A model of ovulatory regulation examining the effects of insulin-mediated testosterone production on ovulatory function

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Abstract

Polycystic ovary syndrome (PCOS), a common cause of infertility in women, is often accompanied by abnormal reproductive and metabolic hormone levels. Specifically, androgens such as testosterone are elevated in many PCOS women, and the syndrome itself is frequently associated with insulin resistance, which leads to hyperinsulinemia, i.e., elevated insulin. Although the precise role of insulin in ovulatory function is unclear, its role in ovulatory dysfunction is often linked to the effects of increased ovarian androgen production. We present a mathematical model of the menstrual cycle that incorporates regulation by the pituitary-ovarian axis and mechanisms of ovarian testosterone production. We determine a physiological role for testosterone in the normal ovulatory cycle and study the role of hyperinsulinemia in pathological regulation of the cycle. Model results indicate increased ovulatory disruption with elevated insulin-mediated testosterone production and suggest that variations in the response of ovarian follicles to essential signals can alter the degree to which hyperinsulinemia disrupts the ovulatory cycle. The model also provides insight into the various PCOS phenotypes and the severity of ovulatory dysfunction.

Keywords: Polycystic ovary syndrome, steroidogenesis

1. Introduction

Polycystic ovary syndrome (PCOS) is a common cause of infertility in women. There are three factors that can lead to PCOS: elevated androgen levels, ovulatory dysfunction, and a polycystic ovary morphology. Of these factors, more than half of women with PCOS exhibit signs of hyperandrogenism [1], which may lead to ovulatory dysfunction [2]. The pathogenesis of PCOS is currently unknown, and because so many women of child-bearing age are affected, it is important to find the most effective treatments for the syndrome to restore normal ovulatory function.

Clinical observations of women with PCOS have shown a frequent association with insulin resistance, i.e. reduced cellular responses to insulin signaling. Insulin resistance is most commonly associated with type 2 diabetes, and its association with PCOS is peculiar. Notably, most women with PCOS and hyperandrogenism are also hyperinsulinemic [1, 3, 4], the latter being a telltale sign of insulin resistance. Although the precise role of insulin in ovulatory function is unclear, its role in ovulatory dysfunction is often linked to the effects of increased ovarian androgen production [1]. In addition, treatments of PCOS symptoms include the use of insulin sensitizing drugs such as

metformin and thiazolidinediones [5, 6]. When successful, these drugs appear to reduce circulating testosterone (T), a common androgen, via a reduction in plasma insulin levels.

The importance of insulin and T in many PCOS cases is evident. In order to identify their role in ovulatory dysfunction more completely, we look to the dynamics underlying normal ovulation. The menstrual cycle itself is regulated by orchestrated feedback in the hypothalamic-pituitary-ovarian axis. Briefly, GnRH neurons in the hypothalamus of the brain are activated to stimulate the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from gonadotropes in the anterior pituitary [7]. Circulating FSH and LH then bind to receptors on ovarian cells to regulate follicle development and production of steroid hormones, namely estradiol (E_2), progesterone (P_4), and T [8, 9].

T concentrations in women are significantly lower than those found in men, and accurate measurements are sparse due to a lack of suitably sensitive assays [10, 11]. This reduces our ability to characterize T dynamics fully during the ovulatory cycle. Although E_2 and P_4 are responsible for much of the feedback to the brain, T is still thought to play an important role in both follicular development and in tonic (basal) levels of luteinizing hormone (LH). Furthermore, even if T does not play a significant role in establishing the physiological ovulatory cycle, it is clear that elevated T can result in dysfunction and infertility.

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It is therefore important, even with limited data, to understand the mechanisms contributing to the behavior of T throughout the ovulatory cycle.

In this work, we model the menstrual cycle, with special consideration of mechanisms of ovarian steroid production. In Sections 2 and 3, we develop the mathematical model. In Section 4, we fit the model to clinical data in the literature. In Section 5, we present simulation results under normal and pathological circumstances. Finally, in Section 6, we discuss model implications on mechanisms and the severity of ovulatory dysregulation.

2. Adaptation of Existing Frameworks: Pituitary Hormone and Follicle Dynamics

Here we develop models of pituitary regulation, follicle maturation, and ovarian steroid production. In particular, we modify and extend existing models of the production and release of pituitary hormones [12–14] and develop a new model of stage-dependent follicle maturation based on [12–15]. We introduce a semi-mechanistic model of ovarian production of T, E₂, and P₄. Because our methodology incorporates cellular mechanisms of production and our primary focus is the ovaries, we assume implicit feedback effects on and from GnRH neurons. The function of these neurons in response to circulating and pituitary hormones is therefore not modeled within this work.

2.1. Pituitary regulation

The dynamics of the pituitary hormones, LH and FSH, are illustrated in Figure 1, following the model of [12]. The potential impact of insulin (I) is also depicted, via its effect on T. The state variables in this subsystem include releasable FSH (FSH_ρ), serum FSH (FSH), releasable LH (LH_ρ), and serum LH (LH). We distinguish between releasable and serum quantities of LH and FSH to reflect the differential effects of ovarian stimulation and inhibition. For further discussion on this framework, see [12, 14].

Inhibins are another class of hormone produced by the ovaries; they primarily suppress FSH synthesis [7]. There are two major types of inhibin. Inhibin A is the dominant inhibin during the luteal stage, whereas Inhibin B

peaks during both the follicular and luteal stages [16]. Although both inhibins reduce FSH production, we focus on the effects sustained during the luteal phase, where FSH inhibition is strongest. We do not model inhibin dynamics explicitly; instead, we incorporate suppression of FSH synthesis through luteal stage (Λ) dynamics (see Equation 1a and Section 2.2, Equation 2c, and Equation 2d). FSH release into the serum is subsequently promoted by P₄ and inhibited by E₂. Once in circulation, FSH decays linearly (see Equation 1b).

Testosterone is an important regulator of LH synthesis by gonadotropes in the pituitary gland. Specifically, T can contribute to a GnRH-mediated increase in LH in both adult female rats [17] and adolescent girls [18]. However, this effect may be reversed with high T levels in normal, but not PCOS, ovaries [18]. The nature of these differential effects is unknown. Therefore, we assume testosterone-dependent LH synthesis simply saturates with maximal rate v_{0L} . It is also hypothesized that hyperandrogenism reduces the ability of P₄ to suppress E₂-stimulated LH synthesis by modulating the sensitivity of the hypothalamus to E₂ and P₄ through an unknown mechanism [18, 19]. Fur-

Figure 1. Schematic of pituitary hormone dynamics, as regulated by ovarian steroids T, P₄, and E₂. A two-compartment model describes the dynamics of releasable (pituitary) and serum hormones. T serves two functions in the pituitary: It allows for basal LH production, and it prevents inhibition of LH synthesis by P₄. P₄ is also important for the release of both LH and FSH. E₂, on the other hand, inhibits this release into the serum. From the serum, both pituitary hormones experience first-order decay. Insulin (I) may affect these processes via its effect on T production. $\rightarrow$ denotes stimulation; $-$ denotes inhibition.

$$\text{Releasable FSH: } \frac{dFSH_\rho}{dt} = \frac{v_F}{1 + c_{F,I} \frac{S\Lambda}{K_{F,I} + S\Lambda}} - k_F \frac{1 + c_{F,P}P_4}{1 + c_{F,E}E_2^2} FSH_\rho \quad (1a)$$

$$\text{Serum FSH: } \frac{dFSH}{dt} = \frac{1}{V} \cdot k_F \frac{1 + c_{F,P}P_4}{1 + c_{F,E}E_2^2} FSH_\rho - \delta_F FSH \quad (1b)$$

$$\text{Releasable LH: } \frac{dLH_\rho}{dt} = \left[v_{0L} \cdot \frac{T}{K_{L,T} + T} + v_{1L} \cdot \frac{E_2^n}{K_{mL}^n + E_2^n} \right] \cdot \frac{1}{1 + P_4/[K_{iL,P}(1 + c_{L,T}T)]} - k_L \frac{1 + c_{L,P}P_4}{1 + c_{L,E}E_2} LH_\rho \quad (1c)$$

$$\text{Serum LH: } \frac{dLH}{dt} = \frac{1}{V} \cdot k_L \frac{1 + c_{L,P}P_4}{1 + c_{L,E}E_2} LH_\rho - \delta_L LH \quad (1d)$$

ther, this effect appears to be reversed, with normal hypothalamic GnRH pulse generator sensitivity, upon treatment with an antiandrogen [20]. We assume this inhibition also exists under normal circumstances and hence use the expression $1 + c_{L,T}T$ to model this effect. P_4 and E_2 have similarly opposing effects in LH release as in FSH release (see Equation 1c).

2.2. Follicle dynamics

The ovulatory cycle can be broadly categorized into three major phases. The follicular phase is a time of growth and development, characterized by selection of a dominant follicle for ovulation [21, 22]. The ovulatory phase is precipitated by a surge in serum LH, which stimulates the rupture of the dominant follicle and ovum release. The luteal phase is characterized by the development and subsequent regression of the corpus luteum, wherein the ruptured follicle undergoes extensive remodeling, becomes highly vascularized, and produces large amounts of P_4 [23].

Previous models have accounted for as many as 12 stages of follicle growth and maturation [12–15]. To reduce the complexity of the present model yet capture essential dynamics, we consider only the three major stages of ovarian development: follicular, ovulatory, and luteal. These theoretical stages reflect a contribution to the ovarian mass (in micrograms) at any given time. We remark that Chen and Ward [15] also consider a three-stage reduction when modeling the menstrual cycle.

Figure 2 illustrates the regulation of follicle development by FSH and LH. Androgens stimulate the growth of small follicles, during the early stages of development [24, 25]. It has been shown that androgens, in particular, increase FSH receptor expression in follicular cells [26, 27]. We therefore assume that T is the androgen responsible for increasing baseline sensitivity to FSH. Once mature enough, follicles increase their mass while in follicular phase Φ in response to growth signals from FSH (see Equation 2a).

LH is the primary ovulation signal, responsible for the transition from stage Φ to ovulatory stage Ω , which may be affected by insulin (discussed in Section 5.3). FSH increases the number of LH receptors during the mid-to-late follicular stage, to prepare follicles for luteinization

[28, 29]. Luteinization is the process through which cells in the pre-ovulatory follicle terminally differentiate into luteal cells, wherein they undergo hypertrophy and accumulate lipids [30]. Follicle sensitivity to LH is therefore mediated by the presence of FSH, resulting in an FSH-induced leftward shift of the dose-response curve of LH-dependent follicular differentiation (see Equation 2a).

Once in the ovulatory phase, Ω , follicle rupture leads to the formation of the corpus luteum and a transition to the luteal stage, Λ (Equation 2b). This is an LH-dependent process, which allows the corpus luteum to both develop and mature [31]. During this stage, LH levels are much lower than surge levels. Still, it is evident LH is required

Figure 2. Schematic of follicular dynamics subsystem. T is responsible for early growth/maturation of immature follicles. It also increases the sensitivity of these follicles to FSH. Later, these follicles respond to LH, progressing from follicular stage Φ to ovulatory stage Ω . LH is further required, in a supporting role, for the transition to the luteal stage Λ and subsequent maintenance of the corpus luteum. Insulin (I) may also play a role in the Φ -to- Ω transition.

$$\text{Follicular phase: } \frac{d\Phi}{dt} = f_0 \cdot \frac{T}{T_0} + \left[\frac{f_1 FSH^2}{\left(\frac{h_1}{1+T/T_0} \right)^2 + FSH^2} - \frac{f_2 LH^2}{\left(\frac{h_2}{1+c_{\Phi,F} FSH} \right)^2 + LH^2} \right] \cdot \Phi \quad (2a)$$

$$\text{Ovulatory phase: } \frac{d\Omega}{dt} = \frac{f_2 LH^2}{\left(\frac{h_2}{1+c_{\Phi,F} FSH} \right)^2 + LH^2} \cdot \Phi - wS\Omega \quad (2b)$$

$$\text{Luteal phase: } \frac{d\Lambda}{dt} = wS\Omega - l(1-S)\Lambda \quad (2c)$$

$$\text{LH Support: } \frac{dS}{dt} = \frac{\hat{s}LH^m}{h_s^m + LH^m} \cdot (1-S) - \delta_s S \quad (2d)$$

to sustain the corpus luteum, as experimental depletion of post-ovulatory LH results in insufficient luteal growth and maturation [31, 32].

We introduce the state variable S to represent LH support. During the luteal stage, support is extremely sensitive to LH, even at very low levels. Under these circumstances, S may approach a maximal value of 1. In the absence of LH, support decays, and S may tend toward 0 (see Equation 2d). Therefore, $0 \leq S(t) \leq 1$ for all t . We regard LH support as a permissive state during which normal luteal activity, specifically luteal growth and hormone production, can occur. Terms involving the luteal stage are multiplied by S to indicate a lack of activity in the absence of sufficient functional support by LH.

The signals that promote luteal regression, or luteolysis, in humans are largely unknown and widely hypothesized [30, 32]. However, because LH is important for the development and maintenance of the corpus luteum, we assume that LH support also prevents premature luteolysis. Thus, the rate of luteal regression is taken to be proportional to $(1 - S)\Lambda$ (see Equation 2c).

3. Model Development: Ovarian Steroidogenesis

Here we introduce a semimechanistic model of ovarian steroidogenesis that incorporates the influence of hyperinsulinemia. We develop the model in three parts; the first presents a preliminary model of intracellular androgen production, the second is a model reduction to testosterone dynamics, and the third extends the reduced model to incorporate follicle dynamics.

Production of any of the ovarian steroids, such as T , E_2 , and P_4 , results from a series of enzymatic conversions of cholesterol and its derivatives. A two-cell hypothesis provides the prevailing explanation of the origins of the major ovarian steroids. The two cell types within follicles

are theca cells and granulosa cells (see Figure 3). In the hypothesis, theca cells are responsible for the production of androgens, especially androstenedione (A), dihydrotestosterone, and T. Granulosa cells, on the other hand, are responsible for generating E_2 and most luteal stage P_4 [32].

Careful consideration of intracellular mechanisms of androgen production in follicles supports a mechanistic role for insulin. Insulin resistance, or impaired insulin signaling, sets the stage for many insulin-mediated defects in the ovulatory cycle. Unlike most tissues in the body, ovarian insulin receptors are predominately regulated by steroids and gonadotropins. Therefore, the ovaries tend to remain sensitive to insulin at the receptor level, even in the midst of systemic insulin resistance [33, 34]. In PCOS, it is unclear what post-receptor defects occur in ovarian insulin signaling. However, it has been proposed that theca cells are actually hypersensitive to insulin signals, resulting in exaggerated androgen synthesis and secretion relative to E_2 and P_4 [35, 36]. Further, through its receptor on ovarian granulosa and theca cells, insulin can act as a co-gonadotropin with LH to increase the expression of steroidogenic enzymes, including androgens [33, 37].

3.1. Preliminary model of ovarian androgen production

We develop the steroidogenesis model under several major assumptions. First, cholesterol, as the primary precursor to all ovarian steroids, is relatively abundant in theca and granulosa cells. Second, the layers of theca cells in a given follicle function uniformly to respond to LH and to synthesize androgens. Finally, the primary effect of elevated insulin is augmented LH-mediated steroidogenic enzyme production within theca cells.

For simplicity, we also begin the steroidogenic cascade with A production. This allows us to focus on the effects of insulin and LH at the level of CYP17 production, which permits the eventual conversion of pregnenolone into androgens [38]. Although several androgens are produced by

Figure 3. Ovarian steroidogenesis model schematic. The two-cell theory of steroid production is taken into account through quantities residing in both theca and granulosa cells. I, LH, and FSH denote processes that are dependent on insulin, LH, and FSH, respectively. The preliminary model takes into account synthesis and secretion of androgens (androstenedione and testosterone) and estradiol. Gray text denotes quantities not explicitly modeled in this subsystem, i.e. intracellular E_2 ($E_{2\gamma}$) and P_4 ($P_{4\gamma}$).

$$\begin{aligned}
\text{Theca cell A:} \quad \frac{dA_\theta}{dt} &= k_0 \mathcal{G}_1(I) \cdot H(LH, \hat{h}_1, 1) - k_1 \mathcal{G}_2(I) \cdot H(LH, \hat{h}_2, 1) \cdot A_\theta - k'_1 A_\theta & (3a) \\
\text{Theca cell T:} \quad \frac{dT_\theta}{dt} &= k_1 \mathcal{G}_2(I) \cdot H(LH, \hat{h}_2, 1) \cdot A_\theta - k'_2 T_\theta & (3b) \\
\text{Granulosa cell T:} \quad \frac{dT_\gamma}{dt} &= q k'_2 T_\theta - k_2 H(FSH, h_3, 1) T_\gamma & (3c) \\
\text{Circulating A:} \quad \frac{dA_\beta}{dt} &= k'_1 A_\theta + k_4 - \delta_A A_\beta - k_3 A_\beta & (3d) \\
\text{Circulating T:} \quad \frac{dT_\beta}{dt} &= k_3 A_\beta + k_5 - \delta_T T_\beta + (1 - q) k'_2 T_\theta & (3e)
\end{aligned}$$

where $H(x, h, n) = \frac{x^n}{x^n + h^n}$ is a Hill function with Hill coefficient n .

theca cells, we take A to be the major precursor and T the major product.

The state variables representing androgens in the intracellular system are theca cell A (A_θ), circulating A (A_β), theca cell T (T_θ), granulosa cell T (T_γ), and circulating T (T_β). The dynamics considered in this subsystem, along with their dependence on insulin, FSH, and LH, are illustrated in Figure 3. To focus on the effects of insulin-mediated ovarian testosterone production in particular, we derive a model only for androgen production. Dynamics for E_2 and P_4 will be considered alongside follicle dynamics in Section 3.3.

In the theca cell model, A is created downstream of intracellular cholesterol through enzyme activity promoted by LH and enhanced by elevated insulin (see Equation 3a). The concentration of A decreases either upon release into circulation or intracellular conversion into T. For simplicity, we assume that T is the only androgen to be aromatized, i.e. converted into E_2 , within granulosa cells.

We define arbitrary functions $\mathcal{G}_1 = \mathcal{G}_1(I)$ and $\mathcal{G}_2 = \mathcal{G}_2(I)$ to represent the influence of insulin, I , in enzyme production for conversion from precursor steroids to A and from A into T, respectively. We assume insulin can augment cellular responses to LH in this capacity, so $\mathcal{G}_1$ and $\mathcal{G}_2$ are increasing functions of I . At basal insulin levels, we take $\mathcal{G}_1 = \mathcal{G}_2 = 1$.

We model theca cell T to be created from its precursor A and subsequently secreted, with a fraction q entering neighboring granulosa cells (see Equation 3b) and the remainder released directly into the circulation. Proximity between theca and granulosa cells is assumed sufficiently small to accommodate rapid transfer between the two cell types. Granulosa cell T transferred from the theca cell is then aromatized via FSH signaling (see Equation 3c).

In Equation 3d, we assume circulating A (A_β) comes directly from theca cells or is produced by the adrenal gland at a constant rate. Once in the bloodstream, A may be converted peripherally into T. This conversion contributes to the circulating T concentration, along with direct theca cell secretion and constant basal production (see Equation 3e).

3.2. Reduction of preliminary model

As defined, Equations (3a)–(3e) represent the dynamics of intracellular androgen concentrations from individual cellular masses. Here we reduce the model to essential dynamics captured by variables T_γ and T_β , under the assumptions that theca cell steroid production is balanced with release and that A, in its various forms, also experiences zero net change. To construct the simplified model for testosterone dynamics, we use these assumptions to solve for A_θ , T_θ , and A_β . Then the reduced model can be written as

$$\begin{aligned}
\frac{dT_\gamma}{dt} &= q k_0 Q_1 - k_2 H(FSH, h_3, 1) T_\gamma, \\
\frac{dT_\beta}{dt} &= k_5 - \delta_T T_\beta + \frac{k_3 k_4}{k_3 + \delta_A} + \frac{k'_1 k_0}{k_1 (k_3 + \delta_A)} \cdot Q_2 \\
&\quad + (1 - q) k_0 Q_1,
\end{aligned}$$

where $Q_1 = \mathcal{G}_1(I) \mathcal{G}_2(I) \cdot LH^2 / \mathcal{D}$, $Q_2 = [\mathcal{G}_1(I) LH^2 + \mathcal{G}_1(I) LH \cdot \hat{h}_2] / \mathcal{D}$,

$$\begin{aligned}
\mathcal{D} &= LH^2 \left[\mathcal{G}_2(I) + \frac{k'_1}{k_1} \right] + \\
&\quad + LH \left[\mathcal{G}_2(I) \hat{h}_1 + \frac{k'_1}{k_1} \cdot (\hat{h}_1 + \hat{h}_2) \right] + \frac{k'_1}{k_1} \cdot \hat{h}_1 \hat{h}_2 \\
&= LH^2 [\mathcal{G}_2(I) + A] + LH [\mathcal{G}_2(I) B + A \cdot (B + C)] + \\
&\quad + A \cdot B \cdot C,
\end{aligned}$$

and where the new parameters are defined by $A = k'_1 / k_1$, $B = \hat{h}_1$, and $C = \hat{h}_2$.

We further define

$$\begin{aligned}
F_1 &= F_1(LH, I) = LH^2 / \mathcal{D} \text{ and} \\
F_2 &= F_2(LH, I) = LH / \mathcal{D},
\end{aligned}$$

so that

$$\begin{aligned}
Q_1 &= \mathcal{G}_1(I) \cdot \mathcal{G}_2(I) \cdot F_1 \quad \text{and} \\
Q_2 &= \mathcal{G}_1(I) [F_1 + \hat{h}_2 F_2].
\end{aligned}$$

The functions F_1 and F_2 reflect the contributions of theca cells and the periphery toward overall concentrations of

Figure 4. Functions used to determine ovarian and peripheral testosterone production. (a) Functions F_1 and F_2 . (b) Peripheral and ovarian production rates.

T. F_1 is a saturating dose-response curve of T production via LH. F_2 is an inverted U-shaped dose-response curve, wherein both low and high levels of LH reduce T production, and maximal stimulation occurs at an intermediate level of LH (see Figure 4(a)). Using F_1 and F_2 , we classify two forms for generalized T production:

1. ovarian T production rate proportional to F_1
2. peripheral T production rate proportional to $F_1 + \hat{h}_2 F_2$.

Under basal conditions, about 60% of circulating T comes from peripheral conversion. In contrast, the ovaries compose the major source of T when LH is elevated [1, 29, 39]. Figure 4(b) illustrates this general notion given F_1 and F_2 .

To complete the reduced model of intracellular testosterone production, we make the following substitutions: $t_{g1} = qk_0$, $t_{g2} = k_2$, $t_0 = k_5 + \frac{k_3 k_4}{k_3 + \delta_A}$, $t_1 = \frac{k'_1 k_0}{k_1 (k_3 + \delta_A)}$, $t_2 = (1 - q)k_0$, and $c_{T,F_2} = \hat{h}_2$. This gives

$$\frac{dT_\gamma}{dt} = t_{g1} \mathcal{G}_1 \cdot \mathcal{G}_2 \cdot F_1 - t_{g2} H(FSH, h_3, 1) T_\gamma, \quad (4a)$$

$$\begin{aligned} \frac{dT_\beta}{dt} = & t_0 - \delta_T T_\beta + t_1 \mathcal{G}_1 [F_1 + c_{T,F_2} F_2] + \\ & + t_2 \mathcal{G}_1 \cdot \mathcal{G}_2 \cdot F_1. \end{aligned} \quad (4b)$$

At baseline, the model only implicitly depends on the basal insulin level, so after setting $\mathcal{G}_1 = \mathcal{G}_2 = 1$, we can

redefine $\mathcal{D} = \kappa_1 LH^2 + \kappa_2 LH + \kappa_3$, with $\kappa_1 = 1 + A$, $\kappa_2 = B + A(B + C)$, and $\kappa_3 = ABC$. Note that $C = c_{T,F_2}$. After estimating κ_1 and κ_2 , the parameters A , B , C , and κ_3 can be obtained by solving a nonlinear system of equations and imposing a uniqueness restriction on the solution. By deriving these four parameters, we reduce the number of unknown parameters in the expression $\mathcal{D}$ from three to two.

Finally, to incorporate insulin-mediated effects beyond baseline, we assume that each insulin-dependent term results in an augmented response modeled by a percentage increase in the LH-dependent effect. To that end, we take $\mathcal{G}_1 = 1 + \alpha$, where α reflects the degree of insulin influence and $\alpha = 0$ at baseline. For simplicity, we set $\mathcal{G}_2 = 1$, noting that this simplification shifts our focus to the upstream effects of insulin, at the level of androgen-producing enzyme CYP17 (17,20) lyase production. With this modification, F_1 and F_2 are now insulin-independent functions. Nevertheless, we do not completely remove $\mathcal{G}_2$ from the model to allow for the possibility of downstream insulin-induced effects.

3.3. Complete model of ovarian steroidogenesis subject to follicular dynamics

With Subsystem 4, we incorporate the effects of elevated insulin on testosterone production in a mechanistic way. We complete the model of ovarian steroid production by subjecting intracellular steroid dynamics to the behavior of the ovarian mass. The state variables in the final steroidogenesis subsystem are serum T (T), intermediate T (T_γ), serum E_2 (E_2) and serum P_4 (P_4).

Follicles that have exited the growth arrest stage are equipped with machinery to produce T; we account for this with the product of intracellular production and the ovarian stages (see Equation 5a). In addition, less mature follicles than those in stage Φ are able to produce testosterone [38]. Therefore, we also assume there is a contribution from the overall ovarian mass not captured within $\Phi + \Omega + \Lambda$.

Unlike the other steroid state variables, T_γ reflects a concentration per unit ovarian mass (see Equation 5b). Intermediate T, whose production depends on a stimulatory response to LH via F_1 , enters the granulosa cell specifically for FSH-dependent conversion into E_2 . We assume E_2 , upon production within granulosa cells, is immediately released into circulation and that this production results only from follicles in the follicular and luteal phases. Any extra-ovarian E_2 is produced peripherally by the adrenal gland at a constant rate. We assume E_2 experiences first-order clearance from serum (see Equation 5c).

The equation for P_4 dynamics (Equation 5d) follows a similar pattern to that of E_2 . Basal production of P_4 outside of the ovaries is negligible [40]; we therefore omit this from the model. In addition, although P_4 is produced upstream of T, we assume theca cells contribute a negligible amount to the serum P_4 level, since most P_4 in theca cells is actually converted into A [41]. We further assume

$$\text{Serum T: } \frac{dT}{dt} = t_0 - \delta_T T + [t_1 \mathcal{G}_1 (F_1 + c_{T,F_2} F_2) + t_2 \mathcal{G}_1 \mathcal{G}_2 F_1] \cdot \left[\Phi + \tau_1 \Omega + \tau_2 S \Lambda + \tau_3 \left(1 - \frac{\Phi + \Omega + \Lambda}{\Psi} \right) \right] \quad (5a)$$

$$\text{Intermediate T: } \frac{dT_\gamma}{dt} = t_{g1} \mathcal{G}_1 \mathcal{G}_2 F_1 - \frac{t_{g2} F S H}{h_3 + F S H} T_\gamma \quad (5b)$$

$$\text{Serum E}_2: \frac{dE_2}{dt} = e_0 - \delta_E E_2 + \frac{t_{g2} F S H}{h_3 + F S H} T_\gamma \cdot [\Phi + \eta \Lambda S] \quad (5c)$$

$$\text{Serum P}_4: \frac{dP_4}{dt} = -\delta_P P_4 + \frac{p L H}{L H + h_p} \cdot \Lambda S \quad (5d)$$

that relevant quantities of P_4 are only produced by the luteal phase in an LH-dependent process. As with other steroids, we assume first-order clearance. Finally, P_4 is assumed to be released into circulation immediately upon production within granulosa cells.

4. Methods

In order to verify the physiological behavior of the current model, we make use of data in the literature and parameter estimation tools. Here we discuss the methodology used to generate model results.

4.1. Comparative data

Currently, there are no studies with published data of women with daily measurements over an entire ovulatory cycle for each of the five major hormones (LH, FSH, E_2 , P_4 , and T) considered in the current model. We therefore combine two sets of published data to verify qualitative dynamics. We use FSH, LH, E_2 , and P_4 data from [42] and T data from [10] for comparison.

Reported average values for each state variable were approximated digitally using the `digitize` package written for MATLAB [43]. The T data used reflects a smoothed average reported by Keefe and colleagues [10]. All data are converted to SI units where applicable.

Data reported in [42] reflect a presumed 31-day cycle, whereas a 28-day cycle is reflected by the T data in [10]. To overcome this discrepancy, we first extrapolate the data to a 31-day cycle, assuming the peak of the data responds to the same day of the preovulatory LH surge reported in [42]. In all results reported here, day 15 responds to the mid-cycle LH surge in a normal ovulatory cycle.

4.2. Parameter estimation

The final model consists of the 12 ordinary differential equations listed in systems (1), (2), and (5) with 40 unknown parameters. In order to achieve good qualitative agreement between model and data, we optimize the parameters of the model using the Nelder-Mead simplex method in MATLAB with weighted least squares. Data for a given state variable were given a baseline weight of $w_d = 1/\text{Var}(d)$, where $\text{Var}(d)$ is the variance of the data

over two cycles, i.e., a 62-day period. Secondary weights were used to ensure that essential events within the cycle, such as the preovulatory LH surge and corpus luteum formation, are captured by the model. The complete list of estimated and fixed parameters, along with initial conditions for state variables, used in the model appear in Table A.1 in Appendix A.

5. Model Behavior and Investigation of Insulin-Mediated Effects

We present numerical simulation results of the model, under both normal ($\alpha = 0$) and pathological ($\alpha > 0$) conditions.

5.1. Dynamics during a normal ovulatory cycle

Figure 5 shows the results of parameter estimation and the model fit to the data, along with the behavior of the three ovarian stages and the LH support variable, S , over time. Although we limit the representative behavior to two cycles, we note that these dynamics reflect stable periodic oscillations with a cycle length of 30.9 days. There is good qualitative agreement between the model and the data. The most significant discrepancy occurs with the variable T, which we attribute to the use of two different data sets. Other substantial differences from the data we attribute to the simplifying assumptions made in the follicular growth subsystem, where we dramatically reduce the number of follicular growth stages. In addition, we have omitted delayed feedback mechanisms, which can provide better agreement with the data [13, 14, 44, 45].

As a novel entity in modeling the ovulatory cycle, S dynamics indicate the support given to luteal growth and function. The support level plateaus at a maximal value once LH concentrations exceed a certain threshold. Support declines with reductions in LH. A loss of S initiates a more rapid decline in Λ .

5.2. Simulated hyperinsulinemia

We can simulate elevations in insulin by varying the parameter α introduced in Section 3.2. Figure 6 shows the model results for selected values of $0 \leq \alpha \leq 5$, demonstrating the changes in dynamics for varying levels of feedback. Results are presented with the first LH surge in

Figure 5. Numerical optimization and simulation results. Comparison to data in [42] (FSH, LH, E₂, P₄) and [10] (T). Lower right panel: simulated ovarian and LH support trajectories for best fit parameters. Results show a 30.9-day cycle, which is repeated for 2 cycles.

each case placed at day 15 for direct comparison. Over all T levels rise in a fairly linear fashion with increased α . LH levels are elevated, particularly at the pre-ovulatory surge and the luteal stage. Peak LH levels increase for small α and less so with larger α . This is due to saturation of basal LH synthesis in the presence of high T. On the other hand, Peak FSH concentrations decline, whereas luteal stage FSH levels are either increased or the same as the baseline case. This suggests an increased LH:FSH ratio with increasing testosterone levels. There is also a slight reduction in cycle length as α increases. This is likely due to feedback from elevated LH, as follicles in stage Φ are prompted to progress to stage Ω more strongly.

E₂ is generally increased with α except at its peak, which occurs just prior to the pre-ovulatory surge. E₂ dynamics are determined by increased T_γ and reduced follicle growth (results not shown). The E₂ peak corresponds to the dip in FSH prior to its pre-ovulatory spike, whereas increased E₂ at ovulation corresponds to reduced peak FSH levels.

Notably, higher α corresponds to a reduced P₄ amplitude. This is the result of reduced follicle growth and excess LH. Further reductions in P₄ (peak less than 3 ng/mL) would likely indicate anovulation, as described in [46]. Collectively, the model produces elevated LH and P₄ deficiency in the presence of insulin-mediated hyperandrogenism.

5.3. Hyperinsulinemia with premature luteinization

A classic characteristic of PCOS is the arrested growth of follicles [47], which prevents the preovulatory LH surge. In the previous section, model exploration with simulated hyperinsulinemia yields a periodic LH surge with possibly normal ovulatory cycles for lower α values. With the

exception of a possible deficiency in P₄, no other obvious dysfunction occurs.

We therefore explore an additional mechanism of ovulatory disruption hypothesized to result from hyperinsulinemia. Premature luteinization (PL) is the process through which follicles become responsive to LH earlier in their maturation. Given the nature of the ovulatory cycle, PL occurs in small follicles, and follicles from individuals with PCOS may be subject to premature luteinization caused by hyperinsulinemia [2, 20, 48].

Mathematically, we simulate PL with an insulin-dependent increase in sensitivity to LH signals during the follicular-to-ovulatory transition. For simplicity, we take α to denote the overall degree of insulin influence in the ovaries. The affected model parameter is h_2 , where increased sensitivity to LH is modeled as $h_2/(1+\alpha)$. Therefore, an increased insulin-mediated effect ($\alpha > 0$) results in a leftward shift of the LH dose-response curve in Equations 2a and 2b.

Figure 7 shows the time courses of selected state variables for $\alpha = 0, 1, 2, 3, 4$, and 5. In the presence of PL, increases in T result in consistent reductions in the pre-ovulatory LH surge until it is completely absent. Because the LH surge is highly variable under PL, the peak of stage Ω —pronounced for every α —is used to synchronize initial cycles. Where applicable, the first LH surge is shown at day 15. For most cases in which the LH surge is absent, e.g. $\alpha = 4$, the LH achieves a maximum value during the height of the luteal stage, which also corresponds to the rise in P₄. As ovulation becomes more dysfunctional, the range of FSH decreases. The system approaches a steady state when $\alpha = 5$. These results indicate that PL exacerbates ovulatory dysfunction and, with elevated T, can eventually eliminate ovulation altogether.

Figure 6. Simulated hyperinsulinemia under normal luteinization. Selected trajectories from numerical simulations shown with $\alpha = 0, 1, 2, 3, 4$, and 5.

Figure 7. Simulated hyperinsulinemia under premature luteinization. Selected trajectories from numerical simulations shown with $\alpha = 0, 1, 2, 3, 4$, and 5.

Figure 8. Comparison between normal (NL) and premature (PL) luteinization. Cycle lengths for both models. Two symbols for a single value of α indicate lengths for two-cycles. For low α , good agreement between NL and PL. Model differences increase with α . NL cycle lengths do not vary significantly, whereas PL lengths increase substantially with intermediate values of α . A steady state is reached for $\alpha = 5$, indicated by a missing point.

5.4. Comparison of hyperinsulinemia models

Here we compare properties of the model under conditions of normal luteinization (NL) and varying levels of PL due to elevated insulin.

Figure 8 compares the effects of PL on cycle length. With increasing α and NL, the cycle lengths do not vary substantially, though there is a tendency toward faster cycles. In contrast, the systemic response to increased insulin under PL is, for the most part, an elongation of the cycle. Period-doubling bifurcations have been observed for related menstrual cycle models [14, 45], and this bifurcation occurs for our model as α changes. We observe cycles alternating between two cycle lengths for $1 \leq \alpha \leq 1.5$ with NL and for $0.75 \leq \alpha \leq 1.5$ with PL, and these cycle pairs (two-cycles) correspond to one periodic solution of period approximately 60 days. Oscillations are stable for all experimental values of α when luteinization is normal. However, the system approaches a non-oscillatory steady state for $\alpha = 5$ under PL. Further, whereas the LH:FSH ratio steadily increases with α during NL, the response of the ratio under PL is non-monotonic, with increases for $2 \leq \alpha \leq 3.5$ and decreases for $\alpha \geq 3.75$ (Figure 8 (??)).

Figure 9(a) illustrates the values of FSH, LH, E_2 , and P_4 at three isolated points during both NL and PL cycles, for various α . We choose the mid-follicular, mid-cycle, and mid-luteal stages because these are important markers throughout a normal ovulatory cycle. To obtain these quantities, model output is normalized to the fraction of an average cycle, with 0 being the start of the cycle and 1 being the end of a cycle, where applicable. The mid-follicular point is assumed to occur at 0.25, the mid-cycle point at 0.5, and the mid-luteal point at 0.75. We are careful to note that the mid-cycle point does not correspond to the preovulatory LH surge. We then determine the value of the state variables at these points for a given α . Since

average cycle lengths are used, we may disregard results from cases in which two-cycles occur (shaded regions in Figure 9a).

During NL, increased α has the largest effect on mid-cycle levels of FSH and mid-luteal levels of LH. The same is true during PL, though there is a more significant decline in mid-cycle FSH. Interestingly, in both cases mid-luteal FSH increases with α .

Higher α results in slightly increased mid-cycle LH under NL, but substantially increased mid-luteal LH. This is in stark contrast to the behavior under PL conditions, in which mid-cycle LH monotonically decreases with α , and mid-luteal LH increases for $\alpha \leq 3.5$ and decreases sharply thereafter.

α also impacts E_2 in NL, most notably by raising mid-follicular E_2 . There is virtually no difference in mid-cycle E_2 , whereas mid-luteal E_2 increases with α . In contrast, during PL, the most drastic effect of α appears to occur during the mid-luteal stage. Mid-cycle E_2 also changes significantly, especially for extreme values of α (either near 0 or near 5). The impact on mid-follicular E_2 is less severe in PL than in NL.

Between the two models, the response of P_4 to changes in α are relatively conserved in all stages, where mid-luteal P_4 decreases as α increases, and P_4 levels at the mid-follicular and mid-cycle points are relatively unchanged.

The LH:FSH ratio has often been cited as being abnormal in PCOS. However, the conditions under which this is true vary according to patient characteristics. For example, thin women with PCOS have more often been found to have higher LH:FSH ratios [4, 49] compared to obese women. In addition, LH levels in PCOS have been found to be inversely correlated with body fat percentage [49]. Further, the LH:FSH ratio itself exhibits time-dependent changes due to variations in LH and FSH dynamics throughout the course of the cycle. In Figure 9(b), we observe changes in the LH:FSH ratio relative to the normal cycle (with $\alpha = 0$) during the mid-follicular, mid-cycle, and mid-luteal points in an average cycle. Under NL, there is a fairly consistent rise with α in the ratio within each phase of the cycle. Under PL, α and the LH:FSH ratio are positively correlated at the mid-follicular time point and negatively correlated at mid-cycle. The changes in the behavior of the ratio at the mid-luteal point is dominated by LH dynamics during both NL and PL.

5.5. Phenotypes of ovulatory dysfunction

The Rotterdam criteria for PCOS is the presence of two or more of the following symptoms: hyperandrogenism (HA), anovulation (AO) or oligo-ovulation, and polycystic ovarian morphology (PCO). Given these symptoms, there are four possible forms that PCOS can take in women: one in which all three symptoms are present, and three possible combinations of pairs of symptoms [46, 50, 51]. Although the present model cannot account for histological characteristics of the ovaries, we can study the

(b)

Figure 9. Endocrine characteristics of representative stages in the ovulatory cycle. All cycles for different values of α have been normalized. Mid-follicular point corresponds to levels at 25% of the total cycle length, mid-cycle at 50% and mid-luteal at 75%. (a) Comparison between normal luteinization (NL) and premature luteinization (PL) dynamics during key times in the course of an average cycle. A subset of state variables in the model is shown. Analysis does not apply to α values that yield a two-cycle (gray shaded regions). (b) Comparison between NL and PL LH:FSH ratio during key times in an average cycle.

Figure 10. Model summary of characteristic phenotypes for ovulatory dysfunction. Region N: normal ovulatory cycle; Region I/II: ovulatory dysfunction with HA and anovulation; Region III: ovulatory cycle with HA; Region IV: ovulatory dysfunction without HA. The values of α satisfying a criterion are indicated with darker shaded rectangles.

major consequences of hyperinsulinemia and premature luteinization by developing a set of relevant phenotypes based on HA and AO alone. We therefore consider three phenotypes to describe generalized ovulatory dysfunction rather than PCOS. Specifically, we define phenotypes I/II (HA^+/AO^+), III (HA^+/AO^-), and IV (HA^-/AO^+). Phenotype I/II is so denoted for consistency with phenotypes I and II defined in [51], which are both HA^+/AO^+ and distinguished by the presence or absence of PCO, respectively. We use N to denote the presence of a regular ovulatory cycle without clinical symptoms. Note that phenotype III describes regular ovulatory function in the presence of HA, and phenotype IV describes ovulatory dysfunction in the absence of HA.

A specific set of markers determines the levels of insulin-mediated feedback required to maintain or disrupt function, as well as the severity of dysfunction. We choose these markers based on their importance in the menstrual cycle and PCOS. They are the day 15 LH level ($LH_\alpha(15)$), after synchronizing the initial cycle for all α to the peak in Ω , the maximum P_4 concentration ($P_{\max}$), and the minimum T concentration ($T_{\min}$). The timing of ovulation is determined by the LH surge. In the absence of a sufficient surge level, we assume ovulation has not occurred. The P_4 level during the luteal stage is typically a marker confirming ovulation, whereas the LH surge initiates the process. A sustained deficiency in P_4 can indicate clinical AO [46]. We use two markers for AO to identify more closely the presence of ovulatory dysfunction. Finally, because T and other androgens play a large role in establishing PCOS, we use hyperandrogenism as an additional factor in dysfunction.

Given these markers, we define a set of threshold quantities to distinguish between normal and abnormal cycles. To allow for mild (M) and severe (S) cases of dysfunction,

we consider two different thresholds for $LH_\alpha(15)$ and for $P_{\max}$. The criteria considered are as follows:

- A. $P_{\max} < 5$ ng/mL;
- B. $P_{\max} < 3$ ng/mL [46];
- C. $T_{\min} > 600$ ng/L [46];
- D. $LH_\alpha(15) < 0.75 \cdot LH_0(15)$;
- E. $LH_\alpha(15) < 0.25 \cdot LH_0(15)$.

We assume I/II-M denotes the mild I/II phenotype, attained when criterion C, along with either A or D, is satisfied. Similarly, we assume I/II-S is the severe I/II phenotype, attained when C, along with both B and E, is satisfied. Further, note that phenotype III is a less severe mode of dysfunction. Phenotype IV, in the absence of HA, we also assume is less severe, as long as neither B or E is satisfied.

In Figure 10, we summarize the results of the NL and PL models to identify the range of α values that satisfy the specified criteria A–E. From left to right, we determine the phenotype of ovulatory dysfunction (regions labeled N, I/II, III, or IV) based on which set of criteria are satisfied for a given α value (patterned rectangles). For example, under PL, there is normal ovulation for $\alpha < 0.75$, mild dysfunction with AN but not HA for $0.75 \leq \alpha < 2$, mild dysfunction with both AN and HA for $2 \leq \alpha < 4.25$, and a transition to severe dysfunction when $\alpha \geq 4.25$.

We find that the presence or absence of premature luteinization determines the likelihood of mild or severe cases of ovulatory dysfunction. Under NL, normal cycles can result for $0 \leq \alpha < 2$ (region N). This window is reduced by more than half in PL. Phenotype III, which is still ovulatory, may occur under NL, but does not with PL.

Phenotype IV can occur in the presence of PL, but not with NL. In both cases, hyperandrogenism occurs when $\alpha \geq 2$. For the α values tested, higher levels of insulin feedback are required for dysfunction under NL than under PL. Based on the defined phenotypes, severe dysfunction can only occur under PL. This suggests that PL is not a requirement for ovulatory dysfunction, but can contribute to its severity with less hyperinsulinemia. In addition, mild hyperinsulinemia does not necessarily result in ovulatory dysfunction, and mild hyperandrogenism can still lead to ovulatory cycles as long as PL is not present.

We remark that with the exception of clinically used thresholds B and C, the distribution of phenotypes is subject to our choice of thresholds, primarily for lower values of α . For example, if criterion D is reduced to 50% of the normal LH peak, the only change would occur to regions N and IV under PL, which would encompass $0 \leq \alpha < 1.75$ and $1.75 \leq \alpha < 2$, respectively; the NL picture would remain unchanged (results not shown). Despite threshold variation, the conclusion that PL increases the severity of dysfunction with less hyperinsulinemia is consistent.

6. Discussion

We have developed a comprehensive mathematical model of the menstrual cycle with a semi-mechanistic model of ovarian steroid production that incorporates the effects of hyperinsulinemia. The model explicitly incorporates a role for testosterone (T) in the normal physiological menstrual cycle, allowing for further exploration in pathology related to ovulatory dysfunction. The model does not explain the origin of hyperinsulinemia, but provides a hypothesized role for the mechanisms that can lead to hyperandrogenism via elevated insulin. Specifically, an important role for insulin is highlighted as a co-gonadotropin with LH and as a mediator of increased LH sensitivity. We do not, however, propose that the only role for insulin is to amplify the effects of LH, as there is likely a clear distinction between mechanisms underlying LH-dependent enzymatic processes and cellular differentiation.

Through its description of essential feedback processes that regulate the ovulatory cycle, the model we present agrees qualitatively with established clinical data. In addition, the model exhibits varying degrees of ovulatory dysfunction depending on the degree of feedback. It also allows us to distinguish between less severe types of ovulatory dysfunction based on a particular profile of model output.

Endocrine profiles have been studied previously in women with PCOS [46, 51]. In [46], $HA^+/AO^+/PCO^+$ women have increased LH and LH:FSH ratios, along with hyperinsulinemia and insulin resistance. Those with $HA^+/AO^+/PCO^-$ have many of the same characteristics, except that LH levels are statistically significantly higher in the group with PCO^+ . Panidis and colleagues also detected higher androgen levels in $HA^+/AO^+/PCO^+$ women

than $HA^+/AO^+/PCO^-$ women [51]. Phenotype I/II of the current work describes these groups collectively, since the model does not capture physical characteristics of the ovaries. We therefore cannot assess differences in androgen levels within phenotype I/II. The model does support the notion that women with I/II have higher levels of insulin, and hence insulin-mediated feedback, than other individuals with PCOS. This is consistent between both the NL and the PL frameworks.

Based on the endocrine data collected in [46] and [51], it appears that the $HA^+/AO^+/PCO^+$ subgroup tends to be more overweight/obese, more insulin-resistant, and have higher LH levels than the $HA^+/AO^+/PCO^-$ subgroup. It is therefore more likely that these individuals are at greater risk for other health complications and may be seen as having a more severe phenotype of PCOS [50]. Although our assessment of ovulatory dysfunction phenotypes indicates that lower levels of $LH_\alpha(15)$ can result in increased severity, we note that clinical studies measure LH concentrations during the follicular phase of a cycle (roughly days 6–8 of a normal cycle). Follicular phase LH increases in both the PL and NL models (see Figure 9a), though to a greater extent under NL. Our results are therefore consistent with these reports.

Also consistent with studies of women with PCOS is phenotype III. Women with ovulatory PCOS have been found to have all of the metabolic and endocrine markers of PCOS, but in a less extreme form [46]. Testosterone, though elevated, seemed to fall between the level in the control group and those with $HA^+/AO^+/PCO^+$. In [51], no differences between insulin resistance markers were found in the ovulatory PCOS group than control individuals. This is in contrast to the present model, which indicates a slight increase in feedback from hyperinsulinemia. Nevertheless, it is clear that this group of individuals is closest to normo-ovulatory.

Normoandrogenic women with PCOS may or may not be more insulin resistant than normally ovulating women [51]. However, ovulatory dysfunction is evident even in the absence of HA. The present model produces these results, with the distinction being determined by the presence of PL. For phenotype IV, the range of insulin resistance, as indicated by insulin-mediated feedback strength α , is higher than normal in the model with PL, but may be higher than or similar to levels in the NL version of the model. Interestingly, there is much debate on whether normoandrogenic PCOS is simply an anomaly that should not be classified beneath the PCOS umbrella. This is certainly possible within the current framework, if we consider the nature of the “mild” endocrine markers used to summarize phenotypes. Reducing the $LH_\alpha(15)$ threshold, which is already arbitrary, would virtually eliminate phenotype IV completely. In this case, there would be no obvious differences between women with NL and those with PL.

Collectively, our results indicate that as long as PL is present, any abnormalities to the cycles result in ovulatory dysfunction. This is in contrast to NL, in which normal

ovulation can persist in the face of HA. This has been observed previously, where prematurely luteinized follicular cells come only from women with PCOS who are also anovulatory, but none who are ovulatory [48].

Many studies of women with PCOS track LH and FSH levels only during the follicular stage of a cycle [46, 50, 51]. This approach does not lend itself to comparison with the full cycle information provided by the model herein. Although we see an increase in the LH:FSH ratio during the follicular phase of both NL and PL, we see drastically different results after examining the LH:FSH ratio during the remainder of the cycle (see Figure 9). In particular, we are interested in the consistent decline in mid-cycle LH during PL, which appears to account for much of the difference. An interesting factor to consider is that in both NL and PL, the Φ -to- Ω transition is accelerated, resulting in reduced follicle growth. In NL, this is due to increased LH; in PL, it is due to premature luteinization. Therefore, the question is why does PL allow for a reduction in the LH surge level with increasing T levels when NL does not. The answer lies in the fact that if follicles are more sensitive to LH earlier in their development, it will take less LH to initiate a progression to the (mathematical) ovulatory phase. As the insulin-mediated feedback increases, less and less LH is required, until the LH surge is absent. Even in these instances, there is a progression through the remaining ovulatory stages of the model. This is unlikely to be the case physiologically, as ovulation would simply not occur at all. One future modification is to separate the processes of luteinization and ovulation. This will effectively incorporate the role of LH as a growth hormone during the late follicular stage. Whether NL or PL is present, the model results suggest that increased LH activity in the ovaries is a significant contributor to ovulatory dysfunction.

An important influence of hyperandrogenism on the disrupted menstrual cycle is, according to the present work, largely dependent on feedback between T and LH. By incorporating a normal physiological role for T in the cycle and by modeling T production in consideration of the underlying biological processes, we are able to observe abnormalities in the system under perturbation of key biological processes. The inclusion of luteinization as a factor underlying androgen-mediated dysfunction also reveals a cause of increased hyperandrogenism when insulin-mediated feedback is excessively high. Collectively, the present model gives insight into the mechanisms leading to ovulatory dysfunction once hyperinsulinemia is in place. The precise role for insulin, however, must be further elucidated, as its function in the current model is as a proposed instigator of hyperandrogenism without the associated mechanisms.

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Appendix A. Model Parameters

Table A.1 lists the parameters used for model simulation, along with the initial conditions of all state variables.

Table A.1. All model parameters. Dimensionless quantities are denoted by #. * denotes fixed (non-estimated) parameters. † denotes derived parameters (see text for details). Note: m and τ_2 are fixed arbitrarily based on model assumptions.

(a) Parameter values used in the pituitary subsystem.

Parameter	Units	Value
$c_{F,E}$	$(\text{ng/L})^{-2}$	2.2729×10^{-3}
$c_{F,I}$	#	1.9488
$c_{F,P}$	$(\mu\text{g/L})^{-1}$	60.428
$c_{L,E}$	$(\text{ng/L})^{-1}$	1.0404×10^{-3}
$c_{L,P}$	$(\mu\text{g/L})^{-1}$	9.9415×10^{-3}
$c_{L,T}$	$(\text{ng/L})^{-1}$	9.5942×10^{-3}
δ_F	1/d	8.21^* [13]
δ_L	1/d	14^* [13]
k_F	1/d	2.5412
$K_{F,I}$	μg	107.01
$K_{iL,P}$	$\mu\text{g/L}$	0.34952
$K_{L,T}$	ng/L	420^* [17]
K_{mL}	$\mu\text{g/L}$	183.56
k_L	1/d	0.74567
n	#	8^* [13]
V	1	2.5^* [13]
v_{0L}	$\mu\text{g/d}$	1051.7
v_{1L}	$\mu\text{g/d}$	34838
v_F	$\mu\text{g/d}$	3236.6

(c) Parameter values used in the steroidogenesis subsystem.

Parameter	Units	Value
c_{T,F_2}	$\mu\text{g/L}$	$123.8136^\dagger$
δ_E	1/d	1.1^* [52, 53]
δ_P	1/d	0.5^* [54]
δ_T	1/d	5.5^* [55, 56]
e_0	$\text{ng/L} \cdot \text{d}$	44.512
η	#	1.1087
h_3	$\mu\text{g/L}$	17.796
κ_1	#	1.09
κ_2	$\mu\text{g/L}$	22.28645
κ_3	$(\mu\text{g/L})^2$	$113.9188^\dagger$
p	$1/\text{L} \cdot \text{d}$	0.3734
t_0	ng/L	741.68
t_1	$\text{ng/L} \cdot \mu\text{g} \cdot \text{d}$	0.57088
t_2	$\text{ng/L} \cdot \mu\text{g} \cdot \text{d}$	1.3481
τ_1	#	5.3989
τ_2	#	0^*
τ_3	μg	430.91
t_{g1}	$\text{ng/L} \cdot \mu\text{g} \cdot \text{d}$	6.6548
t_{g2}	1/d	186.27
Ψ	μg	2004.3

(b) Parameter values used in the follicular dynamics subsystem.

Parameter	Units	Value
$c_{\Phi,F}$	$(\mu\text{g/L})^{-1}$	0.01127
δ_s	1/d	0.74702
f_0	$\mu\text{g/d}$	2.5112×10^{-3}
f_1	1/d	4.3764
f_2	1/d	27.812
h_1	$\mu\text{g/L}$	590.32
h_2	$\mu\text{g/L}$	1815.3
h_p	$\mu\text{g/L}$	20.764
h_s	$\mu\text{g/L}$	12.329
l	1/d	0.49017
m	#	4^*
$\hat{s}$	1/d	2.378
w	1/d	0.23173

(d) Initial conditions used in the physiological model.

Variable	Units	Initial Value
FSH	$\mu\text{g/L}$	142.5
LH	$\mu\text{g/L}$	25.34
FSH_ρ	μg	116.82
LH_ρ	μg	250.35
Φ	μg	0.50185
Ω	μg	9.7509
Λ	μg	4.102
S	#	0.050498
T_γ	$\text{ng/L} \cdot \mu\text{g}$	0.003999
T	ng/L	273.67
E_2	ng/L	56.387
P_4	ng/mL	0.468

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