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ULTRASTRUCTURAL CHANGES IN THE SPERMATOGENIC EPITHELIUM OF THE TESTES OF YOUNG RATS WITH EXPERIMENTAL DIABETES MELLITUS

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Abstract. Diabetes mellitus (DM) is one of the most common endocrine pathologies globally, accompanied by numerous complications including reproductive disorders in men, particularly pathological changes in the spermatogenic epithelium leading to infertility. Streptozotocin-induced diabetes mellitus (SDM) in experimental animals serves as an adequate model for studying the pathogenesis of diabetic complications. The aim of this study was to establish the features of pathomorphological changes in the spermatogenic epithelium of 2-month-old rats' testes with streptozotocin-induced diabetes mellitus in the dynamics of the disease over a period of 42 days. The study included 50 white outbred male rats (2 months old, initial weight 150-180g), divided into control (n=20) and experimental (n=30) groups. SDM was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg). Material was collected on days 14, 28, and 42 of the experiment. Histological, electron microscopic, and morphometric research methods were employed to determine the diameter of convoluted seminiferous tubules, the diameter of their lumen, the height of the spermatogenic epithelium, the tubule differentiation index (TDI), and the spermatogenesis index (SI). The results showed that pathomorphological changes developed in stages. On day 14, testicular histostructure remained mostly preserved with minor changes in spermatogenic epithelium height (decrease to $63.78 \pm 8.15 \mu\text{m}$ compared to control $70.33 \pm 9.81 \mu\text{m}$, $p < 0.05$) and ultrastructural dystrophic changes were detected in supporting epitheliocytes and spermatogenic cells, primarily in mitochondria. By day 28, interstitial tissue edema appeared, cells of the adluminal compartment lost connection and exfoliated into the lumen of the convoluted seminiferous tubule. The height of the spermatogenic epithelium continued to decrease to $57.16 \pm 10.17 \mu\text{m}$ (control $99.33 \pm 16.77 \mu\text{m}$, $p < 0.05$), while the tubule lumen diameter increased by 16.2 % ($p < 0.05$). The TDI was 81.56 ± 9.52 % (control 90.34 ± 5.59 %, $p < 0.05$) and the SI was 15.25 ± 17.05 % (control 22.65 ± 17.95 %, $p < 0.05$). By day 42, pronounced atrophic-destructive changes were observed: vacuolar and hydropic dystrophy of spermatogonia, apoptosis and necrosis of spermatocytes, and near-complete absence of spermatids. The diameter of convoluted seminiferous tubules decreased to $237.37 \pm 43.39 \mu\text{m}$ (control $308.57 \pm 34.87 \mu\text{m}$, $p < 0.05$), epithelium height to $53.29 \pm 4.08 \mu\text{m}$ (control $109.74 \pm 17.54 \mu\text{m}$, $p < 0.05$), and lumen diameter increased to $130.78 \pm 43.82 \mu\text{m}$ (control $89.11 \pm 4.76 \mu\text{m}$, $p < 0.05$). In conclusion, pathomorphological changes in the spermatogenic epithelium of the testes of 2-month-old rats with SDM are characterized by progressive deterioration, transforming from minimal ultrastructural disorders in early stages (day 14) to pronounced dystrophic-destructive changes with atrophy of the spermatogenic epithelium and impaired spermatogenesis at the late stage (day 42). The height of the spermatogenic epithelium decreases by 51.4% compared to the control, while SI decreases by 33 %, indicating a significant impairment of reproductive function in experimental diabetes.

Keywords: streptozotocin-induced diabetes mellitus, spermatogenic epithelium, testes, ultrastructural changes, morphometry, experimental animals, reproductive function, atrophic-destructive changes, spermatogenesis index, convoluted seminiferous tubules.

Introduction. Diabetes mellitus (DM) is one of the most common endocrine pathologies, affecting approximately 537 million people worldwide, with a projected increase to 783 million by 2045 [1]. According to the International Diabetes Federation, the prevalence of DM among the adult population of Ukraine is 7.1%, which corresponds to approximately 2.3 million patients [2, 3]. The problem of reproductive function disorders in men with DM deserves special attention, as they occur in 35-55 % of cases [4, 5].

Among the complications of DM that affect the male reproductive system, the most common are diabetic neuropathy and microangiopathy of the reproductive organs [5, 6]. The pathomorphological substrate of these disorders includes changes in the spermatogenic epithelium, which lead to oligozoospermia, asthenozoospermia, and teratozoospermia [7-9]. However, despite a significant number of studies dedicated to the problem of diabetic gonadopathy, the pathomorphological changes in the

spermatogenic epithelium during the dynamics of DM development remain insufficiently studied.

Experimental models of DM in animals are an important tool for understanding the pathogenesis of diabetic complications. Streptozotocin-induced diabetes mellitus (SDM) is considered an adequate model that reproduces the main pathogenetic mechanisms of type 1 DM development and its complications [10, 11]. The study of the effect of SDM on young sexually immature animals is particularly relevant, as it allows for the assessment of the impact of metabolic disorders on the formation of the reproductive system [12-14].

The study of pathomorphological changes in the spermatogenic epithelium of the testes in SDM has not only theoretical but also practical significance, as it can contribute to the development of new methods for diagnosing and treating reproductive function disorders in men with DM.

The aim of the study. To establish the features of pathomorphological changes in the spermatogenic

epithelium of the testes of 2-month-old rats with streptozotocin-induced diabetes mellitus in the dynamics of the disease over 42 days, which will deepen the understanding of the pathogenesis of diabetic complications in the reproductive system. This study aims to identify sequential structural changes in testicular tissues at different stages of diabetes development, which has not only theoretical significance for understanding the mechanisms of spermatogenesis impairment but also practical value for developing new methods for early diagnosis and treatment of reproductive function disorders in young men with diabetes mellitus.

Materials and methods. The study was conducted on 50 white outbred male rats aged 2 months with an initial weight of 150-180 g, obtained from the vivarium of Ivano-Frankivsk National Medical University. The animals were kept in standard vivarium conditions with free access to water and food. All experiments were conducted in accordance with the "General Ethical Principles of Animal Experiments" (Ukraine, 2001) and the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

The animals were divided into 2 groups: control (n=20) and experimental (n=30). SDM was modeled in rats of the experimental group by a single intraperitoneal injection of streptozotocin (Sigma, USA) at a dose of 60 mg/kg, dissolved in 0.1 M citrate buffer (pH 4.5). The control group of animals was administered an equivalent volume of citrate buffer. The development of diabetes was verified by determining blood glucose levels using an Accu-Chek Active glucometer (Roche Diagnostics, Germany) on day 3 after streptozotocin administration. A glucose level above 13 mmol/L was considered the criterion for DM development.

Material for the study was collected on days 14, 28, and 42 of the experiment after euthanasia of the animals by decapitation under thiopental anesthesia. The testes were fixed in a 10% solution of neutral formalin and Bouin's fluid for light optical examination, as well as in a 2.5% solution of glutaraldehyde followed by post-fixation in a 1% solution of osmic acid for electron microscopic examination.

Paraffin sections with a thickness of 5-7 μm were stained with hematoxylin and eosin according to the standard technique. Semi-thin sections with a thickness of 1 μm were stained with methylene blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate according to Reynolds' method and examined under a PEM-125K electron microscope.

Morphometric analysis was performed using ImageJ software. The following parameters were determined: diameter of convoluted seminiferous tubules, diameter of their lumen, height of the spermatogenic epithelium. The tubule differentiation index (TDI) was calculated as the percentage of convoluted seminiferous tubules containing more than 3 rows of spermatogenic cells. The spermatogenesis index (SI) was defined as the percentage of convoluted seminiferous tubules containing spermatozoa.

Statistical processing of the results was carried out using Statistica 10.0 software. The normality of data distribution was checked with the Shapiro-Wilk test. For normal distribution, Student's t-test was applied; for non-normal distribution, the non-parametric Mann-Whitney U-

test was used. Differences were considered statistically significant at $p < 0.05$.

Results of the research and their discussion. In the control group of 2-month-old rats at the ultrastructural level, spermatogenic epithelium and Sertoli cells (sustentocytes) were located on the basal membrane of the convoluted seminiferous tubules. In the basal part of the convoluted seminiferous tubules, spermatogonia were located, and closer to their lumen - second-order spermatocytes and spermatids. The spermatogenic epithelium was divided by lateral contacts of sustentocytes into two compartments: basal and adluminal.

In the connective tissue layers between the convoluted seminiferous tubules, there were vessels, fibroblasts, and groups of interstitial endocrinocytes. The latter were characterized by electron-dense cytoplasm, numerous elongated young mitochondria, densely packed cisterns of smooth endoplasmic reticulum, and isolated secretory granules of moderate electron density.

On day 14 of SDM development, the histostructure of the testes of 2-month-old rats remained predominantly preserved. In some convoluted seminiferous tubules, a decrease in the height of spermatogenic epithelium and less dense cell packing were observed. According to morphometric analysis, the diameter of the convoluted seminiferous tubules and their lumen did not significantly differ from the control indicators and amounted to $209.28 \pm 19.84 \mu\text{m}$ (control - $219.28 \pm 21.79 \mu\text{m}$, $p > 0.05$) and $81.72 \pm 7.94 \mu\text{m}$ (control - $78.62 \pm 3.81 \mu\text{m}$, $p > 0.05$), respectively. There was a tendency to decrease the height of the spermatogenic epithelium to $63.78 \pm 8.15 \mu\text{m}$ (control - $70.33 \pm 9.81 \mu\text{m}$, $p < 0.05$). The tubule differentiation index (TDI) did not change significantly and was $87.64 \pm 5.5\%$ (control - $91.71 \pm 7.18\%$, $p > 0.05$) (Fig. 1).

On the ultrastructural level in the convoluted seminiferous tubules of diabetic rats, dystrophic changes were observed both in supporting epitheliocytes and in spermatogenic cells. In the Sertoli cells, the most pronounced changes occurred in mitochondria, which became rounded due to edema, their cristae were disorganized and partially destroyed, forming electron-transparent voids inside the organelles. The nuclei of spermatogonia were lightened due to chromatin condensation into separate clumps, and the cisterns of the smooth endoplasmic reticulum were expanded. In some spermatocytes and spermatids, apoptotic bodies were detected, indicating activation of cellular death processes in DM.

On day 28 of SDM development, edema of the interstitial tissue of the testes was noted. The cells of the adluminal compartment lost connection with each other and were exfoliated into the lumen of the convoluted seminiferous tubule. In the spermatogenic epithelium of the testes of rats in the experimental and control groups, it was still possible to trace the process of spermatogenesis and visualize spermatogonia and spermatocytes at different stages of the cell cycle.

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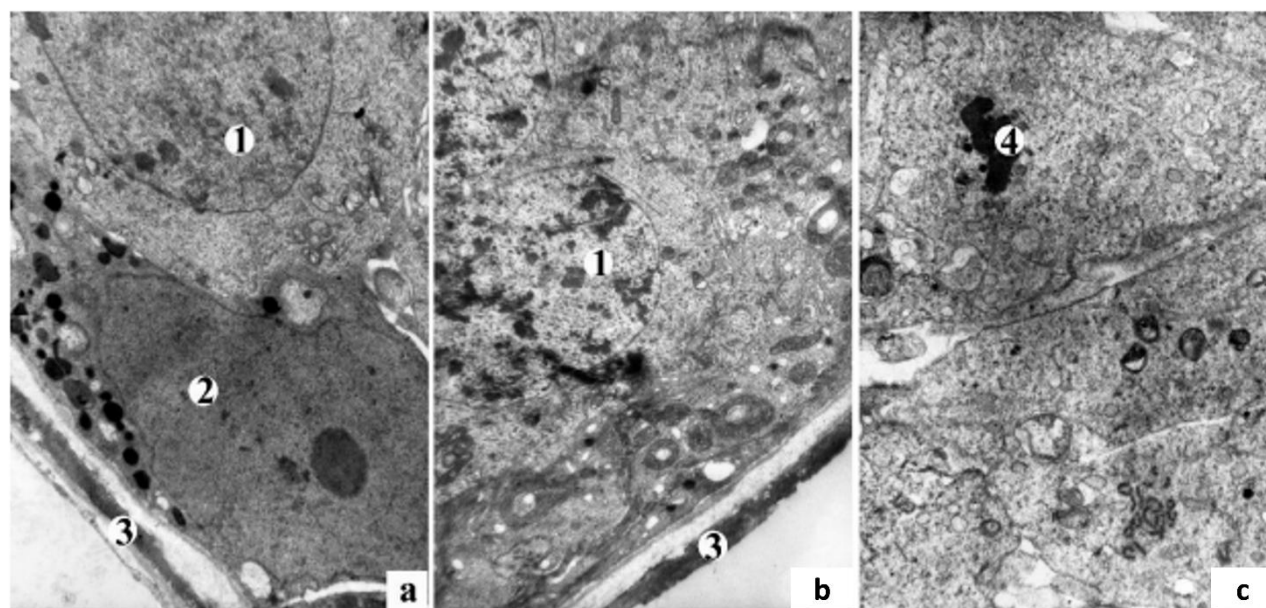


Fig. 1. Features of the ultrastructural organization of convoluted seminiferous tubules of a control rat (a) and with SDM (b, c) on day 14 of the experiment. Electron micrographs. Magnification: a, b) x4800, c) x6400

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In spermatocytes, apoptotic bodies, karyopyknosis and karyolysis, destruction of structural components of the Golgi complex and smooth endoplasmic reticulum were observed. During this period of SDM development, intercellular edema was detected in the spermatogenic epithelium, which often led to desquamation of cells of the luminal compartment into the lumen of the convoluted seminiferous tubules.

At day 42 at the ultrastructural level in the testicular parenchyma of 2-month-old rats, the development of vacuolar dystrophy in most cells of the spermatogenic epithelium is notable. In Sertoli cells, vacuolar dystrophy, expansion and destruction of smooth endoplasmic reticulum cisterns, disorganization and destruction of mitochondrial cristae, appearance of vacuoles and lipid droplets are observed (Fig. 3 a). The most pronounced changes in the spermatogenic epithelium of the testes occur in spermatogonia. The latter show development of vacuolar and

hydropic dystrophy, which ultimately leads to colliquative necrosis of these cells. In spermatocytes at various stages of maturation, the following are noted: nuclear edema and karyolysis, apoptosis, vacuolar dystrophy, proliferation and expansion of smooth endoplasmic reticulum cisterns, increased number of lysosomes, and appearance of autophagosomes (Fig. 3 b). In spermatocytes, apoptotic changes are often detected alongside necrotic changes. In 2-month-old rats with SDM, spermatids are practically not detected, whereas in the control group they are differentiated even at the histological level, indicating impairment of the spermatogenesis process and atrophy of the spermatogenic epithelium.

Conclusion. The progressive nature of morpho-functional changes in the spermatogenic epithelium of the testes of 2-month-old rats with streptozotocin-induced diabetes mellitus has been established: from minimal ultrastructural disorders of mitochondria in Sertoli cells and spermatogenic cells (day 14) to pronounced destructive changes with disruption of intercellular contacts, reduction of TDI to 81.56% and SI to 15.25% (day 28), and at the late stage (day 42) - to severe atrophic-destructive changes of all cellular components with the development of necrosis, karyolysis and cell apoptosis, reduction of spermatogenic epithelium height by 51.4% and increase of tubule lumen diameter by 46.8%. These changes are irreversible and indicate a significant impairment of reproductive function in young animals, which can serve as a basis for developing methods for early diagnosis of reproductive function disorders in young men with diabetes mellitus.

Conflict of interest: absent.

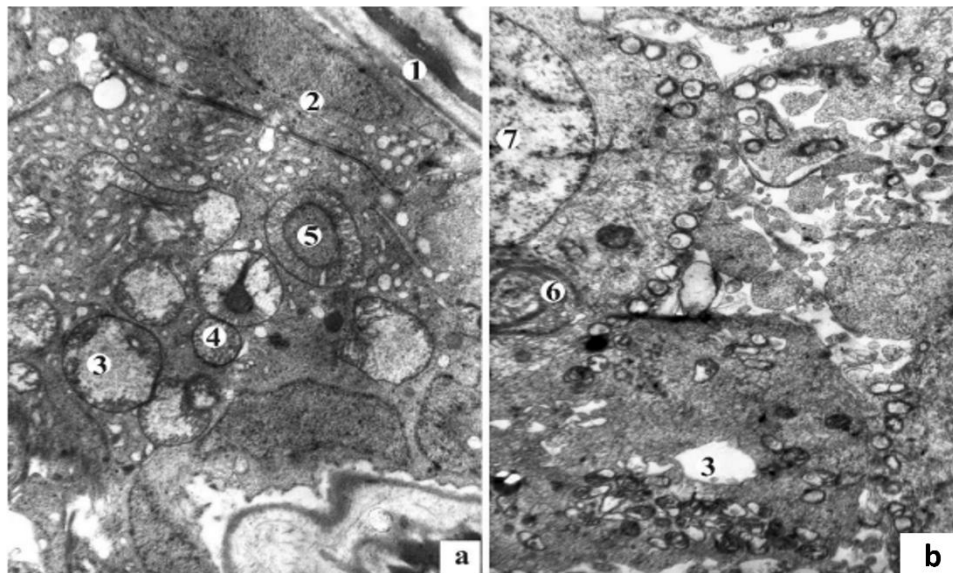


Fig. 2. Vacuolar dystrophy of Sertoli cells (a), spermatocytes and spermatids (b) on day 28 of SDM. Electron micrographs. Magnification: a, b) x6400

Designations: 1 – basal membrane; 2 – Sertoli cell, 3 – vacuole, 4 – mitochondria, 5 – myelin-like inclusion in mitochondria, 6 – Golgi complex, 7 – karyolysis in spermatocyte.

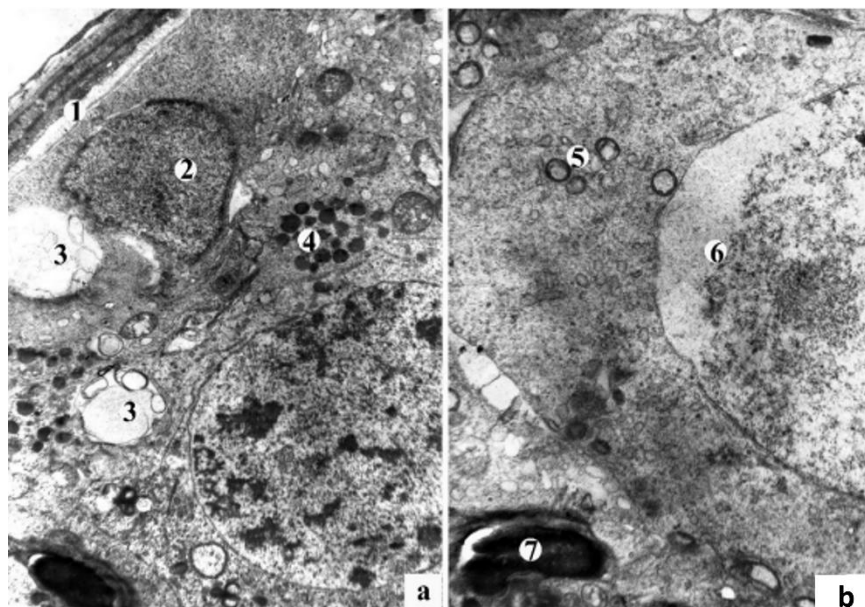


Fig. 3. Ultrastructural changes in the testes of 2-month-old rats on day 42 of SDM. Electron micrographs. Magnification: a) x4800, b) x6400

Designations: 1 – basal membrane; 2 – Sertoli cell, 3 – vacuole, 4 – lysosomes, 5 – proliferation and expansion of smooth endoplasmic reticulum cisterns in spermatogonia, 6 – karyolysis in spermatocyte, 7 – lipid inclusions.

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УЛЬТРАСТРУКТУРНІ ЗМІНИ В СПЕРМАТОГЕННОМУ ЕПІТЕЛІЇ ЯЄЧОК МОЛОДИХ ЩУРІВ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ЦУКРОВОМУ ДІАБЕТИ

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Резюме. Цукровий діабет (ЦД) є однією з найпоширеніших ендокринних патологій у світі, що супроводжується численними ускладненнями, у тому числі порушення репродуктивної функції у чоловіків. Метою дослідження було встановити особливості патоморфологічних змін у сперматогенному епітелії яєчок 2-місячних щурів при стрептозотоцин-індукованому цукровому діабеті (СЦД) протягом 42 діб. Дослідження проведено на 50 білих безпородних щурах-самцях, розподілених на контрольну (n=20) та експериментальну (n=30) групи. СЦД викликали одноразовим введенням стрептозотоцину (60 мг/кг). Матеріал забирали на 14, 28 та 42 добу експерименту, застосовуючи гістологічні, електронно-мікроскопічні та морфометричні методи. Встановлено стадійність розвитку патоморфологічних змін. На 14 добу гістоструктура яєчка залишалася переважно збереженою з незначним зменшенням висоти сперматогенного епітелію та ультраструктурними змінами в підтримуючих епітеліоцитах. На 28 добу спостерігався набряк інтерстиційної тканини, відшарування клітин адлюменального компартменту, зменшення висоти сперматогенного епітелію до 57,16±10,17 мкм (контроль – 99,33±16,77 мкм) та зниження індексу сперматогенезу. На 42 добу виявлено виражені атрофічно-деструктивні зміни: вакуольна та гідропічна дистрофії сперматогоній, апоптоз і некроз сперматоцитів, зменшення діаметру звивистих сім'яних трубочок до 237,37±43,39 мкм (контроль – 308,57±34,87 мкм), зниження висоти сперматогенного епітелію на 51,4 % та індексу сперматогенезу на 33%. Таким чином, патоморфологічні зміни у сперматогенному епітелії при СЦД прогресують від мінімальних структурних порушень до виражених дистрофічно-деструктивних змін з атрофією та значним порушенням репродуктивної функції.

Ключові слова: стрептозотоцин-індукований цукровий діабет, сперматогенний епітелій, яєчка, ультраструктурні зміни, морфометрія, експериментальні тварини, репродуктивна функція, атрофічно-деструктивні зміни, індекс сперматогенезу, звивисті сім'яні трубочки.

Конфлікт інтересів: відсутній.

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