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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.

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

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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,

which leads to infertility in 50-80% of cases. The study of ejaculate in infertile men is one of the main examinational methods, but the nature of ultrastructural changes in the testis in orchiepididymitis remains poorly studied.

The purpose of the study was to determine the nature of cytological and ultrastructural changes in the testis and ejaculate of infertile mature men (aged 22-35 years), who had orchiepididymitis.

Materials and methods. The study material included 14 testicular biopsy specimens from men with a history of orchiepididymitis sampled at the urology hospital. Laboratory analysis of ejaculate in this group of men was performed before surgical intervention. The Medical Ethics Committee of Ivano-Frankivsk National Medical University did not find any violations of moral and ethical standards during the study (Protocol No. 3 of 16.10.2018).

In the studied ejaculate was determined its volume, color, pH, sperm count in 1 ml, the content of living, motile and pathological forms. The calculations were performed in the Goryaev chamber at x400 magnification [6]. Statistical analysis of the obtained indicators was performed using the Stat. Soft. Inc., Tulsa, OK, USA; Statistica 6 software.

Electron microscopic study of testicular biopsy specimens was performed according to the conventional method. Ultrathin sections were studied in the PEM-125k electron microscope [4].

Results of the study and their discussion. According to electron microscopy in the testis of men with orchiepididymitis, the basement membrane of the coiled seminiferous tubules was unevenly thickened and twisted (fig. 1).

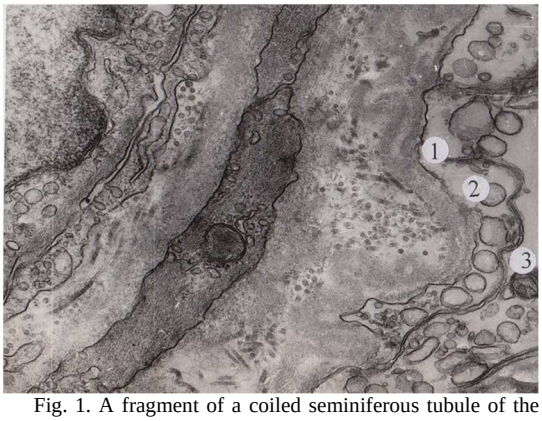

Fig. 1. A fragment of a coiled seminiferous tubule of the 30 years-old man's testis after having orchiepididymitis. x16000.
1 – twisting of basement membrane of the germinal epithelium;
2 – cytoplasm vacuolization in the peritubular myoid cell;
3 – homogenization of mitochondrial cristae.

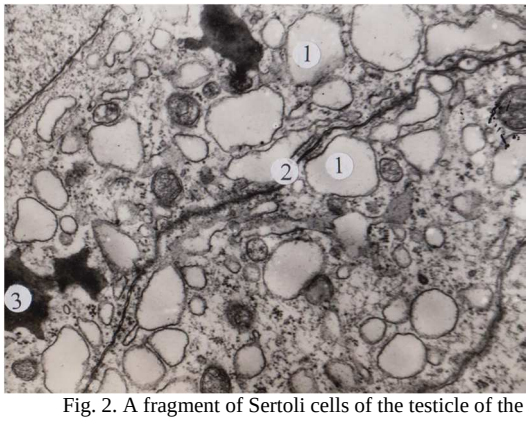

Fig. 2. A fragment of Sertoli cells of the testicle of the 30 years-old man's testis after having orchiepididymitis. x16000.
1 – significant cytoplasm vacuolization;
2 – convergence of the cell membranes of Sertoli cells;
3 – lipid droplets.

Peritubular myoid cell nuclei are hyperchromic with unevenly spaced chromatin. Micropinocytotic vesicles are predominant in the cytoplasm; myofilaments are not found (fig. 2). The cell membrane integrity of the Sertoli cells is impaired, the nucleus is impaired by deepened nuclear envelope invaginations, and the chromatin is rarefied. The cytoplasm of these cells is cleared out and vacuolated, with a large number of lysosomes and lipid inclusions. The mitochondria are small, their matrix is homogenized, the endoplasmic reticulum tubules and elements in the Golgi apparatus are unevenly expanded. In the connective apparatus, the cell membranes of Sertoli cells are converged, the filiform filaments are reduced, and the endoplasmic reticulum tubules are significantly expanded (fig. 2).

Karyorrhexis, uneven expansion of perinuclear space, cytoplasm vacuolization, homogenization of mitochondrial cristae were found in spermatids (fig. 3). The nuclei in the Leydig's cell are deformed, with peripheral chromatin condensation (fig. 4). The cell membrane contours are not clear, the cytoplasm is cleared-out, and the mitochondria have reduced cristae and a vacuolated matrix. The basement membrane of testicular blood capillaries is thickened, the cytoplasmic matrix is vacuolated, and macroclasmatosis is present. Nuclei are with peripheral chromatin condensation.

During the study of the ejaculate, in 20% of men who had orchiepididymitis, any sperm cells were not detected. In other men, the ejaculate volume was reduced to 2.57±0.24 ml, and the sperm concentration was decreased five times in 1 ml to 21.6±3.70 million/ml. It has a 67% and 73% reduction in the number of live and actively motile sperm. At the same time in the ejaculate the content of pathological forms of sperm was increased by 72%, in 90% of them the combined pathology (head, middle piece and flagellum) was detected. The content of fructose in the ejaculate decreased by 2 times. The number of white blood cells in the ejaculate increased significantly (up to 100 per field of view).

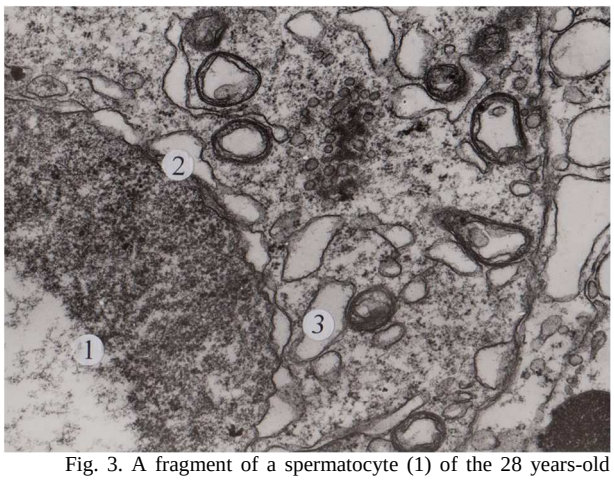

Fig. 3. A fragment of a spermatocyte (1) of the 28 years-old man's testis after having orchiepididymitis; 2 – uneven expansion of the perinuclear space; 3 – cytoplasm vacuolization. x16000.

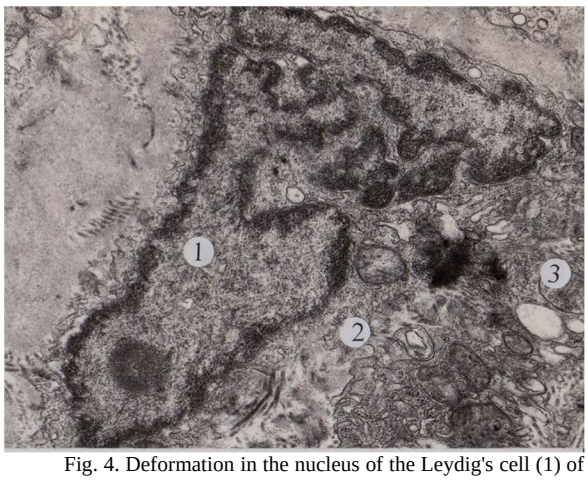

Fig. 4. Deformation in the nucleus of the Leydig's cell (1) of the 28 years-old man's testis after having orchiepididymitis; 2 – cytoplasmic reduction; 3 – homogenization of mitochondrial cristae. x16000.

In our previous studies of testicular blood vessels in men who had orchiepididymitis [3], the microvasculature blood vessel wall was deformed. Blood capillaries have an uneven lumen, their inner diameter was reduced to 4.0±0.3 μm, and focal reduction of capillaries was detected.

Diameter of the coiled tubules in the studied testicular biopsy specimens of men who had orchiepididymitis, was reduced to 156.15±5.0 μm. Counting the germ cells number revealed that only 5.6% of the seminiferous tubules retained their normal structure. Severe degree of damage to the germinal epithelium was found in 44.3%, and 31.2% of the tubules were emptied [3].

Along with atrophic changes in the testicular parenchyma, there was revealed pathology in the interstitium, which consists in the proliferation of connective tissue elements with local lymphocytic infiltration, hyalinosis and sclerosis of the fibrous connective tissue. The volume of the Leydig's cell nuclei decreased to 68.49±3.6 μm^3 versus 97.56±1.60 μm^3 in the control with a parallel decrease in testosterone concentration in the blood. Thus, the ultrastructural changes revealed in the hemocapillaries wall, the coiled seminiferous tubules lining and Sertoli cells with their connective apparatus, which are the components of the blood–testis barrier [1, 2], have led to disorders of spermatogenesis in the testis of men who had orchiepididymitis.

According to other authors, in acute orchiepididymitis was recorded thermal asymmetry, the difference in temperature between the affected and contralateral testis was $2\,^0C$, which also adversely affected spermatogenesis and, in particular, spermogram [5, 7]. In our study, there was a sharp decrease in the concentration of spermatozoa, an increase in the number of pathological forms and a decrease in their motility.

Conclusions

1. Past orchiepididymitis leads to profound ultrastructural changes in the endothelial cells of the testicular hemocapillaries, peritubular myoid cells of the coiled seminiferous tubules lining, Sertoli cells and Leydig's cells with damage to their cytoplasmic organelles.

2. In the ejaculate of men with this testicular pathology decreases the spermatozoa concentration, decreases the number of living and actively mobile spermatozoa, increases the number of dead and pathological spermatozoa forms.

Prospects for further research are to find the means of stimulating spermatogenesis in infertile men who had orchiepididymitis.

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ХАРАКТЕР УЛЬТРАСТРУКТУРНИХ ЗМІН, ЗУМОВЛЕНИХ ОРХОЕПІДИДИМІТОМ, В ЯЄЧКУ І ЕЯКУЛЯТІ ЧОЛОВІКІВ
Глодан О.Я., Грицуляк Б.В., Грицуляк В.Б., Івасюк І.Й.

В 14 біоптатах яєчка неплідних чоловіків віком 22-35 років, що перенесли орхоепідидиміт, досліджено характер ультраструктурних змін та їх вплив на показники еякуляту. Встановлено деформацію і пікноз ядер, нерівномірне розширення перинуклеарного простору, вакуолізацію цитоплазми, гомогенізацію крист мітохондрій, розширення цистерн ендоплазматичної сітки та елементів комплексу Гольджі в ендотеліоцитах гемокапілярів, міоїдних клітинах власної оболонки звивистих сім'яних трубочок, підтримувальних епітеліоцитах, сперматоцитах і сперматидах, які супроводжувались в еякуляті зменшенням вдвічі концентрації сперматозоїдів в 1 мл, збільшенням у 3 рази кількості патологічних форм та зниженням їх рухливості.

Ключові слова: яєчко, орхоепідидиміт, клітини сперматогенного епітелію, еякулят.

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ХАРАКТЕР УЛЬТРАСТРУКТУРНЫХ ИЗМЕНЕНИЙ В ЯИЧКЕ И ЭЯКУЛЯТЕ МУЖЧИН, ОБУСЛОВЛЕННЫХ ОРХОЭПИДИДИМИТОМ
Глодан О.Ya., Hrytsuliak Б.В., Грицуляк V.В., Ивасюк И.И.

В 14 биоптатах яичка бесплодных мужчин в возрасте 22-35 лет, перенесших орхоэпидидимит, исследован характер ультраструктурных изменений и их влияние на показатели эякулята. Установлены деформация и пикноз ядер, неравномерное расширение перинуклеарного пространства, вакуолизация цитоплазмы, гомогенизация крист митохондрий, расширение цистерн эндоплазматической сети и элементов комплекса Гольджи в эндотелиоцитах гемокапилляров, миоидных клетках собственной оболочки извитых семенных трубочек, поддерживающих эпителиоцитах, сперматоцитах и сперматидах, которые сопровождались в эякуляте уменьшением концентрации сперматозоидов вдвое в 1 мл, увеличением в 3 раза количества патологических форм и снижением их подвижности.

Ключевые слова: яичко, орхоэпидидимит, клетки сперматогенного эпителия, эякулят.

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EXPERIMENTAL MORPHOLOGICAL STUDY OF DENTAL PULP LESIONS AT DIFFERENT STAGES OF DENTAL CARIES

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Numerous studies show that in the structure of the affection of deciduous teeth by dental caries, a high percentage of complicated dental caries is observed. Paper was aimed at the study of morphological lesion of the dental pulp in experimental dental caries of various severity in rats with theoretical rationale of the principles of treatment of pulpitis. 50 outbred albino male rates were involved into study. Experimental dental caries in rats was induced by special cariesogenic diet. At the first stage, the depth of carious lesions of the enamel and dentin was measured. The second stage was performed on decalcified blocks of teeth, serial sections were made from the resulting blocks, stained with hematoxylin and eosin. It revealed the potential occurrence of more frequent complications in the conservative treatment of deep carious lesions of approximal surfaces in comparison with deep lesions of chewing surfaces. Dental pulp carious lesion depends not only on the degree of carious process, but also on its localization, which should be taken into account when choosing the optimal method of treatment of dental caries, which also prevents the development of its complications.

Key words: experimental caries, tooth pulp, morphology**.**

The work is a fragment of the research project "Morphogenesis patterns of organs, tissues and vascular-nerve formations in normal, in pathology and under the influence of external factors", state registration No. 0118U004457.

Notwithstanding the rapid development of dental science, the emergence of the advanced technologies and capabilities, the high prevalence of dental caries, especially in childhood, has prompted the WHO to include it into the six global contemporary diseases. Numerous studies show that in the structure of the affection of deciduous teeth by dental caries, a high percentage of complicated dental caries is observed [2, 3].

The main feature of the dental caries occurrence in deciduous teeth is rapid development of the pathological process. This is due to a thin enamel of deciduous teeth, a smaller volume of dentin, the presence of low-mineralized zones in it, which reach the pulp by broad bands in the form of interglobular dentin.

The dentin pulp complex responds to the microorganism's invasion into deep layers of the dentin by the formation of tertiary dentine, which is a protective barrier, since it reduces the diffusion of the latter in the direction of the pulp [7, 10]. However, the feature of the pulp of deciduous teeth is in its insignificant functional ability to produce dentin substitute, due to the minimum expressed protective and adaptive properties. At the same time, according to [9] the deciduous teeth pulp is quite often in a state of chronic