Morphological Changes in Ovaries in Rats with Experimental Polycystic Ovary

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Abstract

Polycystic ovary syndrome is one of the important causes of infertility, metabolic syndrome. Complications of PCOS are a serious health hazard. The relevance of the study of morphological changes in the ovaries in rats is justified by the need to improve the treatment of PCOS. The experiment consisted in the fact that rats from the experimental group received testosterone for 7 days and were in constant artificial daylight for 60 days. As a result of histological examination of the ovaries in this group, multiple cysts, foci of fibrosis were found. Vaginal smears in rats from the experimental and control groups were collected and compared. The extracted ovaries of rats of the experimental and control groups were compared, the differences in colour according to Van Gieson X 100, haematoxylin-eosin X 40, haematoxylin-eosin X 100, B-G X 100 were analysed. Studies indicated oestrous cycle disturbance, hormonal changes in rats with experimental polycystic. The work can serve as material for further studies aimed at identifying fast and easily accessible methods for the diagnosis of metabolic syndrome predictors.

Key words: *morphology, endocrine infertility, experimental and control groups, fibrosis, cyst.*

Introduction

Infertility remains one of the most pressing problems of modern medicine. A significant contribution to the issue of infertility is made by polycystic ovary syndrome (PCOS). It is believed that it causes more than half of all cases of endocrine infertility (56.2%).[1,2] The problem is that PCOS with its manifestations is only the tip of the iceberg, and the metabolic complications constitute the most serious threat, the most noticeable of which are metabolic syndrome, obesity, and insulin resistance.[3,4] Up to 47% of women with polycystic ovary syndrome have metabolic syndrome.[5,6] The discovery of the relationship of chronic anovulation within the framework of both metabolic syndrome (MS), PCOS and IR (insulin resistance) was an important step in the therapeutic effect on this condition.[7]

Systemic inflammation, insulin resistance, and hyperinsulinemia are constant components of the pathogenesis of PCOS in obese women and most women without obesity, due to the presence of a multitude of factors that initiate systemic inflammation and IR.[8] Women with PCOS, both obese and without obesity, have elevated levels of monocytes and lymphocytes in the blood, the activity of nuclear transcription factors kappa B (NFkB), which control the expression of pro-inflammatory cytokines and markers of inflammation: IL-6, TNF-a, C reactive protein.[9]

Although there are many clinical and experimental studies, the aetiology and pathogenesis of chronic anovulation remain unclear.[10] Many factors are involved in the pathogenesis of PCOS: genetic, endocrine, and environmental factors. Ideas about it changed with the accumulation of knowledge about the disease development mechanisms.[11] Congenital genetic condition of PCOS contributes 79% to the total risk of the syndrome, the remaining 21% are epigenetic factors (environmental and lifestyle influences). The familial nature of the disease speaks in favour of the leading role of heredity; in girls with vague symptoms, but with a burdened family history, PCOS should be a priori assumed. The principles of inheritance of the syndrome have not yet been fully studied, however, most likely,

the syndrome has a polygenic nature.[12]

Epi- and ontogenetic factors ("unwanted" genes are activated by the influence of the external environment, for example, folate deficiency), as previously mentioned, account for one fifth of all cases of the disease. Sometimes this happens even in utero (due to adverse environmental factors, micronutrient deficiency). Girls born to mothers with PCOS have more follicles (in diameter) from birth than their peers, and a detailed examination during the pre-pubertal period reveals moderate metabolic disturbances. The syndrome manifests during puberty, when the synthesis of androgens in the body increases.[13] The aim of our work was to study the morphological changes in female rats with experimental polycystic.

Materials and Method

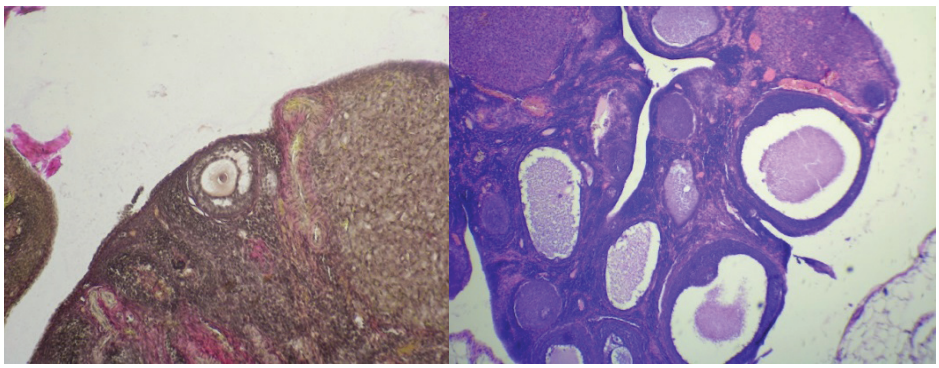
For the experiment, sexually mature female Wistar rats were taken. Experimental animals were divided into the following groups: control (n = 20) – group I, experimental (n = 25) – group II with experimental polycystic. Rats from the experimental group received testosterone intramuscularly (400 mg) for 7 days and were kept for 60 days under constant artificial daylight. Vaginal swabs were taken daily for 30 days to study the oestrous cycle. Stained using Giemsa method. Rectal temperature was measured daily.

The cytological evaluation of rat vaginal smears was performed according to the classification of Geist, Salmon and 2 indices were determined: Maturation Index (MI) and the kariopycnotic index (KPI). MI is the percentage of three types of epithelial cells – basal/parabasal, intermediate and superficial. KPI is the percentage of surface cells with a pycnotic nucleus to all other cells. An increase in the number of surface cells with a pycnotic nucleus correlates with increased oestrogen stimulation. Blood was also taken at the end of the experiment (on day 60) to determine the lipid spectrum and the level of hormones.[14,15] Testosterone, oestradiol, FSH and LH were determined, including the lipid spectrum (atherogenic index, low density lipoproteins, high density lipoproteins, triglycerides and total cholesterol) in the Express Plus laboratory in Bishkek.[16,17]

Animals were taken out of the experiment with an overdose of diethyl ether and ovaries were extracted for further histological examination. Morphological research was conducted on the basis of Republican Anatomic-Pathological Bureau. The material was fixed in a solution of 10% formalin, embedded in paraffin, histological sections were made and stained with haematoxylin and eosin, also according to Van Gieson. Statistical processing of the obtained data was performed using the Statist software package.

Results and Discussion

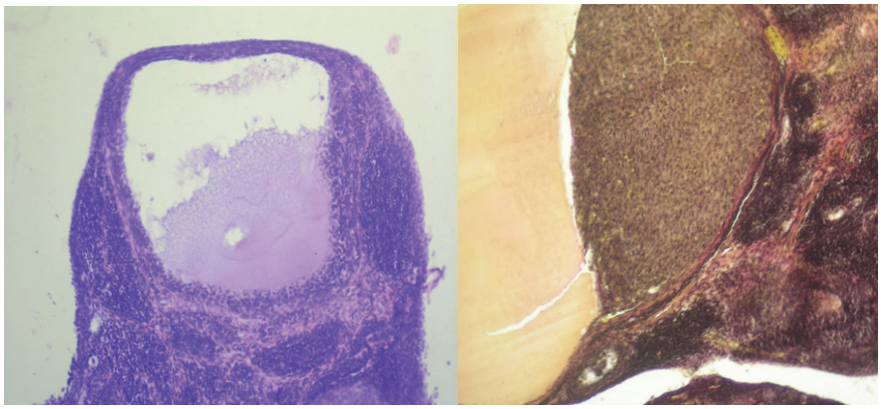
In the control group, the results of histological examination of the ovaries display the normal structures of the ovaries with a maturing egg, the absence of zones of haemorrhage, fibrosis and the formation of cysts (Figure 1A). In the group with experimental polycystic disease, multiple cysts and foci of fibrosis are found (Figure 1B).



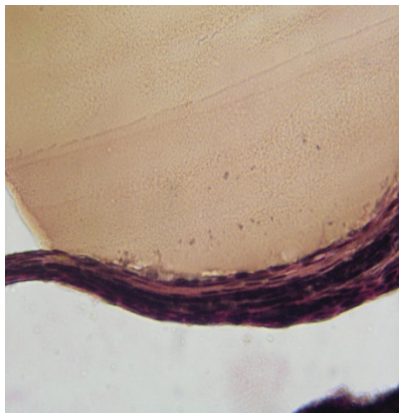
A B

Figure 1. A – rat ovary from the control group, Van Gieson stain X 100; B – rat ovary from the experimental group, haematoxylin-eosin stain X 40

Figure 2B and 2C display a dense protein coating, which is characteristic of PCOS when stained according to Van Gieson. This indicates the absence of ovulation and the formation of multiple cysts in both ovaries.



A **B**



C

Figure 2. A – rat ovary from the experimental group, haematoxylin-eosin X 100 staining, first ovary; B – the ovary of a rat from the experimental group, the colouring V-G X 100; first ovary; C – rat ovary from the experimental group, colour V-G X 100, second ovary

Table 1 reflects changes in hormone levels. In the group with experimental polycystic disease, there is an increase in the level of testosterone, oestradiol and LH (luteinizing hormone) compared with the control, a decrease in the level of FSH (follicle-stimulating hormone).

Table 1. Hormone level changes

	Testosterone (nmol/l)	**Oestradiol (pg/ml)**	**FSH (IU/L)**	**LH (IU/L)**
Control group n=25	1.66±0,04	639.59±22.97	0.2±0.01	0.1±0.03
Experimental group n=25	23±0.61*	1228.7±38.74*	0.1±0.02	0.4±0.02*

Note: *- P<0.05 significantly with respect to the control group

There are many squamous cells in the vaginal smear of rats from the control group, which indicates the presence of ovulation and good oestrogen saturation (Figure 3A). Parabasal cells and white blood cells appear in the stage of meta-oestrus, that is, the epithelium is renewed and their maturation begins (Figure 3B). A large number of leukocytes was found in the di-oestrus phase and epithelial cells in the pro-oestrus phase increase in size, the cytoplasm becomes more transparent, and the nucleus begins to decrease under the influence of oestrogens (Figure 3C and 3D).

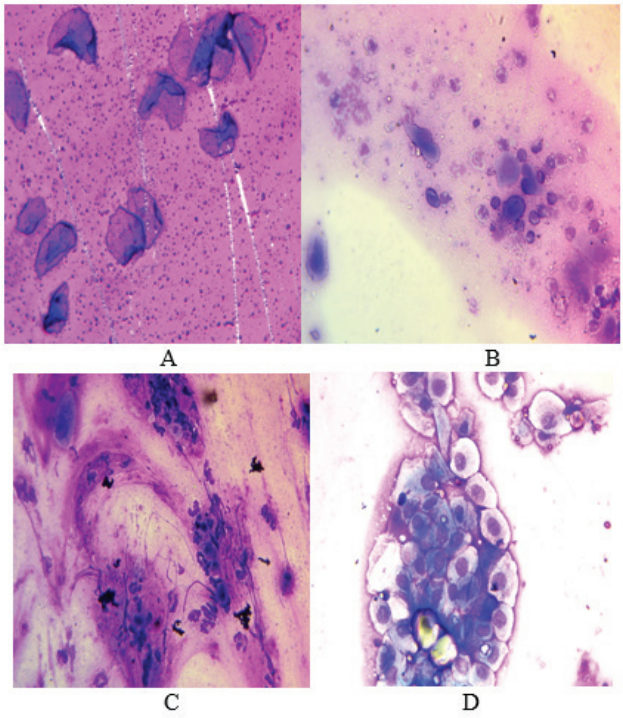


Figure 3. A – vaginal smear in the oestrus phase in rats in the control group; B – a vaginal smear in the meta-oestrus phase in rats in the control group; C – vaginal smear in the di-oestrus phase in rats in the control group; D – vaginal smear in pro-oestrus phase in rats in the control group

Figures 4A, 4B, 4C show meta-oestrus, di-oestrus, and pro-oestrus stages in rats with experimental polycystic disease wherein a large number of leukocytes, parabasal cells and only a small number of epithelial cells were detected. Cell maturation does not occur, which indicates reduced oestrogen stimulation and anovulation. Impaired epithelial cell maturation due to excess testosterone is a major feature of polycystic ovary. In vaginal smears in rats from the experimental group, no pycnotic nuclei and squamous cells were found.

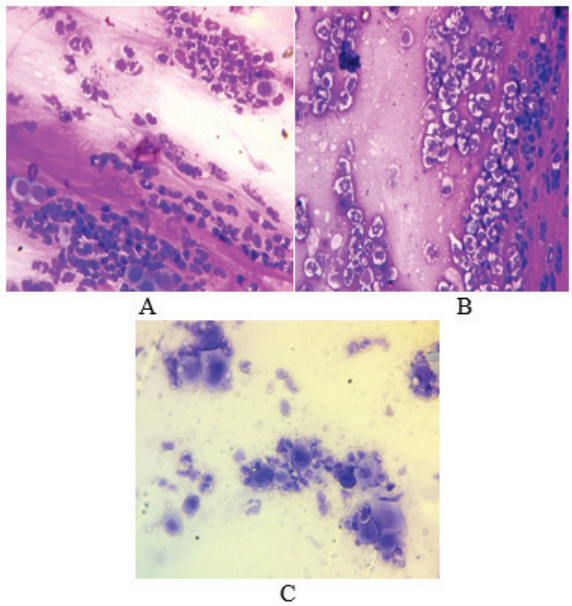


Figure 4. A – vaginal smear in the meta-oestrus phase in the rats in the experimental group; B – vaginal smear in di-oestrus phase in rats in the experimental group; C – vaginal smear in the pro-oestrus phase in rats in the experimental group

(Table 2).

Table 2. Lipid level changes

	Atherogenic index	LDL (mmol/l)	HDL (mmol/l)	Total cholesterol (mmol/l)	Triglycerides (mmol/l)
Control group =25	1.52±0.5	0.17±0s,08	1.43±0.89	1.04±0.13	0.52±0.15
Experimental group n=25	2.36±0.83	0.48±0.18*	0.40±0.9	1.68±0.14*	0.63±0.19

Note: * – P <0.05 significantly with respect to the control group

Conclusions

Despite intensive studies in the field of PCOS, the mechanisms underlying the formation of this pathology are still understudied and debatable, which indicates a multifactorial aetiology and the lack of a single opinion on this pathology, which is very difficult for diagnosis and treatment. In women with menstrual irregularities, metabolic disorders and an increase in low-density lipoproteins (LDL) are significantly more common. These lipid abnormalities, namely an increase in LDL, suggest that women with PCOS have a risk of developing cardiovascular disorders of up to 70%. Furthermore, PCOS is associated with increased sympathicotonia, which is associated with cardiovascular complications. Scientists at experimental PCOS have demonstrated that low-frequency electric acupuncture has a significant effect in rats with PCOS – it reduces heart rate and sympathetic activity.

However, according to other authors, obesity, insulin resistance, impaired glucose tolerance and dyslipidaemia are not pathognomonic signs of PCOS. Overweight is of primary importance in the formation of metabolic disorders, and against the background of endocrine changes characteristic of PCOS, disturbances in carbohydrate and lipid metabolism are aggravated. Violation of lipid and carbohydrate metabolism plays an important part in the pathogenesis of PCOS. A carbohydrate diet can activate lipogenesis in the liver and activate the enzymes involved in lipogenesis, while starvation works the opposite. Our studies indicated a violation of the oestrous cycle in animals with experimental polycystic disease, as evidenced by a change in vaginal epithelial cells.

Our studies demonstrated hormonal changes upon violation of the oestrous cycle in animals with experimental polycystosis, which is confirmed by the morphological change in the ovaries. Further studies aimed at identifying fast and readily available diagnostic methods for the predictors of metabolic syndrome underlying PCOS are necessary.

Acknowledgment: None.

Conflict of Interest: There is no conflict of interests.

Ethical Clearance: All procedures were performed in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. A study was approved by Animal Research Ethics Committee of the St. Petersburg State University, October 25, 2019, No 1947-IL.

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