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Changes in some parameters of the reproductive system of male rats following lead exposure and the effect of bioprophylaxis

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ABSTRACT

Introduction. Current challenges in toxicology include the development and modernization of biological prevention strategies to reduce the risks of long-term toxic effects of lead.

The aim of this study was to determine changes in certain biochemical and histomorphological parameters of the reproductive system of male rats following subchronic exposure to lead acetate and to establish efficacy of a bioprophylactic complex (hereinafter referred to as BPC) specially developed to reduce the severity of toxic effects.

Material and methods. The experiment was conducted on albino outbred male rats aged from 4 to 6 months. The administration was carried out orally, daily for 45 days. The dose of lead acetate was 819 mg/L. Some of the animals received a vitamin and mineral complex, the composition of which was selected based on the mechanisms of lead toxicity. The effectiveness of BPC and the toxic effects of lead acetate were evaluated 21 days after the end of the exposure.

Results. According to the obtained data, lead exposure resulted in a significant decrease in the lumen of the seminiferous tubules and an increase in the proportion of Sertoli cells with degenerative and dystrophic changes. A significantly higher number of abnormal spermatozoa in testicular smears was detected in rats treated with lead acetate compared with control. An increase in the activity of the enzyme β -hydroxysteroid dehydrogenase was observed in exposed rats compared to controls.

Although this study did not measure sex hormone levels in the blood of laboratory animals, we believe that the observed increase in HSD3b1 is more likely a consequence of compensatory responses to the destruction and/or dysfunction of cells responsible for androgen synthesis than a direct consequence of lead toxicity.

Limitations. We did not assess sex hormone levels or the activity of the HSD3b2 isoform of the 3bHSD enzyme.

Conclusion. Male rats exposed to lead acetate showed a slight increase in the activity of β -hydroxysteroid dehydrogenase, an enzyme that plays an important role in regulating sex hormone synthesis and delta-ALA metabolism. The observed histomorphometric changes included a decrease in the lumen of the seminiferous tubules, an increase in the number of abnormal Sertoli cells and degenerated spermatozoa. Our findings prove the protective potential of biological prevention strategies but highlight the importance of further profound studies of the mechanisms of lead toxicity on the male reproductive system and the effects of BPC.

Keywords: lead; long-term effects; bioprophylaxis; BPC; reproductive system; in vivo; β -hydroxysteroid dehydrogenase; 3bHSD; HSD3b1

Compliance with ethical standards. The study was approved by the local Bioethics Committee of the Yekaterinburg Medical Research Center for Prophylaxis and Health Protection of Industrial Workers of Rospotrebnadzor (protocol 1A of February 3, 2025) and conducted in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) and Directive 2010/63/EC of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes.

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Изменение некоторых показателей мужской половой системы крыс при токсическом действии свинца и эффект биопротекции

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РЕЗЮМЕ

Введение. Разработка и модернизация стратегий биологической профилактики, позволяющих снижать риски отдалённых токсических эффектов свинца, относятся к актуальным задачам токсикологии.

Цель исследования — определение изменений некоторых биохимических и гистоморфологических показателей мужской половой системы крыс на фоне субхронического действия ацетата свинца и оценка эффективности разработанного биопротективного комплекса (далее — БПК), предназначенного для уменьшения выраженности этих токсических эффектов.

Материал и методы. Эксперимент проведён на белых беспородных крысах-самцах в возрасте от 4 до 6 месяцев. Введение осуществлялось перорально, ежедневно на протяжении 45 дней. Доза ацетата свинца составила при оральном приёме 819 мг/л. Часть животных получала витаминно-минеральный комплекс, состав которого подбирался исходя из особенностей механизмов токсического действия свинца. Эффективность БПК и токсические эффекты ацетата свинца оценивали через 21 день после окончания экспозиции.

Результаты. Согласно полученным данным, воздействие свинца привело к значимому уменьшению просвета семенных канальцев и увеличению доли клеток Сертоли с дегенеративно-дистрофическими изменениями. У крыс, получавших ацетат свинца, выявлено значимо большее количество патологических форм сперматозоидов в мазках-отпечатках семенников по сравнению с контрольными значениями. Наблюдалось увеличение активности фермента β -гидроксистероиддегидрогеназы у экспонированных крыс по сравнению с контролем.

В настоящем исследовании не было определено содержание половых гормонов в крови лабораторных животных, однако мы полагаем, что наблюдаемое повышение активности $HSD3\beta1$ является следствием компенсаторных реакций организма на разрушение и (или) дисфункцию клеток, ответственных за синтез андрогенов, а не прямым следствием токсического воздействия свинца.

Ограничения исследования. Нами не были оценены уровни половых гормонов, а также активность изоформы $HSD3\beta2$ фермента β -гидроксистероиддегидрогеназы.

Заключение. У крыс-самцов, экспонированных ацетатом свинца, наблюдалось некоторое повышение активности фермента β -гидроксистероиддегидрогеназы, играющего важную роль в регуляции синтеза половых гормонов, а также метаболизме дельта-АЛК. Выявлены гистоморфометрические изменения: уменьшение просвета семенных канальцев, увеличение количества патологических форм клеток Сертоли и дегенеративно изменённых сперматозоидов. Результаты подтверждают наличие протекторного потенциала стратегии биологической профилактики, однако сохраняется необходимость дальнейшего, более глубокого и детального изучения механизмов токсичности свинца на мужскую половую систему и эффектов БПК.

Ключевые слова: свинец; отдалённые эффекты; биопротекция; БПК; половая система; *in vivo*; β -гидроксистероиддегидрогеназа; $3\beta HSD$; $HSD3\beta1$

Соблюдение этических стандартов. Исследование одобрено локальной комиссией по биоэтике ФБУН ЕМНЦ ПОЗРПП Роспотребнадзора (протокол ЛЭК № 1А от 03.02.2025). Условия проведения эксперимента соответствовали Европейской конвенции о защите позвоночных животных ETS N 123 и директиве Европейского парламента и Совета Европейского союза 2010/63/ЕС от 22.09.2010 г. о защите животных, используемых для научных целей.

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Финансирование. Исследование не имело спонсорской поддержки.

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Introduction

Among the reasons for Russia's low demographic potential, exposure to harmful chemical factors of various origins occupies a significant place. The population of regions with developed production is traditionally exposed to a high complex chemical load in the habitat and working area¹. In this case, lead acts as one of the most widespread industrial pollutants that contaminate the environment, causing health risks for both humans and their offspring. Many studies have been conducted on the toxic effects of inorganic lead compounds, but the long-term effects as a serious threat to the health of future generations have not been sufficiently studied.

A key role in combating high chemical exposure is played by the strategy of biological prevention on the population, which increases the body's resistance, adaptation, and elimination capabilities. The developed bioprophylactic complexes (hereinafter referred to as BPC), which include harmless substances with protective properties, have demonstrated their effectiveness in previous experiments implemented on the basis of the Federal State Scientific Institution 'Yekaterinburg Medical Research Center for Prevention and Health Promotion of Industrial Workers' of Rospotrebnadzor [1–3]. Current challenges in toxicology include the development and modernization of biological prevention strategies to reduce the risks of long-term toxic effects of lead.

The aim of this study was to determine changes in certain biochemical and histomorphological parameters of the reproductive system of male rats following subchronic exposure to lead acetate and to establish efficacy of a bioprophylactic complex (hereinafter referred to as BPC) specially developed to reduce the severity of these toxic effects

Material and methods

The study was approved by the Local Bioethics Committee of the Federal State Scientific Institution 'Yekaterinburg Medical Research Center for Prevention and Health Promotion of Industrial Workers' of Rospotrebnadzor (protocol LEC No. 1A

dated February 3, 2025). The experiment was conducted on albino outbred male rats aged from 4 to 6 months (Andreevka branch of the Federal State Scientific Institution 'Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency of Russia', 141551, Moscow Region, Solnechnogorsk, Andreevka settlement, bldg. 49. Certificate No. 181164, issue date: 09.12.2024) Before the experiment, the rats were kept in quarantine, after which they were allowed to enter the vivarium, which was equipped as per GOST 33216–20142. The rats received specialized diet as per GOST R 50258–92³ and purified drinking water. The temperature, humidity, and air flow were carefully controlled to ensure comfort and safety for the laboratory animals during the experiment. A day-night cycle was artificially created in the animal housing areas, simulating sunset and sunrise. Daily veterinary inspections were conducted throughout the experiment.

Four groups, each consisting of eight male rats, were formed for the experiment.

1. The control group was not exposed.
2. The *Pb* group received lead acetate orally for 45 days at a dose of 819 mg/L.
3. The *Pb* + *BPC* group received the bioprophylactic complex along with lead acetate exposure.
4. The *BPC* group received the bioprophylactic complex without lead acetate exposure.

For the composition of the bioprophylactic complex and dosages for laboratory animals, see **Table**.

Collected whole blood from the rats twenty-one days after the end of exposure for subsequent assessment of the content of the HSD3b1 isoform of the enzyme 3 β -hydroxysteroid dehydrogenase (hereinafter referred to as 3bHSD) in the serum, as well as the testes for morphometric analysis of histological specimens (hematoxylin and eosin staining) and cytomorphological analysis of smears (Leishman staining). Determined HSD3b1 isoform content using an enzyme-linked immunosorbent assay according to the kit instructions. Evaluated during the histomorphological analysis changes in

¹ On the State of Sanitary-Epidemiological Well-Being of the Population in the Russian Federation in 2024: State Report. Moscow: Federal Service for Supervision in Healthcare Federal Service for Supervision on Consumer Rights Protection and Human Wellbeing, 2025. 424 p.

² GOST 33216–2014 Guidelines for Accommodation and Care of Animals. Species-Specific Provisions for Laboratory Rodents and Rabbits.

³ GOST R 50258–92 Complete Compound Feeds for Laboratory Animals. Specifications.

Composition and dosage of the bioprophyllactic complex**Состав и дозировка биопрофилактического комплекса**

Препарат Preparation	Доза на 1 крысу в сутки Dose per rat a day
Кальций, мг / Calcium, mg	160
Железо, мг / Iron, mg	1
Йод, мкг / Iodine, µg	4
Глицин, мг / Glycine, mg	12
Глутаминовая кислота, мг / Glutamic acid, mg	160
Рыбий жир с содержанием 35% ПНЖК класса омега-3, мг Fish oil containing 35% omega-3 PUFAs, mg	133
Пектин, мг / Pectin, mg	200
Витамин А, мкг / Vitamin A, µg	35.2
Витамин С, мг / Vitamin C, mg	3.0
Витамин Е, мг / Vitamin E, mg	0.27
Витамин В1, мг / Vitamin B1, mg	0.038
Витамин В2, мг / Vitamin B2, mg	0.08
Витамин РР, мг / Vitamin PP, mg	0.3
Витамин В6, мг / Vitamin B6, mg	0.08
Витамин В9, мкг / Vitamin B9, µg	4
Витамин В12, мкг / Vitamin B12, µg	0.12
Витамин D3, мкг / Vitamin D3, µg	1.7

seminiferous tubule lumen, sperm head angle, and counted abnormal sperm forms and Sertoli cells with degenerative-dystrophic changes.

Performed statistical analysis using the Student's t-test and the Mann-Whitney U-test. Evaluated data normality using the Shapiro-Wilk test. The differences were considered statistically significant with a probability of at least 95% ($p < 0.05$).

Results

The study revealed that content of the HSD3b1 isoform of the 3bHSD enzyme exhibited a 1.5-fold increase in the *Pb* rat group compared to the *Control*, though these differences did not attain statistical significance. The rats of the *Pb + BPC* and *BPC* groups demonstrated values of this indicator equivalent to those of the *Control* (Fig. 1).

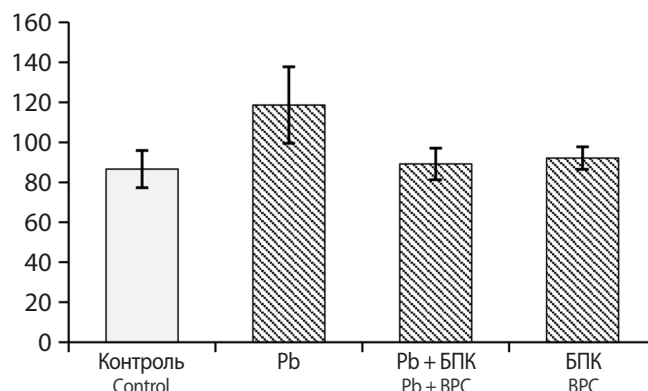


Fig. 1. Activity of the HSD3b1 enzyme in blood of experimental animals, pg/mL.

Рис. 1. Активность фермента HSD3b1 в крови экспериментальных лабораторных животных, пг/мл

A comparison of the histological pattern of the testes from the *Control* and the *BPC* group demonstrated no significant differences. Pathological morphological changes were not observed in either group. Microscopic analysis of histological sections showed that the testicular fibrous membrane consists of a thin layer of connective tissue. The organ's parenchyma is composed of convoluted seminiferous tubules with rounded or ellipsoid shapes, depending on the orientation of the passing section (either longitudinal or transverse). The outer sheath of the tubules is a very thin layer of annular connective tissue, with a smooth layer of myoid cells along the inner contour, including an inner myoid layer directly adjacent to the basement membrane and a layer of fibroblast-like cells. The spermatogenic epithelium layer is evenly distributed. Different stages of spermatogenesis were observed depending on the level of the sections. Nevertheless, all layers were preserved in almost all tubules: spermatozoa and late forms of spermatids were present in the greatest number, and Leydig cells were detected in the periphery of blood vessels in the amount of 3–5 cells per field of view.

The histological analysis of the *Pb* group showed a slight reduction in the number of spermatozoa and spermatocytes compared to the *Control*. This suggested a marginally lower level of spermatogenesis. In contrast to the *Control* and *BPC* groups, fewer Leydig cells were observed in the interstitium: 2–3 per field of view. At the same time, in the *Pb + BPC* group, the changes were less pronounced than in the *Pb* group.

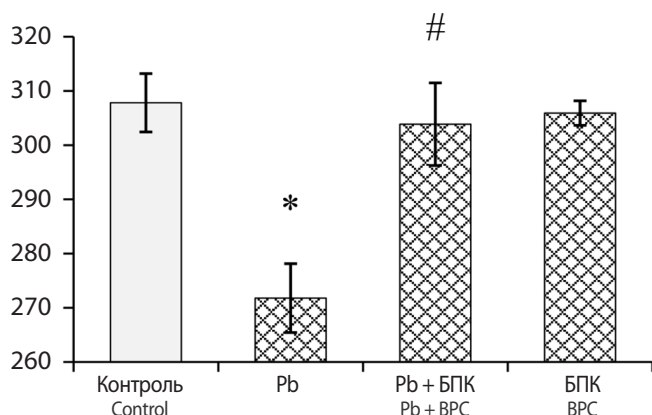


Fig. 3. Morphometry of the lumen of the seminiferous tubules, μm . * – statistically significant difference compared to the Control group; # – statistically significant difference compared to the Pb exposure group.

Рис. 3. Морфометрическая оценка просвета семенных канальцев, мкм. * – статистически значимое изменение по сравнению с группой *Контроль*; # – статистически значимое изменение по сравнению с группой *Pb*.

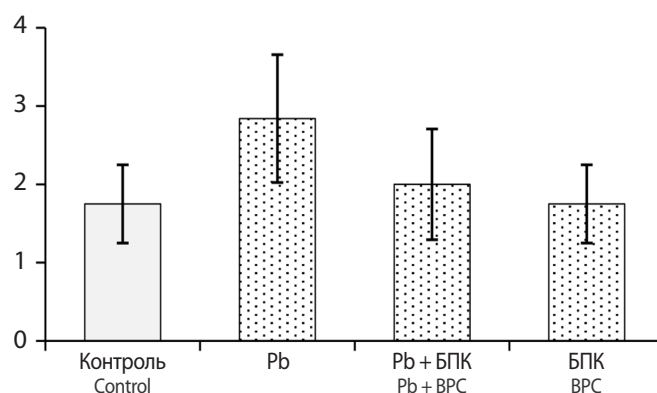


Fig. 4. Results of counting pathological forms of Sertoli cells with degenerative dystrophic changes in a testicular smear, units per 10 fields of view. * – statistically significant difference compared to the Control group.

Рис. 4. Результаты подсчета патологических форм клеток Сертоли с дегенеративно-дистрофическими изменениями в мазке-отпечатке семенников, ед. в 10 полях зрения. * – статистически значимое изменение по сравнению с группой *Контроль*.

The histological analysis showed that the number of spermatozoa and spermatocytes was slightly lower than in the *Control*, but higher than in the experimental group. A higher number of Leydig cells was also observed compared to the *Pb* group: 3–5 per field of view (**Fig. 2**, see on the insert).

According to the obtained data, lead exposure resulted in a significant decrease in the lumen of the seminiferous tubules in the *Pb* group. In the *Pb + BPC* group, changes were minor and consistent with those in the *Control* and *BPC* group (**Fig. 3**).

Analysis of testicular smears in the *Pb* group revealed a tendency to increase the number of degeneratively altered Sertoli cells. The *Pb + BPC* and *BPC* groups demonstrated values equivalent to those of the *Control* (**Fig. 4**).

A significant increase in abnormal sperm forms, particularly cells with altered head angles, was observed in the *Pb* group, compared to the control. However, changes in the *Pb + BPC* group were less pronounced or did not differ at all from control values (**Fig. 4, a, b**).

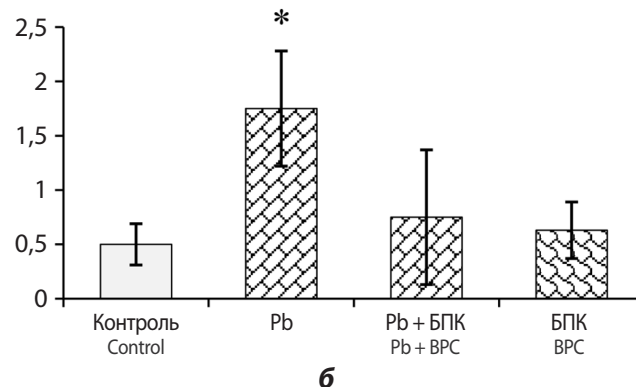
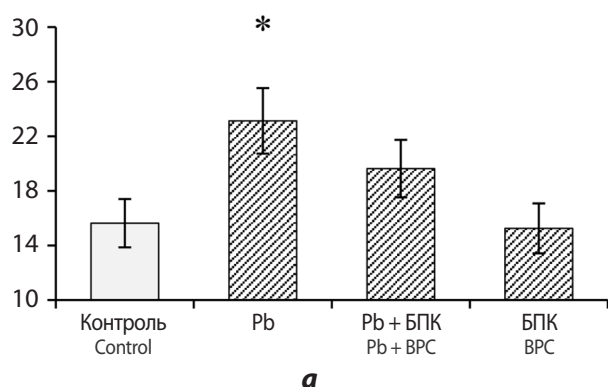


Fig. 5. Abnormal cell count in a testicular smear (U/200 cells): *a* – total number of abnormal spermatozoa; *b* – number of spermatozoa with a change in head angle. * – statistically significant difference compared to the Control group.

Рис. 5. Результаты подсчета патологических форм клеток в мазке-отпечатке семенников (ед./200 кл): *a* – общее количество патологических форм сперматозоидов; *b* – количество сперматозоидов с изменением угла головки. * – статистически значимое изменение по сравнению с группой *Контроль*.

Discussion

One of the key regulators of sex hormone synthesis is the membrane-bound enzyme 3 β HSD from the oxidoreductase class [4,5]. Several studies demonstrate that exposure to inorganic lead compounds results in a reduction of the 3 β HSD enzyme level in the testes [6–8]. Meanwhile, the increase in the content of the HSD3 β 1 isoform of the 3 β HSD enzyme in blood serum observed in the current study could be the result of the destruction of cells involved in androgen synthesis due to toxic lead exposure.

Leydig cells are known to be responsible for the synthesis of male sex hormones, particularly testosterone [10, 11]. The highest number of Leydig cells is observed during puberty, but their number gradually decreases with age. Testosterone is one of the main regulators of spermatogenesis and sexual function in general [12, 13]. In this study, we did not determine sex hormone levels, which does not allow us to objectively assess the disruption of androgen synthesis. Nevertheless, the results of the histomorphometric analysis suggest a positive effect of BPC on the functional activity of spermatogenesis mechanisms in exposed male rats.

It should be noted that changes in spermatogenesis parameters are likely related not only to the direct effect of lead acetate on seminal cells, but also to disturbances in the hypothalamic-pituitary system, which is responsible for neuroendocrine regulation of the reproductive system. Lead is known to inhibit the synthesis of luteinizing hormone [14, 15] in the anterior pituitary gland, one of whose main functions is to stimulate testosterone synthesis. This hypothesis is also supported by the results of our previous studies, which demonstrated the pronounced toxic effect of lead acetate on the central nervous system of laboratory animals and the neuroprotective effect of BPC [16,

17]. The identified changes only provide indirect evidence of impaired neurohumoral regulation, and confirmation of this hypothesis requires determination of sex hormone levels. However, based on the parameters studied, the developed BPC demonstrated a protective effect against lead acetate exposure.

Limitations of the study. We did not assess sex hormone levels, as well as the content of the HSD3 β 2 isoform of the 3 β HSD enzyme. To complete the study and substantiate the genotoxic risks of the factor under study, it is advisable to determine the expression of the *HSD3B1* and *HSD3B2* genes in the testes and adrenal glands. To determine the possible reasons of an increase in the content of the HSD3 β 1 enzyme in the blood, it is necessary to assess its level in tissues involved in the synthesis of androgens. We were limited to the use of a single test substance, route of administration, and exposure time.

Conclusion

According to the study results, male rats exposed to lead acetate showed a slight increase in the blood content of the enzyme 3 β -hydroxysteroid dehydrogenase, which plays an important role in regulating sex hormone synthesis. The observed histomorphometric changes included a decrease in the lumen of the seminiferous tubules, an increase in the number of abnormal Sertoli cells and degenerated spermatozoa. As a whole, the experimental findings confirmed the protective effect of the bioprophylactic complex based on histomorphometric parameters. The findings revealed the protective potential of biological prevention strategies but highlight the importance of further profound studies of the mechanisms of lead toxicity on the male reproductive system and the effects of the developed bioprophylactic complex (BPC).

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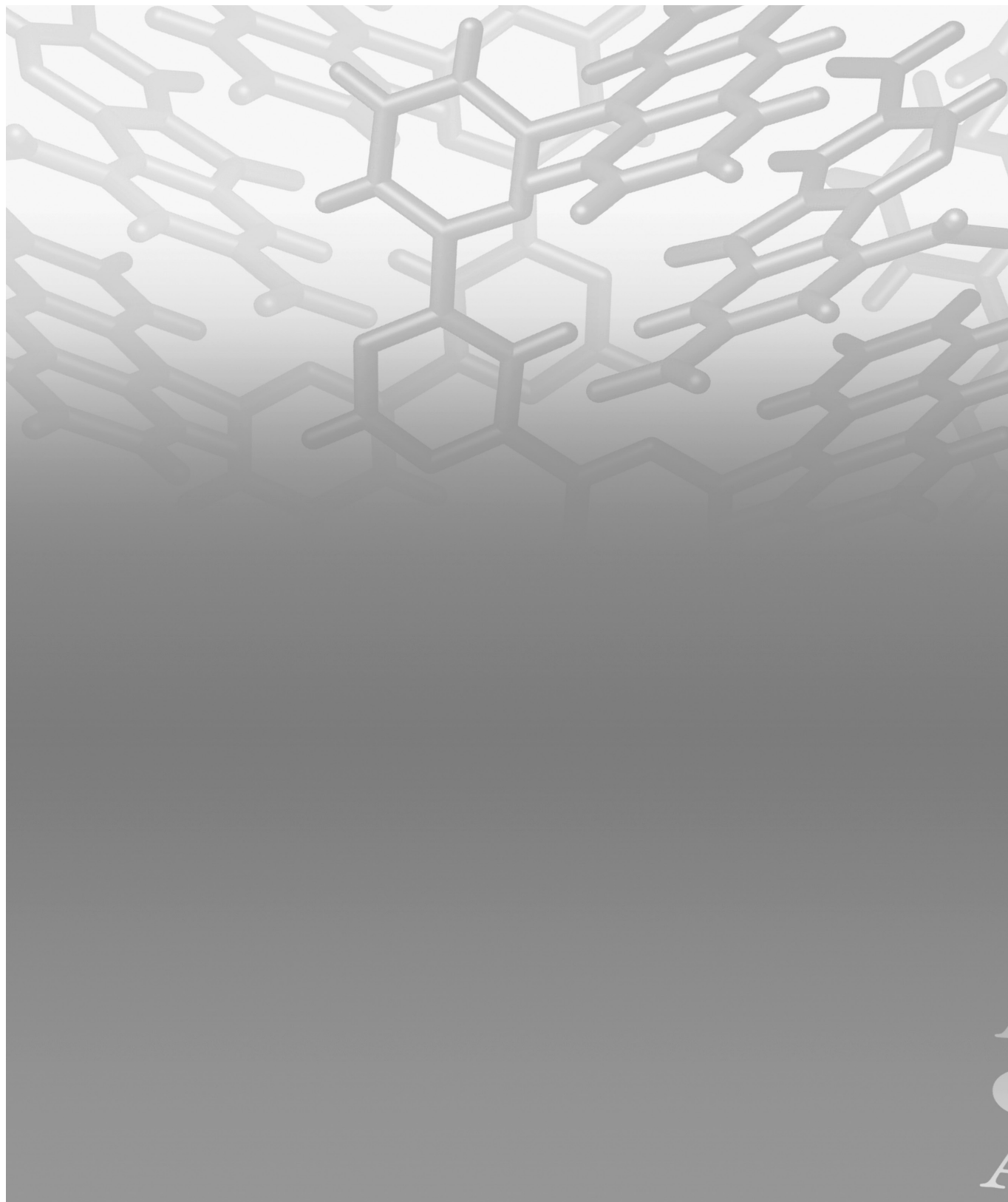
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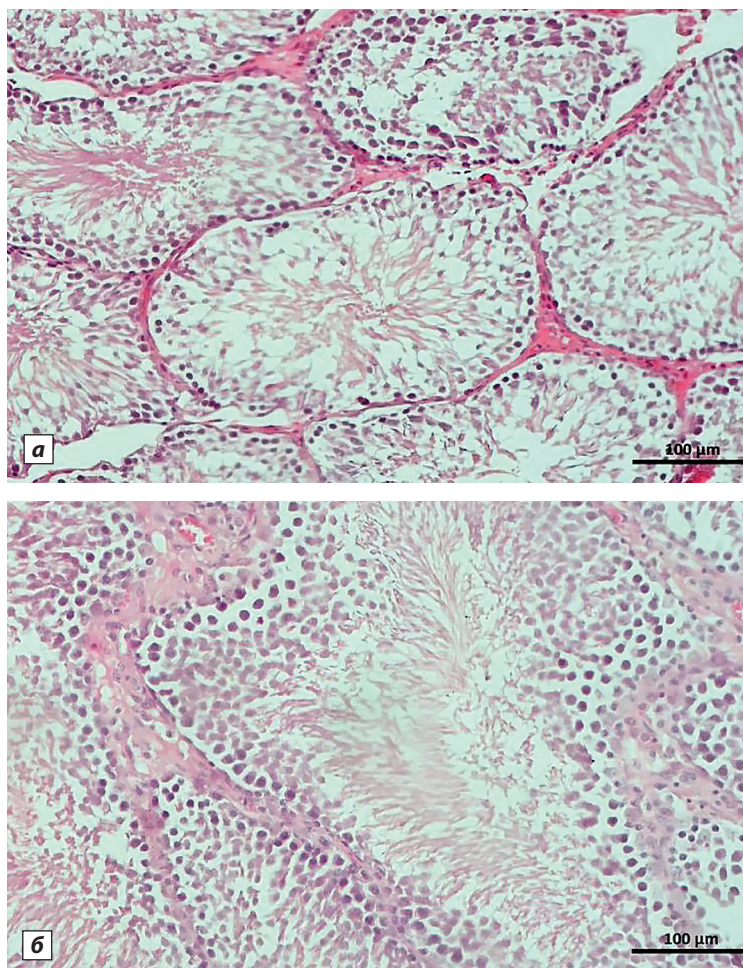


Fig. 2. Testes of the rats from the *Pb* exposure (a) and *Pb* + *BPC* (b) groups. Hematoxylin and eosin staining; $\times 200$ magnification.

Рис. 2. Семенники крыс группы *Pb* (a) и группы *Pb* + БПК (b). Окраска гематоксилином и эозином. Увеличение $\times 200$.