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Review

# Reproductive neuroendocrine dysfunction in polycystic ovary syndrome: Insight from animal models

Alison V. Roland, Suzanne M. Moenter *

*Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109, USA*

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrinopathy with elusive origins. A clinically heterogeneous disorder, PCOS is likely to have multiple etiologies comprised of both genetic and environmental factors. Reproductive neuroendocrine dysfunction involving increased frequency and amplitude of gonadotropin-releasing hormone (GnRH) release, as reflected by pulsatile luteinizing hormone (LH) secretion, is an important pathophysiologic component in PCOS. Whether this defect is primary or secondary to other changes in PCOS is unclear, but it contributes significantly to ongoing reproductive dysfunction. This review highlights recent work in animal models, with a particular emphasis on the mouse, demonstrating the ability of pre- and postnatal steroidal and metabolic factors to drive changes in GnRH/LH pulsatility and GnRH neuron function consistent with the observed abnormalities in PCOS. This work has begun to elucidate how a complex interplay of ovarian, metabolic, and neuroendocrine factors culminates in this syndrome.



## 1. Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of female infertility. The estimated proportion of women affected varies with the criteria used for diagnosis, with the NIH and Rotterdam criteria cited most frequently. NIH criteria, encompassing 6–10% of women (Fauser et al., 2012), are the most strict and require oligo/anovulation and clinical or biochemical signs of hyperandrogenism, with the exclusion of other causes of androgen excess. The Rotterdam consensus workshop defined PCOS as the demonstration of two of three of the following: oligo- or anovulation, hyperandrogenemia or related symptoms such as hirsutism, and polycystic ovarian morphology, thereby including patients without hyperandrogenism and expanding the percent of affected women to 15% (Fauser et al., 2012). In addition to these diagnostic phenotypes, PCOS encompasses a range of other common accompanying abnormalities, in particular metabolic disorders (Dunaif, 1997; Lim et al., 2012; Randeva et al., 2012). Common findings are obesity, insulin resistance, abdominal adiposity, and in some cases glucose intolerance, which often progresses to type 2 diabetes mellitus. These metabolic comorbidities raise the risk for cardiovascular disease, a major cause of premature death. PCOS also elevates the risk of endometrial cancer (Dumesic and Lobo, 2013). Added to the distress associated with infertility, the potential health risks of these sequelae reinforce the need for novel preventative strategies and treatments for this disorder.

Despite intense research efforts, the causes of PCOS remain an enigma, due in part to diagnostic inconsistencies, the diversity of clinical phenotypes, as well as the complicated endocrine feedback and feedforward loops involved in the pathophysiology. Existing evidence suggests that PCOS can be caused by both genetic and environmental factors. PCOS clusters in families, implying at least a partial genetic basis. Several genomic variants have been identified as conferring increased susceptibility to PCOS; these include mutations in genes related to insulin resistance, obesity, gonadotropin receptors, and steroidogenesis (Hughes et al., 2006; San Millán et al., 2004). Recently, the association of two single-nucleotide polymorphisms with PCOS was replicated across multiple ethnic populations in genome-wide association studies. Polymorphisms in THADA and DENND1A were associated with endocrine and metabolic disturbances in Han Chinese (Rotterdam criteria; Cui et al., 2013) and European (NIH criteria; Goodarzi et al., 2012) PCOS cohorts. DENND1A was also associated with hyperandrogenemic PCOS in women of European ancestry (NIH criteria; Welt et al., 2012). THADA encodes thyroid adenoma-associated protein and was previously identified as a type 2 diabetes risk locus (Zeggini et al., 2008). The function of the DENDD1A gene is undetermined; it is expressed in androgen-generating theca

* Corresponding author. Address: Department of Molecular and Integrative Physiology, 7725 Medical Science II, 1137 E. Catherine St., Ann Arbor, MI 48109-5622, USA.
*E-mail address:* smoenter@umich.edu (S.M. Moenter).

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cells as well as in the brain (Strauss et al., 2012), and potentially may modulate androgen production or GnRH release. Nevertheless, these mutations cannot account for all cases of PCOS. Environmental influences, such as fetal and postnatal diet and hormone exposures, are also likely to be important etiological factors in PCOS.

The more restricted number of patients exhibiting stringent NIH PCOS criteria offers a population with relatively consistent phenotypes for study. In this population, when women who have recently spontaneously ovulated (and hence have elevated serum progesterone) are excluded, most exhibit increased LH pulse frequency (Marshall and Eagleson, 1999), which is determined directly by the GnRH pulse frequency. This indicates that neuroendocrine changes, involving alterations in GnRH neuronal activity, are an important aspect of this disorder.

## 2. Neuroendocrine dysfunction in PCOS

GnRH neurons are the final central drivers of the reproductive system. A critical feature of these neurons is their ability to secrete GnRH in discrete pulses; the frequency of these pulses is decoded by the pituitary to evoke preferential synthesis and release of LH or FSH (Wildt et al., 1981). In a healthy woman, the frequency and amplitude of GnRH release undergo cyclical changes across the menstrual cycle in response to steroid feedback (Reame et al., 1984). In the mid follicular phase, estradiol feedback augments GnRH pulse frequency while reducing amplitude (Moenter et al., 1991). The increased frequency drives steroidogenesis, and subsequent rising levels of estradiol provoke a switch in central feedback from negative to positive. This culminates in a surge of GnRH and LH release at mid-cycle that triggers ovulation. Following ovulation, increasing progesterone from the corpus luteum provides negative feedback to reduce GnRH pulsatility. These dynamic changes in the pattern of GnRH release are critical to produce the proper levels of gonadotropins necessary for follicular development and ovulation.

In PCOS, there is substantial evidence for hypothalamic neuroendocrine dysfunction involving disruptions in the pattern of GnRH, and thereby LH and FSH, release. Since GnRH cannot be measured in peripheral serum due to its short half-life and dilution, observations about GnRH release in PCOS are inferred from measures of LH/FSH levels and pulse frequency. Multiple studies have demonstrated increases in LH levels, LH/FSH ratio, and LH pulse frequency and amplitude in women with PCOS (Taylor et al., 1997; Waldstreicher et al., 1988). Downstream at the ovary, the relative suppression of FSH precludes proper follicular maturation, and LH predominance contributes to an overproduction of androgens by theca cells (Gilling-Smith et al., 1994). In sum, these observations indicate that heightened GnRH release, both in frequency and quantity, is an important pathophysiologic aspect in many cases of PCOS. However, whether this defect is primary or secondary to other changes in PCOS remains unclear.

Clinical studies have begun to dissect pharmacologically this abnormal neuroendocrine function in PCOS. One study demonstrated that physiologic levels of circulating progesterone, which are effective to reduce the GnRH/LH pulse frequency in healthy women, failed to reduce the LH pulse frequency in women with PCOS, indicating a failure of normal steroid negative feedback mechanisms (Pastor et al., 1998). In a subsequent study, chronic administration of the androgen antagonist flutamide had no effect on LH pulse frequency or amplitude, LH levels, or response to exogenous GnRH in PCOS patients, indicating that androgen is not a direct cause of these abnormalities (Eagleson et al., 2000). Flutamide did, however, restore the ability of estradiol and progesterone to provide negative central feedback to reduce the LH pulse frequency, suggesting that androgen excess may indirectly contribute to neuroendocrine disruption by interfering with steroid negative feedback.

Clinical studies have also begun to examine the development of PCOS across puberty, when PCOS symptoms are typically first manifested. Hyperandrogenemia in adolescents was found to impede progesterone negative feedback, similar to its action in adult women; however, this effect was only observed in a subpopulation of hyperandrogenemic girls, with progesterone sensitivity being inversely related to fasting insulin levels (Blank et al., 2009; Chhabra et al., 2005). Obese adolescent girls exhibit marked hyperandrogenemia (Coviello et al., 2006; McCartney et al., 2006), as well as elevated free testosterone that is predicted by morning LH and insulin levels (Knudsen et al., 2010; McCartney et al., 2007). Together these findings suggest that peripubertal obesity, by triggering a series of endocrine changes that lead to a reduction in hypothalamic sensitivity to progesterone, may be an environmental factor contributing to the genesis of PCOS during puberty. Recently, several of these adolescent patients were followed longitudinally; hyperandrogenemia and resultant impaired progesterone feedback were found to progress into late puberty and remain stable thereafter, suggesting that early pubertal disruptions may program lasting changes in neuroendocrine function (Beller et al., 2012).

Despite these advances in understanding the etiology and pathophysiological mechanisms of PCOS, clinical studies face inherent limitations with respect to examining neurobiological changes in this disorder. Manipulation of environmental factors, specifically the prenatal administration of androgens, has allowed the generation of animal models that bear many phenotypic similarities to PCOS in species ranging from rodents to ungulates to primates. Additionally, the development of transgenic mice expressing green fluorescent protein in GnRH neurons has enabled the study of steroidal and metabolic regulation of reproductive function directly at the neuronal level. With these models, several laboratories have begun to dissect the complex interplay of multiple systems that culminates in reproductive neuroendocrine dysfunction in PCOS.

## 3. Developmental models for the study of PCOS

While no animal model can perfectly recapitulate a human disease, they are invaluable for enabling manipulations not possible in human subjects and isolating physiologic variables to garner insight into disease pathophysiology. Perhaps the best-studied animal models for PCOS are based on prenatal androgen exposure. Early observations in female pseudohermaphrodite monkeys, initially studied for behavioral outcomes (Goy and Resko, 1972), showed that testosterone exposure *in utero* resulted in PCOS-like symptoms such as hyperandrogenemia in adulthood (Abbott et al., 1998), suggesting a developmental etiology for this disorder. This model was further characterized in the monkey and subsequently replicated across several species to yield analogous phenotypes. Women with congenital adrenal hyperplasia, who produce excessive androgens *in utero* that are subsequently normalized after birth, often manifest PCOS symptoms in adulthood, lending etiological relevance to these models (Barnes et al., 1994). Women with PCOS exhibit elevated androgen levels during gestation (Maliqueo et al., 2013; Sir-Petermann et al., 2002), and placental tissue from these patients exhibits higher 3β-HSD-1 and lower P450 aromatase activities, which could increase androgen production (Maliqueo et al., 2013). A recent study showed that umbilical vein testosterone in female fetuses of PCOS women is elevated to male levels (Barry et al., 2010), although this has not been a consistent finding (Anderson et al., 2010). The sum of these observations, however, suggests a possible mechanism of excess fetal androgen exposure in PCOS offspring, who are at elevated risk for developing the syndrome.