INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder seen in the clinic, with a prevalence depending on the diagnostic criteria utilized. PCOS affects approximately 10% of women according to the currently-recommended Rotterdam diagnostic criteria, which include evidence of at least two of the following: clinical and/or biochemical hyperandrogenism, chronic oligo- or anovulation, and polycystic ovarian morphology. The prevalence of PCOS is approximately 6% according to the classic National Institutes of Health (NIH) definition of PCOS, which mandates both hyperandrogenism and ovulatory dysfunction. \(^1\)\(^2\) Finally, PCOS affects approximately 10% of women according to the Androgen Excess and PCOS Society criteria: hyperandrogenism plus either ovulatory dysfunction or polycystic ovarian morphology. \(^3\) PCOS has also been associated with several comorbidities, including obesity, insulin resistance and type 2 diabetes, depression and anxiety, obstructive sleep apnea, and endometrial cancer. \(^4\)\(^5\)\(^6\)\(^7\)

Although the characteristics that define PCOS (i.e., androgen excess, oligo-/anovulation and polycystic ovarian morphology) are most directly related to ovarian function, the central reproductive neuroendocrine system, particularly the gonadotropin-releasing

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Abstract

Given the critical central role of gonadotropin-releasing hormone (GnRH) neurons in fertility, it is not surprising that the GnRH neural network is implicated in the pathology of polycystic ovary syndrome (PCOS), the most common cause of anovulatory infertility. Although many symptoms of PCOS relate most proximately to ovarian dysfunction, the central reproductive neuroendocrine system ultimately drives ovarian function through its regulation of anterior pituitary gonadotropin release. The typical cyclical changes in frequency of GnRH release are often absent in women with PCOS, resulting in a persistent high-frequency drive promoting gonadotropin changes (i.e., relatively high luteinizing hormone and relatively low follicle-stimulating hormone concentrations) that contribute to ovarian hyperandrogenemia and ovulatory dysfunction. However, the specific mechanisms underpinning GnRH neuron dysfunction in PCOS remain unclear. Here, we summarize several preclinical and clinical studies that explore the causes of aberrant GnRH secretion in PCOS and the role of disordered GnRH secretion in PCOS pathophysiology.

KEYWORDS
gonadotropin-releasing hormone, hyperandrogenemia, luteinizing hormone, polycystic ovary syndrome
hormone (GnRH) pulse generator, ultimately drives ovarian function through its regulation of gonadotropin release, and altered GnRH secretion plays a prominent role in the pathophysiology of PCOS.

The present review aims to:

1. Detail the clinical evidence supporting GnRH pulse generator dysregulation as a prominent player in the pathophysiology of PCOS;
2. Discuss evidence from preclinical animal models of PCOS identifying potential mechanisms underpinning disordered GnRH secretion in PCOS; and
3. Review clinical trial data regarding recently-developed pharmacological agents targeting the GnRH neuronal network in the treatment of PCOS.

2 | NEUROENDOCRINE DYSFUNCTION IN PCOS

The functionally-coordinated assembly of hypothalamic GnRH neurons represent the final node for the neural control of reproductive function. GnRH is secreted in a pulsatile fashion into the hypothalamic portal system, with GnRH pulse frequency largely reflecting the presence and degree of ovarian steroid (progesterone, estradiol) negative feedback. GnRH stimulates pituitary gonadotropes to synthesize and secrete the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Importantly, high and low GnRH pulse frequencies favor LH or FSH secretion, respectively;13-14 these effects are largely mediated at the level of gonadotropin gene transcription.15 Notably, although GnRH release cannot be directly measured in humans, animal studies confirm that each GnRH pulse elicits a secretory burst of LH16,17; thus, patterns of pulsatile GnRH secretion can be inferred from patterns of pulsatile LH secretion in human studies.

2.1 | Altered gonadotropin secretion in PCOS

The majority of women and adolescents with hyperandrogenic PCOS exhibit an increased LH (and by inference GnRH) pulse frequency, an increased LH pulse amplitude and exaggerated LH responses to exogenous GnRH.18-27 By contrast, such patients demonstrate a relative deficiency of FSH, as is expected under persistently high-frequency GnRH stimulation.13 In one study, when excluding those who had recently ovulated, serum LH concentrations and the LH:FSH ratio were elevated in 75% and 94% of women with PCOS, respectively.24 Persistently elevated LH pulse frequency and an elevated LH:FSH ratio indicate hyperactive GnRH pulse secretion,13 implicating dysfunction of the GnRH pulse generator in PCOS pathology.

The aforementioned abnormalities of gonadotropin secretion materially contribute to the ovarian hyperandrogenemia and ovulatory dysfunction of PCOS. LH is the primary stimulus for ovarian androgen production, and the ovarian hyperandrogenemia of PCOS is clearly LH-dependent. The hyperandrogenemia of PCOS typically does not manifest until after the pubertal increase in LH secretion.28 In women with PCOS, long-acting GnRH agonists, which suppress gonadotropin secretion, markedly reduce circulating androgen concentrations.29,30 Likewise, gonadotropin suppression partly accounts for the efficacy of combined oral contraceptives as a treatment for the hyperandrogenism of PCOS. In addition, relative FSH deficiency limits follicular development, contributing to ovulatory dysfunction in PCOS. These well-documented alterations in gonadotropin secretion in PCOS do not depend on gonadotropin-related genetic variants; nonetheless, the functional importance of such changes is supported by studies associating PCOS with variants in a number of gonadotropin-related genes such as FSHB (FSH beta subunit), FSHR (FSH receptor), LHB (LH beta subunit) and LHCGR (LH/choriogonadotropin receptor).31,32

2.2 | Altered GnRH pulsatility in PCOS

Detailed investigations into the hormonal intricacies of PCOS began to be expanded around four decades ago. Initially, increased LH secretion in PCOS was considered to reflect the positive feedback actions of increased serum estrone concentrations. Exogenous estrone does not, however, appear to alter circulating LH concentrations in women with or without PCOS, forcing investigators to look elsewhere for a mechanism.33 The central role of altered GnRH secretion in PCOS became apparent after the changing patterns of LH pulses, presumably reflecting changes in pulsatile GnRH release, pulse frequency in particular, were delineated throughout ovulatory cycles.34,35 In normal ovulatory menstrual cycles, LH pulse frequency is approximately one pulse every 90-100 min in the early follicular phase and one pulse every 60 min in the late follicular phase. Following ovulation, LH pulse frequency falls to approximately one pulse every 4-6 h by the mid-luteal phase; this reduction primarily reflects the negative feedback actions of progesterone.35-39 The ability of progesterone to reduce LH pulse frequency requires the permissive presence of estradiol,40,41 in line with observations that the progesterone receptor is an estrogen-dependent gene. LH pulse frequency increases again across the luteal–follicular transition, reflecting the loss of inhibition by progesterone, so that, by the early follicular phase, frequency has increased once again. This increase in GnRH pulse frequency across the luteal–follicular transition is important for the early-follicular increase in FSH secretion that promotes follicular development.42 The subsequent increase in pulse frequency across the follicular phase (from one pulse every 90-100 min to one pulse every 60 min) partly accounts for the transition from FSH predominance (early follicular phase) to LH predominance (late follicular phase), as is required for successful ovulatory cycles.

By contrast to these typical cyclic events, both hyperandrogenic adolescents and women with PCOS demonstrate persistently high LH pulse frequency, approximating one pulse per hour, similar
to that in the typical late follicular phase. A high LH pulse frequency is a consistent finding in PCOS regardless of obesity status, although obesity per se is associated with relative reductions in mean LH and LH pulse amplitude. A persistently high GnRH pulse frequency is a prominent contributor to the LH excess and relative FSH deficiency of PCOS.

2.3 Causes of elevated GnRH pulse frequency in PCOS

Although a persistently high LH (GnRH) pulse frequency is an expected consequence of anovulation because of a paucity of progesterone in the circulation, anovulation alone does not explain why many women with PCOS never establish regular cycles during puberty. Importantly in this regard, high LH pulse frequency in PCOS also reflects a relative resistance to negative feedback by estradiol and progesterone. Specifically, even when present, progesterone does not suppress LH pulse frequency in women with PCOS to the same extent as in women undergoing typical cycles. In one study, 7 days of exogenous estradiol and progesterone administration, which produced typical luteal phase levels, reduced LH pulse frequency by 60% in normally-cycling controls, but by only 25% in women with PCOS. Similar findings have been observed in studies of adolescents. LH pulse frequency was increased by 25%–40% in mid- to late pubertal adolescents with hyperandrogenism compared to pubertal stage-matched controls. Furthermore, some 35%–50% of hyperandrogenic adolescents are resistant to suppression of the GnRH pulse generator by combined progesterone/estradiol treatment.

Gonadotropin-releasing hormone pulse generator resistance to negative feedback restraint in part reflects the central actions of hyperandrogenemia because feedback suppression can be normalized with androgen-receptor blockade. In a study of adult women with PCOS, pretreatment with the androgen-receptor antagonist flutamide did not alter baseline LH pulse frequency, but it normalized GnRH pulse generator sensitivity to combined progesterone/estradiol negative feedback. Other studies support the hypothesis that hyperandrogenemia per se contributes to the development of neuroendocrine abnormalities in PCOS. For example, adolescent girls with congenital adrenal hyperplasia or exaggerated adrenarche may develop LH excess, ovarian hyperandrogenism and PCOS. As described below, prenatally-androgenized (PNA) female monkeys, sheep and rodents demonstrate increased LH pulse frequency and LH excess. Similarly, androgens increase GnRH neuron activity in adult mice, and prepubertal testosterone administration can increase post-pubertal LH pulse frequency in female rhesus monkeys. Also of interest in this regard, female rhesus monkeys with naturally higher testosterone levels exhibit higher circulat- ing LH concentrations and LH:FSH ratios, comprising findings that again suggest GnRH pulse generator dysfunction in the context of elevated androgens. Taken together, these findings are consistent with the hypothesis that hyperandrogenemia plays a causative role in PCOS, which is supported by a recent genome-wide association analysis suggesting that higher genetically-determined testosterone levels increase the risk for PCOS.

Thus, PCOS appears to be characterized by a vicious cycle in the hypothalamic–pituitary–ovarian axis (Figure 1). Androgen excess, primarily of ovarian origin, impairs GnRH pulse generator sensitivity to negative feedback suppression, leading to a persistently high GnRH pulse frequency, which in turn enhances LH secretion and limits FSH secretion, both of which contribute to ovarian hyperandrogenemia and ovulatory dysfunction.

2.4 Pubertal genesis of abnormal GnRH secretion in nascent PCOS

Prepubertal children exhibit a low-frequency LH pulse frequency, a low LH pulse amplitude and a low LH:FSH ratio. The onset of puberty is characterized by sleep-associated increases in LH pulse frequency and LH pulse frequency when awake gradually increases across puberty, whereas nocturnal LH pulse frequency changes little across puberty. Thus, in late pubertal girls, LH pulse frequency when awake exceeds sleep-associated LH pulse frequency. Higher GnRH pulse frequencies are presumably important for enhancing LH secretion in pubertal girls, whereas periods of relatively low GnRH pulse frequency, when awake during early puberty and when asleep in later puberty, may be important for maintaining adequate FSH secretion. These differential changes in sleep- vs. wake-associated LH pulse frequency across puberty may partly reflect greater sensitivity to progesterone negative feedback on LH release during daytime (vs. night-time) as described in both early and late pubertal girls. In this regard, McCartney and colleagues proposed a working model regarding the typical maturation of LH/GnRH pulse frequency across puberty: (1) in the state of wakefulness, LH (GnRH) pulse frequency is primarily determined by sex steroid (progesterone) negative feedback, and the GnRH pulse generator is exquisitely sensitive to low progesterone concentrations in early puberty (when androgen concentrations are low); (2) the physiologic and gradual pubertal increase in androgen concentrations antagonizes the negative feedback effects of progesterone, resulting in a gradual increase in waking GnRH pulse frequency; and (3) sleep-associated pulse frequency remains relatively constant across puberty because it is not readily influenced by low (non-luteal) progesterone concentrations.

Neuroendocrine dysfunction appears to be an early finding in some girls at risk of developing PCOS. For example, infant daughters of women with PCOS, who have a five- to 10-fold increased risk of being diagnosed with adult PCOS, demonstrate exaggerated LH responses to acute GnRH agonist stimulation. Daughters of women with PCOS may also exhibit lower serum FSH concentrations during childhood, although available studies are not consistent in this regard. Some of the classic neuroendocrine findings of PCOS (i.e., elevated basal LH, basal LH:FSH ratio, GnRH agonist-stimulated LH and GnRH agonist-stimulated LH:FSH ratio) are
not observed in early puberty in daughters of women with PCOS.
Studies in Chilean daughters of women with PCOS suggest that these aspects emerge toward the end of puberty (e.g., Tanner stage 4). In addition, in peripubertal girls with hyperandrogenism, increased LH (GnRH) pulse secretion can be detected prior to the onset of menarche, suggesting that hyperandrogenemia may modulate the normal evolution of LH (GnRH) secretion across pubertal maturation. However, the mechanisms underlying the emergence of neuroendocrine dysfunction across puberty in those who go on to develop PCOS remain unclear.

Although high LH pulse frequency is independent of obesity, obesity appears to feed into the vicious cycle of hormonal interactions in PCOS and may be an important risk factor for the development of PCOS. Peripubertal girls with obesity exhibit two- to four-fold elevated serum free testosterone concentrations compared to pubertal stage-matched controls without obesity. In such girls, circulating LH concentrations predict elevated free testosterone better than circulating insulin concentrations. As a group, girls with obesity develop elevated LH pulse frequency by mid- to late puberty. Also of interest, although non-hyperandrogenemic late pubertal girls with obesity exhibit the expected overnight decrease in LH pulse frequency, late pubertal girls with both obesity and hyperandrogenemia demonstrate high-frequency LH pulses during daytime and night-time hours without the expected overnight decrease.

The above evidence supports the hypothesis that androgen excess modulates the pubertal maturation of GnRH secretion, and McCartney and colleagues proposed a working model regarding...
the pubertal genesis of abnormal GnRH pulse generator function in those with peripubertal hyperandrogenemia. In particular, and in contrast to the typical maturational changes in LH/GnRH pulse frequency across female puberty (described above), when neuroendocrine puberty occurs in the setting of hyperandrogenemia (from any cause), atypically high androgen concentrations markedly antagonize progesterone negative feedback. This causes a rapid transition from low 24-h GnRH pulse frequency to high 24-h GnRH pulse frequency, without the prominent sleep–wake changes that may be important for appropriate balance of LH and FSH secretion. A high 24-h GnRH pulse frequency would be expected to cause LH excess and relative FSH deficiency, which would support a progression to full-blown PCOS.

3 | ABERRANT REPRODUCTIVE NEUROENDOCRINE ACTIVITY IN PRECLINICAL ANIMAL MODELS WITH PCOS-LIKE FEATURES

Ethical constraints prohibit direct scientific assessment of GnRH release in humans. However, prenatal exposure to androgens programs a number of PCOS-like features in several animal species. For example, in addition to exhibiting ovarian hyperandrogenism and ovulatory dysfunction, PNA female monkeys demonstrate central resistance to the negative feedback effects of sex steroids, increased LH (GnRH) pulse frequency, increased circulating LH concentrations, and an increased LH:FSH ratio. PNA rodents and sheep exhibit similar findings. Although PNA mice exhibit PCOS-like neuroendocrine dysfunction, it remains unclear to what degree similar in vivo abnormalities (e.g., elevated serum LH, elevated LH pulse frequency) are observed in postnatally-androgenized mice. However, in female monkeys, experimentally producing mild hyperandrogenemia (3.7-fold elevated testosterone concentration) beginning prepubertally produced elevations in post-pubertal LH pulse frequency.

We note that the degree to which such animal models are relevant to human PCOS remains controversial, in part because no animal model perfectly replicates any human disorder, including PCOS, and in part because various aspects of reproductive physiology can differ by species. Further complicating this, PCOS is heterogeneous in its presentation, and different pathogenic factors likely play different roles in different subsets of patients. With regard to PNA models, it remains unclear whether women with PCOS were exposed to excess androgens in utero. For example, some but not all studies suggest that cord blood androgen concentrations are elevated at the time of delivery in daughters of mothers with PCOS. Although direct surveillance of in utero androgen exposure is exceedingly difficult in humans, anogenital distance (a surrogate measure of intrauterine androgen exposure) appears to be longer in women with PCOS, although the results are mixed in newborn daughters of mothers with PCOS. Similarly, a recent study suggested that sebum production is temporarily increased in newborn daughters of women with PCOS, consistent with in utero exposure to maternal androgen excess.

Much of our understanding of the likely neurobiological mechanisms leading to androgen-mediated neuroendocrine dysfunction in PCOS is derived from rodent models. Studies performing electrophysiologic recordings of GnRH neurons in murine brain slices from control vs. dihydrotestosterone (DHT)-treated mice suggest that DHT (a non-aromatizable androgen) increases GnRH neuron firing rates. GnRH neuron firing frequency is similarly increased in adult PNA mice, which have elevated endogenous testosterone production. Consistent with these observations, LH pulse frequency is elevated and relatively resistant to progesterone negative feedback in PNA mice and sheep, as it is in women with hyperandrogenic PCOS. Such resistance to progesterone negative feedback in these animal models likely reflects reduced progesterone receptor expression in the arcuate nucleus.

Interestingly, conditional neuron-specific knockout of the androgen receptor in mice reduces the ability of postnatal DHT administration to induce PCOS-like features such as ovulatory dysfunction, polycystic ovaries and obesity. These data implicate the importance of neuroendocrine androgen action in the development of PCOS-like features in this model. Also of interest in this regard are mice treated with long-term with the aromatase inhibitor letrozole. These mice exhibit PCOS-like features such as hyperandrogenemia, ovulatory dysfunction and polycystic ovaries. Many of the neuroendocrine changes observed in letrozole-treated rodents (i.e., higher serum LH and lower serum FSH concentrations, reduced progesterone receptor mRNA expression in the mediobasal hypothalamus, higher numbers of arcuate nucleus kisspeptin neurons) reflect reduced estrogen negative feedback per se. However, co-treatment with fluoxetine improves estrous cyclicity and reduces both hyperandrogenemia and pituitary expression of LHβ mRNA, suggesting that some of the neuroendocrine findings in this model likely reflect letrozole-induced hyperandrogenemia.

3.1 | Potential role of GABAergic neurons in PCOS-related GnRH neuron dysfunction

The pharmacological agent valproate increases GABAergic tone, and long-term therapeutic use of valproate for epilepsy and bipolar disorder has been associated with an increased risk for PCOS. In addition, cerebrospinal fluid GABA concentrations may be elevated in women with PCOS. Although one study suggested that valproate administration to normal women for 1 month did not increase LH pulse frequency, studies in preclinical animal models suggest that GABAergic neurons play a role in the disordered GnRH secretion characteristic of PCOS.

The influence of sex steroids on GnRH secretion appears to be substantively mediated indirectly through neuronal systems afferent to GnRH neurons. Thus, neuronal circuits afferent to GnRH neurons likely mediate hyperandrogenemia-related GnRH neuron dysfunction in PCOS. Because GnRH neurons have high intracellular chloride concentrations, GABA A receptor stimulation depolarizes GnRH neurons and can induce action potential firing.
in these cells.\textsuperscript{113,114} GABAergic transmission to GnRH neurons, as well as the amplitude of the GABAergic postsynaptic currents, is decreased and increased by progesterone and DHT, respectively, suggesting that GABA neurons mediate progesterone-mediated suppression and androgen-mediated stimulation of GnRH neuron activity.\textsuperscript{88,115} In PNA mice, anatomical GABAergic innervation onto GnRH neurons is increased, as is functional excitatory GABAergic drive.

These GABAergic neurons, originating largely from the arcuate nucleus, demonstrate less colocalization with progesterone receptors compared to control mice, suggesting a possible mechanism for increased GABAergic drive that would potentially be associated with progesterone resistance.\textsuperscript{102} Long-term selective activation of arcuate nucleus GABAergic neuron terminals in the rostral preoptic area, where GABAergic terminals densely contact GnRH neurons, leads to a PCOS-like phenotype including hyperandrogenemia and disrupted estrous cycles, along with a possible increase in LH pulse frequency.\textsuperscript{119} In addition to influencing GnRH neurons via direct synaptic inputs, GABAergic neurons may influence GnRH release indirectly via arcuate nucleus KNDy (i.e., kisspeptin, neuropekinin B and dynorphin) neurons. For example, PNA ewes exhibit increased GABAergic appositions onto both mediobasal hypothalamus GnRH neurons and arcuate nucleus KNDy neurons.\textsuperscript{120} Overall, these studies imply that PNA causes organizational and functional changes within the GABAergic neuronal networks that, in turn, promote GnRH neuron overactivity and LH excess, in addition to other PCOS-like characteristics.

Although the specific mechanisms by which pathological GABA signaling develops remains to be determined, impaired microglia pruning of GABAergic synapses in early development has been implicated.\textsuperscript{121} In the PNA mouse model, fewer “sculpting” microglia populate the rostral preoptic area during adolescent development, and microglia in this region are found to engulf fewer GABAergic synapses. Whether prenatal androgen excess directly or indirectly drives changes in microglia behavior remains to be determined, although these data suggest that the PNA catalyzes a cascade of events shaping the developing PCOS-like brain prior to disease onset. Also of interest, even though atypically high GABAergic input onto GnRH neurons is observable before puberty and before the emergence of PCOS-like findings in PNA mice,\textsuperscript{116,118} both the atypical GABAergic input onto GnRH neurons and the PCOS-like findings can be reversed after puberty with androgen-receptor blockade.\textsuperscript{117,118}

### 3.3 | Potential role of kisspeptin neurons in PCOS-related GnRH neuron dysfunction

The neuropeptide kisspeptin potently stimulates GnRH neuron activity and GnRH release. Most arcuate nucleus kisspeptin neurons co-express neurokinin B and dynorphin and have thus been called KNDy (kisspeptin/neurokinin B/dynorphin) neurons. A number of studies suggest that arcuate nucleus KNDy neurons form an extensively interconnected autoregulatory network, with neurokinin B augmenting and dynorphin reducing KNDy neuron activity.\textsuperscript{127-130} Accordingly, arcuate kisspeptin neurons are postulated to be a fundamental component of the GnRH pulse generator.\textsuperscript{128,131-133} In addition, KNDy neurons are considered to at least partly mediate sex steroid negative feedback on GnRH secretion.\textsuperscript{129,132,134}

Women with PCOS appear to have elevated circulating kisspeptin levels (standardized mean difference 1.15 with 95%
4 | POTENTIAL EFFICACY OF PHARMACOLOGICAL AGENTS TARGETING THE GNRH-RELATED NEURONAL NETWORK IN PCOS

The previously-described data, which suggest that hyperandrogenemia per se causes dysregulated GnRH secretion, imply the potential utility of androgen-receptor blockade in restoring normal GnRH secretion in PCOS. For example, although the androgen-receptor antagonist flutamide did not alter baseline LH pulse frequency in PCOS, it normalized GnRH pulse generator sensitivity to estradiol and progesterone negative feedback. However, when used in isolation, the overall therapeutic value of androgen-receptor blockade remains unclear. For example, studies are mixed on whether flutamide improves ovulation rates in PCOS. Additionally, androgen-receptor antagonists may adversely affect the development of male offspring, limiting their therapeutic potential in potentially-fertile women. It remains possible that such agents could have unique benefits during critical developmental windows. For example, a recent retrospective study of adult women with PCOS suggested that, compared to antiandrogen initiation in adulthood, the initiation of antiandrogen treatment during adolescence is associated with a greater likelihood of first childbirth after spontaneous (unassisted) conception during adulthood.

Pharmacological agents targeting higher-order neuronal control of GnRH secretion (e.g., the KNDy neuronal network) may prove useful in the future. For example, a study in adults with PCOS suggested that the selective NK3R antagonist pavinetant (formerly MLE4901 and AZD4901) administered at a dose of 80 mg day\(^{-1}\) for 1 week reduced LH pulse frequency (by 3.55 LH pulses over 8 h), circulating LH concentrations (50% reduction in LH area under the curve) and basal (i.e., non-pulsatile) LH secretion (80% lower), at the same time as preserving FSH secretion. Although the efficacy of NK3R blockade appeared to be diminished over time in this study (i.e., changes were not statistically significant after 28 days of use), the reduction in LH area under the curve, LH:FSH ratio, LH pulse frequency and basal LH secretion remained significantly lower at 28 days when analysis was restricted to non-ovulatory patients. In another study of women with PCOS, 40 mg of pavinetant administered twice daily for 7 days reduced both circulating LH concentrations and LH pulse frequency by almost 40%, at the same time as reducing FSH concentrations by 20%. Although these are interesting proof-of-concept studies, the clinical development of pavinetant was abandoned, at least in part because of the potential for liver toxicity. A related, recently-published phase 2a multicenter randomized controlled trial in PCOS demonstrated that 12 weeks of administration of fezolinetant (ESN364), another NK3R antagonist, at 180 mg day\(^{-1}\) reduced serum testosterone by approximately 35%, LH and FSH by approximately 60% and 18%, respectively, and LH:FSH ratio by almost 60%. Although LH pulse frequency was not assessed in this study, reductions in LH:FSH ratio and testosterone were sustained for 12 weeks of treatment. No clear changes in circulating estradiol concentrations or ovulatory function were observed.
in this relatively short-term study.\textsuperscript{153} The success of longer-term NK3R antagonism in PCOS remains to be determined. The potential impact of chronic NK3R antagonism on gonadotropin surge generation and ovulation is unknown. Of note, it is possible that long-term, continuous NK3R antagonist administration could promote hypogonadotropic hypogonadism, as occurs in some individuals with homozygous loss-of-function variants of TACR3, the gene encoding NK3R.\textsuperscript{154}

The site of action of NK3R antagonists may be the KNDy neuron as discussed above, although it is important to bear in mind that this receptor has also been reported in the terminal regions of GnRH neurons in the rat, and that the NK3R agonist senktide increases GnRH release when applied to the median eminence, even in kispeptin knockout mice, suggesting that GnRH neurons themselves could also be targeted.\textsuperscript{155,156}

5 | SUMMARY AND FUTURE DIRECTIONS

It has long been recognized that PCOS is associated with a persistently high LH (GnRH) pulse frequency and disordered gonadotropin secretion, with LH excess and a high LH-to-FSH ratio in particular. Although the translational research community has uncovered some of the mechanisms accounting for aberrant GnRH secretion in PCOS, much remains unclear. Hyperandrogenemia per se contributes to GnRH pulse generator overactivity, at least in part by reducing GnRH pulse generator sensitivity to sex steroid (progesterone) negative feedback. This leads to a persistently high GnRH pulse frequency, which preferentially favors LH production and limits FSH production. In turn, these alterations in gonadotropins bolster ovarian androgen production and contribute to ovulatory dysfunction. The degree to which these alterations of GnRH secretion originate in fetal development remains unclear, although the endogenous androgen excess that develops in prenatally-androgenized animals appears to maintain such abnormalities in prenatally-androgenized animals.\textsuperscript{50,98,117,118} In addition, androgen-receptor antagonism normalizes GnRH pulse generator sensitivity to negative feedback in women with PCOS\textsuperscript{50} and rescues at least some of the neuroendocrine defects identified in preclinical models.\textsuperscript{117,118} These findings suggest the possibility that androgen-receptor blockade can normalize GnRH secretion in PCOS, although the results to date are mixed and additional studies are needed. Early studies of agents that modulate GnRH secretion via higher-order neuronal inputs (e.g., selective neurokinin-3 receptor antagonists) also suggest potential promise as future treatments for PCOS. Preclinical models will continue to play an important role in improving our understanding of neuroendocrine dysfunction in PCOS. Future directions should include studies to define the pathogenic neuroendocrine changes occurring during critical developmental windows in addition to the initial testing of novel therapeutics for PCOS.

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CONFLICT OF INTERESTS

The authors have no conflicts to declare.

AUTHOR CONTRIBUTIONS

Christopher R. McCartney: Conceptualization; Writing – original draft; Writing – review & editing. Rebecca E Campbell: Conceptualization; Writing – original draft; Writing – review & editing. John C Marshall: Conceptualization; Writing – review & editing. Suzanne Moenter: Conceptualization; Writing – review & editing.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

1. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod. 2016;31(12):2841-2855.
2. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19(1):41-47.
3. Teede HJ, Misso ML, Costello MF, et al. International PN. Recommendations from the international evidence-based guide-line for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110(3):364-379.
4. Zawadski JK, Dunai A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunai A, Givens JR, Haseltine FP, Merriam GR, eds. Polycystic ovary syndrome. Blackwell Scientific Publications; 1992:377-384.
5. Azziz R, Carmina E, Dewailly D, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006;91(11):4237-4245.
6. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2010;16(4):347-363.
7. Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(6):618-637.
8. Barry JA, Azziza MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome:...
a systematic review and meta-analysis. *Hum Reprod Update.* 2014;20(5):748-758.

9. Blay SL, Aguiar JV, Passos IC. Polycystic ovary syndrome and mental disorders: a systematic review and exploratory meta-analysis. *Neuropsychiatr Dis Treat.* 2016;12:2895-2903.

10. Cassar S, Misso ML, Hopkins WG, Shaw CS, Teede HJ, Stepto NK. Insulin resistance in polycystic ovary syndrome: a systematic review and meta-analysis of euglycaemic-hyperinsulinaemic clamp studies. *Hum Reprod.* 2016;31(11):2619-2631.

11. Wekker V, van Dammnen L, Koning A, et al. Long-term cardiometabolic risk in women with PCOS: a systematic review and meta-analysis. *Hum Reprod Update.* 2020;26(6):942-960.

12. Kahal H, Kryou I, Uthman OA, et al. The prevalence of obstructive sleep apnoea in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Sleep Breath.* 2020;24(1):339-350.

13. Wildt L, Hausler A, Marshall G, et al. Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology.* 1981;109(2):376-385. [https://doi.org/10.1210/endo-109-2-376](https://doi.org/10.1210/endo-109-2-376)

14. Dalkin AC, Haisenleder DJ, Ortolano GA, Ellis TR, Marshall JC. The frequency of gonadotropin-releasing-hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression. *Endocrinology.* 1989;125(2):917-924.

15. Dalkin AC, Burger LL, Aylor KW, et al. Regulation of gonadotropin subunit gene transcription by gonadotropin-releasing hormone: measurement of primary transcript ribonucleic acids by quantitative reverse transcription-polymerase chain reaction assays. *Endocrinology.* 2001;142(1):139-146.

16. Moenter SM, Brand RM, Midgley AR, Karsch FJ. Dynamics of gonadotropin-releasing hormone release during a pulse. *Endocrinology.* 1992;130(1):503-510.

17. Moenter SM. Leap of Faith: Does Serum Luteinizing Hormone Always Accurately Reflect Central Reproductive Neuroendocrine Activity? *Neuroendocrinology.* 2015;102(4):256-266.

18. Zumoff B, Freeman R, Coupey S, Saenger P, Markowitz M, Kream J. A chronobiologic abnormality in luteinizing hormone secretion in teenage girls with the polycystic-ovary syndrome. *N Engl J Med.* 1983;309(20):1206-1209.

19. Waldstreicher J, Santoro NF, Hall JE, Filicori M, Crowley WF Jr. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization. *J Clin Endocrinol Metab.* 1988;66(1):165-172.

20. Venturroli S, Porcu E, Fabbri R, et al. Longitudinal evaluation of the different gonadotropin pulsatile patterns in anovulatory cycles of young girls. *J Clin Endocrinol Metab.* 1992;74(4):836-841.

21. Berga SL, Guitzk DS, Winters SJ. Increased luteinizing hormone and alpha-subunit secretion in women with hyperandrogenic anovulation. *J Clin Endocrinol Metab.* 1993;77(4):895-901.

22. Apter D, Butzow T, Laughlin GA, Yen SS. Accelerated 24-hour luteinizing hormone pulsatile activity in adolescent girls with ovarian hyperandrogenism: relevance to the developmental phase of polycystic ovarian syndrome. *J Clin Endocrinol Metab.* 1994;79(1):119-125.

23. Porcu E, Venturroli S, Longhi M, Fabbri R, Paradisi R, Flamigni C. Chronobiologic evolution of luteinizing hormone secretion in adolescence: developmental patterns and speculations on the onset of the polycystic ovary syndrome. *Fertil Steril.* 1997;67(5):842-848.

24. Taylor AE, McCourt B, Martin KA, et al. Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1997;82(7):2248-2256.

25. Arroyo A, Laughlin GA, Morales AJ, Yen SS. Inappropriate gonadotropin secretion in polycystic ovary syndrome: Influence of adi- posity. *J Clin Endocrinol Metab.* 1997;82(11):3728-3733.

26. Garcia-Rudaz MC, Ropelato MG, Escobar ME, Veldhuis JD, Barontini M. Augmented frequency and mass of LH discharged per burst are accompanied by marked disorderliness of LH secretion in adolescents with polycystic ovary syndrome. *Eur J Endocrinol.* 1998;139(6):621-630.

27. Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F. Characterization of the inappropriate gonadotropin secretion in polycystic ovarian syndrome. *J Clin Invest.* 1976;57(5):1320-1329.

28. Burt Solorzano CM, McCartney CR. Polycystic ovary syndrome: Ontogeny in adolescence. *Endocrinol Metab Clin North Am.* 2021;50(1):25-42.

29. Chang RJ, Laufer LR, Meldrum DR, et al. Steroid secretion in polycystic ovarian disease after ovarian suppression by a long-acting gonadotropin-releasing hormone agonist. *J Clin Endocrinol Metab.* 1983;56(5):897-903.

30. Steingold K, De Ziegler D, Cedars M, et al. Clinical and hormonal effects of chronic gonadotropin-releasing hormone agonist treatment in polycystic ovarian disease. *J Clin Endocrinol Metab.* 1987;65(4):773-777.

31. Hiam D, Moreno-Asso A, Teede HJ, et al. The genetics of polycystic ovary syndrome: An overview of candidate gene systematic reviews and genome-wide association studies. *J Clin Med.* 2019;8(10):1606.

32. Deswal R, Nanda S, Dang AS. Association of Luteinizing hormone and LH receptor gene polymorphism with susceptibility of Polycystic ovary syndrome. *Syst Biol Reprod Med.* 2019;65(5):400-408.

33. Chang RJ, Mandel FP, Lu JK, Judd HL. Enhanced disparity of gonadotropin secretion by estrone in women with polycystic ovarian disease. *J Clin Endocrinol Metab.* 1982;54(3):490-494.

34. Reame N, Sauder SE, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion during the human menstrual cycle: evidence for altered frequency of gonadotropin-releasing hormone secretion. *J Clin Endocrinol Metab.* 1984;59(2):328-337.

35. Filicori M, Santoro N, Merriam GR, Crowley WF Jr. Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. *J Clin Endocrinol Metab.* 1986;66(6):1136-1144.

36. Hall JE, Schoenfeld DA, Martin KA, Crowley WF Jr. Hypothalamic gonadotropin-releasing hormone secretion and follicle-stimulating hormone dynamics during the luteal-follicular transition. *J Clin Endocrinol Metab.* 1992;74(3):600-607.

37. Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S, Brenner WJ. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab.* 1984;58(2):378-383.

38. Gill S, Lavoie HB, Bo-Abbas Y, Hall JE. Negative feedback effects of gonadal steroids are preserved with aging in postmenopausal women. *J Clin Endocrinol Metab.* 2002;87(5):2297-2302.

39. Cagnacci A, Melis GB, Paololetti AM, et al. Influence of oestradiol and progesterone on pulsatile LH secretion in postmenopausal women. *Clin Endocrinol (Oxf).* 1989;31(5):541-550.

40. Karsch FJ, Weick RF, Hotchkiss J, Dierschke DJ, Knobil E. An analysis of the negative feedback control of gonadotropin secretion utilizing chronic implantation of ovarian steroids in ovariec-tomized rhesus monkeys. *Endocrinology.* 1973;93(2):478-486.

41. Nippoldt TB, Reame NE, Kelch RP, Marshall JC. The roles of estradiol and progesterone in decreasing luteinizing hormone pulse frequency in the luteal phase of the menstrual cycle. *J Clin Endocrinol Metab.* 1989;69(1):67-76.

42. Welt CK, Martin KA, Taylor AE, et al. Frequency modulation of follicle-stimulating hormone (FSH) during the luteal-follicular transition: evidence for FSH control of inhibin B in normal women. *J Clin Endocrinol Metab.* 1997;82(8):2645-2652.

43. Kazer RR, Kessel B, Yen SS. Circulating luteinizing hormone pulse frequency in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1987;65(2):233-236.
Pagan YL, Srouji SS, Jimenez Y, Emerson A, Gill S, Hall JE. Inverse relationship between luteinizing hormone and body mass index in polycystic ovarian syndrome: investigation of hypothalamic and pituitary contributions. J Clin Endocrinol Metab. 2006;91(4):1309-1316.

Morales AJ, Laughlin GA, Butzow T, Maheshwari H, Baumann G, Yen SS. Insulin, somatotropic, and luteinizing hormone axes in lean and obese women with polycystic ovary syndrome: common and distinct features. J Clin Endocrinol Metab. 1996;81(8):2854-2864.

Daniels TL, Berga SL. Resistance of gonadotropin releasing hormone drive to sex steroid-induced suppression in hyperandrogenic anovulation. J Clin Endocrinol Metab. 1997;82(12):4179-4183.

Pastor CL, Griffin-Korf ML, Aloi JA, Evans WS, Marshall JC. Polycystic ovary syndrome: evidence for reduced sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab. 1998;83(2):582-590.

Chhabra S, McCartney CR, Yoo RY, Eagleson CA, Chang RJ, Marshall JC. Progesterone inhibition of the hypothalamic gonadotropin-releasing hormone pulse generator: evidence for varied effects in hyperandrogenemic adolescent girls. J Clin Endocrinol Metab. 2005;90(5):2810-2815.

Blank SK, McCartney CR, Chhabra S, et al. Modulation of gonadotropin-releasing hormone pulse generator sensitivity to progesterone inhibition in hyperandrogenic adolescent girls: implications for regulation of pubertal maturation. J Clin Endocrinol Metab. 2009;94(7):2360-2366.

Eagleson CA, Gingrich MB, Pastor CL, et al. Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab. 2000;85(11):4047-4052.

Rosenfield RL. Clinical review: Identifying children at risk for polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92(3):787-796.

Barnes RB, Rosenfield RL, Ehrmann DA, et al. Ovarian hyperandrogenism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women. J Clin Endocrinol Metab. 1994;79(5):1328-1333.

Ibanez L, Dimartino-Nardi J, Potau N, Saenger P. Premature adrenarche–normal variant or forerunner of adult disease? Endocr Rev. 2000;21(6):671-696.

Dumesic DA, Abbott DH, Eisner JR, Goy RW. Prenatal exposure of female rhesus monkeys to testosterone propionate increases serum luteinizing hormone levels in adulthood. Fertil Steril. 1997;67(1):155-163.

Robinson JE, Forsdike RA, Taylor JA. In utero exposure of female lambs to testosterone reduces the sensitivity of the gonadotropin-releasing hormone neuronal network to inhibition by progesterone. Endocrinology. 1999;140(12):5797-5805.

Foeccking EM, Szabo M, Schwartz NB, Levine JE. Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. Biol Reprod. 2005;72(6):1475-1483.

Melrose P, Gross L. Steroid effects on the secretory modalities of gonadotropin-releasing hormone release. Endocrinology. 1987;121(1):190-199.

Pielecka J, Quaynor SD, Moenter SM. Androgens increase gonadotropin-releasing hormone neuron firing activity in females and interfere with progesterone negative feedback. Endocrinology. 2006;147(3):1474-1479.

McGee WK, Bishop CV, Bahar A, et al. Elevated androgens during puberty in female rhesus monkeys lead to increased neuronal drive to the reproductive axis: a possible component of polycystic ovary syndrome. Hum Reprod. 2012;27(2):531-540.

Abbott DH, Rayome BH, Dumesic DA, et al. Clustering of PCOS-like traits in naturally hyperandrogenic female rhesus monkeys. Hum Reprod. 2017;32(4):923-936.

Ruth KS, Day FR, Tyrell J, et al. Using human genetics to understand the disease impacts of testosterone in men and women. Nat Med. 2020;26(2):252-258.

Apter D, Butzow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. J Clin Endocrinol Metab. 1993;73(4):940-949.

McCartney CR, Prendergast KA, Blank SK, Helm KD, Chhabra S, Marshall JC. Maturation of luteinizing hormone (gonadotropin-releasing hormone) secretion across puberty: evidence for altered regulation in obese prepubertal girls. J Clin Endocrinol Metab. 2009;94(1):56-66.

Collins JS, Marshall JC, McCartney CR. Differential Sleep-Wake Sensitivity of Gonadotropin-Releasing Hormone Secretion to Progesterone Inhibition in Early Pubertal Girls. Neuroendocrinology. 2012;96(3):222-227.

Kim SH, Lundgren JA, Bhabhra R, et al. Progesterone-Mediated Inhibition of the GnRH Pulse Generator: Differential Sensitivity as a Function of Sleep Status. J Clin Endocrinol Metab. 2018;103(3):1112-1121.

Rital S, Pei Y, Lu H, et al. Prenatal androgen exposure and trans-generational susceptibility to polycystic ovary syndrome. Nat Med. 2019;25(12):1894-1904.

Crisosto N, Echiburu B, Maliqueo M, et al. Improvement of hyperandrogenism and hyperinsulinemia during pregnancy in women with polycystic ovary syndrome: possible effect in the ovarian follicular mass of their daughters. Fertil Steril. 2012;97(1):218-224.

Sir-Petermann T, Codner E, Maliqueo M, et al. Increased anti-Mullerian hormone serum concentrations in prepubertal daughters of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91(8):3105-3109.

Sir-Petermann T, Codner E, Perez V, et al. Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2009;94(6):1923-1930.

Sir-Petermann T, Ladron de Guevara A, Codner E, et al. Relationship between anti-Mullerian hormone (AMH) and insulin levels during different Tanner stages in daughters of women with polycystic ovary syndrome. Reprod Sci. 2012;19(4):383-390.

Crisosto N, Ladron de Guevara A, Echiburu B, et al. Higher luteinizing hormone levels associated with antimullerian hormone in post-menarchal daughters of women with polycystic ovary syndrome. Fertil Steril. 2019;111(2):381-388.

Christensen SB, Black MH, Smith N, et al. Prevalence of polycystic ovary syndrome in adolescents. Fertil Steril. 2013;100(2):470-477.

Day FR, Hinds DA, Tung JY, et al. Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. Nat Commun. 2015;6:8464.

Day F, Karaderi T, Jones MR, et al. Large-scale genome-wide meta-analysis of polycystic ovary syndrome suggests shared genetic architecture for different diagnosis criteria. PLoS Genet. 2018;14(12):e1007813.

Brower MA, Hai Y, Jones MR, et al. Bidirectional Mendelian randomization to explore the causal relationships between body mass index and polycystic ovary syndrome. Hum Reprod. 2019;34(1):127-136.

Reinehr T, de Sousa G, Roth CL, Andler W. Androgens before and after weight loss in obese children. J Clin Endocrinol Metab. 2005;90(10):5588-5595.
77. McCartney CR, Blank SK, Prendergast KA, et al. Obesity and sex steroid changes across puberty: evidence for marked hyperandrogenemia in pre- and early pubertal obese girls. *J Clin Endocrinol Metab.* 2007;92(2):430-436.

78. Knudsen KL, Blank SK, Burt Solorzano C, et al. Hyperandrogenemia in obese peripubertal girls: correlates and potential endocrinological determinants. *Obesity (Silver Spring).* 2010;18(11):2118-2124.

79. Kang MJ, Yang S, Hwang IT. The impact of obesity on hyperandrogenemia in Korean girls. *Ann Pediatr Endocrinol Metab.* 2016;21(4):219-225.

80. Torchen LC, Legro RS, Dunaif A. Distinctive reproductive phenotypes in peripubertal girls at risk for polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2019;104(8):3355-3361.

81. Abbot DH, Rogers J, Dumesic DA, Levine JE. Naturally occurring androgen exposure alters KNDy neurons and their afferent network in a model of polycystic ovarian syndrome. *Proc Natl Acad Sci U S A.* 2015;112(2):596-601.

82. Moore AM, Prescott M, Campbell RE. Estradiol negative and positive feedback in a prenatal androgen-induced mouse model of polycystic ovarian syndrome. *Endocrinology.* 2013;154(2):796-806.

83. Collins JS, Beller JP, Burt Solorzano C, et al. Blunted day-night changes in luteinizing hormone pulse frequency in girls with obesity: The potential role of hyperandrogenemia. *J Clin Endocrinol Metab.* 2019;104(10):4390-4397.

84. Cardoso RC, Padmanabhan V. Developmental programming of PCOS traits: Insights from the sheep. *Med Sci (Basel).* 2017;9(7).

85. Stener-Victorin E, Padmanabhan V, Walters KA, et al. Animal models to understand the etiology and pathophysiology of polycystic ovary syndrome. *Endocr Rev.* 2020;41(4):bnaa010.

86. Abbott DH, Rogers J, Dumesc DA, Levine JE. Naturally occurring and experimentally induced rhesus macaque models for polycystic ovarian syndrome: Translational Gateways to Clinical Application. *Med Sci (Basel).* 2019;7(12).

87. Ruddenklau A, Campbell RE. Neuroendocrine impairments of polycystic ovary syndrome. *Endocrinology.* 2019;160(10):2230-2242.

88. Conte-DeJong A, DeLamater J, Chen C, et al. The kisspeptin/neurokinin B/dynorphin (KNDy) cell population of the arcuate nucleus: sex differences and effects of prenatal testosterone in sheep. *Endocrinology.* 2010;151(1):301-311.

89. Aliabadi E, Namavar MR, Marconato K, et al. Kisspeptin expression features in the arcuate and anteroventral periventricular nuclei of hypothalamus of letrozole-induced polycystic ovarian syndrome in rats. *Arch Gynecol Obstet.* 2017;296(5):957-963.

90. Stein AY, Heryer I, de la Garza M, et al. Increased cerebralspinal fluid levels of GABA, testosterone and estradiol in women with polycystic ovary syndrome. *Hum Reprod.* 2017;32(7):1450-1456.

91. DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Mol Endocrinol.* 2002;16(12):2872-2891.
114. Herbison AE, Moenter SM. Depolarising and hyperpolarising actions of GABA(A) receptor activation on gonadotrophin-releasing hormone neurones: Towards an emerging consensus. J Neuroendocrinol. 2011;23(7):557-569.

115. Sullivan SD, Moenter SM. GABAergic integration of progesterone and androgen feedback to gonadotropin-releasing hormone neurones. Biol Reprod. 2005;72(1):33-41.

116. Berg T, Silveira MA, Moenter SM. Prepubertal development of GABAergic transmission to gonadotropin-releasing hormone (GnRH) neurons and postsynaptic response are altered by prenatal androgenization. J Neurosci. 2018;38(9):2283-2293.

117. Sullivan SD, Moenter SM. Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurones: implications for a common fertility disorder. Proc Natl Acad Sci U S A. 2004;101(18):7129-7134.

118. Silva MS, Prescott M, Campbell RE. Ontogeny and reversal of brain circuit abnormalities in a preclinical model of PCOS. JCI Insight. 2018;3(7):e99405.

119. Silva MSB, Desroziers E, Hessler S, et al. Activation of arcuate nucleus GABA neurons promotes luteinizing hormone secretion and reproductive dysfunction: Implications for polycystic ovary syndrome. EBioMedicine. 2019;44:582-596.

120. Porter DT, Moore AM, Cobern JA, et al. Prenatal testosterone exposure alters GABAergic synaptic inputs to GnRH and KNDy neurons in a sheep model of polycystic ovarian syndrome. Endocrinology. 2019;160(11):2529-2542.

121. Sati A, Prescott M, Holland S, Jasoni CL, Desroziers E, Campbell RE. Morphological evidence indicates a role for microglia in shaping the PCOS-like brain. J Neuroendocrinol. 2021;e12999.

122. Tata B, Mimouni NEH, Barbotin AL, et al. Elevated prenatal anti-Mullerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. Nat Med. 2018;24(6):834-846.

123. Piltonen TT, Giacobini P, Edvinsson A, et al. Circulating antimullerian hormone and steroid hormone levels remain high in pregnant women with polycystic ovary syndrome at term. Fertil Steril. 2019;111(3):588-596 e1.

124. Detti L, Christiansen ME, Francillon L, et al. Serum Anti-Mullerian hormone (AMH) in mothers with polycystic ovary syndrome (PCOS) and their term fetuses. Syst Biol Reprod Med. 2019;65(2):147-154.

125. Cimino I, Casoni F, Liu X, et al. Novel role for anti-Mullerian hormone in the regulation of GnRH neuron excitability and hormone secretion. Nat Commun. 2016;7:10055.

126. Mimouni NEH, Paiva I, Barbotin AL, et al. Polycystic ovary syndrome is transmitted via a transgenerational epigenetic process. Cell Metab. 2021;33(3):513-530 e8.

127. Wakabayashi Y, Nakada T, Murata K, et al. Neurakin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci. 2010;30(8):3124-3132.

128. Herbison AE. The gonadotrophin-releasing hormone pulse generator. Endocrinology. 2018;159(11):3723-3736.

129. Moore AM, Coolen LM, Porter DT, Goodman RL, Lehman MN. KNDy cells revisited. Endocrinology. 2018;159(9):3219-3234.

130. Lehman MN, He W, Coolen LM, Levine JE, Goodman RL. Does the KNDy Model for the Control of Gonadotropin-Releasing Hormone Pulses Apply to Monkeys and Humans? Semin Reprod Med. 2019;37(2):71-83.

131. Plant TM. The neurobiological mechanism underlying hypothalamic GnRH pulse generation: the role of kisspeptin neurons in the arcuate nucleus. F1000Res. 2019;8:982.

132. Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: A central node in the control of gonadotropin-releasing hormone secretion. Endocrinology. 2010;151(8):3479-3489.

133. Maeda K, Ohkura S, Uenoyma Y, et al. Neurobiological mechanisms underlying GnRH pulse generation by the hypothalamus. Brain Res. 2010;1364:103-115.

134. McCartney CR, Marshall JC. Neuroendocrinology of Reproduction. In: Strauss JF, Barbieri RL, eds. Yen & Jaffe’s reproductive endocrinology: Physiology, pathophysiology, and clinical management. Elsevier; 2019:1-24.

135. Perez-Lopez FR, Ornat L, Lopez-Baena MT, Santabarbara J, Savorin-Cornudella R, Perez-Roncero GR. Circulating kisspeptin and anti-mullerian hormone levels, and insulin resistance in women with polycystic ovary syndrome: A systematic review, meta-analysis, and meta-regression. Eur J Obstet Gynecol Reprod Biol. 2021;260:85-98.

136. Albalawi FS, Daghestani MH, Daghestani MH, Eldali A, Warsy AS. rs488 polymorphism in KISS1 gene, its effect on polycystic ovary syndrome development and anthropometric and hormonal parameters in Saudi women. J Biomed Sci. 2018;25(1):50.

137. Yan X, Yuan C, Zhao N, Cui Y, Liu J. Prenatal androgen excess enhances stimulation of the GNRH pulse in pubertal female rats. J Endocrinol. 2014;222(1):73-85.

138. Caldwell AS, Eid S, Kay CR, et al. Haplosufficient genomic androgen receptor signaling is adequate to protect female mice from induction of polycystic ovary syndrome features by prenatal hyperandrogenization. Endocrinology. 2015;156(4):1441-1452.

139. Osaka S, Iwase A, Nakahara T, et al. Kisspeptin in the hypothalamus of 2 rat models of polycystic ovary syndrome. Endocrinology. 2017;158(2):367-377.

140. Cernea M, Padmanabhan V, Goodman RL, Coolen LM, Lehman MN. Prenatal testosterone treatment leads to changes in the morphology of KNDy neurons, their inputs, and projections to GnRH cells in female sheep. Endocrinology. 2015;156(9):3277-3291.

141. Weems PW, Witty CF, Amstalden M, Coolen LM, Goodman RL, Lehman MN. Kappa-Opioid receptor is colocalized in GnRH and KNDy cells in the female ovine and rat brain. Endocrinology. 2016;157(6):2367-2379. https://doi.org/10.1210/en.2015-1763

142. Weems PW, Coolen LM, Hileman SM, et al. Evidence that dynorphin acts upon KNDy and GnRH neurons during gnrh pulse termination in the ewe. Endocrinology. 2018;159(9):3187-3199.

143. Berga SL, Yen SS. Opioidergic regulation of LH pulsatility in women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 1989;30(2):177-184.

144. McCarthy EA, Dischino D, Maguire C, et al. Inhibiting kiss1 neurons with kappa opioid receptor agonists to treat polycystic ovary syndrome and vasomotor symptoms. J Clin Endocrinol Metab. 2022;107(1):e328–e347.

145. Gibson AG, Jaime J, Burger LL, Moenter SM. Prenatal androgen treatment does not alter the firing activity of hypothalamic arcuate kisspeptin neurons in female mice. eNeuro. 2021;8(5):ENEURO.0306-21.2021.

146. De Leo V, Lanzetta D, D’Antona D, la Marca A, Morgante G. Hormonal effects of flutamide in young women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1998;83(1):99-102.

147. Ibanez L, Valls C, Ferrer A, Ong K, Dunger DB, De Zegher F. Additive effects of insulin-sensitizing and anti-androgen treatment in young, nonobese women with hyperinsulinism, hyperandrogenism, dyslipidemia, and anovulation. J Clin Endocrinol Metab. 2002;87(6):2870-2874.

148. Paradisi R, Fabbri R, Battaglia C, Venturini S. Ovulatory effects of flutamide in the polycystic ovary syndrome. Gynecol Endocrinol. 2013;29(4):391-395.

149. Elenis E, Desroziers E, Persson S, Sundstrom Poromaa I, Campbell RE. Early initiation of anti-androgen treatment is associated with increased probability of spontaneous conception leading to childbirth in women with polycystic ovary syndrome: a...
population-based multicentre cohort study in Sweden. *Hum Reprod*. 2021;36(5):1427-1435.

150. George JT, Kakkar R, Marshall J, et al. Neurokinin B receptor antagonism in women with polycystic ovary syndrome: A randomized, Placebo-Controlled Trial. *J Clin Endocrinol Metab*. 2016;101(11):4313-4321.

151. Skorupskaite K, George JT, Veldhuis JD, Millar RP, Anderson RA. Kisspeptin and neurokinin B interactions in modulating gonadotropin secretion in women with polycystic ovary syndrome. *Hum Reprod*. 2020;35(6):1421-1431.

152. Modi M, Dhillo WS. Neurokinin B and neurokinin-3 receptor signaling: Promising developments in the management of menopausal hot flushes. *Semin Reprod Med*. 2019;37(3):125-130.

153. Fraser GL, Obermayer-Pietsch B, Laven J, et al. Randomized controlled trial of neurokinin 3 receptor antagonist fezolinetant for treatment of polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2021;106(9):e3519-e3532.

154. Topaloglu AK, Reimann F, Guclu M, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet*. 2009;41(3):354-358.

155. Krajewski SJ, Anderson MJ, Iles-Shih L, Chen KJ, Urbanski HF, Rance NE. Morphologic evidence that neurokinin B modulates gonadotropin-releasing hormone secretion via neurokinin 3 receptors in the rat median eminence. *J Comp Neurol*. 2005;489(3):372-386.

156. Gaskins GT, Glanowska KM, Moenter SM. Activation of neurokinin 3 receptors stimulates GnRH release in a location-dependent but kisspeptin-independent manner in adult mice. *Endocrinology*. 2013;154(11):3984-3989.

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