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INFLUENCE OF HORMONAL STIMULATION AND INTERMITTENT COLD EXPOSURE ON THE MECHANISMS OF FORMATION OF POLYCYSTIC OVARIAN SYNDROME

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Introduction. *Polycystic ovary syndrome (PCOS) is one of the most pressing problems in gynecological endocrinology.*

The aim *of the study was to elucidate the morphological changes in the ovaries of rats under various types of hormonal exposure in experimental modeling of PCOS.*

Materials and methods*. To study three working experimental models were developed, associated with the introduction of various hormonal drugs into the body of an animal.*

Model I-st consisted of subcutaneous administration of an estrogen-containing drug at a dose of 3 mg for 25 days. Model II-d involved the development of PCOS by daily subcutaneous administration of 3 mg of dehydroandrostenediol sulfate. The III model - the formation of PCOS in 5 immature rats using dopamine phytoanalogues at a dose of 3 mg subcutaneously for 25 days. The animals were divided into 3 groups: group 1 - rats that were induced with experimental PCOS and kept in a vivarium at a temperature of +23°C (n=8), group 2 - animals that, against the background of experimental PCOS, were exposed to cold exposure (CE) for 4 hours in a chamber with a constant light regime and a temperature of + 4 °C for 25 days (n = 8); group 3 - intact control (n = 8) - healthy rats without PCOS, which were kept in a vivarium at a temperature of + 23°C.

Results*. Histological examination of the ovaries of group 1 rats revealed morphological changes in the ovaries characteristic of PCOS. The morphological structure of the ovaries corresponded to type II PCOS. In rats of group 3, macroscopically, the ovaries were slightly reduced in size compared to the control group. The levels of adiponectin and leptin as markers of the functional ovarian tissue of PCOS model experiments.When studying the effect of CE on the body of experimental animals, their effect on the formation of tissue cell hyperplasia as a characteristic sign of PCOS was determined.*

Conclusions*. Studies have shown that the formation of PCOS, as well as structural changes in the ovaries, significantly depend on hormonal regulation disorders.*

Keywords: Polycystic ovary syndrome, hormonal exposure, experimental modeling

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most pressing problems in gynecological endocrinology. Often, ovarian pathology develops during puberty and is accompanied by functional disorders, sometimes turning into severe morphological changes. In this regard, the clarification of the etiopathogenesis of the development of this pathology and the formation of various types of PCOS remains a topical issue [1].

Ovulatory dysfunction in PCOS is due to an increase in the number of follicles that occurs in the early stages of their development, as well as a violation of the maturation of the dominant follicle. The mechanisms underlying the formation of PCOS are not well understood and debatable, which indicates a multifactorial etiology and the lack of a unified view of this pathology, which is difficult to diagnose and treat. According to modern concepts, the primary cause of the formation of PCOS is hyperandrogenism (HA),

which prevents normal folliculogenesis and promotes cystic atresia of the follicles. Lack of growth and maturation of follicles further inhibits follicle-stimulating hormone (FSH) secretion [2].

Excess androgens in the peripheral tissue undergo conversion to estrone, an increase in the level of which leads to hypersensitization of gonadotrophs to GnRH and stimulation of GnRH release. In addition, PCOS is characterized by an increase in the level of total and free testosterone (T) with a normal content of dehydroepiandrosterone sulfate (DHEAS) and 17-hydroprogesterone (17-DHP), as well as a decrease in blood sex steroids [3].

The first clinical symptoms of hyperandrogenism (HA) are observed at puberty, however, the increase in androgens is secondary to a state of immaturity of the reproductive system and usually becomes reversible with the onset of regular ovulation. At the same time, the state of stable HA creates the prerequisites for the early formation of PCOS. In this connection, the study of PCOS is extremely important and can be the key to understanding the problem of pathogenesis and stages of development of this pathology [4].

In solving the problem of the pathogenesis of PCOS, biological modeling occupies an important place, while the commonality of the physiological processes of regulation of the reproductive system in humans and rats makes it possible to use these animals to study the mechanisms of PCOS development. The creation of a PCOS model is extremely important, since it allows not only to study the mechanisms of development of this pathology in more detail, but also to determine the prevailing significance of various hormonal factors in the formation of various types of PCOS.

The aim of the study was to elucidate the morphological changes in the ovaries of rats under various types of hormonal exposure in experimental modeling of PCOS.

Materials and methods

According to the biosynthesis and properties of gonadotropins and sex hormones, the estrous cycle of the chris can serve as an experimental model of the ovarian cycle of a woman and a basis for studying the mechanisms of the occurrence of impaired ovarian functions of neuroendocrine etiology, accompanied by anovulation [5]. Determining the features of the formation of polycystic ovaries in various variants of biological modeling is of current importance, since it reveals more specific mechanisms of the pathogenesis of anovulatory disorders and allows obtaining a more adequate human pathology.

To study the formation of PCOS, three working experimental models were developed, associated with the introduction of various hormonal drugs into the body of an animal, causing stimulation of cyst formation in the ovaries and having a different effect on the ovarian tissue.

Model I-st consisted of subcutaneous administration of an estrogen-containing drug (17β-estradiol) at a dose of 3 mg for 25 days to 5 immature Vistar rats (Group 1).

Model II-d involved the development of PCOS by daily subcutaneous administration of 3 mg of dehydroandrostenediol sulfate (DHA sulfate) to 5 immature rats (Group 2).

The III model - the formation of PCOS in 5 immature rats using dopamine phytoanalogues at a dose of 3 mg subcutaneously for 25 days (group 3).

The control group consisted of 5 immature rats who did not undergo PCOS simulation. On the 26th day, the animals were sacrificed, the ovaries were fixed in 4% paraformaldehyde, frozen, and stored in liquid nitrogen until the preparation of cryostat sections. Tissue sections were made with a thickness of 5 μm, stained with hematoxylin and eosin according to the standard method. 24 female Wistar rats were involved in the experiment. The animals were divided into 3 groups: group 1 - rats that were induced with experimental PCOS and kept in a vivarium at a temperature of +23°C (n=8), group 2 - animals that, against the background of experimental PCOS, were exposed to cold exposure (CE) for 4 hours in a chamber with a constant light regime and a temperature of + 4 °C for 25 days (n = 8); group 3 - intact control (n = 8) - healthy rats without PCOS, which were kept in a vivarium at a temperature of + 23°C.

It was found that in group 1 rats with experimental PCOS, after the administration of DHEA, a significant increase in the number of preantral and antral follicles was observed, which confirms the development of PCOS. It was found that the number of corpus luteum in group 1 of rats with PCOS model was significantly less than in the control. In the ovaries of the 2nd group, in which the animals were subjected to CE on the background of experimental PCOS, the number of preantral and antral follicles also increased, but no

cysts were observed in the ovaries. This may indicate a protective effect of CE on the regulation of the process of maturation of follicles. When measuring the thickness of the theca cell layer in the ovaries of rats, it was found that this indicator tends to increase in the 1st group compared to the 2nd, in which the rats were subjected to CE.

Thus, the results indicate that the introduction of androgens into the body of experimental animals leads to tissue cell hyperplasia as a characteristic feature of PCOS. The thickness of the thecal cell layer after the application of CE (group 2 of observations) practically does not differ from the intact control group, which confirms the good clinical effect of CE in preventing the development of PCOS. Our data suggest that under conditions of cold exposure, the secretion of endogenous adrenal androgens is activated, which leads to slight hyperplasia of ovarian theca cells.

Results

In the control group, in 100% of rats, the ovaries were not enlarged; on the section, most of the ovary was occupied by fully formed corpus luteum and follicles of various stages of maturity. Histological examination of the ovaries of group 1 rats revealed morphological changes in the ovaries characteristic of PCOS. The ovaries were hypertrophied, tuberous, on the section, most of the ovary is occupied by multiple mature, diffusely located follicles, with atresia phenomena, while a pronounced proliferation and hyperplasia of thecalutein cells of the stroma are revealed. Yellow bodies were not found. The morphological structure of the ovaries corresponded to type 1 PCOS. In animals of the 2nd group, on macroscopic examination, the ovaries were enlarged 4-5 times compared to the control, stromal hyperplasia was noted, the presence of many cystic-atretic, peripherally located follicles, 5-8 mm in diameter, located under the compacted capsule. The morphological structure of the ovaries corresponded to type II PCOS.

In rats of group 3, macroscopically, the ovaries were slightly reduced in size compared to the control group, they were elongated and contained multiple punctate cavities in the cortical layer. There are a large number of follicles at various stages of maturity with atresia phenomena and the formation of layers of collagen fibers. Yellow bodies were not found. Thus, the dependence of the formation of certain morphological variants of polycystic ovaries was established when different types of sex steroids were used in modeling the pathological process. Experimental models of polycystic ovaries indicate the important role of hormonal disorders in the formation of this pathology.

The discussion of the results

In the development of various variants of PCOS, biological modeling is of great importance, while the commonality of the physiological processes of regulation of the reproductive system in humans and rats makes it possible to use these animals to study the mechanisms of the development of pathological processes [6]. The creation of a PCOS model allows not only to study the mechanisms of development of this pathology, but also to determine the formation of various hormonal types of PCOS. With the development of experimental PCOS, a combination of hyperandrogenism and insulin resistance is observed, which leads to chronic compensatory hyperinsulinemia and the development of obesity. Obesity leads to serious complications and further disruption of their menstrual and reproductive functions. Against the background of steroidogenesis in the granulosa and theca-cell elements of PCOS, the processes of anovulation and the activity of the hypothalamus-pituitary-ovaries system take place [7].

In the study of the pathogenesis of PCOS development, a direct relationship with the disease was established at the level of adiponectin, leptin and resistin in the body, which are actively involved in the energy metabolism of ovarian tissue [8].

Leptin is a hormone of peptide nature, which is synthesized by adipocytes and affects the satiety center, reduces the body's need for food. Leptin regulates fatty acid homeostasis and thereby protects tissues from ectopic lipid accumulation (lipotoxicosis). Due to leptin resistance and hyperleptinemia, disturbances in the processes of steroidogenesis in the granulosa and theca cells of the ovaries, anovulation, and a decrease in the activity of the hypothalamus-pituitary-ovaries system occur. Low plasma levels of another

adipokine, adiponectin, precede the onset of insulin resistance [9].

Adiponectin regulates hormone production and gene expression in somatotrophs and gonadotrophs of the pituitary gland, inhibiting the secretion of luteinizing hormone, but does not affect the concentration of follicle-stimulating hormone. Currently, a direct relationship has been established between the level of adiponectin and PCOS, which indicates the important role of processes occurring in adipose tissue and important in the pathogenesis of this disease. It is known that the level of adiponectin increases under conditions of prolonged cold exposure or acclimatization [10].

A low level of the second adipokine - adiponectin in blood plasma is a preliminary occurrence of insulin resistance. Adiponectin regulates hormone production and gene expression in somatotrophs and gonadotrophs of the pituitary gland, inhibiting the secretion of luteinizing hormone, but does not affect the concentration of follicle-stimulating hormone [11]. In the vast majority of cases, obesity is combined with insulin resistance, arterial hypertension, and dyslipidemia, which is the so-called metabolic syndrome [12]. Adiponectin increases tissue sensitivity to insulin, a protein synthesized by adipocytes, and is involved in the development of insulin resistance. The development and progression of the metabolic syndrome is based on the production of adipose tissue of biologically active substances, which lead to its manifestations and initiate the processes of burning and thrombogenesis [13].

Insulin resistance and associated hyperinsulinemia is another nutritional feature that can lead to reproductive failure. The mechanisms of influence of these factors on the reproductive function of the study is not enough, however, it is assumed that the leading role also belongs to adipocytes and the violation of their condition [14].

At the level of the ovaries, hyperinsulinemia provides an increase in androgen synthesis: steroidogenesis is stimulated in granulosa and theca cells, the sensitivity of pituitary gonadotroph cells to the hypothalamic gonadotropin-releasing hormone, which actively activates steroidogenesis in the testicles, increases. The result of these processes is hyperandrogenism, which negatively affects the processes of folliculogenesis. On the other hand, peripheral conversion produces androgens into estrogen in adipose tissue, which causes the development of hyperestrogenism, which leads to inhibition of normal secretion of gonadotropins and, as a result, dysregulation of ovulatory ovarian function [15].

The levels of adiponectin and leptin as markers of the functional usefulness of the ovarian tissue were studied in various types of PCOS model experiments. When studying the effect of CE on the body of experimental animals, their effect on the formation of tissue cell hyperplasia as a characteristic sign of PCOS was determined. The thickness of the thecal cell layer after the application of CE did not practically differ from the intact control group, which confirms the good clinical effect of CE in preventing the development of PCOS. Our data suggest that under cold conditions, the secretion of endogenous adrenal androgens is activated, which leads to slight hyperplasia of ovarian theca cells.

Conclusions. Studies have shown that the formation of PCOS, as well as structural changes in the ovaries, significantly depend on hormonal regulation disorders.

Modeling PCOS, which develops from puberty, is extremely important, since it allows us to study the pathogenesis of this complication, as well as to develop modern methods for correcting, preventing the development and rehabilitation of reproductive function. More pronounced changes in the ovarian tissue occur in HA, leading to the development of type II PCOS.

With an increased concentration of estrogen in the ovaries, changes characteristic of PCOS occur. Elevated levels of androgens cause an increase in luteinizing hormone (LH), promotes hyperproduction of androgenic steroids in tissues and leads to the formation of PCOS. In addition, the anovulatory state in rats, against the background of multiple follicular atresia, causes negative proliferative changes in the endometrium.

When studying the effect of intermittent cold exposure on the body of experimental animals, it was found that the formation of tissue cell hyperplasia contributes to the development of PCOS. The thickness of the theca cell layer after the application of intermittent cold exposures practically does not differ from the intact control group, which confirms a good clinical effect in preventing the development of PCOS. Our data suggest that under cold conditions, the secretion of endogenous adrenal androgens is activated, which

leads to a slight hyperplasia of the theca cells of the ovarian tissue.

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ВПЛИВ ГОРМОНАЛЬНОЇ СТИМУЛЯЦІЇ ТА ПЕРЕРИВЧАСТОГО ХОЛОДУ НА МЕХАНІЗМИ ФОРМУВАННЯ СИНДРОМУ ПОЛІКІСТОЗУ ЯЄЧНИКІВ

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Вступ. *Синдром полікістозних яєчників є однією з найактуальніших проблем гінекологічної ендокринології. У зв'язку з цим, вивчення синдрому полікістозних яєчників є надзвичайно важливим і може стати ключем до розуміння проблеми патогенезу та стадій розвитку цієї патології.*

Метою *дослідження було з'ясування морфологічних змін в яєчниках щурів при різних типах гормонального впливу при експериментальному моделюванні синдрому полікістозних яєчників.*

Матеріали та методи. *Для дослідження були розроблені три робочі експериментальні моделі, які пов'язані з введенням в організм тварин різних гормональних препаратів. І-я модель полягала у підшкірному введенні естрогенвмісного препарату в дозі 3 мг протягом 25 днів. Модель ІІ передбачала розвиток синдрома полікістозних яєчників шляхом щоденного підшкірного введення 3 мг дегідроандростендіолу сульфату. ІІІ модель – сприяла формуванню синдрома полікістозних яєчників у 5 статевонезрілих щурів із застосуванням фітоаналогів дофаміну в дозі 3 мг підшкірно протягом 25 днів.*

Тварин розділили на 3 групи: 1 група - щури, які індукували експериментальний СПКЯ при утримуванні у віварії температури +23°С (n=8), група 2 - тварини, які на фоні експериментального синдрома полікістозних яєчників піддавали впливу холоду протягом 4 год у камері з постійним світловим режимом і температурою +4 °С протягом 25 діб (n = 8); 3 група – інтактний контроль (n = 8) – здорові щури без полікістозних яєчників, яких утримували у віварії при температурі +23°С.

Результати. *При гістологічному дослідженні яєчників щурів 1 групи виявлено морфологічні зміни в яєчниках, що були характерні для синдрома полікістозних яєчників. Морфологічна структура яєчників відповідала II типу синдрома полікістозних яєчників. У щурів 3 групи макроскопічно яєчники були дещо зменшені в розмірах порівняно з контрольною групою. Рівні адипонектину та лептину використовувались як маркери функціональної тканини яєчників у модельних експериментів із полікістозних яєчників. При вивченні впливу холоду на організм піддослідних тварин визначено їх вплив на формування гіперплазії тканинних клітин як характерної ознаки полікістозних яєчників Створення моделі синдрома полікістозних яєчників дозволяє не тільки вивчити механізми розвитку даної патології, а й визначити формування різних гормональних типів полікістозних яєчників*

При розвитку експериментального синдрому полікістозних яєчників спостерігається поєднання гіперандрогенії та інсулінорезистентності, що призводить до хронічної компенсаторної гіперінсулінемії та розвитку ожиріння.

Висновки. *Дослідження показали, що формування синдрома полікістозних яєчників, а також структурні зміни в яєчниках істотно залежать від порушень гормональної регуляції. Моделювання полікістозних яєчників, що розвивається з пубертатного періоду, є надзвичайно важливим, оскільки дозволяє вивчити патогенез цього ускладнення, а також розробити сучасні методи корекції, попередження розвитку та реабілітації репродуктивної функції. Більш виражені зміни в тканині яєчника відбуваються при гіперандрогенії, що призводить до розвитку синдрома полікістозних яєчників II типу.*

Ключові слова: синдром полікістозних яєчників, гормональний вплив, експериментальне моделювання

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