

MORPHOFUNCTIONAL STATE TESTES OF MATURE RATS ON THE BACKGROUND OF DRUG USE ASPARTAT OF ARGININE

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Лужин В.И. Морфофункциональное состояние семенников половозрелых крыс на фоне употребления аспартата аргинина // Украинський морфологічний альманах. – 2011. – Том 10, №4. – С. 152-154.

В работе проведено комплексное морфологическое исследование семенников интактных крыс и животных, которые получали аспарат аргинина, с помощью анатомических, гистологических, цитологических, электронно-микроскопических и статистических методов. Было установлено, что применение препарата аспартата аргинина («Тивортин») не приводит к статистически достоверным структурным и функциональным изменениям семенников животных контрольной группы. Основываясь на морфологической картине можно сделать вывод о стабильном функционировании половых желез.

Ключевые слова: семенники, строение, «Тивортин»®, спермограмма.

Лужин В.И. Морфофункціональний стан сім'яників статевозрілих щурів на тлі застосування аспартату аргініну // Український морфологічний альманах. – 2011. – Том 10, №4. – С. 152-154.

У роботі проведено комплексне морфологічне дослідження сім'яників інтактних щурів та тварин, що отримували аспарат аргініну, за допомогою анатомічних, гістологічних, цитологічних, електронно-мікроскопічних та статистичних методів. Було встановлено, що застосування препарату аспартату аргініну («Тивортину») не веде до статистично вірогідних структурних та функціональних змін сім'яників тварин контрольної групи. Виходячи з морфологічної картини, можна зробити висновок про стабільне функціонування статевих залоз.

Ключові слова: сім'яники, «Тивортин»®, спермограма.

Luzin V.I. Morphofunctional state testes of mature rats on the background of drug use aspartat of arginine // Український морфологічний альманах. – 2011. – Том 10, №4. – С. 152-154.

This work analyzes a comprehensive morphological study of the testes of intact rats and animals, that were received aspartat of arginine, using the anatomical, histological, cytological, electron microscopic and statistical methods. It was indicated that the usage of medicine aspartat of arginine doesn't lead to a statistically significant structural and functional changes of the testes of animals' control group. The stable functioning of the gonads can be concluded, based on the morphological picture.

Key words: testes, structure, aspartat of arginine, semen.

The work is a part of the research on the theme "Morphofunctional features of adjustment of the skeleton and internal organs in conditions of disturbed homeostasis" (№ state registration 0107U001287).

One of the main reasons of the male infertility is the high sensitivity of the male reproductive system to various chemical and physical agents. The generative structures of testis are the faultiest, which at certain stages of spermatogenesis react to even minor changes in the environment [1]. Therefore, the development of current issues of etiology, pathogenesis, diagnosis and treatment of male infertility in the influence of various environmental factors, is exposed as a priority of modern medicine. [2]

The most common environmental factors that have an effect on the structure and function of the reproductive system are the compounds of some heavy metal salts (HMS), which have a direct and an indirect cytotoxic effect on cells of the testes. [1]

Various drugs are used to reduce the negative influence of HMS on various organs and tissues. In our previous studies we used aspartat of arginine (the domestic production drug "Tivortin" - pharmaceutical company "Yurii Farm", Ukraine). Selection of "Tivortin" for the correction HMS damaging effect on the body in general, is due to the fact that the first of influenced structure is blood capil-

lary's endothelium of microvasculature, including the testes. An active substance of investigational drug is arginine, which was formed by the dissociation of salts of L-arginine aspartate. L-arginine is the only substrate for the synthesis of NO: this physiological process is intended to the support of normal functioning of the endothelium [3, 4]. We conducted experimental studies for exploration of the possibilities of arginine aspartate as a corrector of the morphological changes in the testes under influence on HMS [5]. For the more correct comparison of the results an experiment of the influence of the drug aspartat of arginine on the morphofunctional state of intact animals was conducted.

The **aim** was to experimental study of the influence of the drug aspartat of arginine on the morphofunctional state of intact testes.

Objects and methods. The survey was conducted on 64 laboratory mature white male rats (5 months old, with initial weight 180-200 g). During the experiment, laboratory animals were kept according to the rules adopted by the European Convention for the Protection of Vertebrate Animals using for that experiment and scientific tasks (Strasbourg, 1986), "General ethical rules of experiments on animals", was approved by the National Congress on Bioethics (Kyiv, 2001) and the Law of

Ukraine "On protection of animals from cruelty» № 3477-IV of 21.02.2006 p.

The first group consisted of control rats, which were injected intraperitoneally 2 ml of saline. The animals of the second group received "Tivortin" in a dose of 336 mg/kg intraperitoneally. The duration of the experiment (48 days) is equal to one cycle of spermatogenesis and the time, which is necessary for the passage of sexual gametes to the testis's appendix [1]. Animals were derived from experimental at 7, 14, 30 and 48 days of the experiment by decapitation under ether anesthesia to study the dynamic of the morphological changes. Testes were isolated, weighed on an analytical balance Axis AD-300 (Poland). During the study anatomical, histological, cytological methods and methods of scanning and transmission electron microscopy, atomic absorption spectrophotometry were used.

Statistical analysis predicted a comparison between groups using tailed Student's t-test, the difference was considered a probable at $p < 0.05$.

Results and their discussion. In series of intact animals a slight increase in all size-weighted indexes of gonads was manifested, compared with an each previous term observation - mass, volume, length, width and thickness of the testis. Thus, during the experiment, the mass of the right seminal gland varies 1,44-1,62 g, volume of cancer - 2,03-2,2 cm³, length - 18,84-19,7 mm, width - 11,03-11,45 mm, thickness - 9,89-10,05 mm.

Dimensional-weight performance series of animals, which received "Tivortin" characterized by a tendency of increase. Right testis's weight during the experiment varied within 1,43-1,63 g, volume - 2,05-2,24 cm³, length - 18,9-19,68 mm, width - 11,05-11,43 mm, thickness 9,91-10,06 mm. Between indicators of intact animals and rats, which were treated with pharmaceuticals, reliable statistical differences were found.

Sex glands of rats are covered by white dense connective tissue sheath, which consists of several rows of collagen, a small amount of elastin fibers, which are closely adjacent to each other. Internal parts of connective tissue membrane contain blood vessels, collagen fibers have more loose arrangement (Fig. 2). In connective tissue membranes or trabeculae, which branch off from the protein shell inside the organ, the number of elastic fibers increase. Loose connective tissue is between the seminiferous tubules, it's poor in fibrous structures. Fibrous component is more pronounced in areas near the adventitial arteries and veins, around the islets of Leydig cells (Fig.1). Cellular structure of testicular stroma is represented by fibroblasts and fibrocytes. Fibroblasts were elongated, spindle-shaped, had basophilic nucleus. In loose connective tissue also mast cells, histiocytes can be found. Blood vessels diffusely permeate testis tissue, they have moderate filling with blood. Lymphatic capillaries are invisible; they are in a state of fervor.

It was established a slight fluctuations of most morphometric parameters and indices in the morphometric study of the structural components of

the testes of intact mature rats and animals, which were treated only by corrector. Thus, the thickness of the shell tunic (TST) during observations of intact animals varied from 18,85-20,05 mcm, diameter of convoluted seminiferous tubules (DST) - from 278.34 to 296.8 mcm, height of spermatogenic epithelium in the convoluted wooden tubules (HSE) - from 61.55 to 64.5 mcm. Spermatogenesis index of intact mature rats ranged between 3,44-3,48. In the group of animals under the influence of the drug "Tivortin" TST during the experiment ranged 19,36-20,11 mcm, DST - 274,89-297,03 mcm, HSE - 61,32-66,51 mcm, index of spermatogenesis - 3,46-3,47.

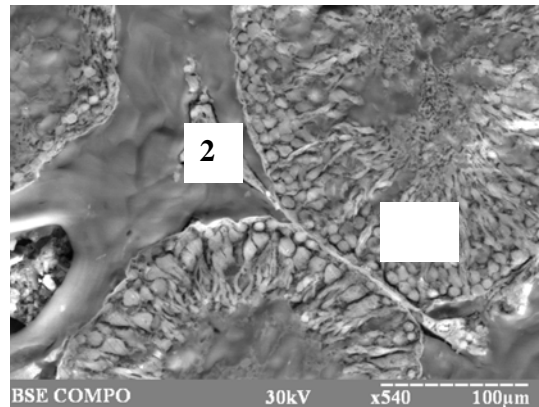


Figure 1. Electronic scans of convoluted seminiferous tubules. Control group animals. 1 - spermatogenic epithelium of the seminiferous tubules, 2 - islet cells of Leydig. x540.

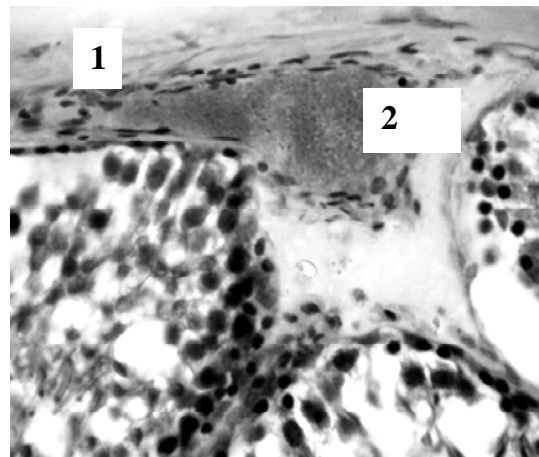


Figure 2. Connective tissue covers mature rat's testis. Control group. Hematoxylin-eosin staining. x400. 1 - outer shell area, 2 - blood capillary.

The average number of Sertoli cells in tubules' cut in the group of intact animals remained constant (89,25-91,5), as well as in the group of animals that received only corrector (90,5-90,75) and it wasn't differ. The larger and smaller diameters of interstitial cells in intact group during the observation period ranged from 4.05 to 4.16 mcm and from 2.39 to 2.51 mcm in the group of animals that received only "Tivortin" - from 4.1 to 4,19 mcm and from 2.4 to 2.49 mcm, respectively, significant difference

between the indices of studied groups was not found.

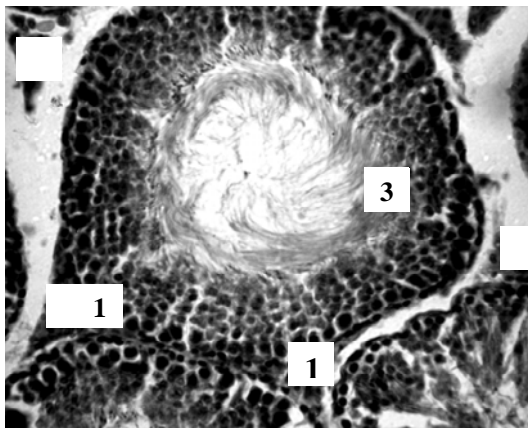


Figure. 3. Interstitial cells around the seminiferous tubules of mature rat. Group of isolated corrector effects. 1 - clusters of Leydig cells (interstitial cells), 2 - blood capillary, 3 - spermatozoa are formed. Hematoxylin-eosin staining. x400.

Correlation between stromal-vascular and glandular component remains relatively stable throughout the experiments' duration in the both groups of animals. For intact rat a stromal area is $16,8 \pm 1,1\%$, parenchyma - $83,2 \pm 1,5\%$, for animals, which were treated by corrector - $16,35 \pm 1,2\%$ and $83,65 \pm 1,3\%$ accordingly.

Semen was performed to identify the morphofunctional status of testes of control series' animals. Using this method, it was found that the slurry, which were obtained from the testes of intact rats, contained an average 49,35-53,6 million spermatozoons in 1 ml, 73,23-76,46% of which are mobile, 14 -14.73% gametes had anomalies morphological structure: a deformation of the head and tail parts, inflection neck. Semen's qualities of rats, which received only drug "Tivortin"®, were not significantly different from that, which were received from intact animals, it indicate any negative or positive corrector's effect on spermatogenesis.

According to the study in 1 ml of semen $35,6 \pm 2,1$ million spermatozoons contained, of which $57 \pm 1,5\%$ live, 45% motile, agglutination was absent.

In determination of the element composition of rat testis tissue of the control group zinc, copper, iron, manganese, chromium and lead at 51,16-56,07 mg / g 1,48-1,51 mg / g, 26 ,13-31, 43 mg / d, 0,96-1,07 mg / g, 1,17-1,2 mg / g and 0,07-0,08 mg / g, respectively were found.

In determination of the element composition of testes tissue of rats, which received only aspartat of arginine, zinc, copper, iron, manganese, chromium and lead was determined at 52,03-58,03 mcg/g, 1,52-1,53 mg / g, 29,17-32,97 mcg/g, 1,06-1,09 mg/g, 1,2-1,22 mg/g and 0,08-0,09 mg/g, respectively were found. These results are not statistically significantly different from the control group in trace elements.

Conclusions:

1. Based on the study morphological picture, we can conclude about the stability of tissue gonads.

2. Use of the drug "Tivortin" does not result in statistically significant structural and functional changes in the testes of animals of the control group.

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