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¹Poltava State Medical University, Poltava, ²Mykolaiv National Agrarian University, Mykolaiv**MORPHOFUNCTIONAL STATE OF THE RATS' EPIPHYSIS UNDER CONDITIONS OF HEAVY METAL SALTS INTOXICATION AND CORRECTION WITH MELATONIN**

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The study presents the data of morphological research of the rats' pineal glands with heavy metal salt intoxication and correction of changes with melatonin. It was established that intoxication with salts of heavy metals leads to a morphological restructuring of the parenchyma and an imbalance in the processes of synthesis and secretion of the pineal gland's metabolites. Morphological changes are expressed by a decrease in the total number of pinealocytes, swelling of the parenchyma, and the appearance of cysts, which indicates the development of hydropic dystrophy, cell necrosis and suppression of the functional activity of the pineal gland. The protective effect of exogenous melatonin consisted in reducing signs of oedema, the number of cysts, and their mean area, increasing the number of cells, changing morphometric indices and bringing them closer to the control indices. Melatonin is a potentially useful agent for neutralizing the toxic effects of heavy metal salts on the pineal gland.

Key words: rats, pineal gland, heavy metals, melatonin, dystrophy, necrosis.

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МОРФОФУНКЦІОНАЛЬНИЙ СТАН ЕПІФІЗУ ЩУРІВ ЗА УМОВ ІНТОКСИКАЦІЇ СОЛЯМИ ВАЖКИХ МЕТАЛІВ ТА КОРЕКЦІЇ МЕЛАТОНІНОМ

У роботі представлені дані морфологічного дослідження епіфізу щурів при інтоксикації солями важких металів та корекції змін мелатоніном. Встановлено, що інтоксикація солями важких металів призводить до морфологічної перебудови паренхіми та дисбалансу процесів синтезу і секреції метаболітів епіфізу. Морфологічні зміни виражаються зниженням загальної кількості пінеалоцитів, набряком паренхіми, появою кіст, що свідчить про розвиток гідропічної дистрофії, некроз клітин та пригнічення функціональної активності епіфізу. Протективна дія екзогенного мелатоніну полягала у зменшенні ознак набряку, кількості кіст, їх середньої площі, збільшенні кількості клітин, зміні морфометричних показників та наближенні їх до показників контролю. Мелатонін є потенційно корисним засобом для нейтралізації токсичного впливу солей важких металів на епіфіз.

Ключові слова: щури, епіфіз, важкі метали, мелатонін, дистрофія, некроз.

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The rapid pace of urbanization and industrialization has led to the rapid spread of heavy metals in the environment, which has become a global problem for the whole world [7, 11]. One of the main sources of exposure to heavy metal salts on humans is contaminated drinking water. Heavy metals in the wastewater of various industries' enterprises pollute the water environment, which causes their high concentration and exceeding the maximum permissible standards [2]. Entering the body with drinking water, heavy metal salts damage cell membranes and lead to toxic damage to tissues of all body systems and are especially dangerous for the brain, liver, and kidneys [4, 5]. The toxicity of heavy metal salts is due to the intensification of free radical oxidation and inhibition of the antioxidant system, which leads to the disruption of metabolic processes at the cellular level, the emergence of pathological conditions, in particular, oxidative stress, cell necrosis, and the development of carcinogenesis [8].

Therefore, for scientists today, the issue of not only providing the population of Ukraine with high-quality drinking water, which directly affects the state of health, but also the search and experimental justification of ways to correct the harmful effects of heavy metal salts on the body is relevant. In literary sources, the correction of the toxic effect of heavy metal salts with the exogenous melatonin hormone is described in sufficient detail, which is due to its antioxidant properties of a wide spectrum of action, the ability to absorb free radicals, remove reactive oxygen species and thereby reduce oxidative damage to target cells and prevent pathomorphological reorganization of organs. [10, 12, 14]. Due to its unique properties, the melatonin hormone in therapeutic doses is successfully used as a drug ("Vita melatonin", Ukraine, "Sigma-Aldrich, Inc.", USA) in various fields of medicine [13]. However, insufficient attention has been paid to the pathohistological changes of the pineal gland as an organ that synthesizes about 80 % of endogenous melatonin in conditions of intoxication with salts of heavy metals. Issues regarding pharmacological correction of pathological conditions of the pineal gland, normalization of synthesis and secretion of endogenous melatonin and restoration of homeostasis remain unresolved.

The purpose of the study was to establish the peculiarities of the structural and functional organization of the laboratory rats' epiphysis during intoxication with salts of heavy metals and correction with melatonin.

Materials and methods. Experimental studies were performed on white adult sexually mature Wistar rats with a body weight of 220–260 g. A total of 18 male rats were involved in all study groups. The choice of males for the study was due to the absence of fluctuations in the level of melatonin in the blood plasma compared to females, in which the concentration of melatonin and the morphofunctional state of the pineal gland depend on the phase of the sexual cycle. The animals were kept in standard vivarium conditions under natural lighting and were divided into three groups. Six animals were selected for each group.

The animals of the group I (control) were housed under normal vivarium conditions and received distilled water. Animals of the group II received distilled water with the addition of heavy metal salts with the following composition: a solution of copper sulfate – 3 mg/kg (1/10 of LD50), a solution of cadmium sulfate – 1.5 mg/kg (1/30 of LD50), a solution of lead nitrate – 1.7 mg/kg (1/50 of LD50) [3]. The study used a combination of heavy metal salts that have a neurotoxic effect. Animals of the group III received distilled water with the addition of the specified amount of heavy metal salts. In order to neutralize the toxic effect of heavy metal salts, the pharmacological preparation Vita-Melatonin (JSC “Kyiv Vitamin Factory”, Ukraine) was used. Melatonin was injected intraperitoneally at a dose of 1.0 mg/kg of body weight in 1.0 ml of solvent once a day at 19.00 for 14 days. [1].

The rats were sacrificed 1 day after the last administration of heavy metal salts by immediate decapitation under thiopentane anesthesia (25 mg/kg, intraperitoneally). All stages of the study, manipulative interventions and euthanasia of animals were carried out in accordance with the provisions of the “General Ethical Principles of Animal Experiments” adopted by the VII National Congress on Bioethics in 2019 and in accordance with other international agreements and current national legislation in the field of medical and biological research.

For histological examination, the pineal gland was fixed in a 10 % solution of neutral formalin. Using standard methods, the material was embedded in paraffin blocks, from which sections with a thickness of 5 μ m were made with a rotary microtome of the semi-automatic type “Microm” (Germany) and stained with hematoxylin and eosin in accordance with generally accepted methods. The obtained histological preparations of the pineal gland were studied under the magnification of binoculars $\times 10$, objective $\times 20$, $\times 40$ of the “Carl Zeiss” microscope (Germany). Photo documentation of the study results was made using a Canon digital SLR camera.

During the histological study of pineal preparations, the number of pinealocytes in the field of view of the microscope (objective $\times 20$, binocular $\times 10$) was counted by analogy with Goryaev's counting chamber. Morphometric measurements were carried out using an eyepiece screw type micrometer MOV 1–16 with an objective magnification ($\times 40$). Large and small diameters of cysts, nucleus and cytoplasm were measured. Given the rounded shape of the nucleoli, their parameters were determined in one direction.

Processing of the obtained study results was carried out using the methods of variational statistics, namely the Student's t-criterion. The difference was considered reliable when the numerical parameters between the groups differed at a value of at least $p < 0.05$. Statistical calculations were performed with a personal computer using the standard software “STATISTICA 6” for computer equipment with the Windows operating system.

Results of the study and their discussion. During the histological examination of the preparations in the control group of animals, it was established that the pineal gland has a typical lobular structure. From the outside, the epiphysis is covered with a capsule of connective tissue. The stroma and parenchyma are visualized in the pineal gland. Parenchyma is represented by two types of pineal cells: light and dark. Bright cells were the most numerous. It was established that the percentage of light cells was 84.12 ± 0.32 % of the total number of pinealocytes, and the percentage of dark cells was 15.88 ± 0.68 %.

Pineal cells were quite densely located in the parenchyma of the organ. The number of pinealocytes in one field of view of the microscope at its total magnification of $\times 200$ was 224.6 ± 8.7 cells. It should be noted that only single cysts and single pinealocytes with manifestations of vacuolization were found in the parenchyma of the gland, which caused the dense arrangement of cells. Another type of epiphyseal cells are gliocytes. Glial cells were diffusely located in histological specimens of rats of the control group (Fig. 1).

After 14 days of intoxication with salts of heavy metals, the epiphysis of the experimental animals retained its typical morphological features of the organization. However, thickening and loosening of the capsule and growth of connective tissue were found in the stromal component of the organ. Edema was observed in the parenchyma, especially in its subcapsular and peripheral parts. In some cases, we found fluid accumulation in the peripheral part of the parenchyma and under the capsule, which led to its exfoliation (Fig. 2).

The parenchyma was represented by two types of pinealocytes. However, it is necessary to note changes in the ratio between dark and light cells. Under the conditions of intoxication with salts of heavy metals, the number of light cells increased by 11.03 %, compared to the intact group, and the number of

dark cells decreased by the corresponding percentage. It was established that the percentage of light cells in the experimental group was $95.15 \pm 1.23 \%$, and the percentage of dark cells decreased to $4.85 \pm 0.51 \%$.

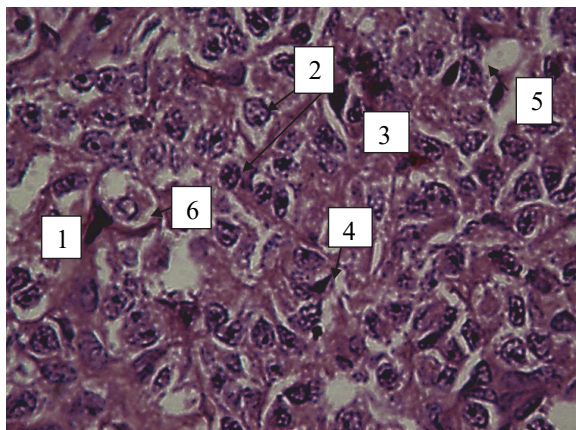


Fig. 1 Fragment of epiphysis parenchyma of a control group rat. Collection: ocul. $\times 10$, ob. $\times 40$. Staining: hematoxylin and eosin. 1 – dark pinealocytes; 2 – light pinealocytes; 3 – blood vessel; 4 – glial cell; 5 – cyst; 6 – a cell with cytoplasmic vacuolization.

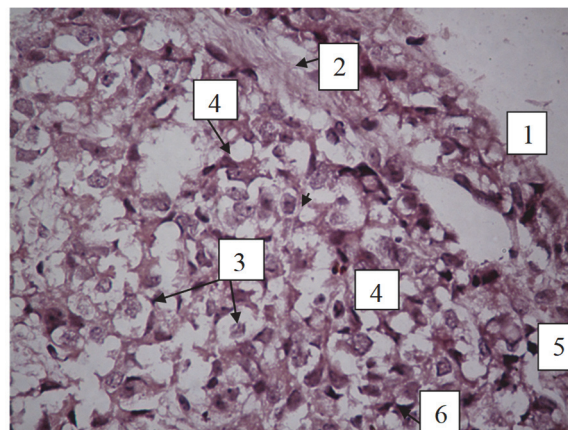


Fig. 2 A fragment of the peripheral region of the rat's epiphysis parenchyma under conditions of 14-day intoxication with heavy metal salts. Collection: approx. $\times 10$, ob. $\times 40$. Staining: hematoxylin and eosin. 1 – capsule of the pineal gland; 2 – growth of connective tissue; 3 – vacuolization of the cytoplasm of light pinealocytes; 4 – cysts; 5 – dark pinealocyte; 6 – gliocyte.

When performing a comparative analysis of histological sections in two groups of animals, it was established that the distribution density of pinealocytes in the parenchyma of the gland changes in the experimental group. There was a significant decrease in the number of cells on the given area of the organ by 29.52% ($p < 0.05$) to the level of 158.3 ± 12.6 cells. The decrease in the density of the location of pinealocytes was explained by the fact that almost all cells were increased in volume due to the increase in morphometric parameters compared to the cells of the control group of animals, and as a result, less of them were located in the field of view of the microscope. Another reason for the sparse arrangement of pinealocytes was the presence of a large number of cysts of different sizes, which increased the distance between neighboring pinealocytes (Fig. 3).

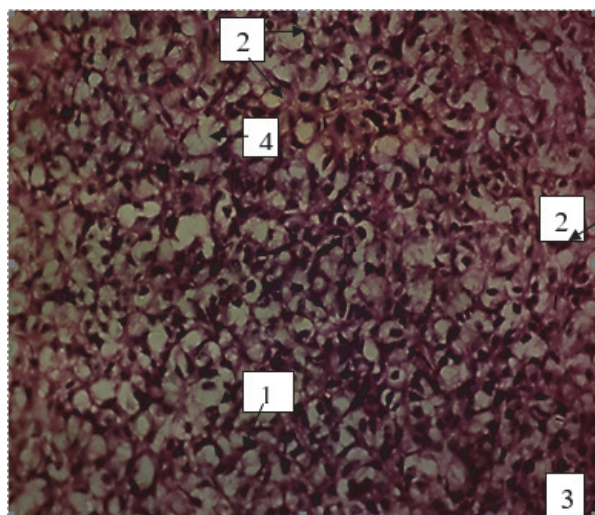


Fig. 3 A fragment of the central zone of the parenchyma of the rat epiphysis under conditions of 14-day intoxication with heavy metal salts. Coll.: approx. $\times 10$, ob. $\times 20$. Staining: hematoxylin and eosin. 1 – vacuolization of the cytoplasm of individual pinealocytes; 2 – cysts; 3 – dark pinealocytes; 4 – vacuolization of the lobule.

The nuclei of light pinealocytes were also different in size and structure. It was established that in cells with a significantly increased area of the cytoplasm, the nucleus was almost completely discolored and turned into a homogeneous mass. In vacuolated pinealocytes, the deformed nuclei were pushed by the vacuole to the periphery of the cell. Signs of pyknosis, swelling and lysis were simultaneously observed in the nuclei of light pinealocytes. The detected morphological changes in the nucleus indicate necrosis of pinealocytes. The mean area of the nucleus increased by 21.44% ($p < 0.05$) relative to the values of the intact group, which indicated the predominance of swelling and hypertrophy of the nuclei.

According to the results of the histomorphometric analysis, it was established that the increase in cell parameters was mainly due to the increase in the area of the cytoplasm. The mean area of the cytoplasm of light pinealocytes increased by 59.66% ($p < 0.05$) compared to the intact ones. Cytoplasm on the preparations looked optically transparent, almost devoid of any structural components. As a result of excessive vacuolization of pinealocytes, the plasmolemma was thinned and stretched, and cell contours became indistinct and blurred. There were cases of disruption of the integrity of the plasmolemma. It should be noted that the processes of vacuolization had a different degree of manifestation and, accordingly, a different volume of liquid accumulated in the cells, as a result of which pinealocytes of different sizes were found. A large number of cysts were found in histological samples, which differed significantly in size depending on the fluid content. The mean area of cysts was $71.42 \pm 12.94 \mu\text{m}^2$.

Nucleoli tended to increase in size. A shift of the nuclear-cytoplasmic index towards the cytoplasm was established, which indicates the hypertrophy of light pinealocytes and is a sign of their swelling. Among the swollen and necrotic light pinealocytes in the parenchyma, individual dark cells were detected. The morphometric parameters of dark cells tended to increase, but the nuclear-cytoplasmic index did not change. Data obtained during morphometric measurements of pineal cells are shown in Table 1.

Table 1

Morphometric parameters of rat pinealocytes

Group of animals	The area of cytoplasm μm^2 M \pm m	The area of nucleus μm^2 M \pm m	The area of nucleolus μm^2 M \pm m	NCI M \pm m
light pinealocytes				
Control		22.76 \pm 0.66 n=50	3.08 \pm 0.14 n=50	0.44 \pm 0.01
Intoxication by heavy metal salts	82.85 \pm 2.46* n=50	27.64 \pm 1.38* n=50	3.26 \pm 0.23 n=50	0.33 \pm 0.01*
Correction by melatonin	63.17 \pm 2.16*/# n=50	23.92 \pm 0.53# n=50	3.21 \pm 0.28 n=50	0.37 \pm 0.01*
Dark pinealocytes				
Control	26.52 \pm 0.71 n=30	12.67 \pm 0.62 n=30	Not determined	0.47 \pm 0.01
Intoxication by heavy metal salts	28.46 \pm 0.93 n=30	13.38 \pm 0.47 n=30		0.47 \pm 0.01
Correction by melatonin	27.95 \pm 0.58 n=30	13.05 \pm 0.94* n=30	Not determined	0.47 \pm 0.01

Note: *significantly with the control ($p<0.05$), #significantly with the group of animals exposed to heavy metal salts intoxication ($p<0.05$)

Microscopic analysis of the pineal gland of animals that were intoxicated with salts of heavy metals for 14 days and under the influence of corrective therapy with the drug "Vita-melatonin" showed a decrease in the intensity of morphological changes compared to the group of rats that were not exposed to the corrector. However, areas of parenchyma with signs of edema continued to be found in the epiphysis. However, the cysts were smaller than in uncorrected rats. It was established that the mean size of cysts decreased by 34.04 % ($p<0.05$) and was $47.11\pm 2.49 \mu\text{m}^2$.

The number of cysts in each field of view of the microscope also decreased visually. At the same time, in the peripheral areas of the pineal gland, groups of light-colored pinealocytes with signs of vacuolization of the cytoplasm were still detected (Fig. 4). However, it is necessary to note a decrease in vacuolization processes, which is confirmed by probable changes in the corresponding morphometric indices. It was established that the mean area of the cytoplasm decreased by 23.75 % compared to the group of animals that were not exposed to melatonin. At the same time, the difference was statistically significant both in relation to the control ($p<0.05$) and in relation to the first experimental group of animals.

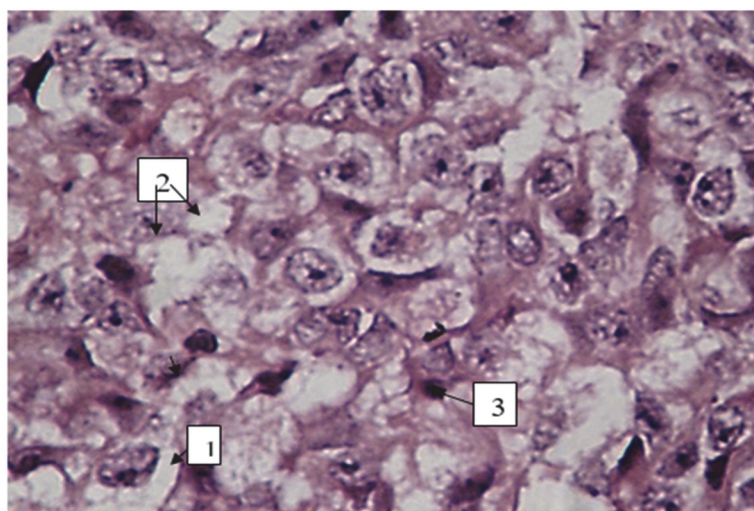


Fig. 4 Photomicrograph of the epiphysis parenchyma of a rat under the influence of 14-day intoxication with heavy metal salts and correction with melatonin. Collection: approx. $\times 10$, ob. $\times 40$. Staining: hematoxylin and eosin. 1 – pinealocytes with vacuolated cytoplasm; 2 – cysts; 3 – dark pinealocytes.

The nuclei of light pinealocytes were located mainly in the center of the cells. A small percentage of nuclei had signs of pyknosis and partial lysis. It was found that the mean area of the nucleus decreased by 13.46 % and was statistically significantly different from the similar index in the group of rats exposed to heavy metal intoxication and approached the intact parameters. Similar changes in parameters indicate an increase in the synthetic activity of light cells. It should be noted that the state of secretory activity of light pinealocytes also improved, which was manifested in a decrease in the area of the cytoplasm and a shift of the nuclear-cytoplasmic index towards the nuclear apparatus.

The protective effect of melatonin is also evidenced by changes in the density of cells in the parenchyma. It was established that the number of pinealocytes in the field of view of the microscope increased by 19.8 % ($p<0.05$) compared to the group exposed to heavy metal salts and amounted to 189.3 ± 6.5 cells. The ratio between the two types of cells has also changed. It was established that the percentage of light pinealocytes decreased to the level of $89.46\pm 0.93\%$, and the percentage of dark cells increased to the level of 10.54 ± 1.21 . The detected changes in the density of the location of cells, morphometric indices, the ratio of active and inactive pinealocytes indicate an approach to the control data and restoration of the parenchyma.

Changes of a similar nature are indicated by a number of other researchers who studied the pathohistological changes of the pineal gland and other parts of the brain under conditions of combined exposure to salts of heavy metals and other toxic substances [5, 6, 9]. Identified violations of the morphology of the pineal gland are manifested by swelling, a decrease in the total number of pinealocytes, an increase in the number of vacuolated cells, cysts, their sizes and a shift of the nuclear-cytoplasmic index towards the cytoplasm, which indicates the development of hydropic dystrophy, which turns into collicative necrosis of cells, especially in the peripheral and subcapsular regions of the parenchyma [8].

The detected morphological changes are accompanied by a decrease in synthesis, a delay in secretory processes in pinealocytes, and a decrease in organ function due to the toxic effect of heavy metal salts on the plasma membrane of cells, a violation of the transmembrane potential and transport of metabolites [4, 9]. At the same time, in addition to destructive processes, changes of an adaptive and compensatory nature were detected, manifested by the inclusion of a reserve supply of pinealocytes in synthetic processes to compensate for their progressive loss, which is evidenced by a decrease in the number of dark cells.

Histological analysis of the pineal gland of animals that were under the influence of corrective therapy with exogenous melatonin for 14 days showed a decrease in the intensity of morphological changes compared to a group of rats that were not exposed to the corrector. The protective effect of melatonin consisted in reducing the signs of edema, improving the synthetic and secretory activity of light pinealocytes, which is confirmed by the shift of the nuclear-cytoplasmic index towards the nucleus. The protective effect of melatonin is also indicated by an increase in the total number of pinealocytes and their quantitative content approaching the control indices, which indicates the restoration of the cellular composition of the epiphysis parenchyma and a decrease in the manifestations of necrosis [1].

Conclusions

1. The toxic effect of heavy metal salts leads to a morphological restructuring of the parenchyma and an imbalance in the processes of synthesis and secretion of metabolites of the pineal gland. Morphological changes are manifested by a decrease in the total number of pinealocytes by 29.52 % ($p < 0.05$), swelling of the parenchyma, due to the appearance of cysts and cells with an increased volume of cytoplasm, an increase in the mean area of the cytoplasm by 59.66 % ($p < 0.05$) and nuclei by 21.44 % ($p < 0.05$), which indicates a violation of synthesis and secretion processes, the development of hydropic dystrophy, necrosis of pinealocytes and inhibition of the functional activity of the pineal gland.

2. Exogenous melatonin reduces the toxic effect of heavy metal salts on pineal cells, promotes the restoration of the processes of synthesis and secretion of endogenous melatonin, which is explained by a decrease in the number of cysts and their mean area by 34.04 % ($p < 0.05$), a probable change in morphometric indices of pinealocytes and their approach to control indices, an increase in the number of cells by 19.8 %, which indicates the development of reparative processes in the parenchyma of the organ.

3. The detected changes indicate the possible reversibility of morphological transformations, which is important for effective pharmacological correction of pathological conditions of the pineal gland, reduction of the toxic effect of heavy metal salts, and restoration of homeostasis. Melatonin is a potentially useful agent in preventing heavy metal toxicity-induced damage to the pineal gland.

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PROOXIDANT-ANTIOXIDANT HOMEOSTASIS IN THE SPERM OF FERTILE BOARS DEPENDS ON THE INFLUENCE OF DIFFERENT FACTORS

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The purpose of the study was to establish the peculiarities of the formation of pro-oxidant-antioxidant homeostasis in sperm and seminal plasma of breeding boars depending on the season and the intensity of their use. 10 adult breeding boars of a large white breed aged from 18 to 36 months were selected for the experiment – analogues in terms of the quality of sperm production. Breeding boars were fed according to the norms of the Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Agrarian Sciences of Ukraine. It has been established that the best indices of sperm production characterize animals in the spring period. In the summer period, the quality of sperm in breeding boars probably decreases. Such changes are accompanied by a significant decrease in catalase activity by 30.6 % ($p < 0.01$) and a significant accumulation of the TBC-active complexes' content by 46.9 % ($p < 0.01$). The quality of sperm production of breeding boars is significantly dependent on the mode of their use. It was found that increasing the intensity of using boars twice a week does not contribute to a significant acceleration of peroxidation processes.

Key words: sperm, boars, sperm production, peroxide oxidation, TBC-active complexes.

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ПРООКСИДАНТНО-АНТИОКСИДАНТНИЙ ГОМЕОСТАЗ У СПЕРМІ КНУРІВ- ПЛІДНИКІВ ЗАЛЕЖНО ВІД ВПЛИВУ РІЗНИХ ФАКТОРІВ

Метою роботи було встановлення особливостей формування прооксидантно-антиоксидантного гомеостазу в спермі і спермальній плазмі кнурів-плідників залежно від пори року та інтенсивності їх використання. Для досліджу відібрані 10 дорослих кнурів-плідників великої білої породи віком від 18 до 36 місяців – аналогів за якістю спермопродукції. Годівлю кнурів-плідників годували згідно з нормами Інституту свинарства і агропромислового виробництва національної академії аграрних наук України. Встановлено, що найкращими показниками спермопродукції характеризуються тварини у весняний період. У літній період якість сперми у кнурів-плідників вірогідно знижується. Такі зміни супроводжуються істотним зниженням активності каталази на 30.6 % ($p < 0.01$) та суттєвим накопиченням умісту ТБК-активних комплексів на 46.9 % ($p < 0.01$). Якість спермопродукції кнурів-плідників перебуває в істотній залежності від режиму їх використання. Виявлено, що підвищення інтенсивності використання кнурів двічі на тиждень не сприяє суттєвому прискоренню процесів пероксидації.

Ключові слова: сперма, кнури, спермопродукція, пероксидне окиснення, ТБК-активні комплекси.

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In the modern pig breeding system, using breeding boars with high breeding value is the most important component of increasing the productivity potential of the herd as a whole [5].

The most intensively highly productive breeding boars are used to produce highly productive offspring [2]. This leads to the search for new approaches in the study of physiological features of the formation of sperm production in these animals [3].