ORIGINAL ARTICLE

CONTENTS

Pathogenetic aspects of the features of androgen deficiency state under the condition of experimental varicocele

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ABSTRACT

Aim: The aim of this article is to study of the main pathogenetic aspects of the features of androgen deficiency state under the condition of experimental varicocele caused by serotonin hydrochloride.

Materials and Methods: Simulation of serotonin testicular damage (experimental varicocele) is used to reproduce gonadal pathology. In the mechanism of the development of this gonadopathy was the vasoconstrictor effect of serotonin hydrochloride plays a leading role. Serotonin hydrochloride (Alfa Aesar®, USA) was administered for 14 days at a dose of 5 mg/kg of body weight ("pathology" group).

Results: Experimental varicocele led to disturbance of structural elements of connective tissue (and the content of connective tissue components of glycosaminoglycans in the testes and chondroitin sulfates in the seminal plasma) as a leading link in the pathogenetic chain which promotes the formation of hormone dependent reproductive diseasess.

Conclusions: Experimental varicocele on the background of the androgen deficiency state led to the disturbance of the morphofunctional state of the mitochondria of testicular cells, damage to the nuclear membrane, a decrease in the content of testosterone in the blood and fructose in the seminal vesicles; a decrease in the concentration and motility of spermatozoa.

KEY WORDS: morphology, connective tissue, varicocele, spermatogenesis, reproductive system

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INTRODUCTION

Ensuring adequate demographic condition is the main task of creating resource potential and the health of the nation as a whole. It is known that demographic policy is focused on the effective regulation processes of the population reproduction . The majority of factors determining depopulation depend on the decline in the birth rate. This is due not only to social and economic conditions, but also to the deterioration of the reproductive health of the population of Ukraine and high rates of reproductive losses, especially during the war years [1]. One of the leading conditions characterizing the deterioration of reproductive health is infertility or hypofertility.

Thus, male infertility is a global medical, socio-economic, demographic, medical-biological and individual marital problem throughout the world and in Ukraine, as well as a problem that requires further study of the etiology and pathogenesis to increase awareness of hypofertility and search for new approaches, methods and mechanisms for the treatment of this common pathological condition of the male body [1, 2].

Among the numerous factors of male fertility disoders the leading place is occupied by the spread of chronic somatic diseases (primarily those accompanied by ischemia of the reproductive system, in particular, varicocele) [1, 2]; an increase in the number of cases of chronic inflammatory diseases of the genital system in man of reproductive age [3]. As already noted, with varicocele blood stasis occurs with the development of hypoxia of the germinal epithelium and a violation of the androgen-estrogen balance, which is the cause of a decrease in the fertilizing ability of sperm [4]. It is well known connective tissue takes an active part in the processes of inflammation, apoptosis, destruction of organs and systems [5, 6]. As for its participation in

the mechanisms of development of pathology of the male reproductive system has not been sufficiently studied to date. It is believed that changes and disturbances in the structure of connective tissue can lead to testicular dysfunctions, including idiopathic male infertility. These configurations are characterized by metabolic disturbances, in particular of collagen and proteoglycans. [7]. Transformation of the connective tissue structures of the venous wall and the uncompensated state of the valve apparatus of the testes cause varicocele [4, 8]. It is well known that changes in blood flow, nutrients and oxygen supply, as well as an increase in local temperature, can adversely affect the functioning of Leydig cells and spermatogenesis, which is a background for male hypofertility or infertility [9]. It is also well known that the detailed etiology and pathogenesis of varicocele-mediated infertility are not fully understood. But heat stress, toxin impact and blood stasis, hormonal imbalance, oxidative stress, and testicular hypoperfusion have been discussed as potential mechanisms. However, none of these mechanisms alone can accurately explain the impairment effects of varicocele on testicular function [8].

AIM

The aim of this article is to study of the main pathogenetic aspects of the features of androgen deficiency state under the condition of experimental varicocele caused by serotonin hydrochloride.

MATERIALS AND METHODS

The studies were carried out in compliance with the rules of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986) [10, 11]. During the experiment, the animals were in the vivarium at t0 18–240C, humidity 50-60 %, natural day-night light mode, on a balanced diet according to the current norms. The experimental investigations were carried on white mature male Wistar rats with basal weight of 340–380 g.

According to the aim and objectives we simulated the androgen deficiency state in male rats taking into account the verified etiopathogenetic role of effects of damaging factors of serotonin hydrochloride in particular.

Simulation of serotonin testicular damage (experimental varicocele) is used to reproduce gonadal pathology. In the mechanism of the development of this gonadopathy was the vasoconstrictor effect of serotonin hydrochloride plays a leading role. As result of the vasoconstriction it is trophic disorders and pathological changes occur, which are usually observed in patients with hypofertility and can occur in case of varicocele, injuries, conditions caused by hypodynamia, etc. [10].

Serotonin hydrochloride (Alfa Aesar®, USA) was administered for 14 days at a dose of 5 mg/kg of body weight *("pathology" group)*. The drug at this dose destroys the metabolism of supporting epitheliocytes (Sertoli cells) and interstitial endocrinocytes of rat testes (Leydig cells), which produce testosterone (Butenko I.V., Laryanovskaya Yu.B., 2010) [10]. To achieve the goal of studying the main pathogenetic features and mechanisms of experimental varicocele caused by serotonin hydrochloride, a series of studies was conducted. Carry out of the mass of androgen-dependent organs as markers of reproductive system function. The levels of intratesticular testosterone in testis homogenates (A. G. Reznikov, 1986) and testosterone in the blood serum was determined by the enzyme immunoassay method using standard kits. The content of fructose in the seminal vesicles (by Bokunyaeva N.I., 1975) , the activity of acid phosphatase (prostatic) in blood serum was determined by the kinetic method (with naphthyl phosphate as a substrate) using a standard kit. After were evaluated features of the morphofunctional state according to the main indicators: the number of spermatozoa (sperm concentration), the percentage of pathological forms, motility and the duration of preservation of their motility according to the generally accepted method (Stefanov O.V., 2001) [10].

A biochemical study of the content of chondroitin sulfate (component of connective tissue) in the spermatoplasm of rats was also carried out according to the Nemeth-Csoka method modified by L.I. Slutsky (Morozenko D.V., Levchenko V.I., Timoshenko O.P., 2012). Morphological examination of spieciments was carried out due to standart method by Merkulov H.P. [10]

Electron microscopic examinations were carried out according to standard methods (Boiko V.V., Prasol V.A., Nevzorova O.F., Nevzorov V.P. et al., 2013). Structural parameters of mitochondria: the number of morphologically normal and altered mitochondria (destruction of the outer membranes, matrix homogenization and edema, lysis of cristae) were determined and counted using an EMV-100BR electron microscope (expressed as a percentage of 100%) (Rozovaya E.V. et al., 2015) [10].

Detection of glycosaminoglycans (GAGs) on sections was shown as a metachromasia reaction by staining with toluidine blue at pH 2.5 (Kern M., Modish L., Dedukh N. V. et al., 1985). The degree of color saturation was assessed according to the principle of counting cells in histological samples, using a semiquantitative method of visual assessment of the intensity of coloration of

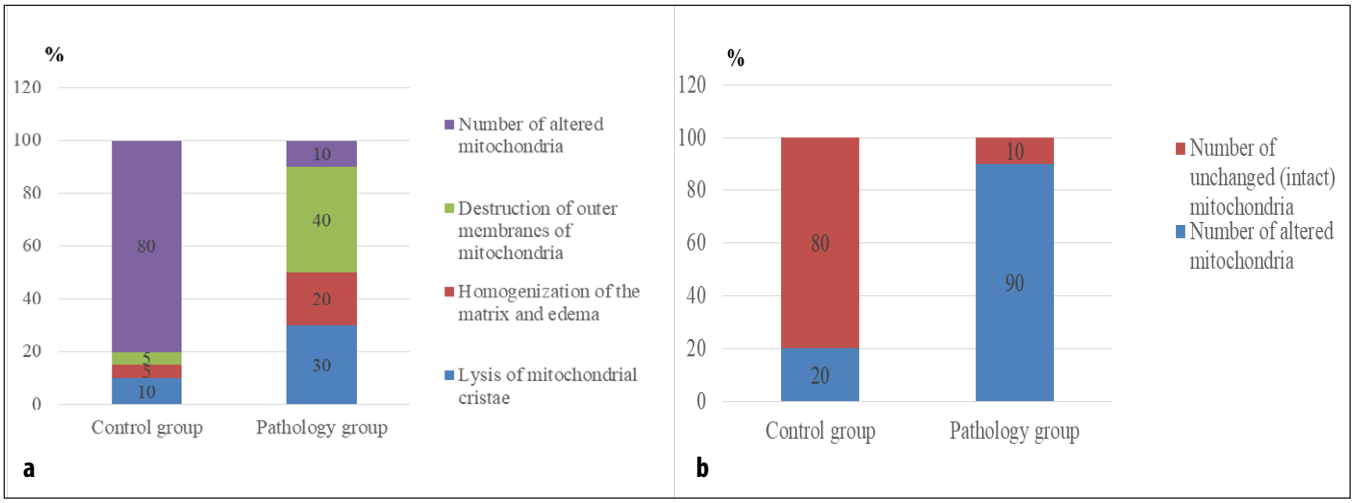

Fig. 1. Frequency of mitochondria structural changes of Leydig cells under the conditions of experimental varicocele
a – structural changes of mitochondria (in %% of the total number of morphologically changed mitochondria, taken as 100%). b – violation of the morphofunctional state of mitochondria.
Picture taken by the authors

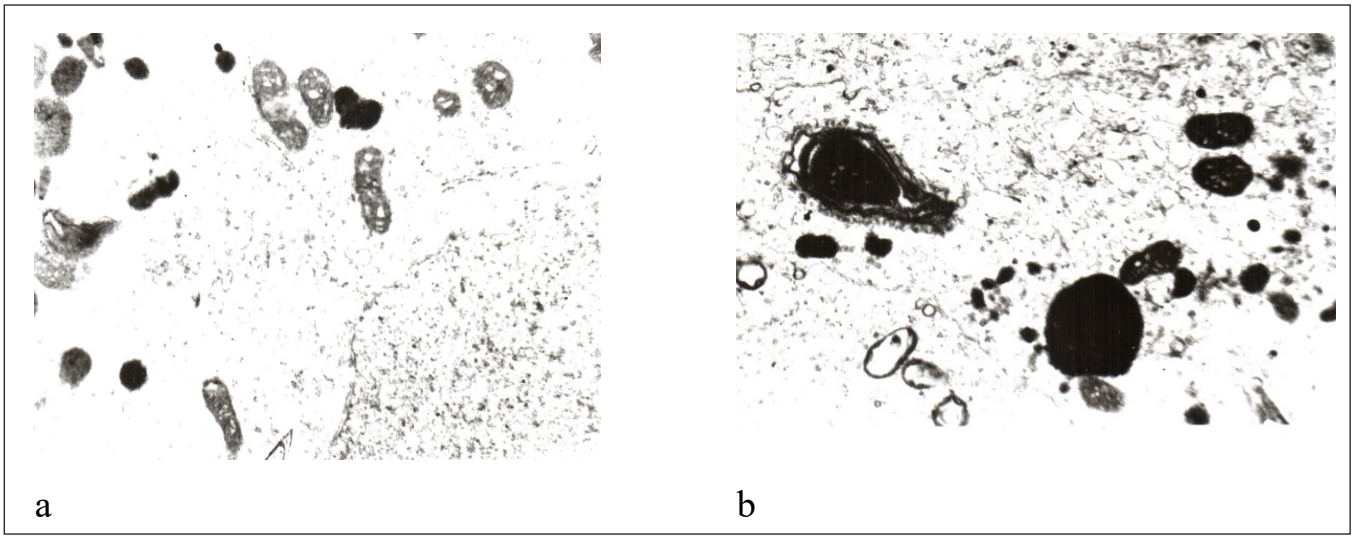

Fig. 2. Ultrastructure of testicles cells of the "pathology" group:
a – Sertoli cells. Lysis of the nuclear membrane. Decondensed chromatin. x32000
b – Leydig cells. Homogenization of the mitochondrial matrix. x35000. Contrasted by lead citrate

microstructures by histochemical reactions (Sokolovsky V.V., 1971). Quantitative assessment of the intensity of staining was expressed in point (+4) [10].

Statistical processing of the obtained results was performed using parametric and non-parametric methods. As a result of the normal distribution, the arithmetic mean and its statistical error were calculated $(\bar{x} \pm s_{\bar{x}})$; if the data were not consistent with the nor mal distribution, the median (Me), minimum and maximum data (min ÷ max) were calculated. Newman-Keuls method, nonparametric Wilcoxon-Mann-Whitney U-test and Kruskal Wallis H-test were used to analyze differences. Critical level of significance was assumed to be equal to or less than 0,05 ($p \leq 0,05$).

FRAMEWORK

The research was carried out as part of investigation works at the SI «V. Danilevsky Institute for Endocrine Pathology Problems of NAMS of Ukraine»: 1) «Mechanisms of development, diagnosis and therapy of infertility in individuals with gonadal dysfunction and comorbid pathology» (state registration number 0116U007259);

2) "Determination of the role of vitamin D deficiency and insufficiency in the development of gonadal dysfunction, substantiation of approaches to their therapy" (state registration number 0119U102387);

3) Determination of the role and deficiency of vitamin D in the development of the sexual glands dysfunctions,

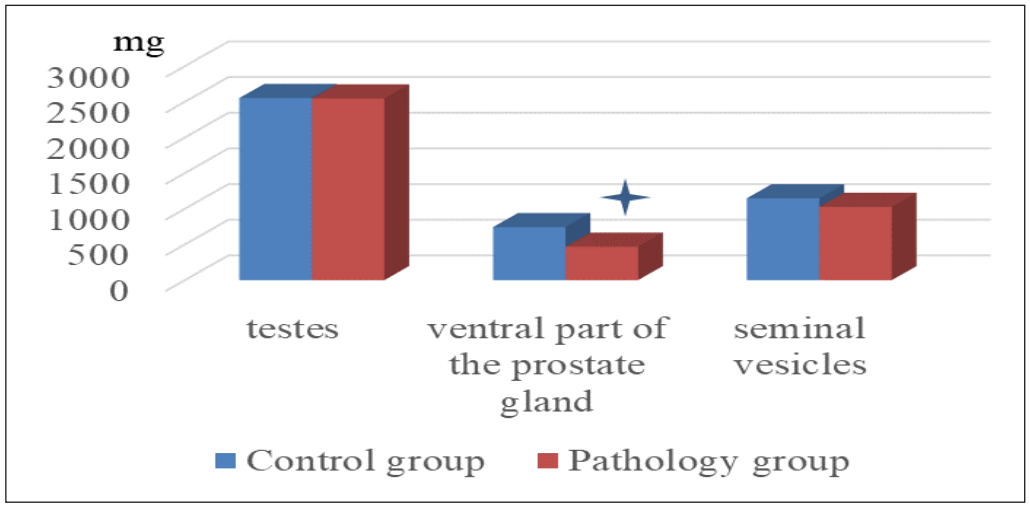

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Fig. 3. Mass of androgen-dependent organs as a markers of reproductive system dysfunction
– statistically significant differences compared to the data for the "Control" group, $p \leq 0.05$

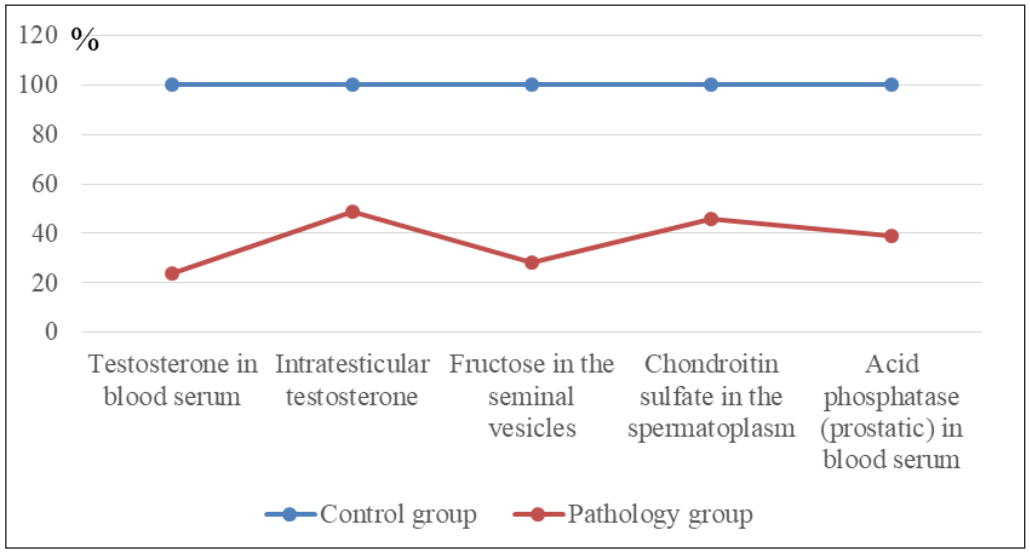

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Fig. 4. Indicators of androgen saturation and the content of chondroitin sulfates in the spermatoplasm of male rats under the conditions of experimental varicocele

definition to ways to their therapies (state registration number 0119U 102387).

RESULTS

The main feature of pathogy of gonad (experimental varicocele) was destructive changes in the organelles of testicular cells. It has been seen the percentage of morphofunctional changes of mitochondria under the simulation of serotonin testicular damage. Thus, a significant number of Leydig and Sertoli cells were found to be foci of cytoplasmic necrosis. The mitochondria of the testis cells had destroyed outer membranes, homogenization of the matrix, edema, and foci of lysis of the cristae, increased a number of altered mitochondria and the cisternae of the endoplasmic reticulum were greatly expanded. A significant increase in the percentage of morphologically altered mitochondria (90 %) was observed. Only 10 % had an unchanged structure (Fig. 1). The cytoplasmic membrane of endotheliocytes had small foci of destruction. The cytoplasm contains a small amount of ribosomes and polysomes. Such changes indicate a slowdown in the recovery of structures against the background of gonadopathy.

nder the simulation of serotonin testicular damage (experimental varicocele) destruction of the nuclear membrane (lysis of the nuclear membrane) of Leydig cells was noted, which can lead to DNA destruction and, as a result, inhibition of transcription and translation processes (Fig. 2). The accumulation of mitochondria and the location of small and large vacuoles, as well as myelin-like structures near the membranes, indicate a violation of synthetic processes in the cell. These factors also caused a violation of the process of spermatogenesis, which is confirmed by anomalies in the ultrastructure of the flagellum, which indicates a violation of the motor apparatus of spermatozoa. Ultramicroscopic examination of testis specimens revealed foci of connective tissue degeneration also.

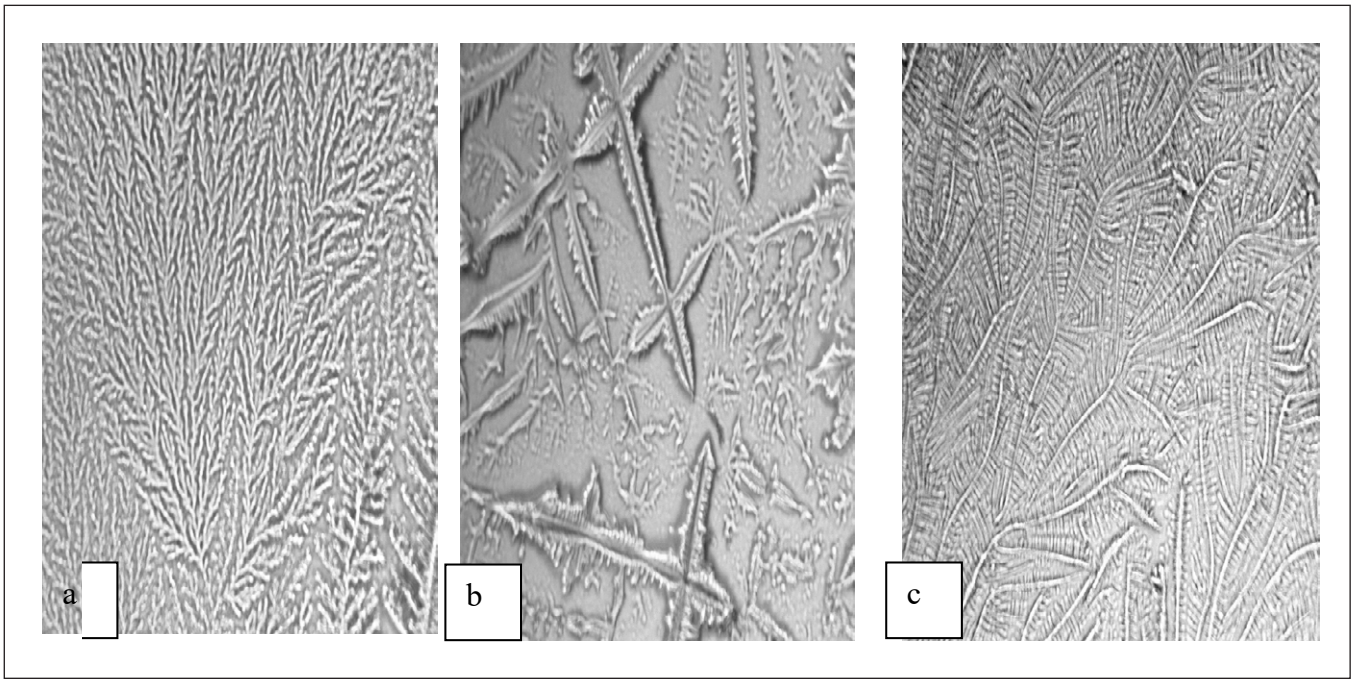

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Fig. 5. Types of crystallization of the prostate gland secretion of rats under the conditions of experimental varicocele:
a - type of crystallization of the secretion of the prostate gland of an intact rat (typical phenomenon of «fern leaves», androgen saturation of the body is normal);
b – single atypical crystals on the background of an amorphous mass;
c – divergence of transverse branching of branches from the main stem at a much greater angle, reduction in size, reduction in the number of side branches.
Androgenic saturation of the body is reduced. Photomicrograph: imprint of the secret. x200

Table 1. Indicators of the spermogram, morphometry of spermatogenesis and the type of crystallizing prostate secretion under the under conditions of experimental varicocele (n=8), $(\bar{x} \pm S_x)$; Me (Q25; Q75)

Indices	"Control" group, n=8	"Simulation of serotonin testicular damage" group ("Pathology" group) n=8
The number of spermatozoa, million/ml	52.42±3.93	25.85±2.42*
Pathological forms, %	21.86±1.97	35.14±4.54*
Sperm motility, %	81.86±3.48	39.57±8.17*
Sperm motility preservation time, min	187.86±12.10	115.71±6.81*
The number of spermatogonia in the seminiferous tubules, pcs	57.12±1.05	13.21±2.03*
The number of seminiferous tubules with the 12th stage of meiosis, %	5.0 (4; 6)	1.0 (0; 7)*
Index of spermatogenesis, points	3.36 (3.28; 3.44)	0.99* (0.36; 2.76)
The type of crystallization of prostate secretion, points,	3.58 (3.2; 3.83)	2.04 (1.33; 2.36)*

notes: * - statistically significant differences compared to the Control group, $p \leq 0.05$
Source: compiled by the authors of this study

Simulation of serotonin testicular damage as well was accompanied by changes in the mass of androgen-dependent organs as markers of reproductive system dysfunction, a decrease in the mass of the ventral part of the prostate gland by 36% was observed compared to the "Control" group, which may be caused by a violation of the morphostructure of the gland as a result of the action of serotonin hydrochloride (Fig. 3).

Experimental varicocele demonstrated the development of androgen deficiency: its formation indicates a

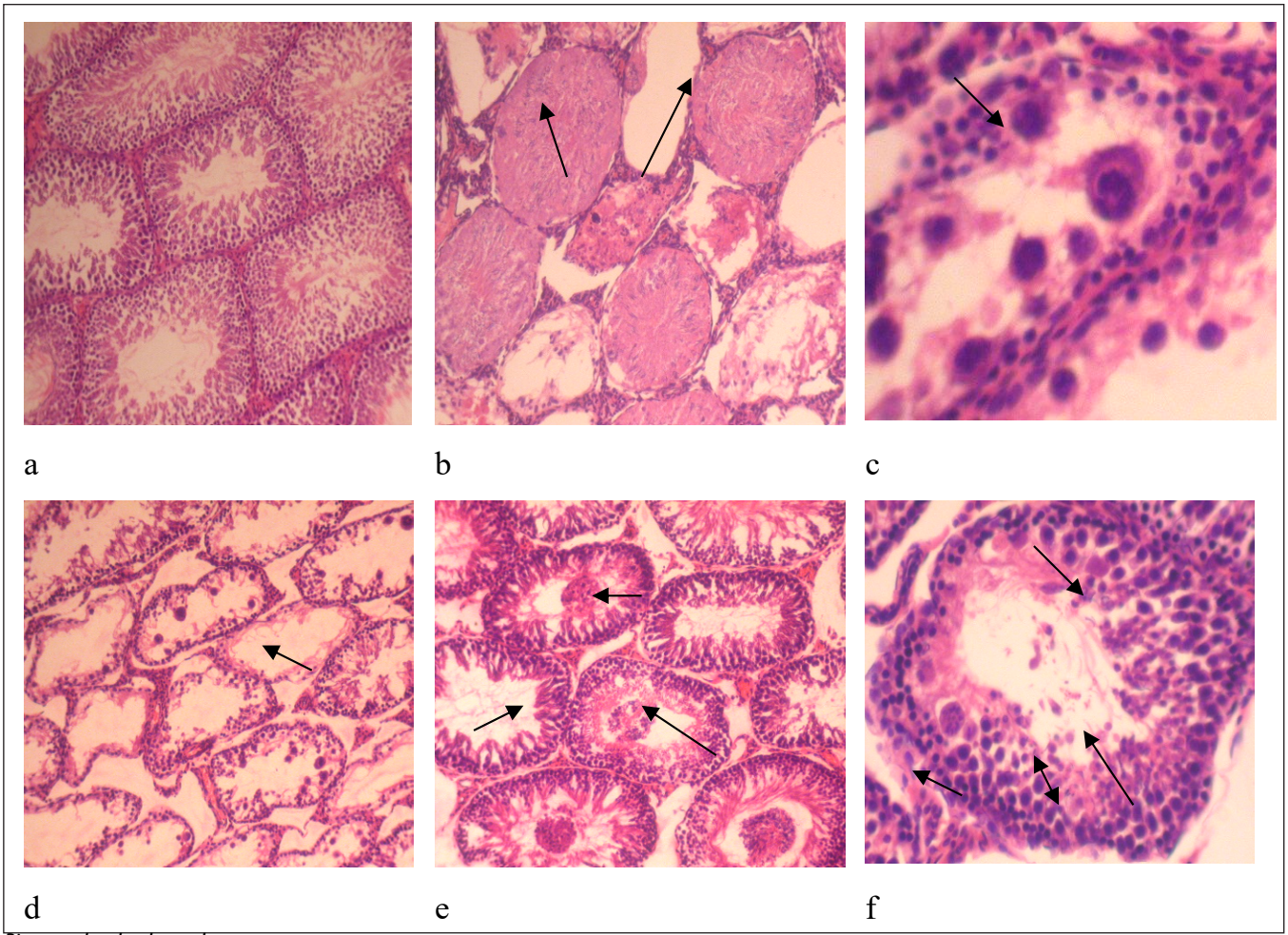

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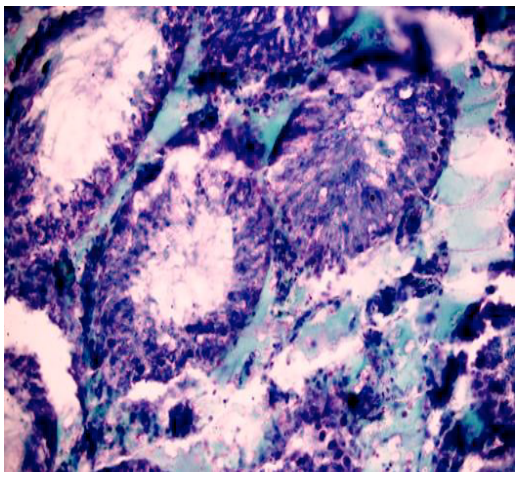
Fig. 6. Testicles of experimental animals (a – "Control" rats; b-e – «Pathology» group (rats with experimental varicocele):
a – normal size of seminiferous tubules, germ cells are presented in full content. x100;
b – necrotic detritus in seminiferous tubules. x100;
c –multinucleated giant cells in the seminiferous tubules. x250;
d – emptying of the seminiferous tubules, reduction in their size, expansion of the intertubular space. x100;
e – desquamation of germ cells in seminiferous tubules (arrow). x100;
f – section of seminiferous tubule wall exposed (arrow), sphere cells (double arrow). x250. Staining with hematoxylin-eosin

decrease in intratesticular testosterone in testicles by 2 times (14.20 (11.60; 17.10) and 28.80 (24.50; 31.30); ($p<0.05$) and general testosterone in blood serum by 4 times (3.84±0.98 and 16.04±0.82; $p<0.05$) in male rats, which leads to decrease in the mass of androgen-dependent organ (ventral part of the prostate gland), disrupting its functioning, as evidenced by a decrease activity of acid phosphatase (prostatic) in blood serum by 2.5 times (8,84±0,70 and 22,46±0,97; $p<0.05$) and content fructose seminal vesicles by 3.6 times (0,98±0,05 and 3,48±0,08; $p<0.05$) and then spermogram parameters (Fig. 4).

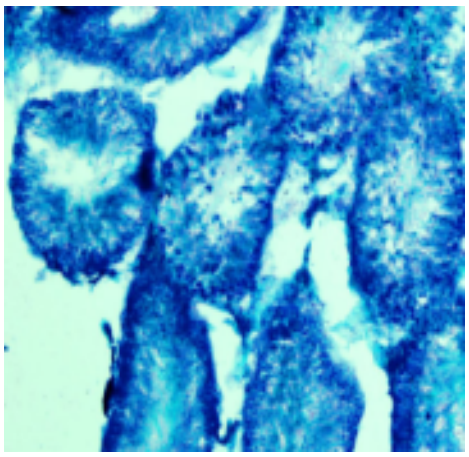
Androgen saturation of the organism which was determined by the type of crystallization of the secretion of the prostate gland of animals, significantly decreased and amounted to 2,04 points ($p<0.05$) (Fig. 5, Table 1).

Against the background of a testosterone decrease impact of serotonin hydrochloride led to damage of spermatogenesis, there was a significant decrease in the number of spermatozoa almost by 2 times (25,85±2,42 and 52,42±3,93; $p<0.05$), their motility and sperm motility preservation time. It has been increased pathologically altered forms of spermatozoa (21,86±1,97 and 35,14±4,54; $p<0.05$) in comparison with "Control" group (Table 1).

According to the obtained data the presence of probable changes in all of the above parameters of the semen analysis which affected the morphometric characteristics of spermatogenesis in the seminiferous tubules, namely, the number of spermatogonia in the seminiferous tubules decreased sharply) and respectively it has been decreased the number of spermato-



A."Control" group (×250)



B."Pathology" group (×250)

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Fig. 7. Histological preparations of rat testicles. Staining with toluidine blue

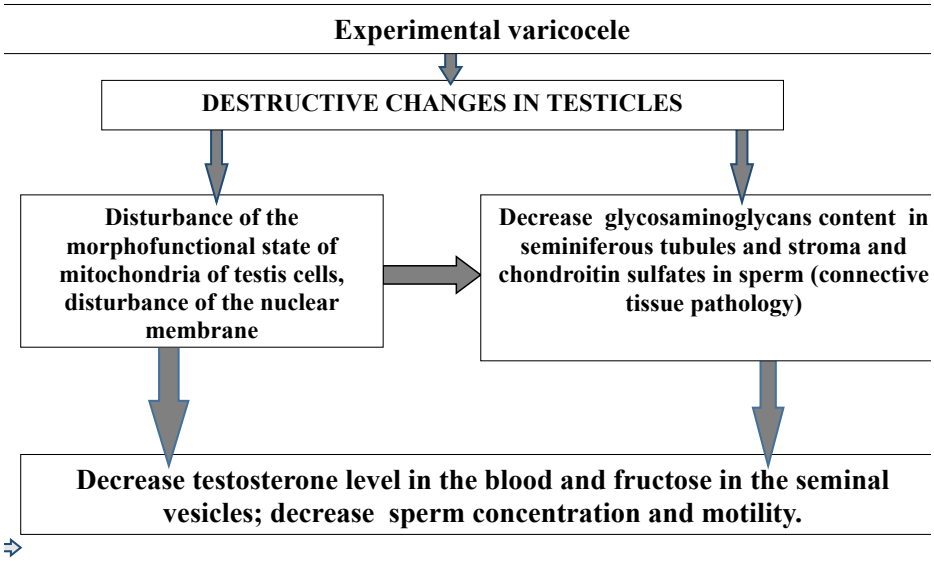

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Fig. 8. Probable diagram of the pathogenetic mechanism of experimental varicocele
Picture taken by the authors

cytes of the second order which entered the metaphase of the second division of maturation (stage 12 of meiosis) - 5 times (5,0 (4; 6) and 1,0 (0; 7) in comparison with "Control" group ($p<0.05$).

All these processes led to a 3-fold drop in index of spermatogenesis (3,36 (3,28; 3,44) and 0,99 (0,36; 2,76) (Table 1). Simulation of serotonin testicular damage confirms the violation of the excretory function of the testes and indicates the development of experimental gonadopathy [9].

It should be noted in animals from the "pathology group" a decrease in total gonadotropic activity by 32% was observed, but this was not reflected in the mass of the testis and pituitary glands.

Therefore, serotonin testicular damage (experimental varicocele) leads to central dysregulation of sexual function.

The histological picture of the testes of experimental groups of rats has been shown at Fig. 6.

Morphological changes of a typical nature were found in the testicles of male rats under the influence of serotonin hydrochloride («Pathology» group). In animals, the vast majority of seminiferous tubules are reduced in size, completely emptied or contain several damaged cells, some of the tubules are filled with necrotic detritus.

Supporting cells are often "bloated", the nucleolus is sharply ectopic or absent. Disorganization of the spermatogenic epithelium was observed in a limited part of the tubules. Spermatids are dystrophically changed, small vacuoles are visible in the nucleus, the shape of the cells is changed. Often, against the background of devastation, spherical cells are visible - giant multinucleated spermatids (containing 3-10 nuclei), formed when their division is disrupted.

The shell of the seminiferous tubules is sclerosed. Interchannel spaces are increased. Interstitial cells (Leydig cells) were characterized by expressive monomorphism in the size

of the nuclei (mostly small), few, in some of them the nuclei were pyknotic. It is possible that this state of interstitial cells is a morphological reflection of a certain decrease of the steroid-producing function of these structures (Fig. 6, b-f).

Under the conditions of simulation of serotonin testicular damage, the content of connective tissue components - chondroitin sulfates in the seminal plasma of rats was reduced by 2,2 times 13,10 (12,40; 13,90) and 28,40 (26,80; 29,30) ($p<0,05$) compared to the "Control" group (Fig. 4).

The results of the study of the content of glycosaminoglycans (GAGs) in testicular samples and seminal plasma under the conditions of serotonin testicular damage are presented in Fig. 7. In the gonads of rats of the "Control" group, when stained with toluidine blue in the seminiferous tubules, purple coloration was detected in the band of the spermatogenic epithelium (see Fig. 7).

Small zones of metachromasia (the same purple color) were also visualized in the intertubular stroma, which indicates the presence of GAG. The degree of color saturation was 4.00±0.53 (4.50; 4.00) points.

During reconstruction of experimental varicocele in the seminiferous tubules and intertubular stroma, an orthochromatic reaction (almost complete absence of purple coloration) was observed when stained with toluidine blue, which indicates a deficiency of GAG compared to the "Control" group (see Fig. 6). The degree of color saturation was 0.00±0.20 (0.00; 0.08) points ($p<0,05$). The obtained results give reason to believe that androgen deficiency caused by serotonin hydrochloride leads to a decrease in the level of GAG in the sperm of rats, which can be a marker of the development of infertility in males.

Such a histochemical pattern in group of "simulation of serotonin testicular damage" can explain the fact that the testosterone level decreases in animals of this group, because the decrease in the amount of loose connective tissue that nourished the Leydig cells led, most likely, to a decrease in the level of their functional activity. Violation of the functional state of the testes under the conditions of "simulation of serotonin testicular damage" was also described by other authors [12].

DISCUSSION

Summing up our long-term research we can conclude that spermatogenesis changes of the "pathology" group (experimental varicocele) associated with change in the morphological structure of the testes, as a result of the effect of vasoconstriction by impact of serotonin hydrochloride, which leads to hypoxia, trophic disorders and pathological conditions that occur in male under the conditions of varicocele, injuries, dysfunctions caused by hypodynamia and lead to hypofertility [12], as well as a decrease in testosterone secretion.

There are data, although sometimes contradictory, about the contribution of connective tissue to the development of many pathological processes in the male reproductive system, disorders of testosterone secretion and spermatogenesis [13]. Thus, sex hormones affect the processes of connective tissue remodeling: estrogens increase the intracellular water content, testosterone causes proliferation of fibroblasts and the synthesis of connective tissue components [10]. Considering that in all types of male infertility there is androgen deficiency of varying degrees of severity [8], a decrease in testosterone level can negatively affect the state of connective tissue. However, in andrological and obstetric-gynecological practice attention is paid to disorders of metabolism connective tissue and its effect on reproductive potential [14].

It has been proven that a decrease in the part of chondroitin sulfate and an increase in the part of keratan sulfates in the composition of glycosaminoglycans of the reproductive system can be used for ecological assessment of areas with probable contamination by toxic pollutants, where an increase in the number of infertile men is observed. The study of the spectrum of proteoglycans in blood serum and seminal plasma can be used as a criterion for predicting fertility (Vetoshkin R.V., Nikolaev A.A., 2015) [7].

Summarizing the data on experimental modeling (simulation) animals pathology of gonad, we can confirm that a general distinguishing feature for experimental varicocele is a disturbance of the morpho-functional state of mitochondria, destruction of the nuclear membrane of Sertoli and Leydig cells, a disturbance of the endocrine and spermatogenic functions of the testes against the background of a decrease in the content of GAGs in the testes and chondroitin sulfates in the spermatozoa. Consequently, we observe the formation of the so-called the "perverse circle": violation of testosterone secretion and pathology of reproductive system → changes in the structure of connective tissue → violation of testosterone secretion and pathology of reproductive system → deepening of violations in the structure of connective tissue. Therefore, the pathogenetic mechanism of experimental varicocele development can be presented schematically (Fig. 8).

CONCLUSIONS

1. Experimental varicocele led to disturbance of structural elements of connective tissue (and the content of connective tissue components of glycosaminoglycans in the testicles and chondroitin sulfates in the seminal plasma) as a leading link in the pathogenetic

chain which promotes the formation of hormone dependent reproductive diseasess.
2. Experimental varicocele on the background of the androgen deficiency state, there is a disturbance of the morphofunctional state of the mitochondria of testicular cells, damage to the nuclear membrane, a decrease in the content of testosterone in the blood and fructose in the seminal vesicles; a decrease in the concentration and motility of spermatozoa.
3. The conducted studies show that under the conditions of experimental varicocele in sexually mature male rats there is a significant impairment of the functional state of the testicles against the background of a decrease in the gonadotropic activity of the pituitary gland.
4. Experimental varicocele associated with violation of the morphofunctional state of the mitochondria of Sertoli and Leydig cells and damage to the nuclear membrane.
5. Pathological changes in the testicular tissue on the background of experimental varicocele are accompanied by suppression of the spermatogenesis process: decrease the number of stem cells (spermatogonia), a delay of the differentiation of germ cells (spermatids → spermatozoa), as a result of these processes spermatogenesis index decreases.
6. Under the condition of experimental varicocele the level of androgen saturation of the body also decreases, there is obviously related to changes in the synthesis of steroidogenesis in glandulocytes.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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