



Review article

Experimental models of polycystic ovary syndrome: An update

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the major endocrine disorders in women. PCOS is a disorder with alterations in the structure as well as functions of female reproductive organs and is also associated with metabolic disorders. Studies on humans have limitations due to ethical issues, hence animal models are used to understand the different aspects of PCOS. Animal models of PCOS aids in studying various facts beginning from etiology to the treatment, hence, several animal models have been developed. Despite of the establishment of several models and a number of studies on PCOS, lacunae exist. This may be due to the failure in selecting a suitable animal model, as all animal models may not exhibit all the key features of the human PCOS condition or may exhibit traits similar to other diseased conditions in addition to the PCOS which should be excluded. Therefore, in this review, we have discussed the different animal models, features they exhibit, their merits and limitations which may aid in the selection of the relevant animal model of PCOS based upon the investigation's focus. In addition, a few nonmammalian models as an alternative to mammalian models have also been discussed which is to be validated further.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common multifactorial endocrine and metabolic disorder in females. In the year 1935, two great researchers Stein and Leventhal reported about the ovarian morphology and clinical findings in women and coined the term polycystic ovary syndrome. Henceforth, PCOS is otherwise called as Stein–Leventhal syndrome [1–4]. It is affecting about 5–20% women of reproductive age as well as premenopausal women [5]. In recent years, PCOS is one of the major causes of infertility or poor fertility [6,7]. PCOS women may show few or all features of the condition viz., hyperandrogenism, anovulation, oligomenorrhea, insulin resistance and presence of cystic follicles.

At a meeting of the National Institute of Health in 1990, experts established and concluded the diagnostic criteria for both research and clinical purposes [8], i.e., PCOS is a condition with the combination of oligomenorrhea or amenorrhea and hyperandrogenism in the absence of nonclassical adrenal hyperplasia, hyperprolactinemia, and thyroid dysfunction. Further, in 2003 during Rotterdam consensus workshop group meeting, the criteria for PCOS diagnosis was revised by the experts as the presence of at least 2 of the three criteria, i.e., oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and the presence of polycystic ovaries. Furthermore, the Androgen Excess-PCOS (AE-PCOS) Society Task Force in 2009 proposed that

PCOS diagnostic criteria are hyperandrogenism, ovarian dysfunction i.e., oligo-anovulation and/or presence of cystic follicles and exclusion of related disorders [9].

2. Signs and symptoms

The PCOS is characterized by three major features [10],

- Oligomenorrhea: Reproductively normal women will have about 12 menstrual cycles per year. However, PCOS women show irregularity in the cycle with a very few number of cycles (< 10 cycles) per year called oligomenorrhea or absence of cyclicity in the absence of pregnancy or lactation is called amenorrhea. Oligomenorrhea/amenorrhea is associated with infertility and high frequency of miscarriages.
- Hyperandrogenism: In females, about 60% from ovarian theca cells and 40% from adrenal zona reticularis secrete androgen [11], comparatively lesser amount than that of male system. In PCOS women, the androgen level is elevated compared to normal women. This may be due to various reasons. Clinical hyperandrogenism is evident by the appearance of acne, hirsutism, etc.
- Presence of cystic follicles: Cystic follicles visualized by the ultrasonography. The number, diameter, and size of the cyst were also determined.

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Other minor features found in PCOS women are.

- d) Insulin resistance and compensatory hyperinsulinemia.
- e) Glucose intolerance.

3. PCOS-associated metabolic syndromes

The PCOS is associated with other metabolic syndromes like cardiovascular disorders, obesity, type 2 diabetes mellitus, etc., [12]. Though it is well known that PCOS has above-mentioned health impacts, the exact etiology of the condition is poorly understood.

The cause of pathology is multifactorial and includes factors like environmental, genetic abnormalities, hormonal imbalance and sedentary lifestyle [13]. Owing to the ethical limitations on human studies, the various animal models for PCOS have been evolved in recent years. The development of experimental animal models would aid in understanding the pathophysiology of the condition, which is in the dark despite several studies. In addition to the pathophysiological understanding, the best possible treatment for PCOS can be determined, since, the treatment being practiced so far is either symptomatic or to induce ovulation. The present study reviews the merits and limitations of various animal models in understanding the different aspects of PCOS condition.

4. Methods

4.1. Search plan and selection of the study

The review of studies in this line was performed following the guidelines of PRISMA. The literature survey was made thoroughly from 1960 to 2018. Briefly, the key words used to search the literature published in english are polycystic ovary syndrome, polycystic ovarian syndrome, PCOS, PCO phenotype, animal model for PCOS, PCOS models, experimental animal models of PCOS, rodent models of PCOS, nonhuman primate models of PCOS, steroid induced PCOS models, EV induced PCOS model, letrozole induced model, genetic models of PCOS, nonmammalian models of PCOS, *in vitro* models of PCOS, PCOS and zebrafish, PCOS and *Drosophila*, BPA and PCOS, Bisphenol A and PCO, DHT and PCOS, DHEA and PCOS, testosterone and PCOS, stress and PCOS, PCOS and ovary, PCOS and hormone levels, PCOS and insulin resistance, etc.

4.2. Selection criteria and exclusion

About 1236 studies were collected and abstracts were reviewed separately by the authors and excluded the studies not relevant based on following criteria,

- a) if the study does not involve induction of PCOS,
- b) if PCOS is not represented with at least two characteristic features,
- c) if the induction of PCOS is influenced by other conditions like cancer.

After exclusion, about 55 full text references were taken for complete review individually and discussed to conclude. The main points focused during the full text review included the following, author name, year of the study published, animal model used, age at which intervention was done, period, type, mode of intervention and variables assessed – serum levels of testosterone, estrous cyclicity and presence of cystic follicles along with metabolic alterations.

5. Animal models

Hyperandrogenism is the hallmark of PCOS [14]. Hyperandrogenic condition is the culprit behind all the consequences of PCOS condition. Hence, various models were developed to increase the total or free

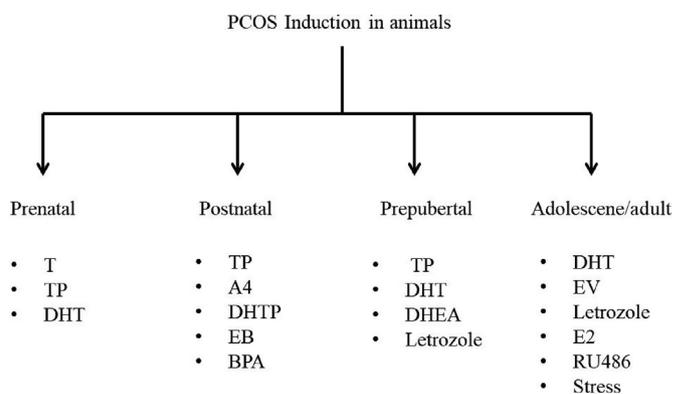


Fig. 1. Flow chart showing the induction of PCOS in an animal model at a different phase of life.

testosterone, which mimic the PCOS condition. The PCOS can be induced at different stages of the development of an organism like prenatal, postnatal, prepubertal and adulthood and it is depicted in the Fig. 1.

5.1. PCOS model induced by environmental factors

It is well established that most of the adverse environmental factors such as varied temperature, irradiation, pesticides, toxic chemicals other than pesticides, endocrine disrupting chemicals (EDCs), etc have various negative health effects. Studies have reported the link between environmental factors and PCOS [15,16]. EDCs are one of the major environmental factors. EDCs are any substance or chemical which affects an individual or an organism by interfering with the synthesis, secretion, transportation, action, metabolism and excretion of endogenous hormones. One such example of EDCs is bisphenol A (BPA). BPA is a synthetic xenoestrogen, used in the production of polycarbonate plastics and epoxy resins [17]. Hence, BPA leaches and reaches our system from the plastic wares used in our daily life, i.e., water bottles, overhead water tank, water pipes, packaging for food and drink [18]. In addition, BPA also reaches our system directly though BPA-free plastics are used since, BPA is also used as the dental sealing agent [18]. Studies have reported that exposure to BPA results in the development of PCOS in rodents. To cite a few, exposure of neonatal rats to high level of BPA (500 µg) resulted in an increased gonadotropin releasing hormone (GnRH) secretory pulse, an increased testosterone concentration and presence of polycystic ovary in adulthood [19]; perinatal exposure of rats [20,21] and mice [22] to BPA resulted in an increased number of antral follicles, the presence of ovarian cysts and reduced number of corpora lutea compared to controls. Further, in sheep, prenatal exposure to BPA showed an increased level of BPA in maternal circulation with reproductive deformities in offspring with elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels similar to that of PCOS women [23]. Furthermore, it is also reported that BPA has been detected in the serum [24,25] and follicular fluid of PCOS women, suggesting the role of BPA in PCOS.

It is well-known that BPA has an estrogenic effect and has an affinity for both alpha and beta receptors of estrogen [26,27]. Further, it is also reported that in addition to the estrogenic effect, BPA also has affinity for androgen receptors and plays a role in the androgen metabolism [28]. This is because, BPA decreases the testosterone catabolism by inhibiting the testosterone hydrolyzing enzymes, i.e., 2-hydroxylase and 4-hydroxylase, which in turn results in the increased level of serum testosterone [29]. Further, BPA stimulates ovarian theca-interstitial cells for the androgen production [30] and it is also a potent steroid hormone binding globulin (SHBG) ligand and hence increase in the concentration of serum BPA replaces the androgen from SHBG and results in a higher level of free testosterone in serum [31]. Furthermore,

Table 1

The PCOS animal models exhibiting the diagnostic features of PCOS women - induced by prenatal androgen treatment.

Animal models of PCOS	Gestation day of intervention	Irregular cyclicity	Oligo-ovulation	Hyper androgenism	Cystic follicles	Insulin resistance	Reference
TP 1 mg (ia) rat	Last 4 days of gestation	-	Yes	-	Yes	-	58
T 3 mg (sc) rat	16th and 19th day of gestation	Yes	Yes	Yes	No	-	56
DHT 3 mg (sc) rat	16th and 19th day of gestation	Yes	Yes	Yes	No	-	56
DHT 250 µg (sc) mouse	Day 16 to day 18 of gestation	Yes	-	Yes	-	-	84

BPA- Bisphenol A; DHEA- Dehydroepiandrosterone; DHT- Dihydrotestosterone; EV- Estradiol valerate; TP- Testosterone propionate; -: Not determined in the publication.

it is also reported that the serum androgen level regulates the BPA concentration. An increased level of androgen downregulates the uridine diphosphate-glucuronosyl transferase activity, a liver enzyme which plays a role in the clearance of BPA from the system/body [32]. These data/studies suggest a significant interaction between androgen and BPA and illustrate the potency of BPA to induce hyperandrogenism.

In addition, BPA also plays a role in the development of PCOS-associated metabolic syndromes. For instance, BPA is known to stimulate pancreatic beta cells for the production of insulin *in vitro*, which may be a cause for insulin resistance [33,34]. BPA may also act as an obesogenic agent, making an individual more prone to obesity [35,36]. This is because, BPA suppresses the release of adiponectin from the adipose tissue, which protects against the insulin resistance [37]. BPA may slow down the metabolism, in turn, decreases the food intake, which further disrupts the insulin signaling [38] and BPA hampers glucose homeostasis as it is reported that adult mice treated with a single dose of BPA showed a decrease in glucose concentration, elevated insulin level and reduced metabolism. This upon prolonged duration resulted in insulin resistance, and compensatory hyperinsulinemia by the action of BPA on pancreatic beta cells [38–40].

Further, Di-(2-ethylhexyl) phthalate (DEHP) is also an endocrine disruptor act as one of the major environmental estrogen. It is reported that DEHP can also induce features similar to PCOS condition as that of BPA. Indeed, couple of studies have shown that exposure of adult female cycling rats to DEHP with 2 g/kg body weight for 12 days resulted in an irregular cyclicity, anovulation, decreased serum estradiol level with a significant increase in the serum FSH levels accompanied by the presence of polycystic follicles compared to controls [41]. The F1 generation offsprings of pregnant mice administered with DEHP perinatally via parenteral route exhibited pathological, genetic and ovarian morphological alterations similar to PCOS [42]. The association between DEHP and PCOS is further confirmed in the detection of DEHP and its metabolites in the serum and urine samples of PCOS women [43].

The mechanism of action of DEHP to induce PCOS is yet to be elucidated clearly. However, it is known that along with estrogen agonist property, DEHP has antiandrogenic property [44]. Additionally, studies have also reported that DEHP can block ovulation by suppressing the transcription of LH-surge response genes [41,45,46]. Further, both *in vitro* and *in vivo* studies have reported that phthalates decrease the mRNA and enzyme levels of aromatase [47–51] an enzyme which convert testosterone to estradiol and maintains hyperandrogenic condition in turn the development of PCOS.

Tributyltin (TBT) is an other endocrine disruptor, widely known for its obesogenic property [52]. Since, obesity is associated with insulin resistance and insulin resistance is one of the features of PCOS, studies have focused on the development of PCOS due to TBT in the recent years. A study by Sena and group [53] reported that administration of TBT to rats showed irregular estrous cycles, hyperandrogenism with few metabolic alterations like elevated serum insulin and leptin levels, however, the serum LH levels were decreased and ovarian morphology with cystic follicles were not observed. The TBT increase the number of fat cells, promote fat accumulation and obesity [35,54]. This in turn lead to insulin resistance and may result in PCOS condition. Although, TBT model exhibit most of the features of PCOS, this model would

mimic obese-PCOS condition, but to understand about lean-PCOS model, this model may not be an ideal one.

To conclude, it is well established that BPA-induced PCOS, but though DEHP and TBT are known to induce PCOS model and exhibit few features of the condition, further studies are required to validate the models for its exploitation in research field.

5.2. PCOS-induced model by hormonal intervention

Altered reproductive hormone levels are one of the characteristic features of the PCOS condition. As mentioned earlier, hyperandrogenism is the hallmark in PCOS women. Hence, hormonal intervention using synthetic hormones, agonists or antagonists, so that it mimics the PCOS features is the concepts for developing alternate animal models for PCOS. Several models have been developed, viz., androgens-induced models, estrogen-related hormones induced models, antiprogestone-induced models, etc.

i. Androgen-induced models:

Though testosterone is the major androgen among major reproductive hormones in both male and female, other androgenic hormones are also present in a significant amount, such as dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT). Thus, testosterone, DHT, DHEA, esters of testosterone (testosterone propionate), etc., are used to induce hyperandrogenism in turn PCOS condition in animals. It is reported that hyperandrogenic environment, both in *in utero* or postnatal condition hampers the normal reproductive physiology and induce PCOS condition. The Table 1 summarise the prenatal androgenized animal models. In addition, *in utero* androgen exposure, i.e., during pregnancy, results in the development of PCOS-associated metabolic syndromes in offsprings [55,56].

i.a) Testosterone or testosterone propionate (TP) induced PCOS models

Testosterone and testosterone propionate is administered in the prenatal or neonatal conditions to induce PCOS. Most of the studies have reported no changes in the estrous cyclicity and ovarian function upon administration of testosterone or testosterone propionate in prenatal mice [57] and rat [58–61], respectively. However, a study by Wu et al. [56] reported an irregular estrous cyclicity, increased serum testosterone, estradiol, progesterone and LH levels accompanied by an increased number of antral and preantral follicles with a significantly decreased number of corpora lutea in rats prenatally (day 16 and 19 of gestation) treated with 3 mg testosterone. Similarly, testosterone treated rats prenatally, during the 20th day of gestation showed features in agreement with the study of Wu et al., [56] along with the presence of cystic follicles [62].

The propionate ester of testosterone is a slow releasing anabolic steroid widely used by athletes and also used in the treatment of breast cancer. Postnatal exposure of 10 mg/kg body weight of TP intraperitoneally to rats showed the characteristic features of PCOS women, i.e., irregular estrous cyclicity, elevated testosterone and insulin levels, presence of cystic follicles with unaltered estradiol levels, reduced progesterone level, blocked ovulation, absence of corpora lutea

compared to controls [63–66]. Further, TP-induced (400 mg) PCOS rats showed an increase in the low-density lipoprotein and very low-density lipoprotein with a concomitant decrease in the high-density lipoprotein indicating the risk of obesity [64]. Furthermore, exposure of rats to 1 mg TP immediately after birth for 5 days showed persistent anovulatory estrus [67], on day 1 or 5 showed acyclicity and the presence of polycystic ovaries, an increase in the number of atretic follicles and elevated levels of testosterone as well as estradiol [68–70]. Though most of the studies report the TP-induced PCOS model during the prepubertal age (around 21 days of age), contrastingly 27 studies reported that rats treated with TP on days between 15–25 did not exhibit the ovarian phenotype similar to PCOS condition.

To sum up, postnatal treatment of rodents with testosterone or TP develops a PCOS model similar to the human condition with reproductive features like altered cyclicity, ovary with cystic follicles, increased testosterone levels, and metabolic features like insulin resistance. However, TP-induced PCOS model lacks detailed evidence of metabolic disruption.

i.b) DHEA-induced PCOS model

The DHEA was the first agent used by scientists to induce PCOS in animals during 1960s. This may be because of an elevated level of DHEA found in PCOS women [71]. Prenatal exposure to DHEA results in the development of PCOS-like features in rats. Wang et al. [72] reported that DHEA injection during the pregnancy for 20 days showed the presence of cystic follicles and elevated levels of testosterone, estradiol, and progesterone in offsprings with compromised fertility. In addition, studies have also reported that the postnatal exposure to DHEA results in the development of PCOS model. A PCOS model produced by injecting with DHEA for 20 days in mice [73] and rat [74,75] exhibited the characteristic features of PCOS. The subcutaneous injection of DHEA to postnatal rats, i.e., at the age of 21 days showed the presence of cystic follicles, elevated plasma testosterone concentration, and increase in the number of atretic follicles compared to that of controls [76]. However, The postnatal treatment of mature mice with 7.5 mg of DHEA for 90 days showed no features of PCOS [77]. Contrastingly, recently Kim et al. [78] reported that administration of DHEA (60 mg/kg bw) by subcutaneous injection to post-pubertal i.e., 42 days old rats for 20–30 days showed improved PCOS model with increased body, ovary and uterine weight, irregular estrous cyclicity as well as more number of ovarian cystic follicles compared to pre-pubertal (21 days old) rats.

Similarly, DHEA treatment showed the presence of cystic follicles, increased number of total follicles and absence of corpora lutea along with the increased incidence of TUNEL-positive follicles compared to controls in immature rats (21 days old) administered with 6 mg/100 g body weight DHEA for 7 or 14 days [79]. Another study on 21 days old Sprague Dawley rats for 20 days showed the key features of PCOS condition along with an elevated fasting blood glucose concentration [80]. Further, with reference to the hormonal levels, the LH was reported to be increased and the FSH level was decreased [81] or both LH and FSH decreased [74] or unchanged in the levels of both gonadotropins [82] in DHEA-treated PCOS model. Also, information on metabolic disturbances in DHEA-induced PCOS model is scarce.

Overall, though DHEA-induced PCOS rodent model exhibited all the key features similar to PCOS women, the cystic follicles in the former were with a thin layer of theca cell layer contrast to thickened theca cell layer in humans [83].

i.c) DHT-induced PCOS model

An enzyme 5 α -reductase irreversibly converts the testosterone synthesized by theca cells of the ovary into DHT. DHT is a non-aromatizable androgen and cannot be converted into estrogen by the action of aromatase, thus enhances the androgen potency. Prenatal

exposure, i.e., exposure of rats (3 mg) [56] and mice (250 μ g) [84] during pregnancy on days 16th, 19th and days 16–18 respectively, showed an increase in the number of preantral and antral follicles with concomitant decrease in the corpora lutea population, elevated levels of serum testosterone and LH. A study by Osuka et al. [85] reported that prenatal exposure of rats to DHT showed elevated levels of LH, irregular estrous cyclicity and normal body weight, whereas, postnatal treatment of DHT showed irregular cyclicity with persistent diestrus, gain in body weight and normal LH levels. They even reported that these contrasting results may be due to elevation in the expression levels of kisspeptin and neurokinin B in the arcuate nucleus. Further, postnatal studies reported that exposure of prepubertal rat (7.5 mg) [86] and mice (2.5 mg) [87] to DHT for 90 days was found to be a better model with features of both reproductive and metabolic disturbances similar to PCOS women. DHT-exposed mice postnatally showed the presence of cystic follicles, increased number of atretic follicles, irregular estrous cycle with higher body weight [77]. It also reported that DHT-exposed rats showed metabolic disturbances associated with PCOS like increased body weight, abdominal fat, elevated cholesterol and leptin levels with insulin resistance [86,88,89]. Disrupted estrous cyclicity with decreased insulin sensitivity and increased body weight was observed in 21-day-old rats implanted with DHT for 12 weeks [90].

DHT-induced PCOS can be used as a model to study the mechanism of altered hormonal regulation and also the ovarian changes. This model is also a suitable model to investigate different aspects of PCOS like ovarian function, pathophysiology, metabolic disturbance, treatment, etc., since this model exhibits most of the reproductive and metabolic alterations of the PCOS condition comparable to PCOS women.

ii) Estrogen-related hormonal intervention

Estrogen is a female sex hormone synthesized in the granulosa cells of the ovary, which is essential for the secondary sexual characters. In PCOS women, serum estrogen levels are found to be elevated. Based on this evidence, estrogen or other forms of estrogen, i.e., estradiol valerate (EV), estradiol benzoate (EB) are used to induce PCOS condition in animal models. For instance, 1-day-old neonatal rats treated with EB showed acyclicity and anovulation. However, the serum LH levels as well as the ovarian weight were decreased and elevated serum FSH levels, contrast to human PCOS condition was observed [91]. In addition, rats exposed to EV (2 mg) failed to exhibit key features of PCOS i.e., rats did not show hypersecretion of LH [92, 93], hyperandrogenism, alteration in the glucose and insulin concentrations contrastly, showing dissimilarity with the human PCOS [94]. Interestingly, adult rats showing regular cycles showed an acyclicity, anovulation, cystic follicles with thin granulosa cell layer and thick theca cell layer similar to cysts observed in PCOS women upon exposure to estradiol implant for 8 weeks [95] or a single injection of 2 mg EV [92,96–98]. In addition, adult rats exposed to EV displayed ovarian and endocrine disruption similar to PCOS women with elevated levels of testosterone, estrogen, LH and a concomitant decrease in FSH and progesterone [99]. This was accompanied by the presence of cystic follicles with thick theca cell layer and decreased number of corpora lutea.

The literature so far suggests the ovarian alterations similar to PCOS women, but the hormonal and metabolic disruptions were not parallel with the PCOS women on exposure to estrogenic conditions.

iii) Letrozole

Letrozole is a nonsteroidal aromatase inhibitor. Aromatase is an enzyme which catalyzes the conversion of androgen to estrogen. The hyperandrogenic condition can be developed not only by administration of exogenous androgens, but also by inhibiting the conversion of testosterone to estradiol or by stimulating the testosterone synthesis. Hence, administration of an aromatase inhibitor blocks the conversion of testosterone to estradiol and maintains the hyperandrogenic

condition (a key feature for the establishment of PCOS model) [100]. Exposure of adult female rats to letrozole exhibited almost all the features of PCOS, i.e., anovulation, acyclicity, elevated levels of testosterone and LH, large follicles with numerous cysts having thin granulosa cell layers and thick theca cells [86,100–105], irregular cyclicity [100] or acyclicity with unchanged estradiol levels [86]. The 21 days old mice treated with letrozole for 90 days exhibited irregular estrous cycles, hyperandrogenic condition with hemorrhagic cysts but no metabolic dysfunction was found [77]. Further, in addition to the reproductive alterations, metabolic disturbance associated with PCOS was also reported in rats treated with letrozole. Rats treated with letrozole displayed elevated levels of glucose, cholesterol, triglyceride [105–107], total protein and globulin [106], increased body weight [74,86,106] and body fat with unaltered insulin sensitivity [86].

Since PCOS model induced by administration of letrozole could exhibit most of both reproductive and metabolic features, this model can be validated for the PCOS studies. However, further studies are required before concluding this as a valid model with respect to the metabolic features and for mechanism exploring studies. This is because, hyperandrogenism, hypersecretion of LH, polycystic ovary, lack of corpora lutea are the features found even in the ER- α (ESR-1) knock-out mice [108–111]. Hence, the key features of PCOS observed in letrozole induced model may be due to impairment in the estrogen action rather than hyperandrogenic condition.

iv) Antiprogestin:

Antiprogestin RU486 is a synthetic steroid also known as mifepristone used widely for abortion during pregnancy, as it has antagonistic potency against progesterone and glucocorticoid receptors. Hence, antiprogestin RU486 antagonizes the action of progesterone in animals/rodents upon treatment and exhibits ovarian and hormonal alterations similar to the PCOS women. Indeed, treatment of adult cycling rats with antiprogestin RU486 resulted in anovulation [112], acyclicity and polycystic ovaries [30,113], elevated levels of serum LH, testosterone and estradiol similar to PCOS women [114]. In addition, though the ovarian and endocrine alterations were exhibited on exposure to antiprogestin similar to human PCOS, they failed to display metabolic disturbances associated with PCOS, as RU486 treatment did not alter the body weight and insulin sensitivity [115]. Hence, further research is required in this line to understand and validate the antiprogestin-induced PCOS models. Furthermore, the effects of RU486 on mice have to be studied since, till date, no data is available on the same.

Table 2

The PCOS animal models exhibiting the diagnostic features of PCOS women - induced by postnatal intervention/treatment.

Animal models of PCOS	Day of intervention	Irregular cyclicity	Oligo-ovulation	Hyper androgenism	Cystic follicles	Insulin resistance	Reference
TP 100 μ g (sc) rat	1 or 5	Yes	Yes	-	Yes	-	[68]
TP 100 μ g (sc) rat	6	Yes	Yes	Yes	Yes	-	[175]
DHT 7.5 mg Pellet rat	21-110	Yes	Yes	No	Yes	Yes	[86]
DHEA 6 mg/100 g bw (sc) rat	27-46	Yes	Yes	Yes	Yes	-	[71]
DHEA 6 mg/100 g bw (sc) rat	27-46	Yes	Yes	-	Yes	-	[75]
DHEA 6 mg/100 g bw (sc) mouse	25-44	Yes	-	-	Yes	-	[83]
DHEA 6 mg/100 g bw (sc) mouse	25-44	Yes	-	-	Yes	Yes	[73]
DHEA 4.5/6 mg/100 g bw (sc)	25-44	-	Yes	Yes	Yes	-	[176]
EV 2 mg (sc) rat	1 injection in young cycling rat	Yes	Yes	-	Yes	-	[92]
EV 2, 4 mg (im) rat	1 injection in young cycling rat	Yes	Yes	No	Yes	-	[96,94]
E2 2 mm long filled implant rat	Chronic exposure	Yes	Yes	-	Yes	-	[95]
Letrozole 0.1, 0.5 mg/kg (po) rat	21 consecutive days in adults	Yes	Yes	Yes	Yes	-	[100]
Letrozole 1 mg/kg bw (po) rat	21 consecutive days in adults	-	Yes	Yes	Yes	-	[101,102]
Letrozole 36 mg pellet rat	Day 21–110	Yes	Yes	Yes	Yes	-	[86]
RU486 4 mg (sc) rat	4 consecutive days	Yes	Yes	Yes	Yes	-	[177,178]
RU 486 2 mg (sc) rat	8 consecutive days	Yes	Yes	Yes	Yes	-	[113]
RU 486 2 mg/100 g bw (sc) rat	7-9 consecutive days	Yes	Yes	Yes	Yes	-	[115]
RU 486 4 mg/100 g bw (sc) rat	9 consecutive days	Yes	-	-	Yes	-	[30]

BPA- Bisphenol A; DHEA- Dehydroepiandrosterone; DHT- Dihydrotestosterone; EV- Estradiol valerate; TP- Testosterone; propionate; -: Not determined in the publication.

The Table 2 outlines the PCOS animal models due to hormonal interventions.

5.3. Nonhuman primate models of PCOS

The PCOS models have also been developed using nonhuman primates by hormonal interventions. Monkeys prenatally treated with testosterone can be used as models of PCOS as they resembles human in the follicular differentiation [116]. The exposure of female monkeys to hyperandrogenic condition during late gestation (100–110 gestation days) also exhibits an adult PCOS-like phenotype, but they do not have obvious abnormalities in LH and insulin secretion or in insulin action [117]. Similarly, fetuses of dams treated with testosterone during the gestation period day 40–44 showed irregular menstrual cycles, elevated levels of ovarian androgen, hypersecretion of LH, and insulin resistance [117,118]. Acute exposure of normal adult female monkeys to testosterone mimic the accelerated early follicular development as found in women with PCOS which is associated with a diminished intraovarian expression of anti-Mullerian hormone [119]. Further, the adult female monkeys chronically exposed to testosterone (10/25 mg) [120], androstenedione [121] or estrone [122] showed hyperandrogenism, irregular estrous cyclicity, however, did not observe PCOS-like ovarian morphology [123]. In addition to testosterone, studies have also reported that the female monkeys exposed to DHT showed a delay in the age at menarche for about 6 months and adults showed irregular menstrual cycles [124,125].

5.4. PCOS induced model by lifestyle

Among the several causative factors of various metabolic syndromes, lifestyle is one of the major factors in this modern society. Lifestyle plays a very important role in an individual's healthy life. In this modern lifestyle, on the one hand, the youths or adolescents are attracted by fast food or food rich in cheese, fried potatoes, refined starch, sugar added beverages, saturated fats and with less fiber. These food styles in addition to increasing the risk of the development of obesity, compromise the diet quality and alters the energy intake [126]. On the other hand, due to competitive and fast-paced society, individuals leading a stressful life inevitably.

In the United States, studies have reported that an individual who consumes junk food consumes more calorie [127,128], which in turn may lead to obesity and other metabolic syndromes including PCOS. Indeed, few studies have reported that PCOS women present a

significantly increased visceral fat compared to normal women [129]. Obesity, sedentary lifestyle, unhealthy food habits, sugar-sweetened beverages are the causative factors of PCOS condition in young and adult girls/women in the present society [130]. Further, recently it has become common for women to work in night shifts, which may alter the hormonal rhythm. Studies have reported that the presence of cystic follicles upon exposure to constant light (24 h) for 74 h [131] and 105 days [132]. Hence, exposure to constant light due to night shift work regime for prolonged duration increases the risk of development of PCOS in women. However, studies have not reported the serum testosterone concentration in constant light exposed model for PCOS, which limits the use of this model for PCOS studies, though it shows other hormonal alterations similar to that of PCOS women along with cystic follicles. This model is also not suitable to study the PCOS-related metabolic disorders as they do not exhibit the same.

An unhealthy diet and sedentary lifestyle can be modified and managed to improve the metabolic health; however, stress, an unescapable factor can not be avoided in this competitive world. An individual is exposed to stressful conditions in their day-to-day life for one or the other reason. Several studies have reported the deleterious effects of stress on one's health. Further, it also reported that chronic stress results in the development of PCOS condition in rats. For instance, exposure to chronic intermittent cold stress for 3 weeks [133,134], 28 days [134–137], cold restraint [138], and restraint and forced swimming exercise for 12 weeks [139] resulted in the development of PCO phenotype.

The stress-induced PCOS may be due to the over stimulation of sympathetic nerve activity of the ovary [135,138]. In addition, Paredes et al. [133] had suggested that the extraovarian factor is acting at the ovarian level to control the release of norepinephrine from the ovary and maintains the ovarian cysts. Further, stress-induced PCOS may also be due to the activation of the hypothalamus-pituitary-adrenal axis, as reported by Divyashree and Yajurvedi, [139]. According to the study, chronic stress for 12 weeks significantly increased the concentration of serum corticosterone, fasting blood glucose and serum insulin levels, accompanied by the presence of cystic follicles and an increase in the number of healthy antral follicles. The study suggested that increased glucocorticoid levels due to stress lead to the hyperglycemic condition. Further, prolonged hyperglycemic condition resulted in an insulin resistant and compensatory hyperinsulinemia which might have finally stimulated the ovarian theca cells for the synthesis of androgen in higher amounts. All these alterations have led to the development of PCOS in the female rat [139].

5.5. PCOS induced model by genetic abnormalities/mutation

In addition to the hormonal and chemical models, several models of genetic mutations have been developed. PCOS women exhibited altered levels of few reproductive and metabolic hormones. Based on which, a genetically mutated model that mimics/exhibit features of the PCOS condition were studied.

For instance, luteinizing hormone (LH) level is elevated in PCOS women [140], hence animal model with LH over expression should rationally satisfy the condition of PCOS and this was investigated by the scientists. As LH stimulates the theca cells for the synthesis and production of androgens, in a LH-overexpressing transgenic mice model [Tg (Cga-LHB/CGB)94Jhn/J], overexpression of LH β subunit exhibited an increase in the testosterone levels with concomitant increase in the estradiol levels with the presence of polycystic ovaries [141,142]. In addition to the characteristic features of PCOS, the above-said transgenic mice also exhibited the metabolic disturbances associated with PCOS, such as elevated insulin levels, obesity and increased abdominal fat [143]. However, this model has limitations that elevated LH levels also exhibited other features including ovarian tumor, increase in the number of corpora lutea, etc., which may interfere with the study related to PCOS.

Similarly, leptin, a hormone synthesized from the adipose tissue is found to be elevated in PCOS women [144] and it is reported that leptin directly stimulates the GnRH secretion [145], which in turn may alter the LH levels and induce PCOS condition. Abnormal GnRH regulation is one of the features of a PCOS condition in women [146,147].

A mouse model with a mutation in the leptin gene (*ob/ob*) which lack endogenous leptin gene showed features similar to that of PCOS women. The *ob/ob* obese female model exhibited acyclicity, anovulation, infertility and increased follicular atresia accompanied by an elevated level of testosterone, estrogen, and progesterone [148] and decreased FSH level [149] similar to that of PCOS women. Likewise, another mouse model of diabetes (*db/db*) lack functional receptors for leptin exhibited characteristic features of PCOS condition similar to that of *ob/ob* mice model, but the serum estradiol and progesterone levels were decreased in *db/db* model in contrast to *ob/ob* model.

In addition, both the *ob/ob* and *db/db* models showed metabolic features found in PCOS women viz., obesity, hyperglycemia, hyperinsulinemia, glucose intolerance [150–153]. However, limitations of these two mutated models for leptin are that both models failed to show polycystic ovary and *ob/ob* model showed no significant change in the LH levels.

Another obese model which exhibit insulin resistance and hyperinsulinemia, the common metabolic features of PCOS is New Zealand obese mouse (*NZO/HLLt*). NZO obese mice though have normal leptin and leptin receptor genes, but have a deficiency in the leptin transport through blood-brain-barrier [154]. NZO obese mice exhibited metabolic features similar to PCOS, reduced LH and elevated serum estradiol levels but failed to show the hallmark of PCOS condition, i.e., hyperandrogenism, increased LH levels and presence of cystic follicles [154].

In addition to letrozole, the inhibition or suppression of aromatase activity/levels can be achieved by aromatase knock out (ArKO) model. The ArKO mice model showed elevated serum LH levels, cystic follicles and decreased serum estradiol concentration [155–158]. The ArKO model also exhibited metabolic alterations like dyslipidemia, high leptin levels, enlarged adipocytes etc., and the metabolic disturbances were normalized upon estrogen supplementation but not the ovarian changes [159,160]. The elevation in the serum LH level due to aromatase gene knock out stimulate androgen synthesis and hyperandrogenic condition mediates the development of cystic follicles [156].

Inhibin is a gonadal protein, which stimulate the FSH synthesis and secretion [161,162]. An increase in the size and number of ovarian follicles was observed upon injection of inhibin into rat ovarian bursa [163]. A positive association was found between elevated inhibin α -sub unit and PCOS [164,165]. Further, McMullen et al. [166] reported that a transgenic mice with overexpression of inhibin α -sub unit showed the presence of cystic follicles, elevated serum testosterone concentration and reduced estradiol level similar to that of PCOS. This model is yet to be validated for further usage as a PCOS model.

To conclude, the genetically mutated models, though show few features of PCOS conditions, these models can be employed to investigate the pathogenesis, treatment and associated metabolic disorders. However, alterations in the ovarian tissues cannot be concluded since, they may exhibit alterations not associated with PCOS like ovarian tumors in the LH overexpressing model.

5.6. Alternate/non-mammalian models

In addition to the animal (mammalian) models for PCOS, recently various other models are being developed with less complexity. As mentioned earlier, animal models have been developed since human studies have ethical limitations. However, even animal studies should also go through the ethical clearance and CPCSEA guidelines should be followed. Interestingly, non-mammalian models are being used, such as cell culture model, *Drosophila melanogaster* and Zebrafish as an alternate model.

D. melanogaster is used as a model organism in various research

Table 3
The summary of animal PCOS models exhibiting the diagnostic features of PCOS women.

Animal models of PCOS	Irregular cyclicity/acyclicity	Oligoovulation/anovulation	Hyperandrogenism	Cystic follicles	Insulin resistance	Obesity
BPA	Yes	Yes	Yes	Yes	*	*
TP	Yes	Yes	Yes	Yes	Yes	*
DHEA	Yes	Yes	Yes	Yes	Yes	No
DHT	Yes	Yes	No	Yes	Yes	Yes
EV	Yes	Yes	No	Yes	No	Yes
Letrozole	Yes	Yes	Yes	Yes	Yes	Yes
RU486	Yes	Yes	Yes	Yes	No	No
Constant light	Yes	Yes	*	Yes	*	*
Stress	Yes	Yes	Yes	Yes	Yes	*

BPA- Bisphenol A; DHEA- Dehydroepiandrosterone; DHT- Dihydrotestosterone; EV- Estradiol valerate; TP- Testosterone propionate; * - Not known.

areas, such as aging [167], neurological disorders [168], developmental [169] studies, etc., since *D. melanogaster* shares about 61% of disease genes with humans. The starvation model of *D. melanogaster* is postulated as a model for PCOS [170]. In PCOS women, testosterone levels are elevated. Similarly, starved fruit flies exhibited an elevation in the ecdysone and its active form 20-hydroxyecdysone homologous hormones with testosterone and 20-hydroxytestosterone, respectively. Indeed, ecdysone and its active form 20-hydroxyecdysone are from the ovarian tissue and plays a role in the follicular development. The other feature of human PCOS is anovulation, which can be determined by monitoring the fecundity of the flies. They found reduced fecundity in starved flies compared to controls [170].

Further, another widely used model organism for various studies is Zebrafish. In addition to the toxicological [171] and fertility [172] studies in Zebrafish, it is hypothesized that this organism can be used as a model to the PCOS. A *gsdf* gene that codes for gonad somatic cell derived factor, controls the ovarian follicle proliferation [173]. A mutation in *gsdf* gene mimics the human PCOS in Zebrafish [174]. This model exhibited premature fertility loss, accumulation of young oocytes, elevated androgen levels, obesity, etc [173].

Furthermore, an *in vitro* model was also proposed using the cell line as a model for human PCOS condition. Here, immortalized steroidogenic granulosa cell lines or primary culture of granulosa cells can be employed, and treatment with a higher concentration of testosterone or insulin may satisfy the condition. However, this model can be used to understand the endocrine abnormalities, mechanism of actions of various drugs or natural herbal extracts or associated disturbances.

To conclude, though these models being postulated and studies are being carried out, more light needs to be shed, and these models are yet to be validated for the use as an alternate for human PCOS model.

6. Conclusion

The present review discussed the different experimental models mimicking key features of PCOS women. The Fig. 1 shows the induction of PCOS condition in animal models using various interventions at different phases/stages of life. Further, the Table 3 summarizes the key diagnostic features of PCOS condition exhibited by different animal models discussed in this review. Though all the experimental models discussed in this review showed features of PCOS women, the androgenized PCOS model is more reliable compared to others. Further, this is the first study to review the BPA, DEHP, TBT-induced model as PCOS model based on the evidence from the literature. Furthermore, recently non-mammalian models have also been established as alternate to PCOS models, which is to be validated in the studies.

7. Future perspectives of investigation

As mentioned earlier, animal models are assisting in understanding the facts about PCOS. Similarly, suitable models should be exploited to determine the best medicine for the same which helps in the cure of

PCOS instead of symptomatic treatment. Further, it is also necessary to shed light on the role of other EDCs other than BPA, like phthalates, dioxin, dioxin-like compounds, polychlorinated biphenyls, etc., in the pathogenesis of PCOS if any. Furthermore, validation of non-mammalian models discussed in this review may also improve the understanding of PCOS.

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Declaration of competing interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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