The effect of intermittent cold effects on the morphometric parameters of the endometrium and rat ovaries in experimental modelling of polycystic ovary syndrome

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Abstract. The morphometric characteristics of the endometrium and ovaries of rats were studied on the background of intermittent cold exposures (ICE) in experimental modeling of polycystic ovary syndrome (PCOS). ICE were performed by daily keeping animals for 4 hours in a chamber in which the light regime and temperature were kept at + 4 ° C. The simulation of PCOS was performed by administering dehydroepiandrosterone (DHEA) to young rats for 25 days. Stimulation of adaptive physiological reactions against the background of ICE blocks the development of signs of PCOS in rats, as evidenced by the morphological parameters of the endometrium and ovaries of rats.

Keywords: *morphometric* parameters, *rat endometrium, polycystic ovary syndrome, cold exposure.*

Introduction. Polycystic ovary syndrome (PCOS) is characterized by a violation of reproductive & menstrual function and is due to a number of factors, the main of which are increased production of androgens, insulin resistance and disruption of the regulation of reproduction hormones [1]. Insulin resistance and violation of the regulation of reproduction hormones underscore the important role of the processes occurring in adipose tissue in the pathogenesis of this disease [2].

Adiponectin, leptin and resistin are important adipokines, associated with energy metabolism [3]. In recent studies, a direct relationship was established between the level of adiponectin and PCOS [4].

It is known that the level of adiponectin increases in conditions of prolonged cold influences or acclimatization [5].

The aim of this work was to study the morphometric parameters of the endometrium and ovary of rats against the background of intermittent cold exposure (ICE) and experimental PCOS modeling by introducing dehydroepiandrosterone (DHEA).

Object and methods of research. Studies were performed on female Wistar rats (n = 32) of 27-day-old age, weighing 30-40 g.

The polycystic process in the ovaries was modeled by daily (for 25 days) subcutaneous administration of DHEA ("Sigma", USA) dissolved in 0,2 ml of purified and sterilized olive oil. The dose of DHEA was 60 mg / kg of body weight.

ICE was carried out by daily keeping the animals for 4 hours in a chamber in which the light regime and the temperature of + 4 ° C were maintained. The remaining 20 h animals were in normal conditions of detention.

Animals were divided into 4 groups: 1 group - animals that were exposed to CCE (n = 8); Group 2 - animals treated with DHEA (n = 8) with ICE; Group 3 - animals who were challenged with experimental PCOS by the administration of DHEA (n = 8); 4 group - intact control (n = 8).

On the 26th day the animals were sacrificed, the uterus and ovaries were taken. The organs were weighed and then fixed in 4% paraformaldehyde (PFA, "Sigma") for 4 hours, after which it was transferred for 12 hours to a 25% sucrose solution on phosphate buffered saline. The organs in the mounting medium of Tissue-Tek ("Sakura", Japan) were frozen and before the preparation of the cryostat sections were stored in liquid nitrogen.

For the preparation of cryostat sections, the organs were removed from the low-temperature storage and fabric sections were made with a thickness of 5 μm on the cryomicrotome MEV (Germany). Sections were stained with hematoxylin and eosin according to a standard procedure.

The microphotography was performed by a light optical microscope with a digital camera Amscope IN300T (China). Morphometric analysis of photos of serial sections stained with hematoxylin and eosin was performed using AxioVision Rel 4.7 image processing software.

Morphometric analysis of the ovaries included counting the number of cysts, yellow bodies and measuring the layer of the tectal cells of the tertiary follicles. Counts were performed on 15 sections of ovarian tissue obtained from each of the experimental animals. Morphometric analysis of uterine tissue included measuring the height of the epithelium, the number of glands per unit area, the thickness of the wall. These masses of ovaries and uterus are represented as a percentage of body weight.

The statistical processing of the results was carried out with the help of the programs "Excel" and "Statictica 10". The data were checked for normal distribution using the Kolmogorov and Smirnov test, single-factor analysis of variance was used to compare two samples, differences at $p < 0,05$ were considered reliable. The quantitative data represented in the form of the mean value ± standard deviation.

Results and discussion. Figure 1 presents data on the relative mass of ovaries and uterus of experimental animals in four groups. It is noteworthy that neither ICE, nor DHEA not influenced on the average relative weight of the ovaries, but the parameters of the uterine mass differed between groups. The relative mass of the uterus significantly increased in the groups with DHEA (groups 2 and 3) in comparison with the intact control.

The same trend towards an increase in uterine weight in rats with an experimental PCOS model was observed by Zhang Y. et al. [6].

It is known that one of the characteristic signs of PCOS is hyperplasia of the ovarian cells [7].

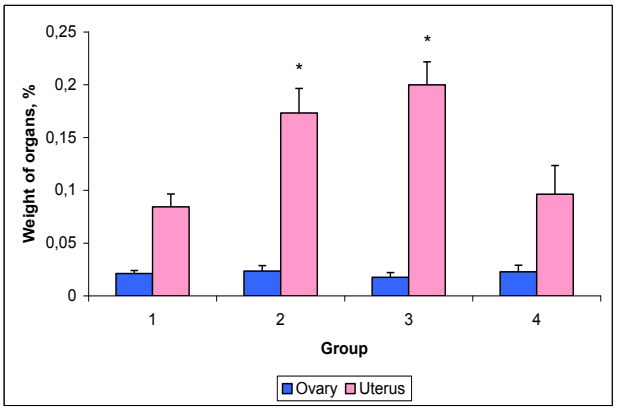


Fig. 1. Relative mass of ovaries and uterus of rats of different experimental groups. * - the index is significantly different from the intact control, P <0,05.

When measuring the thickness of the layer of the cells in the ovaries of rats (Figure 2), it was found that this index tends to increase in groups with ICT (groups 1 and 2) and significantly increases in the 3rd group (introduction of DHEA) compared with intact control. Thus, the results indicate that the exogenous introduction of androgens into the body of experimental animals leads to hyperplasia of the thekal cells as a characteristic feature of PCOS.

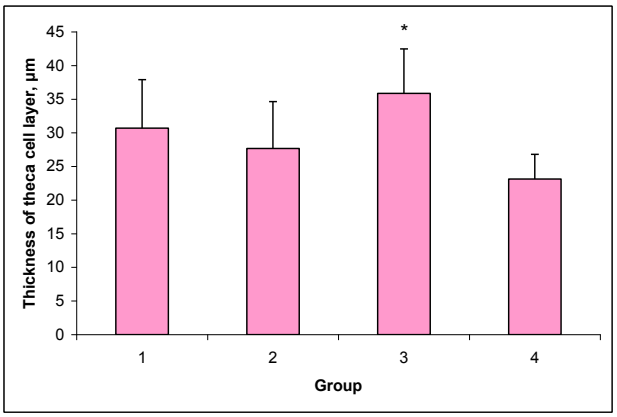


Fig. 2. The thickness of the layer of the thekal ovarian cells of different experimental groups. * - the index is significantly different from the intact control, P <0.05.

However, the fact of a certain expansion of the layer of the thekal cells in the 1 st group (without the administration of DHEA, but with ICE) is of interest. The data obtained by us suggest that under the conditions of cold stress, the secretion of endogenous androgens by the adrenals is activated, which leads to a slight hyperplasia of the thekal ovarian tissue cells [8].

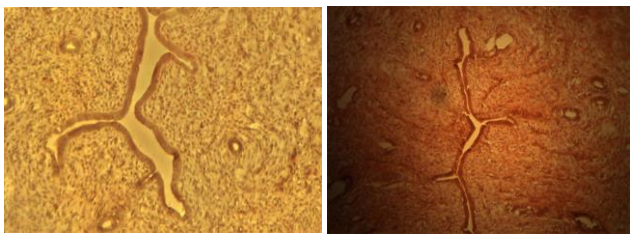
It is known, that ovarian dysfunction in PCOS is characterized by a decrease in the number of mature follicles and, accordingly, yellow bodies. In our experiments it was found that the number of yellow bodies in the group with the PCOS model was significantly less than in the control (0,1 ± 0.05 in group 3 against 0,2 ± 0.01 in the control, p <0.05). In addition, it was the only group of animals, which in the ovaries the formation of cysts was observed. Despite the fact that in groups with ICE (groups 1 and 2) there was a tendency to increase the thickness of the layer of the thekal cells, we were not found in the ovaries of the cysts, and the number of yellow bodies did not differ from the intact control.

Endometrium is a mucous membrane lining the uterine cavity, which reacts to cyclic changes in estrogen and progesterone in the menstrual cycle of the ovaries. The follicular (proliferative) phase of the endometrium is associated with the growth of the follicle in the ovary and the increased secretion of estrogens. In PCOS, in the absence of ovulation and regulation through progesterone secreted by the yellow body, the endometrium is constantly exposed to the mitogenic effects of estrogens, which leads to its proliferation. Previous studies have shown an increase in the thickness of the endometrium in women with PCOS, or in experimental models of PCOS in rats [9].

Microscopic examination of uterine tissue in a group of rats with a PCOS model (Group 3) showed an increase in the height of the endometrial epithelium, the number of glands and the thickening of the uterine wall (Table 1). In the group with PCOS simulation against the background of ICE, as in the group with ICE, these indicators were at the level of intact control. Visually, the histological pattern in the groups of rats with PCOS differed from the other groups (Figure 3) and was characterized by an increase in the number of glands and an expansion of their lumens.

Table 1.

Group	Epithelial cell height, µm	Uterine wall thickness, µm	Lumens of glands, number/field
1	5,6±0,2	496,2±39,4	5,4±1,4
2	5,8±0,3	481,8±36,3	5,3±0,9
3	6,1±0,4	675,4±40,6*	27,8±5,9*
4	5,7±0,1	498,7±23,5	5,0±1,3



A B

Fig. 3. Histological samples of the uterus of rats of the group with the introduction of DHEA (A) and the group with the introduction of DHEA on the background of ICE (B). Color: hematoxylin and eosin x 20.

Conclusions. The introduction of DHEA to young rats for 25 days leads to the appearance in the ovaries and uterus the characteristics of PCOS: thickening of the layer of the cells, a decrease in the number of yellow bodies, the appearance of cysts, thickening of the uterine wall and endometrial hyperplasia. Stimulation of adaptive physiological reactions against the background of intermittent cold effects blocks the development of signs of PCOS in rats with the administration of DHEA.

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