

## MORPHOMETRIC EVALUATION OF SPERMATOGENIC EPITHELIOCYTES STRUCTURE UNDER THE RUBOMYCIN INFLUENCE IN RATS

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**Background.** Over the last 20 years, the quality of men's sperm around the world has deteriorated twice. The causes are poor environment, stress, sedentary lifestyle, prevalence of sexually transmitted infections, obesity, alcohol abuse and various stimulants. The structure and function of the testes, when exposed to various drugs and chemicals, attracts the attention of present researchers.

**Objectives.** The aim of the research was to study the structural changes of the spermatogenic epithelium in testicular damage caused by rubomycin hydrochloride.

**Methods.** The spermatogenic epithelium of the testes of 30 white adult white male rats, which were divided into 2 groups, was morphometrically examined. The 1<sup>st</sup> group comprised 15 experimental intact animals, the 2<sup>nd</sup> – 15 rats, in which testicular damage with rubomycin hydrochloride was simulated. Euthanasia of rats was performed by bloodletting under thiopental anesthesia one month after the experiment beginning. Quantitative morphological parameters were statistically processed. In spermatocytes of the 1<sup>st</sup> P-order, spermatogonia and spermatids of testes, their height, diameter of nuclei, nuclear-cytoplasmic ratio and relative volumes of damaged cells of spermatogenic epithelium were evaluated.

**Results.** It was established that spermatogenic epithelial cells were damaged in the simulated experimental conditions, which was morphometrically confirmed by changes in nuclear-cytoplasmic relations in the 1<sup>st</sup>, 2<sup>nd</sup> order spermatocytes, spermatogonia and spermatids. The relative volumes of damaged spermatogenic epithelial cells also increased significantly. The most significant morphometric parameters were altered in spermatids. Thus, the nuclear-cytoplasmic ratio in the studied cells was statistically significantly ( $p < 0.001$ ) increased by 7.4% compared with the similar control morphometric parameter. The relative volume of damaged spermatids in these experimental conditions reached  $(32.50 \pm 0.18) \%$ , which with a high statistically significant difference ( $p < 0.001$ ) exceeded the same control value in almost 15.8 times. Optically, histological preparations of the testes showed severe vascular disorders, characterized by dilation and plethora mostly of venous vessels, which pointed to violation of venous drainage of the studied organs.

**Conclusions.** According to the attained research results it has been established that nuclear-cytoplasmic relations are an objective and valuable informative indicator of a functional condition of cells and their structural changes in pathological conditions. When rubomycin hydrochloride is exposed to experimental animals, the most significant changes in nuclear-cytoplasmic relations are found in spermatid spermatozoa.

**KEY WORDS:** rubomycin hydrochloride, testes, spermatogenic epithelium, white rats, morphometry.

### Introduction

It is established that in the structure of infertile marriages, the male factor is 20% [2, 4, 8, 12, 17]. The reason for this is a decrease in spermatogenic and hormonal function of the testes. Often the reason for this is the effect of various toxic factors on the body [2, 13, 14, 19]. In recent years, many researchers are interested in changes in the structure and function of the testes in different physiological and pathological conditions [10, 11, 15, 19].

Rubomycin is an antitumor antibiotic of the anthracycline series with a significant cytostatic

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effect, which when administered to men can lead to azoospermia (absence of sperm in the ejaculate) [5]. The effect of rubomycin on the structure of the spermatogenic epithelium has been studied insufficiently.

The aim of our study was to investigate the features of structural changes in the spermatogenic epithelium in cases of testicular damage by rubomycin hydrochloride.

### Methods

The testicular structures of 30 laboratory adult white male rats, which were divided into 2 groups, were studied by morphometric methods. The 1<sup>st</sup> group comprised 15 experimental intact animals, the 2<sup>nd</sup> – 15 rats with simulated

testicular damage by rubomycin hydrochloride [18]. One month after the beginning of the experiment, euthanasia of experimental animals was performed by bloodletting under conditions of thiopental-sodium anesthesia. Pieces were cut from the testes, which were fixed in 10 % neutral formalin solution, passed through ethyl alcohols of increasing concentration and placed in paraffin. Microtome sections 5-6 mcr thick after dewaxing were stained with hematoxylin-eosin, according to van Gizon, Mallory, Weigert, toluidine blue [7]. Histological micropreparations determined the height of spermatogenic cells, the diameter of their nuclei, nuclear-cytoplasmic ratios, the relative volume of damaged epitheliocytes (VODE) [1, 6, 16]. Morphometry of these structures was performed using a light microscope "Olimpus BX-2" with a digital video camera and application package "Video Test 5.0" and "Video size 5.0". Quantitative values were processed statistically by STATISTIKA software package. The differences between the comparative values were determined by the Mann-Whitney and Student's tests [9]. The studies were performed according to national and international regulations on carrying out experimental tests ("European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments", adopted by the First National Congress on Bioethics (Kiev, 2001)) [8], as well as the Law of

Ukraine "On Protection of Animals from Cruelty", dated February 21, 2006.

### Results

The attained morphometric parameters are presented in Table 1. A comprehensive analysis of the data showed that most of the studied morphometric parameters of the spermatogenic epithelium changed significantly. Thus, the height of spermatocytes of the 1<sup>st</sup> order decreased slightly by only 0.65% ( $p < 0.05$ ). The diameters of the nuclei of these cells with a high statistically significant difference ( $p < 0.001$ ) increased from  $(5.25 \pm 0.03) \mu\text{m}$  to  $(5.76 \pm 0.04) \mu\text{m}$ , i.e. by 9.7%. Uneven changes in the spatial characteristics of the nucleus and cytoplasm of spermatocytes of the 1<sup>st</sup> order led to violations of nuclear-cytoplasmic relations in them. This morphometric index increased by 3.2% ( $p < 0.05$ ), which pointed to violation of the studied elements of structural cellular homeostasis [1]. The relative volume of damaged first-order spermatocytes under these experimental conditions with a high statistically significant difference ( $p < 0.001$ ) increased almost in 9.7 times.

The morphometric parameters of P-order spermatocytes changed almost similarly under the influence of rubomycin hydrochloride. The height of these cells was almost the same as in the control, the diameters of the nuclei were statistically significantly ( $p < 0.05$ ) increased by 2.4%, and the nuclear-cytoplasmic ratio – by 4.9% ( $p < 0.01$ ). The relative volume of damaged

**Table 1. Morphometric characteristics of cells of spermatogenic epithelium of testes of the experimental animals ( $M \pm m$ )**

Cells	Morphometric parameter			
	Height, microns	Diameter of the core, microns	Nuclear-cytoplasmic relations	VODE, %
Control group				
Spermatocytes of the first order	7.65±0.05	5.25±0.03	0.556±0.003	2.20±0.01
Spermatocytes of the second order	5.88±0.04	3.76±0.03	0.410±0.002	2.26±0.02
Spermatogonia	6.12±0.04	4.80±0.03	0.615±0.004	2.15±0.02
Spermatids	4.98±0.03	3.07±0.03	0.389±0.002	2.06±0.02
Experimental group				
Spermatocytes of the first order	7.60±0.05	5.76±0.04***	0.574±0.004*	21.30±0.15***
Spermatocytes of the second order	5.85±0.04	3.85±0.03*	0.430±0.003**	25.60±0.15***
Spermatogonia	6.05±0.04	4.79±0.03	0.628±0.004*	29.80±0.15***
Spermatids	4.92±0.03	3.18±0.02*	0.418±0.002***	32.50±0.18***

Notes. \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ . VODE – volume of damaged epitheliocytes.

P-order spermatocytes under these experimental conditions was equal to  $(25.60 \pm 0.15)\%$ . This morphometric parameter with a high statistically significant ( $p < 0.001$ ) difference exceeded the similar quantitative morphological index in 11.3 times.

The height of spermatogonia of the testes in the experimental animals affected by the studied drug medium decreased by only 1.1% ( $p < 0.05$ ), the diameters of their nuclei did not change significantly, and nuclear-cytoplasmic ratios increased by 2.1% ( $p < 0.05$ ). The relative volume of damaged spermatogonia in these experimental conditions statistically significantly ( $p < 0.001$ ) increased in 13.8 times and reached  $(29.80 \pm 0.15)\%$ .

Analysis of the obtained data revealed that under the influence of rubomycin hydrochloride the height of testicular spermatids decreased by only 1.2% ( $p < 0.05$ ), the diameters of their nuclei increased by 3.6% ( $p < 0.05$ ). Nuclear-cytoplasmic ratios in the studied cells statistically significantly ( $p < 0.001$ ) increased by 7.4% compared with the similar control morphometric parameter. The relative volume of damaged spermatids under these experimental conditions reached  $(32.50 \pm 0.18)\%$ . This morphometric parameter with a high statistically significant difference ( $p < 0.001$ ) exceeded the similar control value in almost 15.8 times.

### Discussion

Analysis of the above morphometric parameters proved that the introduction of rubomycin hydrochloride into the body of experimental animals led to structural rearrangement and damage to cells of spermatogenic epithelium of the testes. The most pronounced changes were found in spermatids in comparison with other studied cells.

According to present researchers, nuclear-cytoplasmic relations are a valuable morphometric indicator that adequately reflects the peculiarities of cell function and metabolism, degree of disturbances of cellular structural homeostasis [1, 6, 16]. The nucleus and cytoplasm of cells are separated from each other, but at the same time they are closely interconnected and integrated and form a single structural and functional system. It follows that the isolated study of quantitative morphological parameters of only the cytoplasm of the cell or only its nucleus allows getting a one-sided idea of these structural components of the cell. The study of nuclear-cytoplasmic relationships makes it possible to obtain more adequate,

objective and in-depth information on the relationship between the nucleus and cytoplasm of cells and their changes in various physiological and pathological conditions. Nuclear-cytoplasmic relations can change at hyperfunction of bodies as well as hypofunction, and also in cases of their damage. It is established that nuclear-cytoplasmic ratios depend on cell differentiation. The specified morphometric parameter decreases in case of maturation of cells and their differentiation that is caused to some extent by increase in cytoplasm and its hyperplasia of specific functioning organelles. The relationship between the spatial characteristics of the nucleus and cytoplasm of the cell can also change with cell division, growth, diploidy [6, 16].

In the optical examination of testicular micro preparations, significant vascular disorders were evidenced, which were characterized by dilation and plethora of mainly venous vessels, which pointed to violation of venous drainage from the studied organs (Fig. 1) [3].

A severe edema of the stroma, foci of dystrophically, necrobiotically altered spermatogenic epitheliocytes, stromal structures, local cellular infiltrates, sclerotic processes, desquamation and proliferation of vascular endothelial cells was present (Pic. 2). The latter pointed to the presence of hypoxia. Foci of vascular endothelial cell edema, permeation of their membrane with plasma proteins were also present. Foci of fibrinoid swelling and necrosis in some vessels were evidenced that indicated their severe damage. Among spermatogenic epitheliocytes in the studied conditions of the experiment, spermatids were the most morphologically altered, in which the degree of disorders

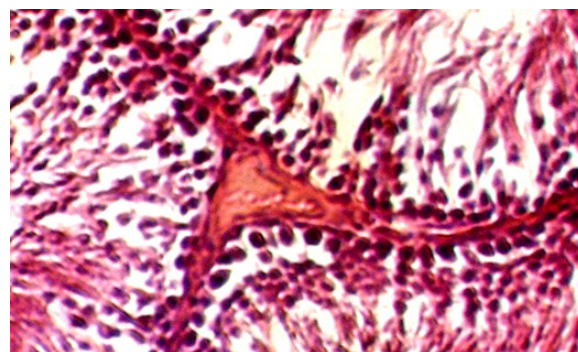


Fig. 1. Stromal edema, partial reduction of layers and damage of spermatogenic epithelial cells, their desquamation, thickening of the membranes of the tortuous seminal tubules of the testis of a white rat under the influence of rubomycin hydrochloride. Hematoxylin-eosin staining.  $\times 140$ .

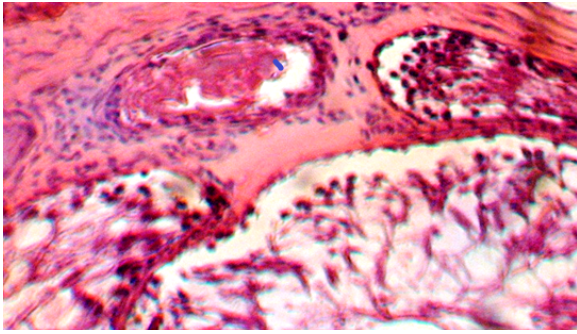


Fig. 2. Structural changes in the vessel wall, perivascular sclerosis, dystrophy, edema, desquamation of spermatogenic epitheliocytes in the tortuous seminal tubules of the testis of experimental animals under the influence of rubomycin hydrochloride. Hematoxylin-eosin staining.  $\times 140$ .

of nuclear-cytoplasmic relations and the relative volume of damaged cells were the largest. It points to the fact that the studied morphometric parameter also reflects the severity and depth of cell damage.

### Conclusions

Nuclear-cytoplasmic relations are an objective and significant informative indicator of functional state of the cells and their structural changes in pathological conditions. When rubomycin hydrochloride is exposed to experimental animals, the most significant changes in nuclear-cytoplasmic relations are found in spermatogenic spermatozoa.

### Funding

This research received no external funding.

### Conflict of Interests

The authors declare no conflict of interest.

### Author Contributions

*Hnatiuk M.S.* – supervision, morphometry, morphological analysis of micropreparations, writing – review and editing; *Konovalenko S.O.* – writing, generalization of results, project administration, *Tatarchuk L.V.* – supervision, review and editing, *Yasinovsky O.B.* – literature analysis, data curation.

## МОРФОМЕТРИЧНА ОЦІНКА СТРУКТУРНОЇ ПЕРЕБУДОВИ КЛІТИН СПЕРМАТОГЕННИХ ЕПІТЕЛІОЦИТІВ СІМ'ЯНИКІВ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН ПІД ВПЛИВОМ РУБОМІЦИНУ ГІДРОХЛОРИДУ

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**Вступ.** За останні 20 років якість сперми чоловіків у всьому світі погіршилась у 2 рази. Причини: погана екологія, стреси, малорухливий спосіб життя, поширеність статевих інфекцій, ожиріння, зловживання алкоголем і різними стимуляторами. Структура та функція сім'яників при дії на організм різних медикаментозних середників та хімічних речовин привертає увагу сучасних дослідників.

**Мета.** Вивчити особливості структурних змін сперматогенного епітелію при ушкодженні сім'яників рубоміцином гідрохлориду.

**Методи.** Морфометрично досліджено сперматогенний епітелій сім'яників 30 білих статевозрілих білих щурів-самців, які були поділені на 2-і групи. 1-а група включала 15 дослідних інтактних тварин, 2-а – 15 щурів, у яких моделювали ушкодження сім'яників рубоміцином гідрохлоридом. Евтаназію щурів здійснювали кровопусканням в умовах тіопенталового наркозу через місяць від початку дослідження. Кількісні морфологічні показники обробляли статистично. У сперматоцитах 1-го. 2-го порядків, сперматогоніях та сперматидях сім'яників вимірювали їх висоту, діаметр ядер, ядерно-цитоплазматичні відношення та відносні об'єми пошкоджених клітин сперматогенного епітелію.

**Результати.** Встановлено, що у змодельованих експериментальних умовах пошкоджувалися клітини сперматогенного епітелію, що морфометрично підтверджувалося змінами ядерно-цитоплазматичних відношень у сперматоцитах 1-го, 2-го порядків, сперматогоніях та сперматидях. Виразено зростали також відносні об'єми пошкоджених вказаних клітин сперматогенного епітелію. Найбільш виражено морфометричні параметри виявилися зміненими у сперматидях. Так, ядерно-цитоплазматичні відношення у досліджуваних клітинах статистично достовірно ( $p < 0,001$ ) збільшилися на 7,4 % порівняно з аналогічним контрольним морфометричним параметром. Відносний об'єм пошкоджених сперматид у даних умовах експерименту досягав ( $32,50 \pm 0,18$ ) %, що з вираженою статистично достовірно різницею ( $p < 0,001$ ) перевищував аналогічну контрольну величину майже у 15,8 рази. Світлооптично на гістологічних препаратах сім'яників спостерігалися виражені судинні розлади, що характеризувалися

розширенням та повнокров'ям переважно венозних судин, що свідчило про порушення венозного дренажу від досліджуваних органів.

**Висновки.** На основі проведених досліджень та отриманих результатів встановлено, що ядерно-цитоплазматичні відношення є об'єктивним та цінним інформативним показником функціонального стану клітин та їх структурних змін в патологічних умовах. При дії на організм дослідних тварин рубоміцину гідрохлориду найвираженіші зміни ядерно-цитоплазматичних відношень виявлені у сперматидях сім'яників.

**КЛЮЧОВІ СЛОВА:** рубоміцин гідрохлорид, сім'яники, сперматогенний епітелій, білі щурі, морфометрія.

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*Received 26 Mar 2020; revised 07 Apr 2020; accepted 29 May 2020.*

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