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EXPERIMENTAL STUDY OF MORPHO-FUNCTIONAL CHANGES IN THE TESTES OF RATS UNDER STRESS

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Annotations
Immobilization stress has both physical and psychoemotional effects on animals. Many authors have noted the negative impact of this type of stress on the state of the male reproductive system. This work shows the negative effect of short-term immobilization (3 hours) on the state of the antioxidant status of spermatozoa and the morphology of the testes of rats. The study was carried out on 24 sexually mature male outbred white rats.

Keywords: rats, stress, testes, antioxidant status, oxidative stress morphology.

Stress is a nonspecific general response of the body to the action of various damaging factors that threaten homeostasis [1]. According to the works of G. Selye, under stress, the systems of neuro-humoral regulation are activated under the influence of the so-called “primary mediator”. According to modern concepts, its role is played by free radicals and lipid peroxidation products. Accordingly, a change in the redox balance triggers a complex of protective and adaptive reactions, and can also negatively affect the morphological characteristics of organs and tissues, causing oxidative stress [2]. In recent years, more and more scientific data have appeared on the influence of various experimental types of stress on the reproductive system of animals [3]. The development of preventive measures for the protection of reproductive health and heredity is currently acquiring special relevance in connection with the increasing adverse effects of a complex of social, environmental and professional factors. Recent studies have shown that the share of the influence of the state of male reproductive health on the reproduction of healthy offspring is more than 50%. At the same time, the frequency of reproductive dysfunctions in men reaches 48% [4]. The reproductive function of men is influenced by social and hygienic factors, the leading of which are the place of work, occupational hazard and work experience in production, the severity of labor, low per capita income, education and marital status. Among the medical and biological factors, the main ones are congenital defects of the reproductive system, past diseases and their complications. Lifestyle and

environmental factors play a significant role, the share of the influence of which, according to WHO estimates, can reach 50 and 18-20%, respectively [8].

The action of pulmonary fibrosis significantly disrupts the balance of metabolism, which maintains the integrity of structures and homeostasis in the cells of various tissues of the body. Of all the organs of the male reproductive system, the testis and epididymis are the so-called universal experimental organs, where the influence of factors, to one degree or another, can be studied quickly and clearly for preliminary reports and also for long-term results. Moreover, according to modern concepts, the epididymis is an accessory gonad, in which the final maturation and accumulation of mature sperm takes place [6].

The negative effect of subacute and chronic stress, which indirectly acts through the development of oxidative stress on the state of the testes in rats and mice, has been studied [7, 8]. However, changes in the organs of the male reproductive system of animals as a result of shorter-term immobilization have not been fully studied.

Purpose of work. To study the state of the antioxidant status of spermatozoa and morphological changes in the testes of outbred white rats under the action of an acute 3-hour immobilization stress.

Materials and methods

An experimental study was carried out on 24 sexually mature male outbred white rats weighing 250.0 g (230.0; 265.0), at the age of 8-10 months. The animals were kept in standard vivarium conditions with free access to food and water and a 12-hour daylight regime. The rats were divided into 2 groups with no statistically significant differences in weight. The animals of the experimental group (n = 11) were exposed to acute immobilization stress. Experimental animals were placed in an individual plastic container (restricting movement), adjusted to the size of the animal, with free access of air. The time spent by the rats in the immobilizers was 3 hours [9]. Intact rats made up the control group (n = 13). In order to level the influence of the time factor on the functional state of animals, all studies were carried out in the first half of the day from 8 to 12 hours. Experiments on animals were carried out in accordance with the Declaration of Helsinki by the World Medical Association on the humane treatment of animals (edition - October 2008) [10].

At the end of the experiment, animals of both groups were weighed, then decapitated. After decapitation, blood was taken from the animals, the testes with their appendages were isolated, the organs were weighed and their weight was assessed with an accuracy of 1 mg. The epididymis was cut longitudinally, and spermatozoa were removed from the tail by dosed washing with distilled water for 2 min. The semen washes were centrifuged at 1800 rpm for 10 minutes in an MPW 210 centrifuge

(Poland). The sedimentary portion of the lysed spermatozoa was collected. Sperm lysis was monitored using microscopy.

The antioxidant status (AOS) was determined in the sperm lysate by the method of T. V. Sirota (patent No. 2144674, Russia, 2000) modified by A. I. Gritsuk et al. at the Department of Biological Chemistry, Gomel State Medical University [11]. In the course of the conversion of adrenaline through adrenaline quinone into adrenochrome, superoxide radicals arise, initiating autooxidation of adrenaline in an alkaline environment. The antioxidant system, intercepting superoxide radicals, inhibits the formation of adrenochrome. The ability of biological fluids (sperm lysate) to inhibit the autooxidation of adrenaline in an alkaline medium was regarded as antioxidant activity (+ 1 c.u.), to activate - as prooxidant (-1 c.u.).

In order to exclude the influence of the anatomical features of the blood supply on the result of the study, the right testis was chosen to assess morphological changes [12]. Testes were fixed in 10% neutral buffered formalin (Lilly) for 24 hours at room temperature. Histological wiring was performed using isopropyl alcohol [13]. Testes were embedded in paraffin and transverse serial sections with a thickness of 5 μm were made using a Leica RM 2125 microtome (Germany). Sections were performed in ethyl alcohol and xylene, stained with hematoxylin (according to Mayer) and eosin. The colored preparations were embedded in polystyrene under a cover glass.

The study of the microstructure of the testes was carried out on a light microscope Nicon Eclipse 50i (Japan) at a total magnification of * 40, x100, * 200. Sections were photographed using a DS-F1 camera.

The number of convoluted seminiferous (ISC) tubules in 10 fields of view was assessed using a magnification of 10 * 10. The area of the visual fields with a magnification of 10 * 10 was 1200.9 * 990.2 = 1189179.2 μm2. We measured the diameter of the transversely cut ISC and the thickness of the germinal layer in μm [14].

To assess the state of spermatogenesis, the number of ISCs with the 12th stage of meiosis was counted in 100 transversely cut ISCs (an increase of 10 * 40) [14, 15].

The average number of Sertoli cells in the ISC was calculated in 20 transversely cut ISC (magnification 10 * 40) [14].

Statistical processing of the research results was carried out using the software package "Statistica", 8.0. Due to the fact that most of the studied characteristics did not obey the law of normal distribution (Shapiro - Wilkie test, W), the nonparametric Mann - Whitney test (U) was used to compare the indicators in two independent groups. The data in the text and the table are given as Me (Qi; Q3), where Me is the median, Q1; Q3 - upper and lower quarters. Differences between indicators were considered statistically significant at $p < 0.05$ [16].

Results and Discussions

Oxidative stress is the result of an imbalance between the activity of the body's antioxidant systems and the amount of reactive oxygen species (ROS) and nitrogen (APA) produced [1, 11]. Determination of the antioxidant status of biological fluids makes it possible to assess the state of the balance between the pro- and antioxidant systems.

In animals that underwent acute immobilization stress, there was a statistically significant decrease in the AOS of spermatozoa - 10.5 (-2.6; 28.3) c.u. as compared with intact animals - 23.9 (19.6; 36.8) conventional units. (p = 0.023). In 36.4% of the rats of the experimental group, prooxidant activity of spermatozoa was observed, which indicates the development of oxidative stress in them. Immobilization stress is a mixed type of stress that has both physical and psychoemotional effects [2]. This type of stress realizes its influence through changes in neuro-humoral regulation mechanisms, causing a decrease in the production of male sex hormones. It also causes the activation of NO synthases, which, in turn, causes the formation of AFA [5]. It has been shown that an excess of ROS and AFA has a different effect on sustentocytes and cells of spermatogenic epithelium at different stages of development [15]. In the early stages of spermatogenesis, oxidative stress can disrupt the process of cell division and cause “meiotic arrest,” and in mature spermatozoa, it can have a damaging effect on the mitochondrial genome, followed by insufficiency of cellular respiration [4].

Macroscopic examination of the rats of the experimental group showed the testes were edematous, of a loose consistency, had a deep red color, and full-blooded blood vessels were detected on the surface. The weight of the testes in animals of the experimental and control groups did not have statistically significant differences.

Microscopic examination of the testes showed that the number of ISCs in rats of the experimental group did not have statistically significant differences in comparison with animals of the control group and amounted, respectively, 122.0 (101.0; 126.0) and 121.0 (112.0; 131 , 0) (p> 0.05).

It is known that an important quantitative indicator indicating the inhibition of spermatogenesis in the testes of rats is the diameter of the ISC [1]. A decrease in the diameter of the testes tubules under the action of extreme factors on the animal organism has been noted by many authors. In studies by J. S. Tash et al. a decrease in the diameter of the ISC in the testes of rats under hypokinetic stress was revealed [12]. During the morphometric analysis of the testes of rats, a statistically significant decrease in the diameter of the ISC was also revealed in animals subjected to 3-hour

immobilization stress - 243.1 (225.8; 252.7) μm versus 283.8 (270.5; 300.7) μm in the control group (p <0.0001).

It is known that the diameter of the ISC is closely related to the number of cells in the epithelialospermatogenic layer [9]. A decrease in this morphometric indicator is accompanied by a decrease in the number of spermatogenic cells in the lumen of the tubule [3]. Our results showed that a decrease in the diameter of the ISC is accompanied by a statistically significant decrease in the thickness of the germinal layer of the ISC in the experimental group of animals - 61.8 (57.5; 65.8) microns versus 93.7 (91.4; 95.6) microns in control group (p <0.0001).

A reduction in the number of spermatogenesis cells, accompanied by a decrease in the thickness of the germinal layer of the ISC, may be associated with disturbances in both karyokinetic and meiotic division of germ cells as a result of oxidative stress. Disorders of the processes of meiotic division of germ cells in our study are evidenced by a statistically significant increase in the number of ISCs with the 12th phase of meiosis in the testes of rats of the experimental group (Table 1).

Table 1 - The number of Sertoli and ISK cells with the 12th phase of meiosis in the testes of rats in the experimental and control groups

Options	Experienced group,	Control group,	p
ISK with the 12th phase of meiosis	6,0 (6,0; 8,0)	3,0 (1,0; 5,0)	< 0,001
Sertoli cells	21,2 (18,7; 21,8)	21,3 (20, 1;22,2)	0,297

An increase in the amount of ISCs with the 12th phase of meiosis in animals subjected to immobilization stress may indicate “meiotic arrest” [5]. This may be due to oxidative stress, accompanied by impaired mitochondrial respiratory activity in developing germ cells [7].

It has been shown that a decrease in the ISC diameter is caused not only by a reduction in the number of germ cells in the epithelialospermatogenic layer, but also by a decrease in the secretion of the liquid medium of the tubule by Sertoli cells [12]. In the studies of O. N. Shevantaeva et al. it was shown that oxidative stress caused by acute hypobaric hypoxia leads to a decrease in the number of Sertoli cells only on the 3rd day after modeling the terminal state in animals [13]. Our studies have shown that short-term immobilization stress does not affect the number of Sertoli cells and it does not differ statistically significantly from the control values. However, the action of immobilization stress leads to changes in the morphological characteristics of Sertoli cells.

Thus, in the testes of the animals of the experimental group, most of the Sertoli cells were dissociated and located in isolation from each other. Many of them lost most of their cytoplasm, which was rejected into the lumen of the ISK. Some Sertoli cells acquired a flattened shape with sometimes flattened hyperchromic nuclei. The cytoplasm of most Sertoli cells had a foamy, cellular character.

Such morphological changes in Sertoli cells can be associated with the phenomena of extracellular and intracellular edema in the spermatogenic epithelium, observed in the testes of rats under conditions of a single immobilization stress [4].

These morphological changes in the testes negatively affect the reproductive capacity of animals and may be a prerequisite for the development of disturbances in the process of spermatogenesis in men under conditions of oxidative stress.

Conclusions

Acute 3 h immobilization stress leads to a decrease in the antioxidant status of spermatozoa in male outbred white rats. Morphological changes in the testes in rats under conditions of acute immobilization stress are accompanied by impaired microcirculation, the development of edema, and degenerative changes in the epithelium of the convoluted seminiferous tubules.

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