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# MACRO-AND MICROSTRUCTURE OF THE SHEEP TESTIS IN THE DYNAMICS OF POSTNATAL ONTOGENESIS

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## Abstract

To study the histologic structure of a sheep testicle of the Dagestan rock breed during various age periods of post-natal ontogenesis.

The material for the study has been selected on farms and at slaughter floors, fixed in Buen's liquid and embedded in paraffin. The sections of 5-6µm thickness were made of paraffin blocks. For histological staining the standard practice of hematoxylin and eosin has been used. Histochemically the black Sudan "B" has been used for lipid detection. The Ascorbic acid has been detected with silver nitrate. The morphometry has been performed with the help of the UCMOS 03100KPA eyepiece camera with the licensed "Altami Studio" software. To determine the percentage of convoluted seminiferous tubules of the testis to the interstitial tissue, RA-6 drawing and projection apparatus has been used. For statistical analysis of the obtained data the MS program "Excel" has been also used.

Carrying out histological analysis, we have obtained the data on the percentage of convoluted seminiferous tubules and interstitial tissue of the testis at various periods of postnatal ontogenesis. So, predominance of the interstitial tissue over the convoluted seminiferous tubules in the newborn period has been noted, whereas in the subsequent age periods the convoluted tubules widely grow. The interstitial endocrinocytes (Leydig cells) are found in all age periods. The maximum number of interstitial endocrinocytes is observed in the newborn period, minimum number of interstitial endocrinocytes is observed in the prepubertal period (3-4 months). These are large-sized cells with clear boundaries of a round, oval and rarely dendritic shape. The cytoplasm is oxyphilic; large amount of the Sudanophil material is accumulated histochemically. The nucleus of a large, light, round shape is located eccentrically.

The obtained results of the research showed that the content of interstitial tissue predominates over the convoluted seminiferous tubules in the testis of the sheep of the newborn period, whereas the convoluted seminiferous tubules grow in the subsequent age periods that leads to a decrease in the content of interstitial tissue. The interstitial endocrinocytes (Leydig cells) are found in all age periods. High functional activity is noted in interstitial endocrinocytes morphologically, histochemically and morphometrically.

***Key words:*** Sheep, testicle morphology, postnatal ontogenesis, Leydig cells, interstitial endocrinocytes, morphometry.

## Introduction

Testicle *(testis)* is a pair body in which male gametes develop. It is well-known that the testicle at the same time is gland of both external, and internal secretion. Among a set of questions of the animals connected with endocrine regulation of an organism and the person, are the most interesting reproduction of healthy posterity. Emitting the hormones in blood, the testicle performs important function, exerting impact on development of secondary sexual characteristics, processes of growth, physical development, a structure of a skeleton (Atagimov, 2013; Khasaev, 2011; Shevlyuk, 2010). A source of formation of male hormones (testosterone, dihydrote stosterone, androstenedione) in a testicle are interstitial endocrinocytes (Leydig cell) which, though have consecration in scientific literature, (Volkov, 2014;

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Kayumov *et al.,* 2016; Barbutska, 2013; Bardin, 1996; de Kretser and Kerr, 1994; Wrobel, 1990) demand additions and specifications in age and pedigree aspects.

The purpose of this work is studying of a histologic structure of a sheep testicle of the Dagestan rock during various age periods of post-natal ontogenesis.

## Research Material and methods

Researches were conducted on clinically healthy animals. Material for a research was selected on farms and in slaughterhouses. After preparation the testicle without appendage was weighed on laboratory scales of CAS XE-300. For a histologic research pieces of a testicle fixed in Buena liquid and filled in in paraffin. Made cuts 5-6 microns thick of paraffin blocks (Korzhevskii, 2013). For histologic coloring the standard technique hematoxylin and eosin was used. Histochemical for identification of lipids used Sudan black "B", revealed Ascorbic acid by means of nitrate silver.

The morphometry was carried out by means of the ocular UCMOS 03100KPA camera with the licensed software of "Altami Studio". Cariometry was seen off in 100 fields of vision at increase in the 600th. Calculation of quantity of interstitial endocrinocytes was carried out in 20 fields of vision.

For definition of a percentage ratio of wavy seed testicle tubules to interstitial fabric used the RA-6 drawing projector. For the statistical analysis of the obtained data used the software package of MS of "Excel".

## Results and discussion

The testicle of a newborn ram with is outside covered with the gentle capsule (*tunica albuginea*). A medial part of the capsule contains many cells and few fibrous structures. In a subcapsular zone a large number of blood vessels of different diameter come to light.

Connective tissue partitions which, passing deep into body, form mediastinum (*mediastinum testis*) from the capsule of a testicle. The parenchima testis is presented by wavy seed tubules and the interstitial fabric consisting of friable connecting fabric among which interstitial endocrinocytes, or Leydig cell (fig. 2) are found small groups. The quantity of Leydig cell in the newborn period in one field of vision equals 7.46±1.4$^0$C. (table 2). These are cells, ramification form, the large sizes roundish, oval less often, with a clear boundary. Cytoplasm is oxyphil is histochemical noted accumulation of a large amount of sudanophil material (fig. 3). The nucleous is large, light rounded shape, it is located excentricly. Diameter of nucleous of Leydig cell averages 8.25±0.18 microns., (table 2). In a nucleous prevails low dispersion euchromatin whereas heterochromatin, in insignificant quantity adjoins to a cariolemma.

**Table 1:** Changes in the weight of the sheep testis at different age periods

| Age of animals | Number of animals | Weight of animals in grams |
|---|---|---|
| 1-10 days | 18 | 0.64±0.3 |
| 3-4 mon. | 17 | 19.7±0.24* |
| 7-8 mon. | 19 | 84.2±1.36 |
| 12-18 mon. | 31 | 135.5±2.03** |

Note: difference with the previous group is statistically significant: * - at P <0.05; ** - at P <0.01; *** - at P <0.001.

**Table 2 :** Morphometric parameters of endocrinocytes of the sheep testis

| Age of an animal | N | Diameter of Leydig cell nuclei in µm. | No. of Leydig cells in the field of view |
|---|---|---|---|
| 1-10 days | 100 | 8.25±0.18 | 7.46±1.44 |
| 3-4 mon. | 100 | 7.19±0,14*** | 2.8±0.78** |
| 7-8 mon. | 100 | 8.5±0.17 | 5.07±1.14 |
| 12-18 mon. | 100 | 9.21±0.13*** | 4.77±1.11 |

Note: statistically significant: * - at P <0.05; ** - at P <0.01; *** - at P <0.001.

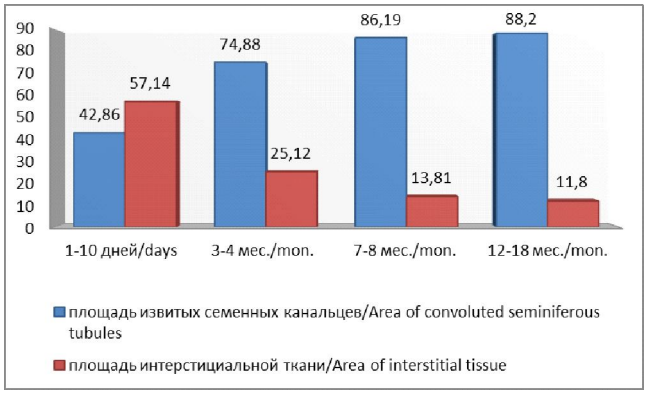


**Fig. 1:** Percentage of convoluted seminiferous tubules of the testis to the interstitial tissue.

At the age of 3-4 months, the mass of a testicle increases repeatedly in comparison with the newborn period and averages 19.7±0.24Γρ. (table 1). Sharp increase in the space occupied by wavy seed tubules in comparison with interstitial fabric (fig. 1) is noted.

The last is presented in the described period by the small maintenance of connective tissue cells which have extended, is more rare rounded shape (fig. 4).

Leydig cell differ from surrounding connective tissue cells in the large sizes, a light nucleous and localization. The nucleous has the central situation, lies excentricly less often. Reduction of diameter of a nucleous at this age in comparison with the newborn period (table 2) is noted. Considerably also the number of Leydig cell under

review decreases (table 2).

In puberty age of 7-8 months, the mass of a testicle increases and averages 84.2±1.36Γρ, (table 1). The percentage ratio of interstitial fabric continues to decrease (fig. 1). Population of Leydig cell is made by mainly highly active average cells which the main form congestions (fig. 5). Cytoplasm has a clear boundary, often on a surface the small granularity is observed. The nucleous of rounded shape, has the central situation. The prevalence of a light euchromatin in a nucleous whereas heterochromatin small sites settle down on the periphery is noted. In this regard are clearly visible several large a nucleous. Cariometry shows small increase in diameter of a nucleous of Leydig cell, the quantity of cells under review also increases (table 2).

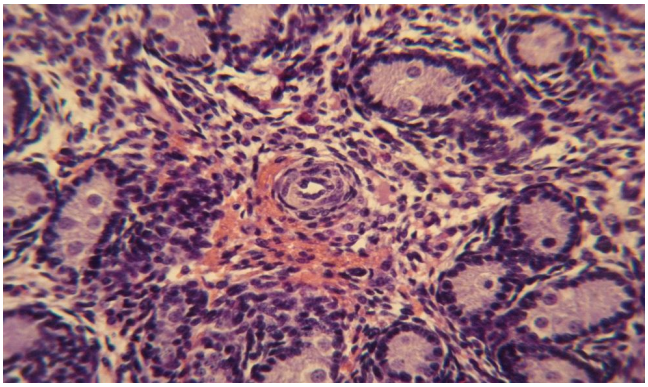

**Fig. 2:** Testis, 1 day. 1-Leidig cells; 2 convoluted tubules; 3 Sertoli cell; 4 - blood vessel. Hematoxylin and eosin. x 400.

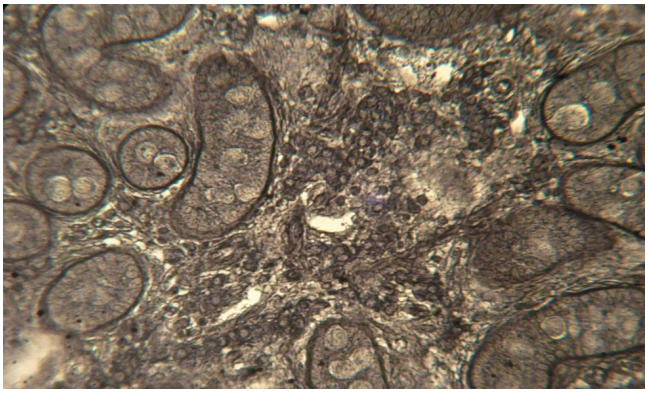

**Fig. 3:** Testis, 2 days. 1 Leydig cells; 2 convoluted tubules; 3-interstitial tissue. Chiachio, Sudan black "B", x400.

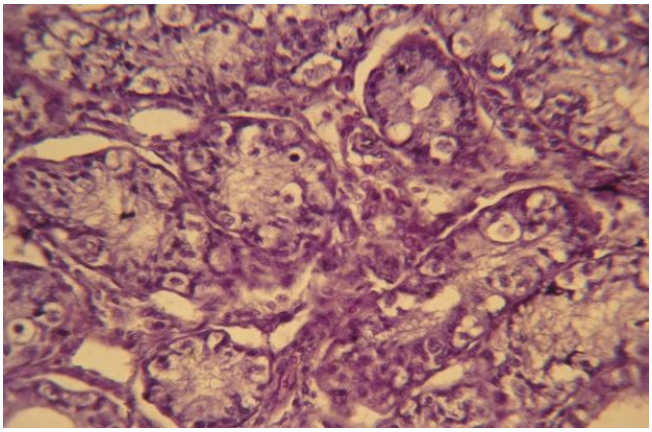

**Fig.4:** Testis, 4 months. 1 Leydig cells; 2 convoluted tubules. Buen, hematoxylin and eosin, x400.

During the definitive age period 1-2 years, the mass of a testicle averages 135.5±2.03 gr. (table 1). When determining a percentage ratio of wavy seed tubules to interstitial fabric becomes clear that wavy tubules of 88.2% whereas the content of interstitial fabric in a testicle is 11.8% (fig. 1). The quantity of interstitial endocrinocytes

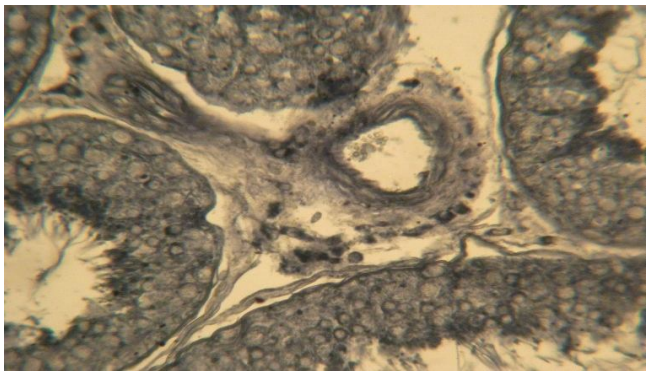

**Fig. 5:** Testis, 7 months. 1 Leydig cells; 2-convoluted tubules; 3-blood vessel. Chiachio, Sudan black "B", x400.

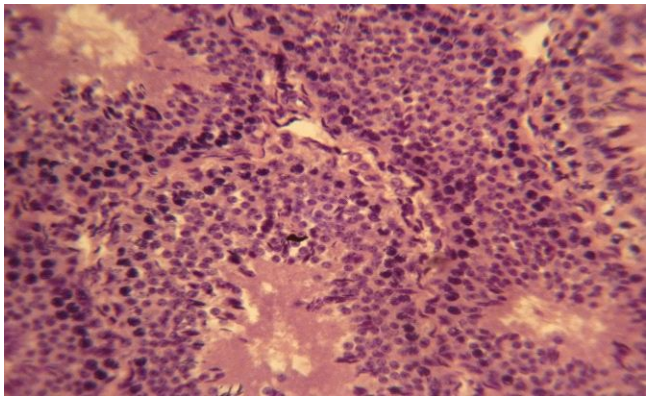

**Fig. 6:** Testis, 1.5 years. 1 Leydig cells; 2-convoluted tubules; Buen, hematoxylin and eosin, x400.

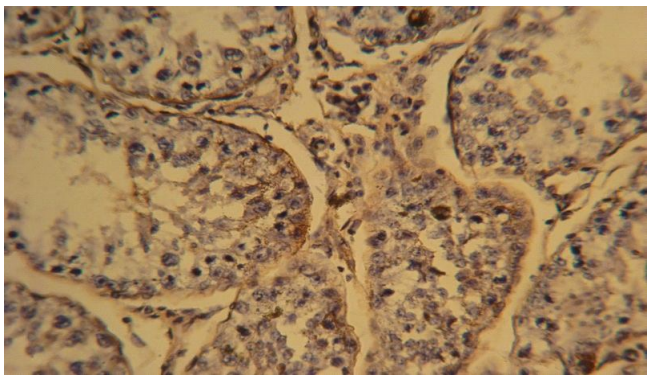

**Fig. 7:** Testis 1 year old. 1 Leydig cells; 2-convoluted tubules. Ascorbic acid according to Kiseli, x400.

at the described age on one field of vision averages $4.77\pm1.11_{KJL}$. (table 2). These are large cells of a rounded, oval or polygonal shape with a clear boundary. Endocrinocytes often form congestions near haemo capillaries (fig. 6).

Cytoplasm is extensive, is plentifully painted with sour dyes, accumulation of granules of ascorbic acid is also noted. The nucleous light heterochromatin adjoins to a cariolemma. Nucleolus comes to light distinctly. Diameter of nucleous averages 9.21±0.13 microns. (table 2).

## Conclusion

The received results of a research showed that in a sheep testicle during the newborn period the content of interstitial fabric prevails over wavy seed tubules whereas the subsequent age periods wavy tubules expand that leads to reduction of content of interstitial fabric. Interstitial endocrinocytes are found in all age periods. Morphologically, histochemical and morphometric in interstitial endocrinocytes the high functional activity is noted.

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