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Authors

Rose, Lillian

Kauffman, Alexander S

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REVIEW ARTICLE

Neuroendocrine Contributions to Polycystic Ovary Syndrome (PCOS)

Divergent effects of hyperandrogenemia on the female reproductive neuroendocrine axis under different physiological conditions

Lillian Rose  | Alexander S. Kauffman 

Department of OBGYN and Reproductive Sciences, University of California, San Diego, La Jolla, California, USA

Correspondence

Alexander S. Kauffman, Department of OBGYN and Reproductive Sciences, University of California San Diego, 9500 Gilman Drive, #0674, La Jolla, CA 92093, USA.
Email: akauffman@ucsd.edu

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Abstract

The hypothalamic–pituitary–gonadal (HPG) axis coordinates reproductive function and incorporates gonadal sex steroid feedback to regulate gonadotropin secretion in a homeostatic manner. In females, ovarian estrogens and progestins act as the primary negative feedback signals to suppress luteinizing hormone (LH) release, whereas in males, androgens, such as testosterone (T), typically provide this feedback. However, androgens can also exert feedback effects on the female HPG axis, particularly in conditions where circulating T levels exceed that of the normal female physiological range. Three clinically relevant examples are anabolic substance abuse, testosterone therapy, and polycystic ovary syndrome (PCOS; recently renamed polycystic metabolic ovarian syndrome [PMOS]). Despite shared exposure to “androgen excess,” these different hyperandrogenic states are associated with strikingly different neuroendocrine phenotypes. In testosterone therapy and anabolic substance abuse, high exogenous androgen exposure in or above the male physiological range typically suppresses LH secretion and disrupts ovulatory cycling, mimicking enhanced negative feedback on the HPG axis. Conversely, in PMOS, endogenous elevations in T, high for a female but below the male physiological range, are associated with increased LH secretion, acyclicity, and anovulation. The mechanisms underlying these divergent LH outcomes remain unclear but may reflect, in part, differences in the source of androgens (exogenous vs. endogenous), circulating androgen concentrations, and development timing and/or duration of androgen exposure. This review synthesizes current clinical and preclinical evidence to compare distinct models of hyperandrogenemia with respect to the female HPG axis and aims to illuminate how endogenous and exogenous androgens with different features differentially alter female neuroendocrine function.

KEYWORDS

androgen, GnRH, kisspeptin, PMOS, testosterone

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1 | INTRODUCTION

The hypothalamic–pituitary–gonadal axis (HPG) is a critical neuroendocrine system that governs reproduction and fertility. Hypothalamic neurons secreting gonadotropin releasing hormone (GnRH) project to the pituitary where they induce the secretion of two gonadotropin hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). These pituitary-derived hormones then act on gonads to drive steroidogenesis (sex steroids) and gonadal function. Gonad-derived sex steroids can circulate back to the hypothalamus, forming a homeostatic negative feedback loop to regulate GnRH neuron activity, GnRH secretion, and subsequent LH and FSH release. Most of the time in females, and all the time in males, GnRH and LH are secreted in a pulsatile manner, with each discrete secretory event termed a GnRH (or LH) pulse; by contrast, FSH secretion is generally not pulsatile in either sex. Sex steroid feedback from the gonads to the hypothalamus typically modulates the frequency and amplitude of GnRH and LH pulses, which in turn affects how the ovary is regulated and functions.

In addition to typical feedback effects of normal circulating levels of sex steroids, certain health or lifestyle conditions can result in exaggerated sex steroid levels which may impact HPG axis function. Under hyperandrogenemic conditions (i.e., androgen excess), circulating levels of androgens, such as testosterone (T), are abnormally increased, which negatively impacts the female reproductive axis, resulting in irregular or absent menstruation, anovulation, and subfertility or infertility.¹ In fact, hyperandrogenemia is a key symptomology of the most common reproductive disorder of adult females, polycystic ovary syndrome (PCOS), which affects approximately 10% of women of reproductive age.¹ Note that recently, the well-documented heterogeneity and complexity of symptoms in this disorder prompted the renaming of PCOS to polyendocrine metabolic ovarian syndrome (PMOS)²; we will therefore use PMOS throughout this article. Importantly, the physiological consequences of hyperandrogenemia with respect to reproductive neuroendocrine function appear to depend strongly on context, as excess endogenous androgens in PMOS is often associated with hyperactive reproductive hormone secretion, while excess exogenous androgens, as occur with anabolic substance abuse or administration for gender-affirming care for transgender men (individuals assigned female at birth), typically results in opposing neuroendocrine outcomes of inhibited reproductive hormones. These divergent physiological outcomes under the general umbrella of “hyperandrogenemia” or “androgen excess” raise important mechanistic questions regarding the source, timing, concentrations, and systemic context of elevated androgen exposure and its effects on the female HPG axis. This review seeks to synthesize recent findings from clinical studies and pre-clinical rodent models to examine how endogenous versus exogenous androgen exposure modulates the female neuroendocrine HPG axis.

2 | SEX STEROIDS AND THE REPRODUCTIVE NEUROENDOCRINE AXIS

In addition to promoting reproductive function, sex steroids secreted from the gonads normally feed back to the hypothalamic brain circuits

that regulate the reproductive axis. In females, estradiol (E₂) and progesterone (P4) are the main steroid hormones produced from the ovaries, whereas in males, testosterone (T) is released from the testes. In the ovaries, E₂ synthesis depends on the coordinated balance between LH-driven androgen synthesis and FSH-supported androgen aromatization. First, LH acts on ovarian theca cells to stimulate the synthesis of androgens, including T.^{3,4} This theca-derived T then diffuses to adjacent granulosa cells, where FSH promotes expression and activity of aromatase, the enzyme that converts T into E₂.^{3,4} Once secreted at sufficient levels, gonadal-derived sex steroids, including E₂ and T, can act in the hypothalamus in a classic “negative feedback loop” to suppress pulsatile GnRH release,^{5,6} which results in a corresponding suppression of pulsatile LH release to keep it within a healthy homeostatic range. Additionally, in females only, there is also a “positive feedback” loop that occurs once per cycle in the late follicular phase (proestrus stage in rodents) when rising ovarian E₂ levels act in the brain to cause a massive, prolonged increase in GnRH secretion (the “GnRH surge”).^{6–11} Correspondingly, this E₂-induced GnRH surge initiates a downstream surge in LH secretion which ultimately triggers ovulation.^{12,13} This positive feedback effect by E₂ is sexually dimorphic and typically only occurs in females, in line with only females ovulating.

Interestingly, GnRH neurons do not have the requisite sex steroid receptors, estrogen receptor α (ER α) and androgen receptor (AR), to directly receive steroid negative or positive feedback signals. Thus, an upstream brain cell population(s) must sense sex steroid signals and then relay that information to GnRH neurons. In the mid-2000s, it was discovered that hypothalamic neurons that make kisspeptin, a neuropeptide encoded by the *Kiss1* gene, express high levels of ER α and AR and act as master stimulators of GnRH neurons.^{14–21} There are two main populations of hypothalamic kisspeptin neurons implicated in mediating positive or negative feedback effects of gonadal sex steroids. One kisspeptin cell population resides in the hypothalamic arcuate nucleus (ARC),^{19,22} a region in which sex steroids act to indirectly suppress GnRH pulse secretion in both males and females. Kisspeptin neurons in the ARC co-express neurokinin B (NKB) and dynorphin and are often termed “KNDy” neurons. KNDy neurons have now been shown to serve as the GnRH pulse generator, guiding downstream GnRH pulse secretion using episodic kisspeptin secretion to stimulate each GnRH pulse event.^{12,23} In the prevailing KNDy model, it is proposed that NKB promotes synchronized neuron activation within the KNDy population, whereas dynorphin inhibitory input helps terminate each episode of KNDy neuron activity, with kisspeptin functioning as the crucial signaling factor released to activate GnRH neurons.^{23,24} KNDy neurons express AR and ER α , and both androgens and estrogens can inhibit KNDy neurons, in part by suppressing *Kiss1* gene expression and kisspeptin synthesis.^{24–28} Decreased kisspeptin signaling from KNDy neurons presumably results in dampened stimulation of GnRH pulse secretion, and hence, reduced LH pulse secretion.

The second main kisspeptin population is located more anteriorly in the hypothalamic preoptic area; in rodents, preoptic kisspeptin neurons are specifically located in the rostral periventricular nucleus of

the third ventricle (RP3V; also termed the AVPV/PeN). Like KNDy neurons, RP3V kisspeptin neurons express both ER α and AR. However, opposite to KNDy neurons, RP3V kisspeptin neurons are stimulated by E₂ (and T) treatment and are thought to mediate E₂ positive feedback induction of the LH surge in females only.^{25–27,29–31}

In males, T secreted from the testes circulates back to the hypothalamus to modulate pulsatile GnRH release and subsequent LH pulses.^{32–36} Classic studies implicated AR signaling as being both necessary and sufficient for proper negative feedback regulation of the male HPG axis. For example, both men and male animals with AR mutations exhibit elevated LH levels,^{37–41} even in the presence of high endogenous T levels,^{41–44} suggesting a necessary role for functional AR signaling in providing negative feedback. Likewise, treatment with AR antagonists like flutamide increases LH pulse frequency in men^{45–48} and mean LH in rodents,^{49–51} similarly indicating a requisite role for AR signaling in LH pulse regulation. Furthermore, treatment with the T-derivative dihydrotestosterone (DHT), a non-aromatizable androgen that cannot be converted into E₂ and therefore cannot indirectly act via ER α , significantly lowers LH secretion in both men and male animals,^{33,34,52–56} indicating that AR signaling is also sufficient to provide negative feedback on GnRH and LH pulses. However, while DHT can only bind to AR, T can be aromatized into E₂, allowing for the possibility that some negative feedback effects of T may indirectly result from ER α signaling in addition to AR signaling. In fact, some evidence supports this, as both men and male mice with mutated ER α exhibit increased mean LH and FSH.^{57–59} Moreover, clinical studies report that treatment with E₂ decreases mean LH and pulse amplitude in men,^{33,45,52–54,60–63} findings supported by animal studies wherein E₂ also lowers mean LH in castrated (CAST) males.^{64–73} This possible dual action of T acting directly via AR and indirectly via ER α in males is particularly interesting when considering how T might regulate the female reproductive neuroendocrine axis in hyperandrogenemic conditions.

3 | EXOGENOUS TESTOSTERONE TREATMENT IN WOMEN AND TRANSGENDER MALES

In both men and male mammals, treatment with exogenous T lowers LH secretion, mimicking normal endogenous T-mediated negative feedback.^{32–36,74,75} Similar to males, exogenous androgen treatments can provide negative feedback-like action on the female HPG axis, as treatment of females with high levels of T (>200 ng/dL) substantially above the normal circulating range in healthy adult women (15–70 ng/dL)⁷⁶ leads to decreased LH levels.^{77–83} High circulating levels of T from androgen-releasing tumors can also inhibit the HPG axis in women, demonstrated by reduced LH secretion and decreased LH pulse frequency.^{79,84,85} Moreover, similar to men,^{33,52–55} acute DHT treatment also decreases LH secretion in healthy women,⁸⁶ suggesting AR signaling is sufficient to mediate androgen suppression of the female reproductive neuroendocrine axis.

3.1 | Exogenous androgen exposure from anabolic substance abuse

Anabolic-androgenic steroid (AAS) abuse is a possible route of exogenous high-level androgen exposure. AAS are synthetic derivatives of T, often used by athletes and body builders. In men, AAS abuse leads to supraphysiological levels of T, often exceeding the normal adult male range, with one study reporting levels reaching 10–100 times higher than endogenous T levels, depending on the compound, dose, and timing of blood sampling.⁸⁷ This high concentration of exogenous androgen exposure suppresses the male HPG axis, leading to reduced LH and FSH levels, testicular atrophy (hypogonadism), and oligospermia.^{88–90} Notably, although gonadotropin suppression in men is most pronounced during active AAS use, LH recovery after discontinuation can be slow and even incomplete.⁹¹ This indicates that prior supraphysiologic androgen exposure may leave prolonged residual effects on HPG axis function.

Women abusing AAS also exhibit suppression of the HPG axis, demonstrated by decreased LH and FSH, as well as amenorrhea and anovulation.^{88,92,93} This suggests that high levels of circulating androgens, in or above the male physiological range, inhibit the female HPG axis, mimicking negative feedback of T in males. However, human studies in women are scarce and often confounded by effects of high-volume exercise, altered body weight, and other training factors,^{92,93} which makes it difficult to isolate the specific contributions of AAS to LH suppression and anovulation. As a result, specifics of LH suppression patterns in women abusing AAS are poorly characterized compared to robust gonadotropin data available in men.

3.2 | Exogenous androgen administration for gender-affirming testosterone

There are over 2.8 million individuals in the United States alone that identify as transgender,⁹⁴ though this reported number is likely confounded by selection bias and social stigma.^{94–99} Approximately 1/3 to 1/2 of these individuals are genetically XX persons assigned female at birth that report a masculine gender identity and identify as transgender men or gender nonbinary (collectively abbreviated here as TGN). Exogenous gender-affirming T treatment (termed GAT) is given to many TGN individuals to mimic the physiologic range of T in cisgender men (320–1000 ng/dL¹⁰⁰) which is 10–15 fold higher than normal T levels in healthy adult women. Such high-concentration T treatment induces desired masculinization of physical characteristics, such as voice deepening, increased facial hair growth, augmented muscle mass, and body fat redistribution.^{78,101–106} Moreover, GAT also reduces menstrual cyclicity, a desired outcome for TGN individuals wanting to minimize female characteristics. While such cessation of menstrual cycles is commonly reported and an expected outcome for TGN individuals taking GAT, fewer studies have rigorously examined how acyclicity is mechanistically induced or the neuroendocrine consequences of GAT with respect to the HPG axis. Single “one-off”

blood samples collected from TGN individuals receiving prolonged male-level GAT reveal lower levels of LH in the presence of the high circulating T levels.^{78,106–109} In contrast to single LH measures, measuring LH pulse secretion patterns in humans is difficult because it typically requires serial blood collection over 8–10 h, and has therefore been performed less often, especially in TGN individuals. Moreover, results from the few LH pulse studies in TGN individuals have been inconsistent due to variability between individuals coupled with very small sample sizes. Nevertheless, while one study on short-term, low dose GAT treatment of just 6 weeks showed no changes to LH pulse frequency,¹¹⁰ another study looking at longer-term, higher dose GAT treatment (>6 months) reported reduced LH pulse frequency.⁷⁸ This difference suggests that the inhibitory effects of exogenous T on the reproductive axis may be time- and dose-dependent, emerging only after sustained exposure of a high enough concentration. Further, as LH pulse frequency is a readout of GnRH pulse generator activity, prolonged high dose GAT treatment appears to mimic T negative feedback observed in males, a conclusion supported by the reported inhibition of mean LH levels by GAT in one-off blood measures.^{78,106–109} However, more studies of GAT effects on LH pulse profile are needed to confirm this.

Other factors possibly influencing the variability of LH results in TGN individuals taking GAT may be due to hormone administration paradigm, dosing, or timing differences. In adult TGN individuals, common dosing regimens include long-acting injectable testosterone cypionate or enanthate (synthetic, long-acting androgen) that can be administered intramuscularly every 2 to 3 weeks at a dose of 100–200 mg.^{111,112} In adolescents, doses start lower at much lower doses, 20–25 mg monthly, and increase slowly to higher levels in adulthood to mimic normal pubertal progression.¹¹¹ Other modes of T administration are considered short-acting, such as oral testosterone undecanoate (TU) which is administered 2–3 times daily at 40–40 mg for adults and 20–40 mg daily for adolescents.¹¹¹ Transdermal administration of T, via T gel or T patches, is also widely available, with adults using 1–2 patches of 5–10 mg T daily and adolescents using 2.5 mg patches every 12 h.^{111,113} The difference in long-acting versus short-acting regimes may cause varied suppression on LH levels, with one meta-analysis concluding that longer-acting T injections reduce LH by 51%–97%, whereas daily gels only reduce LH levels by roughly 35%–85%.¹¹⁴ Furthermore, T injections may suppress FSH more profoundly than short-acting regimes, with reported FSH reductions of 75%–95% compared with 29%–83% for shorter-acting paradigms.¹¹⁴ The variability in LH and FSH suppression suggests that T formulation and dosing frequency may differentially affect gonadotropin suppression. Together, these findings highlight the need for more rigorous, standardized studies that account for treatment paradigm and dose in order to fully clarify the extent of gonadotropin suppression associated with different T regimes.

In addition to inhibiting basal LH secretion and LH pulses, prolonged GAT treatment may also disrupt the estrogen-induced LH surge and subsequent ovulation, as one study reported only one ovulation event in 20 TGN individuals receiving GAT over a 12-week observation period.¹¹⁵ Androgen-mediated inhibition of ovulation may occur indirectly through suppression of GnRH/LH pulsatility and

downstream follicular development and ovarian E₂ production, thereby preventing the sustained preovulatory E₂ rise required to initiate the LH surge. Another possibility is that GAT may directly impair central reproductive circuit responsiveness to E₂ positive feedback, such that an LH surge does not occur even in the presence of sufficient circulating E₂. Indeed, whether or how GAT interferes with E₂-mediated LH surges remains highly limited, as only one small study has probed this question. In that study, exogenous E₂ failed to elicit the normal positive-feedback LH surge responses in TGN individuals receiving GAT, suggesting that GAT may disrupt E₂-mediated LH surge mechanisms.⁴⁵ Additional large-scale studies in TGN individuals are needed to better elucidate these effects.

Notably, the cessation of GAT treatment can lead to the return of ovulation in some TGN individuals, though the length of time to resume normal cyclicity and menses is variable, with some studies documenting a broad range of 5 to 9 months.^{116–118} This is important because many TGN individuals report a desire for biological children,^{119,120} highlighting a need for further research on the reversibility of GAT and its inhibitory effects on reproduction. Although current guidance raises concerns about potential birth defects caused by GAT, emerging evidence suggests that successful oocyte retrieval through IVF may be possible after a period of GAT cessation,^{117,121} and in some cases, even without the return of menses or prior discontinuation of therapy.^{122–124} However, some individuals treated with GAT require higher gonadotropin doses during IVF to achieve desired ovarian outcomes, suggesting that GAT may have enduring inhibitory effects on the female HPG axis,^{117,118} but the mechanisms for this are unknown. Together, these findings underscore the need for further research to better define underlying neuroendocrine mechanisms during GAT exposure.

3.3 | Preclinical rodent models of GAT

Recently developed mouse models of GAT exposure have been useful to study physiological effects of high, male-level T on the female HPG axis and reproductive function. Most previous animal studies examining androgen effects on the female HPG axis were developed to model PMOS, which does not reflect either the high male-level T of GAT or the early adulthood onset of excess androgen induced by GAT. Therefore, newer androgen-treated rodent models have begun to specifically address the reproductive effects of GAT-like therapy. One such model was developed by Kinnear et al.,¹²⁵ wherein young adult female mice are subcutaneously injected twice weekly with T enanthate (which is used in humans) for 6 weeks. This 6-week treatment results in physiological male levels of circulating T (mimicking GAT), loss of estrous cycling, smaller ovaries, and fewer corpora lutea.¹²⁵ From a neuroendocrine perspective, this study also reported a significant suppression of mean LH, as measured in single one-off blood samples.¹²⁵ Similarly, Bartels et al. subcutaneously injected young adult female mice with T cypionate once weekly for 6 weeks and also observed physiological male levels of circulating T, loss of estrous cyclicity, and smaller ovaries with fewer corpora lutea 1 week after the last injection.¹²⁶ Subcutaneous implants of T enanthate

pellets have also been used to model prolonged GAT exposure in adult female mice; such treatment also results in physiological male levels of circulating T and inhibited estrous cycles during 6 weeks of T treatment.¹²⁷ Mean LH from one-off samples was not different several weeks after cessation of T treatment; however, LH was not measured during T exposure.¹²⁷ While these various female mouse models of GAT treatment exhibit cessation of cyclicity and anovulation,^{125–131} similar to the observed effects of exogenous male-level T in TGN individuals, there are no studies yet in such models on in vivo LH pulse patterns during prolonged male-level T exposure, with studies only reporting decreased mean LH from single, one-off samples.^{125,128} More studies are therefore needed to better characterize the effects of male-level T on possible changes in female LH pulse dynamics, which can reveal possible differences in basal LH levels, LH pulse frequency, and/or pulse amplitude to provide valuable insight on the underlying mechanisms.

3.4 | Possible neuroendocrine mechanisms underlying GAT reproductive phenotypes

It is currently unknown which sex steroid receptor signaling pathway(s) or which cell targets are directly mediating exogenous male-level T effects, since T can both act via AR and be aromatized into E₂ to then act via ERs. Researchers have begun to probe this question by treating young adult female mice with male levels of DHT. Though TGN individuals do not take DHT or other nonaromatizable androgens, DHT studies in preclinical models are useful to isolate and target AR pathways specifically, which is helpful to elucidate underlying molecular mechanisms of T action. Young adult female mice that were ovariectomized and received 3 weeks of male-level DHT treatment via a subcutaneous implant showed robust decreases in mean LH as well as decreased in vivo LH pulse parameters, including inhibited LH pulse frequency and amplitude.¹³² These findings indicate that AR signaling is sufficient to suppress the adult female HPG neuroendocrine axis, supporting the idea that inhibitory effects of T may not be solely due to aromatization to E₂. Another study demonstrating sufficiency of AR signaling in suppressing the female reproductive axis was conducted in knockout mice lacking ER α , which have high levels of LH owing to lack of E₂ negative feedback. When these adult ER α KO females were treated with E₂, their mean LH levels remained high as expected, since ER α was absent. However, when these females were treated with DHT, their mean LH levels decreased,¹³³ indicating that AR signaling pathways are sufficient to suppress the adult female HPG axis. However, these findings implicating AR signaling do not rule out a possible involvement of ER α signaling in GAT inhibition of LH after possible aromatization of T to E₂.

The target cell types involved in male-level T suppression of LH pulse secretion have yet to be fully elucidated. Since normal androgen feedback in males, and presumably GAT-mediated inhibition in females, acts to inhibit GnRH pulse output,^{32–34,36,74} ARC kisspeptin neurons have been demonstrated to be a strong candidate, as they express AR and ERs and directly activate GnRH pulse secretion.^{14–}

16,19–21,134–136 Indeed, ARC kisspeptin neurons, aka KNDy neurons, are now considered to be a main component of the brain's "GnRH pulse generator," which controls downstream GnRH and LH pulses.¹³⁷ Thus, a logical hypothesis is that ARC kisspeptin neurons may be a direct target of endogenous or exogenous androgens for mediating inhibition on GnRH pulses. Supporting this, exogenous treatment with high levels of T, DHT or E₂ decreases *Kiss1* mRNA levels in the ARC of female rodents, which could be expected to lead to decreased kisspeptin synthesis and signaling and, hence, decreased GnRH and LH pulses.^{25,26,28,138} Interestingly, treatment with exogenous T in both ER α KO or ARKO male mice leads to decreased *Kiss1* in the ARC,²⁶ demonstrating how T can act via AR as well as indirectly act via ER after aromatization to E₂, at least in males. However, studies are still needed in females to determine whether similar ER- and AR-mediated pathways operate in the female HPG axis. In addition, while no studies have yet assessed T effects on in vivo kisspeptin neuron activity of females, a recent study measured KNDy neuron electrical firing in vivo in female mice given prolonged DHT treatment.¹³⁹ Although the DHT was initiated pre-pubertally (at 3 weeks of age), which is earlier than GAT treatment typically starts, some insight may still be gleaned from the effects on kisspeptin neuron activity that was studied in adulthood: GCaMP fiber photometry recording of KNDy neurons in DHT-treated female mice showed significantly reduced KNDy neuron synchronous firing events, indicating a suppression of the GnRH pulse generator.¹³⁹ Unfortunately, LH was not measured in these DHT-treated females. However, in another study administering chronic DHT to ovary-intact female mice beginning at 3 weeks of age, serial blood sampling of adults found no differences in basal LH, LH pulse frequency, or pulse amplitude.¹⁴⁰ This lack of effect by DHT contrasts both the inhibited KNDy neuron activity reported in the fiber photometry study and the marked inhibition of LH pulses in adult OVX + DHT female mice.¹³² The reason for the discrepancy is unknown but could reflect technical differences or the challenges of detecting low-level LH pulses in ovary-intact mice.

GAT likely affects positive feedback actions within the HPG axis as well. As noted previously, one study reported that in 20 individuals on GAT, only a single ovulation was detected out of a total of 61 combined months of GAT treatment.¹¹⁵ This lack of ovulation may be attributed to HPG axis suppression, and is supported by preclinical mouse models wherein twice weekly T enanthate treatment leads to absence of ovarian corpora lutea.^{125,128} While such an anovulatory phenotype is consistent with impaired LH surge generation, a direct inhibitory effect at the level of the ovary has not yet been ruled out. Interestingly, female rats treated with DHT (at a concentration lower than male physiological levels) similarly exhibit diminished corpora lutea and fail to generate LH surges when exposed to an exogenous E₂ treatment that is known to induce LH surges.¹⁴¹ This suggests a disruption of E₂ positive feedback mechanisms at the central level. Although the cell types and receptor targets mediating this androgen-induced LH surge impairment are unknown, one plausible mechanism may include inhibition of RP3V kisspeptin neurons, which have been strongly implicated as critical players for the LH surges.^{8,30,142–145} and express AR.¹⁴⁶ This raises the possibility that high, male levels of T could act directly on RP3V kisspeptin

neurons to blunt the coordinated GnRH surge release that governs ovulation. Though this possibility requires further testing, the study discussed above in which female rats were treated with mid-range dose of DHT reported reduced RP3V Kiss1 mRNA and RP3V kisspeptin neuronal activity as determined by decreased c-Fos.¹⁴¹ Together, these emerging findings support a model in which male-level androgen excess impairs ovulation though disruption of RP3V kisspeptin neuron-mediated positive feedback signaling, though mechanisms and potential ovarian effects still need to be delineated.

4 | ENDOGENOUS ANDROGEN EXCESS (HYPERANDROGENEMIA) IN PMOS AND PMOS-LIKE PRECLINICAL MODELS

Chronic elevations in endogenous androgens in females are associated with some pathological hyperandrogenemic states, most notably PCOS, which has now been re-termed polyendocrine metabolic ovarian syndrome (PMOS). PMOS affects ~10% of reproductive age women, with diagnosis criteria typically including two of the following phenotypes: (1) oligo- or anovulation and irregular menstruation, (2) clinically high levels of androgens (hyperandrogenemia), and (3) polycystic ovaries.¹⁴⁷ Recently, a fourth criterion has been added: elevated anti-Müllerian hormone (AMH), which is secreted from ovarian follicles.¹⁴⁷ Because these diagnostic features vary between individuals, PMOS is a highly heterogeneous condition, making it challenging to both treat and accurately model to investigate its underlying mechanisms. The pathology of this disorder is not fully understood but may be driven by high levels of T and high levels of circulating LH, as many women with PMOS have also been shown to have abnormally increased basal LH levels and LH pulse frequency, resulting in a hyperactive exaggerated LH pulse profile.^{148,149} Since LH stimulates androgen synthesis in ovarian theca cells, this elevated LH may contribute to the excessive androgen production seen in PMOS. These alterations are likely driven in part by hyperactive hypothalamic GnRH neuron activity perhaps due, in part, to impaired sex steroid feedback sensitivity.¹⁵⁰ Finally, as will be discussed more later, it is also important to note that although T is often elevated in women with PMOS compared to levels in healthy women,^{151,152} the circulating levels of T in PMOS do not typically exceed 200 ng/dL and are much lower than the physiological T range in adult men (320 to 1000 ng/dL),⁸⁰⁻⁸³ the latter of which is the circulating target range for GAT treatment; hence, elevated T levels in PMOS women tend to be much lower than elevated T levels in TGN individuals taking GAT, an important difference in “androgen excess.”

4.1 | Prenatal androgen exposure models of PMOS

Given the complexity of PMOS pathophysiology, and the practical limitations of human neuroendocrine studies, preclinical animal models have become an essential tool for dissecting the mechanistic underpinning of PMOS-like conditions. One proposed etiology of PMOS is

prenatal exposure of females to excess androgens in utero.¹⁵³ This hypothesis is supported by evidence that women with PMOS exhibit elevated levels of androgens during pregnancy, including high T levels in the umbilical vein blood¹⁵⁴ and amniotic fluid,¹⁵⁵ and that their daughters also have an increased likelihood of developing PMOS.¹⁵⁶ Therefore, animal models of prenatal exposure of androgens were developed wherein pregnant dams are treated with T or DHT during gestation. The female offspring from these prenatally androgenized (PNA) models often display PMOS-like hyperandrogenemia in multiple species, such as monkeys, rodents, and sheep.¹⁵⁷⁻¹⁶⁴ In both mice and rats, female PNA offspring have been reported to exhibit increased LH levels and LH pulse frequency in adulthood,^{159,165,166} as well as estrous cycle disruption and follicular atresia,^{157,158,160} as occurs in PMOS women, though some key facets of PMOS like elevated circulating T levels are not consistently recapitulated in PNA mice.^{139,157} For example, recent analysis of PNA mice found elevated mean LH concentrations but no increase in circulating T at 5–7 months.¹³⁹ However, other groups reported normal T levels at 5 months but elevated at 8 months,¹⁵⁷ while another reported normal T levels at 1 month but elevated at 3 months.¹⁶⁷ This inconsistency may reflect, in part, differences in androgen quantification methods, as enzyme-linked immunosorbent assay-based measures can be affected by limited specificity, whereas liquid chromatography-mass spectrometry measurements are more specific but may lack sensitivity at the low T concentrations normally found in female mice.¹⁶⁸⁻¹⁷⁰ Therefore, cross-study comparisons of circulating T concentrations in mice should be interpreted cautiously.

Another newer mouse model of inducing in utero androgen exposure is prenatal treatment with AMH.¹⁷¹ AMH, which is secreted by the ovaries and elevated in many women with PMOS,¹⁷¹ has been shown to drive GnRH neural activity and induce LH secretion.¹⁷² Thus, it is proposed that abnormally elevated AMH in PMOS women may over-activate GnRH neurons, resulting in hyperactive LH secretion and consequently increased LH stimulation of T synthesis by ovarian theca cells. In the prenatal AMH mouse model (pAMH), AMH treatment to pregnant females therefore results in in utero androgen excess by stimulating the HPG neuroendocrine axis. Indeed, adult female offspring of dams exposed to AMH during late pregnancy exhibit impaired estrous cyclicity along with elevated T levels and increased LH pulse frequency.¹⁷¹ Both PNA and pAMH models similarly share in-utero exposure to excess androgens, but whether the hyperandrogenemia state is of the same concentration in the two models has not been determined, and it remains unknown if they rely on the same underlying molecular and physiological mechanisms. For example, T levels are elevated in female offspring from the pAMH model but not always elevated in offspring from the PNA model, suggesting there may be some difference in in utero androgen action between the two.

4.2 | Postnatal androgen exposure models of PMOS

Other preclinical rodent models of PMOS initiate excess androgen exposure peri-pubertally, as this is when PMOS symptoms often first

emerge in girls. One pubertal induction model uses letrozole (LET), a non-steroidal inhibitor of the aromatase enzyme. Treatment with LET inhibits the conversion of T to E₂, thus increasing T secretion and creating a hyperandrogenic state reminiscent of PMOS. Chronic treatment with LET initiated during puberty in female rodents leads to many PMOS-like phenotypes, including impaired estrous cycles, increased levels of both T and LH, polycystic ovaries, and infertility, along with some metabolic phenotypes such as increased body weight and insulin resistance.^{173–176} Furthermore, in vivo LH pulse frequency is robustly elevated in LET females,^{132,177} mimicking the hyperactive LH pulse pattern in many PMOS women. The metabolic phenotypes seen with LET rodent models also closely resemble the metabolic impairments and obesity observed in many PMOS women.¹⁷¹ By contrast, prenatal PNA and AMH rodent models may more closely mirror lean PMOS women because those prenatal models often do not induce overweight or obese phenotypes. Thus, LET and PNA/AMH models provide a complementary framework for dissecting the divergent neuroendocrine and metabolic mechanisms driving obese versus lean PMOS phenotypes.

In another postnatal androgen exposure model, female rodents treated chronically with DHT beginning before (3 weeks old) or at the age of puberty exhibit some PMOS-like characteristics in adulthood, such as disrupted estrous cycles, polycystic ovaries, insulin resistance, and increased adiposity.^{174,178} However, these chronically DHT-treated females fail to display increased blood levels of LH or T and fail to develop enlarged ovaries (another PMOS phenotype). In fact, reduced LH was reported in chronic DHT-treated female rats,¹⁷⁹ and in other studies, mean LH was unchanged in postnatal DHT-treated female mice.^{140,178,180} Thus, these postnatal DHT models do not induce a PMOS-like endocrine state with respect to elevated LH or T, and may more closely resemble the “negative feedback” endocrine profile of anabolic steroid abuse or GAT therapy.

4.3 | Possible neuroendocrine mechanisms underlying PMOS and PMOS-like phenotypes

Many women with PMOS exhibit abnormally elevated LH pulse frequency and increased pulse amplitude.^{148,149} Alterations in LH pulse amplitude suggest changes in either the brain and/or pituitary. Conversely, changes in LH pulse frequency indicate an alteration in the brain, specifically in some aspect of the hypothalamic GnRH pulse generator system. Given the enhanced LH pulse secretion, it has been suggested that PMOS may have neuroendocrine dysfunction at the level of sex steroid negative feedback, which would permit hyperactivity of GnRH and LH secretion. This is supported by findings that women with PMOS require higher levels of estradiol and progesterone treatment to lower their LH levels (negative feedback action) compared to healthy women,¹⁸¹ suggesting disrupted negative feedback of the GnRH pulse generator.

As noted earlier, the GnRH pulse generator is thought to be comprised of ARC kisspeptin neurons that also co-express NKB and Dynorphin (Dyn) (aka, KNDy neurons). Indeed, experimental

activation of KNDy neurons using optogenetics can increase LH secretion in mice,¹⁸² whereas optogenetic inhibition of KNDy neurons reduces LH pulses.¹⁸³ Under normal conditions, sex steroids are proposed to feed back to KNDy neurons to decrease kisspeptin and NKB synthesis as well as lower neuronal activity, culminating in decreased stimulation of downstream GnRH pulse secretion. Given this, it has been proposed that women with PMOS may have hyperactive KNDy neuron function which is somehow resistant to typical androgen negative feedback and which drives their abnormally elevated LH pulse secretion.^{1,177} Congruently, female PNA rats show increased *Kiss1* mRNA levels in the ARC,¹⁶⁶ and in pubertal exposure models, both female rats and mice treated with LET have increased *Kiss1* and *Tac2* (NKB) mRNA levels in the ARC along with elevated LH levels.^{132,184} However, these neuroendocrine features are not identical across PMOS-like rodent studies. For example, while one study reported a 40% increase in LH pulse frequency in PNA mice,¹⁶⁰ another recent PNA mouse study found elevated mean LH but unchanged LH pulse frequency and pulse amplitude¹³⁹; the latter study also reported little to no change in ARC NKB and kisspeptin levels.¹³⁹ However, despite the lack of demonstrated change in LH pulse frequency, the latter study did report that the frequency of KNDy neuron synchronous activity was significantly increased in diestrus PNA mice compared to control females, along with reduced feedback suppression of such KNDy neuron activity by progesterone.¹³⁹ Collectively, most of these findings support the hypothesis that PMOS-like neuroendocrine dysfunction likely involves impaired sex steroid negative feedback and/or hyperactive KNDy neurons, contributing to the observed LH hypersecretion. Further, despite their disrupted estrous cyclicity, PNA mice retain the capacity to generate an E₂-induced LH surge and exhibit corpora lutea, indicating that the positive feedback surge mechanism is not uniformly abolished in that particular PMOS model.^{139,159,167,185}

Exaggerated GABAergic signaling has also been implicated in PMOS pathophysiology. In contrast to its typical inhibitory role in the brain, GABA depolarizes (activates) GnRH neurons due to their elevated intracellular chloride, thereby enhancing GnRH secretion.¹⁸⁶ Consistent with this, some women with PMOS exhibit elevated GABA levels,¹⁸⁷ and it is possible that this may contribute to elevated LH pulses in PMOS. However, such elevated GABA levels have not yet been directly linked to the hyperactive LH or downstream hyperandrogenemia, nor is it known which specific GABA neuron population might be involved. Some prenatal female PMOS mouse models demonstrate increased ARC-derived GABAergic input to GnRH neurons.^{160,171} In addition, selectively activating ARC GABA neurons with chemogenetics or optogenetics in female mice can recapitulate some PMOS-like symptoms, such as increased levels of T, heightened LH secretion, impaired estrous cycles, and decreased corpora lutea.¹⁸⁸ However, another study found that oral administration of GABA to LET-induced PMOS females actually decreases T levels, body weight, and cystic follicles,¹⁸⁹ improving their PMOS phenotypes. Because orally administered GABA may act through peripheral targets, this finding should not necessarily be interpreted as evidence against a possible role of central GABAergic signaling activating GnRH neurons. Rather, this variability across models suggests that GABA's net effect

within the reproductive neuroendocrine axis remains context- or site-dependent, reflecting its diverse actions across many cell populations.

Although elevated androgen levels are well established in most women with PMOS, there remains the caveat regarding exactly which sex steroid receptors are at play, since some T can be aromatized to E_2 and therefore can cause AR or ER signaling. Evidence supporting an essential role for AR signaling comes from studies in women with PMOS, where hyperactive LH levels decreased only when treatment with the AR antagonist flutamide was combined with E_2 and P treatment, but not with E_2 and P treatment alone.^{190,191} Because E_2 and P alone were insufficient to reduce LH levels, these findings suggest that AR signaling contributes to the elevated LH secretion observed in PMOS, perhaps by interfering with E_2 and P negative feedback. Congruently, PNA female mice given flutamide also displayed restored estrous cyclicity and decreased T levels, though LH was not measured,^{158,192} and LET mice treated with flutamide also showed decreased T and LH levels.¹⁹³ Additionally, in prenatal AMH mice or female mice treated with LET, selective knock out of AR from kisspeptin cells results in restored estrous cycles¹⁹⁴ and fertility¹⁹⁵; however, the effects of this conditional AR KO on PMOS-like elevations in LH and T were unfortunately not studied. Given the established role of ARC kisspeptin neurons in regulating LH pulses, these findings suggest that AR action within this neuronal population may perturb GnRH/LH pulse generation in PMOS-like conditions.

In addition to hypothalamic neurons, pituitary gonadotropes also express AR¹⁹⁶ and may represent another target of excess androgen signaling. Androgen actions at the pituitary could alter gonadotrope LH synthesis or secretion, thereby influencing blood LH levels independently of, or in addition to, changes in hypothalamic GnRH pulse generation. Supporting a functional role for pituitary AR signaling, DHT can lower GnRH-induced LH secretion of cultured pituitaries in vitro,¹³² and gonadotrope-specific deletion of AR in female mice attenuates chronic DHT-induced impairments in estrous cyclicity and fertility, along with reduced pituitary responsiveness to GnRH.¹⁹⁷ However, whether gonadotrope AR signaling contributes somehow to the elevated LH hypersecretion characteristic of PMOS remains unknown and needs to be better studied.

For reasons discussed above, targeting the GnRH pulse generator (i.e., KNDy neurons) may represent a promising therapeutic approach for the treatment of PMOS. Indeed, as proof of concept, a recent pre-clinical study found that targeted chemogenetic inhibition of KNDy neurons in PMOS-like female mice (induced via the LET paradigm) could reduce both hyperactive LH pulse frequency and elevated T levels, bringing both endocrine measures down to the healthy control range.¹⁷⁷ Such findings suggest that KNDy neurons are critical in driving PMOS-like endocrine phenotypes and could be important novel targets of future therapeutics for the treatment of PMOS. Indeed, this notion is supported by several recent clinical trials in PMOS women in which KNDy neurons were specifically targeted by drugs inhibiting NKB action. Since KNDy neurons are thought to create “pulse-like” stimulation of GnRH neurons in part through synchronized paracrine NKB signaling acting on neighboring KNDy neurons via the neurokinin 3 receptor (NK3R),^{198,199} researchers investigated whether NK3R

antagonists could suppress the hyperactive LH pulse secretion in PMOS. Treatment with NK3 antagonist AZD4901 resulted in reduced LH pulses and decreased circulating T concentrations in women with PMOS.²⁰⁰ Overall, these promising findings support exaggerated KNDy neuron signaling as a crucial upstream instigator of neuroendocrine dysfunction in PMOS.

5 | COMPARING NEUROENDOCRINE PHENOTYPES IN PMOS VERSUS EXOGENOUS T EXPOSURE

In both GAT and PMOS, levels of androgens are elevated above the normal female range, giving rise to “androgen excess.” However, the elevated androgens in these two scenarios are associated with different physiological outcomes with respect to neuroendocrine hormone secretion (LH) and ovarian growth (Figure 1). For example, although both GAT and PMOS result in impaired cycles and anovulation, in GAT (and GAT animal models), elevated androgens lead to inhibition of LH secretion and smaller ovaries, while in PMOS (and preclinical PMOS models) there is significantly increased LH secretion and larger ovaries. Understanding the differences between the two conditions of hyperandrogenemia could ultimately lead to key mechanistic clues on how androgens operate in the female neuroendocrine axis.

With GAT, exogenous male-level T suppresses mean LH levels and LH pulse frequency in TGN individuals,⁷⁸ which is consistent with

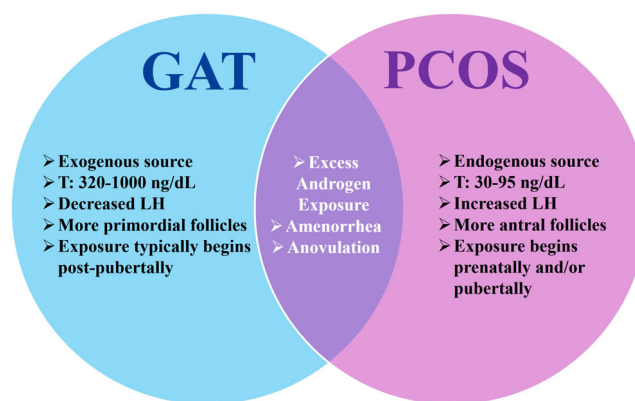


FIGURE 1 Summary comparison of key endocrine and reproductive features of gender-affirming testosterone therapy (GAT) and polyendocrine metabolic ovarian syndrome (PMOS, formerly called PCOS). GAT and PMOS share androgen excess (hyperandrogenemia), impaired menstrual cycles and anovulation, but differ in the androgen source, developmental timing, androgen concentrations, and downstream reproductive effects. GAT is characterized by exogenous T administration, typically in the male physiological range, and is often initiated post pubertally, leading to decreased LH secretion and follicles arrested in the primordial state. In contrast, PMOS is marked by endogenous hyperandrogenism, usually elevated compared to healthy women but not as high as in men, and exposure can begin prenatally or peri-pubertally. By contrast to GAT, PMOS is often associated with increased LH levels and antral follicle numbers.

the expected and documented inhibitory actions of androgens on ARC kisspeptin neurons which comprise the GnRH pulse generator. This coincides with mouse models in which male-level T (or male-level DHT) also leads to suppressed LH levels in adult females.^{125–127,132} By contrast, individuals with PMOS, along with PMOS-like mouse models including LET, PNA, and AMH, all display the opposite LH phenotype, wherein despite markedly elevated T, LH concentrations and/or pulse frequency are increased.^{148,149,165,173,177,201} Under normal physiological conditions, this elevated level of T should lead to suppressed LH output, since T is known to inhibit LH secretion in both sexes.^{26,125,127,138,202,203} However, in PMOS and PMOS models, the elevated T does not result in inhibited LH as expected. This paradox suggests there is a level of androgen feedback “resistance” in PMOS whose mechanism remains unexplained.

One important difference between PMOS and GAT is the source and dose of excess androgen exposure. In GAT, androgens are delivered exogenously to achieve male level T (typically between 320 and 1000 ng/dL),¹⁰⁰ whereas in PMOS, androgens are endogenously sourced from the ovaries and do not reach levels as high as in males, instead usually being between 30 and 95 ng/dL.^{80–83} Therefore, while GAT and PMOS both exhibit hyperandrogenic, the actual levels of circulating T are not similar between the two, being much higher in GAT. Further, GAT treatment typically involves T dosing every 2 to 3 weeks, while individuals with PMOS experience constantly elevated androgens due to persistently hyperactive GnRH/LH secretion. Depending on the formulation and route of administration (e.g., injections versus transdermal), GAT can produce pronounced peaks and troughs corresponding to dosing intervals,²⁰⁴ which contrasts the tonic T levels seen in PMOS. These differences in both magnitude and consistency of the elevated T could contribute to the divergent LH responses observed in these two conditions. However, how the source, timing, and concentration of T interact to produce LH suppression in GAT yet paradoxical LH elevation in PMOS remains unclear.

Another key distinction between GAT and PMOS is the developmental onset of excess androgen exposure. In GAT, exogenous administration of T often first occurs in late puberty or adulthood, whereas individuals with PMOS can be first exposed to elevated T prenatally and/or peri-pubertally, with such exposure persisting into adulthood. This earlier onset of T exposure in PMOS implicates possible developmental effects that could drive the adult differences in LH secretion under hyperandrogenemic conditions. Consistent with this, preclinical models of PMOS initiate excess androgens prenatally or peri-pubertally to model these early developmental influences, while GAT models often begin androgen treatments after puberty in young adult mice. This difference in androgen exposure onset suggests that early androgen exposure in PMOS may permanently alter development or function of brain circuits controlling GnRH pulse generation, leading to a pulse generator that either is immune to androgen negative feedback effects or responds aberrantly to high levels of T later in life. This is in contrast to the more predictable negative feedback-like suppression of LH seen when T is first administered in adulthood to individuals receiving GAT (or AAS).

6 | CONCLUSIONS

Overall, as in males, androgen excess is a powerful regulator of the female reproductive neuroendocrine axis, but the physiological outcome with respect to LH secretion may depend on the source, concentration, timing, onset, and/or context of androgen exposure. In both GAT and PMOS, excess androgens disrupt normal reproductive function, yielding impaired cyclicity and anovulation, yet the neuroendocrine consequences differ substantially. With GAT treatment, male-level androgen exposure generally suppresses LH secretion mimicking enhanced sex steroid negative feedback, whereas in PMOS, endogenous hyperandrogenemia is more often associated with increased LH pulse frequency and impaired sex steroid feedback regulation. These opposing LH phenotypes suggest that T signaling is not acting in isolation, but instead is shaped by development, endocrine environment, and the specific hypothalamic circuits involved in negative feedback regulation. In particular, ARC kisspeptin neurons remain a strong candidate for mediating these divergent outcomes, but the precise receptor pathways and mechanisms responsible for androgen-sensitive changes in LH pulse secretion have yet to be fully elucidated. Future work should focus on directly comparing these two hyperandrogenic states using longitudinal approaches. In PMOS patients, studies tracking hormonal changes over time are needed to better understand how androgen excess relates to LH dynamics and ovulatory dysfunction. Similarly, longitudinal studies in TGN individuals receiving GAT should examine how treatment affects LH secretion and cyclicity across different durations of exposure. Additional neuroendocrine studies are also needed in TGN individuals who discontinue GAT, as recovery of the HPG axis may reveal important insights about the reversibility of neuroendocrine changes.

A challenging limitation of the current clinical literature for androgen effects on LH pulses is small sample sizes, which reduces statistical power and makes it difficult to generalize variable findings across heterogeneous populations. This is in part because accurately measuring LH pulses in humans is technically challenging, requiring a long duration of serial blood sampling over many hours. As a result, many human studies rely on single “one-off” measurements which miss important dynamic changes of LH secretion. Addressing these challenges with adequately powered cohorts is essential for comparing different hyperandrogenic states and for delineating how endogenous versus exogenous androgens at different concentrations differentially regulate the HPG axis. Moreover, since neuronal mechanistic studies cannot be performed in humans, parallel advances in animal models are needed to parse out exactly which receptors, pathways, and brain cell types mediate divergent effects of androgen excess within HPG axis circuits. Together, integrated clinical and preclinical approaches have the potential to move the field toward a mechanistic understanding of how androgen excess drives reproductive dysfunction in females.

AUTHOR CONTRIBUTIONS

Alexander S. Kauffman: Conceptualization; writing – original draft; writing – review and editing; funding acquisition; supervision. **Lillian**

Rose: Conceptualization; writing – original draft; writing – review and editing.

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ORCID

Lillian Rose  <https://orcid.org/0000-0002-0597-3421>

Alexander S. Kauffman  <https://orcid.org/0000-0001-8631-6097>

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