травматизацію і порушення цілісності окремих адипоцитів при підготовці до трансплантації. Однак, аналіз структурної організації адаптованої жирової тканини встановив, що зменшення об'єму адипоцитів при цьому склало 19,4 % - 23,1%. Потовщення прошарків сполучної тканини в адаптованій жировій тканині обумовлено компенсаторно-репаративними процесами і дозріванням волокнистого компоненту і аморфної речовини сполучної тканини при приживлені трансплантата, асептичним запаленням . Означені явища супроводжуються збільшенням кількості клітин-мігрантів в периваскулярній тканині. З боку судин гемомікроциркуляторного русла встановлені зміни мають стереотипний характер на дію екзогенних чинників.

Ключові слова: жирова тканина, ауто пересадка, асиметрія молочних залоз.

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частичной травматизации и нарушении целостности отдельных адипоцитов при подготовке к трансплантации. Однако, анализ структурной организации адаптированной жировой ткани установил, что уменьшение объема адипоцитов при этом составило 19,4 % - 23,1%. Утолщение слоев соединительной ткани в адаптированной жировой ткани обусловлено компенсаторно-репаративными процессами и созреванием волокнистого компонента и аморфного вещества соединительной ткани при приживлении трансплантата. Указанные явления сопровождаются увеличением количества клеток-мигрантов в периваскулярной ткани. Со стороны сосудов гемомикроциркуляторного русла установлены изменения имели стереотипный характер на действие экзогенных факторов.

Ключевые слова: жировая ткань, аутопересадка, асимметрия молочных желез.

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MORPHOFUNCTIONAL FEATURES OF RAT TESTES INTERSTITIAL ENDOCRINOCYTES AND SUSTENTOCYTES AFTER 90 DAYS OF CENTRAL TESTOSTERONE SYNTHESIS DEPRIVATION

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With the social system development, there is a tendency to change attitudes towards family and family values. In developed European countries, there is a trend towards high sexual activity in elderly men and late creation of a family with children, which undergoes certain difficulties in connection with a decreased testosterone production in later years. The purpose of the study was to establish the microscopic organization of rat interstitial endocrinocytes and sustentocytes, to determine the sources of nitric oxide production and the intensity of oxidative stress in the testes with experimental central deprivation of testosterone synthesis with diphereline on the 90th day of the experiment. The experiments were carried out on 20 sexually mature male white rats of the Wistar line. Rats were divided into 2 groups: the control group (10) and the experimental group (10), which were injected subcutaneously with diphereline (Triptorelin embonate) at a dose of 0.3 mg / kg of the active substance for 90 days. Prolonged central deprivation of testosterone synthesis in animals leads to emergence of functional stress structural signs in population of sustentocytes and interstitial endocrinocytes, which are aimed to support testicular secretion. Central deprivation of testosterone synthesis within 90 days causes oxidative stress development owing to reactive oxygen species hyperproduction and nitrite accumulation in testicular tissue due to increased inducible NO-synthase activity.

Key words: testes, interstitial endocrinocytes, sustentocytes, NO-synthase, iNOS, cNOS, L-arginine, superoxide dismutase, rats.

The study is a fragment of the research project "Experimental morphological study of cryopreserved placenta transplants action diphereline, ethanol and 1% methacrylic acid on the morphofunctional status in a number of internal organs", state registration No. 0119U102925.

With the development of the social system, there is a tendency to change attitudes towards family and family values. In developed European countries, there is a trend towards high sexual activity in elderly men and late creation of a family with children, which causes certain difficulties in connection with a decreased testosterone production in older age [2]. Testosterone deficiency leads to infertility and redox imbalance, which further downgrades sperm quality and even causes inflammation in different organs [3, 4]. Besides inflammation and redox imbalance testosterone deficiency may lead to apoptosis in testicular tissues [5].

At the same time, the use of testosterone drugs in uncontrolled quantitative doses may worsen spermatogenesis by suppressing the components of the hypothalamic-pituitary system according to the feedback principle [11].

In our previous article, we showed that 30-days central deprivation of testosterone synthesis had led to the development of compensatory reactions in sustentocytes and interstitial endocrinocytes, which had been accompanied by morphological signs of functional overstrain. Development of oxidative stress and a change in the source of nitric oxide production from constitutive forms of NO synthase (cNOS) to an inducible form (iNOS) were also reported [12].

The effect of a longer central testosterone synthesis deprivation on the testes structure and functional status is not studied sufficiently at the moment.

The **purpose** of the study was to establish the microscopic organization of rat interstitial endocrinocytes and sustentocytes, to determine the sources of nitric oxide production and the intensity of oxidative stress in the testes in experimental central deprivation of testosterone synthesis with diphereline on the 90th day of the experiment.

Materials and methods. The experiments were carried out on 20 sexually mature male white rats of the Wistar line. Rats were divided into 2 groups: the control group (10) and the experimental group (10), which were injected subcutaneously with diphereline (Triptorelin embonate) at a dose of 0.3 mg of the active substance / kg of body weight for 90 days [9]. Animals were kept in standard vivarium conditions of the Ukrainian Medical Stomatological Academy. Experimental animals were sacrificed in strict compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), as well as with the “General Ethical Principles of Animal Experiments” adopted by the First National Congress on Bioethics (Kyiv, 2001).

After an overdose of ketamine, the animals were decapitated, the prepared small pieces of the testes were fixed in a 2.5% glutaraldehyde solution (pH=7.2-7.4). Postfixation of the material was carried out with 1% solution of osmium (IV) oxide, followed by dehydration in propylene oxide and sample was embedded into the epoxy resins mixture. Ultrathin sections made with an ultramicrotome were contrasted with a 1% aqueous solution of uranyl acetate and lead citrate according to the Reynolds method and studied with an electron microscope [1]. Using standard methods, the material was imbedded in paraffin blocks, of which sections 4 μm thick were made and stained with hematoxylin and eosin. Histological preparations were examined using Olympus C 3040-ADU light microscope with digital microfilter with software adapted for these studies (Olympus DP - Soft, license No. VJ285302, VT310403, 1AV4U13B26802) and Biorex 3 (serial No. 5604).

All biochemical studies were carried out in 10% homogenate of testis tissue using Ulab 101 spectrophotometer.

General NO-synthase activity (gNOS), cNOS, iNOS and nitrite concentration was determined according to the method described by Akimov O.Ye., Yelinska A.M. and Kostenko V.O. [14]. Activity of arginases was determined by the increase in the concentration of L-ornithine after a 20-hour incubation in phosphate buffer medium (pH = 7.0) in the presence of a 24 mM solution of L-arginine [14].

General activity of NO-synthase (gNOS), cNOS, iNOS and the activity of arginases was determined by the increase in the concentration of L-ornithine after a 20-hour incubation in phosphate buffer medium (pH = 7.0) in the presence of a 24 mM L-arginine solution [12].

Basic production of superoxide anion radical ($O_2^{\bullet-}$), its production by the mitochondrial electron transport chain (ETC) and microsomal ETC was determined by the growth of diformazan concentration, formed in the reaction of $O_2^{\bullet-}$ with nitro blue tetrazolium [14]. Superoxide dismutase (SOD) and catalases activity was determined according to guidelines [14]. The concentration of free malondialdehyde (MDA) was determined by reaction with 1-methyl-2-phenylindole [14].

Statistical processing of the research results was carried out using the Microsoft Office Excel software and the Real Statistics 2019 extension to it. The nonparametric Mann-Whitney test was used to determine the statistical significance of differences between the groups. The difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion.

In a micropreparation of the rat seminiferous tubules in the control group, all cellular associations characteristic of spermatogenic epithelium were noticeable: spermatogonia are located on the basal membrane, farther from it spermatocytes are located, spermatids and spermatozoa are located in the lumen of the tubules. Their cellular composition indicated various stages of normal spermatogenesis. In the cross section of the rats’ testes in the control group, the seminiferous tubules had a rounded shape. Closer to the basal membrane, the nuclei of the sustentocytes were well visualized. The testicular stroma was represented by loose fibrous connective tissue, in which groups of interstitial endocrinocytes of 3-5 cells in the field of vision, blood vessels and cellular elements were clearly distinguished.

In the experimental group of animals (on the 90^{th} day of the experiment), we found that the testes interstitial space was represented by loose fibrous connective tissue, which between the convoluted seminiferous tubules was enlarged in comparison with the control group. The vessels were full-blooded, the leukocytes margination was determined inside the vessels with elements of migration activity through the vessel wall into the space of interstitial tissue (fig. 1a).

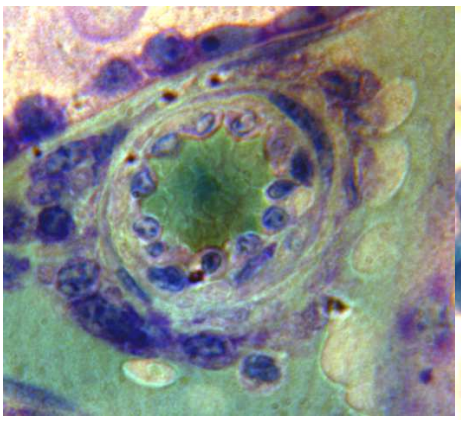

Fig. 1a. Blood vessel of experimental rat on the 3rd month. Microimage. H&E stain: Lens: 80: Ocular lens:15.

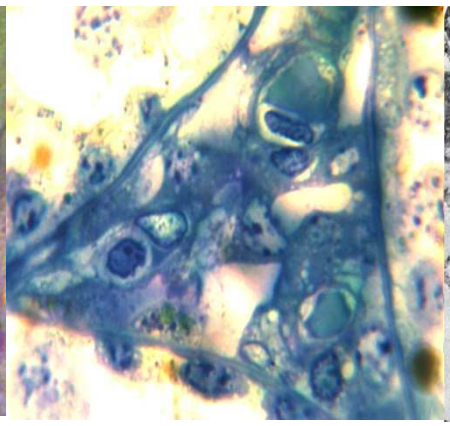

Fig. 1b. Interstitial space of experimental mouse on the 3rd month. Microimage. H&E stain: Lens: 80: Ocular lens:15.

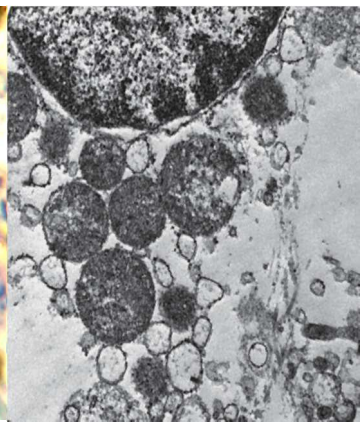

Fig. 1c. Electron micrograph of interstitial endocrinocytes of experimental rat on the 3rd month. Exp. x 12000.

Fig. 1. Blood vessels, interstitial space and interstitial endocrinocyte after 90 days central deprivation of testosterone synthesis.

Around the blood vessels, groups of interstitial endocrinocytes of 1-3 cells were determined in the field of vision (in the control group 3-5 in the field of vision). Interstitial endocrinocytes had round or oval shaped nuclei, 1-2 nucleoli were clearly distinguished (fig.1.b). In the cytoplasm of endocrinocytes, smooth endoplasmic reticulum was determined, which was represented by numerous tubules that branched and were filled with a fine fiber substance, on the membranes of which there were numerous ribosomes. Mitochondria were small, with dense matrix and a small number of cristae. A characteristic feature was the presence of secretory granules of various sizes and electron densities in the cytoplasm; they were localized in a well-developed plate apparatus of the Golgi cytoplasmic complex. Most cells developed destructive disorders in the ultrastructural organization of the plate cytoplasmic Golgi complex, which could be well seen in electron micrographs in comparison with the control group of animals (fig.1.c). In some interstitial endocrinocytes, the smooth membranes of the Golgi complex were randomly oriented and surrounded by single large electron-transparent vacuoles, lipid inclusions and secretory granules. Most interstitial endocrinocytes had fragmented smooth endoplasmic reticulum. The hyaloplasm of glandulocytes was significantly cleared and contained very few free ribosomes and polysomes, compared to the control group of animals. The cytoplasmic membrane of glandulocytes is dissolved, thickened, has high electron density.

In the interstitial space of the experimental group of animals well visualized macrophages were determined. In a micropreparation, two populations of macrophages could be easily distinguished. Depending on the location, macrophages could be divided into parietal (near the convoluted tubule) and actually interstitial, which were located individually or in groups of 1-3 in the field of vision near the blood vessel. Parietal macrophages had a flattened nucleus and cytoplasm, while interstitial macrophages, on the contrary, had a large nucleus of a round or oval shape (fig. 1.b).

When we studied micropreparations of the convoluted seminiferous tubules in the experimental group, it was found that the wall of the tubules is compacted, swollen, with large number of parietal macrophages in comparison with the control group of animals.

Two populations of cells were determined in the lumen of the convoluted tubules: the sustentocytes and cells of the spermatogenic series at different stages of differentiation (fig. 2a, 2b).

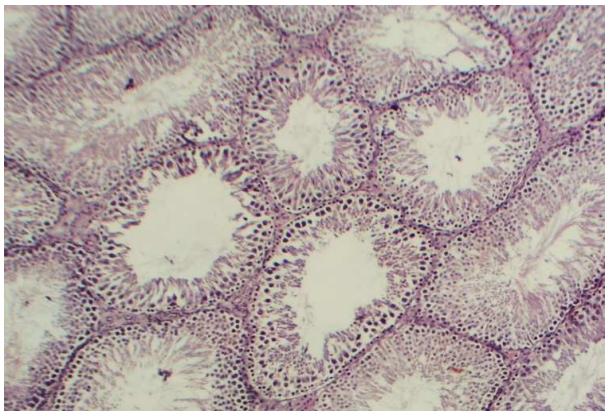

Fig. 2.a. Seminiferous tubules of experimental rat on the 3rd month. Microimage. H&E stain: Lens: 10: Ocular lens: 15.

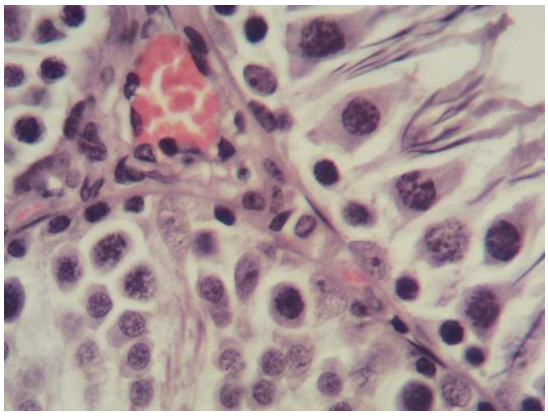

Fig. 2.b. Seminiferous tubules of experimental rat on the 3rd month. Microimage. H&E stain: Lens: 40: Ocular lens: 15.

Fig. 2.

When we studied the structural organization of rat sustentocytes in the experimental group, it was found that most of the cells experienced adaptive reactions, which should be discussed in more detail.

Sustentocytes with hyperplasia of the elements of the smooth endoplasmic reticulum in the cytoplasm, the morphological equivalent of which are numerous small and large dilated round vesicles, localized mainly in the apical sections of cells, were visualized. The heads of maturing spermatids with the impaired structures of the tubulo-bulbar complex with primary signs of degeneration were also found in these fragments of sustentocytes. Quite large phagosomes containing fragments of dead cells occurred in the cytoplasm of the sustentocytes. The intercellular contacts between the round spermatids and sustentocytes were not broken, but the deformation of the inner membranes and vacuolization of mitochondria occurred in the cytoplasm of some spermatocytes, round and maturing spermatids. The number of mitochondria in the cytoplasm of the sustentocytes decreased, electron density of the mitochondrial matrix decreased. Ultrastructural signs of cytoplasm membrane structures degradation in the form of concentric electron-dense formations were found in the cytoplasm of vacuolated sustentocytes, protein structures were present either inside the vacuoles or independently located in the cell cytoplasm. The number of lipid droplets in the cytoplasm of sustentocytes was significantly increased compared to the control group.

In the structure of convoluted seminiferous tubules, an increase in the height of the spermatogenic layer was detected. The presence of small and medium vacuoles, with a tendency to increase and merge in the contact zone between the spermatogonia and the basal membrane of the convoluted seminiferous tubules, was observed.

In some convoluted seminiferous tubules, first there was discomplexation and disorientation, and then desquamation of spermatids. The volume of their cytoplasm decreased in comparison with the control group. Hypochromia and pycnosis were observed in the nuclei. There was also disorientation, discompletion of second-order spermatocytes, then those of the second order, and sometimes their desquamation. Due to the complete or partial desquamation of the sperm layer cells from the basement membrane, “seed layers” were formed in the lumen of the tubules. The number of type A and B spermatogonia decreased.

Table 1

Oxidative stress markers in rat testes during 90-day central testosterone synthesis deprivation (M±m)

Groups	Parameters					
	SOD activity, c.u.	Catalase activity, nkat/g of tissue	Basic $O_2^{\bullet-}$ production, nmol/s per g of tissue	Production of $O_2^{\bullet-}$ from mithochondrial ETC, nmol/s per g of tissue	Production of $O_2^{\bullet-}$ from microsonal ETC, nmol/s per g of tissue	Free MDA, µmol/g of tissue
Control	1.87 ±0.11	182 ±17	0.26 ±0.01	7.84 ±0.13	9.55 ±0.19	6.64 ±1.44
Experimental	2.35 ±0.25*	253 ±20	3.87 ±0.08*	42.08 ±1.62*	36.53 ±0.42*	18.25 ±0.89*

Note:* - indicates that the difference is statistically significant when compared to the control group ($p<0.05$).

Against the background of the described morphological changes, an increase in SOD activity by 25.67% is observed without statistically significant changes in catalase activity (tab. 1). Basic production of $O_2^{\bullet-}$ increased 14.88 times, production of $O_2^{\bullet-}$ from mitochondrial ETC was elevated by 5.37 times and from microsomal ETC – by 3.83 times. The concentration of free MDA increased by 2.75 times. Thus, it can be noted that the development of oxidative stress is observed in the testes.

Table 2

Nitric oxide cycle function during 90-day central testosterone synthesis deprivation (M±m)

Groups	Parameters				
	gNOS activity, µmol/min per g of protein	iNOS activity, µmol/min per g of protein	cNOS activity, µmol/min per g of protein	Arginase activity, µmol/min per g of protein	NO^{2-} concentration, nmol/L
Control	0.54 ±0.04	0.13 ±0.02	0.41 ±0.03	2.48 ±0.05	3.83 ±0.25
Experimental	1.38 ±0.18*	1.23 ±0.17*	0.15 ±0.02*	0.24 ±0.03*	28.45 ±0.46*

Note:* - indicates that the difference is statistically significant when compared to the control group ($p<0.05$).

The total production of NO under these conditions increased 2.56 times (tab. 2). The activity of iNOS increased by 9.46 times, while cNOS activity decreased by 63.41%. Arginase activity dropped by

10.33 times. The concentration of nitrites increased by 7.43 times. Therefore we can state, that the functioning of the nitric oxide cycle shifts toward the predominance of NOS-dependent cleavage of L-arginine.

NO hyperproduction's effect on testicular function significantly depends on the source of NO production. If the source of its production is endothelial NO-synthase, then NO improves testicular function and spermatogenesis [8]. However, in case of iNOS as its main producer, excessive NO has damaging effects on the testicular function [13]. Decrease of arginase activity also worsens the testicular function since polyphenols produced in arginase pathway are necessary for testicular cell division and differentiation [6, 7].

Dilated vascular lumens in the testes are the result of nitric oxide overproduction. An increase in iNOS activity, margination of leukocytes in the testes vessels, and decrease in the activity of arginases may indicate a change in the polarization of macrophages in the testes from anti-inflammatory (M2) to pro-inflammatory (M1) phenotype. Whereas under physiological conditions, the testes macrophages must have immunosuppressive function and the M2 phenotype [10]. Zhao Y. et al. showed, that NF-κB → COX2 signal pathway contributes greatly to testosterone reduction [15]. Activation of NF-κB during testosterone deficiency caused by central deprivation may be one of the reasons of macrophage M1 polarization. Role of NF-κB activation during the prolonged testosterone synthesis deprivation and its mechanisms demand further studies.

Destructive disorders in the ultrastructural organization of the plate cytoplasmic Golgi complex in intestinal endocrinocytes are explained by the increased production of $O_2^{\bullet-}$ by microsomal ETC.

Changes in the mitochondria of sustentocytes and spermatocytes correlate with the increased production of $O_2^{\bullet-}$ by mitochondrial ETC. Therefore, interstitial endocrinocytes increase production of $O_2^{\bullet-}$ at the expense of microsomal ETC, and sustentocytes and spermatocytes due to mitochondrial ETC under the conditions of testosterone deficiency.

Analyzing the dynamics of changes in biochemical parameters when compared to the results of the previous work, it should be noted that there is a compensatory increase in the activity of antioxidant enzymes, which, however, does not prevent further exacerbation of oxidative stress [12].

With a 30-day deprivation of testosterone synthesis, a change in the source of nitric oxide production was observed without increase of its production amount. Continuation of testosterone synthesis deprivation for up to 90 days increases nitric oxide production while maintaining a leading role of iNOS in its production. [12].

An increase in the concentration of nitrites in the testes can lead to the development of nitritive stress, which is accompanied by nitration of the thiol groups of proteins with the formation of modified cysteine.

Conclusion

On the 90th day of the experiment including central deprivation of testosterone synthesis in animals, we found that most of the cells in the sustentocytes population had destructive changes in mitochondria. An opposite tendency was revealed in the interstitial endocrinocyte population. They had structural signs of functional stress aimed at supporting the secretory function of the testes.

Central deprivation of testosterone synthesis for 90 days leads to the development of oxidative stress due to overproduction of reactive oxygen species and the accumulation of nitrites in the testes tissues due to the increased activity of inducible NO synthase.

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Реферати

МОРФОФУНКЦІОНАЛЬНІ ОСОБЛИВОСТІ ІНТЕРСТИЦІЙНИХ ЕНДОКРИНОЦИТІВ ТА СУСТЕНТОЦИТІВ ЯЄЧКА ЩУРІВ ПРИ ЦЕНТРАЛЬНІЙ ДЕПРИВАЦІЇ СИНТЕЗУ ТЕСТОСТЕРОНА ПРОТЯГОМ 90 ДІБ

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На сьогоднішній день в світі спостерігається тенденція до зміни ставлення до сім'ї та сімейних цінностей. У розвинених європейських країнах спостерігається тенденція до високої сексуальної активності у чоловіків літнього віку і пізнього створення сім'ї з дітьми, що має певні труднощі у зв'язку зі зниженням вироблення тестостерону в літньому віці. Метою дослідження було встановити мікроскопічну організацію інтерстиціальних ендокріноцітов і сустентоцітов щурів, визначити джерела продукції оксиду азоту і інтенсивність окисного стресу в яєчках з експериментальної центральної деприваціі синтезу тестостерону з диферелін на 90-ту добу експерименту. Експеримент був проведений на 20 зрілих білих щурах-самцях. Щури були розділені на 2 групи: контрольна група (10), експериментальна група (10), яким підшкірно вводили диферелін (трипторелін ембонат) в дозі 0,3 мг / кг активної речовини протягом 90 днів. Тривала центральна депривація синтезу тестостерону у тварин призводить до появи структурних ознак функціонального стресу в популяції сустентоцітов і інтерстиціальних ендокріноцітов, які спрямовані на підтримку секреції яєчка. Центральна депривація синтезу тестостерону протягом 90 днів викликає розвиток окисного стресу за рахунок гіперпродукції активних форм кисню і накопичення нітритів в тканини яєчка через підвищену індуцібельной активності NO-синтази.

Ключові слова: сім'яники, інтерстиційні ендокриноцити, сустентоцити, NO- синтаза, iNOS, cNOS, L- аргінін, супероксиддисмутаза, мітохондріальна ЕТЛ, М1, М2, щури.

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МОРФОФУНКЦИОНАЛЬНЫЕ ОСОБЕННОСТИ ИНТЕРСТИЦИАЛЬНЫХ ЭНДОКРИНОЦИТОВ И СУСТЕНТОЦИТОВ ЯИЧКА КРЫС ПРИ ЦЕНТРАЛЬНОЙ ДЕПРИВАЦИИ СИНТЕЗА ТЕСТОСТЕРОНА В ТЕЧЕНИИ 90 ДНЕЙ

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По мере развития социума наблюдается тенденция к изменению отношения к семье и семейным ценностям. В развитых европейских странах наблюдается тенденция к высокой сексуальной активности у пожилых мужчин и позднему созданию семьи с детьми, что имеет определенные трудности в связи со снижением выработки тестостерона в пожилом возрасте. Целью исследования было установить микроскопическую организацию интерстициальных эндокриноцитов и сустентоцитов крыс, определить источники продукции оксида азота и интенсивность окислительного стресса в яичках с экспериментальной центральной депривацией синтеза тестостерона с диферелином на 90-е сутки. эксперимент. Эксперименты проводились на 20 зрелых белых крысах-самцах линии Вистар. Крысы были разделены на 2 группы: контрольная группа (10), экспериментальная группа (10), которым подкожно вводили диферелин (трипторелин эмбонат) в дозе 0,3 мг / кг активного вещества в течение 90 дней. Длительная центральная депривация синтеза тестостерона у животных приводит к появлению структурных признаков функционального стресса в популяции сустентоцитов и интерстициальных эндокриноцитов, которые направлены на поддержку секреции яичка. Центральная депривация синтеза тестостерона в течение 90 дней вызывает развитие окислительного стресса за счет гиперпродукции активных форм кислорода и накопления нитритов в ткани яичка из-за повышенной индуцибельной активности NO-синтазы.

Ключевые слова: семенники, интерстициальные эндокриноциты, сустентоциты, NO-синтаза, iNOS, cNOS, L-аргинин, супероксиддисмутаза, митохондриальная ЭТЦ, М1, М2, крысы.

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