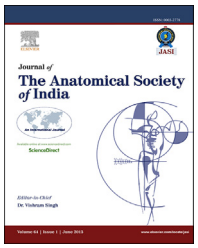
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jasi



Original Article

The morphology of the testes of male albino rats at postnatal ontogenesis

Olga Sergeevna Shubina [a,*], *Natalia Anatolievna Dudenkova* [b], *Leonid Petrovich Teltsov* [c]

[a] *Doctor of Biological Sciences, Professor of Biology, Geography and Teaching Methods, Federal State Budgetary Educational Institution of Higher Professional Education «Mordovian State Pedagogical Institute named after M. E. Evsevjev», Studencheskaya Street, 11A, Mordovia 430007, Saransk, Russia*

[b] *Assistant Professor of Biology, Geography and Teaching Methods, Federal State Budgetary Educational Institution of Higher Professional Education «Mordovian State Pedagogical Institute named after M. E. Evsevjev», Studencheskaya Street, 11A, Mordovia 430007, Saransk, Russia*

[c] *Doctor of Biological Sciences, Professor of the Department of Morphology and Physiology of Animals, Federal State Budgetary Educational Institution of Higher Professional Education «Mordovian State University named after N. P. Ogarev», Bolshevitskaya Street, 68, Mordovia 430005, Saransk, Russia*

ARTICLE INFO

Article history:
Received 14 October 2014
Accepted 18 April 2015
Available online 9 May 2015

Keywords:
Seminal glands (testis)
Convoluted seminiferous tubules
The spermatogenic epithelium
Spermatogenous cells
Interstitial tissue (the interstitium)

ABSTRACT

Introduction: The necessity of studying the peculiarities of the structural organization of the testes is determined by its participation in performing important functions for the body – making spermatozoa and the production of male sex hormones. Despite the presence of works devoted to the study of the peculiarities of the structural organization of the testes, many questions remain unresolved or require clarification.

Materials and methods: As a biological test object used white Mature rats male Wistar rats at the age of 2 months. Seminal glands of male rats were used as a trial material for study. Tissue samples was investigated using a digital microscope Axio Imager.M2 (ZEISS, Japan) with software for image analysis AxioVision SE64 Rel. 4.8.3 and ZEN 2011. When sight-seeing microscopy studied morphological features of the structure of the testes, and then determined their morphometric parameters.

Results: Found that in rats-males aged 2 months in the testes, the proportion of interstitial tissue to the area of convoluted seminiferous tubules is about 1:30. It is shown that in the period of puberty of male white rats the highest percentage of the total amount of the spermatogenic cells are Mature male sex cells – spermatozoa. Second in quantitative terms are male stem cells spermatogonia.

Discussion: Development and sexual differentiation of the testes is a complex process. The most important indicator of the structural and functional development of the testis is characteristic of the spermatogenic reservoir. The obtained results clearly demonstrate the maturity of experimental animals.

* *Corresponding author.*
E-mail address: o.shubina@mail.ru (O.S. Shubina).

http://dx.doi.org/10.1016/j.jasi.2015.04.014

1. Introduction

The necessity of studying structural organization peculiarities of testes is determined by their participation in performing important organism functions – the production of semen and the pathway of male sex hormones.[1–3]

Despite the presence of some works devoted to the study of the testis structural organization peculiarities,[4–7] many questions remain unresolved or require clarification.[8–11]

The aim of this work was to study morphological features of the male albino rat testes in connection with the formation of reproductive function.

2. Materials and methods

White mature male Wistar rats aged 2 months of postnatal development, weighing 200–250 g were used as a biological test object. Altogether 50 animals were used. Seminal glands of male rats were used as a data for study.

The experiment was conducted during the year indoors when the temperature of 22–25 °C and a relative humidity of 67–70%. The animals were housed at the General mode of the vivarium, had free access to feed and water.

The animals were killed by decapitation under ether anesthesia with chloroform (1:1) in compliance with the principles of humanity as set out in the directives of the European Community (86/609/EES) and the Declaration of Helsinki and in accordance with the rules of carrying out the works using experimental animals.

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

Seminal glands of male rats were used as a trial material for study. For histological examination, tissue samples were preserved in 10% solution of neutral formalin. Preserved samples after rinsing in running water were dehydrated by placing in alcohols of increasing concentration and embedded into paraffin according to the conventional methodology. Histological cross-sections of seminal glands were prepared 10–15 microns thick, stained with haematoxylin-eosin and examined by a digital microscope Axio Imager.M2 with the image analysis software AxioVision SE64 Rel. 4.8.3 and ZEN 2011.

Photography products produced with a digital camera AxioCam MRc5 (ZEISS, Japan), followed by image processing in Abode Photoshop Elements 11.

The morphological features of the seminal gland structure were studied during summarizing microscopy with the subsequent determination of the following morphometric parameters:

1. The thickness of the testis tunica albouginea;
2. The number of convoluted seminiferous tubules in the same visual field, the cross-section of the convoluted seminiferous tubule and its lumen, as well as the surface of seminiferous epithelium and its thickness;
3. The number of interstitial sites between convoluted seminiferous tubules in the same visual field and their surface;
4. The number of myoid cells in the wall of a convoluted seminiferous tubule and the surface of myoid cells and their nuclei;
5. The number of Sertoli cells in the spermatogenetic epithelia of a convoluted seminiferous tubule, the width of the basal part of Sertoli cells and the length of their apical part, as well as the surface of these cells and their nuclei;
6. The number of spermatogenic cells of different kinds (spermatogonia, spermatocytes and spermatids) in the seminiferous epithelium of a convoluted seminiferous tubule, the surface of spermatogenic cells and their nuclei, the length and the thickness of the flagellum of late spermatids;
7. The number of spermatozoa in the lumen of a convoluted seminiferous tubule, the area of the head and the nucleus, cervix width and tail length;
8. The number of Leydig cells in the interstitial site; and the surface of Leydig cells and their nuclei;
9. Ratio of the interstitial tissue surface to the surface of convoluted seminiferous tubules in one visual field of the specimen.

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

Resolution of the resulting images was 1300 × 1030 pixels.

A number of informative indicators characterizing the state of the spermatogenesis in the testes of male albino rats were counted on the basis of quantitative data obtained by cytological examination of the testes.

These indicators include:

1. Spermiogramma – percentage distribution of spermatogenic epithelium cells in one convoluted seminiferous tubule[12];
2. Index of spermatogenesis – ratio of the sum of all counted cell layers in one tubule to the number of all counted tubules.

Spermatogenesis index was calculated by the formula: $Is = \sum a/N$, where "a" is the number of layers selected in each tubule (first layer is spermatogonia, the second layer is spermatocytes, the third layer is spermatids and the fourth layer is spermatozoa); "N" is the number of counted tubules[13];

3. Index of relaxation (tension of spermatogenesis) – the ratio of all spermatogenous cells to Sertoli cells in one convoluted seminiferous tubule[12];
4. Index of maturation – the ratio of young (spermatogonia, spermatocytes) and mature forms of seminiferous epithelium (spermatids, spermatozoa) in a convoluted seminiferous tubule;
5. Index of meiotic activity – the ratio of meiotic cells (spermatocytes) to the sum of the remaining germ cells in convoluted seminiferous tubules;
6. Germinative index – the ratio of spermatogonia to Sertoli cells in one convoluted seminiferous tubule.[14]

Statistical processing of digital data was performed using the FStat and Excel program codes. Testing of statistical hypothesis was carried out by Student's t-test. When testing

statistical hypotheses, the accepted significance points were $p \leq 0.05$. Mathematical treatment of morphometric studies was performed using the correlation analysis method.

3. Results

The external inspection of the rat seminal glands showed that they are pinkish-white in colour, with smooth and elastic consistency and have elliptical shape. The weight of testes was 0.588 ± 0.014 g. Plus, the weight of the left testis is a little bit higher than of the right one.

With low power of microscope a pink stripe that goes along the edge of the specimen is noticeable. That is the tunica albuginea composed of indurated cribriform tissue. The bulk of the testis is formed from convoluted seminiferous tubules of spherical or ellipsoidal shape, cut transversely or obliquely (tangentially). Seminiferous tubules are separated from each other by a thin shell of interstitial connective tissue, which is a thick wall of spermatogenic epithelium at different stages of development. Sites of interstice between the convoluted seminiferous tubules are sited evenly, mostly triangular in shape. In the center there is a convoluted tubule lumen, where produced spermatozoa emerge. Even with low power it is noticeable that different stages of spermatogenesis are happening in different tubules (Fig. 1).

With high power it can be seen that the genuine tubule membrane consists of connective tissue fibers. There is a layer of loose connective tissue outwards of the basal membrane, in which the layer of myoid cells of scaly, lunate and elongated shape is sited. Myoid cells are evenly sited over the entire surface of convoluted seminiferous tubules. Inward of its own shell, separated by a basal membrane there is the spermatogenic epithelium.

The histological examination of rat testes showed that the first outer layer of the spermatogenic epithelium in the convoluted seminiferous tubules consistes of sperm cells with a dark optically dense nucleus and a narrow cytoplasm rim lying on the basal membrane of spermatogonia.

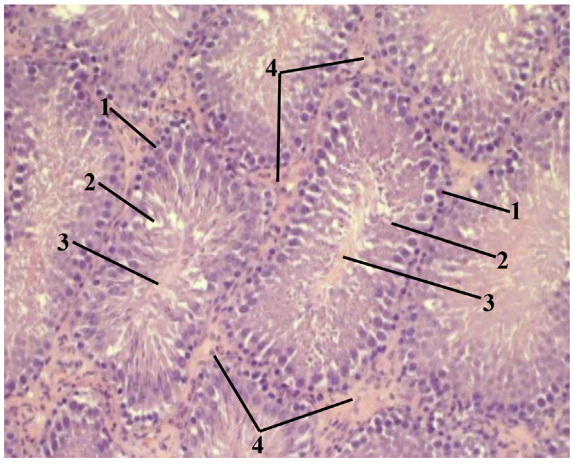


Fig. 1 – A cross section of seminal glands. Stained with haematoxylin-eosin; Zooming 10 × 10: 1 – convoluted seminiferous tubule, 2 – seminiferous epithelium, 3 – tubule lumen, 4 – interstitial tissue.

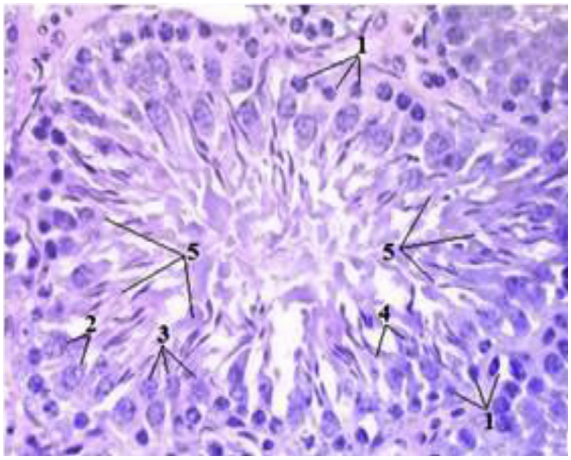


Fig. 2 – Convoluted seminiferous tubule. Stained with haematoxylin-eosin. Zooming 40 × 10: 1 – spermatogonia, 2 – spermatocytes, 3 – early spermatids, 4 – late spermatids, 5 – spermatozoa.

There are spermatocytes closer to the center of the tubule. They are large cells with a large nucleus and a broad round cytoplasm rim.

The innermost layer of convoluted tubules is presented by spermacides. They are small cells lying in several rows with bright nuclei. Early round spermatids with spherical nuclei are in the central layers of the spermatogenic epithelium. Late spermatids are in the layer adhering the tubule lumen and have an elongated shape. Some late spermatids have flagella.

Fully-fledged spermatozoa can be seen in some tubules. Their dark elongated heads are directed to the tubule periphery, and their tails hang down to the tubule lumen (Fig. 2).

Spermatozoa in the lumen of convoluted seminiferous tubules are arranged in groups of 6–8 along the outline of the lumen. The head of the spermatozoa has the form of a hook (Fig. 3).

In the testis interstitial tissue consisting of loose connective tissue, blood vessels were detected. There are large oval or

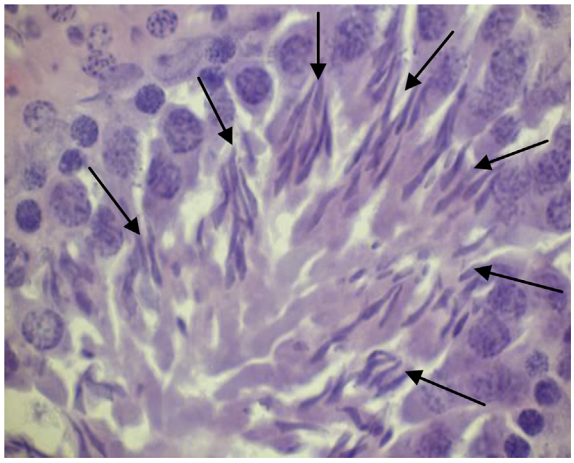


Fig. 3 – Spermatozoa in the lumen of convoluted seminiferous tubule. Stained with haematoxylin-eosin. Zooming 100 × 10.

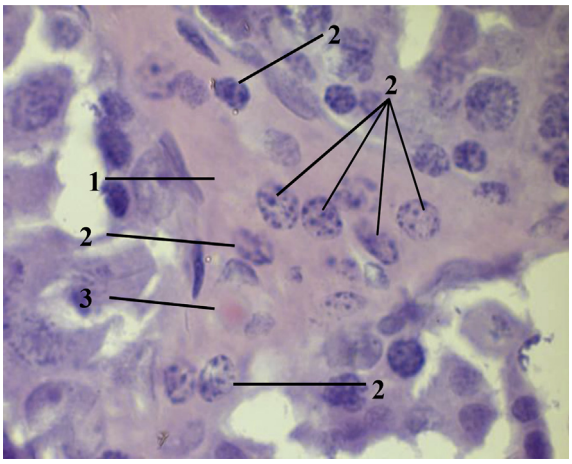


Fig. 4 – The interstitial tissue of the testes of male albino rats. Stained with haematoxylin-eosin. Zooming 100 × 10: 1 – interstitial tissue; 2 – Leydig cells; 3 – blood vessel.

polygonal Leydig cells with large spherical nuclei along of them. They are sited alone or more commonly in groups of 5–7 cells. The total number of glandulocytes in one site of interstice ran up to 10–12 cells (Fig. 4).

The morphometric studies have shown that the thickness of the testis tunica albuginea of male albino rats is equal to 35.23 ± 3.42 μ.

The morphometric characteristics of convoluted seminiferous tubules of male albino rat testes and the interstitial tissue sites surrounding them are given in Tables 1 and 2.

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.

Table 2 – Morphometric indicators of the interstitial tissue of albino rat seminal glands.

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

4. Discussion

Development and sex differentiation of testes is a complex process. The most important indicator of the structural and functional development of testes is a characteristic of the spermatogenic layer.[1,15]

The total content of spermatogenous cells including spermatogonia of different degrees of maturity, spermatocytes, spermatids and spermatozoa was counted to assess the formation of the spermatogenic layer of experimental animals. Plus, indexes of spermatogenesis, relaxation, maturation, meiotic activity and germinative index were taken in consideration.

The count of spermatogonia started from the 60th day of postnatal ontogenesis because according to modern ideas of the spermatogenesis processes of rats in the neonatal period almost all the spermatogenous cells are gonocytes.[16] The formation of the first spermatogonia dates back to 3–6 day of

Table 1 – The morphometric characteristics of convoluted seminiferous tubules of albino rat seminal glands.

1	2	3	4
Indicators	The digital value	Indicators	The digital value
The number of convoluted seminiferous tubules in the same visual field	34.68 ± 0.94	The surface of a spermatogonium nucleus, μ^2	5.55 ± 1.52
The cross-section of a convoluted seminiferous tubule, μ^2	45469.74 ± 1746.76	The number of spermatocytes in the seminiferous epithelium of a convoluted seminiferous tubule	40.80 ± 1.97
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 surface of a spermatocyte, μ^2	41.19 ± 5.86
The seminiferous epithelium thickness, μ	36.62 ± 2.34	The surface of a spermatocyte nucleus, μ^2	3.35 ± 0.43
The number of myoid cells in the wall of a convoluted seminiferous tubule	19.44 ± 1.42	The number of spermatids in the seminiferous epithelium of a convoluted seminiferous tubule	34.80 ± 1.52
The surface of a Myoid cell, μ^2	10.63 ± 2.55	The surface of a spermatid, μ^2	32.69 ± 4.36
The surface of a myoid cell nucleus, μ2	1.14 ± 0.30	The surface of a spermatid nucleus, μ^2	2.93 ± 0.52
The number of Sertoli cells in the seminiferous epithelium of a convoluted seminiferous tubule	23.84 ± 3.16	The flagellum length of late spermatids, μ	10.08 ± 2.15
The surface of a Sertoli cell, μ^2	189.73 ± 18.59	The flagellum thickness of late spermatids, μ	3.17 ± 0.75
The width of the basal part of a Sertoli cell, μ	13.39 ± 1.04	The number of spermatozoa in a lumen of a convoluted seminiferous tubule	304.52 ± 13.14
The height of the apical part of a Sertoli cell, μ	15.78 ± 4.14	The surface of a spermatozoon head, μ^2	17.48 ± 2.12
The surface of a Sertoli cell nucleus, μ^2	15.82 ± 0.73	The width of a spermatozoon cervix, μ	2.97 ± 0.23
The number of spermatogonia in the seminiferous epithelium of a convoluted seminiferous tubule	52.44 ± 1.46	The length of a spermatozoon tail, μ	20.11 ± 0.96
The spermatogonium surface, μ^2	27.58 ± 2.07	The surface of a spermatozoon nucleus, μ^2	1.81 ± 0.56

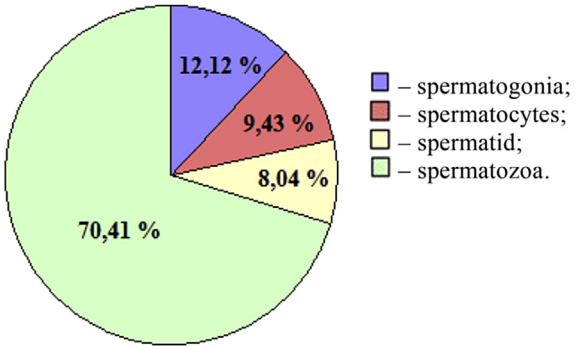


Fig. 5 – Spermiogramma male white rats.

Table 3 – Proportion of individual types of spermatogenic cells in the convoluted seminiferous tubule seminal glands of male white rats.

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.

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

postnatal ontogenesis.[16–18] According to the data of literature, the first spermatozoa in seminal convoluted tubules of rats are formed to 43rd day of life.[17]

The analysis of the total content of spermatogenous cells in seminiferous convoluted tubules showed that spermatozoa, mature male sex cells, have a greater representativeness among all the spermatogenous cells in testes of experimental animals at the age of 2 months of postnatal development. Male stem cells, spermatogonia, take the second place in quantitative terms. Spermatocytes and spermatids have equal percentage ratio to the total number of spermatogenous cells in convoluted seminiferous tubules that likely can be explained by the fact that the maximum tempos of premeiotic spermatogenesis is achieved by this period. It leads to the stabilization of cell numbers.[17,18]

Spermatozoa have the largest percentage; it obviously shows that mature animals go through the stopping of the spermatocyte apoptosis.[18–20]

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.[21]

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 of the generative activity of testes is the index of spermatogenesis reflecting the number of genitures of the spermatogenous cells in the wall of convoluted seminiferous tubules.[22] The study of the index started from the 60th day of postnatal ontogenesis, as the migration of monoblasts and the first wave of spermatogenesis are over by this time.[16]

This indicator, as the analysis showed, was of high level (3.32 ± 0.15), that is obviously related to the transition to the full-fledged spermatogenesis process, culminating in the formation of spermatozoa.

The obtained results clearly demonstrate the maturity of experimental animals.

5. Conclusion

1. The structural features of the male albino rat testes in connection with the formation of spermatogenesis process were showed by means of histological and morphometric research methods.
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 was showed that spermatozoa, mature male sex cells, have a greater representativeness among all the spermatogenous cells in testes of experimental animals at the age of 2 months of postnatal development. Male stem cells, spermatogonia, take the second place in quantitative terms. These data were confirmed by such indicators as: the index of maturation, the index of meiotic activity and the germinative index.
4. Such calculated parameters characterizing the state of the spermatogenesis in the testes of male albino rats as the index of spermatogenesis and the index of relaxation (tension of spermatogenesis) point at the high reproductive activity of the seminal glands.

Conflicts of interest

All authors have none to declare.

Acknowledgments

The study was carried out with the financial support of the Ministry of Education and Science of the Russian Federation within the state programme of FSBEI HPE "Mordovian State Pedagogical Institute named after M.E. Evsevyev" (project "The

influence of anthropogenic factors on the morphofunctional state of the organism").

REFERENCES

1. Ruzen-Range E. Spermatogenesis in animals. *World*. 1980:259.
2. Bagatska NV. *Genetic Factors in the Occurrence of Disorders of Sexual Development in Children Adolescents, Institute of Hygiene and Medical Ecology Named after*. Kiev: Ommerse of Medical Sciences of Ukraine; 2004.
3. Potapov SN, Gorgol' NI, Andreev AV. Morphological features of Leydig cells of fetuses and newborns from mothers with preeclampsia. *Med Today Tomorrow*. 2011;4:23–26.
4. Samusev RP, Kapitonova MYu. General and special histology. *Peace and Education*. 2010:336.
5. Liu HX, Qin WH, Wang GR, et al. Some altered concentrations of elements in semen of workers exposed to trinitrotoluene. *Occup Environ Med*. 1995;52:842–845.
6. Samusev RP, Zubareva EV. The endocrine glands. *Peace and Education*. 2011:144.
7. Nishlag E, Bere GM. Andrology. Men's health and dysfunction of the reproductive system. *Medicine*. 2005:54.
8. Voloshin NA, Topolenko TA. Morphofunctional peculiarities of formation of the testes of rats from birth until the second month of life. *Ukr Morphol Alm*. 2009;7:32–34.
9. Morteza K, Mansoureh M, Seyed JM, Hamid G. Autologous transplantation of adult mice spermato-gonial stem cells into gamma irradiated testes. *Cell J*. 2012;14:82–89.
10. Chang C, Chen YT, Yen SD, et al. Infertility with defective spermatogenesis and hypotestos-teronemia in male mice lacking the androgen receptor in sertoli cells. *Proc Natl Acad Sci USA*. 2004;101:6876–6881.
11. Hess RA, Franca RL. *Spermatogenesis and Cycle of the Seminiferous Epithelium. Molecular Mechanisms in Spermatogenesis*. Austin. TX: Landes Bioscience/Springer Science; 2008:1–15.
12. Shejko LD. *The Influence of Small Doses of Hexavalent Chromium on the Reproductive Function of Small Mammals: A Model Experiment*. Ekaterinburg: Ural Research Institute of Maternity and Infancy; 1998.
13. Narbutova TE. Dynamics of structural and functional changes of the testes of the mice of the second generation with the accumulation of lead in the body and the introduction of alpha-tocopherol. *Biomed Biosoc Anthropol*. 2011;16:27–31.
14. Shevantaeva ON. *Spermatogenesis after Extreme Hypoxic and Ischemic Effects and the Possibility of Correct Medication in the Experiment*. Moscow: State budgetary Educational Institution of Higher Professional Education "Nizhny Novgorod State Medical Academy" of the Ministry of Health and Social Development of the Russian Federation; 2012.
15. Rajcina SS. Spermatogenesis and structural basis of its regulation. *Science*. 1985:207.
16. Zogbi C, Tesser RB, Encinas G, Miraglia SM, Stumpp T. Gonocyte development in rats: proliferation, distribution and death revisited. *Histochem Cell Biol*. 2012;138:305–322.
17. Morales A, Mohamed F, Cavicchia JC. Apoptosis and Blood–Testis barrier during the first spermatogenic wave in the Pubertal rat. *Anatomical Rec*. 2007;290:206–214.
18. Van Haaster LH, De Rooij DG. Spermatogenesis is accelerated in the immature Djungarian and Chinese hamster and rat. *Biol Reproduction*. 1993;49:1229–1235.
19. Moreno RD, Lizama C, Urzúa N, Vergara SP, Reyes JG. Caspase activation throughout the first wave of spermatogenesis in the rat. *Cell Tissue Res*. 2006;325:533–540.
20. Jahnukainen K, Crhysis D, Hou M, Parvinen M, Eksborg S, Söder O. Increased apoptosis occurring during the first wave of spermatogenesis is stage-specific and primarily affects midpachytene spermatocytes in the rat testis. *Biol Reproduction*. 2004;70:290–296.
21. Sizonenko ML, Briukhin GV. The male gonads endocrine compartment of the posterity of female rats with chronic medical hepatobiliar system injury. *Russ J Hum Reproduction*. 2012;1:31–34.
22. Sayapina IYu, Ogorodnikova TL. Oxidative stress in the testes of rats, induced by adaptation to low temperatures, and its correction dihydroquercetin. *Multisubject Netw Electron Sci J Kuban state Agrar Univ (Sci J Kuban state Agrar Univ)*. 2013;5. URL: http://ej.kubagro.ru/2013/05/pdf/39.pdf.