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Impact of stress and diabetes mellitus on ovarian disruption during puberty

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The objective: **to determine how stress affects the histo-ultrastructure of the ovaries of healthy and diabetic peripubertal rats.**
Materials and methods**. The study included 20 two-month-old female albino laboratory rats, which were equally divided into 4 groups: Group 1 – rats with simulated streptozotocin-induced diabetes mellitus (SIDM) and chronic immobilization stress (CIS), Group 2 – rats with SIDM, Group 3 – rats with CIS, Group 4 – control animals. The material (blood and ovarian tissue) was taken on the 28th day from the beginning of the experiment. Levels of cortisol and glycated hemoglobin were determined in the blood of the animals. Histological, electron microscopy, biochemical and statistical research methods were used.**
Results**. Under conditions of SIDM accompanied by hyperglycemia and elevated glycated hemoglobin levels in experimental Groups 1 and 2, the development of diabetic microangiopathy and remodeling of the histoarchitectonics of the ovarian cortex were observed. The number of primordial and primary follicles decreased by 3.1- and 1.7-fold, respectively, in Group 1, and by 2.3- and 1.6-fold in Group 2, against the background of an increased incidence of atretic and cystic follicles. The general morphology of the ovaries of rats in Groups 2–4 was similar to the morphology of the ovaries of rats with polycystic ovary syndrome (PCOS). At the ultrastructural level, oocytes exhibited pathological processes such as apoptosis and necroptosis, and less frequently, colliquative necrosis. In granulosa cells, pathological accumulation of lipid granules (observed exclusively in rats with comorbid pathology) and destructive changes consistent with partial necrosis were found. Intercellular space widening in the granulosa layer and detachment of granulosa follicular epitheliocytes into the follicular antral were also observed.**
CIS exerted its most pronounced effects on primordial follicles, leading to oocyte loss through apoptosis and necroptosis, with a subsequent reduction in their number and in the number of primary follicles by 1.5- and 1.4-fold, respectively. In granulosa follicles, dilatation of the endoplasmic reticulum cisternae, focal lysis of mitochondrial cristae, and lipid droplet accumulation were recorded.
Conclusions**. According to our research, SIDM and CIS lead to the appearance of cystic follicles and an increase in the number of atretic follicles against the background of follicle morphogenesis abnormalities, which indicates the development of PCOS. Under these conditions, the pool of primordial and primary follicles decreased significantly, indicating depletion of the ovarian reserve and a possible decrease in fertility in peripubertal rats. Moreover, the greatest decrease in the reserve of primordial, primary and secondary follicles was observed in rats with comorbid pathology. This indicates that stress significantly worsens the course of diabetes mellitus and reduces reproductive potential at pubertal age.**
Keywords: *diabetes mellitus, stress, polycystic ovary syndrome, female reproductive system, ovaries, gynecological pathology, infertility.*

Вплив стресу та цукрового діабету на функціональні розлади яєчників у період статевого дозрівання

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Мета дослідження: встановити вплив стресу на гістоультраструктуру тканини яєчників здорових та діабетичних щурів у перипубертатному періоді.
Матеріали та методи. У дослідженні використано 20 двомісячних білих лабораторних щурів-самок, яких було рівномірно розподілено на 4 групи: група 1 – щури з індукованим стрептозотоцин-індукованим цукровим діабетом (СІЦД) та хронічним іммобілізаційним стресом (ХІС), група 2 – щури із СІЦД, група 3 – щури з ХІС, група 4 – контрольні тварини. Матеріал (кров і тканину яєчників) забирали на 28-й день від початку експерименту. У крові тварин визначали рівні кортизолу й глікованого гемоглобіну. Використовували гістологічний, електронно-мікроскопічний, біохімічні та статистичні методи дослідження.
Результати. У разі СІЦД на тлі гіперглікемії й високих рівнів глікованого гемоглобіну в досліджуваних групах 1 та 2 відбувається розвиток діабетичної мікроангіопатії та перебудова гістоархітектоніки кори яєчників. Зменшується кількість примордіальних і первинних фолікулів у 3,1 і 1,7 раза відповідно в групі 1 та у 2,3 і 1,6 раза у групі 2 на тлі збільшення атретичних та кістозних фолікулів. Загальна морфологія яєчників щурів у групах 2–4 була подібною до морфології яєчників щурів із синдромом полікістозних яєчників (СПКЯ). На ультраструктурному рівні в яйцеклітинах

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спостерігалися такі патологічні процеси, як апоптоз та некроптоз, рідше – колікваційний некроз. У зернистих клітинах відзначали патологічне накопичення ліпідних гранул (тільки у щурів із коморбідною патологією) та деструктивні зміни за типом парціального некрозу. Розширення міжклітинного простору в зернистому шарі та відшарування зернистих фолікулярних епітеліоцитів у порожнину антрума.

Вплив ХІС призводить до найбільш виражених змін у примордіальних фолікулах унаслідок загибелі яйцеклітин шляхом апоптозу і некроптозу та зменшення їхньої чисельності, а також первинних фолікулів у 1,5 та 1,4 раза відповідно. У зернистих фолікулах спостерігали розширення цистерн ендоплазматичної сітки, вогнищевий лізис крист мітохондрій, накопичення ліпідних крапель.

Висновки. За даними наших досліджень, СІЦД та ХІС призводять до появи кістозних фолікулів і збільшення кількості атретичних фолікулів на тлі аномалій морфогенезу фолікулів, що свідчить про розвиток СПКЯ. За таких умов значно зменшується пул примордіальних та первинних фолікулів, що вказує на виснаження овариального резерву та можливе зниження фертильності у щурів перипубертатного віку. Крім того, найбільше зниження резерву примордіальних, первинних та вторинних фолікулів спостерігалось у щурів із коморбідною патологією. Це вказує на те, що стрес значно погіршує перебіг цукрового діабету та зменшує репродуктивний потенціал у пубертатному віці.

Ключові слова: *цукровий діабет, стрес, синдром полікістозних яєчників, репродуктивна система жінки, яєчники, гінекологічна патологія, безпліддя.*

Type 1 diabetes mellitus (DM) is one of the most common chronic diseases in childhood, with an annual incidence of 2–5% [1]. Due to the increase in the incidence of DM in childhood, the number of women of reproductive age diagnosed with DM is also increasing [2]. 40% of women with DM experience reproductive dysfunction, including delayed menstruation, all types of menstrual disorders, such as amenorrhea, oligomenorrhea, cycle disorders, as well as menorrhagia, infertility, polycystic ovary syndrome (PCOS) and early (or rarely late) menopause [3, 4]. Most studies have found that there is a delay in the age of menarche if diabetes onset occurs before 10 or 11 years of age or closer to the onset of puberty, and that this delay increases with poor glycemic control [5].

Several retrospective studies have shown menstrual cycle disorders in adolescents with type 1 DM [2, 6, 7]. It was revealed that in girls with type 1 DM, the duration of the menstrual cycle was 48 ± 39 days compared to 32 ± 7 days in the control group; oligomenorrhea was diagnosed in 58.9%, and amenorrhea in 10.7% [6]. Girls with HbA1c levels between 7.6% and 8.9% had longer cycles, more variability in their menstrual cycles, and a higher prevalence of oligomenorrhea compared to the control group. Regression analysis showed that for each point increase in HbA1c, the duration of the menstrual cycle increased by 5.1 days [6].

This chronic disease affects various stages of life for those who suffer from it. Adolescence is considered one of the most difficult stages due to all the changes that occur, both physiological and psychosocial, which make young people with diabetes prone to higher levels of stress and psychological disorders. Treatment includes diet, physical activity, insulin administration and proper self-monitoring. The self-monitoring can be difficult, leading to children, adolescents and their families suffering from a variety of psychosocial complications. There is an inverse relationship between self-control and psychosocial complications, the main problems of which are anxiety and depression, with adolescents being 2.3 times more likely to have mental health problems [8]. As is known, the main hormonal systems that mediate the response to stress are the hypothalamic-pituitary-adrenal axis. During stress, there is increased activity of glucocorticoids that affect the hypothalamic-pituitary-ovarian axis. It has been proven that during stress, glucocorticoids suppress the secretion of gonadotrophin-releasing hormone and luteinizing hormone (LH), as well as suppress the biosynthesis of estrogen and progesterone in the ovaries and the action of estrogen in target tissues, resulting in hypothalamic amenorrhea occurs, which is observed in cases of anxiety and depression [9].

Based on the above, **the objective** of this study was to determine how stress affects the histo-ultrastructure of the ovaries of healthy and diabetic peripubertal rats.

MATERIALS AND METHODS

In this study, we used a total of 20 two-month-old female albino laboratory rats, equally divided into four groups (five rats per group): Group 1 included rats with comorbid pathology – streptozotocin-induced DM (SIDM) combined with chronic immobilization stress (CIS); Group 2 included rats with SIDM only; Group 3 included rats subjected to CIS only and Group 4 (Control) consisted of healthy rats. In Groups 1 and 2, SIDM was simulated by a single intraperitoneal injection of streptozotocin "SIGMA" (USA), which was diluted in 0.1M citrate buffer with a pH of 4.5 (at the rate of 7 mg per 100 g of body weight). In Groups 1 and 3, CIS was simulated by placing the animals in a closed plastic container for 5 hours a day. In Group 1, SIDM was simulated and starting from the 14th day of the experiment CIS was added.

The experiment on animals was conducted in the vivarium of the Ivano-Frankivsk National Medical University (IFNMU). The rat cages were kept in a room with a controlled environment, in the following standard conditions (12/12 light/dark cycle with 22 ± 3 °C room temperature). All rats were provided with standard laboratory chow and water ad libitum. At the end of the experiment, the animals were euthanized under ketamine anesthesia. Immediately after euthanasia, blood samples were collected into test tubes for further analysis. Glucose levels were measured using the glucose oxidase method at the Center of Bioelementology of IFNMU. The levels of glycated hemoglobin (HbA1c) and cortisol were determined in the clinical diagnostics laboratory "Medlux". The concentration of HbA1c in the blood was determined using "ACCENT-200 HbA1c DIRECT" diagnostic kit (PZ Cormay S.A., Poland). Serum cortisol levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit ("EIA-1887, Cortisol ELI-

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SA", DRG International, Germany). Tissue samples (ovaries and blood) were collected on the 28th from the beginning of the experiment in Groups 1, 2, and 4. In Group 3, the materials were collected on the 14th day from the beginning of CIS induction. Material collection was performed in the morning (between 7:00 and 8:00 am), before feeding, to exclude the influence of daily rhythm and biological activity on rat metabolism. The estrous cycle stage was determined via vaginal cytology and monitored for at least 7 days before experiments involving the natural estrous cycle [10].

We used histological methods (samples stained with hematoxylin-eosin and Masson's trichrome) and electron microscopy. To evaluate ovarian architecture and follicle number, we conducted samples stained by hematoxylin-eosin. Ten fields from three randomly selected sections of each ovary were examined at an original magnification of × 200 to calculate the number of follicles at different stages of maturation [11].

All sections were evaluated with an optical microscope (Leica DM750) and monitored with attached digital camera (ToupCam 5.2M UHCCD C-Mount Sony sensor, ToupTek Photonics, China). Statistical analysis was performed using the Statistica 12 software (StatSoft Inc., Tulsa, OK, USA). To assess differences between Groups, the Mann–Whitney U test was used. The sample parameters presented in the text are denoted as M ± SD, where M represents the sample mean, SD the standard deviation, and p the achieved level of statistical significance.

RESULTS AND DISCUSSION

When studying biochemical changes in the blood, a sharp increase in glucose and HbA1c was observed in Groups 1 and 2 of rats, compared with the Control Group, respectively: in Group 1 – 20.56±1.78 mmol/L (Control – 3.36 ± 0.19 mmol/L, $p < 0.05$) and 8.45 ± 0.41% (Control – 2.13 ± 0.21%, $p < 0.05$); in Group 2 – 17.25 ± 1.15 mmol/L ($p < 0.05$) and 8.09 ± 0.46% (in all cases $p < 0.05$). Such biochemical changes in Groups 1 and 2 of rats indicate the development of decompensated SIDM. In Group 3, glucose and HbA1c levels did not significantly change compared to the control and were, respectively: 4.26 ± 0.32 mmol/L and 2.31 ± 0.18% (in all cases $p > 0.05$). As for the level of cortisol in blood plasma, it was significantly higher in all experimental groups of animals compared to control values, and amounted to: in Group 1 – 40.34 ± 2.26 ng/mL, in Group 2 – 35.46 ± 1.52 ng/mL, in Group 3 – 25.74 ± 1.98 ng/mL (Control – 10.96 ± 1.14 ng/mL, in all cases $p < 0.05$ compared to control values). Thus, all experimental groups were under chronic stress conditions.

A study of the histostructure of the ovaries of peripubertal rats showed that the ovary is covered externally by a single layer of cubic epithelium, under which there is a protein membrane rich in connective tissue fibres. All stages of folliculogenesis can be traced in the ovarian cortex (Fig. 1a). Under the protein membrane, primordial follicles were sometimes visualised, forming clusters known as egg nests (Fig. 1b). The primordial follicles consisted of an oocyte surrounded by a single layer of flat follicular epithelium. The primary follicles contained an oocyte surrounded by cubic follicular epithelium in the centre (Fig. 1c). Preantral follicles in the ovarian cortex had a normal structure: an oocyte was located in the centre, surrounded by a transparent zone (*zona pellucida*) and granulosa cells (Fig. 1c). Secondary follicles were characterised by different sizes and had several layers of granulosa cells. A characteristic feature of tertiary or Graafian follicles is a fluid-filled cavity, known as the antral follicle. The egg cell lies on the edge of a mound formed by granulosa epithelial cells, called the cumulus oophorus. The theca cells of the inner and outer theca were outside the granulosa cells (Fig. 1a). Blood vessels were located in the loose connective tissue of the ovary. It should be noted that only in two of the ovaries studied in the Control Group did we find a *corpus luteum*; in the other ovaries, there were none, which is obviously related to the age of the animals (Table).

In rats of Groups 1 and 2 with SIDM, the vascular hyperemia of the ovarian medulla is noteworthy. Most of the vessels are filled with erythrocyte sludge and microthrombi. The majority of follicles retain their structure. However, in the ovarian cortex, there is a sharp decrease in the number of primordial follicles by 3.1 times in Group 1 of rats with comorbid pathology and by 2.3 times in Group 2 of rats with SIDM, compared with the control indicators (Table). Primordial follicles do not form clusters but are located singly in the cortical substance under the protein membrane. In some of them, oocyte shrinkage and separation from follicular cells are observed. Secondary follicles undergo degeneration not into Graafian follicles but into cystic follicles (Fig. 1d, e). In tertian follicles, granulosa cells lost their connection with each other and detached into the follicle antrum, forming clusters there. Some granulosa cells apparently underwent irreversible destructive changes. In Groups 1 and 2 of rats, the number of atretic and cystic follicles increased (Table), which may indicate the development of PCOS. Some of the follicles underwent hyaline degeneration, which was most pronounced in rats in Group 1.

In rats of Group 3, which were subjected to CIS for 14 days, the cytoarchitecture of the ovarian cortex underwent less pronounced changes. The number of primordial and primary follicles decreased compared to the control (Table). At the same time, the number of primordial and primary follicles was higher than in Groups 1 and 2, and the number of secondary follicles did not differ statistically significantly from other groups of animals. The number of Graafian follicles and *corpus luteum* remained at the control level, indicating that the ovulation process was preserved. Cystic follicles appeared, but their number was lower than in rats with DM (Table). In secondary and tertiary follicles, intercellular edema appears between the granular cells of the granular layer, leading to detachment and the formation of spaces between them (Fig. 1f). The distance between the cells of the corona radiata was also greater. In the cytoplasm of the oocytes, a reduced density of organelles and a shrivelled nucleus were observed. The structure of the corpus luteum did not differ from that in the Control Group of animals (Fig. 1f).

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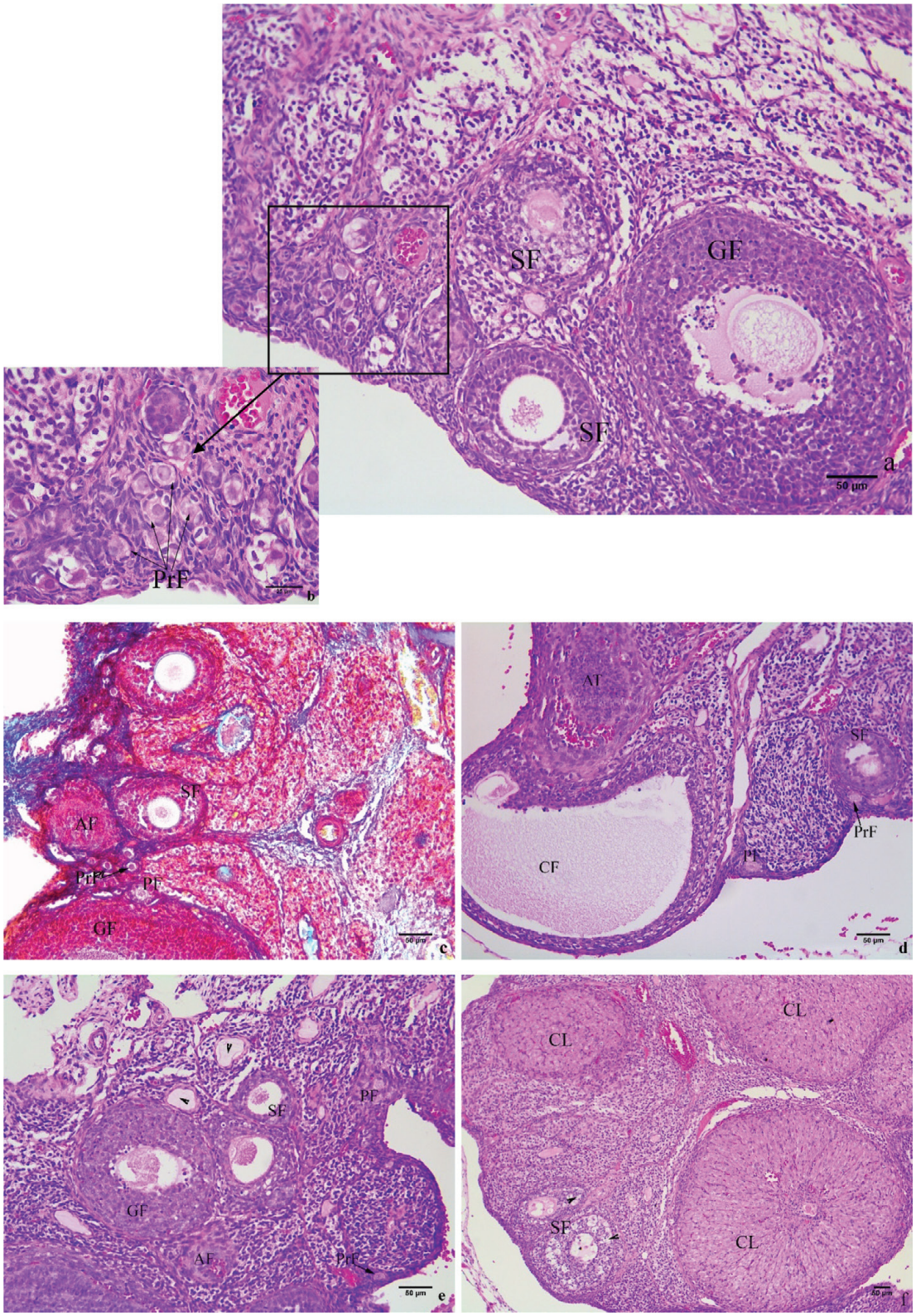

Fig. 1. Histological architecture of the ovaries of 2-month-old rats in the Control Group (a–c), SIDM + CIS (d), SIDM (e), CIS (f). Clusters of primordial follicles in the ovarian nests of the Control Group (b). Appearance of cystic follicles and increase in the number of atretic follicles in rats with comorbid pathology (d). Development of cystic follicles (indicated by an arrow) in rats with SIDM (e). Delamination of the granulosa layer in the secondary follicle and the appearance of spaces between granulosa cells (indicated by an arrow, f). Staining: H & E (a, b, d–f), Masson's trichrome (c). Magnification: a, c–e) × 200; b) × 630; f) × 100. CF indicates cystic follicle; AF – atretic follicle; CL – corpus luteum; PrF – primordial follicle; PF – primary follicle; SF – secondary follicle; GF – Graafian follicle; O – oocyte; H & E – hematoxylin-eosin staining

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Quantitative characteristics of the ovarian cortex in peripubertal rats under various pathological conditions

Follicular development	Group 4 (Control)	Group 1 (SIDM + CIS)	Group 2 (SIDM)	Group 3 (CIS)
Primordial	9.0 ± 2.0	2.90 ± 0.87*β	3.92 ± 1.12*#	6.00 ± 3.35*#
Primary	3.43 ± 1.01	2.00 ± 0.85*	2.08 ± 0.95*	2.44 ± 0.53*
Secondary	2.78 ± 0.89	2.30 ± 0.82	2.60 ± 0.87	2.67 ± 0.60
Graafian	0.93 ± 0.62	0.30 ± 0.48*	0.54 ± 0.78	0.44 ± 0.53
Atretic	0.36 ± 0.49	1.70 ± 1.06*	1.08 ± 0.76*	0.89 ± 0.78
Cystic	ND	1.10 ± 0.73*β	1.38 ± 1.12*β	0.44 ± 0.52*#
Corpus luteum	0.50 ± 0.65	0.50 ± 0.53	0.31 ± 0.63	0.88 ± 1.05

Notes: all results are expressed as mean ± SD, $p < 0.05$ is significant; * – $p < 0.05$, comparison with Control Group; # – $p < 0.05$, comparison with SIDM + CIS; β – $p < 0.05$, comparison with CIS; ND – not detected.

At the ultrastructural level, the ovary of 2-month-old rats has a normal structure (Fig. 2a). As can be seen in the figure, the secondary follicle contains an egg cell with a round nucleus with diffusely located chromatin granules and a nucleolus. The ooplasm contains mitochondria, short parallel cisternae of the granular endoplasmic reticulum, electron-dense cortical granules, and primary lysosomes. The plasma membrane of the oocyte forms numerous microvilli that penetrate the *zona pellucida*. Granulosa cells have a normal structure, are tightly packed together, and are separated from the cells of the inner theca by a basal membrane. The theca cells of the inner shell contain numerous lipid droplets of varying size and electron density.

At the ultrastructural level, the ovaries of rats with comorbid pathology undergo the most pronounced changes. First of all, the development of diabetic microangiopathy in Groups 1 and 2 of animals is noteworthy, which is characterised by hemorrhological disorders in the microhemovessels of the ovarian medulla and cortex: adhesion of erythrocytes and platelets to the luminal surface of endothelial cells, erythrocyte sludge, microthrombi, development of vacuolar dystrophy of endothelial cells of capillaries and arterioles. In some places, destruction of capillaries with diapedesis of erythrocytes into the interstitial space is noted.

In rats with SIDM + CIS, oocyte apoptosis (Fig. 3a) was most often detected in primordial, primary and secondary follicles, with the latter transforming into atretic follicles. Granulosa cells underwent changes of the pyroptosis and necrotic types. In these cells, karyopyknosis, karyorrhexis and accumulation of lipid droplets in the cytoplasm were observed (Fig. 3b). An enlarged intercellular space appeared between the granular cells, whereas in the Control Group it was absent and the cells were tightly adjacent to each other. Such ultrastructural rearrangement of the granulosa layer cells led to their detachment and exfoliation into the follicular lumen. Pathological lipid droplets with scalloped edges also accumulated in the cells of the inner theca. There was an overgrowth of collagen fibres around the follicles and capillaries, as well as fibrosis of the ovarian medulla.

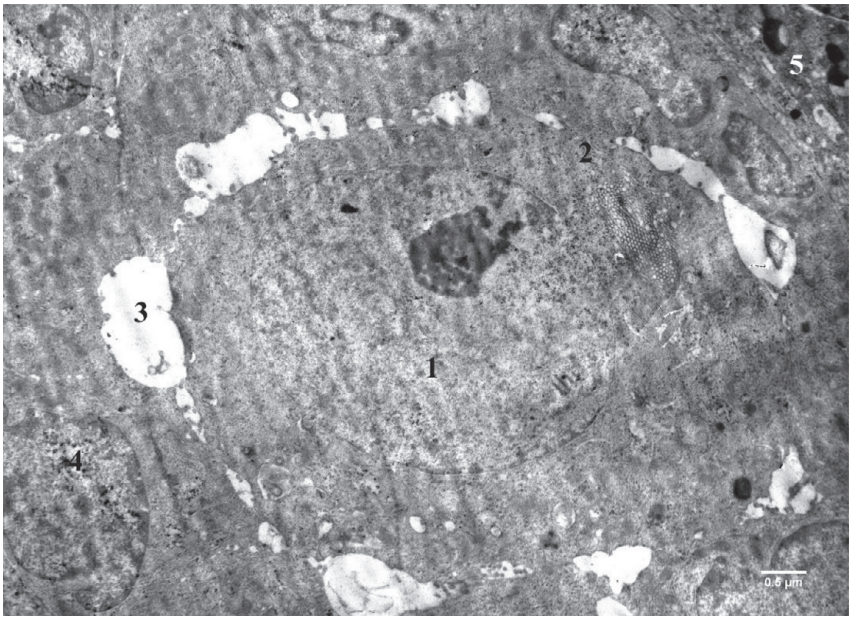

Fig. 2. Ultrastructure of the secondary follicle of a 2-month-old rat in normal condition. Electronogram. Magnification: × 8000. Designations: 1 – egg nucleus; 2 – cisternae of the granular endoplasmic reticulum; 3 – transparent zone; 4 – granulosa cell, theca cell of the inner membrane with lipid droplets

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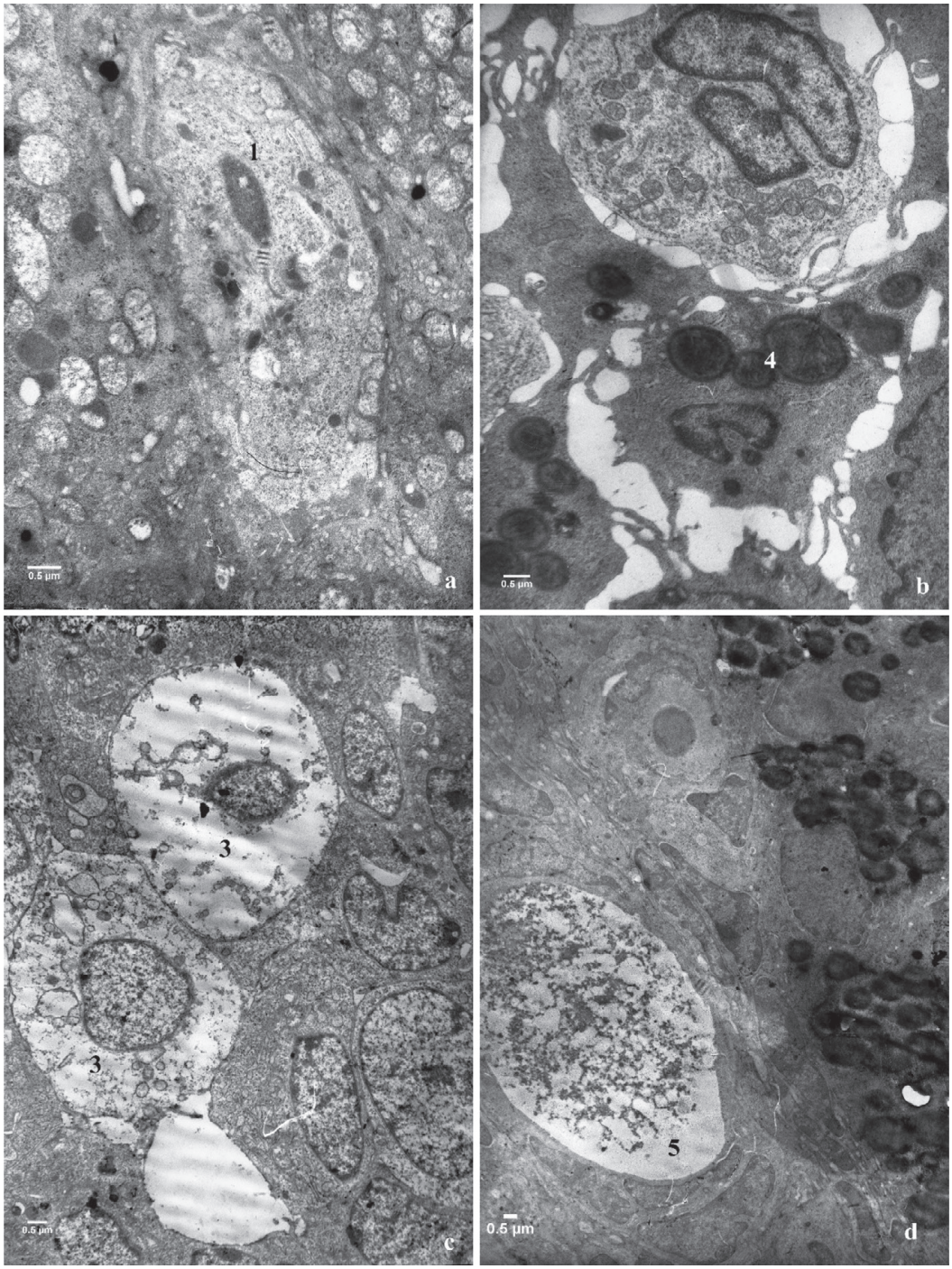

Fig. 3. Ultrastructural reorganization of the ovarian cortex in rats with SIDM + CIS (a, b), SIDM (c), CIS (d). Electronograms. Magnification: a, b) × 8000, c) × 4800, d) × 3200. Designations: 1 – oocyte apoptosis; 2 – oocyte necrosis; 3 – partial necrosis of granulosa cells; 4 – lipid granules in granulosa cells; 5 – partial oocyte necrosis.

Rats with SIDM against a background of microangiopathy also show destructive changes in the ovaries. First of all, this is vacuolar dystrophy of egg cells and granular cells of the granulosa layer of the follicle. Granulosa cells undergo pronounced destructive changes such as vacuolar dystrophy, partial and colliquative necrosis (Fig. 3c). However, we were unable to detect the accumulation of lipid droplets in their cytoplasm, as in rats with comorbid pathology. It should be noted that a small proportion of granulosa cells underwent changes of the type of apoptosis. Vacuolar dystrophy and colliquative necrosis were observed in luteocytes and theca cells.

In rats with CIS, the most pronounced changes occurred in primordial follicles due to the death of oocytes by apoptosis and necrotic apoptosis (Fig. 3d). In granular follicles, enlargement of the endoplasmic reticulum cisternae, focal lysis of mitochondrial cristae, and accumulation of lipid droplets were observed (Fig. 3d).

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As is well known, DM is one of the main causes of reproductive system dysfunction and infertility in both women and men. It can contribute to ovarian ageing, irregular or absent menstruation, and early onset of menopause [12]. Studies also show that there is a link between PCOS and an increased risk of developing diabetes, which is a consequence of insulin resistance [13, 14]. According to our research, SIDM increased the number of cystic and atretic follicles against the background of follicular development abnormalities, indicating the development of PCOS [15, 16].

At the same time, the pool of primordial and primary follicles decreased significantly, indicating depletion of the ovarian reserve and a possible decrease in fertility in peripubertal rats. Conversely, the number of preantral follicles and degenerative *corpus luteum* increased in animals with DM, again indicating a decrease in ovulation. The degeneration of ovarian follicles and disruption of their development in diabetes is consistent with the results of other studies [17]. These changes may be associated with hormonal dysregulation [18–20]. In animals with diabetes, the concentration of sex hormones was significantly lower. As is known, follicle-stimulating hormone (FSH) and LH are necessary for follicle development, oocyte maturation, and normal ovulation. Decreased levels of LH, FSH, and estradiol in animals with diabetes may be partially related to hypothalamic dysfunction caused by hyperglycemia [13, 21]. Recently, J. M. Castellano et al. demonstrated that mice with insulin deficiency and SIDM exhibit hypogonadism due to reduced expression of kisspeptin in the hypothalamus, and that administration of kisspeptin restores gonadotropin and steroid levels in these animals [22].

Insulin is the most important regulator of the hypothalamic-pituitary-gonadal axis. It has been reported that insulin controls secretion by acting directly on gonadotrophin-releasing hormone neurons [3]. In patients with type 1 DM, delayed puberty in adolescents and a higher prevalence of hirsutism and PCOS symptoms are observed [7, 15, 23]. It has been hypothesised that even the administration of exogenous insulin therapy in type 1 DM, which can lead to elevated systemic insulin levels, may contribute to ovarian hyperandrogenism by stimulating direct or indirect release of androgens by theca cells. Studies have shown that insulin acts as a co-gonadotropin, enhancing steroidogenic responses to gonadotropins and promoting ovarian cyst formation in experimental models [24]. According to our research at the ultrastructural level, an increase in lipid droplets in the granular cells of the follicles was observed, which may indicate a change in their reception and the influence of the hypothalamic-pituitary axis on them, and as a result, a disruption in the process of folliculogenesis. Moreover, our hypotheses are confirmed by data from other researchers who have proven that insulin can act almost like co-gonadotropin by binding to cell receptors on the surface of the theca, granulosa and stroma of the ovaries [25]. Hyperglycemia can negatively affect ovarian function due to advanced glycation end products (AGEs) and the formation of reactive oxygen species. Increased oxidative stress and AGE accumulation cause overexpression of the receptor of AGE (RAGE). These receptors have been identified in granulosa and theca cells of healthy women. Activation of RAGE leads to the activation of multiple signaling pathways, resulting in the expression of adhesion molecules, Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB), and many inflammatory cytokines. Activation of the NF-kB pathway also stimulates the release of transforming growth factor beta, which subsequently triggers tissue fibrosis, contributing to the development of pathological processes in the ovary such as stromal and follicular degeneration and stromal fibrosis, as well as significant follicle loss [4, 23, 26].

Several endocrine changes are also associated with menstrual cycle disorders in women with type 1 DM. Menstrual cycle disorders were associated with functional ovarian hyperandrogenism, decreased levels of sex hormone-binding globulin and insulin-like growth factor, increased LH/FSH ratio, and high prevalence of polycystic ovaries [7].

We would like to draw attention to the fact that during the experiment, no statistically significant difference between the number of *corpus luteum* in the study groups and the Control Group was found, which can obviously be explained by the fact that in prepubertal rats, the processes of ovulation and *corpus luteum* maturation are just beginning to form, on the one hand, on the other hand, there is a delay in sexual maturation due to a decrease in the number of Graafian follicles and destructive changes in secondary follicles, which turn into atretic and cystic follicles. Unfortunately, we couldn't find data in the literature that would refute or confirm our opinion concerning the rats of this age. However, in sexually mature rats, SIDM leads to a 4.8-fold decrease in the number of *corpus luteum* [21]. In contrast, in girls, the functioning of the *corpus luteum* plays a special role in the pathogenesis of DM. Female adolescents with type 1 DM have elevated levels of high-sensitivity assays C-reactive protein, which increase even more during the luteal phase, especially in overweight patients. Type 1 DM reduces the natural increase in insulin growth factor 1 (IGF-1) levels observed in the luteal phase in the Control Group. The association between elevated C-reactive protein and reduced IGF-1 during the luteal phase may be a mechanism leading to metabolic complications in women with type 1 DM. Excess weight may exacerbate these disorders and be an important modifiable factor in the prevention of cardiovascular disease in women with type 1 DM [27].

CONCLUSIONS

According to our research, SIDM and stress lead to the appearance of cystic follicles and an increase in the number of atretic follicles against the background of follicle morphogenesis abnormalities, which indicates the development of PCOS. Under these conditions, the pool of primordial and primary follicles decreased significantly, indicating depletion of the ovarian reserve and a possible decrease in fertility in peripubertal rats. Moreover, the greatest decrease in the reserve of primordial, primary and secondary follicles was observed in rats with comorbid pathology, indicating that stress significantly worsens the course of DM and reduces reproductive potential at pubertal age.

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