Title

Androgen Inhibition of Reproductive Neuroendocrine Function in Females and Transgender Males

Journal

Endocrinology, 165(10)

ISSN

0013-7227

Author

Kauffman, Alexander S

Publication Date

2024-08-27

DOI

10.1210/endocr/bqae113

Peer reviewed

Endocrinology, 2024, **165**, bqae113
https://doi.org/10.1210/endocr/bqae113
Advance access publication 29 August 2024
Mini-Review

Androgen Inhibition of Reproductive Neuroendocrine Function in Females and Transgender Males

Alexander S. Kauffman[1]

[1]Department of OBGYN and Reproductive Sciences, University of California San Diego, La Jolla, CA 92093, USA

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

Abstract

Ovarian function is controlled by pituitary secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn are governed by gonadotropin releasing hormone (GnRH) secreted from the brain. A fundamental principle of reproductive axis regulation is negative feedback signaling by gonadal sex steroids back to the brain to fine-tune GnRH and gonadotropin secretion. Endogenous negative feedback effects can be mimicked by exogenous steroid treatments, including androgens, in both sexes. Indeed, a growing number of clinical and animal studies indicate that high levels of exogenous androgens, in the typically male physiological range, can inhibit LH secretion in females, as occurs in males. However, the mechanisms by which male-level androgens inhibit GnRH and LH secretion still remain poorly understood, and this knowledge gap is particularly pronounced in transgender men (individuals designated female at birth but identifying as male). Indeed, many transgender men take long-term gender-affirming hormone therapy that mimics male-level testosterone levels. The impact of such gender-affirming testosterone on the reproductive axis, both at the ovarian and neuroendocrine level, is a long-understudied area that still requires further investigation. Importantly, the few concepts of androgen actions in females mostly come from studies of polycystic ovary syndrome, which does not recapitulate a similar androgen milieu or a pathophysiology of inhibited LH secretion as occurs in testosterone-treated transgender men. This review summarizes clinical evidence indicating that exogenous androgens can impair neuroendocrine reproductive function in both female individuals and transgender men and highlights emerging experimental data supporting this in recently developed transgender rodent models.

Key Words: GnRH, kisspeptin, testosterone, LH, transgender, GAHT

Abbreviations: AR, androgen receptor; ARC, arcuate nucleus; ARKO, androgen receptor knockout; DHT, dihydrotestosterone; E_2, estradiol; ERα/β, estrogen receptor alpha/beta; FSH, follicle stimulating hormone; GAT, gender-affirming testosterone; GnRH, gonadotropin releasing hormone; GnRHa, gonadotropin releasing hormone agonist; HPG, hypothalamic-pituitary-gonadal; IVF, in vitro fertilization; KNDy, kisspeptin, neurokinin B, and dynorphin; KO, knockout; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; RP3V, rostral periventricular nucleus of the third ventricle; T, testosterone; TGN, transgender men (or gender nonbinary people assigned female at birth).

Ovarian function is controlled, in part, by luteinizing hormone (LH) and follicle stimulating hormone (FSH) secreted from the pituitary. Secretion of these gonadotropins is governed by upstream secretion of gonadotropin releasing hormone (GnRH) from neurons in the forebrain. A fundamental tenet of hypothalamic-pituitary-gonadal (HPG) axis regulation is negative feedback by gonadal sex steroids, including estrogens, progestins, and androgens, to the brain to fine-tune GnRH and gonadotropin secretion. Although often informally considered male hormones, androgens are also synthesized in females and can have important physiological actions in both sexes. While the concept of androgen negative feedback was first proposed in the 1930s (1), the actual physiological and molecular mechanisms by which endogenous—or exogenous—androgens act to inhibit GnRH and LH secretion still remain poorly understood, and this knowledge gap is particularly pronounced in females. This issue is of emerging importance for transgender males (designated as female at birth) taking high levels of exogenous androgens for gender-affirming therapy. This mini-review will briefly summarize clinical evidence indicating that androgens can act to alter neuroendocrine reproductive function in both females and transgender males (individuals assigned female at birth but having male identity) and will highlight emerging experimental data supporting such neuroendocrine effects in recent transgender rodent models.

Androgens and Reproductive Neuroendocrine Function in Healthy Females

Testosterone can induce physiological effects by either directly binding and signaling via the androgen receptor (AR) or, after first being aromatized to estradiol (E_2), by activating estrogen receptors (eg, ERα, ERβ). The androgen receptor (*AR*) gene, located on the X chromosome, is pivotal in male reproduction and development. AR mutations in males result in female genitalia (testicular feminization; Tfm) and infertility (2), and androgen deficiency causes infertility in men (3-5). Along with driving sperm production, testicular testosterone (T) feeds back to the male brain to inhibit the secretion of GnRH, and hence LH, in a classic negative feedback loop. This endogenous negative feedback effect is mimicked by exogenous T treatment which inhibits LH pulse secretion in both men and male animals (6-12). This androgen effect in males is

Received: 5 July 2024. **Editorial Decision:** 23 August 2024. **Corrected and Typeset:** 12 September 2024

likely due, at least in part, to AR signaling, as nonaromatizable androgens like dihydrotestosterone (DHT) can also inhibit LH secretion, including LH pulse frequency, in both men (7, 13-16) and male animals (17, 18), and AR antagonists can correspondingly increase LH pulses in men (19-24). However, how this androgen negative feedback action occurs is still poorly understood.

As in males, androgens also have important actions on the female HPG axis, with both androgen excess and androgen deficiency impacting female fertility. Although endogenous androgens are normally "low" in healthy females vs males (general range in healthy women: 15 to 60 ng/dL; general range in healthy men: 320 to 1000 ng/dL (25-28)), clinical evidence suggests that hypo-androgenemia in females (below their normal levels) can impact their neuroendocrine regulation of gonadotropins, cyclicity, and ovulation (29-33). Similarly, global knockout (ie, all cells in the body) of AR in female mice ("ARKO" mice) induces prolonged estrous cycles, fewer and smaller litters, and subfertility (34-38). Importantly, reciprocal ovary transplant studies show that ARKO female mice receiving wild-type ovary transplants still exhibit abnormal estrous cycles (39), indicating their subfertility due to AR deficiency is at the neuroendocrine rather than ovarian level, but the mechanisms for this are still unknown.

Separate from an important role of low levels of endogenous androgen on the female HPG axis, multiple lines of clinical evidence indicate that elevated levels of *exogenous* androgens can potently inhibit the HPG axis, including pituitary LH secretion, in normal females, similar to what occurs in males. Indeed, in women without polycystic ovary syndrome (PCOS), LH levels in the blood are reduced by exposure to high T levels in the normal male physiological range (40-42) (ie, circulating T levels that are much higher than elevated levels typically seen in PCOS, the latter of which are typically < 200 ng/dL (43-46)). LH is normally secreted in pulses, of which pulse frequency, amplitude, pulse peak concentrations, and intervening basal levels can each be modulated by different factors, including influences at both the level of brain and pituitary. Given the need to collect many serial blood samples over the course of 6 to 12 hours to properly measure LH pulse patterns, clinical studies of LH pulse secretion are less common than measuring LH in single "one-off" samples. Still, several clinical investigations in healthy women have reported that inhibitory effects of male-level androgens include decreasing LH pulse frequency (40-42), indicating a suppressive action specifically on some component of the brain's "GnRH pulse generator" mechanism (discussed more below). Similar findings of androgen inhibition on LH pulses were reported in healthy ovulatory adolescent girls: while infusion of a low T dose similar to PCOS levels resulted in slightly greater LH levels, an infusion of a higher T dose closer to the male physiological range suppressed mean and basal LH release and reduced LH pulse frequency (47). Mimicking these inhibitory effects of acute exogenous T treatment, women with markedly elevated endogenous T due to androgen-secreting tumors can similarly show decreased LH, including reduced LH pulse frequency (42, 48, 49).

Currently, it is unknown which specific steroid receptor pathway(s) mediate exogenous T inhibition of LH secretion in females. Importantly, clinical studies demonstrate that like T, nonaromatizable androgens (eg, DHT) can similarly decrease LH pulse secretion in women (50) (as in men (7, 13-16)). These DHT effects in females suggest a sufficient role for AR signaling pathways in mediating the observed LH inhibition after exogenous androgen treatment, though it does not exclude a possible important role for ERα or ERβ signaling after potential aromatization of T to E_2.

Androgen Inhibition of the HPG Axis and LH Secretion in Transgender Men

Exogenous T exposure may have inhibitory effects on the reproductive axis in transgender individuals (gender identity differing from assigned sex at birth). In the United States alone, ~1.6 million adults self-identify as transgender (51) and this prevalence is not only growing but likely higher than reported due to selection bias and social stigma (52-57). Among transgender individuals, 1/3 to 1/2 report masculine gender identity after female sex assignment at birth and therefore identify as transgender men (or gender nonbinary people; collectively designated TGN hereafter). Gender-affirming hormone therapy, typically testosterone treatment producing circulating T levels in the physiological range of adult men, is the mainstay of gender-affirming medical care in adult (postpubertal) TGN (25, 58, 59). Such gender-affirming T (termed hereafter as GAT) treatment is used to masculinize physical characteristics, including facial hair growth, voice deepening, body fat redistribution, and muscle mass, and to also suppress female characteristics such as menstrual cycles and breast growth (59-65). Aligning gender presentation with gender identity via GAT therapy has also been shown to improve overall quality of life (25). The Endocrine Society recommends that GAT treatments, typically via intramuscular or subcutaneous injections or transdermal applications (66, 67), produce circulating serum T levels within the typical physiological range of cisgender men (320 to 1000 ng/dL) (25). Serum T values in GAT-treated TGN are therefore elevated as much as 10- to 15-fold over pre-GAT levels and commonly induce menstrual suppression by 3 to 6 months (60-62, 64, 68).

Importantly, from an HPG axis perspective, many TGN may desire childbearing and may even engage in vaginal intercourse with the potential to conceive spontaneously (69-75). Yet, the majority of TGN undergoing GAT often become amenorrheic (61-63) and cannot easily conceive, if at all, while on GAT. Indeed, high anovulation rates are reported in TGN undergoing GAT (76), although it is currently unknown how high-level T contributes to such pathophysiology or to inhibiting menstrual cycles. Apart from conceiving spontaneously, in vitro fertilization (IVF) may be an option for some TGN, but usually requires unwanted, prolonged cessation of GAT to reverse the HPG inhibition, the time course and precise mechanisms of which are poorly understood. More clinical studies are needed to better understand how GAT in TGN impacts LH secretion and ovarian function, both during and after cessation of such chronic, male-level androgen exposure. While a number of recent studies have begun to look in detail at TGN ovarian biology in this regard (77-81), GAT effects on neuroendocrine brain and pituitary function in TGN have been largely understudied.

To date, very few clinical studies have compared serum LH levels in TGN before and after initiation of GAT, and the limited reports have yielded inconsistent results, in large part due to very small sample sizes, variability in T doses and durations studied, and historical RIA technology that limited assay sensitivity (41, 60, 64, 65, 82-84). However, the collective evidence suggests that unlike some TGN sampled after short-term GAT and/or lower GAT doses, most TGN receiving

prolonged GAT producing circulating *high T levels* in the male range show inhibited LH levels (41, 60, 65, 84, 85). Even fewer TGN studies have analyzed measures of LH pulsatility during GAT. Although one study reported no change in LH pulse pattern after short-term (6 weeks) low-dose oral T (83), another study by the same group reported a trend for reduced LH pulse frequency after longer GAT (6 months; given intramuscularly) at a higher male-like T dose considered standard for TGN (60). While promising, the data in both LH pulse studies was quite variable, likely due to inadequate sample sizes (n = 5-6/group), and thus statistical analyses were unfortunately underpowered. In fact, most prior studies of LH pulses in T-treated women or TGN unfortunately had heterogeneity of T treatment and very small sample sizes and, hence, even moderate variability limited conclusions. More LH pulse studies are needed to rectify this with larger sample sizes and enhanced statistical power, along with more consistent T dosing and duration.

Circulating FSH in TGN before and after GAT initiation has been studied less than LH, and there are conflicting reports of either reduced or unaffected FSH levels owing to GAT treatment, again likely reflecting inconsistent T dosing, duration, and ovarian status between studies (41, 60, 85-87). However, as with LH, studies using higher or longer GAT doses are more likely to report inhibited FSH levels in TGN (41, 60, 85). This matches reports of inhibited FSH in men and women treated with T (40, 88-90), but more evidence is needed to rigorously assess GAT effects on FSH secretion over time and determine whether this mirrors the typically observed reduction in LH secretion.

It is critical to emphasize that although androgens are often elevated several-fold in many women with PCOS vs healthy women (91-97), circulating T levels in PCOS women are not usually above 200 ng/dL and therefore not nearly as high as the typical physiological range of androgens in men (320 to 1000 ng/dL), the latter of which is the target range for GAT. Therefore, circulating T levels in PCOS are often 3- to 10-fold *lower* than in most transgender men taking GAT (60-63, 81, 98). This notable difference in the degree of "androgen excess" between PCOS women and GAT-treated TGN is critical, given the different neuroendocrine profiles of elevated LH in the former vs suppressed LH in the latter. Indeed, the dysfunctional relationship between *moderate* hyperandrogenemia and abnormally *high* LH secretion in PCOS (99, 100) sharply contrasts with the observed *lower* (inhibited) LH secretion induced by higher male-level T in healthy females and TGN (6, 7, 13, 14, 40, 50). This distinction is important, because much of what little is known about androgen excess effects on LH in females has traditionally come from studies of PCOS (reviewed in (94, 101-105)), which does not mirror the developmental or physiological endocrine status of healthy women or TGN. Thus far, T effects on female neuroendocrine reproductive function in non-PCOS women remains understudied but is important to pursue, especially for the rapidly growing TGN population. It is also worth noting that some TGN individuals also have PCOS (106, 107), which may produce a complex endocrine condition if exogenous GAT is also layered on; this might complicate interpretations if not properly accounted for in clinical studies. However, possible interactions between GAT and PCOS physiology in TGN have not yet been studied long term and remains incompletely understood.

Besides potential inhibitory effects of high androgen levels on LH pulse release, prolonged GAT in TGN may also possibly disrupt LH surge secretion (which triggers ovulation) by interfering with ovarian steroid positive feedback, but this possibility has not yet been systematically evaluated. Importantly, a recent study of ovarian function in TGN identified only a single ovulatory event in a total of 61 person-months of GAT, along with demonstrated menstrual irregularity (76). Moreover, transient rises in pregnanediol-3-glucoronide (a hormonal marker that can indicate ovulation if sustained at high levels for several days) followed by irregular bleeding episodes were suggestive of dysfunctional ovulatory cycles in 22% of these TGN subjects (76). Whether this reflects absent or diminished E_2 positive feedback induction of LH surges during GAT is not known, and at present only one small study has examined LH surges during GAT. Importantly, in that study, normal positive feedback responses of LH secretion to exogenous E_2 treatment were absent in GAT-treated TGN (108); this suggests that the ability of E_2 to trigger the LH surge may be disrupted by GAT. Further detailed investigation in larger, prospective TGN cohorts is warranted to determine the impact of prolonged GAT on both LH pulses and LH surges.

It is worth noting that some TGN discontinue GAT for the purpose of achieving pregnancy or pursuing IVF. Interestingly, studies indicate that circulating T levels in TGN return to physiologic female range within 8 to 12 weeks of GAT cessation (109), yet retrospective cohort studies in amenorrheic TGN pursuing fertility treatment suggest that 5 to 9 months of T cessation are often required for return of normal menstruation (110-112). LH surges or ovulation prior to bleeding was not documented in these studies, so the actual time required for resumption of normal HPG neuroendocrine function after stopping GAT is unknown. Interestingly, TGN who discontinued GAT prior to fertility treatment also required higher gonadotropin doses to achieve similar ovarian stimulation outcomes compared to TGN who had not been on GAT, suggesting a lingering inhibitory effect on the ovaries (111, 112). Overall, these observations suggest a potential enduring effect of GAT on HPG suppression, with serum androgen levels returning to physiologic normal levels several months (or more) before resumption of menstruation and possible ovulation. Although restored LH pulse secretion likely precedes the restoration of menses and ovulation following GAT cessation, the time course and mechanisms for reversibility of each has not been rigorously studied.

Emerging Animal Models of Androgen Action on the Reproductive Axis of Transgender Males

As noted above, circulating androgens in PCOS women are several-fold lower than in TGN taking GAT, and the abnormally high LH secretion in the face of moderate hyperandrogenemia in PCOS contrasts the decreased LH in healthy females and TGN treated with exogenous male-level androgens (6, 7, 13, 14, 40, 50, 98). Moreover, in most animal models of PCOS, androgen excess is initiated prenatally or peripubertally (to model PCOS in women) and this developmental timing of androgen excess does not mimic postpubertal (young adult) initiation of GAT in most TGN. These critical differences necessitate the use of non-PCOS animal models to study mechanisms of high-concentration T effects on neuroendocrine function in TGN. To meet this experimental gap, a slowly growing number of studies have invoked animal models of male-level androgens in adult females (reviewed in (113-115)). To date, most of these have been in rodents, especially with respect to HPG and reproductive neuroendocrine

axis effects (the focus of this review). However, one of the first animal studies of male-level T effects on female reproductive neuroendocrine function was in 1985 in adult female monkeys and clearly demonstrated a large inhibitory effect of T treatment on LH pulse secretion, including LH pulse frequency (116). While this outcome was later mirrored by another study showing exogenous androgens can inhibit overall LH levels in female monkeys (117), fewer non-rodent transgender models exist overall and expanding to other species is an important future avenue of research.

Most rodent models for mimicking reproductive axis effects of GAT in TGN administer chronic T to young adult female mice or rats, using treatments that produce circulating T levels in the male physiological range rather than lower, moderately elevated T levels typical of PCOS (Fig. 1). The route and dose of the T treatment in these rodent models varies between studies, with administration methods including repeated T injections or various T-containing implants. Likewise, durations of T treatment vary depending on outcomes being measured, ranging from several weeks to several months. The onset of treatment typically begins in postpubertal, young adults to mirror the age when TGN typically begin GAT therapy. It is important to note that while these transmasculine animal models mimic the hormonal and physiological conditions of genetic XX individuals exposed to exogenous androgens and are therefore valuable to study biological and physiological impacts of chronic androgen exposure, gender identity–related measures have not been studied; however, gender identity itself is not expected to change in such postpubertally treated rodents.

Two initial studies conducted by Goetz and colleagues sought to mimic transgender male phenotypes in female mouse models. These studies treated either early postpubertal (6 weeks old) or young adult (10 weeks old) female mice with recurring weekly subcutaneous T injection (31 µg/week), termed cross-sex therapy, and focused on bone and atherosclerosis outcomes (118, 119). However, reproductive outcomes or reproductive hormone measures were not assessed, and the circulating T levels produced by the injections, while

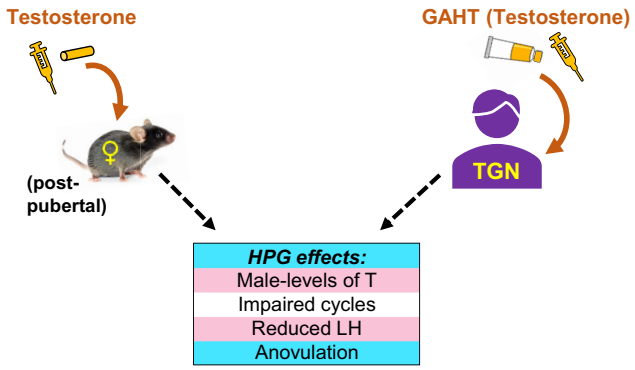

Figure 1. Mouse models of transgender men typically utilize young adult females treated for weeks or months with exogenous androgens, typically T, in the male physiological range. Such T treatments are given as either injections or pellet implants and initiated in young adulthood. As discussed in the text, such emerging transgender male mouse models mimic many of the known reproductive axis impairments reported in transgender men taking gender-affirming T (GAT) therapy, including impairments of cycles and reduction in circulating LH levels. These similarities suggest that these animal models may be useful for further mechanistic studies of androgen actions in XX individuals, especially with respect to neuroendocrine aspects which are more difficult to study in humans.

higher than that of oil control mice (118, 119), did not appear to be elevated enough to reach adult male mouse levels, although this was not tested to be certain. Subsequently, Kinnear and colleagues provided one of the first detailed reports of a transgender male mouse model studying male-level T effects on HPG axis parameters (120). This study tested several doses of T enanthate, delivered via twice-weekly injections for 6 weeks, to young adult (~ 2 months old) female mice. All females had their estrous cycles assessed, via daily vaginal cytology, before and after initiation of T treatment. Overall, the HPG axis of T-treated females was significantly inhibited. Both the 0.225 mg or 0.45 mg dose of T rapidly induced cessation of cyclicity and persistent diestrus (120). Moreover, at 6 weeks of treatment, T-treated mice exhibited elevated circulating T levels in the male physiological range along with significantly reduced circulating LH levels but no major change in FSH levels. T-treated mice also had increases in clitoral area and uterine size, and atretic cyst-like late antral follicles. However, there was no reduction in primordial, primary, secondary, or total antral follicle counts, indicating that T therapy did not deplete ovarian reserves. Importantly, ovaries of T-treated mice demonstrated a complete absence of corpora lutea (120), indicative of anovulation. These phenotypic outcomes nicely mirror similar changes in TGN after prolonged GAT, including absent menstrual cycles, decreased LH, and indices of anovulation. However, while a major focus of this study was on ovarian phenotype and histology, the only neuroendocrine assessments were the single measurement of LH and FSH.

Following the report by Kinnear et al (120), several additional studies from the same investigators, as well as other labs, similarly reported inhibition of the HPG axis in young adult female mice given male levels of T via weekly injections or subcutaneous T implants (Fig. 1) (121-126). Most of these studies focused on ovarian outcomes, with confirmation of inhibited corpora lutea in T-treated mice along with detailed analyses of ovarian follicle development and histology, oocytes, and ovulation induction efficacy (reviewed in detail in (114)). Many of these studies also confirmed that estrous cycles were severely impaired or absent (persistent diestrus) following male-level T treatment (121-126). Importantly, a few of these studies also reported that T-induced inhibition of cycles was reversible upon cessation of T treatment (121, 122, 124). Interestingly, the T treatment paradigm influenced the time course with which estrous cycles resumed following T cessation, with cycles resuming within just a few days after subcutaneous T pellet removal but taking several weeks or longer after stopping injections of T in oil (124). This time course difference likely reflects the much slower decrease in circulating T levels and return to baseline after stopping the T injections vs T pellet removal (days vs weeks) (124), although the reason why injected T took so many more weeks to fully clear the circulation is not known. Regardless, this time course should be considered in future experiments studying physiological outcomes after cessation of T treatment. Finally, it should be emphasized that most of these transmasculine mouse studies did not assess reproductive neuroendocrine measures. Indeed, only one of these subsequent mouse studies measured LH or FSH during GAT treatment but confirmed the prior report by Kinnear et al (120) that a single measure of serum LH was significantly suppressed by GAT whereas FSH was not notably changed (123). Given the paucity of current information on FSH responses to GAT in rodent models, it is not clear if the lack of detected change in

FSH in T-treated female mice reflects a technical limitation (eg, low physiological range in female mice or limited sensitivity of the mouse FSH assay) or a true biological difference between how the 2 gonadotropins are regulated.

Importantly, many TGN youth delay starting GAT until postpubertal or young adult ages; before that time, during adolescence, TGN may take GnRH agonist medication (GnRHa; also known as puberty blockers) to suppress endogenous reproductive hormones in order to delay or prevent incongruous puberty (25, 66, 127, 128). Such GnRHa puberty-blocking treatment is typically replaced later, at an age-appropriate (young adult) time, with GAT to induce gender-congruent masculine characteristics. Several recent studies in female mice and rats have characterized the effects of peripubertal GnRHa treatment on delaying sexual maturation and subsequent adult bone health or cognitive or sexual behaviors (129-133). However, few studies have followed the puberty-blocking GnRHa with GAT in young adulthood and then assessed HPG parameters. Dela Cruz and colleagues recently reported a female mouse model assessing reproductive effects of peripubertal GnRHa treatment prior to young adult GAT treatment (weekly T injections or subcutaneous T implants). Such GAT following adolescent GnRHa treatment was sufficient to suppress adult reproductive status, with female cycles being completely arrested in persistent diestrus and reduced ovarian corpora lutea (123, 134), as previously shown for GAT alone (120, 121, 126). A related study similarly treated female mice with puberty-blocking GnRHa and then GAT in young adulthood and reported that IVF was not greatly impaired by such hormonal intervention (125). This contrasts with a different report of diminished IVF outcomes during GAT treatment (134), although these GAT-treated mice were still able to produce some viable oocytes for fertilization and both studies agree that peripubertal GnRHa treatment does not adversely affect IVF outcomes. Additional studies in transgender male rodent models are needed to better understand the impact of long-term GAT, either alone or in combination with peripubertal GnRHa, on HPG axis parameters, including LH secretion and fertility.

Possible Mechanisms Underlying Androgen Inhibition of LH Secretion in Females and TGN

The physiological, cellular, and molecular mechanisms by which male levels of androgens inhibit the neuroendocrine axis, in particular GnRH and LH secretion, are still poorly understood in both sexes, and greatly understudied in healthy women and TGN. Animal models can offer potential insights into how androgens may inhibit LH levels in females and TGN, and this has begun to be addressed with respect to 2 mechanistic aspects: the receptor signaling pathway(s) and specific cellular targets mediating androgen effects.

With respect to the first aspect, a key mechanistic question is whether the observed inhibition of LH by GAT reflects androgen actions directly via AR pathways vs actions indirectly via ER pathways after T is aromatized to estradiol. Indeed, the contributions of AR- and/or ER-mediated effects of GAT on the HPG axis of TGN currently remains unclear, though it is possible that AR signaling may be one mechanism by which T suppresses HPG function in TGN, consistent with observed LH inhibition by DHT (which acts exclusively via AR signaling pathways) in both men and women (7, 13-16, 50). While TGN currently do not take DHT treatment or other nonaromatizable androgens for their gender-affirming hormone therapy, the experimental assessment of DHT effects in humans and female animal models permits selective isolation of just AR-activated pathways, separate from any possible effects via ERα that may occur after T aromatization. One recent study chronically treated young adult ovariectomized female mice for 3 weeks with physiological male levels of DHT, the dose of which was determined based on the ability of such DHT implants to induce androgen-sensitive measures (eg, seminal vesicle weights) in the normal adult male range. Matching what occurs in humans given nonaromatizable androgens (7, 13-16, 50), this DHT treatment in female mice strongly inhibited both mean LH and endogenous LH pulse secretion vs vehicle-treated controls, including robust decreases in LH pulse frequency and basal LH (Fig. 2) (135). Moreover, mean LH levels (measured in single, one-off samples) are inhibited by 10 days of either T or DHT treatment in adult female ERα knockout (KO) mice, also demonstrating efficacy of DHT to mimic inhibitory T effects on LH secretion (136). While rat models of transgender males have yet to test T or DHT effects on LH secretion, several older studies in adult ovariectomized female rats reported that LH was strongly inhibited by short-term (4, 7, or 14 days), high-dose DHT treatment (presumed in at least one study to produce physiologically functional male levels owing to normal seminal vesicle weights in similarly treated males) (137-140). More recently, female rats chronically treated with a lower, sub-male dose of DHT similarly show strongly inhibited mean LH and suppressed pulsatile LH secretion, along with persistent diestrus (141); while this rat study did not utilize male-level androgens that mimic GAT in TGN, the fact that lower levels of chronic DHT treatment could, like higher DHT in other studies, markedly lower LH secretion further supports AR pathways as a possible mediator of androgen inhibition in females.

Collectively, the DHT effects in female rodents discussed above (Fig. 2), along with similarly observed DHT inhibition of LH in healthy women (50), indicate that AR signaling is sufficient for androgen inhibition of the female neuroendocrine reproductive axis. This suggests that AR signaling may be one critical pathway for GAT's inhibition of LH secretion in TGN. Supporting this, AR is reported in multiple brain areas and cell types in the female brain, including kisspeptin neurons as discussed below. However, such a possible AR effect does not rule out involvement of ERα signaling in also modulating, fully or in part, LH inhibition during treatment with aromatizable androgens, like T. Indeed, the potential participation of AR and ERα in androgen inhibition in females and TGN is not mutually exclusive and requires more investigation. In both humans (142-145) and rodents (146-151), aromatase in expressed in cells in many parts of the female brain, including subregions and nuclei of the hypothalamus, amygdala, thalamus, hippocampus, and cortex, providing a possibility that some circulating GAT may be converted into E_2 at a local neural level, although this has not yet been functionally studied in TGN models. In males, there is support from studies in ERα KO and ARKO mice (lacking ERα or AR, respectively) for preferential AR involvement over ERα in androgen regulation of LH secretion (136, 152, 153), but little data exists for AR vs ERα contributions in females, especially during chronic male-level T exposure. Moreover, one recent study in ovary-intact female mice reported no effect of chronic (90 day) DHT treatment on LH pulses (154), contrasting previously-reported inhibitory effects of shorter-term DHT treatment in female mice (135, 136); whether this conflicting

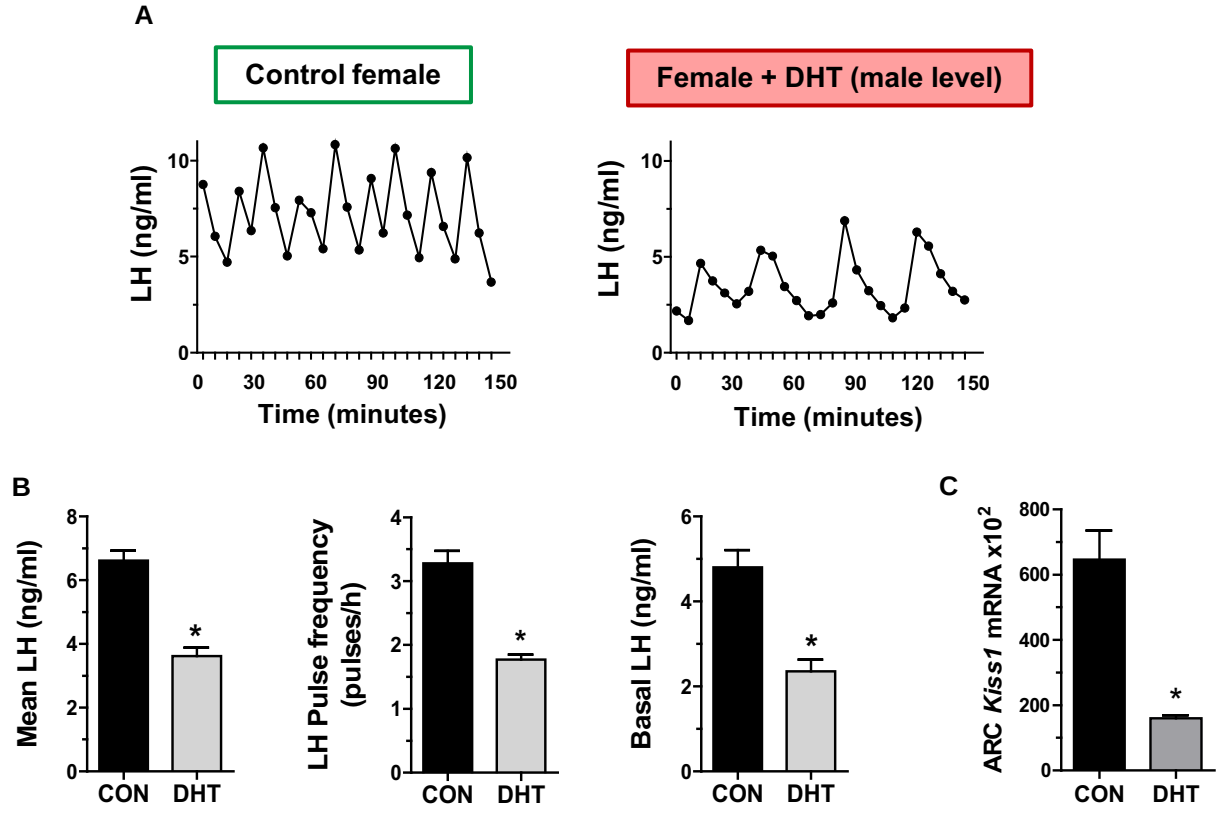

Figure 2. Exogenous chronic treatment of ovariectomized, young adult female mice with a nonaromatizable androgen (DHT) in the physiological functional male range results in large decreases in LH pulse secretion (A), including inhibition of multiple pulse parameters like pulse frequency and basal LH levels (B). This androgen inhibition of LH pulse secretion in ovariectomized female mice is accompanied by large reductions in their kisspeptin (*Kiss1*) gene expression in the hypothalamic arcuate (ARC) nucleus (C), a brain region implicated in controlling GnRH and LH pulse patterns. [Adapted with permission from Esparza et al, *Endocrinology*, 2020 (135).].

data reflects technical, methodological, or age differences, or known limitations of quantifying low-level LH pulses in ovary-intact mice, is unclear but suggests this issue still requires further investigation.

A second mechanistic question regarding androgen inhibition of LH is where such androgen action occurs, in terms of target tissues and cell types. To this end, assessment of specific LH pulse parameters can begin to identify possible target tissues of action within the neuroendocrine axis. The frequency of LH pulse events is determined exclusively within the brain, by components of the neural GnRH pulse generator mechanism, whereas pituitary gonadotropes are not considered to play a critical role in setting the speed of LH pulses. Conversely, the concentration of secreted LH, including basal LH and pulse peak LH levels (and hence pulse amplitude, the difference between the two), can be regulated at the level of both the brain and pituitary, due to changes in the degree of kisspeptin, GnRH, or LH synthesized or released. Thus, if only some LH pulse parameters are affected (eg, pulse frequency but not basal or amplitude, or vice versa) it can give insight into possible targets of androgen action (eg, effects on the brain's GnRH pulse generator vs direct pituitary gonadotrope effects). It should be noted that brain and pituitary actions are not mutually exclusive, especially since AR is expressed in both tissues. Indeed, in females, AR mRNA or protein has been reported in both gonadotropes (155, 156) and numerous brain regions, including, but not limited to, multiple hypothalamic nuclei and neuronal populations (157-165).

In both sexes, a consistently observed change in LH pulse secretion across androgen studies is a decrease in LH pulse frequency, which indicates inhibition within the brain on some aspect of the GnRH pulse generator neural network. This is supported by classic animal studies showing that sex steroids, including androgens, can act in the brain, including the medial basal hypothalamus, to provide negative feedback on GnRH and LH pulses (166-176). However, GnRH neurons do not express AR (or ERα) in either sex. Thus, endogenous or exogenous androgen-mediated inhibition on GnRH pulses must occur indirectly in "upstream" neurons expressing AR (or ERα, if aromatization of T has occurred). The identities of the specific neurons mediating inhibitory androgen effects on GnRH and LH secretion is still not fully known but may involve hypothalamic kisspeptin neurons. Kisspeptin, encoded by *Kiss1*, is a neuropeptide that potently stimulates GnRH neurons to release GnRH (177-180), thereby increasing LH secretion (181-183). Kisspeptin-synthesizing neurons are located in 2 hypothalamic regions, the preoptic area (the rostral periventricular nucleus of the third ventricle [RP3V] in rodents) and the medial basal hypothalamus (arcuate [ARC] nucleus in rodents) (183-189), and project directly to GnRH neurons (186, 190, 191). ARC kisspeptin cells are often also called KNDy neurons based on their co-expression of the neuropeptides neurokinin B and dynorphin (192-194). Importantly, unlike GnRH neurons, kisspeptin neurons in both the ARC and RP3V (termed ARC^{KISS} and $RP3V^{KISS}$ neurons, respectively) express ERα and AR (141, 195-201), suggesting that these populations are possible targets for sex

steroids, including androgens. Indeed, the 2 kisspeptins populations are regulated by sex steroids but in an opposite manner: E_2 and T each inhibit *Kiss1* gene expression in the ARC but stimulate *Kiss1* expression in the RP3V (188, 195, 196, 202). The differential effects of sex steroids on ARC^{KISS} and $RP3V^{KISS}$ cells may functionally relate to these populations' proposed roles in sex steroid feedback control of LH pulses and LH surges, respectively (203, 204).

Importantly, with respect to androgen inhibition of LH pulse frequency in women and TGN, recent evidence strongly suggests that ARC^{KISS} neurons themselves comprise the GnRH pulse generator (205-208), making these neurons prime candidates for direct androgen action to modulate LH pulse secretion. Supporting this possibility, male levels of nonaromatizable androgen (DHT) strongly inhibit both ARC *Kiss1* and *Tac2* (neurokinin B) mRNA levels in the brains of adult female mice, by ~70% and ~40%, respectively, correlating with inhibited LH pulse frequency in these females (Fig. 2) (135). Likewise, diminished ARC *Kiss1* levels in the female brain, along with inhibited LH pulses, were similarly reported for rats receiving chronic DHT treatment (141), although a caveat is that those DHT levels were below the male rat physiological range and were initiated during puberty, which does not mimic current GAT paradigms in TGN. Regardless, these findings support the possibility that exogenous androgens may slow LH pulses in females and TGN by inhibiting ARC^{KISS} neurons (Fig. 2). Whether such inhibition of female ARC^{KISS} neurons occurs via androgens acting directly in these cells vs indirectly in other AR-expressing neurons that communicate with ARC^{KISS} cells is currently unknown, although the possibility for the former is supported by reported AR expression in most female ARC^{KISS} neurons (141, 159, 199, 200). Moreover, it has not yet been assessed whether chronic exogenous male-level T treatment, which better resembles GAT in TGN, also inhibits female ARC^{KISS} neurons as DHT does, although this seems likely given prior reports that short-term T can inhibit ARC *Kiss1*, at least in males (196, 202). If so, such T action could, in theory, inhibit ARC *Kiss1* via either AR or ERα signaling after first being aromatized to E_2, a possibility bolstered by known inhibitory effects of E_2 on ARC^{KISS} neurons (188, 195).

Potential Mechanisms of Male-Level Androgen Inhibition of the LH Surge in Females and TGN

Separate from inhibiting GnRH and LH pulses in females or transgender males, exogenous androgens might also impact the preovulatory GnRH and LH surges during E_2-positive feedback, a critical endocrine process for triggering ovulation. While there are very few studies that have directly assessed endogenous ovulations or LH surges in TGN during GAT, one recent study reported an ovulatory rate of only 4.5% (1 of 22 subjects) in TGN with a median 11 months on GAT therapy (76). Likewise, fewer corpora lutea and other indices of ovulatory impairment were reported in GAT-treated TGN or women abusing anabolic steroids (high-concentration androgens) (60, 209-211), although it also interesting to note that some ovulatory events occasionally occur in a minority of GAT-treated TGN (211), for reasons currently unknown. Matching these few clinical reports, corpora lutea are notably absent in ovaries of transgender male mouse models (T-treated female) (120, 123). However, in all cases, it is unknown if the observed anovulation reflects impaired upstream secretion of LH surges and/or ovarian-specific impairments that prevent ovulation. In both TGN and rodent models, endogenous E_2-generated preovulatory LH surges during GAT are difficult to study since the exogenous male-level T dampens basal LH drive to the ovaries and arrests menstrual/estrous cycles such that the specific cycle stage for endogenous LH surges no longer occurs. Evaluating whether the neuroendocrine component of LH surge generation is compromised by GAT therefore needs to be studied under experimental conditions that maintain (or experimentally provide) positive feedback levels of E_2 during GAT. Intriguingly, one clinical study employed such a paradigm and determined that prolonged GAT blocked E_2-induced LH surges in TGN who had previously shown normal E_2-induced LH surges prior to GAT (108). This suggests that GAT may impair the neuroendocrine mechanisms generating LH surges in response to elevated E_2. This has yet to be studied in TGN animal models, although a similar finding of absent LH surges in response to elevated exogenous E_2 treatment was reported in female rats given a lower, sub-male androgen dose (along with a lack of corpora lutea) (141). This lower-dose androgen blockade of LH surges potentially suggests that higher GAT-like doses of androgens may similarly inhibit the LH surge in female rodents, supporting the few findings thus far in TGN.

Precisely how exogenous androgens might inhibit GnRH and LH surges is not yet known but AR is expressed in pituitary gonadotrope cells (155, 212-214) and it is possible that GAT could act directly in the pituitary to block or lower LH surge release. Indeed, direct pituitary action of androgens have been shown to moderately lower exogenous GnRH-induced LH secretion (either pulses of GnRH or a large GnRH bolus) (135, 214). However, in mice, this pituitary-localized androgen action only partially reduces LH release by ~25% to ~35% (135, 214) and it is unclear if such minor reductions mimic the degree of LH surge inhibition possibly induced by GAT. Relatedly, in female rats given chronic sub-male levels of DHT, a bolus injection of GnRH agonist failed to elicit a robust increase in LH as occurred in control females, supporting a possible pituitary site of action for androgen inhibition of LH in females, although adult, male-level T was not similarly tested. Interestingly, a selective pituitary AR knockout in female mice ameliorated inhibitory effects of a sub-male DHT dose (PCOS-level) on LH surges and corpora lutea formation (214). However, such pituitary AR knockout did not rescue all reproductive measures back to healthy wild-type levels, suggesting additional sites of androgen action beyond the pituitary. Moreover, male-level androgens implemented in adulthood were not tested, precluding comparison to GAT effects in TGN.

While AR in gonadotropes may mediate part of androgen's potential blockade of LH surges, upstream androgen actions in the brain may also play a role. Indeed, androgen actions in the brain and pituitary are not mutually exclusive and both may occur simultaneously. Because GnRH cells lack AR, other brain targets for exogenous androgens would possibly be involved, including $RP3V^{KISS}$ neurons, which are normally activated by elevated E_2 to drive GnRH and LH surge generation (187, 197, 204, 215). $RP3V^{KISS}$ neurons express ERα and progesterone receptor (PR), both essential for generating LH surges (216-219), and also express AR (201). Yet, the function of AR in $RP3V^{KISS}$ neurons is currently unknown. Although short-term T treatment can, like E_2, increase *Kiss1* mRNA levels in the RP3V, activation of AR pathways by DHT treatment has no such stimulatory effect on RP3V *Kiss1* levels (196, 202),

suggesting that the *Kiss1* gene may not be AR-regulated in these neurons. It remains possible that AR signaling affects other genes in female RP3V^{KISS} cells to inhibit these neurons' function, but this needs to be determined. Relatedly, chronic implantation of sub-male levels of DHT in female rats inhibits RP3V *Kiss1* mRNA levels and also partially lowers RP3V^{KISS} neuronal activation (*cfos* induction) during experimental E_2 positive feedback, correlating with impaired LH surges in these DHT-treated females (141). This interesting finding requires further investigation, both with male-level androgen treatment and in additional species, before definitive conclusions can be made with respect to possible mechanisms occurring in TGN. Interestingly, in the DHT rat study, very few RP3V^{KISS} neurons expressed AR (~2%) despite reduced RP3V^{KISS} activation and suppressed LH surges (141). This low AR co-expression differs from higher expression reported in mouse RP3V^{KISS} neurons measured via RNAseq (201). Whether this difference reflects the different hormonal milieu at the time the mouse and rat brains were collected or other technological or species differences is unclear. Yet, the reported low AR levels in female rats could suggest that suppression of LH surges by androgens might be due to androgen action in non-RP3V^{KISS} cells, including possible pituitary action.

Conclusions

Clinical and mechanistic understanding of androgen inhibition on the neuroendocrine reproductive axis in females and TGN in healthy, nondisease states is a long-overlooked area. Historically, there has been a dearth of credible, rigorous clinical investigation on androgen effects on LH pulses, LH surges, and ovulatory function in TGN during GAT, and many concepts of androgen actions in women come primarily from PCOS which does not recapitulate a similar pathophysiology for inhibited LH secretion as in GAT-treated TGN. Only a small handful of prior and recent clinical studies have tackled this issue in TGN, and while several experimental and sample size limitations exist in those studies, the emerging consensus indicates that exogenous male-level androgens can inhibit LH secretion, including LH pulse secretion parameters and perhaps LH surge magnitude, especially with prolonged GAT durations. These critical findings provide an important neuroendocrine link to common menstrual cycle impairments and likely anovulation in a majority of TGN taking prolonged GAT. More clinical studies with greater sample sizes and statistical power, and consistent dosing and durations of GAT, are needed to understand male-level androgen effects on specific LH pulse parameters, including pulse frequency. Future neuroendocrine clinical studies also need to consider evaluating both longer term effects of GAT (beyond just 6-12 months as has been most common) and neuroendocrine effects of GAT withdrawal. Indeed, long-term effects of chronic high-level androgen exposure in females are not well studied but are important considering that most TGN will likely take GAT for most of their adult lives.

The clinical observations of lower LH in TGN during GAT therapy necessitates experimental interrogation of possible underlying mechanisms by which male-level androgens can inhibit, directly or indirectly, LH secretion and menstrual cyclicity in females and TGN. To this end, emerging animal models (thus far, primarily rodents) are starting to assess the impact and mechanisms of male-level androgen action on the adult female HPG axis. While most studies have evaluated ovary alterations, only a few assessed neuroendocrine parameters (eg, GnRH, LH, FSH). Based on the limited current data available, a working model of androgen action in healthy females and TGN posits that male-level exogenous androgens may inhibit the female reproductive neuroendocrine axis by acting, directly or indirectly, on hypothalamic kisspeptin neurons to thereby alter downstream GnRH and LH pulse secretion and possibly GnRH and LH surges, as well as (to a lesser degree) direct action in pituitary gonadotropes (Fig. 3). Future studies testing and refining this model in transmale

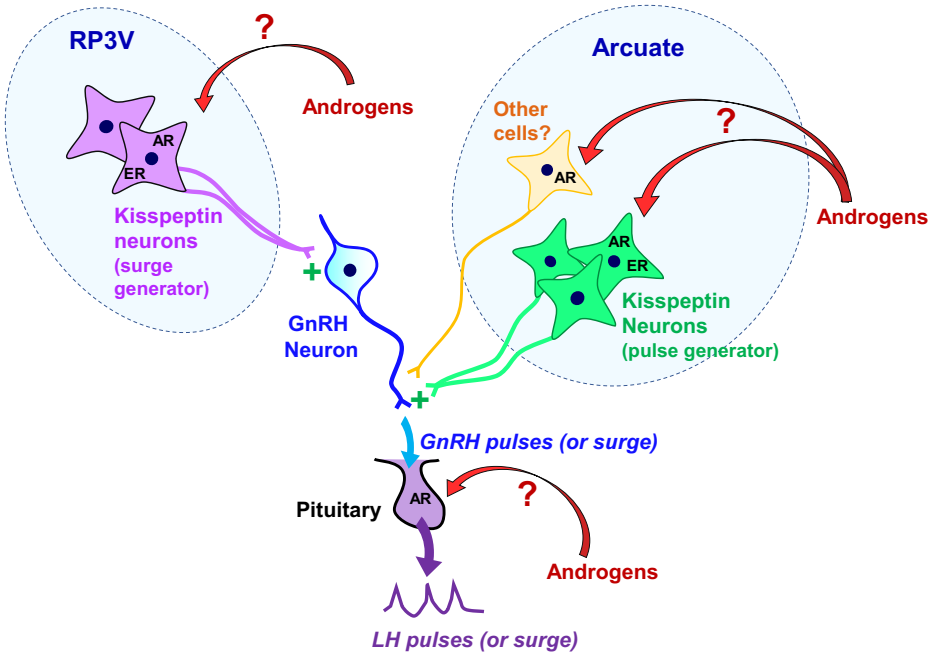

Figure 3. Working model of potential neuroendocrine mechanisms and target sites for exogenous androgen inhibition of LH secretion in females and, possibly, TGN. The model includes possible target sites directly in the pituitary along with AR-expressing kisspeptin neurons and/or other AR-expressing neurons in hypothalamic regions implicated in regulating LH pulses or LH surges (arcuate and RP3V nuclei, respectively).

animal models across different species will be informative to our understanding of the impact of high androgens on reproductive physiology in genetically XX individuals. In addition, further rigorous comparison of T (AR and/or ERα signaling after possible aromatization) vs DHT (only AR signaling) will provide valuable insight into intracellular mechanisms of male-level androgen inhibition of LH secretion and specific cell targets. Ultimately, such mechanistic studies in transgender animal models could have high clinical importance to the rapidly growing, yet currently understudied, TGN population.

Acknowledgments

The author thanks fellow departmental faculty members of the REI Division, including Antoni Duleba, Tracy Harrison, Pamela Mellon, Kellie Breen Church, Mark Lawson, and Kina Thackray, for fruitful prior discussions on this general topic.

Funding

Dr. Kauffman is supported by National Institutes of Health grants R01 HD111650, R01 HD100580, and R01 AG078185.

Disclosures

The author declares no competing financial interests and has nothing to disclose.

Data Availability

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

References

1. Moore CR, Price D. Gonad hormone functions, and the reciprocal influence between gonads and hypophysis with its bearing on the problem of sex hormone antagonism. *Am J Anatomy*. 1932;50(1):13-71.
2. Lyon MF, Glenister PH. Reduced reproductive performance in androgen-resistant Tfm/Tfm female mice. *Proc R Soc Lond B Biol Sci*. 1980;208(1170):1-12.
3. Wylie K, Froggatt N. Late onset hypogonadism, sexuality and fertility. *Hum Fertil (Camb)*. 2010;13(3):126-133.
4. Bhasin S. Approach to the infertile man. *J Clin Endocrinol Metab*. 2007;92(6):1995-2004.
5. Petak SM, Nankin HR, Spark RF, Swerdloff RS, Rodriguez-Rigau LJ, American Association of Clinical Endocrinologists. American Association of Clinical Endocrinologists Medical Guidelines for clinical practice for the evaluation and treatment of hypogonadism in adult male patients–2002 update. *Endocr Pract*. 2002;8(6):440-456.
6. Matsumoto AM, Bremner WJ. Modulation of pulsatile gonadotropin secretion by testosterone in man. *J Clin Endocrinol Metab*. 1984;58(4):609-614.
7. Santen RJ. Is aromatization of testosterone to estradiol required for inhibition of luteinizing hormone secretion in men? *J Clin Invest*. 1975;56(6):1555-1563.
8. Plant TM. Effects of orchidectomy and testosterone replacement treatment on pulsatile luteinizing hormone secretion in the adult rhesus monkey (Macaca mulatta). *Endocrinology*. 1982;110(6):1905-1913.
9. Steiner RA, Bremner WJ, Clifton DK. Regulation of luteinizing hormone pulse frequency and amplitude by testosterone in the adult male rat. *Endocrinology*. 1982;111(6):2055-2061.
10. D'Occhio MJ, Schanbacher BD, Kinder JE. Relationship between serum testosterone concentration and patterns of luteinizing hormone secretion in male sheep. *Endocrinology*. 1982;110(5):1547-1554.
11. Tilbrook AJ, Clarke IJ. Negative feedback regulation of the secretion and actions of gonadotropin-releasing hormone in males. *Biol Reprod*. 2001;64(3):735-742.
12. Plant TM. Gonadal regulation of hypothalamic gonadotropin-releasing hormone release in primates. *Endocr Rev*. 1986;7(1):75-88.
13. Stewart-Bentley M, Odell W, Horton R. The feedback control of luteinizing hormone in normal adult men. *J Clin Endocrinol Metab*. 1974;38(4):545-553.
14. Winters SJ, Janick JJ, Loriaux DL, Sherins RJ. Studies on the role of sex steroids in the feedback control of gonadotropin concentrations in men. II. Use of the estrogen antagonist, clomiphene citrate. *J Clin Endocrinol Metab*. 1979;48(2):222-227.
15. Veldhuis JD, Rogol AD, Samojlik E, Ertel NH. Role of endogenous opiates in the expression of negative feedback actions of androgen and estrogen on pulsatile properties of luteinizing hormone secretion in man. *J Clin Invest*. 1984;74(1):47-55.
16. Vigersky RA, Easley RB, Loriaux DL. Effect of fluoxymesterone on the pituitary-gonadal axis: the role of testosterone-estradiol-binding globulin. *J Clin Endocrinol Metab*. 1976;43(1):1-9.
17. Swerdloff RS, Walsh PC, Odell WD. Control of LH and FSH secretion in the male: evidence that aromatization of androgens to estradiol is not required for inhibition of gonadotropin secretion. *Steroids*. 1972;20(1):13-22.
18. Tilbrook AJ, de Kretser DM, Cummins JT, Clarke IJ. The negative feedback effects of testicular steroids are predominantly at the hypothalamus in the ram. *Endocrinology*. 1991;129(6):3080-3092.
19. Gooren L, Spinder T, Spijkstra JJ, *et al.* Sex steroids and pulsatile luteinizing hormone release in men. Studies in estrogen-treated agonadal subjects and eugonadal subjects treated with a novel nonsteroidal antiandrogen. *J Clin Endocrinol Metab*. 1987;64(4):763-770.
20. Knuth UA, Hano R, Nieschlag E. Effect of flutamide or cyproterone acetate on pituitary and testicular hormones in normal men. *J Clin Endocrinol Metab*. 1984;59(5):963-969.
21. Balzano S, Migliari R, Sica V, *et al.* The effect of androgen blockade on pulsatile gonadotrophin release and LH response to naloxone. *Clin Endocrinol*. 1987;27(4):491-499.
22. Migliari R, Balzano S, Scarpa RM, Campus G, Pintus C, Usai E. Short term effects of flutamide administration on hypothalamic-pituitary-testicular axis in man. *J Urol*. 1988;139(3):637-639.
23. Urban RJ, Davis MR, Rogol AD, Johnson ML, Veldhuis JD. Acute androgen receptor blockade increases luteinizing hormone secretory activity in men. *J Clin Endocrinol Metab*. 1988;67(6):1149-1155.
24. Takahashi PY, Liu PY, Veldhuis JD. Distinct roles of age and abdominal visceral fat in reducing androgen receptor-dependent negative feedback on LH secretion in healthy men. *Andrology*. 2014;2(4):588-595.
25. Hembree WC, Cohen-Kettenis PT, Gooren L, *et al.* Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2017;102(11):3869-3903.
26. Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Position statement: utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *J Clin Endocrinol Metab*. 2007;92(2):405-413.
27. Santoro N, Torrens J, Crawford S, *et al.* Correlates of circulating androgens in mid-life women: the study of women's health across the nation. *J Clin Endocrinol Metab*. 2005;90(8):4836-4845.
28. Wang C, Catlin DH, Demers LM, Starcevic B, Swerdloff RS. Measurement of total serum testosterone in adult men: comparison of current laboratory methods versus liquid chromatography-tandem mass spectrometry. *J Clin Endocrinol Metab*. 2004;89(2):534-543.

29. Gleicher N, Kim A, Weghofer A, *et al*. Hypoandrogenism in association with diminished functional ovarian reserve. *Hum Reprod*. 2013;28(4):1084-1091.
30. Erichsen MM, Husebye ES, Michelsen TM, Dahl AA, Lovas K. Sexuality and fertility in women with Addison's disease. *J Clin Endocrinol Metab*. 2010;95(9):4354-4360.
31. Bachelot A, Chakhtoura Z, Plu-Bureau G, *et al*. Influence of hormonal control on LH pulsatility and secretion in women with classical congenital adrenal hyperplasia. *Eur J Endocrinol*. 2012;167(4):499-505.
32. Matsumoto T, Sakari M, Okada M, *et al*. The androgen receptor in health and disease. *Annu Rev Physiol*. 2013;75(1):201-224.
33. Arlt W, Callies F, van Vlijmen JC, *et al*. Dehydroepiandrosterone replacement in women with adrenal insufficiency. *N Engl J Med*. 1999;341(14):1013-1020.
34. Lin HY, Xu Q, Yeh S, Wang RS, Sparks JD, Chang C. Insulin and leptin resistance with hyperleptinemia in mice lacking androgen receptor. *Diabetes*. 2005;54(6):1717-1725.
35. Yeh S, Tsai MY, Xu Q, *et al*. Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. *Proc Natl Acad Sci U S A*. 2002;99(21):13498-13503.
36. Hu YC, Wang PH, Yeh S, *et al*. Subfertility and defective folliculogenesis in female mice lacking androgen receptor. *Proc Natl Acad Sci U S A*. 2004;101(31):11209-11214.
37. Shiina H, Matsumoto T, Sato T, *et al*. Premature ovarian failure in androgen receptor-deficient mice. *Proc Natl Acad Sci U S A*. 2006;103(1):224-229.
38. Walters KA, Allan CM, Handelsman DJ. Androgen actions and the ovary. *Biol Reprod*. 2008;78(3):380-389.
39. Walters KA, McTavish KJ, Seneviratne MG, *et al*. Subfertile female androgen receptor knockout mice exhibit defects in neuroendocrine signaling, intraovarian function, and uterine development but not uterine function. *Endocrinology*. 2009;150(7):3274-3282.
40. Serafini P, Silva PD, Paulson RJ, Elkind-Hirsch K, Hernandez M, Lobo RA. Acute modulation of the hypothalamic-pituitary axis by intravenous testosterone in normal women. *Am J Obstet Gynecol*. 1986;155(6):1288-1292.
41. Spinder T, Spijkstra JJ, Gooren LJ, Hompes PG, van Kessel H. Effects of long-term testosterone administration on gonadotropin secretion in agonadal female to male transsexuals compared with hypogonadal and normal women. *J Clin Endocrinol Metab*. 1989;68(1):200-207.
42. Bachelot A, Laborde K, Bresson JL, *et al*. Luteinizing hormone pulsatility in patients with major ovarian hyperandrogenism. *J Endocrinol Invest*. 2007;30(8):636-646.
43. Legro RS, Schlaff WD, Diamond MP, *et al*. Total testosterone assays in women with polycystic ovary syndrome: precision and correlation with hirsutism. *J Clin Endocrinol Metab*. 2010;95(12):5305-5313.
44. Penttila TA, Anttila L, Torma A, Koskinen P, Erkkola R, Irjala K. Serum free testosterone in polycystic ovary syndrome measured with a new reference method. *Fertil Steril*. 1996;65(1):55-60.
45. Winters SJ, Talbott E, Guzick DS, Zborowski J, McHugh KP. Serum testosterone levels decrease in middle age in women with the polycystic ovary syndrome. *Fertil Steril*. 2000;73(4):724-729.
46. Pinola P, Piltonen TT, Puurunen J, *et al*. Androgen profile through life in women with polycystic ovary syndrome: a nordic multicenter collaboration study. *J Clin Endocrinol Metab*. 2015;100(9):3400-3407.
47. Ropelato MG, Rudaz MC, Escobar ME, *et al*. Acute effects of testosterone infusion on the serum luteinizing hormone profile in eumenorrheic and polycystic ovary syndrome adolescents. *J Clin Endocrinol Metab*. 2009;94(9):3602-3610.
48. d'Alva CB, Abiven-Lepage G, Viallon V, *et al*. Sex steroids in androgen-secreting adrenocortical tumors: clinical and hormonal features in comparison with non-tumoral causes of androgen excess. *Eur J Endocrinol*. 2008;159(5):641-647.
49. Marcondes JA, Barcellos CR, Rocha MP, Bisi H. Changing pattern of gonadotropins in a patient with an adrenal-androgen secreting tumor. *Clinics (Sao Paulo)*. 2011;66(2):355-357.
50. Vermesh M, Silva PD, Lobo RA. Endogenous opioids modulate the inhibitory effects of androgen on the hypothalamic-pituitary axis of normal women. *J Clin Endocrinol Metab*. 1987;65(6):1183-1186.
51. Herman JL, Flores AR, O'Neil KK. *How Many Adults and Youth Identify as Transgender in the United States?*. UCLA: The Williams Institute; 2022. https://escholarship.org/uc/item/4xs990ws.
52. Meerwijk EL, Sevelius JM. Transgender population size in the United States: a meta-regression of population-based probability samples. *Am J Public Health*. 2017;107(2):e1-e8.
53. Arcelus J, Bouman WP, Van Den Noortgate W, Claes L, Witcomb G, Fernandez-Aranda F. Systematic review and meta-analysis of prevalence studies in transsexualism. *Eur Psychiatry*. 2015;30(6):807-815.
54. Meyer IH, Brown TN, Herman JL, Reisner SL, Bockting WO. Demographic characteristics and health status of transgender adults in select US regions: behavioral risk factor surveillance system, 2014. *Am J Public Health*. 2017;107(4):582-589.
55. De Cuypere G, Van Hemelrijck M, Michel A, *et al*. Prevalence and demography of transsexualism in Belgium. *Eur Psychiatry*. 2007;22(3):137-141.
56. Kuyper L, Wijsen C. Gender identities and gender dysphoria in The Netherlands. *Arch Sex Behav*. 2014;43(2):377-385.
57. Zucker KJ, Lawrence AA. Epidemiology of gender identity disorder: recommendations for the standards of care of the world professional association for transgender health. *Int J Transgenderism*. 2009;11(1):8-18.
58. Coleman E, Bockting W, Botzer M, *et al*. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. *Int J Transgenderism*. 2012;13(4):165-232.
59. T'Sjoen G, Arcelus J, Gooren L, Klink DT, Tangpricha V. Endocrinology of transgender medicine. *Endocr Rev*. 2019;40(1):97-117.
60. Spinder T, Spijkstra JJ, van den Tweel JG, *et al*. The effects of long term testosterone administration on pulsatile luteinizing hormone secretion and on ovarian histology in eugonadal female to male transsexual subjects. *J Clin Endocrinol Metab*. 1989;69(1):151-157.
61. McFarland J, Craig W, Clarke NJ, Spratt DI. Serum testosterone concentrations remain stable between injections in patients receiving subcutaneous testosterone. *J Endocr Soc*. 2017;1(8):1095-1103.
62. Nakamura A, Watanabe M, Sugimoto M, *et al*. Dose-response analysis of testosterone replacement therapy in patients with female to male gender identity disorder. *Endocr J*. 2013;60(3):275-281.
63. Deutsch MB, Bhakri V, Kubicek K. Effects of cross-sex hormone treatment on transgender women and men. *Obstet Gynecol*. 2015;125(3):605-610.
64. Pelusi C, Costantino A, Martelli V, *et al*. Effects of three different testosterone formulations in female-to-male transsexual persons. *J Sex Med*. 2014;11(12):3002-3011.
65. Mueller A, Kiesewetter F, Binder H, Beckmann MW, Dittrich R. Long-term administration of testosterone undecanoate every 3 months for testosterone supplementation in female-to-male transsexuals. *J Clin Endocrinol Metab*. 2007;92(9):3470-3475.
66. Salas-Humara C, Sequeira GM, Rossi W, Dhar CP. Gender affirming medical care of transgender youth. *Curr Probl Pediatr Adolesc Health Care*. 2019;49(9):100683.
67. Moravek MB, Kinnear HM, George J, *et al*. Impact of exogenous testosterone on reproduction in transgender men. *Endocrinology*. 2020;161(3):bqaa014.
68. Pache TD, Chadha S, Gooren LJ, *et al*. Ovarian morphology in long-term androgen-treated female to male transsexuals. A human model for the study of polycystic ovarian syndrome? *Histopathology*. 1991;19(5):445-452.

69. Cipres D, Seidman D, Cloniger C, 3rd, Nova C, O'Shea A, Obedin-Maliver J. Contraceptive use and pregnancy intentions among transgender men presenting to a clinic for sex workers and their families in San Francisco. *Contraception*. 2017;95(2):186-189.
70. Moseson H, Fix L, Hastings J, *et al.* Pregnancy intentions and outcomes among transgender, nonbinary, and gender-expansive people assigned female or intersex at birth in the United States: results from a national, quantitative survey. *Int J Transgend Heal*. 2020;22(1-2):30-41.
71. Wierckx K, Van Caenegem E, Pennings G, *et al.* Reproductive wish in transsexual men. *Hum Reprod*. 2012;27(2):483-487.
72. Kerman HM, Pham A, Crouch JM, *et al.* Gender diverse youth on fertility and future family: a qualitative analysis. *J Adolesc Health*. 2021;68(6):1112-1120.
73. Light A, Wang LF, Zeymo A, Gomez-Lobo V. Family planning and contraception use in transgender men. *Contraception*. 2018;98(4):266-269.
74. Light AD, Obedin-Maliver J, Sevelius JM, Kerns JL. Transgender men who experienced pregnancy after female-to-male gender transitioning. *Obstet Gynecol*. 2014;124(6):1120-1127.
75. Morong JJ, Class QA, Zamah AM, Hinz E. Parenting intentions in transgender and gender-nonconforming adults. *Int J Gynaecol Obstet*. 2022;159(2):557-562.
76. Taub RL, Ellis SA, Neal-Perry G, Magaret AS, Prager SW, Micks EA. The effect of testosterone on ovulatory function in transmasculine individuals. *Am J Obstet Gynecol*. 2020;223(2):229.e1-229.e8.
77. Coskun ADE, Koc N, Vural F, Donmez EE. Ovarian histology of removed ovaries after gender-affirming testosterone therapy. *Int J Surg Pathol*. 2023;31(6):1093-1098.
78. Christodoulaki A, He H, Zhou M, *et al.* Characterization of ovarian tissue oocytes from transgender men reveals poor calcium release and embryo development, which might be overcome by spindle transfer. *Hum Reprod*. 2023;38(6):1135-1150.
79. Bailie E, Maidarti M, Jack S, *et al.* The ovaries of transgender men indicate effects of high dose testosterone on the primordial and early growing follicle pool. *Reprod Fertil*. 2023;4(2):e220102.
80. De Roo C, Lierman S, Tilleman K, *et al.* Ovarian tissue cryopreservation in female-to-male transgender people: insights into ovarian histology and physiology after prolonged androgen treatment. *Reprod Biomed Online*. 2017;34(6):557-566.
81. Yaish I, Tordjman K, Amir H, *et al.* Functional ovarian reserve in transgender men receiving testosterone therapy: evidence for preserved anti-Mullerian hormone and antral follicle count under prolonged treatment. *Hum Reprod*. 2021;36(10):2753-2760.
82. Dewis P, Newman M, Ratcliffe WA, Anderson DC. Does testosterone affect the normal menstrual cycle? *Clin Endocrinol* 1986;24(5):515-521.
83. Scheele F, Hompes PG, Gooren LJ, Spijkstra JJ, Spinder T. The effect of 6 weeks of testosterone treatment on pulsatile luteinizing hormone secretion in eugonadal female-to-male transsexuals. *Fertil Steril*. 1991;55(3):608-611.
84. Pache TD, Hop WC, de Jong FH, *et al.* 17 beta-Oestradiol, androstenedione and inhibin levels in fluid from individual follicles of normal and polycystic ovaries, and in ovaries from androgen treated female to male transsexuals. *Clin Endocrinol*. 1992;36(6):565-571.
85. Caanen MR, Schouten NE, Kuijper EAM, *et al.* Effects of long-term exogenous testosterone administration on ovarian morphology, determined by transvaginal (3D) ultrasound in female-to-male transsexuals. *Hum Reprod*. 2017;32(7):1457-1464.
86. Ikeda K, Baba T, Noguchi H, *et al.* Excessive androgen exposure in female-to-male transsexual persons of reproductive age induces hyperplasia of the ovarian cortex and stroma but not polycystic ovary morphology. *Hum Reprod*. 2013;28(2):453-461.
87. Borras A, Manau MD, Fabregues F, *et al.* Endocrinological and ovarian histological investigations in assigned female at birth transgender people undergoing testosterone therapy. *Reprod Biomed Online*. 2021;43(2):289-297.
88. Lee PA, Jaffe RB, Midgley AR, Jr., Kohen F, Niswinder GD. Regulation of human gonadotropins. 8. Suppression of serum LH and FSH in adult males following exogenous testosterone administration. *J Clin Endocrinol Metab*. 1972;35(5):636-641.
89. Marynick SP, Loriaux DL, Sherins RJ, Pita JC, Jr., Lipsett MB. Evidence that testosterone can suppress pituitary gonadotropin secretion independently of peripheral aromatization. *J Clin Endocrinol Metab*. 1979;49(3):396-398.
90. Sherins RJ, Loriaux DL. Studies of the role of sex steroids in the feedback control of FSH concentrations in men. *J Clin Endocrinol Metab*. 1973;36(5):886-893.
91. Zadwadski J, Dunaif A. Diagnostic cirteria for polysystic ovary syndrome: toward a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, eds. *Polycystic Ovary Syndrome*. Blackwell Scientific; 1992:377-384.
92. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polysystic ovary syndrome. *Fertil Steril*. 2004;81(1):19-25.
93. Azziz R, Carmina E, Dewailly D, *et al.* The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril*. 2009;91(2):456-488.
94. Burt Solorzano CM, Beller JP, Abshire MY, Collins JS, McCartney CR, Marshall JC. Neuroendocrine dysfunction in polycystic ovary syndrome. *Steroids*. 2012;77(4):332-337.
95. Escobar ME, Ropelato MG, Ballerini MG, *et al.* Acceleration of luteinizing hormone pulse frequency in adolescent girls with a history of central precocious puberty with versus without hyperandrogenism. *Horm Res*. 2007;68(6):278-285.
96. Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F. Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. *J Clin Invest*. 1976;57(5):1320-1329.
97. Baird DT, Corker CS, Davidson DW, Hunter WM, Michie EA, Van Look PF. Pituitary-ovarian relationships in polycystic ovary syndrome. *J Clin Endocrinol Metab*. 1977;45(4):798-801.
98. Amirikia H, Savoy-Moore RT, Sundareson AS, Moghissi KS. The effects of long-term androgen treatment on the ovary. *Fertil Steril*. 1986;45(2):202-208.
99. Lawson MA, Jain S, Sun S, Patel K, Malcolm PJ, Chang RJ. Evidence for insulin suppression of baseline luteinizing hormone in women with polycystic ovarian syndrome and normal women. *J Clin Endocrinol Metab*. 2008;93(6):2089-2096.
100. Rosenfield RL, Bordini B. Evidence that obesity and androgens have independent and opposing effects on gonadotropin production from puberty to maturity. *Brain Res*. 2010;1364:186-197.
101. Blank SK, McCartney CR, Helm KD, Marshall JC. Neuroendocrine effects of androgens in adult polycystic ovary syndrome and female puberty. *Semin Reprod Med*. 2007;25(5):352-359.
102. Coyle C, Campbell RE. Pathological pulses in PCOS. *Mol Cell Endocrinol*. 2019;498:110561.
103. Gurule S, Sustaita-Monroe J, Padmanabhan V, Cardoso R. Developmental programming of the neuroendocrine axis by steroid hormones: insights from the sheep model of PCOS. *Front Endocrinol (Lausanne)*. 2023;14:1096187.
104. Szeliga A, Rudnicka E, Maciejewska-Jeske M, *et al.* Neuroendocrine determinants of polycystic ovary syndrome. *Int J Environ Res Public Health*. 2022;19(5):3089.
105. Coutinho EA, Kauffman AS. The role of the brain in the pathogenesis and physiology of polycystic ovary syndrome (PCOS). *Med Sci (Basel)*. 2019;7(8):84.
106. Liu M, Murthi S, Poretsky L. Polycystic ovary syndrome and gender identity. *Yale J Biol Med*. 2020;93(4):529-537.
107. Gezer E, Piro B, Canturk Z, *et al.* The comparison of gender dysphoria, body image satisfaction and quality of life between treatment-naive transgender males with and without polycystic ovary syndrome. *Transgend Health*. 2022;7(6):514-520.
108. Gooren L. The neuroendocrine response of luteinizing hormone to estrogen administration in the human is not sex specific but

dependent on the hormonal environment. *J Clin Endocrinol Metab*. 1986;63(3):589-593.
109. Turner L, Ly LP, Desai R, *et al*. Pharmacokinetics and acceptability of subcutaneous injection of testosterone undecanoate. *J Endocr Soc*. 2019;3(8):1531-1540.
110. Adeleye AJ, Cedars MI, Smith J, Mok-Lin E. Ovarian stimulation for fertility preservation or family building in a cohort of transgender men. *J Assist Reprod Genet*. 2019;36(10):2155-2161.
111. Amir H, Yaish I, Samara N, Hasson J, Groutz A, Azem F. Ovarian stimulation outcomes among transgender men compared with fertile cisgender women. *J Assist Reprod Genet*. 2020;37(10):2463-2472.
112. Leung A, Sakkas D, Pang S, Thornton K, Resetkova N. Assisted reproductive technology outcomes in female-to-male transgender patients compared with cisgender patients: a new frontier in reproductive medicine. *Fertil Steril*. 2019;112(5):858-865.
113. Aghi K, Goetz TG, Pfau DR, Sun SED, Guthman EM, Roepke TA. Using animal models for gender-affirming hormone therapy. *J Endocr Soc*. 2023;8(1):bvad144.
114. Raja NS, Rubin ES, Moravek MB. A review of animal models investigating the reproductive effects of gender-affirming hormone therapy. *J Clin Med*. 2024;13(4):1183.
115. Tassinari R, Maranghi F. Rodent model of gender-affirming hormone therapies as specific tool for identifying susceptibility and vulnerability of transgender people and future applications for risk assessment. *Int J Environ Res Public Health*. 2021;18(23):12640.
116. Dubey AK, Plant TM. Testosterone administration to ovariectomized female rhesus monkeys (Macaca mulatta) reduces the frequency of pulsatile luteinizing hormone secretion. *Biol Reprod*. 1985;32(5):1109-1115.
117. Kowalski W, Chatterton RT, Jr. Effects of subchronic infusion of dehydroepiandrosterone sulfate on serum gonadotropin levels and ovarian function in the cynomolgus monkey. *Fertil Steril*. 1992;57(4):912-920.
118. Goetz TG, Mamillapalli R, Sahin C, *et al*. Addition of estradiol to cross-sex testosterone therapy reduces atherosclerosis plaque formation in female ApoE-/- mice. *Endocrinology*. 2018;159(2):754-762.
119. Goetz TG, Mamillapalli R, Devlin MJ, Robbins AE, Majidi-Zolbin M, Taylor HS. Cross-sex testosterone therapy in ovariectomized mice: addition of low-dose estrogen preserves bone architecture. *Am J Physiol Endocrinol Metab*. 2017;313(5):E540-E551.
120. Kinnear HM, Constance ES, David A, *et al*. A mouse model to investigate the impact of testosterone therapy on reproduction in transgender men. *Hum Reprod*. 2019;34(10):2009-2017.
121. Bartels CB, Uliasz TF, Lestz L, Mehlmann LM. Short-term testosterone use in female mice does not impair fertilizability of eggs: implications for the fertility care of transgender males. *Hum Reprod*. 2021;36(1):189-198.
122. Kinnear HM, Hashim PH, Dela Cruz C, *et al*. Reversibility of testosterone-induced acyclicity after testosterone cessation in a transgender mouse model. *F S Sci*. 2021;2(2):116-123.
123. Dela Cruz C, Kinnear HM, Hashim PH, *et al*. A mouse model mimicking gender-affirming treatment with pubertal suppression followed by testosterone in transmasculine youth. *Hum Reprod*. 2023;38(2):256-265.
124. Hashim PH, Kinnear HM, Cruz CD, Padmanabhan V, Moravek MB, Shikanov A. Pharmacokinetic comparison of three delivery systems for subcutaneous testosterone administration in female mice. *Gen Comp Endocrinol*. 2022;327:114090.
125. Godiwala P, Uliasz TF, Lowther KM, Kaback D, Mehlmann LM. Puberty suppression followed by testosterone therapy does not impair reproductive potential in female mice. *Endocrinology*. 2023;164(11):bqad145.
126. Schwartz AR, Xu M, Henderson NC, *et al*. Impaired in vitro fertilization outcomes following testosterone treatment improve with washout in a mouse model of gender-affirming hormone treatment. *Am J Obstet Gynecol*. 2023;229(4):419.e1-419.e10.
127. Conflitti AC, Spaziani M, Pallotti F, *et al*. Update on bioethical, medical and fertility issues in gender incongruence during transition age. *J Endocrinol Invest*. 2023;46(9):1725-1736.
128. Rew L, Young CC, Monge M, Bogucka R. Review: puberty blockers for transgender and gender diverse youth-a critical review of the literature. *Child Adolesc Ment Health*. 2021;26(1):3-14.
129. Anacker C, Sydnor E, Chen BK, *et al*. Behavioral and neurobiological effects of GnRH agonist treatment in mice-potential implications for puberty suppression in transgender individuals. *Neuropsychopharmacology*. 2021;46(5):882-890.
130. Guarraci FA, Avendano L, Kelly M, *et al*. Chronic periadolescent leuprolide exposure affects the development of reproductive physiology and behavior of female and male rats differently, but both mature after treatment termination. *Biol Sex Differ*. 2023;14(1):1.
131. Guarraci FA, Avendano L, Kelly M, *et al*. Daily GnRH agonist treatment effectively delayed puberty in female rats without long-term effects on sexual behavior or estrous cyclicity. *Physiol Behav*. 2022;254:113879.
132. Roth CL, Neu C, Jarry H, Schoenau E. Different effects of agonistic vs. antagonistic gnrh-analogues (triptorelin vs. cetrorelix) on bone modeling and remodeling in peripubertal female rats. *Exp Clin Endocrinol Diabetes*. 2005;113(8):451-456.
133. Roth C, Leonhardt S, Seidel C, Luft H, Wuttke W, Jarry H. Comparative analysis of different puberty inhibiting mechanisms of two GnRH agonists and the GnRH antagonist cetrorelix using a female rat model. *Pediatr Res*. 2000;48(4):468-474.
134. Dela Cruz C, Wandoff A, Brunette M, Padmanabhan V, Shikanov A, Moravek MB. In vitro fertilization outcomes in a mouse model of gender-affirming hormone therapy in transmasculine youth. *F S Sci*. 2023;4(4):302-310.
135. Esparza LA, Terasaka T, Lawson MA, Kauffman AS. Androgen suppresses in vivo and in vitro LH pulse secretion and neural Kiss1 and Tac2 gene expression in female mice. *Endocrinology*. 2020;161(12):bqaa191.
136. Wersinger SR, Haisenleder DJ, Lubahn DB, Rissman EF. Steroid feedback on gonadotropin release and pituitary gonadotropin subunit mRNA in mice lacking a functional estrogen receptor alpha. *Endocrine*. 1999;11(2):137-143.
137. Ghraf R, Schneider K, Kirchhoff J, Hiemke C. The action of 5 alpha-dihydrotestosterone and antiandrogens on the activities of 5 alpha-reductase and 3 alpha-hydroxysteroid dehydrogenase in the pituitary gland of gonadectomized rats. *J Steroid Biochem*. 1982;16(4):545-552.
138. Nolin JM, Campbell GT, Nansel DD, Bogdanove EM. Does androgen influence prolactin secretion? *Endocr Res Commun*. 1977;4(1):61-70.
139. Beyer C, Jaffe RB, Gay VL. Testosterone metabolism in target tissues: effects of testosterone and dihydrotestosterone injection and hypothalamic implantation on serum LH in ovariectomized rats. *Endocrinology*. 1972;91(5):1372-1375.
140. Wierman ME, Gharib SD, Wang C, LaRovere JM, Badger TM, Chin WW. Divergent regulation of gonadotropin subunit mRNA levels by androgens in the female rat. *Biol Reprod*. 1990;43(2):191-195.
141. Iwata K, Kunimura Y, Matsumoto K, Ozawa H. Effect of androgen on Kiss1 expression and luteinizing hormone release in female rats. *J Endocrinol*. 2017;233(3):281-292.
142. Azcoitia I, Mendez P, Garcia-Segura LM. Aromatase in the human brain. *Androg Clin Res Ther*. 2021;2(1):189-202.
143. Alia-Klein N, Preston-Campbell RN, Kim SW, *et al*. Human cognitive ability is modulated by aromatase availability in the brain in a sex-specific manner. *Front Neurosci*. 2020;14:565668.
144. Jonasson M, Nordeman P, Eriksson J, *et al*. Quantification of aromatase binding in the female human brain using [(11) C]cetrozole positron emission tomography. *J Neurosci Res*. 2020;98(11):2208-2218.
145. Stoffel-Wagner B, Watzka M, Schramm J, Bidlingmaier F, Klingmuller D. Expression of CYP19 (aromatase) mRNA in

different areas of the human brain. *J Steroid Biochem Mol Biol*. 1999;70(4-6):237-241.
146. Stanic D, Dubois S, Chua HK, *et al*. Characterization of aromatase expression in the adult male and female mouse brain. I. Coexistence with oestrogen receptors alpha and beta, and androgen receptors. *PLoS One*. 2014;9(3):e90451.
147. Karolczak M, Kuppers E, Beyer C. Developmental expression and regulation of aromatase- and 5alpha-reductase type I mRNA in the male and female mouse hypothalamus. *J Neuroendocrinol*. 1998;10(4):267-274.
148. Beyer C, Green SJ, Barker PJ, Huskisson NS, Hutchison JB. Aromatase-immunoreactivity is localised specifically in neurones in the developing mouse hypothalamus and cortex. *Brain Res*. 1994;638(1-2):203-210.
149. Wartenberg P, Farkas I, Csillag V, Colledge WH, Hrabovszky E, Boehm U. Sexually dimorphic neurosteroid synthesis regulates neuronal activity in the murine brain. *J Neurosci*. 2021;41(44): 9177-9191.
150. Li J, Oberly PJ, Poloyac SM, Gibbs RB. A microsomal based method to detect aromatase activity in different brain regions of the rat using ultra performance liquid chromatography-mass spectrometry. *J Steroid Biochem Mol Biol*. 2016;163:113-120.
151. Roselli CE, Abdelgadir SE, Ronnekleiv OK, Klosterman SA. Anatomic distribution and regulation of aromatase gene expression in the rat brain. *Biol Reprod*. 1998;58(1):79-87.
152. Eddy EM, Washburn TF, Bunch DO, *et al*. Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. *Endocrinology*. 1996;137(11):4796-4805.
153. Naik SI, Young LS, Charlton HM, Clayton RN. Pituitary gonadotropin-releasing hormone receptor regulation in mice. I: males. *Endocrinology*. 1984;115(1):106-113.
154. Coyle CS, Prescott M, Handelsman DJ, Walters KA, Campbell RE. Chronic androgen excess in female mice does not impact luteinizing hormone pulse frequency or putative GABAergic inputs to GnRH neurons. *J Neuroendocrinol*. 2022;34(4):e13110.
155. Wu S, Chen Y, Fajobi T, *et al*. Conditional knockout of the androgen receptor in gonadotropes reveals crucial roles for androgen in gonadotropin synthesis and surge in female mice. *Mol Endocrinol*. 2014;28(10):1670-1681.
156. Ryan GE, Bohaczuk SC, Cassin J, *et al*. Androgen receptor positively regulates gonadotropin-releasing hormone receptor in pituitary gonadotropes. *Mol Cell Endocrinol*. 2021;530:111286.
157. Dart DA, Bevan CL, Uysal-Onganer P, Jiang WG. Analysis of androgen receptor expression and activity in the mouse brain. *Sci Rep*. 2024;14(1):11115.
158. Denney KA, Wu MV, Sun SED, Moon S, Tollkuhn J. Comparative analysis of gonadal hormone receptor expression in the postnatal house mouse, meadow vole, and prairie vole brain. *Horm Behav*. 2024;158:105463.
159. Decourt C, Watanabe Y, Evans MC, *et al*. Deletion of androgen receptors from kisspeptin neurons prevents PCOS features in a letrozole mouse model. *Endocrinology*. 2023;164(6):bqad077.
160. Watanabe Y, Fisher L, Campbell RE, Jasoni CL. Defining potential targets of prenatal androgen excess: expression analysis of androgen receptor on hypothalamic neurons in the fetal female mouse brain. *J Neuroendocrinol*. 2023;35(6):e13302.
161. Jahan MR, Kokubu K, Islam MN, *et al*. Species differences in androgen receptor expression in the medial preoptic and anterior hypothalamic areas of adult male and female rodents. *Neuroscience*. 2015;284:943-961.
162. Brock O, De Mees C, Bakker J. Hypothalamic expression of oestrogen receptor alpha and androgen receptor is sex-, age- and region-dependent in mice. *J Neuroendocrinol*. 2015;27(4): 264-276.
163. Dart DA, Waxman J, Aboagye EO, Bevan CL. Visualising androgen receptor activity in male and female mice. *PLoS One*. 2013;8(8):e71694.
164. Tsai HW, Taniguchi S, Samoza J, Ridder A. Age- and sex-dependent changes in androgen receptor expression in the developing mouse cortex and hippocampus. *Neurosci J*. 2015;2015:525369.
165. Watanabe Y, Prescott M, Campbell RE, Jasoni CL. Prenatal androgenization causes expression changes of progesterone and androgen receptor mRNAs in the arcuate nucleus of female mice across development. *J Neuroendocrinol*. 2021;33(12):e13058.
166. Toranzo D, Dupont E, Simard J, *et al*. Regulation of pro-gonadotropin-releasing hormone gene expression by sex steroids in the brain of male and female rats. *Mol Endocrinol*. 1989;3(11):1748-1756.
167. Selmanoff M, Shu C, Petersen SL, Barraclough CA, Zoeller RT. Single cell levels of hypothalamic messenger ribonucleic acid encoding luteinizing hormone-releasing hormone in intact, castrated, and hyperprolactinemic male rats. *Endocrinology*. 1991;128(1):459-466.
168. Kalra PS, Kalra SP. Modulation of hypothalamic luteinizing hormone-releasing hormone levels by intracranial and subcutaneous implants of gonadal steroids in castrated rats: effects of androgen and estrogen antagonists. *Endocrinology*. 1980;106(1):390-397.
169. Gross DS. Effect of castration and steroid replacement on immunoreactive gonadotropin-releasing hormone in hypothalamus and preoptic area. *Endocrinology*. 1980;106(5):1442-1450.
170. Roselli CE, Kelly MJ, Ronnekleiv OK. Testosterone regulates progonadotropin-releasing hormone levels in the preoptic area and basal hypothalamus of the male rat. *Endocrinology*. 1990; 126(2):1080-1086.
171. Smith ER, Davidson JM. Location of feedback receptors: effects of intracranially implanted steroids on plasma LH and LRF response. *Endocrinology*. 1974;95(6):1566-1573.
172. Plant TM, Dubey AK. Evidence from the rhesus monkey (Macaca mulatta) for the view that negative feedback control of luteinizing hormone secretion by the testis is mediated by a deceleration of hypothalamic gonadotropin-releasing hormone pulse frequency. *Endocrinology*. 1984;115(6):2145-2153.
173. Plant TM, Krey LC, Moossy J, McCormack JT, Hess DL, Knobil E. The arcuate nucleus and the control of gonadotropin and prolactin secretion in the female rhesus monkey (Macaca mulatta). *Endocrinology*. 1978;102(1):52-62.
174. Alcin E, Sahu A, Ramaswamy S, *et al*. Ovarian regulation of kisspeptin neurones in the arcuate nucleus of the rhesus monkey (macaca mulatta). *J Neuroendocrinol*. 2013;25(5):488-496.
175. Scott CJ, Kuehl DE, Ferreira SA, Jackson GL. Hypothalamic sites of action for testosterone, dihydrotestosterone, and estrogen in the regulation of luteinizing hormone secretion in male sheep. *Endocrinology*. 1997;138(9):3686-3694.
176. Ferin M, Carmel PW, Zimmerman EA, Warren M, Perez R, Vande Wiele RL. Location of intrahypothalamic estrogen-responsive sites influencing LH secretion in the female rhesus monkey. *Endocrinology*. 1974;95(4):1059-1068.
177. Messager S, Chatzidaki EE, Ma D, *et al*. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc Natl Acad Sci U S A*. 2005;102(5): 1761-1766.
178. Irwig MS, Fraley GS, Smith JT, *et al*. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. *Neuroendocrinology*. 2004;80(4):264-272.
179. d'Anglemont de Tassigny X, Fagg LA, Carlton MB, Colledge WH. Kisspeptin can stimulate gonadotropin-releasing hormone (GnRH) release by a direct action at GnRH nerve terminals. *Endocrinology*. 2008;149(8):3926-3932.
180. Han SK, Gottsch ML, Lee KJ, *et al*. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci*. 2005;25(49):11349-11356.
181. Navarro VM, Castellano JM, Fernandez-Fernandez R, *et al*. Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology*. 2005;146(1):156-163.
182. Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM. Increased hypothalamic GPR54 signaling: a potential

mechanism for initiation of puberty in primates. *Proc Natl Acad Sci U S A*. 2005;102(6):2129-2134.
183. Gottsch ML, Cunningham MJ, Smith JT, *et al*. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*. 2004;145(9):4073-4077.
184. Hrabovszky E, Takacs S, Rumpler E, Skrapits K. The human hypothalamic kisspeptin system: functional neuroanatomy and clinical perspectives. *Handb Clin Neurol*. 2021;180:275-296.
185. Rumpler E, Skrapits K, Takacs S, *et al*. Characterization of kisspeptin neurons in the human rostral hypothalamus. *Neuroendocrinology*. 2021;111(3):249-262.
186. Lehman MN, Hileman SM, Goodman RL. Neuroanatomy of the kisspeptin signaling system in mammals: comparative and developmental aspects. *Adv Exp Med Biol*. 2013;784:27-62.
187. Herbison AE. Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: the case for the rostral periventricular area of the third ventricle (RP3V). *Brain Res Rev*. 2008;57(2):277-287.
188. Kauffman AS, Gottsch ML, Roa J, *et al*. Sexual differentiation of Kiss1 gene expression in the brain of the rat. *Endocrinology*. 2007;148(4):1774-1783.
189. Clarkson J, de Tassigny XD, Colledge WH, Caraty A, Herbison AE. Distribution of kisspeptin neurons in the adult female mouse brain. *J Neuroendocrinol*. 2009;21(8):673-682.
190. Yip SH, Campos P, Liu X, Porteous R, Herbison AE. Innervation of GnRH neuron distal projections and activation by kisspeptin in a new GnRH-Cre rat model. *Endocrinology*. 2021;162(1):bqaa186.
191. Yip SH, Boehm U, Herbison AE, Campbell RE. Conditional viral tract tracing delineates the projections of the distinct kisspeptin neuron populations to gonadotropin-releasing hormone (GnRH) neurons in the mouse. *Endocrinology*. 2015;156(7):2582-2594.
192. Wakabayashi Y, Nakada T, Murata K, *et al*. Neurokinin 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.
193. Goodman RL, Lehman MN, Smith JT, *et al*. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. *Endocrinology*. 2007;148(12):5752-5760.
194. Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *J Neurosci*. 2009;29(38):11859-11866.
195. Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. *Endocrinology*. 2005;146(9):3686-3692.
196. Smith JT, Dungan HM, Stoll EA, *et al*. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. *Endocrinology*. 2005;146(7):2976-2984.
197. Poling MC, Luo EY, Kauffman AS. Sex differences in steroid receptor coexpression and circadian-timed activation of kisspeptin and RFRP-3 neurons may contribute to the sexually dimorphic basis of the LH surge. *Endocrinology*. 2017;158(10):3565-3578.
198. Clarkson J, Shamas S, Mallinson S, Herbison AE. Gonadal steroid induction of kisspeptin peptide expression in the rostral periventricular area of the third ventricle during postnatal development in the male mouse. *J Neuroendocrinol*. 2012;24(6):907-915.
199. Ho EV, Shi C, Cassin J, *et al*. Reproductive deficits induced by prenatal antimullerian hormone exposure require androgen receptor in kisspeptin cells. *Endocrinology*. 2021;162(12):bqab197.
200. Moore AM, Lohr DB, Coolen LM, Lehman MN. Prenatal androgen exposure alters KNDy neurons and their afferent network in a model of polycystic ovarian syndrome. *Endocrinology*. 2021;162(11):bqab158.
201. Stephens SBZ, Kauffman AS. Estrogen regulation of the molecular phenotype and active translatome of AVPV kisspeptin neurons. *Endocrinology*. 2021;162(9):bqab080.
202. Kim J, Semaan SJ, Clifton DK, Steiner RA, Dhamija S, Kauffman AS. Regulation of Kiss1 expression by sex steroids in the amygdala of the rat and mouse. *Endocrinology*. 2011;152(5):2020-2030.
203. Goodman RL, Herbison AE, Lehman MN, Navarro VM. Neuroendocrine control of gonadotropin-releasing hormone: pulsatile and surge modes of secretion. *J Neuroendocrinol*. 2022;34(5):e13094.
204. Kauffman AS. Neuroendocrine mechanisms underlying estrogen positive feedback and the LH surge. *Front Neurosci*. 2022;16:953252.
205. Herbison AE. The gonadotropin-releasing hormone pulse generator. *Endocrinology*. 2018;159(11):3723-3736.
206. Plant TM. The neurobiological mechanism underlying hypothalamic GnRH pulse generation: the role of kisspeptin neurons in the arcuate nucleus. *F1000Res*. 2019;8:F1000 Faculty Rev-982.
207. Wakabayashi Y, Yamamura T, Sakamoto K, Mori Y, Okamura H. Electrophysiological and morphological evidence for synchronized GnRH pulse generator activity among Kisspeptin/neurokinin B/dynorphin A (KNDy) neurons in goats. *J Reprod Dev*. 2013;59(1):40-48.
208. Clarkson J, Han SY, Piet R, *et al*. Definition of the hypothalamic GnRH pulse generator in mice. *Proc Natl Acad Sci U S A*. 2017;114(47):E10216-E10223.
209. Vorona E, Nieschlag E. Adverse effects of doping with anabolic androgenic steroids in competitive athletics, recreational sports and bodybuilding. *Minerva Endocrinol*. 2018;43(4):476-488.
210. Nieschlag E, Vorona E. Mechanisms IN ENDOCRINOLOGY: medical consequences of doping with anabolic androgenic steroids: effects on reproductive functions. *Eur J Endocrinol*. 2015;173(2):R47-R58.
211. Asseler JD, Del Valle JS, Chuva de Sousa Lopes SM, *et al*. One-third of amenorrheic transmasculine people on testosterone ovulate. *Cell Rep Med*. 2024;5(3):101440.
212. Okada Y, Fujii Y, Moore JP, Jr., Winters SJ. Androgen receptors in gonadotrophs in pituitary cultures from adult male monkeys and rats. *Endocrinology*. 2003;144(1):267-273.
213. Stefaneanu L. Pituitary sex steroid receptors: localization and function. *Endocr Pathol*. 1997;8(2):91-108.
214. Wang Z, Feng M, Awe O, *et al*. Gonadotrope androgen receptor mediates pituitary responsiveness to hormones and androgen-induced subfertility. *JCI Insight*. 2019;5(17):e127817.
215. Robertson JL, Clifton DK, de la Iglesia HO, Steiner RA, Kauffman AS. Circadian regulation of Kiss1 neurons: implications for timing the preovulatory GnRH/LH surge. *Endocrinology*. 2009;150(8):3664-3671.
216. Stephens SB, Tolson KP, Rouse ML, Jr., *et al*. Absent progesterone signaling in kisspeptin neurons disrupts the LH surge and impairs fertility in female mice. *Endocrinology*. 2015;156(9):3091-3097.
217. Wintermantel TM, Campbell RE, Porteous R, *et al*. Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. *Neuron*. 2006;52(2):271-280.
218. Mohr MA, Esparza LA, Steffen P, Micevych PE, Kauffman AS. Progesterone receptors in AVPV kisspeptin neurons are sufficient for positive feedback induction of the LH surge. *Endocrinology*. 2021;162(11):bqab161.
219. Wang L, Vanacker C, Burger LL, *et al*. Genetic dissection of the different roles of hypothalamic kisspeptin neurons in regulating female reproduction. *Elife*. 2019;8:e43999.