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Morphological and morphometric features of the testis structure of male white rats during postnatal ontogenesis

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Abstract

Using histological and morphometric research methods, the features of the spermatogenesis process in the convoluted seminal tubules of the testes of male white rats are shown. As a biological test object, white mongrel sexually mature male rats weighing 200-250 grams were used in the work. The material of the study is the testes of white male rats. Based on the morphometric measurements carried out – counting different types of spermatogenic cells in one convoluted seminal tubule, a table of their ratio was compiled. In the course of the conducted studies, it was found out that during the period of sexual maturity of male white rats, the largest percentage of the total number of spermatogenic cells in the spermatogenic epithelium are the earliest forming germ cells – spermatogonia, and when studying the quantitative change of various types of spermatogenic cells in the convoluted seminal tubule of the testes of male white rats, the largest number are mature male germ cells – spermatozoa.

Keywords: testes, convoluted seminal tubules, spermatogenesis, spermatogenic cells.

1. Introduction

The need to study the features of the structural organization of the testes is determined by its participation in the performance of important functions for the body - the production of spermatozoa and the production of male sex hormones **(Potapov S.N. et al., 2011; Shubina, O.S., 2016)**.

Despite the presence of works devoted to the study of the features of the structural organization of the testes **(Nishlag E. et al., 2009; Samusev R.P. et al., 2010; Samusev R.P. et al., 2011)**, many questions remain unresolved or require clarification **(Voloshin N.A. et al., 2009; Hess R.A. et al., 2008; Morteza K. et al., 2012)**.

The aim of the work was to study the morphological features of the testes of male white rats in connection with the development of the reproductive function.

2. Material and research methods

White sexually mature male rats weighing 200-250 g at the age of 60 days were used as a biological test object in the work, since according to the literature, the period of puberty in rats begins on the 60th day **(Chaliapina V.G., 1991).**

A total of 50 animals were used.

The testicles of male white rats served as the material for the study.

Animals were sacrificed by decapitation under anesthesia of ether with chloroform (1: 1) in compliance with the principles of humanity set forth in the directives of the European Community (86/609 / EEC) and the Declaration of Helsinki, and in accordance with the requirements of the rules for working with experimental animals.

The weight of the testes was measured using a Sartorius analytical balance (Germany).

For histological examination, tissue samples of the seminal glands were fixed in a 10% solution of neutral formalin. The fixed samples, after washing in running water, were subjected to dehydration by placing the test material in alcohols of increasing concentration and embedded in paraffin according to the standard technique. Prepared histological cross sections of the seminal glands with a thickness of 10-15 microns, stained them with hematoxylin-eosin according to the standard technique.

Tissue samples were examined using an Axio Imager.M2 digital microscope (ZEISS, Japan) with AxioVision SE64 Rel. 4.8.3 and ZEN 2011. The preparations were photographed with an AxioCam MRc5 digital camera (ZEISS, Japan) with subsequent image processing in Abode Photoshop Elements 11.

During the survey microscopy, the morphological features of the structure of the testes were studied, after which the following morphometric parameters were determined:

1. The thickness of the tunica albuginea of the testes.

2. The number of convoluted seminiferous tubules in one field of view, the cross-sectional area of the convoluted seminiferous tubule and its lumen, the area of spermatogenic epithelium and its thickness.

3. The number of areas of interstitium between the convoluted seminiferous tubules in one field of view, as well as their area.

4. The number of myoid cells in the wall of the convoluted seminiferous tubule, the area of myoid cells and their nuclei.

5. The number of Sertoli cells in the spermatogenic epithelium of the convoluted seminiferous tubule, the width of the basal and the length of the apical parts of Sertoli cells, the area of cells and their nuclei.

6. The number of different types of spermatogenic cells (spermatogonia, spermatocyte and spermatid) in the spermatogenic epithelium of the convoluted seminiferous tubule, the area of spermatogenic cells and their nuclei, the length and thickness of the flagellum of late spermatids.

7. The number of sperm in the lumen of the convoluted seminiferous tubule, the area of the head and nucleus, the width of the neck and the length of the tail.

8. The number of Leydig cells in the area of the interstitium; area of Leydig cells and their nuclei.

9. The ratio of the area of interstitial tissue to the area of convoluted seminiferous tubules is in one field of view of the preparation.

Morphometric measurements were performed with a zooming in 10×10, 40×10 and 100×10.

The resolution of the received images is 1300×1030 pixels.

Based on the quantitative data obtained in the cytological study of the testes, a number of informative indicators were calculated that characterize the state of spermatogenesis in the testes of male white rats.

These indicators include:

1. Spermatogram – the percentage distribution of spermatogenic epithelial cells in one convoluted seminiferous tubule **(Shejko L.D., 1998)**.

2. Spermatogenesis index – the ratio of the sum of all counted cell layers in one tubule to the number of all counted tubules.

The spermatogenesis index was calculated by the formula: Is = ∑a/N, where a is the number of layers isolated in each tubule (the first layer is spermatogonia, the second is spermatocytes, the third is spermatids, the fourth is spermatozoa); N is the number of counted tubules **(Narbutova T.E., 2011)**.

3. The index of relaxation (tension of spermatogenesis) – the ratio of the sum of all spermatogenic cells to the sum of Sertoli cells in one convoluted seminiferous tubule **(Shejko L.D., 1998)**.

4. Maturation index – the ratio of the sum of young (spermatogonia, spermatocytes) and mature forms of spermatogenic epithelium (spermatids, spermatozoa) in one convoluted seminiferous tubule.

5. Index of meiotic activity – the ratio of the sum of meiotic cells (spermatocytes) to the sum of the remaining germ cells in one convoluted seminiferous tubule.

6. Germinal index – the ratio of the sum of spermatogonia to the sum of Sertoli cells in one convoluted seminiferous tubule **(Shevantaeva, O.N., 2012)**.

Statistical processing of digital data was carried out using the FStat and Excel programs. Statistical hypotheses were tested using the Student's t-test.

When evaluating statistical hypotheses, the following significance levels were taken: $p \leq 0.05$.

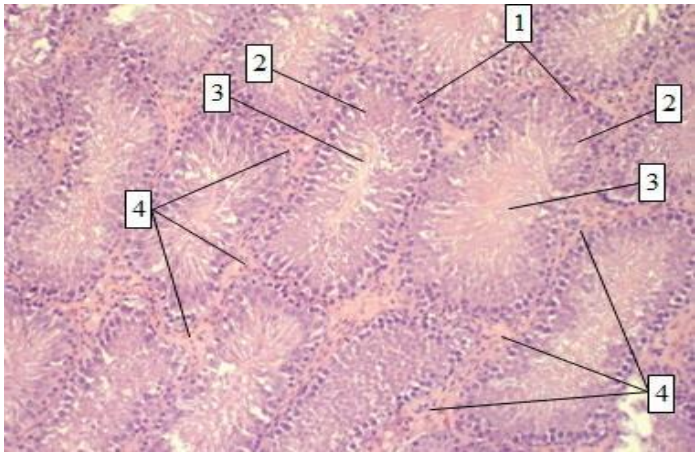
3. Research results

External examination of the testes of male rats showed that they are pinkish-white in color, soft-elastic consistency, elliptical in shape. The weight of the testes was 0.588 ± 0.014 g. Moreover, the mass of the left testes was several masses of the right one.

At low magnification of the microscope, a pink stripe is noticeable along the edge of the specimen - this is a tunica albuginea, consisting of dense loose connective tissue. The bulk of the testis is formed by seminiferous convoluted tubules, cut across or obliquely (tangentially), round or elliptical in

shape. The seminiferous tubules are separated from each other by a thin layer of interstitial connective tissue, under which there is a thick wall of spermatogenic epithelium at different stages of development. The areas of the interstitium between the convoluted seminiferous tubules are evenly spaced, predominantly triangular in shape. In the center of the convoluted tubule there is a lumen where the formed sperm are released. Even with low magnification, it is noticeable that different stages of spermatogenesis go through different tubules (Fig. 1).

Figure.1 Cross section of the seminal glands. Stained with haematoxylin-eosin. Zooming 40×10: 1 – convoluted seminiferous tubule; 2 – spermatogenic epithelium; 3 – tubule lumen; 4 – interstitial tissue.

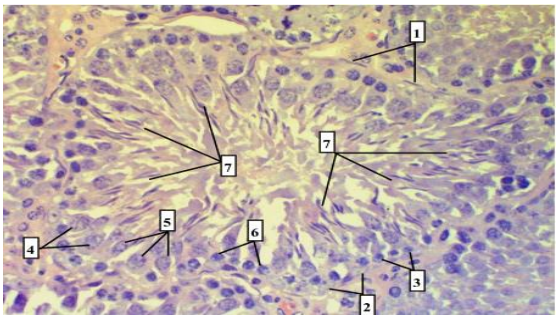


At high magnification, it can be seen that the tubule's own membrane is built of connective tissue fibers. Outside of the basement membrane is a layer of loose connective tissue, which contains a layer of myoid cells, which have a squamous, lunate and elongated shape. Myoid cells are distributed evenly along the entire contour of the convoluted seminiferous tubule. Inside of its own membrane, separated by the basement membrane, is the spermatogenic epithelium.

Histological studies of the testes of white rats showed that the first outer layer of the spermatogenic epithelium in the convoluted seminiferous tubules is composed of spermatogonia lying on the basement membrane with a dark optically dense nucleus and a narrow rim of the cytoplasm. Spermatocytes are located closer to the center of the tubule. These are large cells with a large nucleus and a wide rim of the cytoplasm, and have a rounded shape.

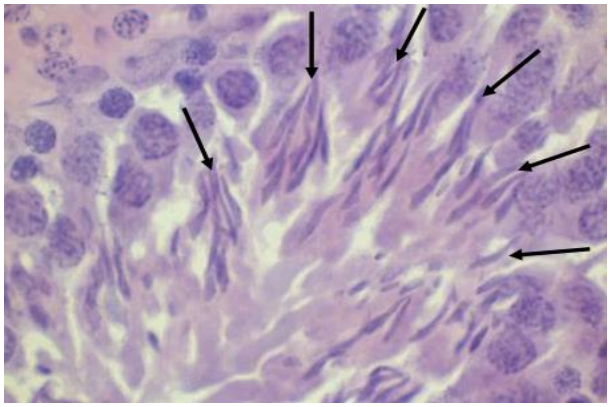
The innermost layer of the convoluted tubule is made up of spermatids, small cells with a light nucleus, lying in several rows. Early spermatids of a rounded shape with a spherical nucleus are located in the middle layers of the spermatogenic epithelium. Late spermatids lie in the layer adjacent to the lumen of the tubule and have an elongated shape. In some late spermatids, a flagellum is found. In some tubules, formed spermatozoa are visible. Their dark elongated heads are directed to the periphery of the tubule, and their tails hang down into the lumen of the tubule (Fig. 2).

Figure.2 The structure of the convoluted seminal tubule of the testis of a male white rat. Stained with haematoxylin-eosin. Zooming 40×10: 1 – myoid cells; 2 – Sertoli cells; 3 – spermatogonia; 4 – spermatocytes; 5 – early spermatids; 6 – late spermatids; 7 – spermatozoa.



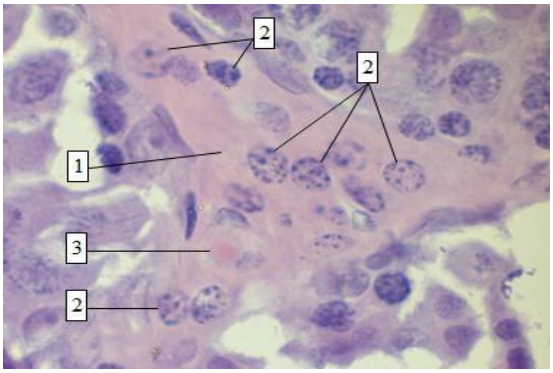
Spermatozoa in the lumen of the convoluted seminiferous tubule are located in groups of 6–8 pieces along the entire contour of the lumen. The sperm head is shaped like a hook (Fig. 3).

Figure.3 Spermatozoa of male white rats in the lumen of the convoluted seminal tubule. Stained with haematoxylin-eosin. Zooming 100×10.



In the interstitial tissue of the testes, consisting of loose connective tissue, blood vessels were found, around which single, or more often in groups of 5–7 cells, large oval or polygonal Leydig cells lie with a large spherical nucleus. The total number of glandulocytes in one area of the interstitium reached 10–12 pieces (Fig. 4).

Figure.4 Interstitial tissue of the seminal glands. Stained with haematoxylin-eosin. Zooming 100×10: 1 – area of the interstitium; 2 – Leydig cells; 3 – blood vessel.



3.1. Morphometric studies

Morphometric studies have shown that the thickness of the albuginea of the testes of male white rats is 35.23±3.42 μ.

Morphometric parameters of the convoluted seminal tubules of the testes and their surrounding areas of interstitial tissue of male white rats are shown in Tables 1 and 2.

Table.1. Morphometric parameters of the convoluted seminiferous tubules of the testes of male white rats (M ± m)

Indicators	The digital value
1	**2**
The number of convoluted seminiferous tubules in the same visual field	34.68±0.94
The cross-section of a convoluted seminiferous tubule, μ^2	45469.74±1746.76
The surface of a tubule lumen, μ^2	8878.17±832.41
The surface of the seminiferous epithelium, μ^2	36591.57±1243.36
The seminiferous epithelium thickness, μ	36.62±2.34
The number of myoid cells in the wall of a convoluted seminiferous tubule	19.44±1.42
The surface of a Myoid cell, μ^2	10.63±2.55
The surface of a myoid cell nucleus, μ^2	1.14±0.30
The number of Sertoli cells in the seminiferous epithelium of a convoluted seminiferous tubule	23.84±3.16
The surface of a Sertoli cell, μ^2	189.73±18.59
The width of the basal part of a Sertoli cell, μ	13.39±1.04
The height of the apical part of a Sertoli cell, μ	15.78±4.14
1	**2**
The surface of a Sertoli cell nucleus, μ^2	15.82±0.73
The number of spermatogonia in the seminiferous epithelium of a convoluted seminiferous tubule	52.44±1.46
The spermatogonium surface, μ^2	27.58±2.07
The surface of a spermatogonium nucleus, μ^2	5.55±1.52
The number of spermatocytes in the seminiferous epithelium of a convoluted seminiferous tubule	40.80±1.97
The surface of a spermatocyte, μ^2	41.19±5.86
The surface of a spermatocyte nucleus, μ^2	3.35±0.43
The number of spermatids in the seminiferous epithelium of a convoluted seminiferous tubule	34.80±1.52
The surface of a spermatid, μ^2	32.69±4.36
The surface of a spermatid nucleus, μ^2	2.93±0.52
The flagellum length of late spermatids, μ	10.08±2.15
The flagellum thickness of late spermatids , μ	3.17±0.75

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The number of spermatozoa in a lumen of a convoluted seminiferous tubule	304.52±13.14
The surface of a spermatozoon head, μ^2	17.48±2.12
The width of a spermatozoon cervix, μ	2.97±0.23
The length of a spermatozoon tail, μ	20.11±0.96
The surface of a spermatozoon nucleus, μ^2	1.81±0.56

Table.2. Morphometric parameters of interstitial testicular tissue of male white rats (M±m)

Indicators	The digital value
The surface of interstitial tissue, μ^2	1226.14±103.75
The number of sites of the interstitial tissue between seminiferous tubules in the same visual field	42.56±2.26
The surface of a Leydig cell, μ^2	40.44±1.30
The surface of a Leydig cell nucleus, μ^2	10.82±1.06
The number of Leydig cells in the interstitial site	9.20±1.20

The ratio of the interstitial tissue surface to the surface of convoluted seminiferous tubules in the same visual field of the specimen is about 1:30.

The study of the spermatogenesis process showed the intensity of this process in male albino rat testes (Fig. 5, Table 3, 4).

Figure.5 Spermiogramma male white rats.

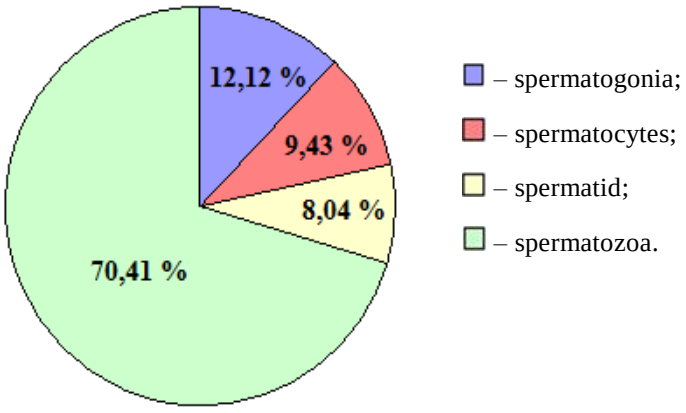

Table.3. Proportion of individual types of spermatogenic cells in the convoluted seminiferous tubule seminal glands of male white rats (M±m)

Indicators	The number of cells in the tortuous seminiferous tubule	% of total number of spermatogenic cells

Spermatogonia	52.44±1.46	12.12±2.71
Spermatocytes	40.80±1.97	9.43±1.61
Spermatid	34.80±1.52	8.04±1.20
Spermatozoa	304.52±13.14	70.41±4.14

Table.4. The measurement of the functional activity of male albino rat testes (M±m)

Indicators	**The digital value**
Index of spermatogenesis	3.32±0.15
Index of relaxation (tension of spermatogenesis)	18.14±1.72
Index of ripening	0.28±0.01
Index of meiotic activity	0.10±0.01
Germinative index	2.21±0.17

4. Discussion

The development and sexual differentiation of the testes is a complex process. The most important indicator of the structural and functional formation of the testis is the characteristic of the spermatogenic layer **(Evdokimov V.V. et all., 2006)**.

To assess the spermatogenic layer of experimental animals, we calculated the total content of spermatogenic cells, including spermatogonia of varying degrees of maturity, spermatocytes, spermatids and spermatozoa, as well as indices of spermatogenesis, relaxation, maturation, meiotic activity and germinal index.

Spermatogonia were counted starting from the 60th day of postnatal ontogenesis, since, according to modern concepts of spermatogenesis, in rats during the neonatal period, almost all spermatogenic cells are gonocytes **(Zogbi C. et al., 2012)**, the formation of the first spermatogonia dates back to 3-6 days of postnatal ontogenesis **(De Rooij, D.G. et al., 2010; Morales A. et al., 2007)**. According to the literature, the first spermatozoa in the seminiferous convoluted tubules of rats are formed by the 43rd day of life **(Zogbi C. et al., 2012).**

Analysis of the total content of spermatogenic cells in seminiferous convoluted tubules showed that in experimental animals at the age of 2 months of postnatal development, the largest percentage of the total number of spermatogenic cells are mature male germ cells – spermatozoa. The second in quantitative terms are male stem cells - spermatogonia. The content of spermatocytes and spermatids in the convoluted seminiferous tubules is approximately the same percentage of the total number of spermatogenic cells, which is obviously explained by the achievement of the maximum rate of premeiotic spermatogenesis by this period, as a result of which these cell populations stabilize in numbers **(Utkina A.S. et all., 2021; Zogbi C. et al., 2012)**.

The highest percentage of spermatozoa, apparently, indicates the suspension of apoptosis of spermatocytes in sexually mature animals **(Van Haaster L.H. et al., 1993; Morales A. et al., 2007; Moreno S.G. et al., 2001**).

The results of quantitative research of tastes showed that the surface of rat convoluted seminiferous tubules increases during the growth of an animal, but it is constant after its coming in sexual maturity, that's why this indicator serves as a reliable measure reflecting the structural and functional state of the male gonads **(Sizonenko M.L. et al., 2012)**.

Our findings coincide with those of the literature. It was found out that when male white rats were at the age of 2 months of postnatal development, the ratio of interstitial tissue surface to the surface of convoluted seminiferous tubules was about 1:30.

The most important quantitative indicator characterizing the generative activity of the testis is the spermatogenesis index, which reflects the number of generations of spermatogenic cells in the wall of the convoluted seminiferous tubules **(Sayapina I.Yu. et al., 2013)**. The index was studied starting from the 60th day of postnatal ontogenesis, since by this period the migration of gonoblasts ends and the first wave of spermatogenesis passes **(Zogbi C. et al., 2012)**.

The analysis of this indicator made it possible to record its high level (3.32±0.15), which is obviously associated with the transition to a full-fledged process of spermatogenesis, which ends with the formation of spermatozoa.

The obtained results clearly demonstrate the maturity of experimental animals.

5. Conclusion

In the course of our research, the following conclusions can be drawn:

1. With the help of histological and morphometric research methods, the structural features of the testes of male white rats in connection with the formation of the process of spermatogenesis are shown.

2. It was found out that when male rats are at the age of 18-20 months, the ratio of interstitial tissue surface to the surface of convoluted seminiferous tubules in testes is about 1:30.

3. It has been shown that during the period of sexual maturity in male white rats, the largest percentage of the total number of spermatogenic cells are mature male germ cells - spermatozoa. The second in quantitative terms are male stem cells - spermatogonia. These indicators confirm such indicators as: the maturation index, the meiotic activity index and the germinal index.

4. The high reproductive activity of the seminal glands is indicated by such calculated indicators characterizing the state of spermatogenesis in the testes of male white rats, such as: the index of spermatogenesis, the index of relaxation (tension of spermatogenesis).

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