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MORPHO-FUNCTIONAL CHARACTERISTICS OF THE TESTICLES OF MEN WITH DIFFERENT FORMS OF PATHOSPERMIA

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Abstract. The most difficult form of male infertility to treat is azoospermia. It is identified as a complete absence of sperm in the ejaculate. Depending on the nature and causes of impaired spermatogenesis, azoospermia is divided into obstructive (excretory, OA) and non-obstructive (secretory, NOA). Testicular biopsy is the most effective method of diagnosis and a component of possible treatment for azoospermia. It is the only objective method of differential diagnosis between non-obstructive and obstructive forms of azoospermia.

The aim: histological analysis of testicular biopsies of men with various forms of azoospermia.

Materials and Methods. 78 infertile men with azoospermia were examined. They were divided into two groups: the first group, men with NOA (n = 28); the second group, men with OA (n = 50). The biopsy was preceded by a mandatory ultrasound diagnosis of the portal system. The biopsy was performed using the method of open operative access. Biopsies were mostly taken from the more palpable testicle or from both testicles. Biopsies were fixed in buffered 10% formalin (pH 7.2). After 1 day, they were dehydrated in 70% ethanol and embedded in paraffin. For histological studies, sections with a thickness of 5 µm were stained with hematoxylin and eosin. Biopsies were evaluated in accordance with previously described methods.

Results. Histological analysis of testicular biopsies from 28.7% of patients with a non-obstructive form of azoospermia showed swelling of the testicular stroma, destructive changes in testosterone-producing cells, disruption of the structure of the syncytial complexes of the spermatogenic epithelium, and the complete absence of the process of spermatogenesis in individual tortuous seminiferous tubules, the absence of contacts between sustentocytes, and in erythrocyte sludge in the lumen of vessels. 42.8% of patients had fibrosis of the testicular stroma, stroma swelling, thinning of the wall of convoluted seminiferous tubules, violation of the structure of the syncytial complexes of the spermatogenic epithelium, proliferation of the wall of the convoluted seminiferous tubules into their lumen, and infiltration of the testicular stroma with lymphocytes. In 54.0% of patients with preserved spermatogenesis and an obstructive form of azoospermia, it was possible to find a history of orchepididymitis in the anamnesis; one patient (2.0%) underwent bilateral orchopexy at the age of 5 years due to cryptorchidism; 6.0% recalled the trauma calculi in the anamnesis; and 38.0% denied any factors affecting fecundity in the anamnesis.

Conclusions. The non-obstructive form of azoospermia is characterized by the following parameters: mostly a violation of the structure of the spermatogenic epithelium, a complete absence of the process of spermatogenesis in individual convoluted seminiferous tubules, a violation of the structure of the hematotesticular barrier, and a violation of blood microcirculation. The histological picture of preserved spermatogenesis is of the same type in 88.0% of patients with an obstructive form of azoospermia. In most tubules, a fixed number of cell rows is preserved, and cells of various stages of spermatogenesis are determined in them: spermatogonies, spermatocytes, a moderate number of spermatids.

In the lumen of the tubules, exfoliated cells and a moderate number of spermatozoa are found.

Keywords: male infertility, azoospermia, testicles, biopsy specimens.

Introduction. According to research by the WHO [3, 28] and the European Association of Urologists [21], the incidence of infertility is increasing worldwide. The male factor causes up to 50% of all cases of infertility in married couples and approximately 7% of men worldwide suffer from infertility [8, 11, 21].

Infertility is defined as the inability to conceive after one year of regular unprotected sex with the same partner [11, 21].

The most difficult form of male infertility to treat is azoospermia [5, 10, 11, 13, 17, 21, 24]. Azoospermia is defined as the complete absence of spermatozoa in the ejaculate. Depending on the nature and causes of impaired spermatogenesis, azoospermia is divided into obstructive (excretory, OA) and non-obstructive (secretory, NOA) [11, 21, 25]. In male infertility, azoospermia is detected in 10-15% of cases, while the share of obstructive and non-

obstructive forms is approximately 40 and 60%, respectively [11, 21, 26].

Background of the research. The problem is the differential diagnosis of OA and NOA. Obstructive azoospermia is the absence of spermatozoa and germinogenic cells in the ejaculate due to bilateral obstruction of the vas deferens [11, 16, 19-21, 26]. OA is less common than non-obstructive azoospermia. Among the causes of excretory aspermia are epididymitis of a specific and non-specific nature. Often, the cause of excretory aspermia is an abnormality in the development of various parts of the vas deferens. Excretory-obstructive infertility in men is also caused by damage to the accessory gonads. The most common cause of this is a chronic inflammatory process with impaired secretory function.

Secretory infertility is caused by hypogonadism, which means a decrease or absence of the hormone-producing function of interstitial endocrinocytes or the spermatogenic function of the germinogenic epithelium with reduced secretory and excretory functions of the testicles [5, 10, 11, 14, 22].

Testicular biopsy is the most effective method of diagnosis and a component of possible treatment of azoospermia [1, 2, 4, 7, 9, 27, 29]. It is the only objective method of differential diagnosis between non-obstructive and obstructive forms of azoospermia. This method can be used for both diagnostic and therapeutic purposes in case of obtaining spermatozoa in sufficient quantity for ICSI (intracytoplasmic sperm injection) [1, 2, 12, 23]. To evaluate the testicular tissue, a testicular biopsy is performed, the stage of spermatogenesis is determined, signs of obstruction of the vas deferens are detected, and the possibility of obtaining material for the ICSI program is evaluated [4, 23, 29].

The aim: histological analysis of testicular biopsies of men with various forms of azoospermia.

Materials and Methods. The material for carrying out the assigned tasks was the results of the examination of patients with infertility who were under observation at the Department of Urology of the Danylo Halytsky Lviv National Medical University, the regional consultative polyclinic of the Lviv Regional Clinical Hospital, and the «Salyutas» Medical Center. 33 men aged 22 to 45 years, average age 32.7 ± 4.3 years (group I), were diagnosed with «idiopathic infertility».

Patients with azoospermia were divided into two groups based on the analysis of spermograms, physical examination methods, and ultrasound diagnostics: those with secretory infertility characterized by a non-obstructive form of azoospermia (group 1, $n = 28$) and excretory-obstructive infertility characterized by an obstructive form of azoospermia (group 1, $n = 28$) group 2, $n = 50$). Inclusion and exclusion criteria were taken into account when selecting the research groups.

These patients did not undergo any treatment course, had regular sexual contact with their partners, but they could not get pregnant within 12 months. There were no varicocele, hypogonadism, or leukocytospermia. The

anamnesis excluded smoking, alcohol, and long-term illnesses. A detailed anamnesis was made for both the husband and the wife.

The diagnosis of infertility was carried out at the outpatient stage according to the standards of the European Association of Urologists [21] and WHO [3, 28].

Biopsy is performed for both diagnostic and therapeutic purposes in the case of obtaining spermatozoa for assisted reproductive technologies. The biopsy was preceded by a mandatory ultrasound diagnosis of the portal system [15, 18]. The biopsy was performed using the method of open operative access. Biopsies were mostly taken from the more palpable testicle, or from both testicles [1]. Biopsies were fixed in buffered 10% formalin (pH 7.2). After 1 day, they were dehydrated in 70% ethanol and embedded in paraffin. For histological studies, sections with a thickness of 5 μm were stained with hematoxylin and eosin. Biopsies were evaluated in accordance with previously described methods [6].

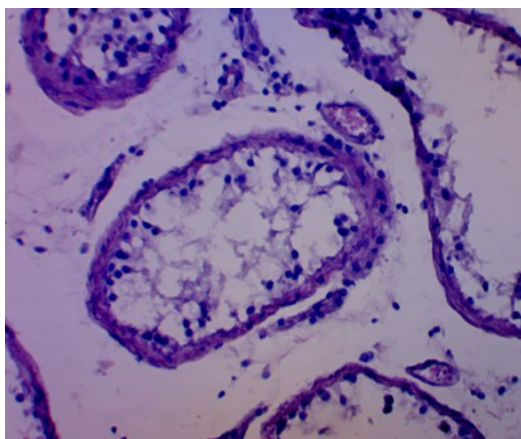
Results are presented as the arithmetic mean (M) \pm standard error of the mean (m). The number of experiments (n) corresponds to the number of persons examined in each case (blood lymphocytes from one patient or a practically healthy donor were used each time). The equation of a straight line, which best approximates the experimental data, was calculated using the method of least squares. The absolute value of the correlation coefficient r was between 0.90 - 0.98. The reliability of the calculated parameters of the straight line was checked by Fisher's F -criterion: an approximation with $p \leq 0.05$ was considered reliable.

The Bioethical Expertise Committee of the Danylo Halytsky Lviv National Medical University did not find any violations of moral and ethical norms during the dissertation work.

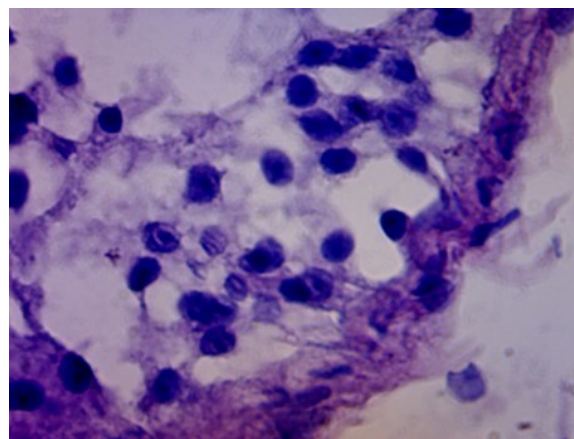
Results. In the first group of studies, all patients with NOA ($n = 28$) had a biopsy performed on one side, with testicles of different sizes and consistency, but from a palpably fuller testicle.

Histological analysis of testicular biopsies from 8 patients (28.7%) with a non-obstructive form of azoospermia showed (Fig. 1): swelling of the testicular stroma, destructive changes in testosterone-producing cells, disruption of the structure of the syncytial complexes of the spermatogenic epithelium, and the complete absence of the process of spermatogenesis in some tortuous families of tubules, absence of contacts between sustentocytes (violation of the structure of the hemato-testicular barrier), and erythrocyte sludge in the lumen of vessels (violation of blood microcirculation).

In the other 12 patients (42.8%) (Fig. 2), fibrosis of the testicular stroma, edema of the stroma, thinning of the wall of the convoluted seminiferous tubules, disruption of the structure of the syncytial complexes of the spermatogenic epithelium, proliferation of the wall of the convoluted seminiferous tubules into their lumen, infiltration were observed testicular stroma lymphocytes.

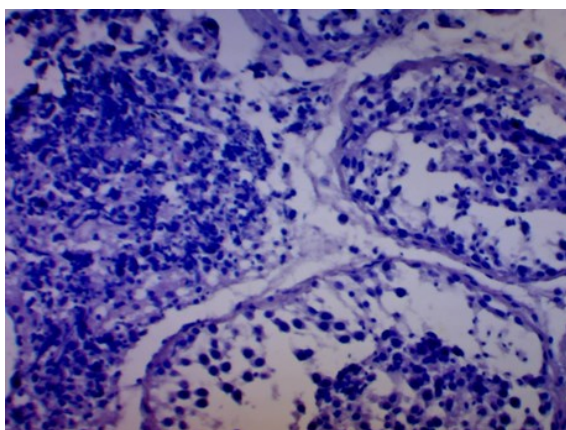


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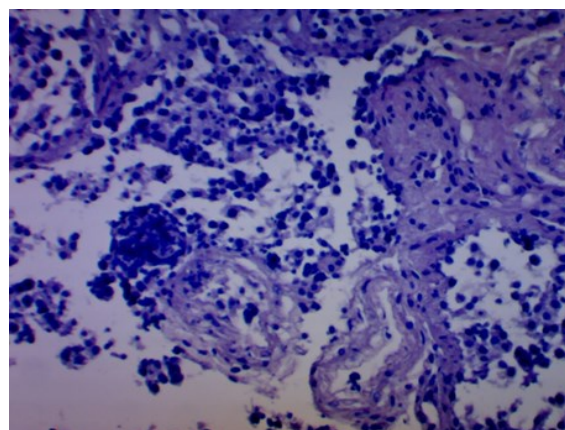


B

Fig. 1. Morphology of the testicle. Staining with hematoxylin and eosin. A - coll. x 300, B - coll. x 600.



A



B

Fig. 2. Morphology of the patient's testicle. Staining with hematoxylin and eosin. A and B - coll. x 300.

Also, 5 patients (17.8%) had destructive changes in the convoluted seminiferous tubules, thickening of the wall of the convoluted seminiferous tubules, proliferation of the wall into the lumen of the tubule, infiltration by lymphocytes, and absence of spermatogenic cells in the lumen of the convoluted tubules. 3 patients (10.7%) had fibrosis of the testicular stroma and lymphocyte infiltration, proliferation of the testicular wall into the lumen of the convoluted seminiferous tubules, and absence of spermatogenic

epithelium in the lumen of the convoluted seminiferous tubules.

50 patients with preserved spermatogenesis were diagnosed with "excretory-obstructive infertility" (obstructive azoospermia). Among the examined, 27 (54.0%) revealed a history of orchepididymitis, one (2.0%) patient underwent bilateral orchopexy at the age of 5 due to cryptorchidism, three (6.0%) recalled the trauma calculus in the anamnesis, the remaining 19 (38.0%) denied any factors affecting fecundity in the anamnesis (Table 1).

Table 1

Indicators of the condition of patients with excretory-obstructive infertility (n = 50)

The diagnosis	Number/percentage
Preserved spermatogenesis	50 (100 %)
Orchoepididymitis	27 (54,0 %)
Orhopexy due to cryptorchidism	1 (2,0 %)
Injury of the wicket	3 (6,0 %)
Unknown anamnesis	19 (38,0 %)

Testicular hypoplasia (less than 4 cm in the largest dimension) was observed in 6 (12.0%) patients (Table 2). Also, only five (10.0%) patients had palpable rosary areas of compaction at the level of the distal parts of the vas deferens and the excretory duct of the epididymis. In 2 (4.0%) patients, according to ultrasound, strongly calcified

vas deferens were visualized. In 6 (8.0%) patients, the distal divisions of the vas deferens were not palpated, in the other 33 (66.0%) patients, palpation of the organs of the portal vein revealed no pathology, even after 16 of them had orchepididymitis.

Table 2

State of the testicles according to ultrasound diagnosis and palpation (n = 50)

State	Number/percentage
Testicular hypoplasia	6 (12,0 %)
Sealing of the vas deferens	5 (10,0 %)
Calcified ducts	2 (4,0 %)
The distal sections of the vas deferens are not palpable	4 (8,0 %)
Palpation of the portal vein revealed no pathology	33 (66,0 %)

In 49 (98.0%) patients in the group with OA, azoospermia was detected, in one (2.0%) - persistent leukocytozoospermia was detected.

According to the results of the histological analysis of testicular biopsies, it was found that in 44 (88.0%) patients of the group with an obstructive form of azoospermia, the histological pattern of preserved spermatogenesis is of the same type (Table 3).

In most of the tubules, the number of cell rows is preserved, and cells of different stages of spermatogenesis

are determined in them: spermatogonies, spermatocytes, and, in particular, with single division figures, a moderate number of spermatids. In the lumen of the tubules, exfoliated cells and a moderate number of spermatozoa are found. In some tubules, the number of cell rows was reduced, although cells of different stages of spermatogenesis were also identified in them, as were spermatozoa in the lumen. Sertoli cells are preserved. Small clusters of Leydig cells are found in the stroma. Interstitial edema was found in three of these 50 patients.

Table 3

Histological analysis of testicular biopsies in excretory-obstructive infertility (obstructive form of azoospermia) (n = 50)

Histological analysis	Number/percentage
Preserved spermatogenesis (cells of different stages of spermatogenesis: spermatogonia, spermatocytes, spermatids, spermatozoa in the lumen of tubules). Sertoli cells are preserved. In the stroma, there are small clusters of Leydig cells.	44 (88,0 %)
Spermatogenesis and sclerosis are preserved.	6 (12,0 %)

Six (12.0%) patients had preserved spermatogenesis and focal tubular sclerosis, probably due to obstruction of the vas deferens. In more than half of the tubules, the number of cell rows is preserved; they contain cells of different stages of spermatogenesis: spermatogonies, spermatocytes, and a moderate number of spermatozoa. Exfoliated cells are found in the lumen of some tubules. In some tubules, the number of cell rows is reduced, and Sertoli cells are preserved; in others, only one cell row is found. The walls of such tubules are thickened and sclerosed. Fibrosis persists around many tubules. Focal lympho-macrophagic inflammatory infiltration, focal sclerosis, and single groups of Leydig cells are determined in the stroma.

Discussion. Patients with an obstructive form of azoospermia are suggested to contact the clinic of reproductive medicine to perform appropriate procedures for obtaining spermatozoa and subsequent in vitro fertilization of partners. Patients with acquired epididymal obstruction can be offered unilateral or bilateral microsurgical end-to-end or end-to-side vasoepididymoanastomoses. Before microsurgery, it is necessary to cryopreserve sperm from the epididymis for further ICSI needs in the event of unsuccessful surgery.

Anatomical recanalization usually occurs at 3–18 months in 60–87% of men, and its success rate for subsequent pregnancies ranges from 10–43% [11, 16].

During the histological examination of testicular biopsies of patients with OA, various caliber and deformed convoluted tubules were found. A significant part of them contained spermatogenic cells at different levels of maturation, including spermatogonia, spermatocytes, and spermatozoa. This histological picture is considered to reflect weakened spermatogenesis [10].

In testicular biopsies of patients with NOA, groups of convoluted tubules with a thin basement

membrane, single Sertoli cells, and spermatogonia were found. Spermatogenic cells at various stages of development were determined in the lumen of tubules, but not beyond spermatids. Therefore, the ultrastructural changes of the testicular parenchyma play a leading role in understanding the prospects for performing in vitro fertilization with one's own spermatozoa.

Conclusions. Obtaining testicular tissue samples is much more difficult than obtaining ejaculate or blood samples for research. Therefore, there is a need to search for biomarkers of spermatogenesis in seminal plasma and venous blood.

Previously, FSH was considered such a marker; currently, additional markers are needed to determine male infertility. Thus, in connection with the polyetiological nature of forms of azoospermia, there is a need to search for universal markers (biochemical, cytogenetic). The change in level of which would allow determining the management tactics of patients with impaired fertility and the perspective of their treatment.

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МОРФО-ФУНКЦІОНАЛЬНА ХАРАКТЕРИСТИКА СІМ'ЯНИКІВ ПРИ РІЗНИХ ФОРМАХ ПАТОСПЕРМІЇ ЧОЛОВІКІВМ.З. Воробець¹, О.В. Мельник², Р.В. Фафула³,
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Резюме. Найскладнішою для лікування формою чоловічого непліддя є азооспермія. Залежно від характеру та причин порушення сперматогенезу азооспермію поділяють на обструктивну (екскреторну, ОА) та необструктивну (секреторну, НОА).

Мета. Гістологічний аналіз біоптатів яєчок чоловіків із різними формами азооспермії.

Матеріали і методи. Обстежено 78 неплідних чоловіків із азооспермією. Вони були розділені на дві

групи: перша група – чоловіки з НОА (n = 28); друга група – чоловіки з ОА (n = 50). Біопсію виконували методом відкритого оперативного доступу.

Результати. Гістологічний аналіз біоптатів яєчок 28,7 % пацієнтів із НОА показав набряк строми яєчка, деструктивні зміни тестостерон-продукуючих клітин, порушення структури синцитіальних комплексів сперматогенного епітелію та повну відсутність процесу сперматогенезу, відсутність контактів між суспендоцитами, у просвітах судин еритроцитарні складжі. У 42,8 % пацієнтів спостерігались фіброз строми яєчка, набряк строми, витончення стінки сім'яних каналців, порушення структури синцитіальних комплексів сперматогенного епітелію, проліферація стінки звивистих сім'яних каналців у їх просвіт. У 54,0 % пацієнтів зі збереженим сперматогенезом при ОА вдалось виявити перенесений орхоепідидиміт в анамнезі. 38,0 % пацієнтів будь-які вражаючи фетильність фактори в анамнезі заперечували.

Висновки:

1. НОА характеризується здебільшого порушенням структури сперматогенного епітелію, повною відсутністю процесу сперматогенезу, порушенням мікроциркуляції крові.

2. У 88,0 % пацієнтів із ОА гістологічна картина збереженого сперматогенезу однотипна. У більшості каналців кількість клітинних рядів збережена, у них визначаються клітини різних стадій сперматогенезу: сперматогонії, сперматоцити, помірна кількість сперматид.

Ключові слова: чоловіче непліддя, азооспермія, сям'яники, біоптати.

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