

УДК 616.697+616-018:616-091.8:616-076

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## MORPHOLOGY OF SPERMATOGENESIS IN OBSTRUCTIVE AZOOSPERMIA

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### МОРФОЛОГИЯ СПЕРМАТОГЕНЕЗА ПРИ ОБСТРУКТИВНОЙ АЗОСПЕРМИИ

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#### РЕЗЮМЕ

Для оценки состояния сперматогенеза исследовали 21 биопт яичка, взятых у мужчин 26-40 лет с диагнозом обструктивная азооспермия. Результаты исследования биоптатов яичек при обтурационной азооспермии позволили выделить 3 гистологические группы, основным критерием которых является разной степени выраженности фиброз и гиалиноз базальных мембран семенеформных канальцев и стромы. В зависимости от выраженности фибробластических реакций определены характерные особенности сперматогенеза в каждой из групп

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#### РЕЗЮМЕ

Для оцінки стану сперматогенезу досліджували 21 біопт яєчка у чоловіків 26-40 років з діагнозом обструктивна азооспермія. Результати дослідження дозволили виділити 3 гістологічні групи, основним критерієм якої є різного ступеню вираженості фіброз і гіаліноз базальних мембран семенеформних канальців і стромі. Залежно від вираженості фібробластичних реакцій визначені характерні особливості сперматогенезу в кожній з груп.

**Key words: morphology, spermatogenesis, azoospermia, diagnostics.**

The problem of male infertility, which develops as a result of a number of diseases or summary of pathological effects on the reproductive system of men, is very relevant in the present all over the world. Its pathogenesis, structure, diagnosis continues to be the subject of many discussions [2,4,8,11].

The variety of factors that lead to male infertility, the possibility of its combining, lead to difficulty of choosing of correct methods of diagnosis and treatment [1,6,7,10,14].

One of the causes of male infertility is obstructive azoospermia, where spermatozoa do not fall in sperm from the testis and epididymis due to obstruction of the seminal tract. [1,4,6,8,13]. Obstructive azoospermia usually develops on the background of chronic inflammatory diseases causing obliteration of the lumen of the seminiferous ways, in rare cases - as a result of its congenital anomalies. In this case, missing the entire vas deferens, or part of it, or the communication breakdown between the tubules and testicular duct epididymis [11]. Only morphological investigation of testicular biopsy can help diagnose these pathological processes, will determine pathology and the level of damage of spermatogenesis, type of azoospermia, with a view to subsequent use of intracytoplasmic sperm injection (ICSI), will successfully achieve fertility of men with the most severe forms of infertility [1,3,5,7,9,10,12,13,14,15].

Aim of study: to determine the morphological

criteria for the diagnosis of obstructive azoospermia in testicular biopsies.

#### MATERIALS AND METHODS

The material for the morphological study - 21 testicular biopsies taken from men 26-40 years old with a diagnosis of obstructive azoospermia.

Fragments of testicular tissue in the size 3x3mm fixed by Bouin's fluid. Formalin should not be used routinely [13]. Fixation time 3-24 h. Embedded in paraffin and sectioned to a thickness of 4 micrometers followed by observation staining with hematoxylin and eosin [HE], histochemical staining for visualisation of a connective tissue with application of picric acid and sour fuchsin (Van Gieson's staining) by a standard technique [13]. As a result of histochemical reaction collagen fibers were stained red colour. Photographing was carried out with a digital camera OLYMPUS C 5050Z, mounted on a microscope OLYMPUS CX 41. Calculation of cells we carried out with the DP SOFT program and statistical methods of research.

#### RESULTS AND DISCUSSION

Histological scoring system by S.G. Johnsen S.G. modified by De Kretser and Holstein [12] was used for morphological assessment of testicular tissue, according to European urological society (tabl. 1).

First of all, we paid attention to the number of seminiferous tubules, which must be around 50,5±0,002 in biopstate, there shapes, the ratio of germ cells and

Table 1

**Histopathological criteria of testicular biopsies in obstructive azoospermia, depending on the modified Johnsen's scoring system**

Histological classification	Number (n)	%	Johensen's
Fibrosis of the tubules with relatively preserved spermatogenesis (n = 11)	5	23,8	9
	4	19,0	8
	2	9,6	7
Fibrosis of the tubules with impaired spermatogenesis (n = 6)	2	9,6	6 и 5
	4	19,0	4
Obstructive tubular fibrosis (n = 4)	4	19,0	3 и 2
Total	21	100,0	

Sertoli cells, basement membranes and stroma condition, presents of Leydig's cells, fibrosis and hyalinosis.

Histological examination of all biopsy specimens show signs of obstructive azoospermia, characterized by fibrosis of the stroma with marked proliferation of fibroblasts, tubular basement membranes hyalinosis and fibrosis. However, despite the prevalence of the fibroblastic reactions, condition of spermatogenesis varied.

The distribution of histologic diagnoses based on histological criteria count according to modified Johnsen's scoring system shown in Table [12].

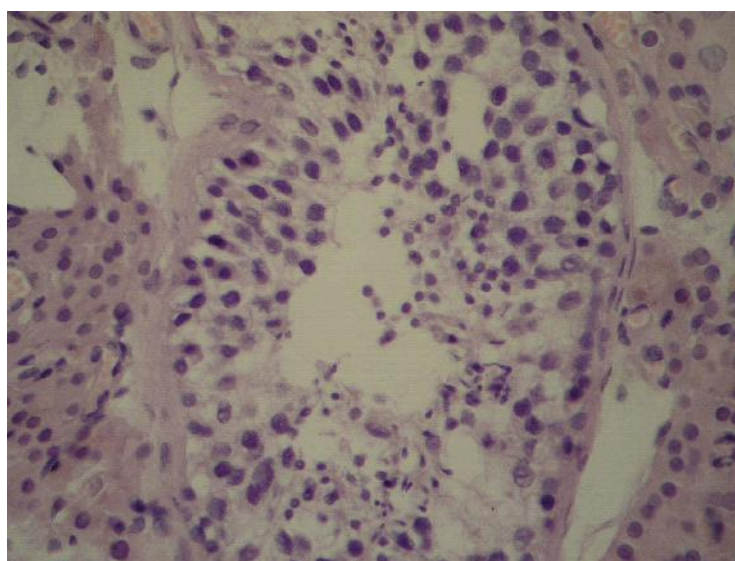
In 11 cases (52.4%) in biopsy of testicular tissue condition of spermatogenesis was assessed as adequate.

In the tubules was determined chaotic arrangement of spermatogenic cells. On the inner side of the basement membrane located spermatogonia, primary and secondary spermatocytes, single Sertoli cells. In some tubules spermatogenic epithelium partially desquamated

and located chaotically.

From these, five testicular biopsies (23.8%) showed more than 20 mature spermatids and from 5 to 10 mature spermatozoa (index of Johnsen's scale - 9 points). At 4 biopsies (19.0%) determined less number of mature spermatids (less than 20) and spermatozoa (less than 5) in the tubule, which corresponds to 8 points according to the modified Johnsen's scoring system. In 2 biopsies (9.6%) immature spermatids amount less than 20 were detected, and generally no spermatozoa (7 points).

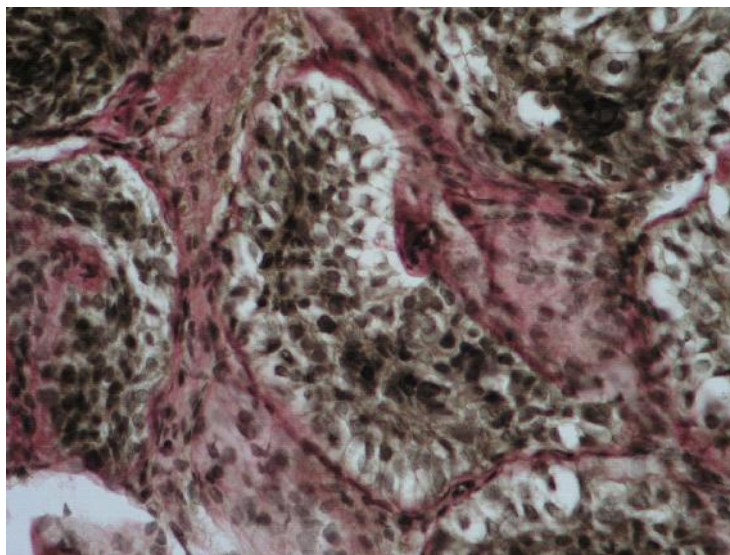
In addition, there is a focal hyperplasia of Leydig's cells localized in intertubular interstitium. Basement membranes were irregularly thickened. Under histochemical reaction with picrofuchsin connective tissue has intense red colored. There is also a moderate fibrosis of the stroma and slight hyperplasia of Leydig's cells (Fig. 1).



**Figure 1. Fibrosis of the tubules with relatively preserved spermatogenesis (9 points according to of modified Johnsen's scoring system). Leydig cell hyperplasia. HE staining (x 40).**

In 6 cases (28.6%) were found signs of impaired spermatogenesis, manifested as peripheral position of spermatogonia along the basement membrane, the presence of single Sertoli cells, chaotic arrangement of spermatocytes. Mature spermatids and spermatozoa were absent. In some tubules remained isolated immature spermatids, which corresponds to 6 points

of Johnsen's scale. In most tubules were determined block of spermatogenesis at the level of spermatocytes. In this case, a 2-biopsy specimens (9.6%) was visualized a large number of spermatocytes, filling the entire lumen of the tubules, with the presence of meiotic and mitotic figures (5 points of modified Johnsen's scoring system) (Fig. 2).



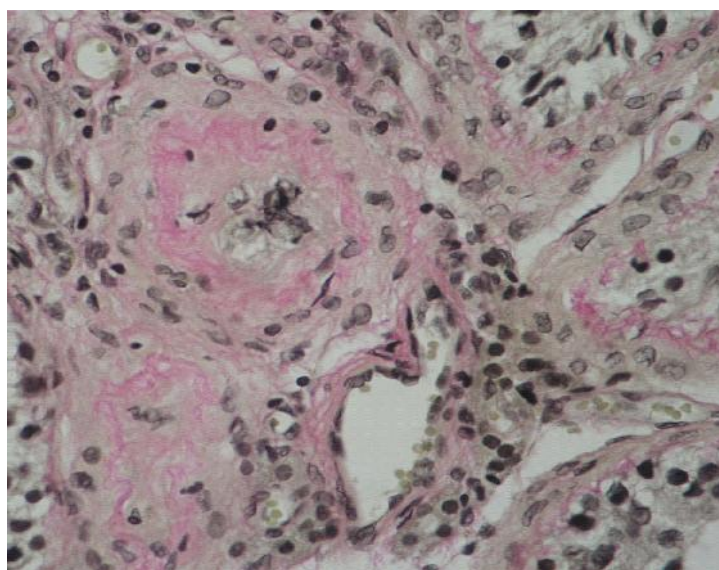
**Fig.2. Fibrosis of the tubules with impaired spermatogenesis. Arrest of maturation of spermatogenesis at the level of spermatocytes (5 points according to of modified Johnsen's scoring system). Picric acid and fuchsin staining (x40).**

In 4 cases (19.0%) number of spermatocytes decreased sharply, rarely localized in the tubules (4 points of of modified Johnsen's scoring system).

Note the sharp uniform thickening of the basal membrane due to fibrosis and hyalinosis, greater amount of fibrous stroma, which is often accompanied by deformation of the tubules. Histochemical reaction with

picrofuchsin has an intense red color. It is noted more pronounced Leydig cell hyperplasia.

In 4 cases (19.0%) showed a so-called «tubular obstructive fibrosis,» manifested as proliferation of fibrous tissue within the stroma and in the basement membranes. Fibrosis and hyalinosis of basement membranes are so pronounced that there has been a sharp



**Figure 3. Obstructive fibrosis of the tubules (1 point according to of modified Johnsen's scoring system). Leydig's cell hyperplasia. Picric acid and fuchsin staining (x20).**

narrowing of the canals up to obliteration, tubular atrophy and deformation. In the stroma, along with an increase in lymphocytic and histiocytic infiltration, reduces the number of Leydig's cells. In the lumen of the tubules were found only spermatogonia (3 points), only Sertoli cells (2 points) or single degenerative Sertoli cells (1 point) (Fig. 3).

#### CONCLUSION

Thus, the results of testicular biopsies in obstructive azoospermia given possibility to distinguish 3 histological groups. The main hallmark of these groups is the varying degree of fibrosis and hyalinosis of the basement membrane and stroma of tubules.

Depending on the severity of the reactions defined fibroblastic characteristics of spermatogenesis in each group:

1. Histological picture of testicular biopsy with index of 9, 8 and 7 points according to the modified Johansen's scoring system defined as «fibrosis of the tubules with relatively preserved spermatogenesis,» which may be prognostically favorable for ICSI.

2. Dedicated histological features and the condition of spermatogenesis with obstructive azoospermia, depending on histological criteria of modified Johansen's scoring system - 6,5 and 4 points named as «fibrosis of the tubules with impaired spermatogenesis.»

3. The presence of deformation, atrophy and obliteration of the testicular tubules, absence of seminiferous epithelium (1,2,3 points of modified Johansen's scoring system) is characteristic of the 3 groups - «obstructive fibrosis of the tubules.» Considering the intensity of fibroblastic reaction, thickening of the basement membrane of tubules, take place a violation of stromal-epithelial relationships, leading to disruption and arrest of the maturation and differentiation of spermatogenic cells, demonstrates the impossibility of extracting spermatozoa for later ICSI.

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