DOI 10.26724/2079-8334-2019-4-70-169-173
UDC 611.018.53.428:611.08:616-056.257

T.V. Harapko
Uzhhorod National University, Uzhhorod

HISTOLOGICAL CHANGES OF STRUCTURAL COMPONENTS IN LYMPH NODES OF RATS AND CHANGES IN BIOCHEMICAL BLOOD INDICES IN EXPERIMENTAL OBESITY

e-mail: garapkotv@gmail.com

The article analyses the results and data of experimental study performed on white female and male rats of reproductive age (2.5-3.5-months). The purpose of the study was to establish the histological changes of rat lymph nodes structural components and changes in biochemical blood parameters through different terms of experimental obesity. The biochemical analysis of white male and female rats' blood for the level of glucose, ALT, AST, cholesterol and triglycerides was carried out throughout the experiment. After one week of experiment, there is an insignificant expansion of the marginal and cortical lymphatic sinuses. With the experiment duration increase all pathological changes exacerbate.

Key words: obesity, experiment, rat, lymph node, lymphocytes

This study is a part of complex projects «Morphological characteristics of internal organs and vascular bed in ontogenesis in the norm and patterns of their restructuring in obesity and effects on the body of physical factors», state registration number 0119U102059.

One of the most topical problems of modern society is the body overweight [6-9]. Obesity is a chronic recurrent disease manifested by excessive accumulation of adipose tissue and is the result of energy intake and discharge imbalance in individuals with hereditary predisposition or in the absence of it [10-11]. According to recent calculations, every third inhabitant of the Earth has overweight, and every tenth one is suffering from obesity [4].

An important role in the whole body homeostasis is played by lymphoid (immune) organs. The secondary lymphoid (immune) organs include lymph nodes. In secondary lymphoid organs, antigen-dependent proliferation is taking place together with differentiation of T-B-lymphocytes entering the blood from the primary lymphoid organs. It provides an adequate immune response of the body to foreign antigens. Changes in lymphoid organs after affecting the body of various harmful factors, including drugs have been studied for a long time [1-3, 5, 10]. In experimental obesity, the results obtained by the authors showed that the thymus and spleen react differently to the long-term use of a high-calorie diet. Changes in the mass and cellularity of the thymus and the spleen were of a multi-directional nature. High-calorie daiet resulted in a decrease in the mass of thymus, cell growth. In the spleen, on the contrary, there was an increase in the mass, a decrease in cellularity [5].

However, there is not enough data on the lymphoid organs changes under the obesity conditions.

The purpose of the work was to study histological changes in rat lymph nodes structural components; to study the biochemical blood indices changes through different terms of experimental obesity.

Materials and methods. We carried out the study on 50 white rats of reproductive age (2.5-3.5 months) weighing 150-180 g. Microanatomy of the lymph nodes structural components in white rats under conditions of physiological norm was studied on 10 intact animals. Experimental animals were divided into 4 groups: the first group (10 animals), being fed a high-calorie diet for one week; the second group (10 animals), fed high-calorie diet for two weeks; the third group (10 animals), fed high-calorie diet for three weeks; the fourth group (10 animals), fed the same diet for four weeks.

Each group included 5 male and 5 female rats. High-calorie diet was achieved due to the fact that glutamate sodium was added into food in a dose of 0.07 g / kg of rat body weight.

The experiment was performed according to the agreement on scientific cooperation between the Department of Human Anatomy and Histology of the Faculty of Medicine of Uzhhorod National University and the Department of Normal Anatomy of Danylo Halytsky National Medical University on November 18, 2013 at the Department of Normal Anatomy of the Danylo Halytsky Lviv National Medical University. The study was performed in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council of Europe Directives 86/609 / EEC (1986), Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty", the general ethical principles of experiments on animals adopted by the First National Congress of Ukraine on Bioethics (2001).

Images from the histological preparations of the club-shaped and mesenteric lymph nodes in the computer monitor were displayed from the MICROmed SEO SCAN microscope by means of the Vision

CCD Camera. The studies were carried out within the established schedule of the trial in samples stained with hematoxylin, eosin and azane.

To control the state of the liver, blood vessels, capillaries and heart, a biochemical analysis of the white male and female rats' blood was performed to determine the glucose, ALT, AST, cholesterol and triglycerides content throughout the experiment.

Results of the study and their discussion.

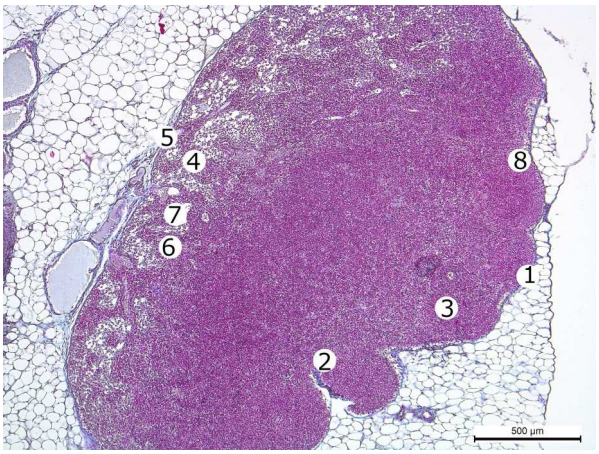

Fig. 1. A mesenteric lymph node of an intact white rat male. Azane stained. Magnif: obj. × 5, ocul. × 10. Designation: 1 - capsule; 2 - trabecula; 3 - cortical substance; 4 - medullary substance; 5 - the hilum of the node; 6 - lymph cord; 7 - medullary lymph sinus 8 - marginal sinus.

In animals of the intact and control groups, according to our histological studies, the structure of the iliac and mesenteric lymph nodes was compliant with the species norm. Externally, the lymph nodes are surrounded by a connective-tissue capsule, from which numerous trabeculae lead inside the node's parenchyma. On the node's concave side a hilum is located. Parenchyma consists of a cortical substance located on the node's periphery, and is closer to the gate of the medullary substance. Under the capsule there is a marginal sinus. In the cortical substance there are primary and secondary lymphoid follicles. The secondary ones contain clearing centers: germinal centers. There is a marginal layer around them. The medullary substance contains lymph cords and medullary intermediate lymph sinuses (fig. 1).

After 1-2 weeks of experiment, both in male and in female rats, the general structure of the lymph node corresponds to that of the intact animal group. There is a slight increase of the marginal and the cortical lymph sinuses (fig. 2A). Two weeks after, the medullary lymph sinuses get expanded. The veins and arteries are full-blooded (fig. 2B). The capsule is somewhat thickened.

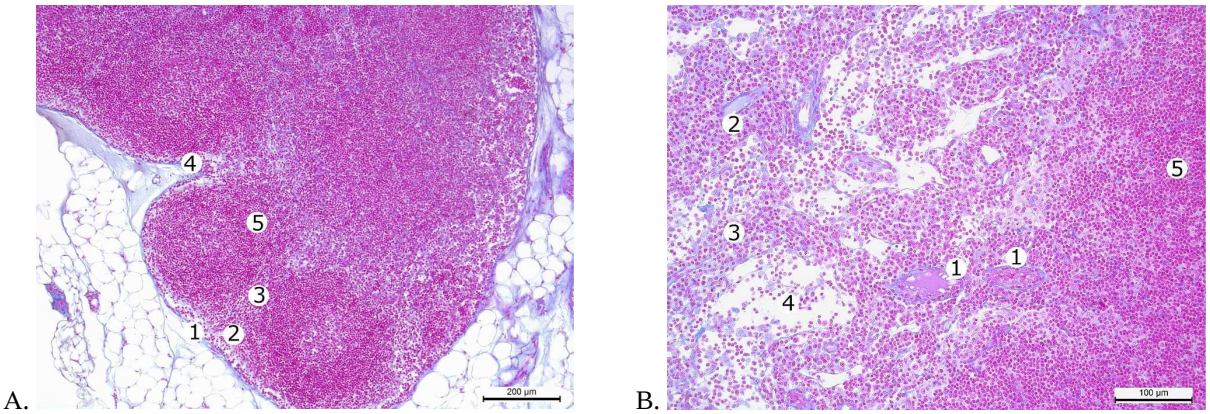

Fig. 2. A fragment of a mesenteric lymph node of a white female rat (A) and white male rat (B) after one (A) and two (B) weeks of the experiment. Azane stained. Magnif: A - obj. × 10, ocul. × 10; B - obj. × 20, ocul. × 10. Designation: A. 1-capsule; 2 - slightly expanded marginal sinus; 3 - expanded cortical lymph sinus; 4 - trabecula; 5 - secondary lymphoid follicle. B. 1 - varicose and full-blooded veins in the paracortical region; 2 - varicose and full-blooded vein in the medulla; 3 - medulla lymph cord; 4 - expanded medullary lymph sinus; 5 - cortical substance.

The level of glucose in the blood of white male rats grows to its maximum after two weeks of the experiment by 72.3%, then gradually reduces and after four weeks it exceeds the intact animals' indices by 16.7%. The level of glucose in the blood of white female rats grows to its maximum after one week of the experiment by 67.7%, then gradually reduces and four weeks after it is lower by 13.7% than in the intact animals (table 1).

Table 1

Indices of blood glucose levels in white rats, mmol/l (M ± m)

Group name	White male rat	White female rat
Intact animals	5.9±0.09	6.0±0.08
Group I	6.14±0.1	10.06±0.11
Group II	10.58±0.12	9.56±0.1
Group III	7.9±0.08	5.76±0.05
Group IV	7.08±0.12	5.18±0.09

The ALT level in the blood of white male and female rats grows to its maximum after two weeks of experiment, which exceeds the rate of intact animals by 57.3% and 40.4% respectively. During the experiment, it gradually reduces and four weeks after it is by 18.2% and 11.6% less than that of intact animals (table 2).

The AST level in the blood of white male rats grows to its maximum after one week of the experiment by 44.2% and 40.4% respectively, exceeding that of intact animals. During the experiment it fluctuates, and four weeks after it exceeds by 8.0% the intact animals' index. The AST level in the blood of white female rats does not change significantly during the experiment (table 2).

Table 2

Indices of ALT and AST levels in the blood of white rats, U/L (M ± m)

Group name	White male rat	White female rat	White male rat	White female rat
	ALT		AST	
Intact animals	76.5±0.2	67.5±0.18	175.7±0.35	168±0.49
Group I	93.4±0.3	58.9±0.21	253.4±0.39	148.7±0.7
Group II	120.3±0.31	94.8±0.29	172.4±0.33	167.8±0.67
Group III	96.7±0.4	61.3±0.22	240.8±0.77	172±0.56
Group IV	62.8±0.08	59.7±0.09	189.7±0.45	168.2±0.44

The level of cholesterol in the blood of white male rats and white feline rats increases as much as two weeks of the experiment, which is 2.5 and 2.9 times correspondingly higher than the rate of intact animals. During the experiment, it decreases somewhat and in four weeks it is 56.7% and 82.8% higher than the rate of intact animals (table 3).

The level of triglycerides in the blood of white male rats at the beginning of the experiment decreases, and from the second week begins to increase and in four weeks it is 11.6% less than the rate of intact animals. The level of triglycerides in the blood of white female rats increases as much as 2.9 times in two weeks of the experiment, then gradually decreases and returns to the level of intact animals four weeks later (table 3).

Table 3

Indices of cholesterol (mmol/l) and triglycerides (mmol/l) in blood of white rats (M ± m)

Group name	White male rat	White female rat	White male rat	White female rat
	cholesterol		triglycerides	
Intact animals	0.9±0.01	0.87±0.02	0.69±0.009	0.67±0.01
Group I	2.22±0.011	2.54±0.011	0.28±0.006	0.96±0.01
Group II	1.77±0.013	1.16±0.01	0.29±0.09	1.92±0.011
Group III	1.95±0.01	1.63±0.013	0.53±0.008	0.62±0.009
Group IV	1.41±0.01	1.59±0.011	0.61±0.007	0.68±0.01

After three weeks of the experiment, the capsule of the lymph nodes is thickened, containing a large amount of adipose tissue. Arteries in the node parenchyma and its hilum with a thickened wall, full blooded; vessels, veins and venules are deformed, dilated and full-blooded (fig. 3). The number of secondary lymphoid follicles in the cortical substance of the mesenteric and iliac lymph nodes grows both in male and in female rats.

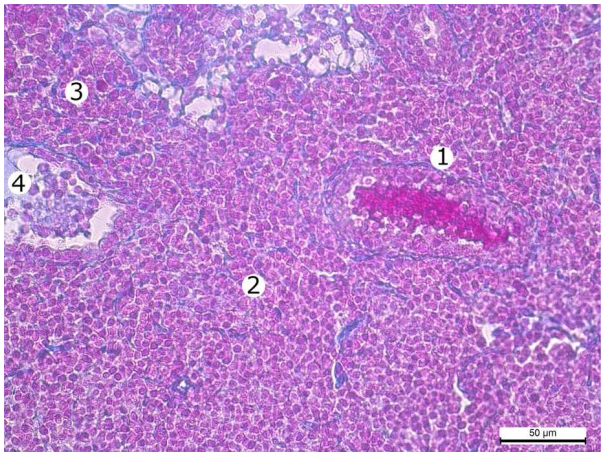

Fig. 3. Venule (1) with a thickened swollen wall in the paracortical region (2) of a white female rat's mesenteric lymph node after three weeks of experiment. Azane stained. Magnif.: obj. × 40, ocul. × 10. Designation: 2 – medullary lymph cord; 3 - a slightly enlarged medullary lymph sinus.

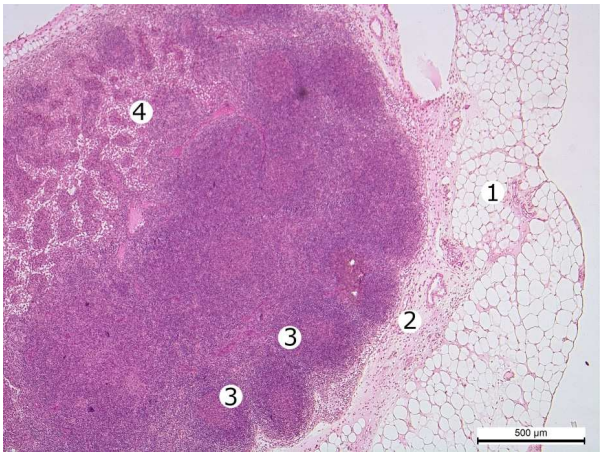

Fig. 4. White female rats' mesentery lymph node after four weeks of the experiment. Stained with hematoxylin and eosin. Magnif.: obj. × 5, ocul. × 10. Designations: 1 - accumulation of adipose tissue around and in the thickness of the capsule; 2- thickened capsule; 3- secondary lymphoid follicles in the cortical substance; 4 – medullary substance.

After four weeks of the experiment, the number of secondary lymphoid follicles in the cortical substance of the mesentery and iliac lymph nodes continues to grow both in male and in female rats. The germinal center is cleared, enlarged (fig. 4). The amount of adipose tissue grows around the body. Trabeculas leaving the capsule are pronounced, thickened. The area of cortical substance reduces, and that of medulla, respectively, grows, which is more pronounced in male rats. Arteries have thickened walls, full-blooded. Veins are varicose, deformed, full-blooded. The marginal sinus is unevenly enlarged. Medullary lymph nodes are enlarged, tortuous (fig. 4). B-lymphocytes, plasmacytes and macrophages are densely located in the medullary lymph cords.

In female rats, a large proportion of adipose tissue has been found around the organ. In male rats, destructurization in the cortical substance is more frequently observed: the integrity of the mantle zone is lost, the elongated germinal center is expanded. There is a large number of vacuole-like structures in the capsule.

As a result of the analysis of modern literature, it has been found that the development of obesity causes pathological changes in the lymphatic system, such as an increase in the average size of the spleen in animals was found to be 38%, indicating splenomegaly. In the study of histological preparations of the spleen there was a significant expansion of venous sinuses (sinusoids), macrophages filled with drops of hemosiderin were revealed, on some sections, especially around small vessels, eosinophilic aggregations and accumulation of lipids in the extended sinusoid were revealed. Consequently, a high-fat diet resulted from splenomegaly, which the authors associate with sinusoidal dilatation and intracellular and intercellular deposits [7].

Expansion of lymph sinuses, expansion and full blooded of vessels is also noted in studies of other authors [5, 7].

With the help of light and electron microscopy of liver preparations of the experimental group of animals, the expression of enlarged sinusoidal capillaries, microvessicular steatosis, an increase in the proportion of connective tissue in the liver, hepatocytes with an excessively extended smooth endoplasmic net, altered mitochondria and signs of necrosis have been revealed [7].

Thus, adipose tissue is a complex endocrine organ whose action on organs and tissues is weighty and diverse, increasing the likelihood of multiple diseases. The study of the dynamics of changes in the structural organization of organs and tissues, as well as possible methods for their correction, remain relevant and important for both theoretical and practical medicine. According to the results of our study, we showed an increase in the relative area of the B-dependent zone and a decrease in the T-dependent. Similar changes can lead to redistribution of activity in the direction of humoral immune response. There are signs of constant immune activity.

Conclusion

As a result of the study performed on male and female rats, we found that histological changes in the structural components of the iliac and mesentery lymph nodes were already observed after a week: a slight expansion of the marginal and cortical lymph nodes. With the duration of the experiment increasing, all changes are worsened. In the cortical substance, the number of secondary lymphoid follicles grows, the marginal sinus expands, the capsule thickens, accumulation of adipose tissue around the organ is gradually increasing.

In female rats, a large proportion of adipose tissue is found around the organ. In male rats, destructurization of cortical substances is more frequently observed.

In the biochemical analysis of the experimental animals' blood, after four weeks of high-calorie diet, it was found that the level of glucose in white male rats exceeds the indices of intact animals by 16.7%, and in female rats it is lower by 13.7%; ALT reduces by 18.2% and 11.6% respectively; AST in the white male rats' blood grows by 8.0%; cholesterol level grows by 56.7% in white male rats and by 82.8% in white female rats; the level of triglycerides in the white male rats' blood grows by 11.6%.

References

1. Holovatskyi AS, Harapko TV. Strukturni zminy v orhanakh i tkanynakh pry diyi na orhanizm opioidnykh analhetykiv. Naukovyi visnyk Uzhhorodskoho universytetu. Seriya "Medytsyna". 2015; 2(52):130-4. [in Ukrainian]

2. Holovatskyi AS, Valko OO. Morfofunktsionalni zminy v limfatychnykh vuzlakh pry diyi na orhanizm khimichnykh i fizychnykh chynnykiv. Naukovyi visnyk Uzhhorodskoho universytetu. Seriya "Medytsyna". 2016; 1(53):131-6. [in Ukrainian]

3. Valko OO, Holovatskyi AS, Nebesna ZM, Volkov KS, Kramar SB. Strukturni zminy limfatychnykh vuzliv bilykh shchuriv pry dvotyzhnevomu ta chotyrytyzhnevomu opioidnomu vplyvi. Naukovyi visnyk Uzhhorodskoho universytetu. Seriya "Medytsyna". 2017; 2(56):10-7. [in Ukrainian]

4. Cherniak SI, redaktor. Osnovni pokaznyky invalidnosti ta diyalnosti medyko-sotsialnykh ekspertnykh komisiy Ukrainy za 2015 rik: analitychno-informatsiynyi dovidnyk. Dnipro: Roial-Prynt; 2016. 157 s. [in Ukrainian]

5. Yakubtsova IV, Khilko TD, Savytska IM, Konopelniuk VV, Preobrazhenska TD, Makai Sh. Vplyv Trigonella foenum graecum L. na stan imunokompetentnykh orhaniv za umov diyetindukovanoho ozhyrinnia u shchuriv. Scientific Journal "ScienceRise: Biological Science". 2016; 3(3):53-60. [in Ukrainian]
6. Alpert MA, Omran J, Bostick BP. Effects of Obesity on Cardiovascular Hemodynamics, Cardiac Morphology, and Ventricular Function. Curr. Obes. Rep. 2016;15:156-171.
7. Andersen CJ, Murphy KE, Fernandez ML. Impact of Obesity and Metabolic Syndrome on Immunity. Adv Nutr. 2016;7(1):66-75.
8. Desai M, Beall M, Ross MG. Developmental Origins of Obesity: Programmed Adipogenesis. Curr Diab Rep. 2013;13(1):27-33.
9. Sansbury BE, Hill BG. Regulation of obesity and insulin resistance by nitric oxide. Free Radical Biology and Medicine. 2014;73:383-399.
10. Shaikh SR, Haas KM, Beck MA, Teague H. The effects of diet-induced obesity on B cell function. Clinical & Experimental Immunology. 2015;179(1):90-5.
11. Wang HJ, Si QJ, Shan ZL, Guo YT, Lin K, Zhao XN, et al. Effects of body mass index on risks for ischemic stroke, thromboembolism, and mortality in Chinese atrial fibrillation patients: a single-center experience. PLoS One. 2015;10(4):231-242.

Реферати

ГІСТОЛОГІЧНІ ЗМІНИ СТРУКТУРНИХ КОМПОНЕНТІВ У ЛІМФАТИЧНИХ ВУЗЛАХ ЩУРІВ ТА ЗМІНИ БІОХІМІЧНИХ ПОКАЗНИКІВ КРОВІ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ОЖИРІННІ

Гарапко Т.В.

У даній статті наведені та проаналізовані результати експериментального дослідження, яке проводилося на білих щурах, самках і самцях репродуктивного віку (2,5-3,5 місяці). Метою дослідження було встановлення гістологічних змін структурних компонентів лімфатичних вузлів щурів і зміни біохімічних показників крові в різні терміни експериментального ожиріння. Проведено біохімічний аналіз крові білих щурів-самців і білих щурів-самок на рівень глюкози, АЛТ, АСТ, холестерину і тригліцеридів протягом всього експерименту. Через один тиждень експерименту спостерігається незначне розширення крайової і коркових лімфатичних пазух. При збільшенні тривалості експерименту всі патологічні зміни поглиблюються.

Ключові слова: ожиріння, експеримент, щур, лімфатичний вузол, лімфоцити.

Стаття надійшла 10.03.2019 р.

ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ СТРУКТУРНЫХ КОМПОНЕНТОВ В ЛИМФАТИЧЕСКИХ УЗЛАХ КРЫС И ЗМЕНЕНИЯ БИОХИМИЧЕСКИХ ПОКАЗАТЕЛЕЙ КРОВИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОЖИРЕНИИ

Гарапко Т.В.

В данной статье приведены и проанализированы результаты экспериментального исследования, которое проводилось на белых крысах самках и самцах репродуктивного возраста (2,5-3,5 месяца). Целью исследования является установление гистологических изменений структурных компонентов лимфатических узлов крыс и изменения биохимических показателей крови в различные сроки экспериментального ожирения. Проведен биохимический анализ крови белых крыс-самцов и белых крыс-самок на уровень глюкозы, АЛТ, АСТ, холестерина и триглицеридов в течение всего эксперимента. Через одну неделю эксперимента наблюдается незначительное расширение краевой и корковых лимфатических пазух. При увеличении продолжительности эксперимента все патологические изменения усугубляются.

Ключевые слова: ожирение, эксперимент, крыса, лимфатический узел, лимфоциты.

Рецензент Єрошенко Г.А.

DOI 10.26724/2079-8334-2020-1-71-173-176
UDC 616-071. 611.631+611.632/617.681

O.Ya. Hlodan, B.V. Hrytsuliak, V.B. Hrytsuliak, I.Y. Ivasiuk
Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk

THE NATURE OF ULTRASTRUCTURAL CHANGES INDUCED BY ORCHIEPIDIDYMITIS IN THE MEN'S TESTES AND EJACULATE

e-mail: glodan.oksana@ gmail.com

In 14 testicular biopsy specimens of men with male infertility, who had orchiepididymitis, aged 22-35 years we studied the nature of ultrastructural changes and their effect on ejaculate values. There were determined: deformity and pycnosis of nuclei, uneven expansion of perinuclear space, cytoplasm vacuolization, homogenization of mitochondrial cristae, expansion of endoplasmic reticulum cisterns and elements in the Golgi apparatus in endothelial cells of the hemocapillaries, peritubular myoid cells of the coiled seminiferous tubules lining, Sertoli cells, spermatocytes and spermatids, which were complemented in the ejaculate with reducing twice the sperm concentration in 1 ml, increasing 3 times the number of pathological forms and reducing the sperm motility.

Key words: testis, orchiepididymitis, germinal epithelium cells, ejaculate.

The work is a fragment of the research project "Topical aspects of andrology and correction of spermatogenesis", state registration No. 0119U103671.

Acute orchiepididymitis is one of the most common genital diseases of men of all ages, and the most common one among the complications of transurethral surgical and instrumental interventions [3, 7]. Half of orchiepididymitis cases involve sexually transmitted infections. Pathogens: Gonococci, Chlamydia, Streptococci, Escherichia coli, etc. penetrate the testis and epididymis through the vas deferens [2, 7]. According to the literature data [3, 6], the sperm pathology was found in all patients with orchiepididymitis,