Rodent Models of Polycystic Ovary Syndrome

BIOLOGY OF REPRODUCTION (2012) **86**(5):149, 1–12
Published online before print 15 February 2012.
DOI 10.1095/biolreprod.111.097808

Minireview

Rodent Models for Human Polycystic Ovary Syndrome[1]

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most frequent female endocrine disorder, affecting 5%–10% of women, causing infertility due to dysfunctional follicular maturation and ovulation, distinctive multicystic ovaries and hyperandrogenism, together with metabolic abnormalities including obesity, hyperinsulinism, an increased risk of type 2 diabetes, and cardiovascular disease. The etiology of PCOS is unclear, and decisive clinical studies are limited by ethical and logistic constraints. Consequently treatment is palliative rather than curative and focuses on symptomatic approaches. Hence, a suitable animal model could provide a valuable means with which to study the pathogenesis of the characteristic reproductive and metabolic abnormalities and thereby identify novel and more effective treatments. So far there is no consensus on the best experimental animal model, which should ideally reproduce the key features associated with human PCOS. The prenatally androgenized rhesus monkey displays many characteristics of the human condition, including hyperandrogenism, anovulation, polycystic ovaries, increased adiposity, and insulin insensitivity. However, the high cost of nonhuman primate studies limits the practical utility of these large-animal models. Rodent models, on the other hand, are inexpensive, provide well-characterized and stable genetic backgrounds readily accessible for targeted genetic manipulation, and shorter reproductive life spans and generation times. Recent rodent models display both reproductive and metabolic disturbances associated with human PCOS. This review aimed to evaluate the rodent models reported to identify the advantages and disadvantages of the distinct rodent models used to investigate this complex endocrine disorder.

animal models, fertility, follicular development, ovary, PCOS

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common causes of anovulation, infertility, and hyperandrogenism in women, affecting 5%–10% of women of reproductive age worldwide [1]. PCOS in women is characterized by reduced fertility, due to dysfunctional follicular maturation and ovulation and miscarriage, dysregulation of reproductive hormones including luteinizing hormone (LH) hypersecretion and hyperandrogenism, causing acne and hirsutism [1–4]. Women with PCOS also often exhibit nonreproductive metabolic abnormalities such as obesity, metabolic syndrome, hyperinsulinemia, insulin resistance, dyslipidemia with an increased risk of cardiovascular disease, and type 2 diabetes [3–5] (Fig. 1). Yet, despite its prevalence and health impact, the etiology of PCOS remains poorly understood. In particular, whether reproductive hormone abnormalities are a primary or secondary reflex remains enigmatic. Etiological hypotheses for the origins of PCOS include hormonal imbalances, epigenetic changes in fetal life, genetic abnormalities, lifestyle, and environmental factors [3, 4]. The heterogeneity of PCOS and lack of consensus on a universally accepted PCOS diagnosis make the unraveling of the etiology and development of optimal or curative treatment of PCOS difficult. Due to the logistic and ethical limitations on human experimentation, appropriate animal models that mimic many or all PCOS traits would facilitate research, leading to improved understanding of the pathogenesis of PCOS and potential for innovative and curative treatments for the PCOS syndrome.

Presently there are 3 different definitions of the clinical diagnostic criteria used to define PCOS is women. The National Institute of Child Health and Human Development Conference in 1990 advised that in order of importance, diagnostic criteria should be defined as hyperandrogenism, menstrual dysfunction, and the exclusion of other known factors [6]. According to the 2003 Rotterdam consensus criteria, the presence of 2 of 3 of oligo-ovulation or anovulation, hyperandrogenism (clinical or biochemical or both), and polycystic ovaries fulfills a diagnosis of PCOS [7]. Whereas in 2006, the Androgen Excess-PCOS Society recommended that PCOS should be defined by the presence of hyperandrogenism and/or oligo-ovulation and polycystic ovaries and the exclusion of other related disorders [8, 9]. The morphological criteria for a diagnosis of polycystic ovaries is based on ultrasonographic data where patients exhibit ovarian enlargement, a thickened outer tunica albuginea, more than 12 follicles per ovary with a diameter of 2 to 10 mm, and an increased density and area of stroma [1, 10, 11]. Human PCOS ovaries also exhibit an increase in the numbers of growing preantral and antral follicles and an arrest in mid-antral follicle growth, which leads to antrum expansion, increased granulosa cell degeneration, and development of cystic follicles with thin granulosa cell walls [10, 12]. On the other hand, the layer of theca cells that surround the follicle is much thicker than in normal follicles [10]. Ideally, animal models of human conditions, such as PCOS, should replicate many or most clinical characteristics of that disorder. Since the 1960s, a range of animal models, including rodents, sheep, and non-human

[1]Supported by National Health and Medical Research Council grants 632678 and APP1022648, Australia.
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Received: 15 November 2011.
First decision: 18 December 2011.
Accepted: 8 February 2012.

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

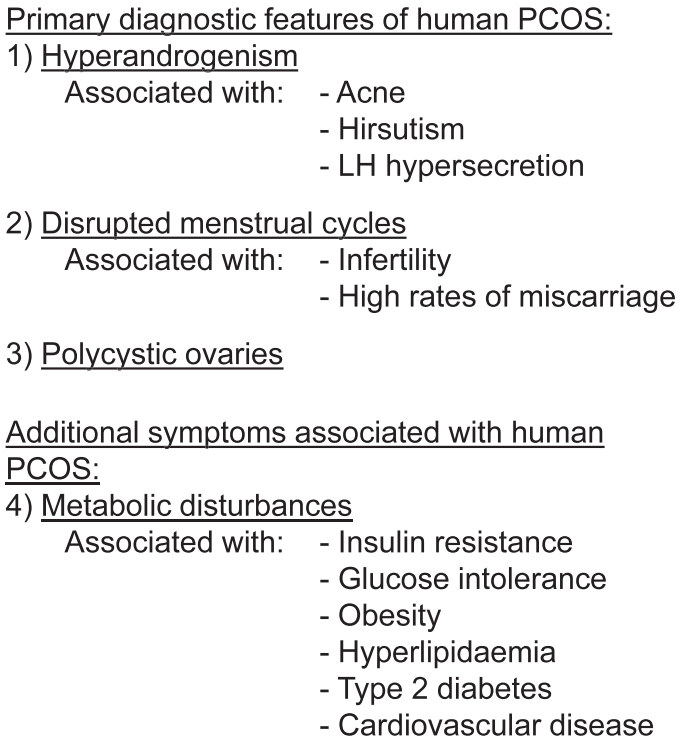

FIG. 1. Primary diagnostic features of human PCOS and common additional symptoms of the condition.

primates, have been used to study the origins and pathology of this condition [13–16]. These models have advanced our understanding of the pathogenesis of PCOS; however, at present, a convincing whole-animal model representing all features associated with human PCOS has not been established. However, a range of characteristics similar to those seen in women with PCOS have been described in distinct animal models. Prenatal exposure of sheep and non-human primates to androgens has provided models that show striking similarities to women with PCOS [15, 17, 18]; however, these models are extremely expensive and are not readily adaptable to the use of genetic manipulations. Rodent models provide a versatile tool for deciphering the precise biological mechanism(s) associated with the development of PCOS. Among the numerous advantages of using rats and mice over other animal species used as in vivo models include their stable genetic backgrounds, ease of handling and maintenance, shorter reproductive lifespan and generation times, short estrous cycles, feasibility of genetic manipulations, and affordability.

The strong evolutionarily conserved similarities in regulation of reproductive function by the hypothalamic-pituitary axis and the process of ovarian follicle development and ovulation allow parallels to be drawn between rodent and human species. Additionally, rodent models of PCOS have shown many characteristics of the human disorder including hyperandrogenism, elevated LH, disrupted cyclicity, presence of follicular cysts/polycystic ovaries, and altered insulin sensitivity. Hence, in terms of exhibiting the majority of reproductive and endocrine symptoms associated with PCOS, rodent PCOS models appear to closely parallel the human condition. This review focuses on outlining the advantages and disadvantages of the numerous rodent models used to investigate this complex endocrine disorder.

HORMONAL METHODS TO INDUCE PCOS IN RODENTS

Androgens

Within the ovary, androgens, mainly androstenedione (A4) and testosterone (T), are synthesized in the theca cells. A direct role for androgen receptor (AR)-mediated effects in the ovary and female reproductive functions has been recently confirmed by findings from AR knockout mouse models, where a loss of AR actions lead to subfertility, predominantly due to defective gonadotropin regulation, follicular development, and ovulation [19, 20].

Testosterone and androstenedione. Although 1 study reported that exposure of rodent fetuses to testosterone propionate (TP) by intra-amniotic administration induced anovulation in 64% of rats [21], in most studies, prenatal treatment of mice with T [22] or rats with TP [21, 23–27] had no effect on cyclicity or ovarian function, inferred by the presence of follicles at various stages and corpora lutea. A detailed study by Wu et al. [28] showed that prenatal treatment of rats with T on Days 16 and 19 of gestation resulted in irregular estrous cycles and an ovarian phenotype of increased numbers of preantral and antral follicles but a decrease in preovulatory follicle and corpus lutea populations. Treated rats also exhibited an increase in T, estradiol (E2), progesterone (P), and LH serum levels and an increase in the frequency of LH pulse secretion [28]. The variation in the findings of the presence of disrupted cyclicity and anovulation appears to be due to the degree of transplacental transfer of the administered steroid into the fetus [21].

A single postnatal treatment of rats with TP during the first 5 days of life completely blocked [23, 25, 29–32] or significantly reduced their ability to ovulate [23]. Rats exposed to TP in the first 5 days of life resulted in persistent anovulatory estrus [33], whereas TP exposure on Day 1 or 5 also caused acyclicity and polycystic ovaries with atretic follicles, cystic follicles exhibiting thin granulosa cell layers [30–32, 34], and increased production of estrogens (estrone [E1] and E2) and androgens (T and A4) [34]. Similarly, mice treated on Days 1–3 with TP or T [16, 31] or with TP on Days 1–24 [27] exhibited anovulation and the presence of polyfollicular ovaries, while most but not all A4-treated females exhibited anovulation [16]. Treatment of older rats (~3 weeks of age) with TP or A4 for 35 consecutive days also caused polycystic ovaries [35, 36], and TP treatment induced anovulation and increased the presence of apoptotic follicles and unhealthy oocytes [35]. Females also exhibited insulin resistance, a characteristic feature of human PCOS, indicating that androgens can lead to insulin resistance [35]. However, unlike the significantly enlarged ovaries from women with PCOS, TP [23, 30, 34, 37] and A4 [36] treatment resulted in smaller ovaries in rats. Interestingly, treatment of rats on Days 15–25 with TP did not induce a PCOS-like phenotype, with rats exhibiting morphologically normal ovaries [27], highlighting the fact that androgen effects, which leads to the development of the PCOS phenotype may occur only during specific times.

In summary, although there are some conflicting findings from fetal exposure to T and TP, generally, prenatal exposure does not consistently affect cyclicity or ovarian function (Fig. 2). On the other hand, postnatal treatment with T and TP induces typical human PCOS features of acyclicity, anovulation, polycystic ovaries, hyperandrogenism, and insulin resistance. In contrast to PCOS ovaries, TP treatment reduced ovary weight, and A4 treatment produced less severe characteristics associated with PCOS (Fig. 3). These model descriptions lack detailed analysis of metabolic disturbances,

Treatment & species	Gestational day of treatment & age collected	Puberty	Estrus cycles	Disrupted ovulation	Multi-cystic ovaries	Ovarian phenotype	Hyper-androgenism	Other hormonal characteristics	Body weight & composition	Metabolic disturbances
TP 1mg (im) Rat [23]	16 & 19 (18-19wks)	Vaginal anomalies	-	No	-	Day 19 ↓ovary weight. CL present.	-	-	-	-
TP 10mg (sc) Rat [21]	1 to 4 days before delivery (Adult)	Vaginal fusion	-	No	No	Normal ovaries with CL.	-	-	-	-
TP 1mg (ia) Rat [21]	1 to 4 days before delivery (Adult)	-	-	Yes	Yes	Two thirds had no CL.	-	-	-	-
TP 20mg/kg (sc) Rat [27]	14.5-21.5 (Day 25 and 90)	Vaginal fusion	-	No	No	Normal ovaries with CL.	-	No change in E2, LH, FSH.	No change in body weight.	-
TP 2mg (sc) Rat [24]	16 – 20 (5-7wks)	Accelerated sexual maturity	Cyclic	No	No	Normal ovaries with CL.	-	-	↓ body weight	-
TP 2.5mg (sc) Rat [25]	19 – 22 (10,21 & 26wks)	Vaginal fusion	-	No	-	Normal ovaries with CL.	-	-	-	-
TP 10, 25mg (sc) Rat [26]	19,20,21 or 22 (8 & 21wks)	Day 19 or 20 vaginal fusion	-	Yes	-	At 8 wks normal ovaries with CL. At 21 wks↓ovary weight & majority exhibit no or ↓ CL.	-	-	-	-
TP 20μg (ia) Rat [26]	17,18,20 or 21 (18wks)	Some vaginal fusion	-	No	-	Normal ovaries with CL	-	-	-	-
T 3mg (sc) Rat [28]	16 &19 (9-10wks)	Some vaginal fusion	Irregular cycles	Yes	No	↑ preantral & antral follicles. ↓ preovulatory follicles and CL.	Yes	↑ T, E2, P and LH. No change in FSH. ↑ frequency of LH secretion.	-	-
T 0.75mg (implant) Mouse [22]	13-18 (12wks)	Vaginal fusion	-	No	-	Normal ovaries with CL.	-	-	-	-
DHT 3mg (sc) Rat [28]	16 &19 (9-10wks)	Some vaginal fusion	Irregular cycles	Yes	No	↑ preantral & antral follicles. ↓ preovulatory follicles and CL.	Yes	↑T, E2, P and LH. No change in FSH. ↑ frequency of LH secretion.	-	-
DHT 250μg (sc) Mouse [38]	16-18 (4-6mths)	-	↑ cycle length	-	-	-	Yes	↑ T and LH at diestrus.	-	-
DHT 250μg (sc) Mouse [39]	16-18 (8-10wks)	Advanced vaginal opening	↑ cycle length	-	-	-	No	↑ LH no change in T or A4.	No change in body and fat mass.	↑ fasting glucose

FIG. 2. Dysfunctional reproductive and metabolic features of human PCOS found in PCOS rodent models, induced by prenatal treatment with androgens. CL, corpus luteum; ia, intra-amniotic injection; im, intramuscular; implant, silicone elastomer (Silastic; Dow Corning) implant; sc, subcutaneous injection; ↓, decrease; ↑, increase; -, not determined in publication(s).

and defining androgen-regulated mechanisms can be difficult to interpret as steroid effects may be induced by either the AR or estrogen receptors (ER), due to the fact that T and A4 can be aromatized to the estrogens E2 and E1, respectively.

Dihydrotestosterone. Dihydrotestosterone (DHT) is a nonaromatizable androgen that is converted irreversibly from T by the enzyme 5α-reductase, a step which enhances its androgenic potency. Fetal exposure of rats to DHT on Days 16 and 19 and mice on days 16–18 of pregnancy resulted in irregular estrous cycles in mature, fetus-exposed female mice [28, 38]. Ovaries from fetal DHT-treated rats exhibited an increase in preantral and antral follicle numbers but a decrease in preovulatory follicle and corpora lutea populations, implying reduced ovulations due to defective follicle maturation to the preovulatory stage [28]. Rats and mice prenatally exposed to DHT exhibited increased T and LH serum levels, replicating the human PCOS traits of androgen and LH hypersecretion [28, 38]. Rats that were exposed also exhibited an increase in the frequency of LH pulse secretion and elevated serum E2 and P levels [28], suggesting excessive androgens may disrupt negative steroidal feedback signaling to the hypothalamus. In addition to reproductive axis abnormalities, prenatally androgenized mice (treated with DHT on Days 16–18 of gestation) exhibit metabolic alterations with impaired glucose tolerance but normal insulin sensitivity and increased adipocyte size, indicating altered adipocyte function; however, body and fat mass were unchanged [39].

Postnatal treatment of rats with DHT propionate (DHTP [DHT ester with prolonged half-life relative to that of DHT]) on Day 1 or 5 had no effect upon cyclicity or the histological appearance of ovarian follicles stages and corpora lutea [30]. On the other hand, 21-day-old (prepubertal) rats treated with 90-day continuous-release pellet containing DHT and collected 11–13 weeks later displayed 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 [40]. However, unlike human PCOS ovaries, ovary weight was reduced. At the estrous stage, plasma T and E2 levels were unaltered, but P was significantly decreased, indicating anovulation. DHT-treated rats also showed many metabolic features also present in human PCOS, including increased body weight, body fat, and abdominal fat; enlarged adipocytes; elevated leptin and cholesterol levels; and insulin resistance [40–42].

In conclusion, prenatal exposure to DHT induced irregular reproductive cycles, indicating this model may be of use in the study of mechanisms leading to disrupted regulation of the hypothalamic-pituitary-gonadal axis (Fig. 2). However, polycystic ovaries were not present and detailed analysis of metabolic features of human PCOS are lacking. Postnatal treatment from 3 weeks of age with 90-day continuous release pellets containing DHT appears to be an attractive model with ovarian morphology and key reproductive and metabolic features closely paralleling the human condition (Fig. 3).

Dehydroepiandrosterone. The observation that dihydrotestosterone (DHEA) levels are increased in women with PCOS [43] led to the development of a PCOS animal model using postnatal DHEA treatment (22- to 23-day-old rats treated with DHEA for 36 days) as the inducer of polycystic ovaries [36]. The DHEA rodent model exhibits some features of the

Treatment & species	Day of treatment & age collected	Puberty	Estrus cycles	Disrupted ovulation	Multi-cystic ovaries	Ovarian phenotype	Hyper-androgenism	Hormonal characteristics	Body weight & composition	Metabolic disturbances
TP 1mg (sc) Rat [29]	1 (13wks)	Vaginal fusion	-	Yes	-	↓ovary weight. No CL.	-	↓ FSH, ↑ PRL. No change in LH.	-	-
TP 100μg (sc) Rat [30]	1 or 5 (9-10wks)	Vaginal fusion (D1). Advanced vaginal opening (D5).	Acyclic	Yes	Yes	↓ovary weight. No CL.	-	-	-	-
TP 1μg (sc) Rat [23]	1 or 5 (18-19wks)	-	-	Yes	-	↓ovary weight. No CL or ↓CL	-	-	-	-
TP 20mg/kg (sc) Rat [27]	1-24 or 15-25 (Day 90)	Vaginal fusion (D1-24)	-	Yes (D1-24)	Yes (D1-24)	↓ ovary weight. & no CL (D1-24).	-	↓ FSH (D1-24). No change in LH or E2.	↑ body weight	-
TP 100μg (sc) Rat [34]	6 (300 days)	Advanced vaginal opening	1 or 2 normal cycles before constant estrus	Yes	Yes	↓ovary weight.	Yes	↑ T, A4, E1 & E2	-	-
TP 1mg/100g BW (sc) Rat [35]	21-55 (8wks)	-	-	Yes	Yes	No CL. ↑apoptotic follicles & unhealthy oocytes.	-	↓ P	-	Insulin resistant
TP 100μg,1mg & **T** 100μg (sc) Mouse [16,31]	1-3 or 5 (9 or 12wks)	-	-	Yes	Yes	No CL.	-	-	-	-
A4 30mg/kg (sc) Rat [36]	22/23-57/58 (8wks)	-	-	-	Yes	↓ovary weight.	-	-	No change in body weight.	-
A4 100μg (sc) Mouse [16]	1-3 (12wks)	-	-	Yes	-	-	-	-	-	-
DHTP 100μg (sc) Rat [30]	1 or 5 (9-10wks)	Vaginal fusion (D1). Advanced vaginal opening (D5).	Cyclic	No	No	Normal ovaries with CL.	-	-	-	-
DHT 7.5mg (pellet) Rat [40]	21-110 (14-16wks)	-	Irregular cycles	Yes	Yes	↓ovary weight. ↓ CL. ↑ atretic follicles. Thin granulosa cell layers.	No	No change in T or E2. ↓ P	↑ body weight, body fat, LBM, BMC. ↑ fat depots weight.	Insulin resistant. ↑ leptin. No change in TC & TG.
DHT 7.5mg (pellet) Rat [41]	21-110 (16wks)	-	Acyclic	-	-	-	-	-	↑ body weight, body fat, LBM, BMC. ↑ fat depots weight.	Insulin resistant. ↑ LDL & TG.
DHEA 30mg/kg (sc) Rat [36]	22/23-57/58 (8wks)	-	-	-	Yes	↓ovary weight.	-	-	No change in body weight.	-
DHEA 6mg/100g BW (sc) Rat [44]	27-46 (6-7wks)	-	Irregular cycles	Yes	Yes	No CL.	Yes	↑ T, A4, DHT, E1, E2, P, PRL & LH. No change in FSH.	-	-
DHEA 6mg/100g BW (sc) Rat [45]	27-46 (5-6wks)	-	Acyclic	Yes	Yes	-	-	-	-	-
DHEA 6mg/100g BW (sc) Mouse [49]	25-44 (6-7wks)	-	Acyclic	-	Yes	↑ atretic follicles. Thin theca cell layers & compacted granulosa cells.	-	↑ E2, P & PGE	-	-
DHEA 6mg/100g BW (sc) Mouse [47]	25-44 (6-7wks)	-	Acyclic	-	Yes	-	-	↑ E2, & P. ↓ PGE	No change in body weight.	Insulin resistant
DHEA 4.5, 6mg/100g BW (sc) Mouse [48]	25-44 (6wks)	-	-	Yes	Yes	↑ atretic follicles. No CL.	Yes	↑ T, A4, DHT, E1, 3α- & 3β-diol.	-	-

FIG. 3. Dysfunctional reproductive and metabolic features of human PCOS present in PCOS rodent models induced by postnatal treatment with androgens. Day of birth is Day 1. A4, androstenedione; BMC, bone mineral content; BW, body weight; CL, corpus luteum; DHTP, dihydrotestosterone propionate; LBM, lean body mass; LDL, low-density lipoprotein; pellet, 90-day continuous release pellet; PRL, prolactin; sc, subcutaneous injection; TC, total cholesterol; TG, triglycerides; ↓, decrease; ↑, increase; -, not determined in publication(s).

human PCOS condition, such as acyclicity, abnormal maturation of ovarian follicles, and anovulation [44–46].

Postnatal treatment of mice [47, 48] and rats [44–46] with DHEA for 20 consecutive days resulted in all or most females exhibiting follicular cysts with a thin granulosa cell layer and anovulation. Ovaries exhibited an increase in fat and stroma tissues and increased numbers of atretic follicles [48, 49], hyperandrogenism, and altered ovarian steroidogenesis with elevated serum levels of androgens [44, 48, 50], estrogens, P, and prostaglandin [44, 47, 49, 50]. In one study, LH levels were elevated while follicle-stimulating hormone (FSH) levels did not change [44], but in other studies, LH and FSH levels were both decreased [46] or unchanged [50]. Limited data are available on whether DHEA treatment induced the metabolic disturbances associated with PCOS. However, DHEA treatment of mice did not affect body weight, but did increase serum fasting insulin levels without affecting fasting glucose levels [47].

In conclusion, postnatal treatment of rodents with DHEA induced human PCOS characteristics of acyclicity, anovulation, polycystic ovaries, and hyperandrogenism (Fig. 3). However, unlike human PCOS cystic follicles, which are characterized by a thickened theca cell layer, cysts in DHEA-treated ovaries exhibited a thin layer of theca cells [49]. Furthermore, the elevation in LH levels was not consistent, and currently, there are limited data pertaining to the presence of associated metabolic characteristics.

Overall, prenatal and postnatal exposure to various androgens can induce both reproductive and metabolic deficits similar to those exhibited in PCOS women (Figs. 2 and 3). However, care must be taken when comparing models, as age of analysis had an effect on the observed phenotype, with differences in the presence of cysts or corpora lutea observed [26, 45] in most but not all studies [25]. Prenatal exposure can lead to vaginal fusion, and although researchers have varied doses of androgens to minimize this effect [38], this is a significant limitation of this model for evaluation of fertility, which is a key feature of PCOS. Furthermore, although some neonatally androgenized rats display elevated androgen and LH levels, this is not consistent, and some models display normal serum levels of LH, FSH, T, and E2 [51], raising doubt about their suitability as models for PCOS. On the other hand, although postnatal treatment with most androgens decreased ovary weight, in contrast to enlarged ovaries in PCOS women, findings from the treatment with androgens later in life, in particular DHT, support the use of this approach in the study of

the etiology and treatment of PCOS, as rodents exhibited many reproductive and metabolic features associated with human PCOS.

Estrogens

Estrogens, are synthesized in the granulosa cells by the conversion of androgens, involving the enzyme P450 aromatase [52], and play a major role in female fertility including normal ovarian and uterine function [53, 54].

Estradiol benzoate, E2, and E2 valerate. Adult rats, postnatally treated with estradiol benzoate (EB) on Day 1, displayed acyclicity, anovulation, and ovarian atrophy [29]. However unlike human PCOS, ovary weight and serum LH levels were decreased. Other hormone differences observed were a significant increase in both FSH and prolactin serum levels [29]. Young cycling adult rats exposed to E2 for 8 weeks via a subcutaneous continuous release implant [55] or a single injection of estradiol valerate (EV) [56–59] exhibited acyclicity, anovulation, and polycystic ovaries, which contained an increased number of atretic follicles and cysts with a thin granulosa cell layer and an abnormally thickened theca layer [55, 57, 60]. However, EV treatment decreased ovary weight and failed to provoke LH hypersecretion [56, 61], hyperandrogenism, obesity, and changes in glucose and insulin concentrations, which differs significantly from human PCOS, but rats did exhibit hypertension and an increase in inguinal fat depot weight [60].

In summary, exposure to E2 resulted in ovarian morphological features of anovulation and polycystic ovaries similar to those of PCOS patients (Fig. 4). However, these models are limited by the lack of endocrine and metabolic features associated with human PCOS.

Aromatase Inhibitors

Polycystic ovaries can be induced by androgen exposure including not only exogenous androgens but also as a result of secondary endogenous androgen excess [2, 62]. The latter includes the rat PCOS model induced by letrozole, a nonsteroidal aromatase inhibitor, which blocks the conversion of androgens to estrogen [63]. Letrozole treatment of adult rats for at least 21 consecutive days induced acyclicity [40] or irregular estrous cycles [63] and anovulation, with ovaries exhibiting many large follicular cysts and either reduced numbers or no corpora lutea [40, 63–65]. Ovaries exhibited increased follicle atresia and multiple cysts with thin granulosa cell layers and thickened theca cell layers [40]. Endocrine disruptions included elevated levels of LH, FSH, and T, reflecting the accumulation of endogenous ovarian androgen secretion due to a block in aromatase activity. In contrast, the decreased P secretion observed is consistent with the observed anovulation [40, 63–65]. E2 levels were either decreased [64, 65] or unchanged [40]. Additionally, treated rats exhibited some metabolic features of human PCOS with increased body weight [40, 66] and body fat but no change in insulin sensitivity or lipid metabolism [40]. However, there is one report of elevated glucose, cholesterol, and triglyceride levels in female rats treat orally with letrozole [66].

In conclusion, the letrozole-induced PCOS rodent models induced many features of human PCOS (Fig. 4), although further work is required to confirm the metabolic disruptions present before this model can be confirmed as a valid and useful model for the metabolic features of PCOS. Furthermore, the reduction in E2 observed [63–65] may be a limitation of this strategy as the polycystic ovaries, anovulation, absence of corpus luteum, and elevation of serum LH and T levels, also present in ER-α (*Esr1*) knockout female mice [67–70] may be a consequence of disruption of E2 action rather than the reflex increase in serum T.

Antiprogestins

The antiprogestin RU486 is a synthetic steroid with a high affinity for progesterone (and glucocorticoid) receptors with potent antagonistic but no agonistic activity [71]. Rodents treated with RU486, hence lacking progesterone action, show numerous endocrine and ovarian morphological features similar to those of human PCOS. Administration of RU486 to adult cycling female rats for 4–9 days resulted in acyclicity, polycystic ovaries [72–74], and anovulation [75]. Ovaries contained an increased number of atretic follicles [72, 74, 75] and thin granulosa cell layers [73, 75]. Similar to human PCOS, serum LH, T, and E2 levels were significantly increased [74–77]. FSH levels were variable, with different models displaying unchanged [77], increased [72], or decreased [74] levels. Whether the differing length or dose of RU486 treatment affected FSH levels requires further assessment. In respect to metabolic abnormalities associated with human PCOS, RU486 treatment did not alter body weight or insulin sensitivity [77].

In summary, rats injected with RU486 displayed many features found in women with PCOS, including acyclicity, anovulation, presence of follicular cysts and elevated androgen and LH levels (Fig. 4). However, for RU486 administration to be validated as a useful PCOS model, metabolic disturbances require further detailed assessment. Furthermore, the effects of RU486 have, to date, not been studied in mice.

PHYSIOLOGICAL MANIPULATION TO INDUCE PCOS IN RODENTS

Changes in Light Exposure

In rodents, the LH surges that trigger ovulation are controlled by cyclic light-dark photoperiods [78]. An absence of these light-dark photoperiods within a 24-hour period can disrupt normal cycling in rats and inhibit ovulation, a key characteristic of PCOS [79]. Such a physical mechanism to induce PCOS may have advantages in avoiding the off-target effects of hormone inducers of PCOS models, which may differ from naturally occurring PCOS in women. For instance, aromatase inhibitors that induced a PCOS phenotype dramatically reduced E2 activity [63–65]. Continual exposure of mature rats to an environment of constant light was developed as an alternative approach to inducing PCOS [80]. Exposure of adult rats to continuous light leads to the gradual development of chronic anovulation. The intensity, duration, and spectral characteristics of the light influence the rate at which acyclicity and anovulation occur [81, 82]. Exposure of 21-day-old rats to constant light for 10 [82] or 12 [81] weeks induced acyclicity and smaller polycystic ovaries. In another rat study acyclicity, anovulation and polycystic ovaries without a reduction in ovary weight were found after continuous light for 74 days [83]. Altered hormones levels are also induced by exposure of rats to constant light with serum FSH and P levels lower, E2 and E1 levels were elevated, but LH level was unchanged compared to those of controls [84]. Surprisingly, androgen levels were not assessed.

In conclusion, although the light exposure approach induced anovulation and disrupted cycles, LH hypersecretion observed in human PCOS was not present in this model. Furthermore, hyperandrogenism, a key characteristic of human PCOS, and

Treatment & species	Treatment regimen & age collected	Estrus cycles	Disrupted ovulation	Multi-cystic ovaries	Ovarian phenotype	Hyper-androgenism	Hormonal characteristics	Body weight & composition	Metabolic disturbances
EB 100μg (sc) Rat [29]	Day 1 (2wks)	Acyclic	Yes	-	↓ovary weight. No CL.	-	↓ LH, ↑ FSH & PRL.	-	-
EV 2mg (sc) Rat [56]	1 injection in young adult cycling rats (8wks after EV)	Acyclic	Yes	Yes	↓ovary weight. Majority exhibit no CL.	-	↑ E2 & PRL. No change in LH & FSH.	-	-
EV 2, 4mg (im) Rat [57,58,60]	1 injection in young adult cycling rats (28 - 56 days after EV)	Acyclic	Yes	Yes	↓ovary weight. ↓ or no CL. ↑ atretic follicles.	No	↓ T. ↑ P. No change in E2. ↓ LH. No change in FSH.	↑ inguinal fat depot weight	No change in glucose & insulin. Hypertension.
E2 2mm long filled implant Rat [55]	Chronic exposure (8wks after E2)	Acyclic	Yes	Yes	No CL. Thin granulosa cell layers.	-	-	-	-
Letrozole 0.1, 0.5 or mg/kg(po) Rat [63]	21 consecutive days in adults (9wks)	Irregular cycles	Yes	Yes	↓ CL.	Yes	↑ T, LH & FSH. ↓ E2 & P.	-	-
Letrozole 1mg/kg (po) Rat [64,65]	21 consecutive days in adults (11wks)	-	Yes	Yes	No CL. Thickened granulosa cell layer.	Yes	↑ T, LH & FSH at pro- & diestrus. ↓ E2 at proestrus. ↓ P at diestrus.	-	-
Letrozole 1mg/kg (po) Rat [66]	28 consecutive days in adults (16wks)	-	-	-	-	-	-	↑ body weight.	Insulin Resistant. ↑ cholesterol & TG.
Letrozole 36mg (pellet) Rat [40]	Days 21-110 (14-16wks)	Acyclic	Yes	Yes	↑ ovary weight. ↑atretic follicles. No CL.	Yes	↑ T, No change in E2. ↓ P.	↑ body weight, body fat, LBM, BMC. ↑ fat depots weight.	-
RU486 4mg (sc) Rat [72,75]	4 consecutive days with 1st day of estrus = day 1 (Adult)	Acyclic	Yes	Yes	↑ ovary weight. ↑atretic follicles. Thin granulosa cell layers.	Yes	↑ T, E2, PRL, LH & FSH.	-	-
RU486 2mg (sc) Rat [74]	8 consecutive days with1st day of estrus = day 1 (day 9 after RU486)	Acyclic	Yes	Yes	↑ ovary weight. ↑atretic follicles. Arrest follicle growth (lack of antral follicles). ↑ CL size.	Yes	↑ T, LH & E2. ↓ FSH.	-	-
RU486 2mg/ 100g BW (sc) Rat [77]	7-9 consecutive days with 1st day of estrus = day 1 (days 7-9 after RU486)	Acyclic	Yes	Yes	↓ CL. ↑atretic follicles.	Yes	↑ T & LH. No change in FSH.	No change in body weight or serum insulin levels.	-
RU486 4mg/ 100g BW(sc) Rat [73]	9 consecutive days with 1st day of estrus = day 1 (9-10wks)	Acyclic	-	Yes	Thin granulosa cell layers. ↑ antral follicles.	-	-	-	-

FIG. 4. Dysfunctional reproductive and metabolic features of human PCOS observed in PCOS rodent models, induced by postnatal treatment with estrogen, letrozole, and RU486. BMC, bone mineral content; BW, body weight; CL, corpus luteum; EB, estradiol benzoate; im, intramuscular; implant, subcutaneous continuous release; LBM, lean body mass; pellet, 90-day continuous release pellet; po, once daily orally; PRL, prolactin; sc, subcutaneous injection; ↓, decrease; ↑ = increase; -, not determined in publication(s).

Treatment	Treatment regimen & age collected	Estrus cycles	Disrupted ovulation	Multi-cystic ovaries	Ovarian phenotype	Hyper-androgenism	Hormonal characteristics	Body weight and composition	Metabolic disturbances
Exposure to constant light Rat [81,82]	10-12 weeks of permanent light (13-15wks)	Acyclic	Yes	Yes	↓ovary weight. No CL.	-	-	-	-
Exposure to constant light Rat [83]	74 days of permanent Light (Day 75 after start of treatment)	Acyclic	Yes	Yes	No CL.	-	No change LH or FSH.	-	-
Exposure to constant light Rat [84]	permanent light (10-13wks)	Acyclic	-	-	-	-	↑ E1 & E2. ↓ P & FSH. No change in LH.	-	-
Exposure to constant light Rat [80]	25-140 days of permanent light (6-14wks)	Acyclic	Yes	Yes	↓ovary weight. No CL.	-	-	-	-

Genetic model	Estrus cycles	Disrupted ovulation	Multi-cystic ovaries	Ovarian phenotype	Hyper-androgenism	Hormonal characteristics	Body weight & composiiton	Metabolic disturbances
Leptin deficient mouse [89,91, 94,95,98]	Acyclic	Yes	No	↓ ovary weight. ↑atretic follicles. ↓ CL.	Yes	↑ T, E2 & P. ↓ FSH. No change in LH.	↑ body weight.	↑ plasma glucose & insulin. Insulin resistance.
New Zealand obese Mouse [99,100]	-	Yes	No	↑ ovary volume. ↑ total follicles. ↑atretic follicles. ↓ CL.	No	↓ LH & ↑ E2. No change in T.	↑body weight.	↑ plasma glucose & insulin. Insulin resistance. ↑ serum cholesterol & TG.
***JCR:LA-cp* Rat** [103]	Irregular cycles	Yes	Yes	↓ovary weight. ↑ cystic follicles. Thin layer of granulosa cells. ↑atretic follicles. ↓ CL.	Yes	↑ T, no change in E2	↑ body weight. ↑ perimetrial & perirenal fat pad weight	↑ fasting plasma glucose & insulin. ↑ cholesterol and TG.
Elevated LHβ transgenic mouse [105,106,107]	-	Yes	Yes	3 types of ovarian morphology: enlarged and packed with CLs, polycystic and ovarian tumors.	Yes	↑ T & E2	↑ body weight. ↑ abdominal fat	↑ leptin & insulin. ↓ cholesterol. No change in TG.
hSERPINE1 transgenic mouse [111]	-	Yes	Yes	Cystic follicles present. Disorganized layers of granulosa cells. Lipid vacuoles in theca. ↓ CL.	Yes	↑ T	-	-

FIG. 5. Dysfunctional reproductive and metabolic features of human PCOS present in rodent models, induced by exposure to constant light or genetic modification. CL, corpus luteum; TG, triglycerides; ↓, decrease; ↑, increase; -, not determined in publication(s).

the presence of metabolic disturbances have not been reported (Fig. 5).

GENETICALLY MODIFIED RODENT MODELS OF PCOS

To date, several rodent models with characterized genetic mutations exhibit many of the reproductive and metabolic characteristics associated with human PCOS (Fig. 5).

Leptin Mutant Rodent Strains

Leptin is synthesized and secreted from fat cells in response to metabolic status and has been found at higher than expected levels in a substantial proportion of women with PCOS for their body mass index, T level, and insulin sensitivity [85]. Altered leptin signaling has been proposed to be involved in the development of the disorder [85]. In support of this, leptin has a direct stimulatory effect on GnRH secretion [86], and an abnormality in the regulation of hypothalamic GnRH secretion is a feature of human PCOS [87, 88].

Leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. Mice with a mutation in the obese (*ob*) or diabetes (*db*) gene lack endogenous leptin or possess a nonfunctional leptin receptor, respectively, and displayed some metabolic and reproductive characteristics of women with PCOS. Adult *ob*/*ob* and *db*/*db* females are infertile and exhibited acyclicity, anovulation, and increased follicular atresia [89–92]. Hormone changes in *ob*/*ob* mice included significantly increased serum T, E2, and P levels [93, 94] and reduced serum FSH levels [95], while *db*/*db* females exhibited a significant decrease in serum E2 and P levels. Metabolic features of PCOS exhibited by both mutant mice include severe obesity, a diabetes-like syndrome of hyperglycemia, glucose intolerance, and elevated plasma insulin [91, 94, 96–98]. However unlike human PCOS, polycystic ovaries were not present in either model, and serum LH levels were unchanged in *ob*/*ob* mice [95].

New Zealand obese mouse (NZO/HlLt). New Zealand obese (NZO) mice represent a model of polygenic obesity. Although they have normal leptin and leptin receptor genes, NZO mice exhibited a defect in leptin transport across the blood-brain barrier. Along with obesity, the NZO mouse exhibited insulin resistance and hyperinsulinemia, all of which are common metabolic abnormal characteristics of PCOS [99]. Furthermore, the mouse displayed dyslipidemia, hypercholesterolemia, and hypertension [100]. The NZO mouse is subfertile, and ovaries displayed increased ovarian volume, reduced numbers of corpora lutea and ovulations, and an increased number of atretic follicles [99]. Hormonal differences included reduced LH levels and increased E2 levels but unchanged T levels. Although NZO mice displayed many metabolic disturbances associated with human PCOS, the key features of polyfollicular ovaries and hyperandrogenism are absent in this model.

JCR:LA-cp corpulent (cp/cp) rat. The JCR:LA-corpulent rat (*cp*/*cp*) incorporates the corpulent (*cp*) gene, isolated by Koletsky [101]. Rats that are homozygous for the *cp* gene (*cp*/*cp*) have a defect in the leptin receptor [102]. Female (*cp*/*cp*) rats have been proposed as a potential PCOS model to investigate the etiology and possible treatment of PCOS, particularly in the context of metabolic disturbances associated with the disease. Adult *cp*/*cp* females displayed irregular estrous cycles and disrupted ovulation [103], but unlike human PCOS ovaries, *cp*/*cp* ovaries were reduced in weight. Additionally, although *cp*/*cp* females had a 2-fold increase in the number of cystic follicles, which were lined with a thin layer of granulosa cells, compared to those of controls, control rats also exhibited cystic follicles. As in human PCOS ovaries, the number of atretic follicles were significantly increased and corpora lutea numbers were decreased. Adult (12-week-old) females exhibited elevated serum T concentrations, while E2 levels were similar to those of controls. Most importantly, *cp*/*cp* females exhibited many metabolic disturbances associated with human PCOS, including obesity, hyperlipidemia, hyperinsulinemia, and an increased risk of cardiovascular disease [103]. This model of a genetically obese rodent with the associated metabolic abnormalities appears to lead to ovarian dysfunction, which may be useful in the investigation of the development of PCOS in women who exhibit obesity, insulin resistance, and dyslipidemia.

Overexpressing Luteinizing Hormone Transgenic Mice [Tg(Cga-LHB/CGB)94Jhn/J]

As LH hypersecretion is a key feature of PCOS [104], the production of a mouse overexpressing LH was a logical model to be evaluated for whether it replicated features of PCOS. Overexpression of human LHβ subunit revealed that continually elevated levels of LH led to infertility, anovulation, elevated T and E2 levels, and polycystic ovaries [105, 106]. These transgenic mice also featured some metabolic alterations associated with PCOS, including obesity, and increase abdominal fat and insulin levels [107]. However, the persistently elevated transgenic LH levels also produced other phenotypes not associated with PCOS, such as ovarian tumors and enlarged ovaries with multiple corpora lutea, suggesting that although LH may be associated with the etiology of PCOS, it is unlikely that LH levels alone trigger the changes leading to the development of the syndrome.

Mice with Transgenic Overexpression of Plasminogen Activator Inhibitor-1 (Tg-Serpine1)

Several studies have supported an association between an elevation in plasma plasminogen activator inhibitor-1 (PAI-1 [official symbol, SERPINE1]) and PCOS [108–110]. SERPINE1 is a member of the superfamily of serine protease inhibitors and prevents plasminogen activation via its inhibition of plasminogen activators. SERPINE1 is the principal inhibitor of tissue plasminogen activator (tPA), which mediates fibrinolysis and urokinase (uPA), which plays a role in cell surface plasminogen activation. Transgenic overexpression of an active form of human SERPINE1 in mice led to alterations in ovarian structure that resembled abnormalities found in human polycystic ovaries, including reduced corpora lutea, a thickened tunica albuginea, and the presence of cysts with a thin granulosa cell layer [111]. Ovaries from Tg-Serpine1 exhibited a thickened tunica and follicular cysts and rarely exhibited corpus lutea, indicating oligo-anovulation. Ovarian stromal volume was increased, theca exhibited large lipid vacuoles, and antral follicles had disorganized granulosa cells layers. Importantly, hyperandrogenism was evident with significantly higher T levels in transgenic mice [111]; however, other hormones were not assessed. This model displayed many reproductive features of human PCOS, and it has been proposed that an excess of SERPINE1 in patients with PCOS may contribute to the development of the disorder [111]. However, a full assessment of hormone profiles and metabolic features associated with human PCOS remains to be characterized in this model.

Postnatal treatments used to induced rodent PCOS models	Human diagnostics traits of PCOS									
	Irregular cycles/ acyclic	Hyper-Androgenism (↑ in T)	Oligo-anovulation/ Anovulation	Polycystic ovaries	↑ follicle atresia	Thin granulosa cell layer	LH hyper-secretion	Insulin resistance	Overweight/ obesity	Dyslipidaemia
T	✓	✓	✓	✓	✓	?	?	✓	?	?
DHT	✓	×	✓	✓	✓	✓	?	✓	✓	✓
DHEA	✓	✓	✓	✓	✓	✓	✓*	✓	×	?
EV	✓	×	✓	✓	✓	?	×	×	✓	?
E2	✓	?	✓	✓	?	✓	?	?	?	?
Letrozole	✓	✓	✓	✓	✓	×	✓	✓	✓	✓
RU486	✓	✓	✓	✓	✓	✓	✓	×	×	?
Constant light	✓	?	✓	✓	?	?	×	?	?	?
Genetically modified rodent models of PCOS										
Leptin deficient mouse	✓	✓	✓	×	✓	?	×	✓	✓	?
New Zealand obese mouse	?	×	✓	×	✓	?	×	✓	✓	✓
JCR:LA-cp Rat	✓	✓	✓	✓	✓	✓	?	✓	✓	✓
hSERPINE1 mouse	?	✓	✓	✓	?	?	?	?	?	?

FIG. 6. Summary of dysfunctional reproductive and metabolic traits in PCOS women and rodent models of PCOS. ✓, present; ×, not present; ?, unknown; *Data are not consistent between publications.

Genetically Modified Rodent Models Exhibiting PCOS-Like Ovarian Cyst Formation

Several genetically modified mouse models developed without PCOS in mind display unexpected PCOS-like ovarian pathology with the formation of ovarian cysts. Some models also exhibit other features associated with human PCOS, such as elevated T and LH levels; for example, some transgenic mice expressing human insulin-like growth factor 1 (hIGF1) under the control of the mouse LH receptor promoter failed to mate and displayed polycystic ovaries. Hormone disruptions were also found with significantly elevated serum T levels and unchanged E2 levels, but unlike PCOS patients, mice exhibited decreased LH levels [112]. Ovarian hemorrhagic cyst formation is a phenotype in several genetic mouse models, including the aromatase knockout [113], *Esr1* knockout [69], transgenic hCG overexpressing mice [114], transgenic FSH overexpressing [115] and mutated FSH receptor (increased receptor activity) [116] mice. The hemorrhagic cystic phenotype, which is not a true PCOS phenotype, appears to be caused by the common feature of increased gonadotropin action in these models, implying that elevated gonadotropins themselves are not the key cause of PCOS development.

In conclusion, genetically modified rodent models of PCOS provide an insight into possible mechanisms or markers for the development of PCOS. The *ob*/*ob*, *db*/*db*, and NZO mouse models and cp/cp rat model all exhibit similar metabolic disturbances; hence, these models may prove to be useful for the investigation of the etiology and treatment of PCOS, particularly in the context of metabolic disturbances associated with human PCOS. However, their ovarian features of ovarian PCOS, presence of cysts and lack of corpora lutea, and altered estrous cycles lack the severity exhibited in many induced models [13, 40], with *ob*/*ob* and *db*/*db* models not exhibiting polycystic ovaries and *cp*/*cp* females displaying irregular estrous cycles rather than being acyclic. On the other hand, the overexpressing Tg-Serpine1 mouse closely correlates reproductive characteristics of human PCOS, but metabolic disturbances remain to be fully characterized. Hence, for future investigations of PCOS, the use of transgenic approaches has the advantage of allowing specific candidate genes to be studied in isolation and/or combinations to identify whether changes in their expression lead to development of features of PCOS that parallel the human disorder.

CONCLUSION

Several hypotheses and speculations surround the etiology of PCOS and, despite it being the most common endocrine condition in women, little information is available on the

mechanisms driving its development. Consequently, logical forms of curative treatment based on its pathogenesis remain lacking. Various animal models have been shown to closely mimic key phenotypes of women with PCOS (Fig. 6) and thus may provide valuable insight into the origins and/or pathogenesis of this enigmatic condition. However, the heterogeneity of PCOS is reflected in the different phenotypes observed in the many different animal models reported so far. Great opportunities remain to unravel the various key features of this syndrome by using animal models to decipher the precise mechanisms involved, and to improve knowledge of the pathogenesis and treatment of PCOS.

Careful critical analysis of the models to date has increased our understanding of the pathogenesis of PCOS. Hyperandrogenism is the most consistent feature of women with PCOS [117]. PCOS rodent models induced by elevated androgen levels clearly show that excess androgen can induced many reproductive and metabolic features of human PCOS. Furthermore, differences are observed in the presence and/or severity of these features according to the timing of the prenatal and postnatal treatment [27] (Figs. 2 and 3). Hence, androgen programming of the adult female that leads to the development of the PCOS phenotype may occur only during specific time windows of prenatal and postnatal life. Estrogens and the antiprogestin RU486 also induce reproductive features found in women with PCOS, such as disrupted ovulation, altered estrous cycles, and changes in hormone levels. However, unlike many of the androgen-induced models, alterations in follicular dynamics, a key feature of human PCOS [12, 118], are not a feature in the estrogen induced models, and both estrogen and RU486 models fail to closely follow the metabolic disturbances associated with human PCOS. Thus, these models may be useful in questions relating to endocrine features of PCOS but are less informative in terms of the primary causes of PCOS. Rodent models with altered leptin activity, which primarily exhibit severe obesity and related metabolic abnormalities, and the double insulin/leptin receptor knockout mouse [119] exhibit some of the reproductive characteristics of human PCOS. This implies that obesity and insulin resistance may play a role in the development of PCOS. However, due to the fact that lean women exhibiting anovulation and androgen excess can have normal insulin insensitivity, it is more likely that obesity and insulin resistance act to amplify features of PCOS, rather than them being primary causes themselves. This conclusion is supported by the findings that not all rodent androgen induced PCOS models exhibited changes in body weight.

In conclusion, key questions remain regarding how PCOS originates, what predisposes women to the condition and its associated metabolic disturbances, and how approaches to innovative treatments based on its pathogenesis may be developed. We have shown that rodent PCOS models do replicate many of the reproductive, hormonal, and metabolic characteristics observed in human PCOS and, hence, may be useful for investigating the pathogenesis of PCOS. However, different models have distinct advantages and disadvantages; thus, no one model provides complete replication of the complex clinical disorder, and more than one single model may be required to make effective progress in understanding this condition. Appropriate animal models should be selected based on which specific facet of PCOS is of interest. Therefore, with careful and thoughtful use of rodent models of PCOS, these in vivo paradigms can provide informative and decisive information on the mechanisms driving the development of PCOS and its consequences.

ACKNOWLEDGMENT

The authors wish to thank Mark Jimenez for help in the preparation of the manuscript.

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