmed and weighed. Length and width of testis were determined by means of digital

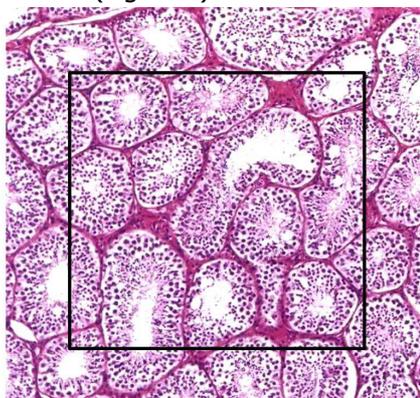
caliper. Testicular volume were determined applying the following equation;  $\frac{4}{3} \pi ABC$ , where A, B and C were half width, half thickness and half length, respectively (Mascarenhas *et al.*, 2006). Testis volume was directly converted into gram, since density of mammalian testis has been established to be close to one (Johnson *et al.*, 1981; Paula, 1999). The weight of testis parenchyma was calculated after exclusion of the tunica albuginea and mediastinum (~10%) from the total testis volume (Becker-Silva, 2000). Slabs of testis (25 mm<sup>2</sup>) were sampled randomly and fixed in 10% formalin, and stored at room temperature for testicular histological study. Fixed slabs of testis were histologically processed and embedded in paraffin. Histological sections (5µm thickness) were prepared using a rotary microtome (4060 cut SLEE). Sections were stained by routine Haematoxylin-Eosin (H&E) method. The mean diameter of seminiferous tubules, lumen and height of seminiferous tubule epithelium (Figure 1) were measured using Motic Image Plus 2 software.



**Figure 1: Transverse section of seminiferous tubules in Lori goat. A. diameter of tubule. B. height of epithelium.**

Sixty seminiferous tubules were randomly selected and measured. The numbers of spermatogonia, spermatocytes and sertoli cell were morphometrically estimated. The loss in spermatogenesis was not significant (Johnson *et al.*, 2000) thus; spermatid reserves of the testis (SRT) were calculated on the basis of the round spermatid populations (Amann, 1962; Berndtson, 1997) as follows:  $SRT = (\text{Round spermatid} \times \% \text{ seminiferous tubules} \times \text{total volume of the testis parenchyma}) / (\text{seminiferous}$

tubule area in transverse section  $\times$  cut thickness  $\times$  100). Transversal section area of the seminiferous tubule was calculated using the equation  $nR^2$  where R was ray obtained from average of 60 seminiferous tubules diameter per animal. The length density of seminiferous tubules (length per unit volume) was estimated stereologically (Howard and Reed, 2010). Briefly microscopic image of testis (total magnification of 25 $\times$ ) was displayed on a computer monitor and superimposed with a 10  $\times$  10 cm frame. The tubules that fell within the frame and do not cross the lower and left lines of the frame were counted (Figure 2).



**Figure 2: Method of random selection of seminiferous tubules for estimation of length tubules of an Iranian indigenous goat (Lori goat).**

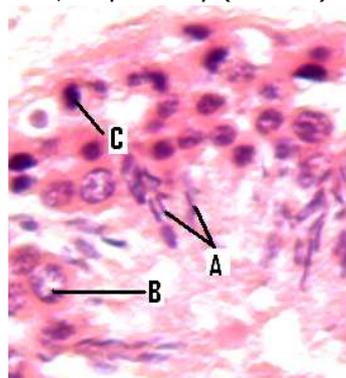
The length of tubules was calculated using the formula:  $L_v(\text{tubule/testis}) = 2 \cdot \Sigma Q / (a/f \cdot \Sigma p)$ , where  $\Sigma Q$  = total number of tubules counted,  $a/f$  = area per frame = frame area/magnification<sup>2</sup> and  $\Sigma p$  = total number of frames applied (Howard and Reed, 2010). The data were analyzed statistically for their central tendencies by using SPSS 11.5. Data are presented as means  $\pm$  standard divisions of means.

## RESULTS

Average of semen volume in each ejaculation was 2-2.5 ml with  $1.28 \times 10^9$  sperm per ml. Motility and normality of sperm were estimated to be 90 and 95 percents, respectively. The means of spermatogonia, primary and secondary spermatocyte, spermatid (Figure 3) and sertoli cell (Figures 4 and 5) per each tubule section were  $140.30 \pm 28.60$ ,  $146.40 \pm$

$019.80$ ,  $163.00 \pm 99.9$  and  $5.50 \pm 2.40$ , respectively (Table 1). Each sertoli cell was sustained approximately 24.4 germ cells.

The average diameter of seminiferous tubules and epithelial height was  $197.00 \pm 1.80$  and  $39.00 \pm 1.00 \mu\text{m}$ , respectively. According to section thickness ( $5 \mu\text{m}$ ) and number of seminiferous tubules per area and length of seminiferous tubules were estimated to be  $87.00 \pm 1.30$  and  $9956 \pm 93$  meters per gram and per testis, respectively (Table 2).



**Figure 3: Transverse section seminiferous tubules, different type of germ cells spermatogonium (C), spermatocyte (B) and spermatozoid (A) of an Iranian indigenous goat (Lori goat).**

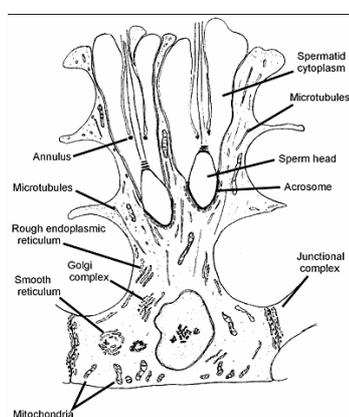


**Figure 4: Transverse section of seminiferous tubules of an Iranian indigenous goat (Lori goat). Sertoli cells shown in tissue section.**

## DISCUSSION

The average of testis weight for Lori goat was about  $114 \pm 27$  grams. In Lori goat, density of testis parenchyma estimated to be about 86.5%. The density of testis parenchyma of 80 – 85 % has been reported (Oke *et al.*, 1984, Yadav and Sharma, 1994; Nishimura *et al.*, 2000). This was in agreement with the finding of Russell *et al.* (1990) that seminiferous tubules occupy between 70 – 90 % of testis parenchyma in most mammals. The recorded tubular diameter of  $197.20 \pm 1.80 \mu\text{m}$  in this

study was in agreement with the range 180 – 350  $\mu\text{m}$  for tubular diameter of most mammals (Roosen-Runge, 1973; Setchell *et al.*, 1994). However, the value of 197.2  $\mu\text{m}$  was smaller than values of  $237.00 \pm 3.00 \mu\text{m}$  reported by Franca and Russell (1998) and Leal *et al.* (2004) for Alpine bucks. The differences may be due to excessive shrinkage in long term storage of samples in formalin. Spermatogenesis is a complex process involving mitotic, meiosis cell divisions and the process of spermiogenesis (De Krestler *et al.*, 1998). Related to testis morphometry, for calculating the testis parenchyma, the mediastinum and Tunica albuginea are reduced from the gonadal mass, because these sections do not directly participate in the spermatogenesis and androgenic function (Johnson *et al.*, 2000).



**Figure 5: Schematic diagram of sertoli cell in testis of an Iranian indigenous goat (Lori goat).**

The volumetric proportion of the testicular albuginea and mediastinum has been reported to be around 10% of testis weight (Franca and Russell, 1998). In African lion, tunica albuginea and mediastinum were calculated to be 18% of testis weight (Barros *et al.*, 2004). In present study tubular length per gram of testis parenchyma was estimated  $87.00 \pm 1.30$  meter. This value was more than 4 times higher than the values reported previously (Setchell *et al.*, 1994; Franca and Russell, 1998; Leal *et al.*, 2004). Leal *et al.* (2004) found that the length of seminiferous tubules per gram testis parenchyma was 20 meter. Seminiferous tubular length in goat was about 85% of testis parenchyma and occupied (Yadav and Sharma, 1994). The differences may be due to variation in goat breeds and can also be attributed to variations in methodological approaches utilized

for histological processing of testis (Okwun *et al.*, 1996). Seminiferous epithelium in Lori goat was estimated to be  $39.2 \pm 0.98 \mu\text{m}$ , this parameter in goat has previously been reported to be  $74.4 \pm 1.0 \mu\text{m}$  (Leal *et al.*, 2004). During spermatogenesis, apoptosis normally occurs (Blanco-Rodriguez, 1998). The kinetic of spermatogenesis wasn't investigated in this study, but in goats it has been shown that 30% cell loss occurred during the second process of meiotic division (Leal *et al.*, 2004).

Sertoli cells play an important role in spermatogenesis. Sertoli cells are mainly responsible for transport of spermatogenic cells from basal seminiferous tubules to the lumen. Sertoli cells are also involved in the phagocytosis of degenerated cells, as well as nutrition and protection of germ cells (Kelly *et al.*, 1984; Jurado *et al.*, 1994). Sertoli cells represent the structural framework that supports the developing germ cells in the seminiferous tubules (Yin *et al.*, 2006). In ruminant, sertoli cells have prominent structure (multivesicular nuclear body) and differ from non-ruminant species (Wroble and Schimmel, 1989). The number of sertoli cell determines the sperm production in sexually mature animals (Orth *et al.*, 1988, Franca *et al.*, 2000). Efficiency of spermatogenesis is usually positively correlated with the number of germ cells supported by each sertoli cell (Russell and Peterson, 1984; Sharpe, 1994; Neves, 2001). It is therefore likely that sertoli cells play a significant role in sequestration and degradation of residual bodies in the goats and sheep after spermiation (Onyango *et al.*, 2000), thus it has been proposed that the number of sertoli cells and their size are important parameters for determination of spermatogenic efficiency in mammals (Franca *et al.*, 2002). Present study showed that the number of sertoli cells per gram in Lori goat was about  $8 \times 10^6$ . This value for was lower than those found in Alpine goat and other domestic mammals. In Alpine goat the number of sertoli cell per gram of testis were found to be  $21.4 \times 10^6$  (Leal *et al.*, 2004). The number of sertoli cell per gram testis of bull, stallion, rabbit and boar were reported to be 29, 28, 25 and  $20 \times 10^6$ , respectively (Franca and Russell, 1998). Lori goat had nearly the

same number of sertoli cell as in ram  $8 - 12 \times 10^6$  (Franca and Russell, 1998). In each tubule section, the number of sertoli cell was estimated to be  $5.5 \pm 2.4$ . This specifically may play a role in sperm release (Ross, 1976) during different seasons. The seasonal variations mainly occur in response to a decline in the levels of FSH and LH and testosterone (Michael and Bonsall, 1997; Elsayed, 2008). In Lori goat, spermatid reserve of the testis (SRT) was estimated to be  $1.7 \times 10^9$ . Thus it seems that high reproductive efficiency of Lori goat can be related to the long seminiferous tubules and the number of sertoli cell. However, further studies are needed on the kinetic of spermatogenesis in Lori goat.

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