МОРФОЛОГИЯ, ФИЗИОЛОГИЯ И ПАТОФИЗИОЛОГИЯ
MORPHOLOGY, PHYSIOLOGY AND PATHOPHYSIOLOGY

EFFECTS OF EMISSIONS FROM FOREST FIRES ON THE MORPHOFUNCTIONAL STATE OF REPRODUCTIVE SYSTEM OF WHITE RATS

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ABSTRACT

Introduction. *At present, considering active participation of people in the elimination of wildfires, the study of the effects of smoke on the reproductive system is an urgent scientific problem.*

Materials and methods. *Sexually mature outbred male rats were exposed to a daily four-hour inhalation exposure to smoke for one month. Immediately after the end of the exposure, some of the animals were removed from the experiment, and some were left to survive for two months. The study on testicular samples included counting the total number of spermatogonia, the number of tubules with desquamated epithelium, as well as determining the spermatogenesis index and the number of Leydig cells.*

Results. *The effect of forest fire smoke on the reproductive system of model animals (outbred laboratory rats, males) was characterized by a decrease in the spermatogenesis index with a simultaneous decrease in the percentage of spermatogonia and the number of Leydig interstitial cells. The indices of the reproductive system of animals left for survival did not differ from the control values.*

Discussion. *The results of this study suggest that prolonged exposure to combustion products released during a forest fire negatively affects the reproductive function of male laboratory rats examined immediately after the end of the exposure. After the course of the standard cycle of spertatogenesis, the indicators characterizing the development of sperm and the generation of male sex hormones are restored to normal, which indicates a high plasticity of the male reproductive system in response to the adverse environmental factor – smoke of forest fires.*

Key words: *fire, smoke, rats, reproductive system*

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ВОЗДЕЙСТВИЕ ЭМИССИИ ОТ ЛЕСНЫХ ПОЖАРОВ НА МОРФОФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ РЕПРОДУКТИВНОЙ СИСТЕМЫ БЕЛЫХ КРЫС

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РЕЗЮМЕ

Введение. *В настоящее время, с учётом активного участия людей в ликвидации природных пожаров, изучение последствий воздействия дыма на репродуктивную систему является актуальной научной проблемой.*

Материалы и методы. *Половозрелых беспородных крыс-самцов подвергали ежедневному четырёхчасовому ингаляционному воздействию дыма в течение одного месяца. Сразу после окончания воздействия часть животных выводили из эксперимента, часть оставляли на дожитие в течение двух месяцев. Исследование на образцах семенников включало в себя подсчёт общего количества сперматогониев, числа канальцев со слущенным эпителием, а также определение индекса сперматогенеза и количества клеток Лейдига.*

Результаты. *Воздействие дыма лесного пожара на репродуктивную систему модельных животных (беспородные лабораторные крысы-самцы) характеризовалось снижением индекса сперматогенеза с одновременным сокращением процентного содержания сперматогониев и количества интерстициальных клеток Лейдига. Показатели репродуктивной системы животных, оставленных на дожитие, от контрольных значений не отличались.*

Обсуждение. *Результаты проведённого исследования позволяют полагать, что длительное воздействие выделяющихся при лесном пожаре продуктов горения негативно сказывается на репродуктивную функцию лабораторных крыс-самцов, обследованных сразу после окончания экспозиции. После протекания стандартного цикла сперматогенеза показатели, характеризующие развитие сперматозоидов и генерации мужских половых гормонов, восстанавливаются до нормальных, что говорит о высокой пластичности мужской репродуктивной системы в ответ на действие неблагоприятного экологического фактора – дыма лесных пожаров.*

Ключевые слова: *пожар, дым, крысы, репродуктивная система*

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INTRODUCTION

One of the most important environmental and technogenic factors, which adversely affect human health, are the products of combustion of organic substances. They pollute the air everywhere with varying intensity in different countries of the world, including Russia. According to Federal Forestry Agency, in 2019 the area covered by natural fires amounted to approximately 1 % of the area of the entire forest fund of the country [1]. In some regions of Russia, natural fires occur with an annual frequency [2], while smoke reaching settlements has a negative impact on the health of the population. All this determines the need to assess the risk of the consequences of the emissions effects from forest fires for the health of various cohorts of the population and to develop new approaches to prevention. Currently, scientific research is mainly aimed at studying the effect of large wildfires on the respiratory and cardiovascular systems [3–5]. The authors report about the sustained increase in hospital admissions in the exposed population with asthma and other concomitant upper respiratory tract diseases. However, one cannot ignore the fact that a significant amount of potential repro- and genotoxicants (polycyclic aromatic hydrocarbons, acetaldehyde, formaldehyde, benzene, toluene, chloromethane, etc.) are observed in the smoke of natural fires, while studies devoted to the effects of products of combustion of forest biomass on the reproductive system are extremely insignificant in the scientific press. There are publications which link wildfires with a decrease in the average weight of fetuses exposed in the womb [6]. However, domestic and foreign publications do not have enough direct evidence of the relationship between the exposure to smoke of combustion products of natural fires and dysfunction of the reproductive system. Considering the fact that the vast majority of the personnel involved in elimination of wildfires are males, the study of the reproductive toxicity of emissions from forest fires to male reproductive system seems to be extremely relevant.

The purpose of this study was to estimate the dynamic of morphofunctional state of reproductive system of male rats exposed to smoke of natural fire.

MATERIALS AND METHODS

Objects of study

Experimental studies were carried out on 60 mature outbred white male rats weighing 180–240 g. An experimental installation for reproducing the conditions of exposure to combustion products included an exposure chamber with a volume of 200 liters, designed to accommodate experimental animals and equipped with devices for controlling the air environment (temperature, humidity), and a chamber where the substrate smoldered. We used biomass as a combustible substrate, consisting of a mixture of forest litter, twigs, pieces of bark and the upper soil horizon. This biomass was sampled in an ecologically favorable area, at a great distance from industrial enterprises and highways.

The animals of the experimental group (n = 30) were subjected to subacute inhalation exposure to smoke for 1 month for 4 hours a day (which corresponds to approximately four months of firefighters' work) in a special exposure chamber with the concentrations of the main components of the smoke presented by us earlier [7]. The air temperature in the exposure chambers was 24–25 °C, the relative humidity was 40–50 %. Rats of the control group (n = 30) were supplied with clean air into the chambers. Fifteen animals from the experimental and control groups were sacrificed by decapitation under light ether anesthesia immediately after the end of exposure to combustion products. These animals constituted the early survey period (ESP). The remaining 15 animals in each group were left to survive for 2 months (to undergo a full cycle of spermatogenesis) and made up a long-term survey period (LTSP). The morphofunctional state of the reproductive system of male rats was assessed using the methods described earlier [7].

All experimental animals were obtained by their own reproduction in the vivarium of the East-Siberian Institute of Medical and Ecological Research and were kept on a standard diet. The work was carried out in compliance with the rules of humane treatment of animals in accordance with the requirements of the International Recommendations for Biomedical Research Using Animals (WHO, Geneva, 1985) and the Rules of Laboratory Practice of the Ministry of Health and Social Development of Russia.

Methods of genotoxicity study

To assess the state of the spermatogenic epithelium, the animals were sacrificed by decapitation under light ether anesthesia for microscopic examination immediately after the exposure. The testicles were removed, their external examination was carried out, then they were fixed in a 10% neutral buffered formalin solution with a pH of 7.4; sections 3–5 microns thick were prepared from paraffin blocks using an MS-1 sledge microtome (Russia), which were stained with hematoxylin and eosin according to the following algorithm: the sections were deparaffinized with xylene or toluene for at least 10 minutes, then they were passed through alcohols of decreasing concentration with an exposure of 3 minutes and brought to the water. Sections were well washed and kept in distilled water for 5–10 minutes. Then hematoxylin was applied for 3–5 minutes. After that, the samples were washed again with tap water and hydrochloric alcohol was applied for 10–15 seconds. To remove excess dye, the samples were left in tap water for 10 minutes and then eosin was applied for 30 seconds. Sections were washed again in tap water and 96% alcohol was applied. At the final stage, the samples were kept in carbol-xylene for 10–20 minutes and in ortho-xylene for 1 hour, after which they were embedded in polystyrene. Stained histological preparations were analyzed at low and high magnification using an Olympus BX 51 microscope. Microimages of brain sections were entered into a computer using an Olympus E420 camera. The obtained images were processed using the techniques included in the Image Scope M software package. On histological sections, the spermato-

genesis index was calculated morphometrically (according to the formula:

$$Is = \sum a/N,$$

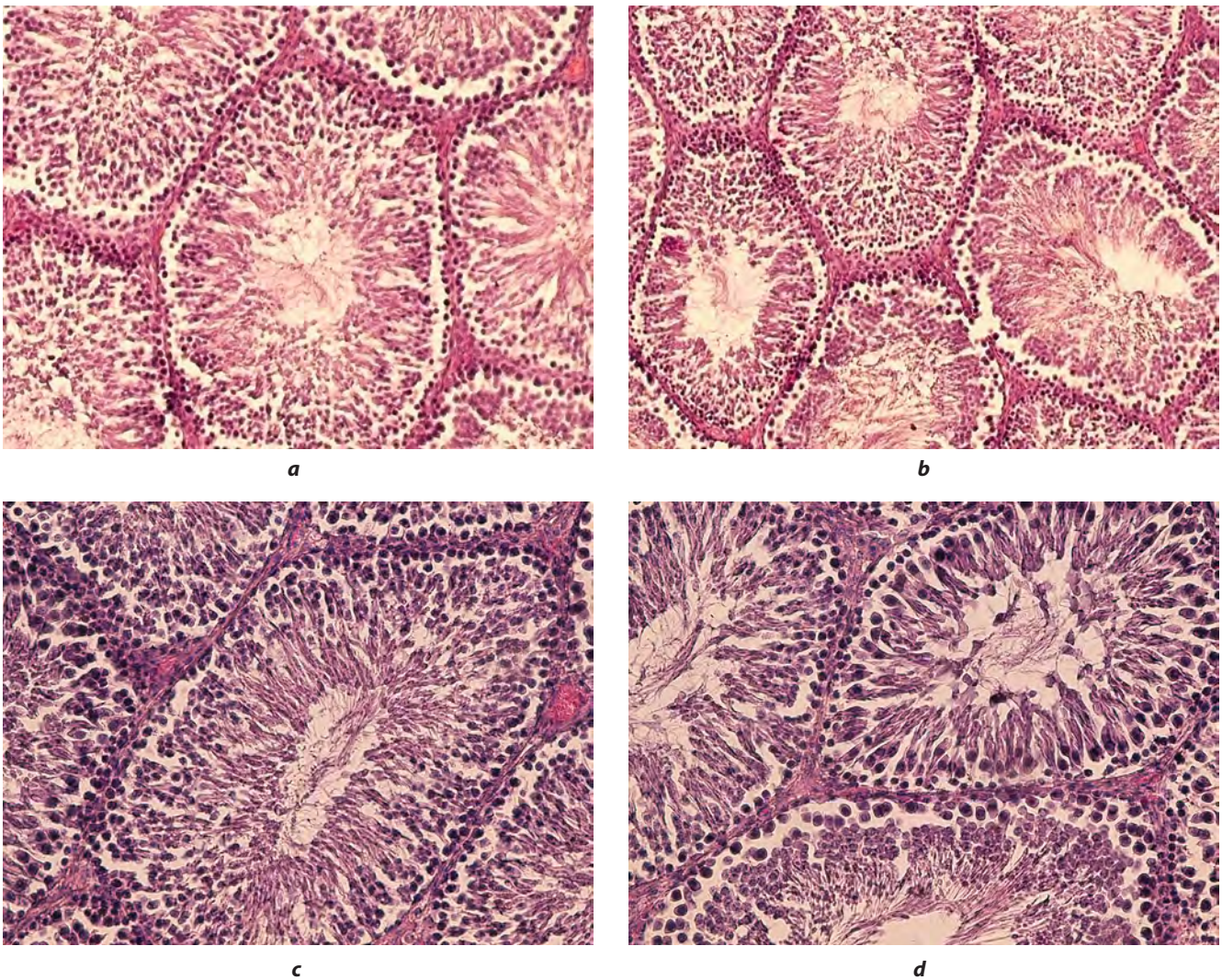
where a is the number of layers isolated in each tubule (the first layer is spermatogonia, the second – spermatocytes, the third – spermatids, the fourth – spermatozoa); N – the number of counted tubules [8]). We also calculated indicators characterizing the functional state of the reproductive system: the number of tubules with desquamated epithelium, the average number of spermatogonia and the relative number of Leydig cells.

Statistical methods

Statistical analysis of the research results was carried out using the Statistica 6.1 software package (StatSoft Inc., USA) (face No. AXXR004E642326FA) [9]. The Shapiro – Wilk W-test was used to make a decision on the type of feature distribution. The Mann – Whitney U-test was used to compare the groups. Null hypotheses about the absence of differences between the groups were rejected at the achieved significance level of $p \leq 0.05$. Results are presented as median and interquartile range (Me (LQ; UQ)).

RESULTS

An external examination of the testes of male rats showed that they were pinkish-white in color, soft-elastic in consistency, and elliptical in shape. With a small magnification of the microscope, a pink stripe is visible along the edge of the preparation – this is a protein membrane, consisting of dense, unformed connective tissue. The bulk of the testis is formed by seminiferous convoluted tubules, cut across or obliquely (tangentially), rounded or elliptical in shape. The seminiferous tubules are separated from each other by a thin membrane of interstitial connective tissue, under which there is a thick wall of spermatogenic epithelium at different stages of development. The areas of interstitium between the convoluted seminiferous tubules are evenly spaced, mostly triangular in shape. In the center



a ***b*** ***c*** ***d***

FIG. 1.

*Microphoto of testes of white rats of the control (**a**, **c**) and experimental (**b**, **d**) groups of early and long-term survey period. Hematoxylin-eosin. Mag. ×400*

TABLE 1

RESULTS OF MORPHOMETRIC STUDY OF THE TESTES OF WHITE MALE RATS

Indicator	Animal groups	
	control	experimental
Spermatogenesis index	4,0 (3,49; 4,21) 4,02 (3,63; 4,31)	3,09 (1,08; 4,09)* 3,78 (2,87; 4,67)#
Number of tubules with desquamated epithelium, %	0,8 (0,6; 1) 0,9 (0,7; 1,1)	0,8 (0,6; 0,9) 0,9 (0,6; 1,0)
Average number of spermatogonia, %	40,3 (38,9; 41,9) 41,1 (36,8; 43,1)	38,7 (29,8; 42,7)* 40,7 (36,1; 43,7)
Relative amount of Leydig cells (in terms of a cross section of one convoluted seminiferous tubule)	11,0 (10,8–13,0) 11,4 (10,4–13,2)	9,0 (8,7–10,0)* 11,0 (9,8–12,6)#

Note. Above the line – early survey period; under the line – long-term survey period; * – differences are statistically significant compared with control at $p \leq 0.05$; # – differences are statistically significant in comparison with the experimental group of the early period of the survey at $p \leq 0.05$.

of the convoluted tubule there is a lumen where the formed spermatozoa exit.

At high magnification, it can be seen that the own shell of the tubule is built from connective tissue fibers. Outside of the basement membrane is a layer of loose connective tissue, which contains a layer of myoid cells that have a scaly, lunate and elongated shape. Myoid cells are located evenly along the entire contour of the convoluted seminiferous tubule. Inside from its own shell, separated by a basement membrane, is the spermatogenic epithelium (Fig. 1).

The results of a morphometric study of the testes of white rats in the early and late periods of the survey are presented in Table 1.

It was revealed that the subacute inhalation effect of forest fire smoke negatively affects the reproductive function and leads to its suppression, which is expressed in a statistically significant decrease in the spermatogenesis index, as well as a decrease in the number of spermatogonia and the relative number of Leydig cells. The values of the indicators of the reproductive system of ESP and LTSP animals did not differ from those in the control animals. However, there was a statistically significant increase in the spermatogenesis index ($p \leq 0.05$) and an increase in the relative number of Leydig cells ($p \leq 0.05$), which indicates the restoration of reproductive function over time.

DISCUSSION

It is known that male reproductive health can be a sensitive marker of the impact of environmental factors, including forest fire smoke [10]. In a real situation, when a natural fire is extinguished, it is males (firefighters) who are most exposed to combustion products. The scientific literature shows the relationship between an unfavorable environmental situation and male infertility [11]. In men who are in a sterile marriage and are exposed to exogenous exposure to toxic factors, morphological disorders and functionally defective spermatozoa have been identified [12].

Previously performed model experiments showed the absence of disturbances in the functional state of the testes during subacute exposure to smoke on male rats for one week for four hours, which in total amounted to 28 hours, and corresponds to 35–40 days of exposure to humans. In real conditions of a fire hazardous period, firefighters and rescuers can be in smoke for up to 2–4 months, since when one fire is eliminated, they are transferred to another. In this regard, we increased the time of inhalation exposure to smoke on male rats for 4 weeks, which can be compared with four months of work of firefighters in the centers of smoke. Of particular interest was the dynamic observation of the state of the testes of rats after the end of the exposure, when the full cycle of spermatogenesis was completed. As a result of the carried out experimental modeling on the effect of forest biomass combustion products on male rats, the gonadotoxic effect of exposure in ESP animals was revealed. It was shown that exposure to forest fire smoke for 4 weeks led to a significant decrease in the spermatogenesis index, which is the most important quantitative indicator and characterizes the generative function of the testes. In the dynamics of observation, the spermatogenesis index practically returns to the initial values, while statistically not differing from the control values in LTSP. Also, in animals in the early period of examination, a decrease in the number of spermatogonia (pluripotent stem cells of the spermatogenic epithelium) and the relative number of Leydig cells – the site of synthesis and secretion of androgens, including testosterone, which plays an important role in the regulation of reproduction processes in males. At the same time, as with the spermatogenesis index, over time, these indicators practically did not differ from the control values. Changes in all of the above indicators indicate the inhibition of the process of spermatogenesis. However, over time and full cycle of sperm development [13], the indicators characterizing the functional state of the reproductive system return to normal, which is confirmed by the results obtained in the long-term period of the examination. This fact indicates that the changes in the studied functional indicators under the influence of forest fire smoke are reversible, and the male reproductive system itself is quite plastic, since the initial indicators are restored after passing through the spermatogenesis phase.

In general, the experimentally revealed effect of air smoke during forest fires on male reproductive health justifies the need for in-depth studies aimed at understanding the mechanisms of action of the components that make up the smoke mixture. In addition, the results obtained in modeling the combustion of forest biomass raise the problem of the long-term effects of exposure to offspring obtained from exposed males. The solution of these issues will reveal the mechanisms of the possible formation of an infertile state in men participating in the elimination of emergency situations associated with natural fires and identify ways to diagnose and prevent adverse effects on the male body of prolonged stay in the center of smoke.

Conflict of interest

The authors declare no conflict of interest.

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