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# Histomorphometric Study of Sheep Fetal Testis

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**Abstract:** The aim of this study was to determine sheep fetal testis developmental aspect. Histomorphometric investigation was carried out on 21 sheep fetal testis aging from 41 to 86 days of age. Foeti were collected from Shahrekord abattoir, Iran and allocated into 4 age groups according to measured CRL. Tissue sections of the organs showed that morphological characteristics of interatubular and intertubular cells of ovine fetal testis are similar to those in other ruminates such as bovine and buffalo fetal testis. Morphometric values showed that with increasing age the number of gonocytes and indifferent supporting cells increases, Leydig cell numbers increases from the first to the third group but decreases suddenly in forth group. The number of pre-Sertoli cells in cross-sectioned cord remains always predominant in all four age groups.

**Key words:** Morphometry, testis, fetal sheep

## INTRODUCTION

The histomorphometric values of prenatal testicular development in domestic animals are still not well understood. Nasr *et al*. (1966) and Khalil (1969) studied the microscopic picture of the buffalo testis. Testicular development of sheep fetus was studied by Hochereau-de Reviers *et al*. (1995). Proper development of testis is critical to establish the male phenotype and attain maximal reproductive capacity (Dufour *et al*., 2002). Disturbance in prenatal development and differentiation of the testis can be responsible for an array of undermusculinisation syndromes, ranging from XY females to males with subnormal fertility (Tohonen *et al*., 2003). Only few reports on testicular development in sheep can be traced in the available literature. The target of this study was to give exact histomorphometric values of ovine embryonic testis of Lori Bakhtiyari breed in Iran.

## MATERIALS AND METHODS

Testes from 21 apparently healthy prenatal Lori Bakhtiyari sheep aging approximately from 41 to 86 days of intrauterine life, were collected from the Shahrekord slaughterhouse for this investigation in September 2007. Immediately after collection samples were taken to Shahrekord campus laboratory and the foeti were measured for their Crown-Rump Length (CRL) in millimeters with the help of a graduated nylon tape. The foeti were then subjected to proper dissection of testes from the body. The testes were cut longitudinally into two halves to allow rapid fixation in 10% buffered formalin; they were then dehydrated and embedded in paraffin. Thin sections of 5 μ were cut and stained with H and E stain. Thirty clearly cut tubules were chosen at random from each testis and examined for type and number of intratubular cells. Intertubular cell count in each viewing field

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(40x magnification) was performed using a 10×10 square graticule. Data were analyzed statistically, using Kruskal-Wallis test. $p<0.05$ was considered as significant. The results are presented as Mean±SE.

The estimation of the fetal age was based on the CRL (McGeady *et al*., 2006).

## RESULTS

The material was distributed according to the approximated ages in 4 groups (Table 1).

### Sex Cords

As early as the day 41, the sex cords are clearly organized, solid and possess no lumina. The cavities of the cords are filled with mesenchymal transparent mass and the cords are widely separated from each other. The forming testicular cords are surrounded by a marked basal lamina and a layer of peritubular cells. With increasing age, as a consequence of the relative expansion in the interstitium, the seminiferous cords are progressively separated from each other.

### Intratubular Cells

In all four groups, two types of cells are observed in the sex cords: a large number of dark polygonal cells with irregular nuclei, pre-Sertoli cells and a small number of large, light, round cells with relatively round nuclei, the Primordial Germ Cells (PGC). The ovine pre-Sertoli cells are mainly arranged on the basal lamina of these cords. With increasing age, the number of pre-Sertoli cells increase and some of them displace more centrally by the close packing of cells within the cords. The pre-Sertoli cells display a polygonal shape with their cytoplasm often oriented radially within the cords. Their nuclei are variable in shape. PGCs are polygonal with light cytoplasm and spherical nuclei. They are located either at the periphery of cords or slightly central. In the first group the average total number of cells within the cross-sectioned sex cord is 8.62. With increasing age, the average number of total cells within the testicular cord cross section considerably increases and approaches their maximum at forth group (Table 2).

The number of the indifferent supporting cells at the first group averages 7.31 per cross-sectioned cord, increasing to 8.98 at the fourth group (Table 2).

The number of gonocytes per cross-sectioned sex cord averages 1.31 and 1.79 at the first and fourth group, respectively (Table 2). The number of pre-Sertoli cells in cross-sectioned cord remains always predominant in all four age groups (Table 2).

### Intertubular Cells

In the first group, the interstitial cells are numerous, filling the relatively wide intertubular spaces. These cells are polygonal with large round nuclei that appear somewhat darker than that of pre-Sertoli

Table 1: Distribution of the material

| Groups | Age (days) | Mean age (days) | Estimated CRL (mm) | No. of fetuses |
|---|---|---|---|---|
| 1 | 41-45 | 43 | 40-55 | 6 |
| 2 | 46-63 | 55 | 61-118 | 6 |
| 3 | 64-70 | 67 | 122-145 | 4 |
| 4 | 71-86 | 80 | 161-200 | 5 |

Table 2: The average number of cells per cross-sectioned sex cord

| Groups | Leydig cells | Gonocytes | Supporting cells | Total |
|---|---|---|---|---|
| 1 | $3.20\pm0.20^{a}$ | $1.31\pm0.03^{b,c,d}$ | $7.31\pm0.26^{e,f,g}$ | $8.62\pm0.27^{h,i,j}$ |
| 2 | $5.40\pm0.31^{a}$ | $1.46\pm0.08^{b}$ | $8.60\pm0.32^{e}$ | $10.06\pm0.39^{h}$ |
| 3 | $7.80\pm0.15^{a}$ | $1.70\pm0.10^{c}$ | $8.75\pm0.27^{f}$ | $10.45\pm0.30^{i}$ |
| 4 | $2.30\pm0.45^{a}$ | $1.79\pm0.12^{d}$ | $8.98\pm0.24^{g}$ | $10.77\pm0.15^{j}$ |

The same letter(s) means significant statistical difference

and germ cells. The cytoplasm of the fetal Leydig cells is mostly acidophilic. These cells are mainly situated between the testicular cords. A few fibroblasts are also found around the sex cords.

The number of Leydig cells in intertubular spaces increases numerically from first to third group, but it shows a sudden decrease in the fourth group (Table 2).

## DISCUSSION

In all four age groups, the testicular cords are solid. This simulates the findings of Abdel-Raouf (1960) in cattle and Abdel-Raouf *et al*. (1974) in buffalo. Results of this study also revealed that the ovine testicular cords are lined by two types of cell population: large number of dark polygonal cells with irregular nuclei, pre-Sertoli cells and small number of large light cells with relatively round nuclei, the PGCs. These findings appear consistent with those of Abdel-Raouf (1960) in fetal bovine testis and Abdel-Raouf *et al*. (1974) in fetal buffalo testis. Ovine pre-Sertoli cells are generally in contact with the basal lamina, despite some are sometimes displaced more centrally by the close packing of cells within the cords especially at the fourth group, where, the number of pre-Sertoli cells has relatively increased. They have polygonal shape with their cytoplasm oriented radially within the cords. Their nuclei are variable in shape. Schrag (1983) identified two types (light and dark) and Abd Elmaksoud (2005) identified only one type of presumptive Sertoli cells within the fetal bovine testis. The present study recognized only one type of these cells within the ovine fetal testis. This finding is supported by the result of Abdel-Raouf (1960) and Abd Elmaksoud (2005) in bovine fetal testis. This study revealed that the germ cells in ovine fetal testis can be identified as polygonal cells with light cytoplasm and spherical nuclei. They are located either at the periphery of cords or slightly central. Generally, these cells are larger than pre-Sertoli cells and are easily recognizable elements of the seminiferous epithelium in all four age groups. The number of presumptive Sertoli cells within an individual cord cross section is always greater than that of germ cells. Abd Elmaksoud (2005) reported the same result in the bovine fetal testis. Schrag (1983) identified two types (light and dark) of bovine germ cells and described these cells as two different cellular functional states. Gaskell *et al*. (2004) identified three types of germ cells in the human fetal testis using single, double and triple immunohistochemistry. Results of this study are inconsistent with the results of Schrag (1983) in cattle and that may be due to inappropriate fixation. In virtually all species so far investigated, recognizable steroid secreting cells, the Leydig cells, appear in the interstitium of fetal testis shortly after the testicular cord development (Sinowatz *et al*., 1987). Results of this study reveals that in ovine fetal testis, Leydig cells are recognizable from the first age group. Therefore, it can be deduced that the appearance of these cells occurs earlier than 41 days of age in sheep fetal Testis. Ovine Leydig cells are polygonal with large spherical nuclei that appear slightly darker than those of the pre-Sertoli cells and germ cells and their cytoplasm appears mostly acidophilic in routine histological staining. These findings appear consistent with those of Abd Elmaksoud (2005) in fetal bovine testis Leydig cells. It was also revealed that the number of Leydig cells progressively increases in the first three age groups but considerably decreases in the fourth group (Table 2). The progressive increase in Leydig cell numbers in the first three age groups resembles the same phenomenon in fetal bovine testis (Abd Elmaksoud, 2005) and because of mitotic figures within the Leydig cell population are rarely observed (Abd Elmaksoud, 2005), this is probably due to further differentiation of mesenchymal cells into Leydig cells. The decrease of Leydig cell numbers in the fourth age group is probably due to the fact that the expansion of intertubular spaces which is per so as a result of testicular growth exceeds mesenchymal cell differentiation into Leydig cells.

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