

Experimental models of polycystic ovary syndrome

Polikistik over sendromu oluşturmak için kullanılan deneysel modeller

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common endocrine metabolic disorder in women. Although many animal and human studies have been conducted, mechanisms involved in polycystic ovary formation and related metabolic dysfunction have not been fully understood. Different techniques have been tried on animals to induce PCOS. Chronologically, the experimental methods used to induce PCOS included dehydroepiandrosterone application, estradiol valerate injection, fetal androgen administration, antiprogesterone (RU486) application and letrozole use. All of these models were described in this review article.

Key words: PCOS, experimental animal models, rat

ÖZ

Polikistik over sendromu (PKOS), kadınlarda en sık görülen endokrin metabolik bozukluktur. Oluşum mekanizması halen net olarak aydınlanmamış olan PKOS ile alakalı pek çok hayvan çalışmaları mevcuttur. PKOS modeli oluşturmak ve hastalık mekanizmasını anlamak amacıyla, sırasıyla dehidroepiandrosteron sülfat uygulaması, östradiol valerate enjeksiyonu, fetal androgen verilmesi, antiprogesterin (RU486) uygulanması ve letrozol uygulaması denenmiştir. Derlememizde, bu modelleri derlemeye çalıştık.

Anahtar kelimeler: PKOS, deneysel hayvan modelleri, rat

INTRODUCTION

Polycystic ovary syndrome (PCOS), is the most common endocrine metabolic disorder in women of reproductive age with a prevalence of 5-20%^{1,2}. Diagnostic criteria for PCOS have been revised in 2003 and new guidelines were issued by the Rotterdam Workshop Group³. According to these guidelines, two out of three of the following should be met to make a of PCOS:

- Hyperandrogenism, as determined by elevated circulating levels of total or free testosterone or clinical signs of hirsutism.
- Intermittent or absent menstrual cycles.
- Polycystic ovaries (as visualized on ultrasonograms).

Reproductive and endocrine disturbances seen in PCOS include hypersecretion of LH, adrenal hyperandrogenism, ovarian hyper-responsiveness to go-

nadotropin in intrauterine insemination and in vitro fertilization, and endometrial hyperplasia with possible progression to endometrial cancer. Increased miscarriage rates, presumably linked to the genetic dysregulation in oocytes and endometrial cells have been also reported in cases with infertility⁴. Obesity and insulin resistance are frequently seen among PCOS patients with a prevalence of 30% and 60-75%, respectively⁵. Compensatory hyperinsulinemia, impaired glucose tolerance and type 2 diabetes mellitus are common metabolic manifestations, accompanying obesity in PCOS patients^{6,7}. Hyperlipidemia together with impaired glucose tolerance contributes to endothelial dysfunction that leads to atherosclerotic vascular changes and myocardial dysfunction, both of which increase the risk for cardiovascular disease in later years⁸⁻¹⁰.

PCOS has a multi-system presentation. The mecha-

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nisms involved in polycystic ovary formation and related metabolic dysfunction have not been fully understood, although many animal and human studies have been conducted. Different techniques were experimented on animals to induce PCOS. Initially, dehydroepiandrosterone (DHEA) was used to instigate PCOS in animal models^{11,12}. This was followed by estradiol valerate injection¹³, fetal androgen application¹⁴ and letrozole administration. Whether PCOS has an in utero etiology or is due to a hormonal disturbance occurring in adolescence or later, has been extensively studied. All of the attempts mentioned above have led to the appearance of PCOS in animals, as evidenced by the classical polycystic morphology of ovaries and most of the hormonal imbalances characteristic of PCOS.

In this review article we aimed to summarize these experiments and their models related to PCOS. These models are;

Hormonal methods to induce PCOS

a) Dehydroepiandrosterone applications

As early as in the 1960s, DHEA application was the first procedure used to induce polycystic ovaries in animals. It was administered to normally cyclic rats that subsequently developed anovulation and cystic degenerations in their ovaries. Roy et al.¹¹ demonstrated ovulation failure and formation of polycystic ovaries following administration of DHEA. The experimental dosage of DHEA ranged from 1.5 mg/kg to 6 mg/kg body weight via subcutaneous injections. Experiments with all of the different doses (1.5 mg/kg; 3 mg/kg ; 4 mg/kg or 6 mg/kg) have led to the typical ovarian appearance of PCOS. Ten or 20 days of DHEA application was equally effective as assessed by the pattern of cystic and degenerative follicles on histological exams.

Upon administration of DHEA, FSH and prolactin levels were shown to be increased, while LH levels were decreased. These changes were similar with both 3 mg/kg or 6 mg/kg doses. Different duration

of DHEA administration (3, 7, 11 and 15 days) have led to similar outcomes in terms of gonadotropin levels. Increased prolactin concentrations following DHEA administration might be the result of increased estrogen production due to conversion of DHEA to estrogen in the ovaries¹⁵.

b) Estradiol valerate applications

Administration of estradiol valerate (EV) to prepubertal rats was shown to disrupt the ovarian cycle via activation of the sympathetic ovarian nerve and increased intra-ovarian expression of norepinephrine (NE). Rosa-E-Silva et al. administered a single intramuscular injection of 2 mg of EV in 0.2 ml of corn oil into the study group of rats via subcutaneous injections. They tried to investigate the effect of prepubertal EV administration by observing for resultant anovulatory state and/or cystic morphologic changes in the ovaries following administration. In the ovaries, EV induced the secretion of tyrosine kinase, which is the rate-limiting enzyme in NE synthesis¹⁶. The subsequent increase in NE interrupted the ovarian cycle and led to ovulatory dysfunction. After sympathetic denervation of the ovaries, they evaluated the impact of decreased levels of intraovarian norepinephrine on prevention of PCOS. They concluded that prepubertal presence of EV in a rat ovary resulted in early pubertal onset, activation of the sympathetic ovarian nerve, and increased NE synthesis and induced formation of an anovulatory state with cystic ovarian morphology. Inhibition of NE release by sympathetic nerve denervation prevented ovarian transformation typical of PCOS, despite administration of EV¹⁷. Administration of EV suppressed circulating LH, FSH and $\Delta 4$ androstenedione levels and increased ovarian norepinephrine and estradiol concentrations. The action of LH was not as significant as that observed in the clinical presentation of PCOS patients. However, increased levels of intraovarian NE were shown to be responsible for cystic ovarian changes.

c) Fetal androgen application

Early embryonic stage is a critical stage of develop-

ment. Any chemical stimulus or injury at this stage may easily affect the ongoing developmental process and carry a life-long impact. An imbalance in androgen concentration at this early embryonic stage was associated with polycystic ovarian formation¹⁸. Widdowson et al.¹⁹ concluded that excess androgen disturbed reproductive functioning by its action on the hypothalamic-pituitary-gonadal axis. Increased androgen administration to pregnant animals induced characteristic PCOS changes, indicated by ovarian morphology and clinical symptoms^{20,21}. Abbott et al.²² demonstrated that female rhesus monkeys with in utero exposure to androgen levels equivalent to those seen naturally in their male counterparts, expressed clinical signs distinctive of PCOS. Wu et al.²³ confirmed the relationship of in utero androgen introduction and cystic ovarian formation in rats. The two rat groups of this study were given 3 mg testosterone (T) and 3 mg dihydrotestosterone (DHT) every day from 16. to 19. days of gestation via subcutaneous injections. Histological examination revealed an increased number of preantral follicles in the study group exposed to T and DHT. Biochemical analysis of the gonadotropins showed increased T and DHT levels. This hyperandrogenic state was similar to that seen in PCOS patients. The experimental group showed increased LH levels, consistent with the hormonal imbalance seen in PCOS patients. Anti-estrogenic effect of the androgens led to decreased E2 levels and the negative feedback of E2 on the hypothalamic receptors was interrupted. This resulted in increased concentrations of LH. Impaired periodicity in response to gonadotropin stimulation and the release of androgens contributed to menstrual dysfunction (oligomenorrhoea and/or amenorrhoea), similar to the clinical presentation of PCOS. The researchers concluded that PCOS is a complex disease and excess androgen is only one factor relevant to the mechanism of PCOS pathogenesis.

d) Letrozole administration

In another experimental model of PCOS an aromatase inhibitor letrozole was used. Manneras et al.²⁴ worked on an animal model of PCOS in order to evaluate

the morphological changes in the ovary and the ensuing metabolic states. Their research focused on adiposity as an etiological factor in the development of PCOS and the resulting metabolic disturbances of animal subjects. They subcutaneously implanted 90-day continuous-release pellets of dihydrotestosterone (DHT, 7.5 mg, daily dose 83 g) and letrozole (36 mg, daily dose 400 g) separately in animals belonging to two different study groups and assessed ovarian morphology, histology, body weight, body fat, mesenteric adiposities and insulin resistance. Menstrual periodicity was disturbed in both study groups. Letrozole administered rats were completely acyclic, while DHT application produced irregular cycles. Morphologic features of the ovaries were similar to those seen in human patients. The number of cystic follicles increased in response to DHT and letrozole when compared with the control group. Atresia of antral follicles and follicular cysts were also dominant in DHT and letrozole implanted rats. The cysts in these rats were located in the periphery of the ovary, similar to the orientation of cysts in PCOS patients. As expected, testosterone levels were increased in rats given DHT and letrozole. E2 levels were unaltered and progesterone levels decreased. Body composition, analyzed by DEXA, was found to be increased upon DHT and letrozole application when compared to controls. Body fat distribution was increased only in the DHT group, while letrozole group was not different from the controls. Insulin sensitivity was found to be decreased in DHT administered rats but not in rats exposed to letrozole. DHT implanted rats showed an increase in the mean mesenteric adipocyte size, which was unaffected in rats that received letrozole. In conclusion, the letrozole model was useful for studies of the ovarian features of PCOS, while the DHT model was useful for studies of both ovarian and metabolic features of the syndrome.

e) Antiprogestin RU486

RU486 is a synthetic steroid that shows a high affinity for progesterone and glucocorticoid receptors. RU486 has potent antagonistic activity without agonistic activity for progesterone receptors²⁵. Application of

Table 1. The association between experimental PCOS models and human diagnostic traits of PCOS.

Experimental PCOS models	Acyclicity	Hyper androgenism	Oligo/ anovulation	Polycystic ovaries	↑Follicle atresia	LH Hyper secretion	Insuline resistance	Obesity
DHEA	+	+	+	+	+	+	+	-
EV	+	-	+	+	+	-	-	+
FAA	+	+	+	+	+	-	+	?
LA	+	+	+	+	+	+	+	+
RU486	+	+	+	+	+	+	-	-

DHES: Dehydroepiandrosterone, EV: estradiol valerate, FAA: fetal androgen application, LA: letrozole administration

RU486 in rats eliminates progesterone activity and creates many endocrine and ovarian morphological features similar to human PCOS. Treatment of RU486 with adult cycling female rats for 4-9 days resulted in acyclicity, polycystic ovaries, and anovulation^{26,27}. Changes in ovaries include increased number of atretic follicles²⁸ and thin granulosa cell layers²⁹. Similar to human PCOS, serum LH, T, and E2 levels were increased²⁹. FSH levels were variable, with different models displaying unchanged, increased, or decreased FSH levels. With regard to metabolic abnormalities related to human PCOS, RU486 application does not cause any change body weight or insulin sensitivity³⁰. As a result, rats treated with subcutaneously implanted RU486 shows many features found in women with PCOS, including acyclicity, anovulation, presence of follicular cysts and elevated androgen and LH levels.

CONCLUSION

Experimental models for induction of PCOS are important in understanding the mechanisms underlying its physiopathology. Table 1 summarized the association between experimental PCOS models and human diagnostic traits of PCOS. Hormonal imbalance during prenatal or postnatal periods may trigger the syndrome. Intraovarian sympathetic nerve stimulation via NE may alter the microenvironment and disturb folliculogenesis. Adiposity may be a cofactor involved in the transformation of a functional ovary into a dysfunctional, cystic one. Despite many experimental studies on PCOS, the exact mechanism is not yet elucidated. Since it is a multifaceted disease and affects various systems, further animal and human studies should be performed.

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