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CHANGES IN TESTICULAR TISSUE IN OBSTRUCTIVE AZOOSPERMIA WITH AN INCREASE IN THE DURATION OF THE DISEASE

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Abstract

To understand the infertility development it is important to study how individual components of the connective complexes of Sertoli's cells are formed and developed in order to allow not only individual cells, but also entire syncytia of germ cells to migrate from the basal to the adluminal compartment of the spermatic epithelium, without causing loss of permeability. Testicular tissue changes in azoospermia with an increase in the duration of the disease and the prognosis of the possibility of conception are of particular interest. **The aim.** To reveal changes in the testicular tissue on azoospermia with an increase in the duration of the disease. **Materials and methods**: to achieve the aim, a pathomorphological study was carried out. The material for the study was testicular biopsies of patients aged 26-45 years with a diagnosis of obstructive azoospermia (OA). The material was divided into three groups: group 1, control: healthy fertile men; group 2 (study group): men of mature age (26–45 y. o.) with a clinical diagnosis of OA up to 5 years of duration. Group 3 (study group): men of mature age (30–45 y. o.) with a clinical diagnosis of OA up to 10 years of duration. The preparations were studied macroscopically, microscopically, morphometrically and immunohistochemically. **Results and discussion.** Healthy spermatogenesis was typical for all

control group samples. In group 2 (disease up to 5 years from the previous conception) there were three spermatogenesis samples out of eight. There were focal processes of fibrosis and hyalinosis, with focal proliferation of interstitial tissue. In the the group of men with OA lasting up to ten years from the previous conception, the absence of healthy spermatogenesis in all cases was noted. **Conclusions**. 1. In the group of men with OA lasting up to five years from the previous conception there were violations of spermatogenesis, a decrease in the transport of biologically active substances through the blood-testicular barrier. The latter leads to degenerative processes in the germ cells. 2. In the group of men with OA lasting up to ten years from the previous conception, there was a pronounced deterioration in spermatogenesis, the transition of OA to NOA with increased duration of the pathological process. The latter significantly worsens the prognosis of the disease course and significantly reduces the possibility of conception.

Key words: development of infertility; obstructive azoospermia; spermatogenesis; Sertoli cells.

Introduction

Azoospermia, a condition manifested by the absence of sperm in the ejaculate, is one of the hardest causes of male infertility is. According to various authors, azoospermia can be detected in 10% of infertile patients [1]..

There are two types of azoospermia - obstructive azoospermia (OA), as a result of which spermatozoa from the testicle do not enter the sperm due to obstruction of the vas deferens; and non-obstructive azoospermia (NOA). In this case sperm are not produced or matured [2].

Sperm maturation requires a specific environment, which is partly created by the blood-testicular barrier. The barrier zone is topographically located at the base of the seminal epithelium and surrounds Sertoli cells. The barrier divides the epithelium into two cell compartments: the basal and the adluminal [3].

The relationship between all the components of the epithelial lining is necessary for the barrier zone to function synchronously with the differentiation of germ cells.

The structural connective complex of Sertoli cells consists of occlusive, fissural, closed and tight contacts. According to Dube et al. in OA men, testicular cells are unable to form tight contacts, which, accordingly, leads to impaired utilization of residual spermatid bodies and atrophy of germ cells [4].

To understand the development of fertility it is necessary to study how individual components of Sertoli cells connective complexes are formed and developed in order to allow not only individual cells, but also entire syncytia of germ cells to migrate from the basal to the adluminal compartment of the spermatic epithelium, without causing loss of permeability [2, 3].

Testicular tissue changes in OA with an increase in the duration of the disease and the prognosis of the conception possibility is of particular interest.

The aim. To reveal changes in testicular tissue in OA with an increase of the disease.

Materials and methods: Path morphological study has been done. Testicular biopsies of OA patients aged 26-45 years were material under study. All the patients were examined at V. I. Shapovalov Kharkiv Regional Clinical Center of Urology and Nephrology (Ukraine).

The case histories data and outpatient follow-up cards have been analyzed. The patients' age, clinical diagnosis, family history, testicular biopsy results, and final histological diagnosis of the surgical material were taken into account.

The material was divided into three groups:

Group 1 - control group consisted of healthy fertile men 26-40 years old, with conditional control of the physiological course of spermatogenesis. There were one or more births in a family history. The object of the study in this group was the autopsy material of the testicles obtained during the autopsy of the men corpses (no more than 6 hours after the ascertaining of biological death). The testicles were examined macroscopically for the presence or absence of inflammatory and tumor processes.

Group 2 - study group. It included mature age men (26–45 y. o.) with a clinical diagnosis of OA (n = 8). OA, lasting up to 5 years from previous conception, was medical indication for obtaining testicular biopsies.

Group 3 – study group. Men aged 30–45 y. o. with a clinical diagnosis of OA (n = 8). OA, lasting up to 10 years from prevous conception was the medical indication for obtaining testicular biopsies.

The causes of azoospermia in both study groups were surgical interventions (hernioplasty, varicocele) and testicular trauma.

Morphometric parameters of the preparations were studied. They measured the stromal-parenchymal index, Leydig's cells density in the field of view x400, Leydig's cells area, proliferation index (Ki-67) of androgen receptors to assess the hormonal activity of Leydig's cells.

In each case, when calculating the cell size, 5 randomly selected fields of view were studied, and 5 measurements were performed in each field of view. To assess the intensity of the immunohistochemical label, a semi-quantitative scale 0-3 + was used: 0 - no expression, + - weak, ++ - moderate, +++ - a pronounced reaction. The percentage of cells expressing the marker in the field of view was taken into account: 0 - no expression, 1 – 1 - 33% of cells, 2 – 34 - 66% of cells, 3 – 67 - 100% of cells. For Ki-67 marker, the expression level of 0% to 1%, up to 5%, 10% and more than 10% of cells was taken into account [5].

Morphological evaluation also included the use of immunohistochemical methods. The expression of such immunohistochemical markers as Ki-67 was used to assess the proliferative activity of cells; MMP-9 - to determine the processes of collagen formation; TGF-β - fibroblast growth factor for stroma assessment; VEGF and CD34 (a marker of vascular endothelium) were examined to assess angiogenesis and the degree of vascularization; the composition of cells responsible for increased collagen formation and fibrosis was assessed using CD68 (a marker of macrophages); PLAP - as a marker of functional activity of epithelial structures; CD44 - as a marker of cell adhesion and cell contacts.

De-masking heat treatment was performed by boiling the sections in citrate buffer (pH 6.0). To visualize primary antibodies, the Mouse / RabbitPolyVue HRP / DAB DetectionSystems (DiagnosticBioSystems, USA) detection system was used. DAB (diaminobenzidine) was used as a chromogen. A complex of morphological studies was carried out on an Olympus BH-2 microscope (Japan) using a Baumer / optronicType: CX05c camera and Olympus DP-Soft (Version 3: 1) and Microsoft Excel 2010 programs.

Results and their discussion

In the study of the control group, attention was drawn to the presence of healthy spermatogenesis in all cases. In the seminiferous tubules, a sequentially located spermatogenic epithelium and a small number of Sertoli cells were found (Fig. 1).

There are the presence of Leydig’s cells and absence of fibrosis and hyalinosis in stroma. An immunohistochemical study high proliferation index, which testifies in favor of active spermatogenesis, focal presence of immunocompetent cells (CD68) and moderate, in some places pronounced expression of CD44, as a marker of cell contacts were revealed. This indicates the ability of testicular cells to make tight contacts, which is necessary for normal spermatogenesis. Leydig’s cells expressed androgen, which indicates the normal endocrine status of men in the control group.

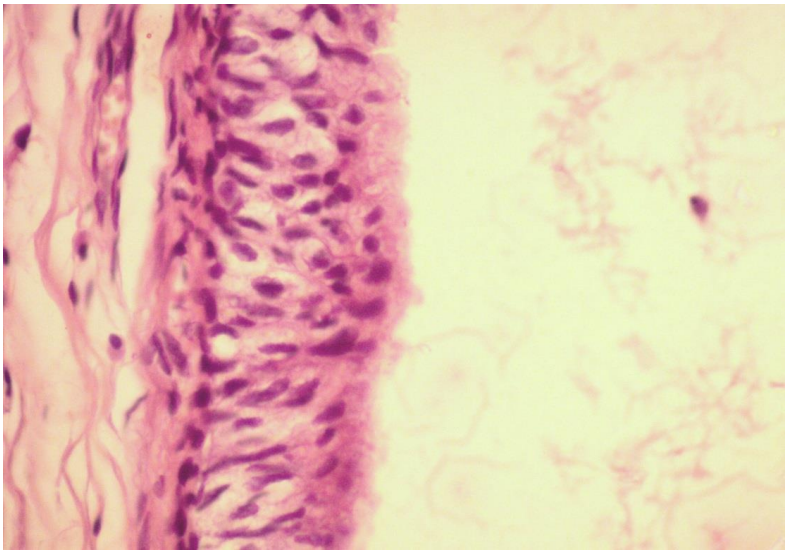

Fig. 1. The preparation demonstrates six typical cellular associations in the seminiferous tubules of the male control group. Spermatids at various stages of spermatogenesis (I), spermatocytes (II), Sertoli cells (III). Staining with hematoxylin and eosin, x400

When studying a group of men with OA lasting up to five years from the previous conception, attention was drawn to the presence of spermatogenesis in three cases out of eight. In the seminiferous tubules, a sequentially located spermatogenic epithelium and a small number of Sertoli's cells were found. However, in contrast to the control group, attention was drawn to desquamation of the embryonic epithelium, its focal atrophy (Fig. 2).

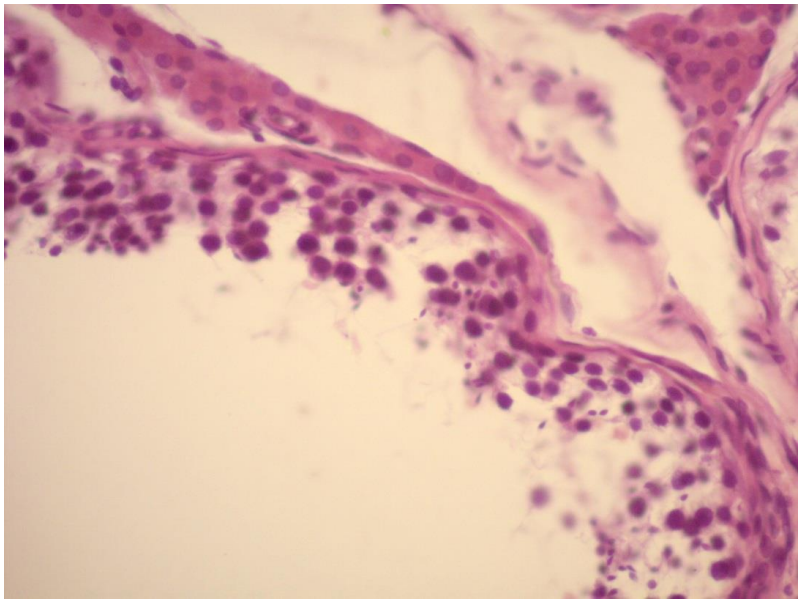

Fig. 2. Chaotic arrangement of germ cells and foci of epithelial desquamation. Staining with hematoxylin and eosin, x 400

In stroma, Leydig's cells were hypertrophic, which was confirmed by an increase in cell area (p = 0.04).

Focal processes of fibrosis and hyalinosis were observed, with focal proliferation of interstitial tissue, which was confirmed by a significant increase in the stromal-parenchymal index (p = 0.004) (Table 1).

Table 1 - The main morphometric parameters of the testicular tissue of the study group

Group	Stroma, %	Parenchyma, %	SPI	Leydig's cells density, sp-s in f/vis x400	Leydig's cells area, Mm^2
Control, n=7	20.3 ± 0.5	79.7 ± 1.8	0.25 ± 0.005	35 ± 0.8	174.3 ± 0.6
OA Group up to 5 years, n=8	27.6 ± 0.6 p = 0.05	72.4 ± 1.6 p = 0.01	0.4 ± 0.01 p = 0.004	0.4 ± 0.01 p = 0.004	229.4 ± 0.9 p = 0.04
OA Group up to 10 years, n=8	36.7 ± 0.8 p = 0.001 p1 = 0.006	63.3 ± 1.4 p = 0.005 p1 = 0.05	63.3 ± 1.4 p = 0.005 p1 = 0.05	57.2 ± 1.3 p = 0.05 p1 = 0.003	57.2 ± 1.3 p = 0.05 p1 = 0.003

p - comparison with the control group
p1 - comparison with OA group up to 5 years

Significant decrease of the proliferation index (p = 0.003) was revealed at immunohistochemical study. This indicates a decrease in the activity of spermatogenesis. An increase in the expression of CD68 was found with the appearance of focal lymphohistiocytic infiltration.

A decrease in the intensity of expression of the CD44 marker was also observed, which indicates a decrease in the ability of testicular cells to make tight contacts, and, accordingly, the possibility of normal natural elimination of residual spermatid bodies. Such changes can lead to severe atrophy of the embryonic epithelium.

Leydig's cells actively expressed androgen, which may indicate an increase in the endocrine function of the testes in response to a blockage of the vas deferens.

The appearance of angiotrophic disorders, confirmed by a decrease in the expression of markers VEGF and CD34, causes a decrease in the transport of biologically active substances across the blood-testicular barrier, leading to pronounced degenerative processes in germ cells.

The main IHI markers are presented in Table 2.

Table 2 - Expression of IHC markers in the groups under study

Marker	group	Control, n=7	OA, up to 5 years, n=8	OA, up to 10 years, n=8	Confidence, p
MMP-9.92kDa	0	0	0	0	p = 0.12
	1+	6 (85.7%)	0	0	p1 = 0.01
	2+	1 (14.3%)	7 (87.5%)	4 (50%)	
	3+	0	1 (12.5%)	4 (50%)	
TGF-β	0	0	0	0	p = 0.02
	1+	7 (100%)	1 (12.5%)	0	p1 = 0.01
	2+	0	5 (62.5%)	4 (50%)	
	3+	0	2 (25%)	4 (50%)	
CD34	0	0	3 (37.5%)	4 (50%)	p = 0.03
	1+	5 (71.4%)	4 (50%)	4 (50%)	p1 = 0.05
	2+	2 (28.6%)	1 (12.5%)	0	
	3+	0	0	0	
VEGF	0	0	0	2 (25%)	p = 0.05
	1+	1 (14.3%)	5 (62.5%)	5 (62.5%)	p1 = 0.01
	2+	4 (57.1%)	3 (37.5%)	1 (12.5%)	
	3+	2 (28.6%)	0	0	
Ki-67	0	0	0	1 (12.5%)	P = 0.02
	1+	1 (14.3%)	3 (37.5%)	5 (62.5%)	p1 = 0.01
	2+	5 (71.4%)	5 (62.5%)	2 (25%)	
	3+	1 (14.3%)	0	0	
CD68	0	1 (14.3%)	0	0	P = 0.05
	1+	4 (57.1%)	3 (37.5%)	0	p1 = 0.02
	2+	2 (28.6%)	5	4 (50%)	
	3+	0	0	4 (50%)	
PLAP	0	6 (85.7%)	0	0	P = 0.01
	1+	1 (14.3%)	1 (12.5%)	0	p1 = 0.01
	2+	0	3 (37.5%)	2 (25%)	
	3+	0	4 (50%)	6 (75%)	
CD44	0	0	1 (12.5%)	3 (37.5%)	P = 0.01
	1+	0	4 (50%)	3 (37.5%)	p1 = 0.02
	2+	2 (28.6%)	3 (37.5%)	2 (25%)	
	3+	5 (71.4%)	0	0	
Androgen	0	0	1 (12.5%)	0	P = 0.1
	1+	5 (71.4%)	1 (12.5%)	2 (25%)	p1 = 0.05
	2+	2 (28.6%)	4 (50%)	2 (25%)	
	3+	0	2 (25%)	4 (50%)	

P - comparison with the control group,
p1 - comparison with the OA group up to 5 years

When study a group of men with OA lasting up to ten years from the previous conception, attention was drawn to the absence of healthy spermatogenesis in all cases. We observed a different morphological picture. 3 patients had hypospermatogenesis (Fig. 3) (spermatogonia and destroyed primary spermatocytes are located in the seminiferous tubules); the 4th patient had a block of maturation with foci of subtotal aplasia of gametes (single

spermatogonia); one patient had tubular atrophy. The data obtained confirm the literature data that with a long course of OA, morphological changes can lead to the development of NOA.

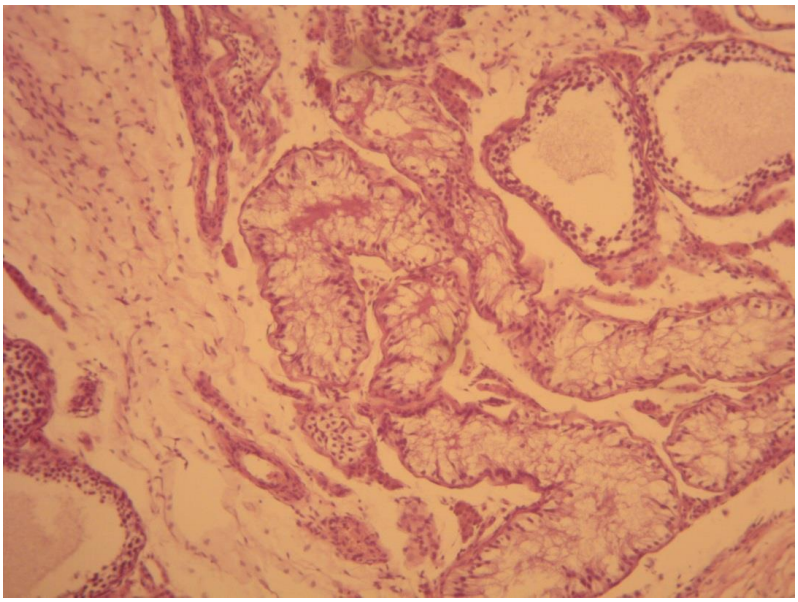

Fig. 3 - Focuses of hypospermatogenesis in testicular tissue in OA group patient. OA lasts up to 10 years. Staining with hematoxylin and eosin, x100

In the seminiferous tubules, a pronounced atrophy of the spermatogenic epithelium and an increase in the number of Sertoli's cells were found. In stroma, Leydig's cells were hyperplasic, which significantly differed from both the control group and the OA group with OA lasting up to 5 years ($p = 0.05$ when compared with the control, $p = 0.003$ when compared with the OA group up to 5 years).

There were pronounced fibrosis and hyalinosis with the proliferation of interstitial tissue, which was confirmed by a significant increase in the stromal-parenchymal index ($p = 0.005$ with the control group, $p = 0.01$ with the OA group up to 5 years). Immunohistochemical study revealed a significant decrease in the proliferation index ($p = 0.0001$ when compared with the control, $p = 0.004$ when compared with OA group up to 5 years), which confirms a decrease in the activity of spermatogenesis.

The number of macrophages (CD68) increased, focal lymphohistiocytic infiltration appeared, which was not observed in the control group. There was a significant ($p = 0.02$) increase in infiltration when compared with OA group up to 5 years.

Leydig's cells actively expressed androgen when compared with the control group, however, when compared with OA group up to 5 years, no significant differences were observed. The data obtained may indicate an increase of the testicles endocrine function as a response to blockage of the vas deferens. Angiotrophic changes were confirmed by a decrease

in the expression of VEGF and CD34. The appearance of angiotrophic disorders causes a decrease in the transport of biologically active substances through the blood-testicular barrier, leading to pronounced degenerative processes in the germ cells.

The stages of hypospermatogenesis and maturation block in OA are characterized by a decrease in the proliferation of spermatogonia, their impaired differentiation and the presence of embryonic PLAP-positive cells.

Conclusions

1. At the examination of OA men with its duration up to five years from the previous conception, the following morphological changes were found: hypertrophy of Leydig's cells with an increase in androgen secretion, initial manifestations of desquamation and atrophy of the embryonic epithelium, initial manifestations of fibrosis and stromal hyalinosis with an increase in the stromal-parenchymal index ($p = 0.005$) and an increase in the expression of markers MMP-9 and TGF-β, a weakening of tight contacts between cells, as well as manifestations of angiotrophic disorders. These data indicate a violation of spermatogenesis, a decrease in the transport of biologically active substances through the blood-testicular barrier, which leads to degenerative processes in the germ cells.

2. In the group of men with OA lasting up to ten years from the previous conception, the following morphological changes were found: hypospermatogenesis, block of maturation with foci of subtotal gamete aplasia (single spermatogonia), tubular tubular atrophy; hyperplasia of Leydig's cells, pronounced manifestations of desquamation and atrophy of the embryonic epithelium, pronounced manifestations of fibrosis and hyalinosis of the stroma with an increase in the stromal-parenchymal index ($p = 0.005$) and an increase in the expression of markers MMP-9 and TGF-β, a pronounced weakening of tight contacts between embryonic cells, the appearance of PLAP-positive cells, as well as pronounced manifestations of angiotrophic disorders. These data indicate a pronounced deterioration in the processes of spermatogenesis, the transition of OA to NOA with an increase in the duration of the pathological process, which significantly worsens the prognosis of the course of the disease and significantly reduces the possibility of conception.

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