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CHANGES IN HEMODYNAMICS IN THE TESTES AND EJACULATES OF ADULT MEN IN CHRONIC DIABETES MELLITUS

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An analysis was performed of echometric, hemodynamic, histological and laboratory changes in the testes and ejaculate of infertile men aged 22–35 years (the first period of adulthood), who were diagnosed with insulin-dependent diabetes mellitus of moderate severity. It is established that under these conditions, the volume of both testicles probably decreases, hemodynamic parameters in them both within the spermatic cord and under the protein shell decrease, there is a reduction of spermatogenic epithelial cells' layers in the vast majority of tortuous seminal tubules, the concentration of testosterone in the blood decreases, and in the ejaculate – the total number of sperm, the number of living and actively moving sperm reduces. The number of pathological forms of sperm doubles, and the number of immobile sperm increases by 5 times.

Key words: diabetes mellitus, testicles, hemodynamics, spermatogenesis.

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ЗМІНИ ГЕМОДИНАМІКИ В ЯЄЧКАХ ТА ЕЯКУЛЯТІ ЧОЛОВІКІВ ЗРІЛОГО ВІКУ ПРИ ХРОНІЧНОМУ ЦУКРОВОМУ ДІАБЕТІ

Проведений аналіз ехометричних, гемодинамічних, гістологічних і лабораторних змін в яєчках та еякуляті неплідних чоловіків віком 22–35 років (перший період зрілого віку), у яких діагностовано інсулінозалежний цукровий діабет середньої важкості. Встановлено, що за цих умов вірогідно зменшується об'єм обох яєчок, знижуються показники гемодинаміки в них як в межах сім'яного канатика, так і під білковою оболонкою, наявна редукція шарів клітин сперматогенного епітелію у переважній більшості звивистих сім'яних трубочок, знижується концентрація тестостерону в крові, а в еякуляті – загальна кількість сперматозоїдів, кількість живих та активно рухливих сперматозоїдів. Вдвоє зростає кількість патологічних форм сперматозоїдів та в 5 разів – кількість нерухомих сперматозоїдів.

Ключові слова: цукровий діабет, яєчка, гемодинаміка, сперматогенез.

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According to the WHO, the problem of male infertility is constantly exacerbated due to the high sensitivity of the testicular parenchyma and spermatogenesis to the action of various factors, both external and many internal factors. This [1, 6, 7] problem has a pronounced social character because in recent years, the number of infertile marriages has been growing, and 50 % of them are due to male fertility disorders.

Spermatogenic and endocrine functions of the testes are often reduced, caused by both local and general circulatory disorders in the testes. Local circulatory disorders occur in oblique inguinal hernias. The contents of the hernia sac not only constantly press on the hernia sac blood vessels' spermatic cord but also violates the temperature in the scrotum [4, 5, 8]. The operation to remove the hernia sac can also injure the elements of the spermatic cord and pull them into the postoperative scar. It disrupts hemodynamics in the testes and varicose veins of the spermatic cord.

The anatomical prerequisite for the development of varicocele in 90 % of patients is the prolapse of the left testicular vein into the left renal one at right angles. Varicocele also occurs due to the insufficiency of the testicular vein valves and the subsequent development of renal-testicular reflux, compression of the sigmoid colon of the left renal vein, which flows into the left testicular vein. Varicocele has been associated with venous hypertension due to congenital left renal vein stenosis [2, 3, 12]. The general injury of blood vessels in the body occurs, particularly at the diagnosed micro-and macroangiopathies of coronary arteries, arteries of the brain, kidneys, lower extremities caused by disorders in the metabolism of carbohydrates.

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They are well described in the literature, as diabetes is one of the most common diseases, affecting more than 15 million people of all ages. According to the International Diabetes Federation, the number of people with diabetes will increase to 642 million in the next 20 years, 10 % of the total population [9, 10, 11].

Therefore, using modern clinical methods to diagnose infertility in adult men with insulin-dependent diabetes mellitus remains relevant.

The purpose of the study was to determine the features of hemodynamic and structural-functional changes in the testicles and ejaculate of infertile men aged 22-35 years with diagnosed insulin-dependent diabetes mellitus of moderate severity.

Materials and methods. Ultrasound scan and colour ultrasound angiography of the testicles of 12 infertile men aged 22–35 years, who were diagnosed with moderate severity diabetes mellitus, was performed at the Clinical Diagnostic Center using SIEMENS G60S apparatus (Siemens AW, Germany). The volume of the testes and hemodynamic parameters in them were determined, the diameter of the testicles and the quality of blood flow were assessed: peak arterial blood flow velocity (cm/s), diastolic blood flow velocity (cm/s), resistance index (relative units, RU), volumetric blood flow (ml/min).

The ejaculate was used to study the concentration of spermatozoa, their morphological forms and motility according to the WHO methodology of 2010, revised by Chornokulsky I.S. (2013). The testosterone concentration in the blood was determined by enzyme-linked immunosorbent assay using an automatic analyser IMMULITE-2000 (Siemens Healthcare Diagnostics Inc., USA).

Cytohistological examination of testicular biopsies was performed from 9 infertile men aged 22-35 years with diagnosed diabetes mellitus of moderate severity, taken in the urology department of the Ivano-Frankivsk Regional Clinical Hospital. Testicular microslides were used to assess the condition of the convoluted seminal vesicles, their diameter, the degree of damage to spermatogenic epithelial cells, the number of spermatogenic epithelial cells, and the volume of interstitial endocrinocyte nuclei.

The ultrathin sections obtained with the “Tesla BS – 490 A” ultramicrotome were contrasted on the grids with lead citrate and studied in a PEM – 125 K electron microscope (Selmi, Ukraine). Violation of moral and ethical norms during the research was not detected (Minutes No. 2 of February 18, 2020, Vasyl Stefanyk Precarpathian National University). The control for ultrasound, cytohistological and laboratory tests was the ejaculate data of 7 mature men with a history of diabetes mellitus.

Data processing. Statistical analysis of the obtained indices was performed using Stat. Soft Inc. software. (Tulsa, Ok, USA) Statistica 10. Nonparametric methods were applied using the Wilcoxon-Mann-Whitney test. The difference was considered significant at $p<0.05$. Farris fertility rate was determined by the formula: $I=V\times N/100$.

Results of the study and their discussion. In men aged 22–35 years of the control group, colour Doppler mapping gave a fairly clear image of the blood vessels of the testicular parenchyma and capsular centripetal and recurrent arteries. In most cases, the veins are better visualised in the upper half of the testicle. With the help of pulsed Doppler sonography, we obtained quantitative indices of blood flow in the testis, which are as follows: the mean maximum linear velocity of blood flow in the testicular artery within the spermatic cord is 19.0±1.5 cm/s, and in the testicular artery – 12, 30±1.8 cm/s, respectively. The volumetric blood flow in the testicular artery was 16.50 ± 1.2 ml / s. The mean linear blood flow velocity in the testicular veins was 8.0±0.3 cm/s, and the volumetric velocity of blood flow was 7.90±0.4 ml/min.

The testis volume in adult men of the control group was 19.60±0.7 cm^3. The mean maximum blood flow velocity in the seminiferous duct artery was 13.60±1.0 cm/s, and the mean minimum blood flow velocity in it was 9.20±0.5 cm/s. At usual scrotal scanning, the dilated veins of a vine-like plexus are found out in the form of multiple echonegative structures of the oval shape of various diameters.

We also studied the rate of reverse blood flow in the testicular veins, but the reverse discharge of blood at the height of the Valsalva test in the vast majority of cases was absent.

According to the cytohistological study of testicular biopsies of mature men, the diameter of the convoluted seminal vesicles was 199.60±5.4 μm. However, 14 % of tortuous seminal vesicles have a mild degree of spermatogenic epithelial cell damage, 8 % have a severe degree of damage, and 5 % have no detectable spermatogenic epithelial cells. In 73% of tortuous seminal vesicles, spermatogonia 7.28±1.88, spermatocytes 160.40±5.64, and spermatids 350.70±7.39 adjoin their own shell in several layers. The volume of interstitial endocrinocyte nuclei was 95.70±1.50 μm^3, and the testosterone level in the blood was 550.0±2.0 ng/dc. According to electron microscopy of testicular biopsies of the male control group, the peculiarity of the structure of tortuous spermatic tubes’ own shell was the presence of several layers (2 to 5) of myoid cells, close contacts between peripheral processes were not determined. The nuclei of the cells were spindle-shaped, mitochondria, tubules of the granular endoplasmic reticulum, elements of the Golgi complex, pinocytic vesicles were determined in the cytoplasm. A feature of the cytoplasm was the presence of myofilaments in it. An inner non-cellular layer of homogeneous substance was determined between myoid cells and supporting epitheliocytes, which in some places protruded into the base of supporting epitheliocytes. The presence of deep intussusception characterised the nuclei of the latter; the cytoplasm was rich in fatty inclusions. There were specialised compounds between the cytoplasms of neighbouring cells, which included tanks of the endoplasmic reticulum and microfilaments. These compounds divided spermatogenic epithelial cells into basal and adluminal compartments.

Layer, between which there are close contacts. The cells' nuclei were oval; the cytoplasm contained organelles. Pores and fenestrae in endotheliocytes were not defined. Interstitial endocrinocytes were characterised by a large oval nucleus. In the cytoplasm – a significant number of lipid inclusions, tubules of the endoplasmic reticulum, mitochondria with a light matrix and lamellar-sensitive crystae. In the ejaculate of men in the control group, the number of spermatozoa with a normal structure was 73.50±3.60 million/ml, the number of pathological forms – 24.10±1.15 %, with the pathology of the head – 11.78 %, with the pathology of the main part of the flagellum – 9 %.

According to the data of colour ultrasound angiography of the testicles in infertile men aged 22–35 years diagnosed with insulin-dependent diabetes mellitus, the mean maximum blood flow velocity in the testicular artery within the spermatic cord decreased to 14.7±0.8 cm/s, and in the testicular artery – up to 9.6±0.5 cm/s, against 19.0±1.5 cm/s and 12.3±1.8 cm/s, respectively, in men of the control group. Under these conditions, the testicular artery's mean minimum blood flow velocity decreased to 5.9±0.3 cm/s and 4.8±0.5 cm/s, respectively. The volumetric blood flow in the testicular artery decreased to 8.2±1.0 ml/s versus 16.5±1.2 ml/s in control. The mean linear velocity of blood flow in the testicular veins decreased to 7.0±0.3 cm/s, and the volumetric velocity of blood flow in them – up to 6.1±0.2 ml/min against 8.0±0.2 cm/c and 7.9±0.4 ml/min in control. Testicular volume in adult men with this pathology decreased to 14.2±1.0 cm^3 against 19.6±0.7 cm^3 in control.

According to the cytohistological study of testicular biopsies of infertile men with diabetes mellitus, the diameter of the tortuous seminal vesicles decreased to 161.3±7.2 μm against 199.6±5.4 μm in control. In the vast majority of them, 1–2 layers of cells – spermatogonia and spermatocytes – adjoin the thickened and hyalinised own membrane (fig. 1).

According to electron microscopy of testicular biopsies, the basement membrane of the spermatogenic epithelium was thickened and twisted. The nuclei of myoid cells were deformed, hyperchromic, cytoplasmic organelles were reduced, myofilaments were not determined (fig. 2).

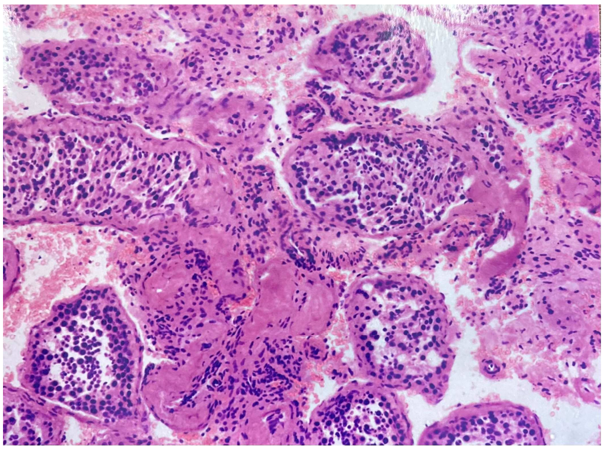


Fig. 1. Significant thickening and hyalinosis of the tortuous seminal tubules own shell and the growth of connective tissue of the testicle of a 35-year-old man with diabetes. Staining with hematoxylin and eosin. Magn.: x80.

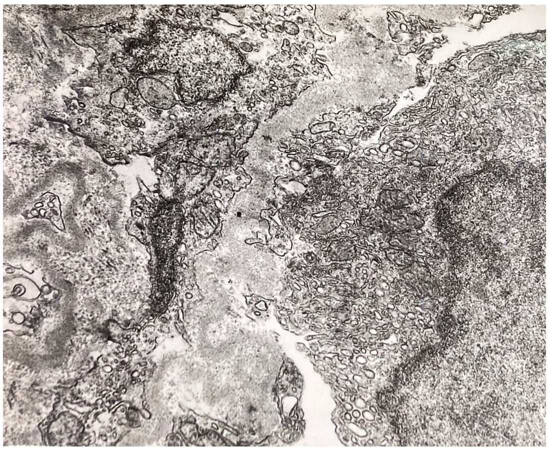
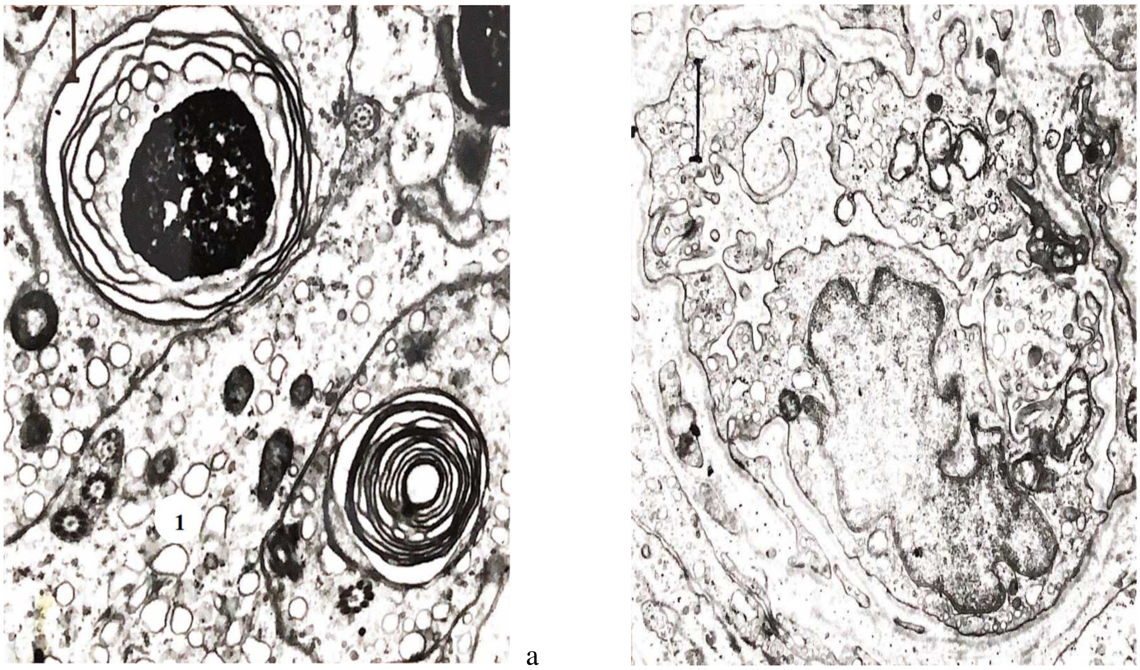


Fig. 2. Deformation of the nucleus of the myoid cell and interstitial endocrinocyte. Twisting of the non-cellular layer of the tympanic membrane of the testis of a 35-year-old man with diabetes mellitus. Magn.: x16000.

The cytoplasm of supporting epitheliocytes was vacuolated, mitochondrial ridges were reduced, lysosomes and myelin-like structures were present (fig. 3a). In the connective tissue of the supporting epitheliocytes, the cytolemmas were close together, the cisterns of the endoplasmic reticulum were unevenly dilated, and the microfilaments were reduced.

Internal cytolema of endothelium with deep intussusception and microclasmatosis. Irregularly shaped endothelial nuclei with peripheral chromatin condensation (fig. 3b).



a b

Fig. 3. Pronounced vacuolation (1) of the cytoplasm of the supporting epitheliocyte and the presence of secondary lysosomes (2) and myelin-like structures (3) (a); deformation of the endothelial cell nucleus of the blood capillary and macroclasmatosis (b). Electronic microphotography.Magn.: (a) x16000; (b) x10000.

In interstitial endocrinocytes, the cytoplasm was reduced, cytoplasmic organelles were deformed nuclei with peripheral chromatin concentration. In the blood capillaries of the testis, the basement membrane was unevenly thickened, the cytoplasm of endothelial cells was vacuolated, mitochondrial cristae were reduced, elements of the endoplasmic reticulum and the Golgi complex were deformed.

Laboratory studies of male ejaculate in diabetes mellitus show that all its indices change significantly (Table 1).

□□bl□1.

Morphological and functional parameters of the spermogram of infertile men aged 22–35 years with insulin-dependent diabetes mellitus (M±m)

Spermogram indicators control	Diabetes mellitus	Control
Spermatozoa concentration (million/ml)	73.50±3.60 p=0.001	17.20±6.50
Number of normal spermatozoa (%)	75.90±4.52 p=0.05	45.72±1.30
Number of pathological forms of spermatozoa (%)	24.10±1.15 p=0.001	51.28±1.00
Pathology of the head (%)	11.78±0.36 p=0.05	20.18±1.70
Pathology of the main part of the flagellum (%)	9.06±0.43 p=0.001	18.70±1.50
Pathology of the intermediate flagellum (%)	3.26±0.50 p=0.001	12.40±0.80
Number of live spermatozoa (%)	76.00±4.30 p=0.05	50.00±5.40
Immobile spermatozoa (%)	9.50±1.34 p=0.001	48.50±2.00
Total sperm motility (%)	76.00±4.20 p=0.05	42.50±4.00
Ferris fertility rates (units)	218.40±7.93 p=0.001	97.80±3.20

According to the literature, the use of non-invasive research methods, in particular, ultrasound scanning and colour ultrasound angiography, are widely used in the clinic in diseases of many internal organs of the genitourinary system, including kidneys, bladder, prostate, testicles and testes [1, 2, 9]. We used these methods to study the bloodstream of the testicles in infertile mature men with insulin-dependent diabetes mellitus due to the possible development of macro- and microangiopathy.

Doppler data revealed changes in hemodynamics in the testes, which are manifested by a probable decrease, up to 14.7 cm/s and up to 5.9 cm/s, indices of arterial blood flow in the testicular artery within the spermatic cord and under the protein shell, which led to partial atrophy of the testes with a decrease in their volume to 14.2 cm^3 against 19.6 cm^3 in men of the control group.

Our cytohistological diagnosis of testicular biopsies of infertile men with diabetes mellitus indicates a probable decrease in the diameters of tortuous spermatic tubes to 161.1 μm against 199.6 μm in the testes of control men and reduction of spermatogenic epithelial cell layers. We and other authors have described such changes in the testes in disorders of blood circulation in the testes with varicose veins of the spermatic cord, oblique inguinal hernias, and after surgery for inguinal canal plastics [3, 7, 11].

According to electron microscopy of male testicular biopsies under these conditions, significant thickening and hyalinosis of the basement membrane of spermatogenic epithelium and deformation of the specialised joints of supporting epitheliocytes are present essential components of the blood-testis barrier and hemocapillary wall.

These changes in the testis led to disorders of spermatogenesis, as evidenced by the results of ejaculate studies, in which the concentration of sperm decreased by 4.5 times, the concentration of sperm with the pathology of the head and main flagellum increased by 2 times, and testosterone in the blood of men decreased by 1.5 times.

Conclusions

1. In infertile men aged 22–35 years diagnosed with insulin-dependent diabetes mellitus of moderate severity according to colour ultrasound angiography, the mean maximum blood flow velocity in the testicular artery within the spermatic cord decreases to 14.7±0.8 cm/s against 19.0±1.5 cm/s. The minimum blood flow velocity is up to 5.9±0.3 cm/s against 8.2±1.0 cm/s in control.

2. In testicular biopsies of men diagnosed with moderate-grade insulin-dependent diabetes mellitus, tortuous seminal vesicles are reduced to 161±7.2 μm versus 199.6±5.4 μm in controls. 1–2 layers of spermatogonia and spermatocytes adjoin their own shell. The basement membrane of the spermatogenic epithelium is thickened and hyalinised. The ultrastructure of specialised connections of supporting epitheliocytes is disturbed, their cytoplasm is vacuolated, cytoplasmic organelles are deformed. Nuclei of myoid cells are of irregular shape, hyperchromic, myofilaments in the cytoplasm are not defined.

3. In the ejaculate of men with this pathology, the concentration of sperm is reduced to 17.2±6.50 million/ml, against 73.50±3.60 million/ml in control. The number of sperm with the pathology of the head increases to 20.18 %, against 11.78 % and up to 18.70 %, against 9.0 % – sperm with the pathology of the main part of the flagellum. The testosterone level in the blood decreases to 390.0±1.7 ng/dc, against 550.0±2.0 ng/dc in control.

Prospects for further research are to study, under these conditions, the nature of structural and functional changes in the prostate of infertile men and their correction.

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