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*Review Article*

# Genetic modeling of ovarian phenotypes in mice for the study of human polycystic ovary syndrome

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**Abstract:** Polycystic ovary syndrome (PCOS) presents with a range of clinical complications including hyperandrogenism, polycystic ovaries, chronic oligo/anovulation, infertility, and metabolic alterations related to insulin resistance. Because the mechanism by which this disorder develops is poorly understood, information from experimental models of human disease phenotypes may help to define the mechanisms for the initiation and development of PCOS-related pathological events. The establishment of animal models compatible with human PCOS is challenging, and applying the lessons learned from these models to human PCOS is often complicated. In this mini-review we provide examples of currently available genetic mouse models, their ovarian phenotypes, and their possible relationship to different aspects of human PCOS. Because of the practical and ethical limitations of studying PCOS-related events in humans, our understanding of the mechanisms that contribute to the etiology of human PCOS may be enhanced through further study of these transgenic and knockout mouse models.

**Keywords:** PCOS, hemorrhagic cystic follicles, transgenic and knockout mice

## Introduction

Polycystic ovary syndrome (PCOS), a complex genetic disorder, is a significant women's health issue due to its high incidence and the fact that it often occurs before puberty [1]. It is estimated to affect 8–17% of women of reproductive age worldwide, and the number of reported cases of human PCOS increases annually [2]. The disorder presents with a wide range of clinical complications including hyperandrogenism, polycystic ovaries, chronic oligo/anovulation, infertility, hyperinsulinemia, insulin resistance, and a higher prevalence of obesity [3]. The etiology of the disease and the mechanisms by which this disorder progresses are still unclear, and this makes understanding the pathophysiology of human PCOS challenging. Although abnormal gene expression profiles in the ovaries and theca cells of women with PCOS have been identified [4, 5], transgenic and knockout (KO) mouse models with pathological ovarian phenotypes mimicking those found in human PCOS are still of great interest. The value of developing such animal models is in their providing a means to systematically analyze the mechanisms underlying the development of PCOS. These models can also provide new insights into the etiology of PCOS and provide opportunities to explore diverse aspects of the disease such as drug development.

A polycystic ovary morphology is consistent with, but not essential for, the diagnosis of human PCOS [3]. Pathological ovarian features include arrest of follicular development, accumulation of multiple follicular cysts, and an increase in ovarian stromal thickness. These features lead to chronic oligo- or anovulation and subsequent infertility [3, 6].

Autocrine, paracrine, and endocrine factors are necessary for normal ovarian function in mammals [6], and proper ovarian function is depen-

dent upon several specialized cell lineages in a spatially ordered configuration. It is expected, therefore, that many genes are required to orchestrate ovarian cellular function and that intercellular signaling events will participate in the development of PCOS. However, the cellular and molecular mechanisms underlying the development of cystic and/or hemorrhagic follicles containing an enlarged theca cell layer are not well defined. This review briefly describes several transgenic and KO mouse models of human PCOS (**Table 1**), and discusses their ovarian phenotypes and the advantages and possible limitations of applying these models to human PCOS.

## Genetic modeling of ovarian phenotypes in mice

Several lines of evidence indicate that changes in the production of luteinizing hormone (LH) in the pituitary gland are important for the development of cystic follicles in human PCOS. For example, women suffering from PCOS exhibit significantly increased levels of circulating LH compared to healthy controls [7]. Ovarian theca cells are the cell type that predominately expresses the LH receptor [8], and these cells become hypersensitive to LH during the development of human PCOS [1, 9]. Furthermore, chronic treatment with human chorionic gonadotropin (hCG) [10] or overexpression of LH and hCG [11-13] in female mice induces cystic follicle formation in the ovary. However, development of cystic or hemorrhagic follicles does not always occur along with an increase in LH levels in female mice that overexpress hCG under the control of different promoters [14, 15]. Although female follicle-stimulating hormone (FSH)β knockout mice have been shown to have increased levels of circulating LH, there is no evidence for the development of cystic or hemorrhagic follicles in FSH-deficient female mice [16]. In addition, overexpression of FSHβ leads to detectable numbers of cystic or hemorrhagic follicles without changes to circulating LH levels [17]. Thus, although LH is essential for the later stages of follicular development (from preovulatory to periovulatory stages) and ovulation, these results argue in favor of a primary role for LH in the formation of cystic or hemorrhagic follicles in the ovary.

It is generally accepted that elevated androgens are the main culprit behind the development of PCOS [1]. It is notable, however, that polycystic ovaries exist in women during pubertal development [18] and in women with hyperandrogenism [1] even when LH secretion and pulsatility is normal [19]. It has been shown that long-term treatment with estradiol valerate (EV) or dihydrotestosterone causes the formation of polycystic ovaries in rats [20-23]. Furthermore, treatment with letrozole, an aromatase cytochrome P450 (P450arom) blocker, inhibits androgen-to-estrogen conversion and leads to the development of massive multiple follicular cysts in rats [23]. Thus, both clinical and experimental studies suggest that a dynamic equilibrium among ovarian steroid hormones plays a significant role in the development of multiple cystic follicles under both physiological and pathological conditions.

Animal studies have demonstrated that both estrogens and androgens contribute to folliculogenesis, ovarian remodeling, and the development of several diseases [24]. There is *in vivo* and *in vitro* evidence to support the idea that the proliferation of theca cells in growing follicles results in significant androgen biosynthesis [1], but estrogens have been shown to inhibit androgen production in estrogen receptor (ER) α-expressing theca cells [25-27]. The absence of P450arom in theca cells is reflective of the paracrine action of ovarian-derived estrogens in the activation of ERα signaling *in vivo* [28, 29]. Understanding the role of ERα in the regulation of theca cell function has been aided by the global and theca cell-specific deletion of the *ERα* and/or *ERβ* gene in mice [30-34]. The initial analyses of the ovaries of these KO mice indicate that formation of hemorrhagic cystic follicles is likely to arise from the loss of ovarian ERα action in theca cells. Similarly to the ERα KO mice (ERαKO) [30-34], female mice overexpressing LH/hCG [11-13] or lacking plasminogen activator inhibitor-1 (PAI-1) [35] also exhibited theca cell hyperplasia that is consistent with that seen in human PCOS [36]. However, although female mice lacking P450arom (ArKO mice) developed hemorrhagic cystic follicles [28, 29], histological examination showed no hyperplasia of the theca cells in the ArKO ovaries. These studies lead to at least two conclusions: (1) there is no cause-and-effect relationship between theca cell hyperplasia and the formation of cystic and hemorrhagic follicles, and (2) altered estrogen

**Table 1.** Published mouse models that have ovarian alterations and their accompanying PCOS-like phenotypes

| Transgenic or knockout models | Ovarian phenotype | | | Ovulation | Infertility | Anterior pituitary | | Sex steroids | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Follicular cysts | Hemorrhagic cysts | TC / IC | | | FSH | LH/hCG | E2 | P4 | T/DHT | A | |
| 1. Overexpression of hCGαβ | + | +, massive | hyperplasia | No | Yes | n.d. | ↑ | ↑ | n.d. | n.d. | n.d. | [11] |
| 2. Overexpression of bovine LHβ | +, large | +, frequent | hyperplasia | No | Yes | n.d. | ↑ | ↑ | ↑ | ↑ | n.d. | [12, 13] |
| 3. ERαβKO | + | + | n.d. | No | Yes | – | ↑ | – | n.d. | ↑ | – | [30, 32] |
| 4. ERαKO | +, large | +, frequent | hyperplasia | No | Yes | – | ↑ | ↑ | – | ↑ | ↑ | [30-32, 34, 37] |
| 5. TC-specific ERαKO with PMSG/hCG stimulation | +, large | +, frequent | hyperplasia | Yes (oocytes ↓) | Age-dependent | – | ↓ | n.d. | n.d. | ↑ | n.d. | [31] |
| 6. Overexpression of NGF | No | No | n.d. | Yes | No | – | – | – | ↑ | – | – | [40] |
| 7. Overexpression of NGF with PMSG/hCG stimulation | +, large | +, frequent | n.d. | Yes (pups ↓) | n.d. | n.d. | n.d. | ↑ | ↓ | ↑ | – | [40] |
| 8. Overexpression of hPAI-1 | +, large | + | hyperplasia | No | n.d. | n.d. | n.d. | – | – | ↑ | n.d. | [35] |

TC, theca cells; IC, interstitial cells; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, 17β-estradiol; P4, progesterone; T, testosterone; A, androstene/androstenedione; PMSG, pregnant mare's serum gonadotropin; hCG, human chorionic gonadotropin; ER, estrogen receptor; NGF, nerve growth factor; hPAI-1, human plasminogen activator inhibitor-1; +, presence; –, no changes vs. wild-type controls; ↑; increased vs. wild-type controls; ↓, decreased vs. wild-type controls; n.d., not determined.

biosynthesis in P450arom-expressing granulosa cells or the estrogenic action on ERα-expressing theca cells is likely involved in the formation of cystic and hemorrhagic follicles.

Because human PCOS is often associated with metabolic disturbances [3, 18], caution should be taken when selecting the appropriate transgenic or KO models based solely on their ovarian phenotype. In addition to impaired ovarian function and fertility, the onset of insulin resistance and diabetes observed in adult female ERαKO mice [37] mimics what is seen in some PCOS patients [1, 18]. Thus, activation of ERα may serve as a critical link between reproduction and metabolic disturbances. Although it remains to be determined what effects theca cell-specific deletion of ERα has on metabolism, the ERαKO mouse model can be useful for progressive studies of ovarian dysfunction and metabolic changes or for expanded studies that seek to understand the complex PCOS signature.

Manipulation of endogenous estrogen levels by treatment with EV or gonadotropin from pregnant mare serum has previously been shown to increase the production of ovarian nerve growth factor (NGF) in rat theca cells and to induce the formation of follicular cysts [38, 39]. Moreover, intraovarian treatment with a neutralizing antiserum to NGF in conjunction with systemic exposure to an antisense oligodeoxynucleotide to the p75 NGF receptor reduces the number of precystic and cystic follicles [39]. This demonstrates that it is possible to prevent the development of cystic follicles by inhibiting NGF signaling. Transgenic NGF female mice, however, are indistinguishable from wild-type animals in all major reproductive functions and a detailed histological analysis did not reveal polycystic ovaries in these animals [40]. Interestingly, these transgenic NGF mice were found to be susceptible to challenge with gonadotropins (FSH and LH). After treatment with gonadotropins, the mice presented with a detectable ovarian phenotype that included the formation of massive follicular cysts [40] suggesting that endogenous steroid hormones participate in NGF-mediated ovarian dysfunction. More research needs to be done to determine whether the abnormal ovarian NGF signaling results in metabolic changes, and to elucidate what the downstream target of the NGF signaling pathway may be in relation to the development of polycystic ovaries.

Another factor implicated in human PCOS is the glycoprotein PAI-1. Several studies have shown that women with PCOS have increased levels of PAI-1 and increased PAI-1 activity [41-44]. It has also been shown that the PAI-1 polymorphism is significantly associated with the risk of developing PCOS [45]. Both pathological and

histological studies have been performed in mice overexpressing PAI-1 [35] and these mice have been found to have polycystic ovaries and increased testosterone levels that are comparable to what are seen in human PCOS. Thus, the transgenic PAI-1 mouse model could be used for future experimental investigations into the many gaps in the understanding of the interactions between reproductive and metabolic processes in human PCOS.

## Concluding remarks

PCOS is a complicated endocrine disorder whose pathophysiology is the result of the interactions, combinations, and contributions of various genetic and environmental factors. Because of the heterogeneous nature of PCOS [3], it is worth noting that the majority of PCOS-like animal models rely upon external chemical treatments to be able to focus on particular aspects of the disease's clinical pathology. As outlined above, transgenic and knockout mouse models do not replicate the full spectrum of human PCOS, but they do provide opportunities to gain deeper insight into the development of PCOS. Polycystic ovaries are the morphological ovarian phenotype in human PCOS [3, 6], and there is evidence for a primary ovarian defect being the root cause of human PCOS. The use of different transgenic and knockout mouse models with their own unique ovarian phenotype(s) may help to identify and quantify changes in reproductive and endocrine networks in these animals that may lead to important clinical insights into the development of PCOS in women.

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## Disclosure statement

The authors have declared that there is no conflict of interest.

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