EXPERIMENTAL RESEARCHES

EFFECT OF A SORBENT COMPOSITION BASED ON ALUMINUM OXIDE AND POLYDIMETHYLSILOXANE ON THE REPRODUCTIVE SYSTEM OF *DB/DB* FEMALE MICE WITH GENETICALLY DETERMINED OBESITY AND TYPE 2 DIABETES MELLITUS

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ABSTRACT

Metabolic syndrome, obesity, type 2 diabetes mellitus are characterized by the accumulation of toxic metabolic products in the internal environment of the organism. The development of innovative medicines based on a sorbent matrix modified with biologically active molecules remains relevant. The sorbent composition from aluminum oxide and polydimethylsiloxane is considered promising.

The aim of the study. *To investigate the effect of the sorbent composition from aluminum oxide and polydimethylsiloxane on the uterus and ovaries of db/db mice with obesity and type 2 diabetes mellitus.*

Materials and methods. *The sorbent composition (0.665 g/kg in 200 µl of distilled water) was administered to 14-week-old animals through an intragastric tube once a day for 7 days. The comparison groups were female rats injected with placebo (daily intragastrical administration of 200 µl of water for 7 days) and intact animals. Digital images of light-optical preparations stained with hematoxylin and eosin were processed using Image-Pro Plus 4.1 software. In the ovaries, the numerical density of primordial, primary, secondary follicles and corpuses luteum was determined. The width of the uterus layers, the diameters of the blood and lymphatic vessels, the width of the interstitial fissures in both organs were measured. The statistical significance of differences was determined using the Mann – Whitney test.*

Results. *In the myometrium and endometrium of the uterus of db/db mice, dilatation of arteries, veins, lymphatic vessels and edema were noted due to the accumulation of tissue fluid in the interstitium layers. There were no tertiary follicles in the ovaries. The introduction of the sorbent composition contributed to a decrease in the diameters of arteries, veins, lymphatic vessels of the uterus, a decrease in edema in both organs due to the narrowing of the prelymphatic slits, and stimulated an increase in the numerical density of secondary follicles.*

Conclusion. *A corrective effect of the sorbent composition of aluminum oxide and polydimethylsiloxane on prelymphatic slits, blood and lymphatic vessels in the uterus and ovaries in db/db mice with obesity and type 2 diabetes mellitus was revealed.*

Key words: *db/db mice, obesity, type 2 diabetes mellitus, uterus, ovaries, blood and lymphatic vessels, prelymphatic slits*

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ВЛИЯНИЕ СОРБЕНТНОЙ КОМПОЗИЦИИ ИЗ ОКСИДА АЛЮМИНИЯ И ПОЛИДИМЕТИЛСИЛОКСАНА НА ПОЛОВУЮ СИСТЕМУ САМОК *DB/DB* МЫШЕЙ С ГЕНЕТИЧЕСКИ ДЕТЕРМИНИРОВАННЫМ РАЗВИТИЕМ ОЖИРЕНИЯ И САХАРНОГО ДИАБЕТА 2-ГО ТИПА

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РЕЗЮМЕ

Метаболический синдром, ожирение, сахарный диабет 2-го типа характеризуются накоплением токсических продуктов метаболизма во внутренней среде организма. Актуальна разработка инновационных лекарственных препаратов на основе сорбентной матрицы, модифицированной биологически активными молекулами. Перспективной является сорбентная композиция из оксида алюминия и полидиметилсилоксана.

Цель исследования. *Изучение влияния данной сорбентной матрицы на матку и яичники db/db мышей с ожирением и сахарным диабетом 2-го типа.*

Материалы и методы. *Сорбентную композицию (0,665 г/кг в 200 мкл дистиллированной воды) вводили 14-недельным животным через внутрижелудочный зонд 1 раз в день в течение 7 суток. Группами сравнения служили самки, которым вводили плацебо (ежедневно внутрижелудочно 200 мкл воды в течение 7 суток), и интактные животные. Цифровые изображения светооптических препаратов, окрашенных гематоксилином и эозином, обрабатывали в программе Image-Pro Plus 4.1. В яичниках определяли численную плотность примордиальных, первичных, вторичных фолликулов и жёлтых тел. Измеряли ширину слоёв матки, диаметры кровеносных и лимфатических сосудов, ширину интерстициальных щелей в обоих органах. Статистическую значимость различий определяли с помощью критерия Манна – Уитни.*

Результаты. *В миометрии и эндометрии матки db/db мышей отмечались дилятация артерий, вен, лимфатических сосудов и отёк за счёт скопления тканевой жидкости в интерстиции слоёв. В яичниках отсутствовали третичные фолликулы. Введение сорбентной композиции способствовало уменьшению диаметров артерий, вен, лимфатических сосудов матки, снижению отёка в обоих органах за счёт сужения прелимфатических щелей, стимулировало увеличение численной плотности вторичных фолликулов. Заключение. Выявлен корригирующий эффект сорбентной композиции из оксида алюминия и полидиметилсилоксана на прелимфатики, кровеносные и лимфатические сосуды в матке и яичниках у db/db мышей с ожирением и сахарным диабетом 2-го типа.*

Ключевые слова: *db/db мыши, ожирение, сахарный диабет 2-го типа, матка, яичники, кровеносные и лимфатические сосуды, прелимфатики*

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INTRODUCTION

Metabolic syndrome, obesity and type 2 diabetes mellitus are an urgent medical and social problem for society associated with a decrease in the quality of life and life expectancy. Metabolic syndrome, the main feature of which is obesity, includes a group of abnormalities (accumulation of abdominal fat, hyperglycemia, hyperinsulinemia, dyslipidemia, hypertension) leading to diabetes and cardiovascular diseases. The prevalence of metabolic syndrome is high and has been found to affect between 17 % and 46 % of the general population in developed countries [1]. In Russia, according to Rosstat estimates, 5.1 million people were diagnosed with diabetes mellitus and 1.9 million were diagnosed with obesity in 2020 [2]. There has been a significant increase in the prevalence of obesity among young people: over the past twenty years, the proportion of 12–19-year-olds suffering from this disease has increased from 5 to 14 % [3].

An increase in the prevalence of obesity among women of reproductive age leads to an increase in infertility. Metabolic syndrome occurs in 30–33 % of women with impaired reproductive function [4]. Among patients with recurrent endometrial hyperplastic processes, the frequency of this pathology reaches 70 % [5]. 30–70 % of women with polycystic ovary syndrome (PCOS) are overweight and obese. At the same time, PCOS is the most common cause (70 %) of anovulatory infertility [6].

The multifactorial pathogenesis of metabolic syndrome, associated with the involvement of many body systems, determines the complexity and effectiveness of therapy, despite clear ideas about the mechanisms of development of reproductive system pathology [4–6]. In metabolic syndrome and type 2 diabetes mellitus, disorders of carbohydrate, fat, protein, mineral and water-salt metabolism are noted. The accumulation of products of impaired metabolism and edema fluid in the interstitium leads to an increase in the load on the regional lymphatic apparatus. Sorption therapy is aimed at removing toxic products from the interstitium by hemo-, plasma-, and lymphosorption. Long-term studies at the Research Institute of Clinical and Experimental Lymphology – Branch of the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (RICEL – Branch of the ICG SB RAS) have shown that correction with sorbents has a positive effect on the lymphatic system, improves its drainage and detoxification functions [7–9]. RICEL – Branch of the ICG SB RAS is developing a direction for the development of innovative medicines based on the embedding of biologically active molecules into the structure of porous carriers in which the pores of sorbents act as containers for active pharmaceutical ingredients. Currently, a sorbent matrix based on aluminum oxide and polydimethylsiloxane is actively used as a carrier of biologically active molecules of melatonin, lithium, etc. However, we have not found studies on the effect of metabolic syndrome, obesity and type 2 diabetes mellitus on the blood and lymphatic vessels of the pelvic organs in the available literature. To study the effect of the «sorbent + medicine» complexes, it is necessary to investigate the effect of the sorbent itself on the reproductive organs in these conditions. The study of the follicular composition of the ovaries, blood and lymphatic vessels, the prelymphatic slits of the ovaries and uterus, as well as the effect of sorption therapy on these structures in women with metabolic syndrome is impossible due to ethical and methodological problems. Therefore, we selected a model of genetically programmed obesity and type 2 diabetes mellitus in homozygous mice of the BKS.Cg-Dock7^{m}+/+Lepr^{db}/J (*db/db* mice) line.

So far, it is known that *db/db* mice have a point mutation in the leptin receptor gene, have pathological obesity and are characterized by chronic hyperglycemia, atrophy of pancreatic beta cells, hypoinsulinemia and dyslipidemia. The hyperlipidemic metabolic microenvironment caused by such a mutation leads to the accumulation of lipids in the theca cells and ovarian interstitium, progressive lipoapoptotic cytoatrophy of the compartments of follicular granulosa cells of the ovaries and epithelial cells of the endometrium of the uterus, which ultimately contributes to the development of infertility and premature organ involution in these mice [10, 11]. Thus, we consider *db/db* mice with obesity and type 2 diabetes mellitus as a suitable model for studying the drainage function of the abovementioned sorbent composition on the reproductive system of females.

THE AIM OF THE STUDY

To identify the effect of a sorbent composition of aluminum oxide and polydimethylsiloxane on the width of the myometrium and endometrium of the uterus, follicular apparatus and corpuses luteum of the ovaries, the state of blood, lymphatic vessels, uterine and ovarian prelymphatic slits of *db/db* mice with genetically determined development of obesity and type 2 diabetes mellitus.

MATERIAL AND METHODS

Experiment design

Female homozygous mice of the BKS.Cg-Dock7^{m}+/+Lepr^{db}/J (*db/db* mice) line at the age of 14 weeks were kept in controlled barrier rooms of the Laboratory Animal Genetic Resource Center of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (RFMEFI62119X0023). The animals had free access to food (SSNIFF (Germany), balanced granulated feed) and water. The experiments were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes and the Principles of Good Laboratory Practice (GLP). The study was approved by the local Ethics committee (Protocol No. 128 dated March 15, 2017).

A powdered sorbent complex of aluminum oxide and polydimethylsiloxane with a particle size up to 0.1 mm, a bulk density close to 1, an average pore volume of up to 0.26 cm^3/g, and a specific surface area of up to 160 m^2/g were used in the work. The sorbent matrix produced in Russia, created on the basis of aluminum oxide and polymethylsiloxane, is a hydrophilic-hydrophobic matrix compatible with biological tissues [12].

The following groups were formed:

1. Intact animals ($n = 6$).
2. Placebo ($n = 7$) – mice who received intragastric 200 µl of distilled water daily for 7 days.
3. Sorbent ($n = 5$) – mice that were injected with a sorbent composition (0.665 g per 1 kg of body weight, diluted in 200 µl of distilled water) through an intragastric tube once a day for 7 days.

Methods

After the animals were removed from the experiment by craniocervical dislocation, the uterus and ovaries were taken for histological examination. The organs were fixed in 10 % neutral formalin, dehydrated in a series of alcohols of increasing concentration and placed in a histomix. Slices with a thickness of 5 µm obtained using the Leica RM2155 microtome (Leica Biosystems, USA) were stained with hematoxylin and eosin. The preparations were examined under an Axioplan light microscope (Carl Zeiss, Germany) connected to a digital camera at magnifications of the eyepiece ×10, lens ×5. Digital images of the preparations were processed using the Image-Pro Plus 4.1 program. The width of the myometrium, endometrium, diameter of blood and lymph vessels, width of interstitial slits (prelymphatic slits) of the uterus and ovaries were measured in microns. To assess the follicular composition of the ovaries of mice, the numerical density of the primordial, primary, secondary follicles and corpuses luteum in ovary slices which passed through the ovarian gate was determined. Follicle identification was carried out according to the classification described in the work of A.E. Katelnikova et al. (2020) [13]. Each class of follicles is characterized by a fixed number of epithelial cells: primordial, consisting of an oocyte and surrounded by a single layer of flattened follicular cells, between which there are slits, and as the follicle forms, epithelial cells connect; primary (single-layered, preantral) and secondary (multilayer, antral) follicles formed by an oocyte and cubic granulosa cells; tertiary (preovulatory) follicles, which later become mature and ready for ovulation.

Statistical processing

Statistical processing was performed using the Statistica 12 software package (StatSoft Inc., USA). The values of the median, the first and third quartiles, and the arithmetic average were determined. The statistical significance of the differences in the compared values was established using the non-parametric Mann – Whitney test. The differences were considered statistically significant at $p < 0.05$.

RESULTS

In female *db/db* mice, unlike normal laboratory mice, no clearly defined inner circular and outer longitudinal layers were found in the uterine myometrium. Between the layers of the myometrium, there was also no expression of the vascular layer between the layers of the myometrium, in which the blood and lymph vessels of the uterus normally pass. Only individual vessels, which were located between edematous muscle fibers and single myocytes, were detected in *db/db* mice. The average diameters of the myometrial vessels in *db/db* mice were: arteries – 11.93 (11.43; 17.46) microns, veins – 20.81 (20.07; 28.39) µm, lymphatic vessels – 30.79 (25.54; 36.86) µm. Average diameters of endometrial vessels: arteries – 11.82 (11.79; 14.18) µm, veins – 24.57 (18.74; 26.35) µm, lymphatic vessels – 23.21 (21.67; 31.50) µm. The muscle layer in the females looked edematous due to dilation of the prelymphatic slits and accumulation of tissue fluid in the interstitium. The average width of the prelymphatic slits in the myometrium was 28.86 (26.66; 32.16) µm, and the average thickness of the myometrium reached 138.61 (125.75; 152.27) µm. The same pattern of edema was detected in the endometrium (Fig. 1a). The average width of the prelymphatic slits in the endometrium was 14.13 (13.78; 14.50) µm, and the average thickness of the endometrium itself reached 347.48 (301.82; 358.44) µm. In the placebo group, the edema pattern persisted and the thickness of the layers did not change.

After treatment with the sorbent composition of aluminum oxide and polydimethylsiloxane of *db/db* mice, the thickness of the uterine layers decreased compared to placebo ($p < 0.0122$) (Fig. 2). Sorbent treatment contributed to the narrowing of the diameters of both the bearing vessels – arteries ($p < 0.0216$) and vessels draining fluid from the organ – veins ($p < 0.0122$), lymphatic vessels ($p < 0.0122$) (Fig. 1b; Fig. 3).

The width of the prelymphatic slits decreased ($p < 0.0122$; Fig. 4a, b), interstitial edema decreased, making the endometrium and muscle layer appear more "dense". A decrease in the diameter of all types of vessels and the width of the prelymphatic slits, a decrease in edema in both layers *db/db* mice uterus after administration of aluminum oxide and polydimethylsiloxane may indicate a pronounced draining effect of this sorbent composition on the interstitium of different uterus layers.

Administration of aluminum oxide and polydimethylsiloxane had no effect on the size of the diameters of blood and lymph vessels in the ovaries, but caused a pronounced tendency to decrease the width of the prelymphatic slits in them ($p < 0.0513$) (Fig. 4b). In the follicular apparatus of the ovaries of female *db/db* mice, tertiary follicles were absent, and the numerical densities of other structures were: primordial follicles – 15.0 (11.75; 17.5) units, primary follicles – 5.0 (3.5; 5.0) units, secondary – 3.0 (3.0; 3.75) units, corpuses luteum – 3.0 (2.25; 3.0) units. The quantitative composition of the ovarian follicular apparatus in animals of the placebo group did not differ statistically significantly.

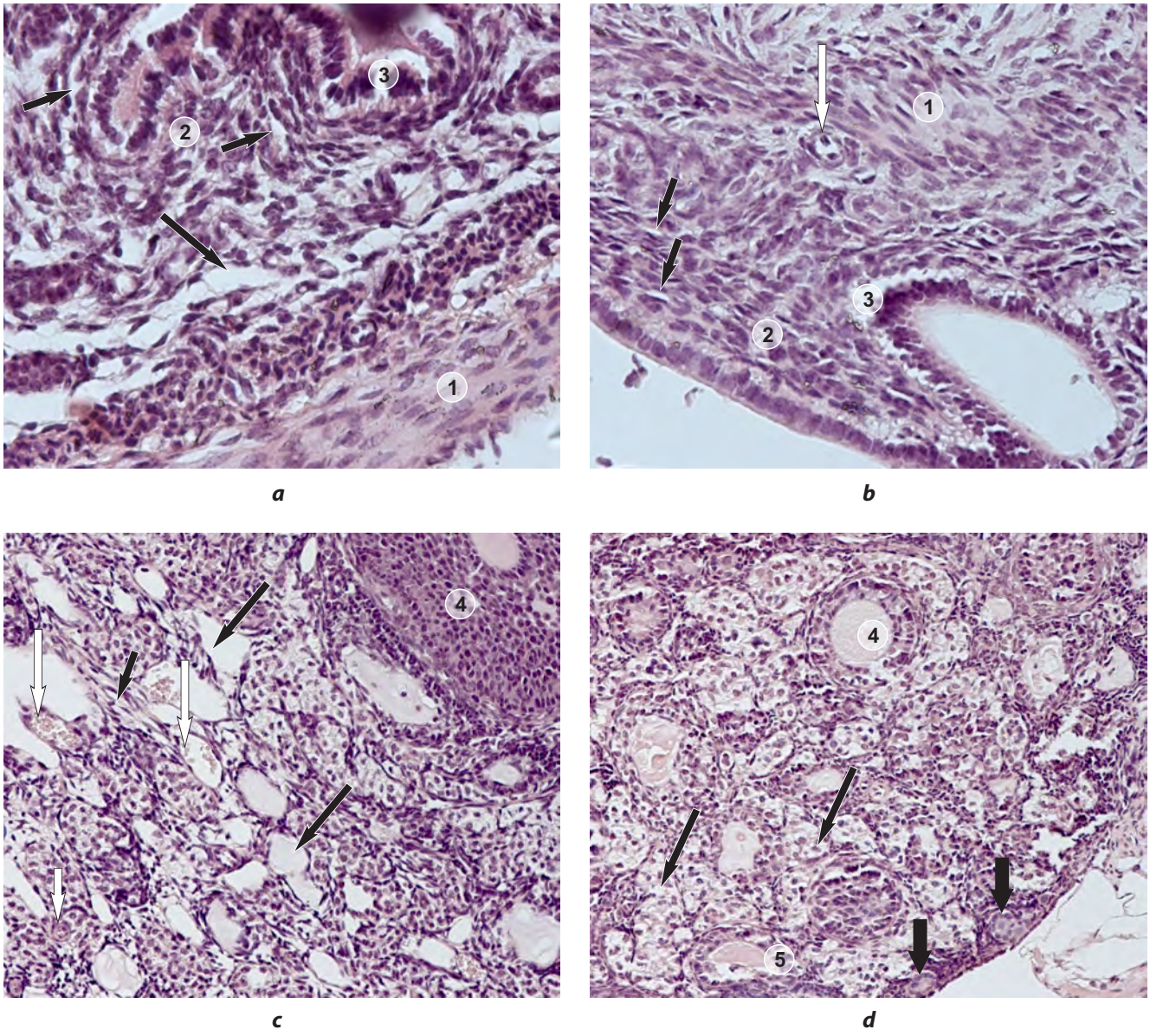

FIG. 1.

*Uterus (**a**, **b**) and ovaries (**c**, **d**) in db/db mice before (**a**, **c**) and after (**b**, **d**) treatment with of the sorbent complex:* 1 *– myometrium;* 2 *– endometrium;* 3 *– uterine glands;* 4 *– secondary follicles; thick black arrows – primordial follicles; long black arrows – lymphatic vessels; short black arrows – prelymphatic slits; long white arrows – veins; short white arrow – artery. Magnification × 400 (**a**, **b**), × 200 (**c**, **d**)*

Administration of the sorbent matrix had no effect on the number of primordial, primary follicles and corpuses luteum, but contributed to an increase in the numerical density of secondary follicles – up to 5.0 (4.0; 6.0) units compared with placebo (2.5 (2.0; 3.0) units; $p < 0.0176$). Perhaps it was the improvement in the drainage of the ovarian interstitium and the removal of toxic metabolic products that contributed to the maturation of secondary follicles.

DISCUSSION

It is known that in obese women, the presence of high levels of lipid droplets in the cumulus and granulosa cells of the ovaries is associated with unfavorable results of gestation and carrying of a pregnancy [14]. Changes in the concentrations of glucose and lipids (free fatty acids/triglycerides) in the intercellular space affect the interstitial and cytoplasmic microchemical environment, which significantly changes the cellular diffusion of nutrients and the rate of active transmembrane flow. For example, it has been shown that a progressive increase in the absorption of interstitial and cytoplasmic lipids is associated with DNA fragmentation of endometrial epithelial cells and follicular granulosa cells of the ovaries in *db/db* mice by lipoinfiltration into the chromatin matrix, that leads to the development of nuclear lipoapoptosis [15]. At the same time, conditions are created in the interstitium to increase the level of free radicals, reactive oxygen/nitrogen species, which in turn contributes to the accumulation of low molecular weight hyaluro-

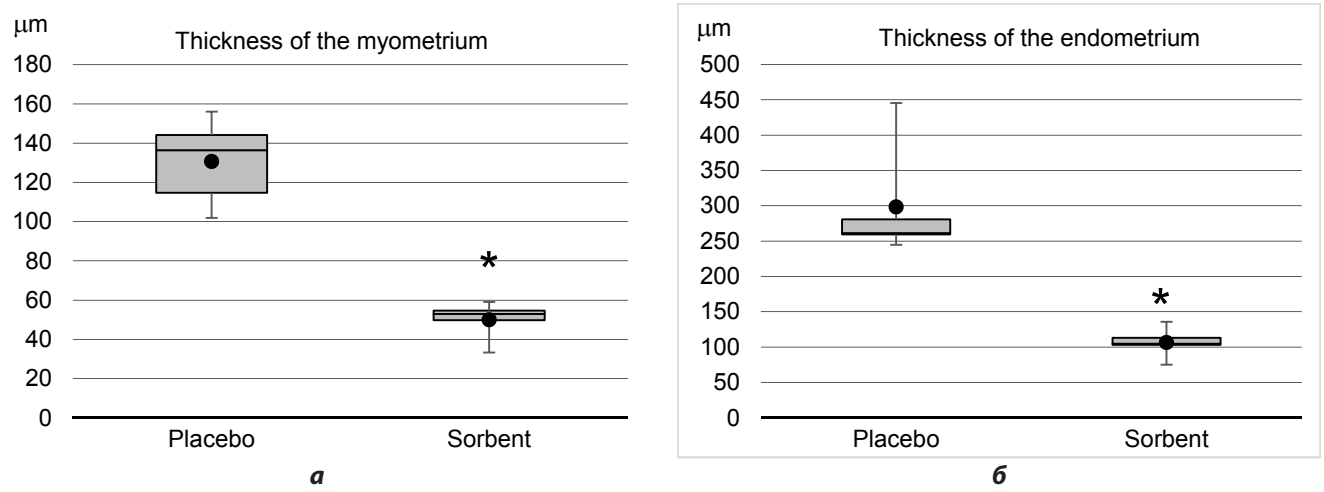

FIG. 2.

*Quantitative assessment of the thickness of the myometrium (**a**) and endometrium (**b**) in the uterus of db/db mice before and after administration of aluminum oxide and polydimethylsiloxane: ▬ – median; ▭ – 25%–75% percentiles; ● – arithmetic average; ✱ – p < 0.05*

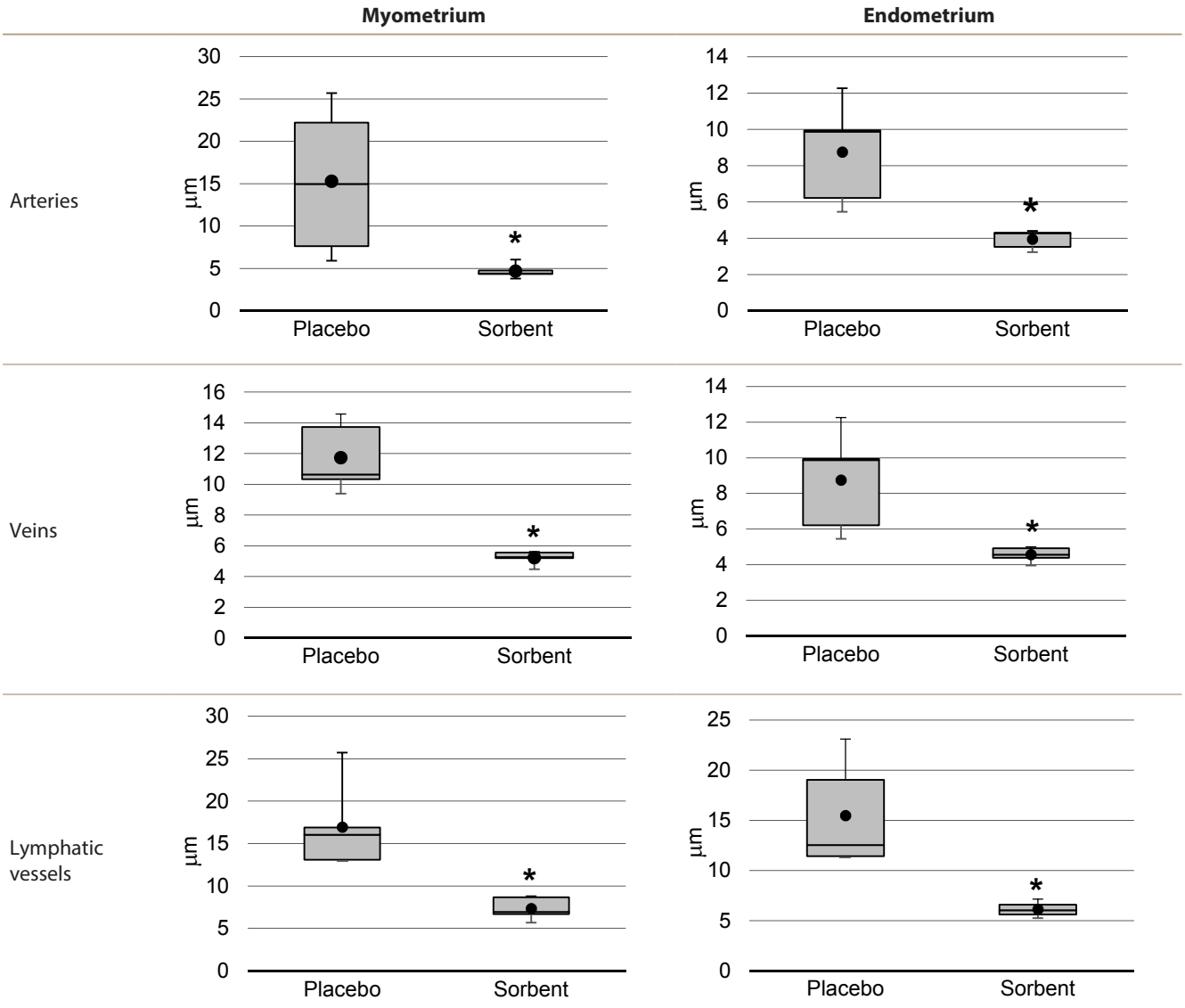

FIG. 3.

Quantitative assessment of the diameters of arteries, veins and lymphatic vessels in the myometrium and endometrium in the uterus of db/db mice before and after administration of aluminum oxide and polydimethylsiloxane: ▬ – median; ▭ – 25%–75% percentiles; ● – arithmetic average; ✱ – p < 0.05

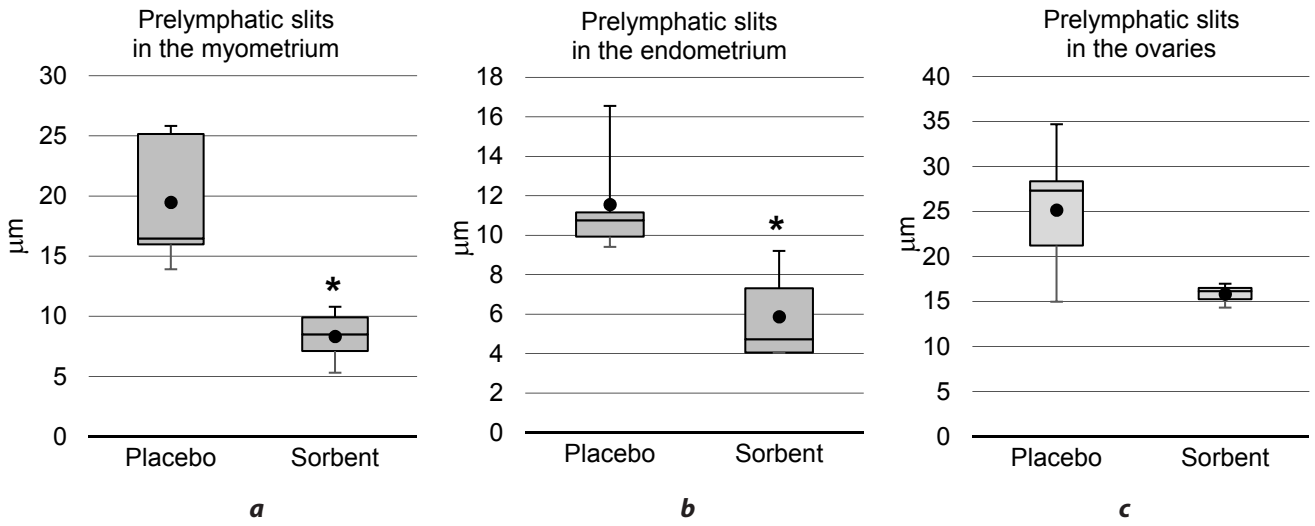

FIG. 4.

*Quantitative assessment of the width of interstitial slits (prelimphatics) in the myometrium (**a**) and endometrium (**b**) of the uterus and in the ovaries (**c**) of db/db mice before and after administration of aluminum oxide and polydimethylsiloxane: ▬ – median; ▭ – 25%–75%th percentiles; ● – arithmetic average; ✱ – $p < 0.05$*

nan of the intercellular matrix. Hyaluronic acid, fragmented to low molecular weight polymers (from 1 to 500 kDa), can not only disrupt the permeability of the blood vessel wall, but also, by binding to the LYVE-1 receptor, destroy the integrity of the endothelial barrier of lymphatic vessels [16, 17]. As a result, we observe dilatation of arteries, veins, lymphatic vessels, interstitial prelymphatic slits of the myometrium and endometrium of the uterus, dilation of the prelymphatic slits in the ovaries and edema due to accumulation of tissue fluid in the interstitial of these organs. Thus, in *db/db* obese mice with type 2 diabetes mellitus, we have already noted an expansion of sinusoidal capillaries and a low level of LYVE-1 expression in the liver [18].

In patients with metabolic syndrome, angiography data are usually provided – when assessing the nature of damage to the coronary arteries, multivessel and polysegmental lesions are more often described [19, 20]. We have not found an analysis of the size of the diameter of veins, lymphatic vessels in the organs of the female reproductive system in the available literature. In our study, it was shown that treatment with the sorbent composition based on aluminum oxide and polydimethylsiloxane has a positive effect on the water homeostasis of reproductive organs, as evidenced by a decrease in the diameters of arteries, veins and lymphatic vessels of the myometrium and endometrium of the uterus, a narrowing of the width of the prelymphatic slits and a decrease of ovarian and uterine tissue edema in *db/db* mice. These results indicate the draining and detoxification effects of aluminum oxide and polydimethylsiloxane.

Obesity affects the hypothalamic-pituitary axis throughout a woman's life. It affects the processes of puberty and is associated with an increased risk of hyperandrogenism and ovarian dysfunction. The association of hyperinsulinemia and hyperandrogenism in disorders of the latters, including PCOS, is well known [5]. The study of the number of follicles in the ovaries of *db/db* mice deserves attention. So, M.V. Denisenko et al. [21] when studying the ovaries of laboratory outbred female mice, counted up to 6 primordial follicles in 1 field of view. It is known that the numerical density of follicles in the ovaries depends on age. A pattern has been traced between the number of primordial follicles in 1 field of vision and the age of a woman: at the age of 18–35, the number of primordial follicles was 7.5 ± 2.5, at the age of 36–55 years – 1 ± 1 (× 240) [21, 22]. The number of all types of follicles in mice decreases significantly every 4 weeks with age [11]. In our study on *db/db* mice, it was revealed that the number of primordial follicles in the ovaries is significantly higher than in ordinary laboratory mice. This is consistent with the results and conclusions of researchers who observed polycystic ovaries in women with metabolic syndrome and type 2 diabetes mellitus [23–25]. Most of the symptoms of polycystic ovaries occur at the beginning of puberty. Persistent hormonal imbalance leads to the formation of multiple small antral follicles and an irregular anovulatory menstrual cycle, that ultimately causes infertility in women. Insulin resistance, cardiovascular diseases, abdominal obesity, psychological disorders, infertility and cancer are also associated with PCOS. Hyperandrogenism causes insulin resistance and hyperglycemia, which leads to oxidative stress and abdominal obesity. As a result, inflammation, production of reactive oxygen species, insulin resistance and hyperandrogenism also increase [26, 27].

Hyperandrogenism affects gene expression in endocrine theca cells, promotes an unbalanced change in the subtypes of granulosa cells [28] and even leads to apoptosis of the latters, whereas the peripheral conversion of androgens to estrogens suppresses the secretion of gon-

adotropins. Q.L. Zhang et al. [29] explain the impaired follicle development (a decrease in the number of preantral and antral follicles) by an increase in insulin-dependent phosphorylation of protein kinase B and levels of caspase-3 in ovaries of mice with hyperinsulinemia. Most of the changes related to ovarian physiology in obesity are the result of transcriptional disorders caused by altered leptin signaling. Increased leptin levels stimulate higher expression of *CART*, which encodes an endogenous neuropeptide that plays a key role in regulating follicle atresia in granulosa cells of overweight and obese animals and humans [30]. Obesity can lead to the activation of inflammatory pathways and the creation of an unbalanced microenvironment around cells, as well as to a higher expression of molecular inflammatory mediators in the follicles themselves, contributing to irreversible damage to the latters [30]. Obese women have elevated levels of C-reactive protein in follicular fluid, indicating that there is a direct effect of the woman's metabolism on the ovarian follicle microenvironment and is a marker of inflammation and oxidative stress. All this contributes to the delay in follicle maturation and the development of anovulation [5, 23, 30]. In our study, it was found that the treatment of *db/db* mice with a sorbent composition based on aluminum oxide and polydimethylsiloxane leads to an increase in the numerical density of secondary follicles, relative to the placebo group. Perhaps it was the improvement in the drainage of the ovarian interstitium and the removal of toxic metabolic products that contributed to the maturation of secondary follicles.

CONCLUSION

Thus, in female *db/db* mice with genetically determined development of obesity and type 2 diabetes mellitus, dilation of the blood and lymph vessels of the myometrium and endometrium in the uterus, expansion of interstitial slits (prelymphatic slits) and edema in the ovaries and both layers of the uterus, a large number of primordial follicles and absence of tertiary follicles in the ovaries are observed. Administration of the sorbent composition based on aluminum oxide and polydimethylsiloxane leads to a decrease in the diameters of the blood and lymph vessels of the uterus, a narrowing of the width of the prelymphatic slits, that contributes to the reduction of tissue edema of reproductive organs, the structuring of the uterine tissue layers and the improvement of the functional activity of the ovaries, as evidenced by an increase in the number of secondary follicles.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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