ISSN 2509-4327 (print)
ISSN 2510-4780 (online)

Deutscher Wissenschaftsherold
German Science Herald

№ 1/2021

Die Zeitschrift „Deutscher Wissenschaftsherold" ist eine Veröffentlichung mit dem Ziel ein breites Spektrum der Wissenschaft allgemeinverständlich darzustellen. Die Redaktionsleitung versteht sich als Vermittler zwischen Wissenschaftlern und Lesern. Durch die populärwissenschaftliche Bearbeitung wird es möglich unseren Lesern neue wissenschaftliche Leistungen am besten und vollständigsten zu vermitteln. Es werden Untersuchungen, Analysen, Vorlesungen, kurze Berichte und aktuelle Fragen der modernen Wissenschaft veröffentlicht.

Impressum
Deutscher Wissenschaftsherold – German Science Herald
Wissenschaftliche Zeitschrift
Herausgeber:
InterGING
Wiesenwinkel 2,
31785 Aerzen
Inhaber: Marina Kisiliuk
Tel.: + 49 5154 567 2017
Fax.: +49 5154 567 2018
Email: info@dwherold.de
Internet:www.dwherold.de
Chefredakeur:
Prof. Zamiatin P.M.
Korrektur:
O. Champela
Gestaltung:
N. Gavrilets

Auflage: № 1/2021 (Februari) – 20
Redaktionsschluss Februar, 2021
Erscheint vierteljährlich
Editorial office: InterGING
Wiesenwinkel 2,
31785 Aerzen
Tel.: + 49 5154 567 2017
Fax.: +49 5154 567 2018
Email: info@dwherold.de
Deutscher Wissenschaftsherold – German Science Herald is an international, German/English language, peer-reviewed journal and is published quarterly.
№ 1/2020
Passed in press in May, 2021
Druck: WIRmachenDRUCK GmbH
Mühlbachstr. 7
71522 Backnang
Deutschland

INDEXING: Index Copernicus, Google Scolar, Ulrich's Periodicals Directory, Fachzeitungen, MIAR.

http://miar.ub.edu/issn/2509-4327

DDC-UDC 611.631.068.08:616.379-008.64 DOI:10.19221/2021110

Kondrat A-I.V.,
Graduate student of the Department of Pathological Anatomy of Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine, e-mail: lilia_kondrat@ukr.net
Zhurakivska O.Ya.
Doctor of Medical Sciences, Professor of the Department of Human Anatomy of Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine, e-mail: zhurakivska.o.ya@gmail.com

AGE FEATURES OF TESTICULAR MORPHOLOGICAL CHANGES IN DIABETES MELLITUS

Abstract. *The paper investigates changes in the testicular structure of 2-month-old and 6-month-old white male rats with streptozotocin-induced diabetes mellitus. It was found that on the 42nd day of streptozotocin-induced diabetes mellitus on the background of hyperglycemia and high levels of glycosylated hemoglobin in the hemomicrocirculatory bed, the development of diabetic microangiopathy was observed, which led to destructive changes in the testicular parenchyma. In 2-month-old and 6-month-old rats, a 1.1–1.6-fold decrease in the diameter of convoluted seminiferous tubules and a 1.6–2-fold decrease in spermatogenic epithelium height were found. At the same time, 6-month-old rats were dominated by processes of spermatogenic epithelial separation and pronounced destructive changes in sustentocytes, spermatogonia, and spermatids, whereas in 2-month-old rats violations of spermatogenesis were revealed (vacuolar dystrophy of spermatogonia, almost complete lack of spermatids). Thus, streptozotocin-induced diabetes mellitus leads to atrophic-destructive changes in the spermatogenic epithelium of the testes, which grow against the background of the development of diabetic microangiopathy and violation of the blood-testis barrier.*
Key words: *testis, hemomicrocirculatory bed, streptozotocin-induced diabetes mellitus.*

Introduction. In the last decade, the most acute medical and social problem of the national health care system of all countries in the world is diabetes mellitus (DM), which is associated with its steady growth, complications, disability and high mortality, especially among the working population [6, 11]. In diabetes, vasculogenic erectile dysfunction is quite common [10, 18]. Erectile dysfunction in men with these diseases often occurs before the development of their clinical signs and is therefore considered to be their early marker [19]. Besides erectile dysfunction, males suffering from diabetes also have retrograde ejaculation or its absence and impaired spermatogenesis, which significantly impairs their quality of life [14, 18]. It has been experimentally proven that in diabetes, insulin deficiency leads to disruption of metabolic processes in Sertoli-Leydig cells [9]. Moreover, the hypothalamic-pituitary-testicular axis is impaired [2, 7], which is manifested by insufficient production of follicle-stimulating and luteinizing hormones, and as a consequence, leads to Leydig cell dysfunction and impaired spermatogenesis [12, 15].

Based on the above, the aim of our work was to investigate the age-related morphological changes in the testes of 2-month and 6-month rats with streptozotocin-induced diabetes mellitus (SIDM).

Material and methods. The material for the study were the testicles of twenty 2-month-old and 6-month-old white outbred male rats, which were equally divided into 2 groups: the control and experimental ones. SIDM in experimental animals was simulated by a single intraperitoneal injection of streptozotocin (“Sigma”, USA) (dissolved in 0.1 M citrate buffer solution with a pH of 4.5) at a dose of 7 mg/100 g of body weight in 2-month-old rats and 6 mg/100 g of body weight of 6-month-old rats. The control group of animals had intraperitoneal injection of citrate buffer at an equivalent dose of 0.1 M. Glucose levels in animals were measured daily by collecting a drop of blood from the tail vein using test strips on an Assu-Schec glucometer (Germany). The level of glycated hemoglobin was measured in a private certified laboratory “Diameb”. The material was collected on the 42nd day of the SIDM development. All manipulations

carried out with animals during the experiment did not contradict the provisions of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), Law of Ukraine "On protection of animals from cruel treatment" of December 15, 2009 and orders of the Ministry of Health of Ukraine No. 690 of September 23, 2009, No. 616 of August 3, 2012.

Histological (staining with hematoxylin and eosin, Trichrome according to Masson, semi-thin sections stained with methylene blue), biochemical and electron microscopic methods were used.

For morphometric studies, photographs of histological and semi-thin sections saved in TIF format were used. Morphometry was performed using ImageJ version 1.47t. The diameter of cross section of the convoluted seminiferous tubules and their lumen, the height of the spermatogenic epithelium were determined. Computer data processing was performed using the statistical package STATISTICA (StatSoft, Inc. (2010), STATISTICA (data analysis software system), version 10. All data were reported as Mean±SD. Differences were considered to be statistically significant if $p<0.05$.

Results of the research. On the 42nd day of SIDM, the level of glucose and glycosylated hemoglobin in 2-month-old rats increased, respectively, to 17.54±1.42 mmol/l (control 3.25±0.57 mmol/l, p=0.0001) and 9.82±0.56% (control 1.89±0.54%, p=0.0001), in 6-month-old rats – up to 17.65±1.84 mmol/l (control 4.85±0.93 mmol/l, p=0.0001) and 9.28±1.02% (control 2.34±0.41%, p=0.0023), respectively which indicates the development of decompensated diabetes mellitus of moderate severity.

On the 42nd day of SIDM, 6-month-old rats have violations in the histostructure of convoluted seminiferous tubules. In most spermatic tubules, there is a separation of the germinal epithelium, vacuolation of spermatogonia, and atrophy of the spermatogenic epithelium (Fig. 1). In the interstitial tissue, the vessels of the hemomicrocirculatory bed are filled with erythrocyte sludges (Fig. 1d). Morphometric analysis shows a probable decrease in the diameter of convoluted seminiferous tubules up

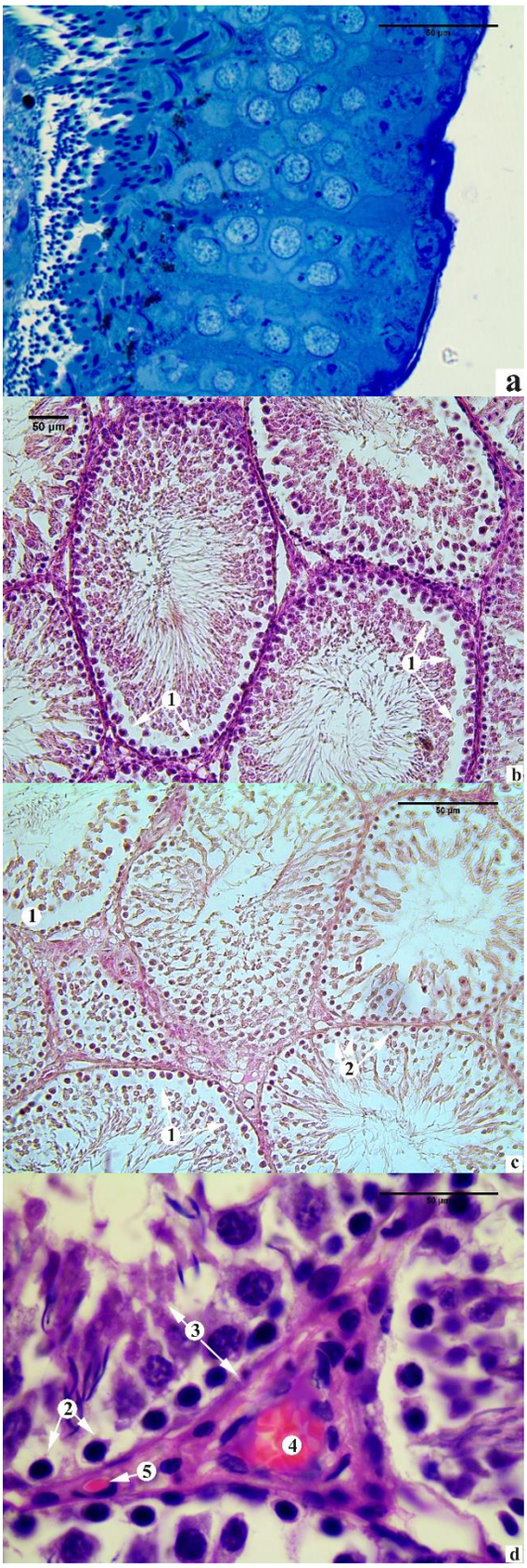

Fig. 1. Testicular histostructure of a 6-month-old rat of the control group (a) and with SIDM (b-d). Separation of spermatogenic epithelium (1), vacuolation of spermatogonia (2), atrophy of spermatogenic epithelium (3), erythrocyte sludges in the venule (4) and adhesion of the erythrocyte in the capillary (5). Staining: a) semi-thin section stained with methylene blue; b-d) H&E.

to 410.72±32.73 µm (control – 470.61±20.16 µm, p=0.0005) and the height of the spermatogenic epithelium up to 92.12±13.04 µm (control – 149.26±14.65 µm), p=0.0002, while the diameter of the lumen of the convoluted seminiferous tubules increases up to 226.48±27.98 µm (control – 172.09±17.27 µm, p=0.0003) (Fig. 2).

In 2-month-old rats with SIDM, atrophic-destructive changes of the spermatogenic epithelium of convoluted seminiferous tubules are observed: vacuolar and balloon dystrophy of spermatogonia (Fig. 3 b), disappearance of spermatids (Fig. 3 b). In the lumen of individual seminal tubules, there are groups of exfoliated cells of the spermatogenic epithelium (Fig. 3 c). In the interstitial tissue, the venules of the hemomicrocirculatory bed are filled with erythrocyte sludges (Fig. 3 c). The morphometric analysis showed a decrease in the diameter of convoluted seminiferous tubules up to 188.18±10.79 µm (control – 293.07±32.15 µm, p=0.0002), the height of the spermatogenic epithelium – up to 50.25±5.27 µm (control – 101.74±14.95 µm, p=0.0002), while the diameter of their lumen probably did not change and was 87.68±4.40 µm (control – 89.60±4.51 µm, p=0.3447) (Fig. 4).

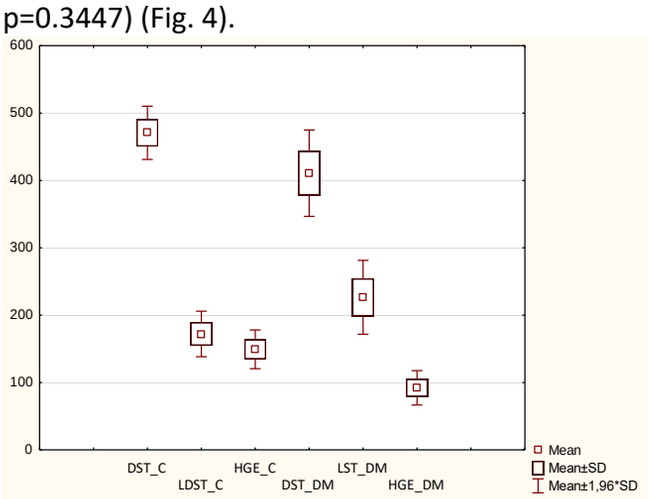

Fig. 2. Morphometric changes in convoluted seminiferous tubules of 6-month-old rats. Designation: DST_C – diameter of convoluted seminiferous tubules of rats of the control group, LDST_C – diameter of the lumen of convoluted seminiferous tubules of rats of the control group, HGE_C – height of the spermatogenic epithelium of rats of the control group, DST_DM – diameter of convoluted seminiferous tubules of rats with SIDM, LDST_DM – diameter of the lumen of convoluted seminiferous tubules of rats with SIDM, HGE_DM – height of the spermatogenic epithelium of rats with SIDM.

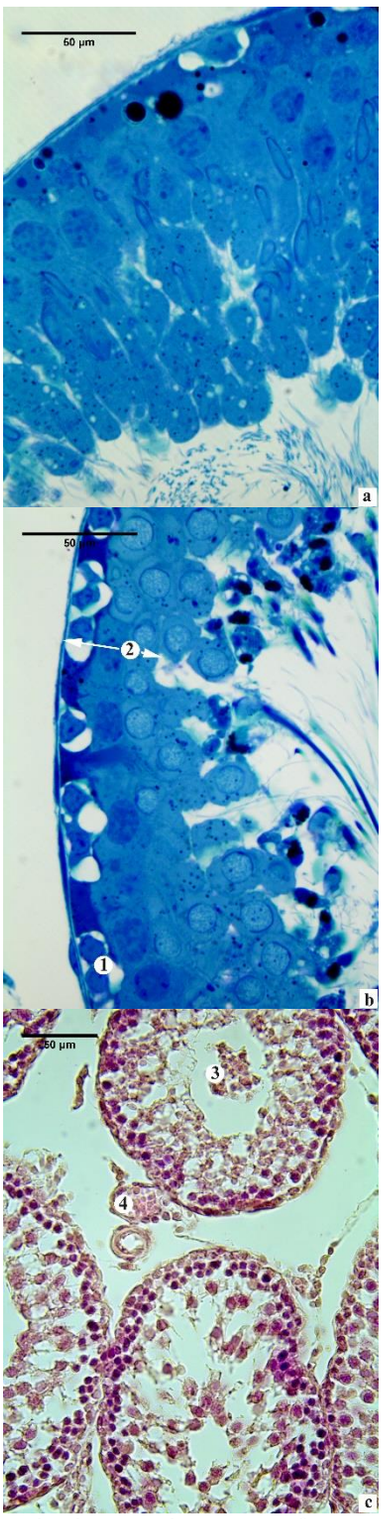

Fig. 3. Testicular histoarchitecture of 2-month-old rats of the control group (a) and with SIDM (b, c). Vacuolization of spermatogonia (1), atrophy of spermatogenic epithelium (2), luminal sloughing of germ cells (3), erythrocyte sludges in the venule (4). Staining: a, b) semi-thin section stained with methylene blue; c) H&E.

At the ultrastructural level, in the testicular parenchyma of 2- month-old and 6- month-old rats the development of diabetic microangiopathy stands out. It is characterized by hemorheological disorders in the vessels of the hemomicrocircula-

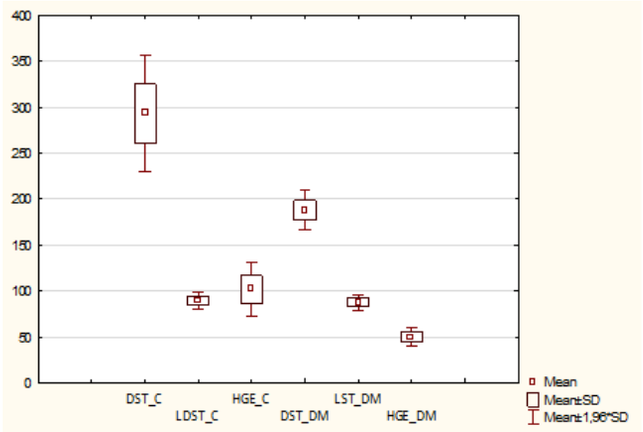

Fig. 4. Morphometric changes in convoluted seminiferous tubules of 2-month-old rats. Designation: DST_C – diameter of convoluted seminiferous tubules of rats of the control group, LDST_C – diameter of the lumen of convoluted seminiferous tubules of rats of the control group, HGE_C – height of the spermatogenic epithelium of rats of the control group, DST_DM – diameter of convoluted seminiferous tubules of rats with SIDM, LDST_DM – diameter of the lumen of convoluted seminiferous tubules of rats with SIDM, HGE_DM – height of the spermatogenic epithelium of rats with SIDM.

tory bed (erythrocyte sludge, microthrombi, adhesion of erythrocytes and thrombocytes to the lumenal surface of endothelial cells) (Fig. 5a), destruction and desquamation of endotheliocytes in capillaries, thickening and proliferation of the basement membrane of capillaries in the form of individual plates. There is a vacuolar dystrophy of expansion and destruction of cisterns of a smooth endoplasmic reticulum, disorganization and destruction of mitochondrial cristae, formation of vacuoles and lipid drops in sustentocytes of different age groups of rats (Fig. 5b). Spermatogonia undergo the most pronounced changes in the spermatogenic epithelium of the testes. In the latter, the development of vacuolar and hydropic dystrophies is observed, which ultimately leads to colliquative necrosis of these cells (Fig. 5 b, d). In spermatocytes, at different stages of their maturation, the following is observed: nuclear edema and karyolysis, apoptosis, vacuolar dystrophy, proliferation and expansion of the cisterns of smooth endoplasmic reticulum, increase in the number of lysosomes and the appearance of autophagosomes (Fig. 5 c, e). In 2-month-old rats with SIDM, the spermatids are practically not detected, while in the control group they are differentiated even at the histological level, which indicates a violation of spermatogenesis and atrophy of the spermatogenic epithelium. Degranulated Leydig cells (Fig. 5 f) with sharply expanded and partially destroyed cisterns of the smooth endoplasmic reticulum, karyorexis and karyolysis are found in interstitial tissue, which may indicate their functional insufficiency.

Discussion. In diabetic rats of different age groups a decrease in the diameter of convoluted seminiferous tubules is noted. It is associated with atrophic and destructive changes in the spermatogenic epithelium. Such changes in diabetes are associated with several factors. First of all, insulin deficiency and hyperglycemia cause dysfunction of the hypothalamic-pituitary axis [4]. This is due to both a decrease in the synthesis of gonadotropins and a violation of the receptor apparatus of correlations between testosterone and gonadotropins [16]. Davoud Kianifard et al [8] proved that SIDM leads to reduced weight of the testes due to their atrophy and impaired spermatogenesis. In the blood of rats with SIDM, a decrease in blood testosterone levels was observed, which may be associated with a decrease in the synthesis of its precursors 17-β estradiol and progesterone [8] and a decrease in the blood of gonadotropins [22]. In diabetes, metabolic changes in the body lead to dysfunction of endocrine organs. Thus, a strong direct relationship is found between the level of follicle-stimulating hormone (FSH) and insulin [20], so the lack of one leads to a decrease in the other in the blood, and as a result low levels of FSH cause decreased synthesis of androgens [8]. DM leads to a decrease in luteinizing hormone (LH) in the blood, which is responsible for the normal functioning of Leydig cells [13]. Insulin is known to maintain LH levels on Leydig cell receptors. LH deficiency provokes a decrease in synthesis and secretion of testosterone by Leydig cells due to a decrease in the number of LH binding sites in Leydig cells in diabetic rats [13, 22].

Impaired synthesis and secretion of FSH and LH are primarily associated with a decreased number of gonadotropic endocrinocytes in the adenohyprosis of diabetic rats [24]. These cells die due to apoptosis and necrosis [3, 25]. Other researchers have noted a decrease in blood levels

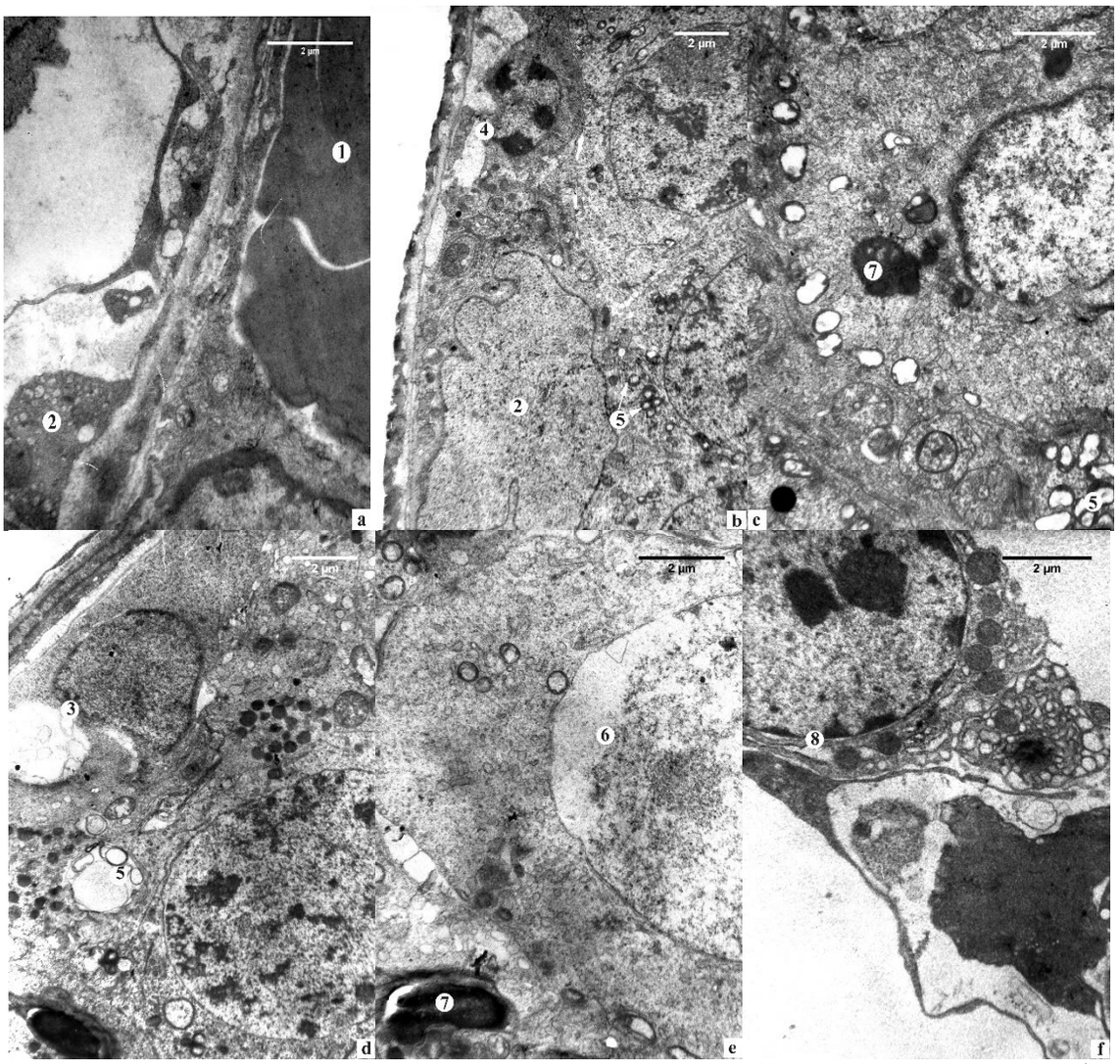

Fig.5. Ultrastructural changes in the testes of 6-month-old (a-c) and 2-month-old (d-f) rats on the 42nd day of SIDM. Electronic microphotographs. Designations: 1 – erythrocyte sludges; 2 – sustentocyte, 3 – vacuolar dystrophy of sustentocytes, 4 – balloon dystrophy of spermatogonia, 5 – proliferation and expansion of cisterns of the smooth endoplasmic reticulum in a spermatocyte, 6 – karyolysis in a spermatocyte, 7 – autophagosomes, 8 – Leydig cell.

of gonado-, somato-, prolactoliberin in diabetic rats, which in immature animals lead to delay of growth and puberty [17, 21] and explains the atrophic-destructive changes in the spermatogenic epithelium of the testes of 2-month-old rats.

Along with changes in the neuroendocrine system, there are violations of testicular blood supply in diabetic rats, which leads to destructive changes in the convoluted seminiferous tubules. Thus, in 2-month-old and 6-month-old rats with SIDM, the development of diabetic microangiopathy was observed. Thus, in microhemovessels the erythrocyte sludges, adhesion of erythrocytes and thrombocytes, microclasmatosis were revealed by us. Hemorreological disorders are a response to hyperglycemia and high levels of glycosylated hemoglobin. It is believed that the initiating factor of endothelial damage in diabetes is the process of glucosylation of hemoglobin, as a result of which erythrocytes change their surface S-charge. This leads to true capillary stasis, erythrocyte sludge and agglutination, and later to microthrombosis, which creates local circulatory and hemic hypoxia and activates a cascade of molecular mechanisms of cell membrane damage [5, 9]. An important factor in endothelial damage in diabetes is the sorbitol pathway of glucose metabolism, which is associated with the activation of aldoreductase with subsequent accumulation of sorbitol in endothelial cells, leading to osmotic edema and destruction of the latter [5, 9, 12]. Such changes in the vessels of the hemomicrocirculatory bed, along with destructive changes in sustentocytes, lead to a violation of the

blood-testis barrier in the testicles and to destructive changes in the spermatogenic epithelium. Hypoxia and hyperglycemia cause oxidative stress and increased formation of reactive oxygen species (ROS) [2, 23], which in turn make a significant contribution to the development of male infertility. Oxidative stress can cause a decrease in testosterone levels, changes in the structure of convoluted seminiferous tubules and impaired spermatogenesis [12]. High concentrations of reactive oxygen species in semen were observed in 30-40% of cases of male infertility [1].

Conclusions. Thus, SIDM leads to diabetic microangiopathy, which is manifested by: hemorheological disorders in microhemovessels, microclasmatosis, thickening and proliferation of the basement membrane of capillaries. Changes in sustenocytes against the background of the development of diabetic microangiopathy lead to a violation of the blood-tests barrier, and as a consequence, to the development of atrophic-destructive changes in the spermatogenic epithelium of convoluted seminiferous tubules and to disruption of spermatogenesis in rats of different ages.

Prospects for further research consist in a comprehensive study of the patterns of changes in the testicular parenchyma in SIDM and its correction by administration of various antidiabetic drugs.

References.

1. Agarwal A, Viek G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014; 32:1–17.

2. Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. Curr Diabetes. 2008; 4:46–54.

3. Arroba AI, Lechuga-Sancho AM, Frago LM et al. Cell-specific expression of X-linked inhibitor of apoptosis in the anterior pituitary of streptozotocin-induced diabetic rats. J. Endocrinol. 2007; 192 (1):215–227. Access to the journ. : http://www.endocrinology-journals.org.

4. Baccetti B, la Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F, De Leo V. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Hum Reprod. 2002; 10:2673–7.

5. Borovkova OS, Iftodii AH. The question of the pathogenesis of diabetic angiopathy. Bukovynskyi medychnyi visnyk. 2006; 10(2):132-135.

6. Chian JL, Kirkman MS, Laffel LM, Peters AL. Type 1 diabetes through the span: a position statement of the American Diabetes Association. Diabetes Care. 2014 Jul;37:2034-54. doi: 10.2337/dc14-1140.

7. Chudhary D, Cleveland DA, Agdere L, Graetz R, Etienne D. Does the Use of Diabetes Electronic Medical Record Template Lead to Improvement of Screening for Complications of Tipe 1 Diabetes Mellitus in the Pediatric Population. Pediatrics. 2018; 141(1): 122–8.

8. Davoud Kianifard, Rajab Ali Sadrkhanlou, Shapour Hasanzadeh. The Ultrastructural Changes of the Sertoli and Leydig Cells Following Streptozotocin Induced Diabetes. Iranian Journal of Basic Medical Sciences. 2012; 15 (1): 623-635.

9. Efymov A, Zueva N, Skrobonskaia N. Diabetic angiopathies: etiology and pathogenesis. Liky. 2004;11:36-38.

10. El–Sakka AI, Anis T, Khadr N et al. Sildenafil for erectile dysfunction in the Middle East: observational analysis of patients with diabetes and/or hypertension treated in the clinical practice setting. J Int Med Res. 2011; 39 (2): 558–568.

11. Gupta S, Gupta N. The Prevalence of Cardiac Autonomic Neuropathy in Type 2 Diabetes Mellitus. J Med Sci Clin Res. 2017; 5(7): 23-8. Available from: https://doi.org/10.18535/jmscr/v5i7.57

12. Kanter M, Aktas C, Erboga M. Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis. Food Chem Toxicol. 2012; 50:719–25.

13. Khaki A, Nouri M, Fathiazad F, Ahmadi-Ashtiani HR, Rastgar H, Rezazadeh S. Protective effect of quercetin on spermatogenesis in streptozotocin-induced diabetic rat. J Med Plan. 2009; 8:57-64.

14. Mallidis C, Agbaje I, McClure N, Kliesch S. The influence of diabetes mellitus on male reproductive function: a poorly investigated aspect of male infertility. Urologe A. 2011; 50 (1): 33-7.

15. Mangoli E, Talebi RA, Anvari M, Pourentezari M. Effect of experimentally-induced diabetes on sperm parameters and chromatin

quality in mice. Iran J Reprod Med. 2013; 11:53–60.

16. Navarro-Cassado L, Juncos-Tobarra MA, Chafer-Rudilla M, Iniguez de Onzono L, Blazquez-Cabrera JA, et al. Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. J Androl 2010; 108:007260.

17. Nishimura E, Söderlund D, Castro-Fernández C et al. In vitro biological-to-immunological ratio of serum gonadotropins throughout male puberty in children with insulin-dependent diabetes mellitus. Endocrine. 2007; 31(1): 18–26.

18. Povorozniuk MV. Prevalence and main causes of male infertility. Medical aspects of men's health. 2012; 5(3): 62-73.

19. Shin D, Pregenzer GJr, Gardin JM. Erectile dysfunction: a disease marker for cardiovascular disease. Cardiol Rev. 2011; 19 (1): 5-11.

20. Sudha S, Valli G, Julie P, Arunakaran J, Govindarajulu P, Balasu-Bramanian K. Influence of streptozotocin-induced diabetes and insulin treatment on the pituitary-testicular axis during sexual maturation in rat. Endocrinol Diabetes 1999; 107:14-20.

21. Sudha S. Valli G, Julie P et al. Influence of streptozotocin-induced diabetes and insulin treatment on the pituitary-testicular axis during sexual maturation in rats. Exp. Clin. Endocrinol. Diabetes. 2000; 108 (1): 14–20.

22. Sureeporn Nak-ung, Nutnicha Nakprom, Chonikan Maneengam, Sutisa Nudmamud-Thanoi, Samur Thanoi. Changes in sperm quality and testicular structure in a rat model of type 1 diabetes. Asian Biomed (Res Rev News) 2018; 12(4 Anat issue Pt 2):141-7.

23. Ullah A, Khan A, Khan I. Diabetes mellitus and oxidative stress—A concise review.Saudi Pharmaceutical Journal. 2016; 24:547–53.

24. Zhurakivska OYa Morpho-functional changes in adenohypophysis gonadotropes in rats of different ages with experimental diabetes mellitus. Bulletin of problems biology and medicine. 2012; 2: 189-194.

25. Zhurakivska OYa, Levytskyi VA. The severity of apoptosis in adenohypophysis cells in experimental type 1 diabetes. Morphology at the present stage of development of science: materials of the scientific and practical conference (Oct. 5-6. 2012, Ternopil), Ternopil, 2012: 120–12.

CONTENT:

Deutscher Wissenschaftsherold
German Science Herald

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet athttp://dnb.dnb.de

№ 1/2021 – 10