CHALLENGES IN ESTABLISHING A RELEVANT MODEL OF POLYCYSTIC OVARY SYNDROME IN RATS – A MINI REVIEW

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IZAZOVI U USPOSTAVLJANJU RELEVANTNOG MODELA SINDROMA POLICISTIČNIH JAJNIKA KOD PACOVA - MINI REVIJSKI PRIKAZ

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common female endocrinopathy and one of the leading causes of infertility. However, the exact etiopathogenetic mechanisms are not discovered yet, while therapeutic strategies in PCOS commonly rely on symptomatic rather than curative. Regarding reasonable ethical limitations in human population, animal experimental studies can provide better insights into mechanisms underlying etiopathogenesis of PCOS, as well as investigations of different therapeutic strategies. Rodent models for PCOS are very useful for experimental studies due to their great genetic similarities with human genome, short reproductive and life span, feasible generating of genetically adapted animals, and convenient and accessible use. To our knowledge, androgens (dehydroepiandrosterone, testosterone propionate, 5 α -dihydrotestosterone), as well as estradiol valerate, represent the most frequently used hormones for PCOS modeling. Furthermore, the administration of antiprogesterone or letrozole has been reported as effective for PCOS induction. In our review, the presented PCOS models were accomplished by the administration of different hormones or drugs and alterations of environment. The main focus of this review was to summarize the alterations in ovarian morphology, hypothalamic-pituitary-ovarian axis, and hormone levels across above-mentioned protocols for postnatal PCOS modeling in rats.

Keywords: Polycystic ovary syndrome, animal model, ovary, hormone status, androgen, estrogen, letrozole, antiprogesterone.

SAŽETAK

Sindrom policističnih jajnika (Polycystic Ovary Syndrome - PCOS) je jedna od najčešćih endokrinopatija kod žena i jedan od vodećih uzroka neplodnosti. Međutim, precizni etiopatogenetski mehanizmi još nisu otkriveni, a terapijske strategije PCOS obično su više simptomatske nego kurativne. Imajući u vidu razumna etička ograničenja u ljudskoj populaciji, eksperimentalne studije na životinjama omogućavaju rasvetljavanje mehanizama koji su u osnovi etiopatogeneze PCOS, kao i ispitivanje efekata različitih terapijskih strategija. Indukovani modeli PCOS na glodarima su veoma korisni za eksperimentalna ispitivanja zbog njihove velike genetske sličnosti sa ljudskim genomom, kratkog reproduktivnog i životnog veka, izvodljivosti genetičkih modifikacija, pogodnosti upotrebe i pristupačnosti. Prema našim saznanjima, androgeni (dehidroepiandrosteron, testosteronpropionat, 5 α -dihidrotestosteron), kao i estrogen valerat, predstavljaju najčešće korišćene hormone za indukciju PCOS. Pored toga, poznato je da primena antiprogesterona, kao i letrozola, efikasno dovodi do indukcije PCOS. U našem preglednom članku, predstavljeni PCOS modeli ostvareni su primenom različitih hormona ili lekova i promenama faktora okoline. Glavni cilj rada bio je sumiranje promena u morfoloiji jajnika, osovini hipotalamus-hipofiza-jajnik, kao i hormonskom statusu u gore pomenutim protokolima za postnatalnu indukciju PCOS kod pacova.

Ključne reči: Sindrom policističnih jajnika, animalni model, jajnik, androgen, estrogen, letrozol, antiprogesteron

INTRODUCTION

A better understanding of the mechanisms underlying Polycystic ovary syndrome (PCOS) is essential to shift therapeutic approaches from incomplete and symptomatic to curative. Studies on human population are ethically restricted and frequently don't provide enough deep insights into molecular basis. However, animal models exhibit numerous PCOS characteristics and represents a paramount importance regarding the studies investigating different aspects on this complex syndrome.

ANIMAL PCOS MODELS: SIMILARITIES AND DIFFERENCES TO PCOS IN WOMEN

Animal models are suitable for studying etiopathogenesis and investigations of novel therapeutic strategies, as they allow the application of invasive procedures, otherwise unethical for humans (1). In animal studies investigating PCOS, the main problem represents its heterogeneous clinical presentation and the fact that its etiology still isn't completely defined (2). In the most studies, morphological changes in the ovaries, mainly represent an increase in the number of antral and cystic follicles and a decrease in the number of corpora lutea. Hyperandrogenic status could be induced using androgens (1,2-6,) and other stimulants, while estrogen usage did not lead to changes in circulating androgens (2,7-10). A better understanding of human disorders is often identified only after discoveries in adapted animal models. Animal models must have 2 or more PCOS-like equivalents in females (11). PCOS rodent models in particular have provided valuable insights into the pathogenesis of PCOS due to their accessibility and ease of use, short reproductive and life span, great genetic similarity to the human genome and feasibility of generating genetically adapted mice.

There are also disadvantages to using rodents for PCOS induction. Female rodents are polyovulatory, while women are (the most commonly) monovulatory, and the follicle stimulating hormone (FSH)-dependent follicle secretion process in rodents differs from that in women (2, 11, 12). The initial stages of follicular growth (from the initial to the preantral stage) are similar in women and rodents, but there are differences in regulation (2, 11). There are also significant differences in the time of onset of folliculogenesis between rodents and women. While folliculogenesis in women begins during intrauterine development and is stopped and postponed until puberty in the diplotene of the first meiotic division after which it continues to develop, folliculogenesis in rodents occurs only during the early postnatal period (12). Therefore, the results obtained in rodents may not be translated directly into women, but nonetheless, will provide easier understanding and better insight into the etiopathogenesis of PCOS.

DEFINITION, ETIOPATHOGENESIS AND CLINICAL PRESENTATION OF PCOS – A BRIEF CONSIDERATIONS

PCOS is one of the most common female endocrinopathies and one of the leading cause of infertility. The clinical presentation of women with PCOS is extremely variable: PCOS women could be obese or lean, with or without insulin resistance, experiencing amenorrhea, oligomenorrhea (irregular or absent menstrual cycles), infertility, hyperandrogenism (clinical or biochemical), with frequently registered polycystic ovaries during ultrasonographical examination (1,13). Clinical hyperandrogenism represents presence of menstrual irregularities, signs of androgen excess, obesity, and hirsutism, while biochemical hyperandrogenism refers to higher serum level of testosterone (T), as well as higher level of calculated free (unbound) T and free androgen index (FAI), and higher level of androstenedione and dehydroepiandrosteronesulphate (DHEA-S).

According to Diagnostic Rotterdam criteria, PCOS includes presence two of the three following characteristics: polycystic ovaries visible on ultrasound, clinical or biochemical hyperandrogenism and ovulatory dysfunction. Clinically, these manifestations could be associated with infertility due to impaired follicle maturation and consequent anovulation.

Occurrence of PCOS in the same family is frequent, because it had a great hereditary rate (14, 15, 16), while about 60% daughters of women affected by PCOS develop manifestations of this syndrome during puberty or early adolescent age, also (17,18).

PCOS represents a complex disorder of uncertain and multifactorial etiopathogenesis. Despite its prevalence and great impact on reproductive health, the etiology of PCOS remains poorly understood. Etiological hypotheses for the origins of PCOS include hormonal imbalances, low grade inflammation, oxidative stress, genetic abnormalities, lifestyle, and environmental factors (19). The heterogeneity of PCOS presentation, and lacking of consensus on generally uniform criteria for PCOS diagnosis, contributed to difficulties in unraveling the etiopathogenetic mechanisms and consecutive development of optimal or curative treatment of PCOS. Women with PCOS have a bigger chance for metabolic syndrome development, while obesity could be potentiated by hyperandrogenism. Oxidative stress (20) and low-grade inflammation (21) are correlated to PCOS development, and both are considered important factors for pathogenesis of this complex syndrome. Women with PCOS have increased oxidative stress, which correlates with central obesity, higher arterial blood pressure levels, elevated glycemia and insulin levels, insulin resistance and hypertriglyceridemia (22). Lipid peroxidation significantly increased in PCOS and this alteration positively correlates with BMI, insulin level and blood pressure. Increase in markers of oxidative stress and inflammation could be related with higher androgen levels (23). The visceral fat adipocytes exert effects in a paracrine and endocrine manner via secretion of a various molecules,

acting as markers of inflammation. Manifestations of chronic inflammation as evidenced by increases of C-reactive protein (CRP), pro-inflammatory cytokines and chemokines, white blood cell count (WBC), oxidative stress and various markers of endothelial inflammation. In contrast, antioxidant status was observed to be depleted in PCOS (23).

Excess in LH levels/pulsatility is the main cause of hyperandrogenism. Hyperinsulinemia due to insulin resistance may accompany hyperandrogenism and involve metabolic dysfunction in women with PCOS. Insulin synergises with LH to potentiate androgen production in theca ovarian cells, while also reducing the level of sex hormone-binding globulin (SHBG), causing elevated levels of total and free T (24). Excess of insulin inhibits the maturation of ovarian follicles causing premature luteinization. Hyperinsulinemia also enhances FSH-induced regulation of LH receptors in granulosa cells during differentiation, which stops cell proliferation and subsequent follicle growth, while increasing the ability of granulosa cells to produce progesterone (P) in response to LH. Moreover, cultured granulosa cells from small PCOS follicles showed a premature response to LH and an extensive steroidogenesis transition from estradiol to P production. PCOS also increases the risk of not only insulin resistance, but also dyslipidemia, abdominal obesity together with altered morphology and function of adipose tissue, diabetes and diabetic complications and cardiovascular diseases. It is of great importance to mention that PCOS women had a risk of myocardial infarction 7 times higher than normal women (25,26). However, regular monitoring of PCOS patients could prevent many long-term complications.

RAT PCOS MODELS

Numerous evidence form literature, summarized in this paper, confirm that PCOS in rats could be induced by different protocols. PCOS in rats could be established by the hormone administration and manipulations (such as inhibition of aromatase), prolonged exposure to light and the by use of transgenic technology. Concerning hormones, androgens such as dehydroepiandrosterone (DHEA), testosterone propionate (TP), 5 α -dihydrotestosterone (DHT), are commonly used. However, long acting estrogen valerate or blockade of progesterone by antiprogesterone could be performed also in order to induce PCOS. Inhibitor of aromatase - letrozole and other medicines represent a known way to produces PCOS in rodent. Hormones and medicines applications can be performed in different routes: orally, subcutaneously, or by usage of subcutaneous implants. In rodents, PCOS induction protocol could be performed prenatally and postnatally. Post-natal protocols could be accomplished treating prepubertal or postpubertal rats. This review will summarize postnatally performed protocols for PCOS induction in rats.

RAT POSTNATAL PCOS MODELS

Rat PCOS induced by androgens

One of the sites in female rats where androgens could be produced are ovaries. Theca cells in ovaries produce mainly androstenedione (A4) and testosterone (T). Hyperandrogenic PCOS animal models provided the most comprehensive insight into the pathogenesis of PCOS. Hyperandrogenism is one of the main manifestations of PCOS. Androgens such as dehydroepiandrosterone (DHEA), testosterone propionate (TP), and 5 α -dihydrotestosterone (DHT) are most commonly used to induce an acute state of PCOS. Routes of androgen administration for inducing PCOS in rats are: daily subcutaneous injections, oral route or application of subcutaneous implants containing androgen.

PCOS induced by dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is an androgen primarily produced in the adrenal glands. The fact that first elevated androgen in plasma of PCOS adolescents represents DHEA, led researchers to develop animal PCOS model using postnatal administration of DHEA (1).

DHEA was first used by Roi et al. (27) to induce PCOS in rats. Prepubertal rats, approximately 22 days old, were used and injected daily with DHEA (6 mg / 100 g body weight, dissolved in 0.2 ml sesame oil) for up to 25 days. The body weight of rats in the DHEA group displayed significant increase 20–30 days after initial DHEA injection, compared with the control group. After treatment, rats become acyclic and anovulatory with abnormal follicles maturation, mostly remaining in estrus phase of cycle and hyperandrogenic (7, 28, 29).

Ovarian morphology

Ovarian weight in postnatal DHEA rats was increased with multicystic structure compared to control group (1,30,31). The increase in weight was accompanied by cystic alteration of the ovaries, ie the number of cystic follicles with a diameter of 0.45 to 2.2 mm increased, the granulosa cell layer was thinned and the granulosa cells were degenerated. (32,33).

Hormonal status

In the serum of DHEA-treated rats, LH levels were lower or the same as in the plasma of the control groups, but the levels of DHEA, estradiol (E2), prolactin (PRL), T and the LH / FSH ratio were higher than in the control rats. (6,28, 33-36).

Summary

Postpubertal exposure to excessive DHEA improved reproducibility, PCOS induction ratio, ovarian cysts formation. Such effect might be closely related to PCOS in adolescents and in young adults. The DHEA-treated rats are useful for investigating the impact of higher T in the ovary, but may not be the optimal model for high LH levels. Regarding glycoregulation, different results could be found in literature. Although some authors showed there were no significant

changes in fasting glucose, fasting insulin and plasma lipid profiles, and that the use of DHEA-treated rats is not a good animal model for the study of metabolic abnormalities in PCOS (37), another registered such a metabolic abnormalities after DHEA administration (38).

PCOS induced by testosterone

Testosterone propionate (TP) treatment induced anovulation and increased the presence of apoptotic follicles and unhealthy oocytes (39). Female rats also exhibited insulin resistance, a characteristic feature of human PCOS, indicating that androgens can lead to insulin resistance (39). Postnatal treatment begins after weaning, when animals are 21 days old, and is performed as daily TP injection in dose of 1 mg/100 g body weight (dissolved in propylene glycol) until 56 days of age. Also, TP could be administrated as 1.25 mg/100 g body weight from the age of 5 to 21 postnatal day, orally (40). Testosterone enanthate induced PCOS causes stereological features in the ovary, increases the oxidative stress and inflammatory markers (41).

Ovarian morphology

Postnatal treatment of TP caused morphological changes of the ovaries that are also seen in the human PCOS phenotypes. TP-treated rats have large or atretic cystic follicles and hypertectosis and luteinization of theca cells in the ovaries (1). The corpus luteum fails to develop even at 56 days of age, unlike control animals which then have well developed corpora lutea. No apoptosis was evident in corpora lutea while the percentage of apoptotic oocytes increased. The proportion of preantral follicles increases after TP treatment (39), which may be associated with increased early follicular development (2).

Hormonal status

Serum T, LH, and PRL concentrations were increased, while FSH, P, and E2 levels were decreased in rats given a single dose treatment (1,42).

Summary

The development of polycystic ovaries in this model involved the of large cystic follicles formation and anovulation. An imbalance between gonadotropins and sex hormones occurs after chronic TP treatment; androgens indirectly promoted a pathological increase in FSH that blocks ovulation and causes cystic follicle in ovaries of. The TP model is a direct model of hyperandrogenemia, T-induced anovulation, and polycystic ovarian morphology, as well as disorders of insulin/glucose metabolism.

PCOS induced by dihydrotestosterone

Dihydrotestosterone (DHT) is a nonaromatizable androgen that is converted irreversibly from T by the enzyme 5α-

reductase, DHT has stronger androgenic potency. Manneras (43) has shown that DHT can induce PCOS in rats. DHT can induce PCOS of both ovaries and metabolic disorders in rodents. DHT can not be converted into E2 by aromatase, so effects of estrogen converted from androgen don't exist in this model. Postnatal treatment begins at 21 days of age. It can be done by subcutaneously administering DHT pellets (7.5 mg/pellet, 90-day release, daily dose is 83 µg) (2,42,43). Plasma DHT concentrations were increased 1.7-fold compared to control animals. Irregular estrous cycles and ovarian features similar to human PCOS, including increased numbers of large atretic follicles and follicular cysts with a thickened theca interna cell layer and thin granulosa cell layer and fewer corpora lutea than controls (43). DHT-treated rats also showed many metabolic features also present in human PCOS, including increased body weight, body fat, and abdominal fat with enlarged adipocytes, elevated leptin and cholesterol levels, and insulin resistance.

Ovarian morphology

However, unlike human PCOS ovaries, ovary weight was reduced. Ovarian volume was reduced in postnatal DHT treatment compared to the control (1, 44), which distinguishes this group from DHEA-induced PCOS models. The ovaries have an increased frequency of atretic, antral and cystic follicles, with a reduced layer of granulosa cells and hypertectosis.

Hormonal status

Concentrations of LH, FSH and E2 are unaltered and not differed significantly in relation to the control group, but the level of progesterone is reduced, which indicates anovulation (1, 43, 44).

Summary

The rat DHT model of PCOS mimics the effects of T and its higher effects on androgen receptors. Irregular cycles and cystic ovaries are present, and this model can be used to represent the hyperandrogenic phenotype of PCOS in women without effects of aromatizable testosterone. The DHT-induced model may be of use in the study of mechanisms leading to disrupted regulation of the hypothalamic-pituitary-gonadal axis. This model can also be a model for investigating the metabolic properties of PCOS, ie the development of disorders in insulin metabolism, glucose, leptin metabolism and obesity.

Antiprogesterone-induced model of PCOS

Antiprogesterone (RU486 or mifepristone) is a synthetic steroid that acts as a P antagonist, blocking its activity very strongly. P is predominantly synthesized in the corpus luteum of ovary after ovulation. P is also secreted by the adrenal

gland and plays a significant role in regulating gonadotropin secretion, follicular development and ovulation. P inhibits the frequency of GnRH pulse and when rats are treated with RU486, the P concentration is reduced, as observed that in the human PCOS model, and the frequency of GnRH pulses is increased (45,46,47,48,49). When the frequency of GnRH is increased, LH secretion predominates and the LH / FSH ratio is increased, leading to increased ovarian T secretion. Excess of T can result in follicular atresia and anovulation, (49) similar to the pathophysiology described in the development of PCOS in women (48,49). In adult cyclic rats, PCOS was induced when RU486 was administered subcutaneously (4 mg) for 8 consecutive days beginning on the first day of estrus (48,49). After 4 days of RU486 treatment, rats showed irregular cycles consisting of persistent estrous (50).

Ovarian morphology

The ovaries of rats treated with RU486 showed arrest of follicular growth and a higher rate of follicular atresia. Cysts were diameter higher than 2 mm (51). The numbers of preantral and small antral follicles and atretic cyst follicles were also increased compared to untreated rats (1). Follicles had varying degrees of luteinization (refers to hypertrophy of P-secreting granulosa cells) (49).

Hormonal status

The higher mean amplitude of LH pulse was noted. LH, T, E2, and PRL concentrations were increased, while FSH levels were reduced in RU486-treated rats compared to control group of animals. The mean amplitude of the LH pulse is increased. The LH / FSH and T / E2 ratio is also increased in this model (48). PRL hypersecretion occurs due to the antagonistic effect of P on E2. Increased PRL stimulates the corpus luteum and granulosa cells of atretic follicles to secrete P and leading to the formation of luteinized cysts (47,48,49). Therefore, the ovaries contain different degrees of luteinized cysts (2).

Summary

The RU486-treated rats represent a specific model due to progesterone-induced antagonism and induction of hyperprolactinemia, and may be a desirable model for investigating the role of PRL and T in the PCOS. This model is suitable for investigating progesterone negative feedback damage in PCOS pathophysiology.

Estrogen-induced PCOS model

Estradiol valerate (EV) is a long-acting estrogen and its administration leads to dysregulation of the hypothalamic-pituitary axis, gonadotropic releasing hormone GnRH, which results in improper release and storage of LH (7). Basal serum LH concentrations are attenuated whereas serum FSH concentrations are higher, but in normal range in these animals (52). PCOS models were established by injecting EV single doses of 2-4 mg to post-pubertal rats in estrus

(53,54,55) causing anovulation and polycystic ovaries within 8 weeks (18,19).

Ovarian morphology

The ovaries of EV-treated rats were reduced in size compared to the control group and were characterized by large cystic follicles with reduced granulosa cell layers and hyperthecosis (9). Also, there are a high incidence of atretic / degenerative secondary follicles (10). Five to 16 days after EV injection, many follicles showed atresia; ovarian weight was reduced 16 days after EV injection, so ovaries had decreased weight compared to control animals (56).

Hormonal status

Basal concentrations of luteinizing hormone (LH) in the serum of EV-treated rats decreased during the early stages of PCOS induction (8–10 weeks after EV treatment). After 22 weeks of treatment, LH levels usually increase, but still remain lower than in control groups of animals. T, E2, and FSH concentrations showed a similar profile, which is not typical of the hyperandrogenemic profile of the PCOS phenotype, and P concentrations were increased in EV-treated rats (9). The EV-induced PCOS model has been compared with polycystic ovaries in women, which are usually characterized by increased plasma LH concentrations, weight gain, and small ovaries with large cystic follicles and no stromal hypertrophy. However, plasma LH concentrations were increased in EV-treated rats compared to the proestrus control group after GnRH injection. Administration of 2 mg EV increased T and E2 levels, while a concentration of 4 mg decreased T and increased LH and P levels, but had no effect on E2 levels (55,56).

Summary

The EV-induced model is anovulatory without hyperandrogenemia and resembles the normoandrogenic PCOS phenotype. Acute estrogen exposure by altering the activity of follicle enzymes reduces androgen production, so this model may not be the most optimal for studying PCOS. Although anatomy and physiology of the ovary resemble those of human PCOS, progressive degeneration of hypothalamus and altered response of pituitary to LHRH rendered this model not be the most optimal for studying hypothalamus-pituitary-ovarian axis in PCOS (1).

Letrozole-induced PCOS model

Letrozole is a non-steroidal aromatase inhibitor that reduces the conversion of androgens to estrogens, stimulating the events that occur in PCOS. Causing the endogenous hyperandrogenemia, decreased production of E2 (57), neuroendocrine alterations, and consecutive reproductive and metabolic abnormalities (43,58,59), this model seems to be very useful tool in PCOS investigations. Aromatase is a key enzyme that converts T and androstenedione to E2. It is produced in a variety of human tissues, such as the placenta, ovaries, and testicles (60). Decreased ovarian aromatase activity

is one of the pathophysiological hypotheses for PCOS development (58). It should be mention that excess of T is likely to cause polycystic ovarian morphology in rats treated by letrozole. Estrogen depletion weakens negative feedback and LH production in the pituitary gland, which increases LH levels (59), further stimulating the secretion of T-secreting cells. 6 weeks aged female rats (puberty) were given letrozole in a variation of 1-3 mg daily orally, for 21 days, up to 100-400 µg / d / 100 g body weight by implantation of subcutaneous pellets. After that application, they become acyclic, with the biochemical characteristics of PCOS. (60)

Ovarian morphology

Morphological changes in ovaries of rats treated with letrozole represent presence of antral follicles and follicular cysts with hyperplasia of internal cells of the theca layer, thickened ovarian capsule and increased area of the dominant follicle. The number of corpora lutea is reduced, indicating oligo-/anovulation. Ovarian weight with letrozole remains unchanged or slightly increased (43,58).

Hormonal status

Serum LH and T levels in letrozole-treated rats were elevated compared to those in control animals in estrus and diestrus. FSH was significantly increased in the higher dose groups (0.5 and 1.0 mg/kg), which are not typical characteristics of human PCOS (54). Serum E2 and P levels were lower in these rats than in proestrus and diestrus controls (56,57).

Summary

The letrozole model this animal model in several ways is similar to the human PCOS. This model allows the investigation regarding the role of aromatase and may be an effective tool for induction the metabolic changes in line with PCOS. Because the administration of aromatase inhibitor can cause ovulatory failure and polycystic ovaries, the substance that increases aromatase activity could be used as a potential treatment protocols investigation.

ALTERATIONS IN ENVIRONMENTAL FACTORS IN ORDER TO INDUCE PCOS IN RATS

PCOS induced by prolonged light exposure

Circadian rhythms are 24-h cycles generated by the suprachiasmatic nuclei (SCN) who induces daily rhythms in hormone concentrations (61), body temperature (62), heart rate and blood pressure (63), and many other parameters. The light regulates the secretion of several pituitary hormones. In view of the correlation of the circadian system with the estrous cycle in the rat, it is proposed the induction of polycystic ovaries by exposing rats to continuous light.

Continuous light exposure impairs the rhythm of melatonin secretion. Inhibition the melatonin effects can induce

PCOS symptoms. Prolonged exposure of rats to light is associated with dysmenorrhea and metabolic syndrome, insulin resistance and deregulation of glucose metabolism, and these symptoms are also symptoms that occur within PCOS. Prolonged exposure to light causes polycystic ovaries and hyperandrogenism in rodents as well, and indicates a connection between circadian rhythm disorders and disorder of melatonin and the manifestation of PCOS (64). Continuous light exposure leads into stress reaction so the pituitary cell starts producing PRL (65).

However, the effect of circadian rhythm disorders on the reproductive characteristics of PCOS and the mechanism underlying this pathogenesis have not been fully discovered and elucidated (66). Model of PCOS developed by chronic exposure of female rats to constant light, proved elevated levels of estradiol and estrone (67). Estradiol inhibits the GH and stimulates the PRL secretion (68, 69). This model can be developed by dividing 6-week-old rats randomly, into two groups: a control group and a group exposed to light. The control group was under a periodic cycle (12/12-hour light/dark group), while the light group was exposed to continuous light (12/12-hour light/light group) for four consecutive weeks.

Ovarian morphology

Both ovaries in the constant light group was heavier than that in the normal light/dark cycle group. Under thickened tunica albuginea, there were several follicles in different developmental phases, including atretic follicles and large monoantral follicles, as well as fewer layers of granular cells and missing oocytes and corona radiating within the follicles (64).

Plasma gonadotropin measurement and histological ovarian analysis showed that 90% of the animals in constant light conditions developed cystic follicles with the absence of the corpus luteum, despite the lack of reduction in ovarian weight commonly (70).

Hormonal status

Levels of FSH, LH, P and E2 were reduced compared to control groups (71) while serum testosterone levels were significantly increased.

Summary

Steroid hormones play an important role in the effects of daylight changes. Melatonin maintains the proper follicular function, while it is and also important for ovulation and progesterone production (72). Continuous exposure to light contributes to the formation and development of PCOS (9). Continuous exposure to light is one of the spontaneous causes of PCOS so this more consistent with the natural disease state. Poor sleep habits may be an important lifestyle factor in pathogenesis of the PCOS (73).

High fat diet induced PCOS

Since there is a high prevalence of diabetes mellitus (DM) and dyslipidemia in women with polycystic ovary syndrome (PCOS) high fat diet model of PCOS is proposed as suitable. PCOS includes disorders of insulin action, insulin resistance (IR), finally leading to diabetes, and it also displays neuroendocrine dysfunction with exaggerated LH pulsatility and higher production of adrenal androgens. High-fat dieting has been shown to impair reproductive health and reduce fertility in rodent subjects (75). PCOS can be induced by injecting the rats with andronate subcutaneously at a dose of 1 mg/100 g body weight/day for 8 weeks and also fed with a lipid-enriched diet, while control group should be fed with standard laboratory diet (75).

Ovarian morphology

Ovaries had many cystic follicles with apoptotic granulosa cells. The treated group had fatty infiltrated larger ovaries than the control group, experimental group had fewer corpus lutea and preovulatory follicles and had more preantral and antral follicles than the controls, while follicular diameters were also higher.

These antral follicles displayed characteristics of precysts but the presence of intact granulosa cell layers excludes it from cysts. Corpora lutea were identified according to tissue density and the presence of a grainy luteinised cell appearance. Corpora lutea were counted only when they exhibited more than a 60% area with higher tissue density (75).

Hormonal status

The FSH levels were not affected, the LH levels were increased in the treated groups. The LH/FSH ratio was elevated in the treated group, testosterone levels were significantly elevated in treated group, indicating that hyperandrogenism was present in this animal model (76). Prepubertal high fat diet in rats led to similar hormonal and metabolic disturbances as human PCOS (77).

Summary

A rat model of PCOS was successfully established with features of polycystic ovaries, obesity, irregular cycles of vaginal smear, increased plasma insulin levels, decreased insulin sensitivity, hyperandrogenism, and increased LH concentrations. The diet itself may play an important role in PCOS pathogenesis. This study demonstrates a rodent model for diet-induced obesity resulting in PCOS with both the metabolic and reproductive phenotype.

Method	Summary		Reference
PCOS induced by DHEA	The DHEA-induced model can be used to reflect early hyperandrogenemia associated with anovulation and cystic ovaries and DHEA-treated rats is not a good animal model for the study of metabolic abnormalities in PCOS.	DHEA, E2, PRL, T LH/FSH ↑ P ↓	(34)
PCOS induced by testosterone	The TP model is a direct model of hyperandrogenemia, anovulation of insulin/glucose metabolism disorders.	T, LH and PRL ↑ FSH, P and E ↓	(39)
PCOS induced by DHT	The DHT-induced model can also be a model for investigating the development of disorders in metabolism, insulin, glucose, leptin metabolism and adiposity in PCOS.	LH, FSH, E2, T = P ↓	(43,44)
Antiprogesterone-induced model of PCOS	A rat treated with RU486 is a specific model due to progesterone-induced antagonism and induction of hyperprolactinemia, and may be a desirable model for investigating the role of PRL and T in the polycystic condition.	LH, T, E2 and PRL ↑ FSH ↓	(48)
Estrogen-induced PCOS model	The EV-induced model is anovulatory without hyperandrogenemia and resembles the normoandrogenic PCOS phenotype. Acute estrogen exposure by altering the activity of follicle enzymes reduces androgen production, so this model may not be the most optimal for studying PCOS. Although anatomy and physiology of the ovary resemble those of human PCOS, progressive degeneration of hypothalamus and altered response of pituitary to LHRH rendered this model not be the most optimal for studying hypothalamus-pituitaryovarian axis in PCOS .	LH, FSH, E, T ↓ P ↑	(7, 2)

Method	Summary		Reference
Letrozole-induced PCOS model	The letrozole model this animal model in several ways is similar to the human polycystic ovary syndrome, it allows the study of aromatase and may be an effective mechanism with other mechanisms that induce metabolic changes to study these factors in the PCOS state because of it recapitulates the metabolic features of human PCOS. Because administration of aromatase inhibitor can cause ovulatory failure and polycystic ovaries, the substance that increases aromatase activity could be used as a treatment.	LH, FSH, T ↑ E2, P ↓	(57)
PCOS induced by prolonged exposure to light	Steroid hormones play an important role in the effects of daylight changes. Melatonin maintains the proper follicular function and also is important for ovulation and progesterone production. Continuous exposure to light contributes to the formation and development of PCOS. Continuous exposure to light is one of the spontaneous causes of PCOS so this more consistent with the natural disease state. Poor sleep habits may be an important lifestyle factor in pathogenesis of the PCOS.	FSH, LH P, E2 ↓ T ↑	(71)
High fat diet induced PCOS	The diet itself may play an important role in PCOS pathogenesis. This study demonstrates a rodent model for diet-induced obesity resulting in PCOS with both the metabolic and reproductive phenotype.	FSH = LH, LH/FSH, T ↑	(76)

CONCLUSION

This review summarized an important data regarding hormonal status and ovarian morphology in postnatally induced PCOS rat models. Hormone-induced PCOS models, as well as environmental and diet alterations such exposure to constant light and high fat diet protocols for induction of PCOS, could be proposed as suitable procedures. Although the perfect animal model which absolutely mimics PCOS milieu in human population, doesn't exist, experimental studies could provide a specific insights to PCOS etiology, pathophysiology and potential therapeutic strategies, frequently impossible to apply in human population. The presented models recapitulate the different aspects of PCOS and researchers should choose such an animal model corresponding to their research objectives. PCOS represents more than a reproductive disorder and its risks should be seriously taken into consideration regarding reproductive, metabolic and cardiovascular consequences. Inflammation and oxidative stress are important to investigate with the purpose of highlighting pathogenetic mechanisms, which could lead to the development of the specific curative treatments. Findings from studies using these animal models may provide insight into the pathophysiology of PCOS in humans. Considering the global significance of PCOS and its prevalence in the female population, it is important to conduct future research on animal models that would contribute to their impact on the human population.

LITERATURE

- Osuka S, Nakanishi N, Murase T, Nakamura T, Goto M, Iwase A, et al. Animal models of polycystic ovary syndrome: a review of hormone-induced rodent models focused on hypothalamus-pituitary-ovary axis and neuropeptides. *Reprod Med Biol*. 2019, 18:151–60. doi: 10.1002/rmb2.12262
- Paixão L, Ramos RB, Lavarda A et al. Animal models of hyperandrogenism and ovarian morphology changes as features of polycystic ovary syndrome: a systematic review. *Reprod Biol Endocrinol* 15, 12 (2017). <https://doi.org/10.1186/s12958-017-0231-z>
- Haning RV Jr, Hua JJ, Hackett RJ, Wheeler CA, Frishman GN, Seifer DB, et al. Dehydroepiandrosterone sulfate and anovulation increase serum inhibin and affect follicular function during administration of gonadotropins. *J Clin Endocrinol Metab* 1994; 78:145–9.
- Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, EscobarMorreale HF, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* 2009; 91:456–88.
- Noroozadeh M, Behboudi-Gandevani S, Zadeh-Vakili A, Ramezani TF. Hormone-induced rat model of polycystic ovary syndrome: A systematic review. *Life Sci*. 2017;191:259-272.

6. Knudsen JF, Costoff A, Mahesh VB. Dehydroepiandrosterone-induced polycystic ovaries and acyclicity in the rat. *Fertil Steril* 1975; 26:807–17.
7. Quandt LM, Hutz RJ. Induction by estradiol-17 beta of polycystic ovaries in the guinea pig. *Biol Reprod* 1993; 48:1088–94.
8. Takeo Y. Influence of continuous illumination on estrous cycle of rats: time course of changes in levels of gonadotropins and ovarian steroids until occurrence of persistent estrus. *Neuroendocrinology* 1984; 39:97–104.
9. Chung, F. F., Yao, C. C., and Wan, G. H. (2005). The associations between menstrual function and life style/working conditions among nurses in Taiwan. *J. Occup. Health.* 47, 149–156. doi: 10.1539/joh.47.149
10. Mesbah F, Moslem M, Vojdani Z, Mirkhani H. Does metformin improve in vitro maturation and ultrastructure of oocytes retrieved from estradiol valerate polycystic ovary syndrome-induced rats. *J Ovarian Res.* 2015; 8:1–10.
11. Franks S. Can animal models of PCOS help in the direction of early and effective therapeutic intervention in women with the syndrome? *Endocrinology.* 2015; doi: 10.1210 / sr.2015-1420.
12. Malikueo M, Benrick A, Stener-Victorin E. Rodent models of polycystic ovary syndrome: phenotypic presentation, pathophysiology and effects of different interventions. *Semin Reprod Med.* 2014; doi: 10.1055/s-0034-1371090.
13. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocr Rev.* 2015; 36(5):487–525.
14. Gorsic LK, Dapas M, Legro RS, Hayes MG, Urbanek M. Functional genetic variation in the anti-Müllerian hormone pathway in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2019; 104(7):2855–74.
15. Gorsic LK, Kosova G, Werstein B, et al. Pathogenic anti-Müllerian hormone variants in polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2017; 102(8):2862–72.
16. Dapas M, Sisk R, Legro RS, Urbanek M, Dunaif A, Hayes MG. Family-based quantitative trait metaanalysis implicates rare noncoding variants in DENND1A in polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2019; 104(9):3835–50.
17. Crisosto N, Codner E, Maliqueo M, et al. AntiMüllerian hormone levels in peripubertal daughters of women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2007; 92(7):2739–43.
18. Crisosto N, Ladrón de Guevara A, Echiburú B, et al. Higher luteinizing hormone levels associated with antimüllerian hormone in postmenarchal daughters of women with polycystic ovary syndrome. *Fertil Steril.* 2019; 111(2):381–8.
19. Walters KA, Allan CM, Handelsman DJ. Rodent models for human polycystic ovary syndrome. *Biol Reprod.* 2012 May 10;86(5):149, 1–12. doi: 10.1095/biolreprod.111.097808. PMID: 22337333.
20. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. *Clin Biochem.* 2001 Jul;34(5):407–13. doi: 10.1016/s0009-9120(01)00245-4. PMID: 11522279.
21. Duleba AJ, Dokras A. Is PCOS an inflammatory process? *Fertil Steril.* 2012 Jan;97(1):7–12. doi: 10.1016/j.fertnstert.2011.11.023. PMID: 22192135; PMCID: PMC3245829.
22. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. *Fertil Steril.* 2003 Jul;80(1):123–127. DOI: 10.1016/s0015-0282(03)00571-5.
23. Colín-González AL, Santana RA, Silva-Islas CA, Cháñez-Cárdenas ME, Santamaría A, Maldonado PD. The antioxidant mechanisms underlying the aged garlic extract- and S-allylcysteine-induced protection. *Oxid Med Cell Longev.* 2012;2012:907162. doi: 10.1155/2012/907162. Epub 2012 May 17. PMID: 22685624; PMCID: PMC3363007.
24. Baillargeon JP, Nestler JE. Commentary: polycystic ovary syndrome: a syndrome of ovarian hypersensitivity to insulin? *J Clin Endocrinol Metab.* 2006; 91(1):22–4.
25. Nestler JE, Powers LP, Matt DW, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1991; 72(1):83–9.
26. Dahlgren E, Janson PO. Polycystic ovary syndrome-long-term metabolic consequences. *Int J Gynaecol Obstet.* 1994 Jan;44(1):3–8. doi: 10.1016/0020-7292(94)90015-9. PMID: 7907056.
27. Roy S, Mahesh VB, Greenblatt RB. Effect of dehydroepiandrosterone and delta4-androstenedione on the reproductive organs of female rats: production of cystic changes in the ovary. *Nature* 1962; 196:42–3.
28. Wang D, Wang W, Liang Q, et al. DHEA-induced ovarian hyper-fibrosis is mediated by TGF- β signaling pathway. *J Ovarian Res.* 2018; 11:1–11.
29. Liu W, Liu W, Fu Y, Wang Y, Zhang Y. BakFoong pills combined with metformin in the treatment of a polycystic ovarian syndrome rat model. *Oncol Lett.* 2015;10:1819–1825
30. Jang M, Lee MJ, Lee JM, et al. Oriental medicine Kyung-Ok-Ko prevents and alleviates dehydroepiandrosterone-induced polycystic ovarian syndrome in rats. *PLoS ONE.* 2014; 9:e87623.
31. Cussons AJ, Watts GF, Burke V, Shaw JE, Zimmet PZ, Stuckey BG. Cardiometabolic risk in polycystic ovary syndrome: a comparison of different approaches to defining the metabolic syndrome. *Hum Reprod* 2008;23: 2352–8
32. Trivax B, Azziz R. Diagnosis of polycystic ovary syndrome. *ClinObstetGynecol*2007; 50:168–77.

33. Apter D, Butzow T, Laughlin GA, Yen SS. Accelerated 24-hour luteinizing hormone pulsatile activity in adolescent girls with ovarian hyperandrogenism: relevance to the developmental phase of polycystic ovarian syndrome. *J Clin Endocrinol Metab* 1994;79:119–25.
34. Anderson E, Lee GY, O'Brien K. Polycystic ovarian condition in the dehydroepiandrosterone-treated rat model: hyperandrogenism and the resumption of meiosis are major initial events associated with cystogenesis of antral follicles. *Anat Rec* 1997; 249:44–53.
35. Misugi T, Ozaki K, El Beltagy K, Tokuyama O, Honda K, Ishiko O. Insulin-lowering agents inhibit synthesis of testosterone in ovaries of DHEA-induced PCOS rats. *Gynecol Obstet Invest.* 2006;61(4):208–15. doi: 10.1159/000091496. Epub 2006 Feb 13. PMID: 16479139.
36. Brawer JR, Munoz M, Farookhi R. Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. *Biol Reprod.* 1986; 35:647–655.
37. Seow KM, Ting CH, Huang SW, Ho LT, Juan CC. The use of dehydroepiandrosterone-treated rats is not a good animal model for the study of metabolic abnormalities in polycystic ovary syndrome. *Taiwan J Obstet Gynecol.* 2018 Oct;57(5):696–704. doi: 10.1016/j.tjog.2018.08.015. PMID: 30342654.
38. Zhang H, Yi M, Zhang Y, Jin H, Zhang W, Yang J, Yan L, Li R, Zhao Y, Qiao J. High-fat diets exaggerate endocrine and metabolic phenotypes in a rat model of DHEA-induced PCOS. *Reproduction.* 2016 Apr;151(4):431–41. doi: 10.1530/REP-15-0542. Epub 2016 Jan 26. PMID: 26814210
39. Beloosesky R, Gold R, Almog B, Sasson R, Dantes A, Land-Bracha A, et al. Induction of polycystic ovary by testosterone in immature female rats: modulation of apoptosis and attenuation of glucose/insulin ratio. *Int J Mol Med* 2004; 14:207–15.
40. Wu C, Lin F, Qiu S, Jiang Z. The characterization of obese polycystic ovary syndrome rat model suitable for exercise intervention. *PLoS One.* 2014 Jun 6;9(6):e99155. doi: 10.1371/journal.pone.0099155. PMID: 24905232; PMCID: PMC4048306.
41. Kalhori Z, Soleimani Mehranjani M, Azadbakht M, Shariatzadeh MA. Ovary stereological features and serum biochemical factors following induction of polycystic ovary syndrome with testosterone enanthate in mice: An experimental study. *Int J Reprod Biomed.* 2018 Apr;16(4):267–274. PMID: 29942935; PMCID: PMC6004592.
42. Ota H, Fukushima M, Maki M. Endocrinological and histological aspects of the process of polycystic ovary formation in the rat treated with testosterone propionate. *Tohoku J Exp Med* 1983; 140:121–31.
43. Mannerås L, Cajander S, Holmång A, et al. A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. *Endocrinology.* 2007; 148:3781–91.
44. Keller J, Mandala M, Casson P, Osol G. Endothelial dysfunction in a rat model of PCOS: Evidence of increased vasoconstrictor prostanoid activity. *Endocrinology.* 2011; 152:4927–36.
45. Liu JH, Garzo G, Morris S, Stuenkel C, Ulmann A, Yen SS. Disruption of follicular maturation and delay of ovulation after administration of the antiprogesterone RU486. *J Clin Endocrinol Metab* 1987; 65:1135–40.
46. Ruiz A, Aguilar R, Tebar AM, Gaytan F, Sanchez-Criado JE. RU486-treated rats show endocrine and morphological responses to therapies analogous to responses of women with polycystic ovary syndrome treated with similar therapies. *Biol Reprod* 1996; 55:1284–91.
47. Corbin CJ, Trant JM, Walters KW, Conley AJ. Changes in testosterone metabolism associated with the evolution of placental and gonadal isozymes of porcine aromatase cytochrome P450. *Endocrinology* 1999; 140:5202–10.
48. Sommers SC. Polycystic ovaries revisited. In: Fenoglio CM, Wolff M, editors. *Progress in surgical pathology.* New York: Masson Publishing; 1980:221–32.
49. Erickson GE, Yen SS. The polycystic ovary syndrome. In: Adashi EY, Leung PCK, editors. *The ovary.* New York: Raven Press; 1993:561–79.
50. Kondo M, Osuka S, Iwase A, Nakahara T, Saito A, Bayasula, Nakamura T, Goto M, Kotani T, Kikkawa F. Increase of kisspeptin-positive cells in the hypothalamus of a rat model of polycystic ovary syndrome. *Metab Brain Dis.* 2016 Jun;31(3):673–81. doi: 10.1007/s11011-016-9807-0. Epub 2016 Feb 11. PMID: 26864582.
51. Sánchez-Criado JE, Sánchez A, Ruiz A, Gaytán F. Endocrine and morphological features of cystic ovarian condition in antiprogesterone RU486-treated rats. *Acta Endocrinol (Copenh).* 1993 Sep;129(3):237–45. doi: 10.1530/acta.0.1290237. PMID: 8212989.
52. Simard M, Brawer JR, Farookhi R. An intractable, ovary-independent impairment in hypothalamo-pituitary function in the estradiol-valerate-induced polycystic ovarian condition in the rat. *Biol Reprod.* 1987 Jun;36(5):1229–37. doi: 10.1095/biolreprod36.5.1229. PMID: 3113503.)
53. Dikmen A, Ergenoglu AM, Yeniel AO, Dilsiz OY, Er-can G, Yilmaz H. Evaluation of glycemic and oxidative/antioxidative status in the estradiol valerate-induced PCOS model of rats. *Eur J Obstet Gynecol Reprod Biol.* 2012 Jan;160(1):55–9. doi: 10.1016/j.ejogrb.2011.09.042. Epub 2011 Nov 8. PMID: 22071112.
54. Linares R, Rosas G, Vieyra E, Ramírez DA, Velázquez DR, Espinoza JA, Morán C, Domínguez R, Morales-Ledesma L. In Adult Rats With Polycystic Ovarian Syndrome, Unilateral or Bilateral Vagotomy Modifies the Noradrenergic Concentration in the Ovaries and the Celiac Superior Mesenteric Ganglia in Different Ways. *Front Physiol.* 2019 Oct 22;10:1309. doi: 10.3389/fphys.2019.01309. PMID: 31695622; PMCID: PMC6817458.
55. Schulster A, Farookhi R, Brawer JR. Polycystic ovarian condition in estradiol valerate-treated rats: spontaneous changes in character-istic endocrine features. *Biol Reprod.* 1984; 31:587–93.

56. Karimzadeh L, Nabiuni M, Sheikholeslami A, Irian S. Bee venom treatment reduced C-reactive protein and improved follicle quality in a rat model of estradiol valerate-induced polycystic ovarian syndrome. *J Venom Anim Toxins Incl Trop Dis*. 2012; 18:384-92.
57. Diamanti-Kandarakis E. Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. *Expert Rev Mol Med* 2008; 10:e3.
58. Baravalle C, Salvetti NR, Mira GA, Pezzone N, Ortega HH. Microscopic characterization of follicular structures in letrozole-induced polycystic ovarian syndrome in the rat. *Arch Med Res*. 2006; 37:830-39.
59. Maliqueo M, Sun M, Johansson J, et al. Continuous administration of a P450 aromatase inhibitor induces polycystic ovary syndrome with a metabolic and endocrine phenotype in female rats at adult age. *Endocrinology*. 2013; 154(1):434-45.
60. Fulghesu AM, Angioni S, Frau E, Belosi C, Apa R, Mioni R, et al. Ultrasound in polycystic ovary syndrome—the measuring of ovarian stroma and relationship with circulating androgens: results of a multicentric study. *Hum Reprod* 2007; 22: 2501–8.
61. Kalsbeek, A., Fliers, E., Romijn, J. A., La Fleur, S. E., Wortel, J., Bakker, O., Endert, E., and Buijs, R. M. (2001) The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142, 2677–2685
62. Scheer, F. A., Kalsbeek, A., and Buijs, R. M. (2003) Cardiovascular control by the suprachiasmatic nucleus: neural and neuroendocrine mechanisms in human and rat. *Biol. Chem.* 384,697–709
63. Scheer, F. A., Ter Horst, G. J., van der Vliet, J., and Buijs, R. M. (2001) Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. *Am. J. Physiol. Heart*
64. Stener-Victorin E, Padmanabhan V, Walters KA, et al. Animal Models to Understand the Etiology and Pathophysiology of Polycystic Ovary Syndrome. *Endocr Rev*. 2020;41(4):538-576. doi:10.1210/edrv/bnaa010
65. Milosević V, Trifunović S, Sekulić M, Sosić-Jurjević B, Filipović B, Negić N, Nestorović N, Manojlović Stojanoski M, Starcević V. Chronic exposure to constant light affects morphology and secretion of adrenal zona fasciculata cells in female rats. *Gen Physiol Biophys*. 2005 Sep;24(3):299-309. PMID: 16308425
66. Ryu Y, Kim SW, Kim YY, Ku SY. Animal Models for Human Polycystic Ovary Syndrome (PCOS) Focused on the Use of Indirect Hormonal Perturbations: A Review of the Literature. *Int J Mol Sci*. 2019 ;20(11):2720.
67. Nestorović N, Ristić N, Ajdžanović V, Trifunović S, Milošević V. Morphological and Functional Changes of Pituitary GH and PRL Cells Following Prolonged Exposure of Female Rats to Constant Light. *Serbian Journal of Experimental and Clinical Research*. 2021;0(0)
68. Meinhardt UJ, Ho KK. Modulation of growth hormone action by sex steroids. *Clin Endocrinol (Oxf)*. 2006; 65(4): 413-22
69. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. *Physiol Rev*. 2000; 80(4): 1523-631
70. Baldissera SF, Motta LD, Almeida MC, Antunes-Rodrigues J. Proposal of an experimental model for the study of polycystic ovaries. *Braz J Med Biol Res*. 1991;24(7):747-51. PMID: 1823293.
71. Chu W, Zhai J, Xu J, et al. Continuous Light-Induced PCOS-Like Changes in Reproduction, Metabolism, and Gut Microbiota in Sprague-Dawley Rats. *Front Microbiol*. 2020;10:3145. Published 2020 Jan 21. doi:10.3389/fmicb.2019.03145
72. Maganhin CC, Fuchs LF, Simões RS, Oliveira-Filho RM, de Jesus Simões M, Baracat EC, Soares JM Jr. Effects of melatonin on ovarian follicles. *Eur J Obstet Gynecol Reprod Biol*. 2013 Feb;166(2):178-84. doi: 10.1016/j.ejogrb.2012.10.006. Epub 2012 Oct 24. PMID: 23102587.
73. Xuezhi K, Lina J, Xueyong S, "Manifestation of Hyperandrogenism in the Continuous Light Exposure-Induced PCOS Rat Model", *BioMed Res.*, vol. 2015, Article ID 943694, 9 pages, 2015. <https://doi.org/10.1155/2015/943694>
74. Soule SG: Neuroendocrinology of the polycystic ovary syndrome. *Baillieres Clin Endocrinol Metab*. 1996, 10 (2): 205-219. 10.1016/S0950-351X(96)80071-1
75. Zhai, HL., Wu, H., Xu, H. et al. Trace glucose and lipid metabolism in high androgen and high-fat diet induced polycystic ovary syndrome rats. *Reprod Biol Endocrinol* **10**, 5 (2012). <https://doi.org/10.1186/1477-7827-10-5>
76. Roberts JS, Perets RA, Sarfert KS, Bowman JJ, Ozark PA, Whitworth GB, Blythe SN, Toporikova N. High-fat high-sugar diet induces polycystic ovary syndrome in a rodent model. *Biol Reprod*. 2017 Mar;96(3):551-562. doi: 10.1095/biolreprod.116.142786. Epub 2017 Jan 27. PMID: 28203719.
77. Patel R, Shah G. High-fat diet exposure from pre-pubertal age induces polycystic ovary syndrome (PCOS) in rats. *Reproduction*. 2018 Feb;155(2):141-151. doi: 10.1530/REP-17-0584. Epub 2017 Dec 1. PMID: 29196492.