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Experimental Animal Models in Obstetrics and Gynecology

Jinekolojik Rahatsızlıklarda Kullanılan Deneysel Hayvan Modelleri

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ABSTRACT

This study focuses on two major diseases affecting women's reproductive health: endometriosis and polycystic ovary syndrome (PCOS). Endometriosis is characterized as an estrogen-dependent condition, highlighting estrogen's role in understanding the disease's development and treatment strategies. Rat and mouse models are crucial for comprehending the pathophysiology of endometriosis and testing new therapeutic approaches. These models are particularly valuable in evaluating the effects of hormones and immune system modulators on endometriosis. Conversely, experimental models of PCOS emphasize the central role of hyperandrogenism in the development of this condition. Models induced by substances like dehydroepiandrosterone, testosterone propionate, and letrozole provide insights into the metabolic and endocrinological disruptions associated with PCOS. The letrozole-induced model, in particular, helps in understanding the relationship between hormonal imbalances and the onset of PCOS. Experimental models of both diseases offer critical knowledge for both basic science research and clinical applications. They provide essential data for understanding the pathophysiology of these conditions and developing new treatment strategies. This study demonstrates how findings from experimental models can improve women's reproductive health and lead to more effective treatments for these diseases. An enhanced understanding of hormonal and immune system mechanisms will guide future research and offer innovative solutions for treating these conditions.
Keywords: Gynecological disorders; endometriosis; polycystic ovary syndrome.

ÖZ

Bu çalışma kadınların üreme sağlığını etkileyen iki önemli hastalık olan endometriyoz ve polikistik over sendromu (PKOS) için oluşturulan hayvan modellerine odaklanmaktadır. Endometriyozun östrojene bağımlı bir durum olarak karakterize edilmesi, östrojenin hastalığın gelişimi ve tedavi stratejilerinin anlaşılmasındaki rolünü vurgulamaktadır. Sıçan ve fare modelleri, endometriyozun patofizyolojisini anlamak ve yeni tedavi yaklaşımlarını test etmek için çok önemlidir. Bu modeller özellikle hormonların ve bağışıklık sistemi modülatörlerinin endometriyoz üzerindeki etkilerinin değerlendirilmesinde önem arz etmektedir. PKOS'un deneysel modelleri bu durumun gelişiminde hiperandrojenizmin merkezi rolünü vurgulamaktadır. Dehidroepiandrosteron, testosteron propiyonat ve letrozol gibi maddelerin neden olduğu modeller, PKOS ile ilişkili metabolik ve endokrinolojik bozulmalara ilişkin öngörü sağlamaktadır. Özellikle letrozolün neden olduğu model, hormonal dengesizlikler ile PKOS'un başlangıcı arasındaki ilişkinin anlaşılmasına yardımcı olmaktadır. Her iki hastalığın deneysel modelleri, hem bilimsel araştırmalar hem de klinik araştırmalar için kritik bilgiler sunmaktadır. Bu hastalıkların patofizyolojisini anlamak ve yeni tedavi stratejileri geliştirmek için gerekli verileri sağlamaktadır. Bu çalışma, deneysel modellerden elde edilen bulguların kadınların üreme sağlığını nasıl iyileştirebileceğini ve bu hastalıklara yönelik daha etkili tedavilere nasıl yol açabileceğini göstermektedir. Hormonal ve bağışıklık sistemi mekanizmalarının daha iyi anlaşılması gelecekteki araştırmalara yol gösterecek ve bu durumların tedavisi için yenilikçi çözümler sunacaktır.
Anahtar kelimeler: Jinekolojik rahatsızlıklar; endometriyoz; polikistik over sendromu.

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INTRODUCTION

Endometriosis is a chronic illness that is influenced by estrogen and affects 5-10% of women who are in their reproductive years (1). While some cases may be asymptomatic, the primary symptoms of this condition include dysmenorrhea, persistent pelvic discomfort, pain during sexual activity, infertility, and uterine bleeding. These symptoms can significantly affect the patient's psychological condition and quality of life (2). The condition is defined by the presence of endometrial-like tissue in abnormal locations, leading to a persistent inflammatory reaction, adhesions, and scar formation that changes the structure of the pelvic region. From a clinical standpoint, endometriosis is classified into three categories: peritoneal surface lesions, ovarian cysts (endometriomas), and deep infiltrating endometriosis. The classification of this condition is commonly based on the updated American Fertility Society classification (1), which divides it into four stages: minimum, mild, moderate, and severe. Retrograde menstruation has been suggested as a possible explanation for the creation of endometriosis in the peritoneal cavity during the menstrual cycle. However, given that retrograde menstruation has been detected in almost 90% of women without health issues, it is plausible that additional elements, such as hormonal, immunological, genetic, and epigenetic pathways, may also play a role in the development and advancement of the condition (3,4).

Polycystic ovarian syndrome (PCOS) is a common metabolic and hormonal condition that affects women during their reproductive years. The syndrome is characterized by metabolic symptoms including insulin resistance, obesity, and an increase in risk factors for cardiovascular disease. It also involves endocrine symptoms such as high levels of male hormones (hyperandrogenemia), irregular or infrequent menstrual periods (oligomenorrhea), absence of menstrual periods (amenorrhea), and hirsutism (5). PCOS patients have disruptions in the mechanisms that regulate follicular growth due to alterations in the endocrine system balance, leading to observable morphological abnormalities in the ovaries. Increased levels of luteinizing hormone (LH) disrupt the communication between granulosa cells and oocytes, as well as the growth of follicles and oocytes. It also results in the antral follicles staying tiny (6). Global standards for the evaluation and treatment of PCOS have advanced the detection of PCOS during the past thirty years. In 1990, the National Institutes of Health (NIH) established criteria for PCOS, including hyperandrogenism, oligo-ovulation, and the elimination of other potential causes such as Cushing’s syndrome and hyperprolactinemia (7). In 2004, the Rotterdam criteria were revised to diagnose PCOS. The current Rotterdam criteria encompass oligo- or anovulation, clinical and/or biochemical indications of hyperandrogenism, and the presence of polycystic ovaries. The International Guidelines for the Evaluation and Treatment of PCOS endorse a clinical diagnosis that requires the presence of at least two out of the three Rotterdam criteria (8). The prevalence of the disease differs among populations depending on whether the NIH or Rotterdam criteria are employed. Treatments for PCOS include blocking excess androgen production, correcting menstrual irregularities, maintaining the endometrium, enhancing fertility, and addressing metabolic issues. Each of these factors is crucial for the well-being of individuals with PCOS. Treatments primarily alleviate symptoms of PCOS (9).

Experimental animal models provide necessary data to understand the pathophysiology of diseases such as endometriosis and PCOS and to develop new treatment strategies. This review aims to demonstrate how findings from experimental models can improve women's reproductive health and shed light on more effective treatments for these diseases.

EXPERIMENTAL MODELS FOR ENDOMETRIOSIS

Uterine tissue has been effectively transplanted to aberrant places in small laboratory animals such as rats, mice, hamsters, and rabbits. This procedure has been documented in various studies conducted by Grümmer (10).

The rat and mouse models have been the main subjects of recent developments among non-primate models. In these experimental models of endometriosis, the uteri are surgically removed and dissected into tiny fragments. These fragments are subsequently reinserted into the peritoneal cavity, often by using sutures. The majority of these investigations did not distinguish the endometrium from other tissues. Both compartments, the endometrium and myometrium, were implanted (11,12). In rats, the uterine tissue undergoes development and forms fluid-filled, oval-shaped, cystic formations consisting of endometrial and myometrial tissue. The cysts experience growth but reach a stable size after approximately 2 months and maintain their viability for a minimum of 10 months (12). The ectopic uterine fragments in mice exhibit histological features resembling those of human disease. These characteristics encompass the formation of several, well-supplied lesions that consist of stroma, cysts, and endometrial glands. Importantly, the localization of these lesions within the abdomen is not dependent on their peritoneal location (11). Only a few experiments have been conducted in rats and mice to separate the endometrium from the myometrium and inoculate the endometrium to ectopic places. These investigations were performed by Katsuki et al. (13) in rats, and by Somigliana et al. (14), Hirata et al. (15), and Yao et al. (16) in mice. In Somigliana et al.'s (14) investigation, they took extracted endometrial tissue and carefully divided it into small fragments before delicately placing them back into the peritoneal cavity of recipient mice that shared the same genetic makeup. Both the donor and recipient mice underwent ovariectomy and were administered estrogen therapy. All animals that received the treatment showed signs of endometriosis in the peritoneum after 3 weeks. Additionally, new blood vessel formation was identified on the surface of the lesions. Nevertheless, the 'take-rate', which refers to the proportion of lesions obtained from a certain number of randomly injected endometrial pieces, averaged 30% of the inoculated tissue. Hirata et al. (15) established a homologous mouse model utilizing 'green mice' to improve the diagnosis of size and location of ectopic endometriotic lesions following transplantation. They were able to demonstrate a substantial correlation between the weight of endometriotic lesions and the assessed fluorescence intensity. The fluorescence

exhibited a notable increase in the mice that received estrogen supplementation, in comparison to the control animals. This finding provides evidence for the reliance of these abnormal endometrial lesions on estrogen.

UTILIZATION OF THE HOMOLOGOUS MODEL

Endometriosis is a condition in women that is influenced by estrogen and the reduction of estrogen in the blood helps to shrink the abnormal growths in other areas of the body (17). The mouse model, in which uterine tissue is transplanted and displays dependence on steroid hormones, has been extensively utilized to assess the responsiveness of lesions to steroid hormones and medications that disrupt steroid activity. The formation of ectopic endometrial tissue in both rodent species was found to be reliant on estrogen, similar to the situation in humans (12,18). In a study conducted by Schor et al. (19) in 1999, it was shown that rats that had their ovaries removed and were implanted with uterine tissue showed superior recovery of ectopic fragments when given with estrogen alone following ovariectomy, compared to those treated with a combination of estrogen and progesterone. In a study conducted by Fang et al. (20), it was found that estrogen has a significant impact on the size of implants in mice. They also showed that progesterone's ability to inhibit the growth of endometriotic tissue that depends on estrogen is due to the progesterone receptor remaining intact. Progesterone was observed to inhibit this growth in the uterine tissues of normal mice, while mice without the progesterone receptor did not show the same suppression.

Creating a hypoestrogenic condition can help promote the regression of uterine ectopic implants in rats. This can be achieved using methods such as ovariectomy or the injection of GnRH agonists, as demonstrated in studies by Kudoh et al. (21) and Sakata et al. (22).

The suppression of ovulation can be achieved by several methods, including the use of natural progestational compounds such as Kudoh et al. (21), synthetic progestational compounds like levonorgestrel or dienogest as mentioned by Jones (23) and Katsuki et al. (13), or through danazol medication as demonstrated by Sakata et al. (22). In this model, the desired effects can be obtained by decreasing the concentration of estrogen by the use of antiestrogens, or by utilizing selective estrogen (24).

The receptor modulator raloxifen or aromatase inhibitors, which disrupt estrogen production can be used for therapy (21,25). Furthermore, the autologous rat model has been widely employed for research on immune-modulating medicines and anti-inflammatory medications in endometriosis. Uchiide et al. (26) showed that when uterine tissue is transplanted in rats, it causes the accumulation of cells associated with allergic inflammation in the peritoneal stroma that is connected to the ectopic uterine tissue. Administering interferon-α-2b through intraperitoneal or subcutaneous treatment in rats resulted in a decrease in the size of induced lesions. This was observed through consecutive laparotomies conducted over a period of up to 4 months (27). Similarly, the use of the immunomodulator loxoribine in rats (28) and the intraperitoneal injection of interleukin-12 in a syngeneic mouse model also led to a reduction in lesion size (14). In addition, the development of artificially induced endometriosis in rats was inhibited by recombinant human tumor necrosis factor (TNF)-binding protein-1 (r-hTBP-1), which is the soluble form of TNF receptor type I (29). The same outcome was observed when rats were treated with pentoxifylline, a substance known for its anti-inflammatory properties and ability to decrease the production of inflammatory cytokines without causing a decrease in estrogen levels (30). Similar results were also observed with ciglitazone, a compound that binds to proliferator-activated receptor-γ (PPAR-γ) (31). In addition, the use of cyclooxygenase-2 (COX-2) inhibitors has been found to decrease the initial development of ectopic implants in rats (32). Similarly, the application of different non-steroidal anti-inflammatory drugs, including celecoxib, indomethacin, sulinac, and ibuprofen (but not aspirin), has also been shown to reduce the development of ectopic implants in a mouse model for endometriosis (33). Moreover, this mouse model offers the chance to study the impact of environmental pollutants on the formation of abnormal uterine implants. Previous studies have shown that prior exposure to dioxin before the surgical production of endometriosis leads to a proportional increase in the size of endometriotic sites in rats and mice. This effect was particularly pronounced in mice, as shown by Cummings et al. (11). In a novel study, Dinulescu et al. (34) introduced the initial mouse model of de-novo endometriosis. When the oncogene K-ras was activated in ovarian surface epithelial cells, it resulted in the development of benign epithelial lesions that showed histomorphological features resembling human endometriosis. However, this activation did not occur in cells of the peritoneal lining. Furthermore, almost 50% of the animals exhibited the formation of peritoneal endometriosis 8 months following the activation of ovarian surface epithelial cells by K-ras. When Pten is conditionally deleted, it can contribute to the development of endometrioid ovarian carcinoma in humans. In addition, the expression of K-ras can lead to the formation of metastatic endometrioid ovarian adenocarcinomas. Thus far, no genetic alterations of K-ras have been detected in cases of human endometriosis (35). Endometriosis in women is characterized by intense pelvic discomfort and a notable decrease in fertility (36). Researchers have examined the impact of abnormal lesions on reproductive ability in laboratory animals. While Cummings et al. (11) did not witness a decline in fertility among mice, they did observe a decrease in reproductive capacity among rats with artificially induced endometriosis. This decline could be attributed, at least in part, to an elevated count of luteinized unruptured ovarian follicles (37). Furthermore, the possibility of pelvic adhesions has not been ruled out. Additionally, the heightened activation of inflammatory cells in the peritoneum might potentially impact fertility in individuals with endometriosis. Steinleitner et al. (38) provided evidence that pentoxifylline, a substance, can counteract macrophage-mediated subfertility in mice. A medication that counteracts the impact of excessive activation of macrophages, and the drug-induced suppression of macrophage activation improved fertility in a hamster model of endometriosis (39). Recently, published research has examined the impact of ectopic endometrial lesions on pain responses in rats with endometriosis. The autotransplanted ectopic endometrial cystic pieces establish their own innervation, consisting of

both sympathetic efferent and sensory fibers (40). This innervation may have a broad impact on the nervous system. This is corroborated by the discovery that vaginal pain sensitivity was heightened in rats with endometrial cysts, mirroring the condition observed in humans with endometriosis. Furthermore, the rats exhibited vaginal hyperalgesia, as shown by Berkley et al. (41), which is a symptom commonly associated with heightened pelvic discomfort in people. There is speculation that neuroactive substances found in the endometrial cysts could stimulate nociceptive responses.

The afferents have an impact on the central brain mechanisms related to vaginal pain perception, as well as on reproductive processes through interactions between internal organs (40). It is yet unclear if the rats in this endometriosis model show any persistent pelvic pain symptoms other than vaginal hyperalgesia.

ANDROGEN-INDUCED PCOS RODENT MODELS

PCOS is primarily characterized by hyperandrogenism. An etiologic theory of PCOS suggests that being exposed to an excessive amount of androgens throughout the early stages of life can result in the development of PCOS during maturity. Over 30 years ago, it was documented that increased levels of circulating androgens in rodents had an impact on the development of ovarian follicles and the production of cysts (42). Various androgens, such as dehydroepiandrosterone (DHEA), testosterone propionate (TP), and 5α-dihydrotestosterone (DHT), have been administered to rats either by daily injections or subcutaneous implants to create an acute form of PCOS. It is crucial to acknowledge that there are variations in the way endocrine hormones and ovarian histology are reported in various models, leading to some discrepancy among research. Furthermore, several studies have failed to evaluate cardiometabolic parameters, and the impact of daily androgen injection and/or therapy on physiological indicators such as body weight, stress markers, or food consumption is typically not documented (43). In these rodent models, the development of PCOS is temporary and relies on the administration of androgen hormones. Therefore, the return to the regular reproductive/ovarian cycle happens after the injection of androgens is stopped.

PCOS INDUCED BY DHEA

DHEA is the initial androgen hormone that increases during the peripubertal period in females (44). Research has shown that approximately 50% of the T hormone produced in the follicles can come from DHEA in the bloodstream (45). Additionally, 25% of individuals with PCOS have higher than usual levels of DHEA in their bloodstream (46). Roy et al. (47) initially employed DHEA to produce PCOS in rats. Normally, prepubertal rats that have not yet reached sexual maturity and are around 22 days old, receive a daily injection of DHEA (6 mg/100 g body weight, diluted in 0.2 mL of sesame oil) for a period of 20-27 days. Following therapy, rats experience a cessation of menstrual cycles and a lack of ovulation (48).

PCOS INDUCED BY TP

Testosterone is administered to young female rats in order to stimulate the development of polycystic ovaries (49). This procedure involves the daily injection of TP (1 mg/100 g body weight dissolved in propylene glycol) into 21-day-old animals for a maximum of 35 days (49).

PCOS INDUCED BY ESTROGEN

Estradiol valerate (EV) is a type of estrogen that has a long-lasting effect. When it is given, it disrupts the normal functioning of the hypothalamus and pituitary gland, which leads to irregular release and storage of LH. LH is recognized as a crucial causative element in the progression of PCOS. Administering a 2 mg dosage of EV to young adult cyclic rats results in anovulation and the development of polycystic ovaries after 8 weeks (50).

PCOS INDUCED BY LETROZOLE

Aromatase is the primary enzyme responsible for converting testosterone and androstenedione into estradiol (E2) and estrone, respectively. The expression of this gene is prevalent in various human organs, including the placenta, ovary, and testis (51). PCOS development may be attributed to reduced ovarian aromatase activity, according to one of the pathophysiologic hypotheses (52). Letrozole is a type of medication known as a nonsteroidal aromatase inhibitor. It works by reducing the conversion of androgens (male hormones) to estrogens (female hormones) in the ovary. This leads to a rise in testosterone levels and a decrease in E2 production (51). Elevated levels of T in the ovaries are very probable to directly induce polycystic ovaries in rats treated with letrozole (53). The decrease in estrogen diminishes the inhibitory effect on LH synthesis in the pituitary, leading to elevated levels of LH (54), which in turn enhances the secretion of T by theca cells. Normally, female rats that are 6 weeks old (at the age of puberty) are given letrozole orally at dosages of 0.1, 0.5, and 1.0 mg/kg every day for a period of 21 days. As a result, they have a lack of menstrual cycles and exhibit histological and biochemical characteristics similar to those seen in human PCOS.

CONCLUSION

This study examines experimental models of two significant diseases affecting women's reproductive health: endometriosis and PCOS. Endometriosis is particularly characterized as an estrogen-dependent disease, highlighting the role of estrogen in understanding the disease's development and treatment strategies through experimental models. Rat and mouse models are crucial in comprehending the pathophysiology of endometriosis and testing new therapeutic approaches. These models are especially valuable in assessing the effects of hormones and immune system modulators on endometriosis. On the other hand, experimental models of PCOS emphasize the central role of hyperandrogenism in the development of this condition. Models induced by substances like DHEA, TP, and letrozole provide valuable insights into the metabolic and endocrinological disruptions associated with PCOS. Particularly, the letrozole-induced model helps in understanding the relationship between hormonal imbalances and the onset of PCOS. The experimental models of both diseases offer critical knowledge for both basic science research and clinical applications. The models of endometriosis and PCOS provide essential data for better understanding the pathophysiology of these conditions and developing new treatment strategies. This

study demonstrates how findings from the use of experimental models can be utilized to improve women's reproductive health and develop more effective treatments for these diseases. Furthermore, a better understanding of hormonal and immune system-related mechanisms will guide future research and offer innovative solutions in the treatment of these diseases. In conclusion, the experimental models of endometriosis and PCOS are indispensable tools for better understanding and treating these diseases. These models reveal the underlying mechanisms of these complex conditions affecting women's reproductive health, contributing significantly to future research and clinical applications.

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