

A Model of Gonadotropin Regulation during the Menstrual Cycle in Women: Qualitative Features

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Increasing concerns that environmental contaminants may disrupt the endocrine system require development of mathematical tools to predict the potential for such compounds to significantly alter human endocrine function. The endocrine system is largely self-regulating, compensating for moderate changes in dietary phytoestrogens (e.g., in soy products) and normal variations in physiology. However, severe changes in dietary or oral exposures or in health status (e.g., anorexia), can completely disrupt the menstrual cycle in women. Thus, risk assessment tools should account for normal regulation and its limits. We present a mathematical model for the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in women as a function of estrogen, progesterone, and inhibin blood levels. The model reproduces the time courses of LH and FSH during the menstrual cycle and correctly predicts observed effects of administered estrogen and progesterone on LH and FSH during clinical studies. The model should be useful for predicting effects of hormonally active substances, both in the pharmaceutical sciences and in toxicology and risk assessment. **Key words:** estradiol, follicle-stimulating hormone, luteinizing hormone, mathematical model, menstrual cycle, progesterone. — *Environ Health Perspect* 108(suppl 5):873–881 (2000).

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There is now significant concern that environmental pollutants may disrupt the endocrine systems in both humans and wildlife. Of particular concern are compounds that can mimic the effects of endogenous estrogen by binding to the estrogen receptor and subsequently activating estrogen-responsive genes or compounds that can antagonize the effects of estrogen by binding the receptor in an inactive conformation and blocking the binding of endogenous estrogen (1,2). Estrogenic materials have been implicated as being responsible both for reported declines in sperm counts and other male reproductive disorders (3) and for reported increases in the incidence of breast cancer (4). However, there is considerable scientific uncertainty and debate about the plausibility of these hypotheses (5,6).

Our ultimate goal is to develop a mechanistic, mathematical model of the human menstrual cycle that can be used to predict the effects of interactions between exogenous compounds and the sexual endocrine system in adult women. Such a model would be directly useful in evaluating hypotheses about the role of xenoestrogens in breast cancer and in the menstrual cycle. The model presented here is considered to be a generic building block that can be further elaborated for specific applications depending on the mechanism and biologic response being considered.

In this article, we describe the structure for a mathematical model of the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and their regulation under normal physiologic

conditions in adult, cycling women. Given representative profiles for ovarian hormones during the menstrual cycle, we show that the model reproduces the correct qualitative behavior. In a previous article (7), we showed that if these input profiles for the ovarian hormones are periodic functions of time of fixed period T , then our system of differential equations has a unique, asymptotically stable solution and that this solution has period T . Hence, if T is taken to be 30 days, our model predicts that all women with the same concentrations of ovarian hormones, which are repeating every 30 days, and with the same volumes of distribution and clearance rates for LH and FSH will have the same LH and FSH concentrations, which also are cycling every 30 days. (It is possible for a model of this complexity to exhibit quasiperiodic or chaotic behavior, but ours does not.) In addition, given altered steroid hormone profiles induced in clinical experiments, our model reproduces the observed patterns of response in LH and FSH levels. Finally, we demonstrate that the observed biphasic response of LH to a bolus challenge of estradiol (E_2) can be explained with a model in which E_2 exerts only negative feedback.

Several models of the menstrual cycle have been described in the literature (8–14). Although we have found the general framework for these models to be a useful starting point, each was deemed inappropriate for our purposes for at least one of the following reasons: aspects or components are descriptive rather than predictive in behavior; they contain switches in regulation rather than

continuous dose–response relationships; or they assume physiologically separate tonic and surge pools of gonadotropins. We seek to develop a model that is predictive, in which each of the variables can be identified with physiologic quantities, and in which the response functions of tissues are continuous, though they may be highly nonlinear.

The issue of switches in tissue response is somewhat contentious. The key distinction seems to be between the behavior of tissues as a whole and that of individual cells. This is best illustrated by the observations of Ferrell and Machleder (15) on the response of *xenopus* oocytes to progesterone (P_4). These investigators found that individual oocytes did indeed exhibit essentially all-or-nothing responses (activation of the mitogen-activated protein kinase phosphorylation) to varying levels of P_4 ; i.e., as P_4 levels were increased, each oocyte either showed no response or was maximally induced. However, different oocytes responded at different levels of P_4 so that when the response of collections of oocytes was quantified, the dose–response function was best described by a Hill equation with an apparent Hill coefficient of 1. Although the system examined by Ferrell and Machleder is very different from and far simpler than the hypothalamic–pituitary axis we seek to describe, we believe that the principle demonstrated by these results still applies: that “all-or-none” switching observed in individual cells does not necessarily imply that the response of the population as a whole (i.e., the entire pituitary) will be all or none. Cells in an intact tissue are each likely to have their own, different microenvironment and biochemical status. Therefore, while individual gonadotrophs may also respond in an all-or-nothing manner to increasing ovarian hormone stimulus, it seems likely that the

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tissue as a whole would respond in a more continuous manner. In fact, the results of Yamaji et al. (16) in which LH surges were induced by injections of estradiol, shown in Figure 1, clearly depict a varying level of response to varying levels of stimulus rather than an all-or-nothing response. Thus, we have chosen to describe our response functions using continuous, Hill-type equations rather than with switches.

Many existing models, such as that of Plouffe and Luxenberg (12), divide pituitary LH synthesis and storage into two separate compartments, one for the relatively low, continual (tonic) release of LH throughout most of the cycle and a second to account for the surge. There does not appear to be any physical evidence for separate pools of LH in the pituitary or separate pathways for its synthesis under different regulation. Thus, this separation appears to be an artificial construct that evades the problem of accounting for the complex regulation of LH synthesis and release. Our presumption is that there is a single pathway for LH synthesis and release, but that this pathway is under somewhat complex control by ovarian hormones.

A hallmark of the complexity of LH regulation is the biphasic response observed after challenge with an estrogen agonist (17), where acute administration of an estrogen agonist causes an initial decline in LH levels, which is evidence of negative feedback (inhibition of LH by E_2), followed by a surge in LH levels above initial or control levels. This subsequent surge has been cited as evidence of positive feedback (18). Through the use of a very simple preliminary model, we demonstrate that this behavior can be explained with a mechanism that only involves negative feedback by E_2 . The conclusion is that this biphasic

response is not, by itself, sufficient evidence that both negative and positive feedback occur. To be clear, there is strong evidence for positive feedback by E_2 , and our full model does include positive feedback (Figure 2). This evidence includes experiments in which E_2 is infused in normal, midfollicular-phase women at a steadily increasing rate, where a surge is induced with little or no initial decline in serum LH (19).

The model described here lumps the hypothalamus and the pituitary into a single "black box." This is done for simplicity, as the level of model complexity required to describe the complex dynamics of gonadotropin-releasing hormone (GnRH) synthesis, release, regulation, and action would be significantly greater than that presented here. In this regard we have applied a modeler's version of Occam's Razor: we are working with a simpler model rather than a more complex one until such time as there is a demonstrated need for the greater complexity (i.e., we will separate out the hypothalamus when it becomes necessary to adequately describe experimental data). The lumped model structure presented here is successful in predicting the menstrual cycle dynamics and certain clinical steroid challenges. Andersen et al. (14) successfully used an even simpler model, which also lumps the hypothalamus and pituitary, to predict the effects of neonatal exposure to estrogen on age of persistent estrus in the rat. Thus, the model need not be more complex for it to be useful.

In synopsis, we describe a mathematical model of gonadotropin synthesis and release in normal cycling women. Although simplistic in structure, the model can also serve as the framework or a building block for more elaborate models in the future. The model is shown to reproduce the correct qualitative behavior during the cycle and to reproduce observed responses to clinical challenges by E_2 and P_4 .

Thus, the model should be useful as is for predicting the effects of exogenous compounds that act (primarily) through activation of the estrogen, progesterone, or inhibin receptors. Finally, we show that a biphasic response of LH to a bolus challenge of E_2 can be explained with a model in which there is only negative feedback from E_2 .

Methods

Model Structure and Assumptions

The full model that we describe here contains both positive and negative feedback and is depicted in Figure 2. A primary feature of this model is that we separate the processes of gonadotropin synthesis and gonadotropin release. Here, synthesis refers to all steps involved in the generation of the mature, heterodimeric proteins and sequestering of the proteins into secretory vesicles, producing a releasable pool. The subsequent release into circulation is treated as a separate step, whose regulation differs from the regulation of synthesis. In particular, because the size of the releasable pool may change with time, the rate of synthesis at any given moment may not be the same as the rate of release. For example, there may be times when the rate of release, and hence the circulating level of LH, is dropping while the rate of synthesis is increasing. There is clear evidence that pituitary LH levels change during the estrus cycles in rats and that these changes are not in parallel with serum levels (20). For example, pituitary levels drop during the first half of the surge (last 4 hr of proestrus), whereas serum levels are rising. Results *in vitro* with quartered rat pituitaries show that GnRH-induced LH release is not coupled to synthesis (21). Similarly, Hotchkiss et al. (22) showed that the pituitary content of LH and FSH in rhesus monkeys varies during the menstrual cycle over periods when blood levels of LH and FSH remain constant, establishing that this type of regulation also occurs in primates.

The release of GnRH has been shown to be regulated by E_2 and P_4 in ewes (23), and the pattern of gonadotropin release in women also indicates regulation by ovarian hormones (24). Release is presumed to occur at a rate proportional to the size of the releasable pool, with the proportionality constant being a function of E_2 and P_4 levels. Synthesis is also presumed to be regulated by E_2 and P_4 as well as by the peptide hormone, inhibin (Ih). There is evidence of both direct effects of these hormones on the pituitary and indirect regulation via the hypothalamus (GnRH). However, we decided not to treat these two levels of regulation separately (in part, due to the complexity of tracking GnRH) but to group together the direct and GnRH-mediated regulation of gonadotropin synthesis

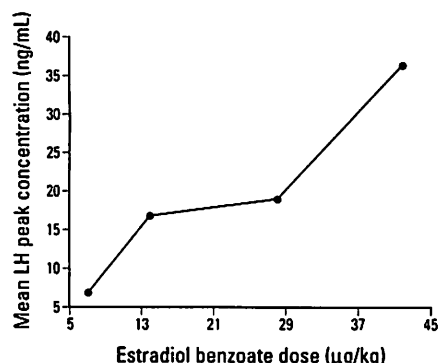


Figure 1. LH response data after estrogen challenge showing a less than all-or-nothing response function. Female rhesus monkeys were given varying doses of estradiol benzoate in oil as a single, subcutaneous injection ($n = 4$ animals per dose). Serum LH levels were then sampled at 12-hr intervals and the mean maximal LH concentration, which occurred 36–72 hr after injection, is shown. Intermediate doses (14 and 28 $\mu\text{g/kg}$) led to intermediate responses. Data from Yamaji et al. (16).

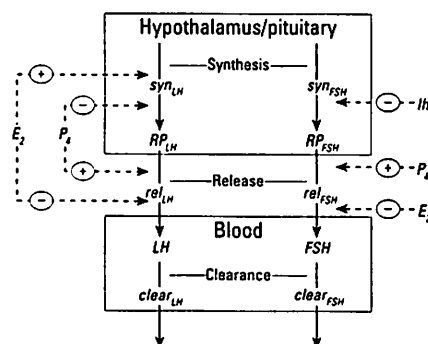


Figure 2. Structure of mathematical model of LH and FSH synthesis and release. Solid arrows represent pathways for synthesis (*syn*), release to the blood (*rel*), and clearance from the blood (*clear*). Dashed arrows represent regulatory influences, with the sign indicating either positive or negative feedback of estradiol (E_2), progesterone (P_4), and inhibin (*Ih*).

and release by E_2 , P_4 , and I_h . Given that synthesis and release are regulated independently, this grouping of direct and indirect regulation leads to a model structure in which one mathematical function describes the regulation of synthesis and a second, independent function describes the regulation of release. In particular, the model assumes that E_2 promotes LH synthesis but inhibits LH release.

The primary evidence that E_2 inhibits both LH and FSH release is the short-term depression in LH and FSH serum levels following administration of ethinyl estradiol (18). The fact that incremental infusion of E_2 over a period of several days causes little or no drop in LH and FSH serum levels (19) is taken as evidence that this short-term effect is on release rather than on synthesis. If the inhibitory effect were on synthesis and not on release, then one would expect that (at short times) when the pituitary pool had changed little, a decrease in synthesis rate would have little or no effect on release, and that serum levels would drop only over longer periods. If this effect is on release instead, then incremental infusion would allow time for pituitary levels to build (due to the inhibition of release) and so compensate for most of the inhibitory effect. We presume here that release rates are proportional to pituitary levels, and that inhibition of release occurs by decreasing the proportionality constant. A sudden drop in this constant would cause serum levels to drop until pituitary levels had time to compensate. A slow drop in this constant allows pituitary levels to build in such a way that there is little effect on release.

The promotional effect of E_2 on LH synthesis follows from the fact that an LH surge can be induced in hypogonadal women as a result of incremental infusion where E_2 levels are raised and maintained at elevated levels (19).

At this point it is appropriate to comment that the current model is not intended to describe gonadotropin synthesis and release for levels of E_2 (in particular) well below the normal range that occurs during the menstrual cycle. The overall relationship between E_2 levels and LH synthesis, as well as the approximation to be used here, is depicted in Figure 3. In particular, there is strong evidence that at very low E_2 levels, such as those occurring in hypogonadal (19) or postmenopausal women (25), E_2 is an inhibitor of LH and FSH synthesis as depicted by the downward-sloping portion of the dashed curve in Figure 3. We have decided to ignore this low-concentration inhibition in the current model, as our primary concern is for subtle effects in normally cycling women and, ultimately, in pregnant women that are well short of complete disruption of the cycle. In both these cases we do not anticipate estrogenic activities dropping to this

inhibitory range. The equation describing synthesis can be altered appropriately in the future should the need arise. Also, while pituitary responsiveness to P_4 has been shown to require induction of P_4 receptors by E_2 in sheep (26), we presume that E_2 levels described by the model are sufficient to maintain normal P_4 responsiveness, so this dependence need not be explicitly included.

The effect of E_2 on the synthesis of LH is assumed to be delayed by some number of hours. An increase in synthesis may first require transcription, translation, and modification of mRNA, whereas a decrease may require time for mRNA degradation before the change is realized. Thus, changes in synthesis rates may not follow instantaneously from changes in E_2 levels. The well-known self-priming effect of GnRH is evidence of a delay in response by the pituitary to regulatory stimuli. There are also clear data showing that GnRH-stimulated LH release from dispersed rat pituitary cells exhibits a biphasic response with respect to progesterone, with the second, inhibitory phase not occurring until some time between 6 and 12 hr of incubation with progesterone (27). Our presumption is that E_2 acts by similar, time-dependent mechanisms. When normal, mid-follicular-phase women are infused with E_2 , there is a period of 24 hr between the time when E_2 levels reach their maximum and the start of the subsequently induced surge (19). Finally, given the fast dynamic of the normal mid-cycle LH surge, the fact that peak LH levels are achieved between 12 and 24 hr after the peak in E_2 levels requires the introduction of such a delay in our model equations. Without a delay, we have been unsuccessful in simulating both the rapid rise and fall of LH during the surge and the time differential between the peaks of E_2 and LH. On the other hand, LH release occurs immediately after GnRH stimulation, so we consider regulation of release to be rapid, and the inhibition of release by E_2 is treated as occurring on a time scale of minutes.

For P_4 , the effect on LH synthesis and release is presumed to be just the opposite of that of E_2 : inhibition of synthesis and promotion of release. The promotion of both LH and FSH release is suggested by the induction of LH and FSH surges in postmenopausal women pretreated with ethinyl estradiol (25). This is further supported by the increased duration of estrogen-induced mid-follicular-phase LH and FSH surges in normal women and the increased size and duration of estrogen-induced surges in hypogonadal women when P_4 is infused during the surge compared to E_2 treatment alone (19). Other evidence comes from the stimulation of GnRH-induced LH and FSH release from primary cultures of rat pituitary cells after

short-term (< 6 hr) preincubation with P_4 (27,28). As discussed above for the effects of E_2 on synthesis, we also presume that the effect of P_4 on synthesis is delayed. In fact, to describe correctly the timing of the surge, a delay of almost 3 days is necessary with the current model equations. In this case, the data of Krey and Kamel (27) provide direct evidence for a delay (though of shorter duration in rat pituitary cells). As for E_2 , the effect of P_4 on release is presumed to be instantaneous.

Although there is some evidence that E_2 and P_4 also alter FSH synthesis, in general this level of control appears to be weak. For example, when ethinyl estradiol was administered to women in the early follicular phase, FSH was initially depressed, as one would expect for a negative effect on release, and then slowly rose back to the normal follicular-phase level without any evidence of surge in contrast to the surge in LH elicited by this treatment (17). The mid-cycle surge of FSH follows a prolonged depression in circulatory levels relative to the early follicular phase, unlike LH for which circulatory levels are virtually flat up to the surge (29). This suggests that the mid-cycle FSH surge largely results from a buildup in pituitary levels due to the negative feedback on FSH release as E_2 levels rise during the follicular phase, followed by an induction of release due to the periovulatory rise in P_4 and the drop in E_2 during the day prior to the FSH peak. In short, much of the response of FSH to changes in steroid hormone levels can be explained by regulation of release (which is not the case for LH). The fact that FSH exhibits a preovulatory depression relative to early follicular phase levels, whereas LH does not, is accounted for in the model by presuming that inhibition of FSH release

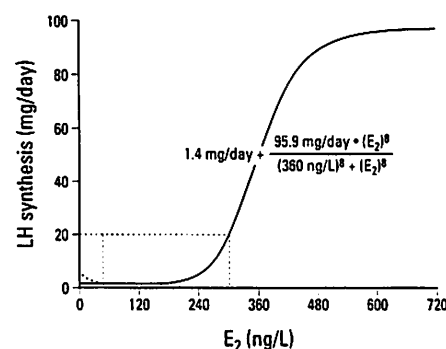


Figure 3. Dose-response curve for LH synthesis as a function of the E_2 blood concentration, given by Equation 1c, with $P_4 = 0$ (solid curve; equation shown is rate of LH synthesis as plotted). The area marked between 50 and 300 ng/L E_2 and up to 20 mg/day LH synthesis is the normal range occurring during the menstrual cycle for which the equation is used. The dashed curve at very low E_2 levels represents the increase in LH levels observed in hypogonadal or postmenopausal women but which is not described by Equation 1c.

increases as the square of E_2 concentration, whereas inhibition of LH release only increases in proportion to E_2 levels.

The model does include a negative feedback term for the effect of Ih on FSH synthesis. The fact that Ih decreases circulating FSH levels has been well demonstrated in the rat (30). A more careful examination of the time course of responses in rats to Ih indicates that the decrement in FSH levels relative to controls increases gradually with time (31), which differs from the very rapid responses resulting from steroid hormone injection. This suggests that the effect of Ih is on synthesis rather than on release. Finally, given the metabolic clearance rate of FSH [half-life ~ 6 hr (32)], the rapid rise and, in particular, the rapid fall of FSH as the surge occurs and ends indicate that the time constant for release of FSH is no more than a day or so. Therefore the extended depression of FSH levels that occurs during the luteal phase, which lasts a week or more and yet is not followed by a surge as Ih levels drop, must be due to a depression in synthesis. Put another way, it is not possible to describe both the fast dynamic of the surge and the slow dynamic of the luteal low (without a postluteal surge) using a model in which only release is regulated and synthesis is constant. Therefore we presumed that the effect of Ih is on synthesis. With this assumption and the regulation of release by E_2 and P_4 , we can simulate the dynamics of FSH throughout the cycle. As for the effects of E_2 and P_4 on LH synthesis, we presume that the effect of Ih on FSH synthesis is delayed, in this case by about 2 days.

Finally, although there is evidence for the inhibition of LH by Ih (31), the effect does not appear to be as dramatic as on FSH. Further, given that we have included a term for inhibition of LH synthesis by P_4 and the close correlation of P_4 and Ih levels during the menstrual cycle [data in McLachlan et al. (29)], distinguishing the effects of P_4 and Ih during parameter estimation with the available data would be rather difficult. Attempting to include a term for both effects could then yield highly erroneous parameter values. Therefore we have not included such a term in the initial model. If we are consistently unable to describe existing data for LH or data clearly distinguishing the effects of Ih and P_4 on LH in humans become available, then a term for this effect will be included.

Model Equations

Two systems of two-dimensional ordinary differential equations are used to model both the LH and FSH processes of synthesis, release, and clearance. Each system is a two-compartment model consisting of the pituitary and the blood, as depicted in Figure 2. (LH and FSH are presumed not to distribute

significantly into tissues.) Gonadotropin synthesis occurs in the pituitary, where it is held in a reserve pool for release into the blood stream. $RP_{LH}(t)$ and $RP_{FSH}(t)$ denote the functions of time t that represent the amounts in the pituitary of LH and FSH, respectively. The notation RP stands for releasable pool. Similarly, $LH(t)$ and $FSH(t)$ denote the concentrations in the blood, which can be measured experimentally. The differential equations for $RP_{LH}(t)$ and $RP_{FSH}(t)$ contain terms for synthesis and for release; the differential equations for $LH(t)$ and $FSH(t)$ contain terms for assimilation into blood and for clearance. Both these systems are linear in the phase variables with time-dependent coefficients that are functions of the ovarian hormones (i.e., E_2 , P_4 , and Ih).

The LH system is:

$$\frac{d}{dt} RP_{LH} = \text{syn}_{LH}(E_2, P_4) - \text{rel}_{LH}(E_2, P_4, RP_{LH}) \quad [1a]$$

$$\frac{d}{dt} LH = \frac{\text{rel}_{LH}(E_2, P_4, RP_{LH})}{v_{dis}} - \text{clear}_{LH}(LH) \quad [1b]$$

where

$$\text{syn}_{LH}(E_2, P_4) = \frac{V_{0-LH} + \frac{V_{1-LH} \cdot E_2(t-d_E)^h}{K_{mLH}^h + E_2(t-d_E)^h}}{1 + P_4(t-d_P)/K_{iLH-P}} \quad [1c]$$

$$\text{rel}_{LH}(E_2, P_4, RP_{LH}) = \frac{k_{LH-rel}[1 + C_{LH-P} \cdot P_4(t)]RP_{LH}}{1 + C_{LH-E} \cdot E_2(t)}, \quad [1d]$$

and

$$\text{clear}_{LH}(LH) = k_{LH-d} \cdot LH. \quad [1e]$$

In these simulations, $E_2(t)$ and $P_4(t)$ are input functions that represent the blood concentrations of E_2 and P_4 in normally cycling women. The term syn_{LH} accounts for the production of LH in the pituitary. The fractional expression in the numerator of Equation 1c is a Hill function. This Hill function increases rapidly as E_2 varies between 250 and 600 ng/L (Figure 3) and reflects the positive effect of large estradiol concentration on the synthesis of LH. The denominator of Equation 1c represents an inhibitory effect of progesterone on LH synthesis. The time-delays d_E and d_P are parameters that describe the period between the time when changes in blood levels of gonadal hormones occur and the time when subsequent changes in synthesis rates

occur. The term rel_{LH} accounts for the release of LH from the reserve pool. This term increases in proportion to the P_4 concentration and is inversely proportional to a linear function of the E_2 concentration. These functions represent the enhancing effect of P_4 and the inhibiting effect of E_2 on LH release. This same term scaled by the volume of distribution, v_{dis} , appears in the second equation to account for LH assimilation into the bloodstream. The term clear_{LH} is simply a first-order LH clearance term.

The FSH system is similar to the LH model, i.e.:

$$\frac{d}{dt} RP_{FSH} = \text{syn}_{FSH}(Ih) - \text{rel}_{FSH}(E_2, P_4, RP_{FSH}) \quad [2a]$$

$$\frac{d}{dt} FSH = \frac{\text{rel}_{FSH}(E_2, P_4, RP_{FSH})}{v_{dis}} - \text{clear}_{FSH}(FSH) \quad [2b]$$

where

$$\text{syn}_{FSH}(Ih) = \frac{V_{FSH}}{1 + Ih(t-d_{Ih})/K_{iFSH-Ih}} \quad [2c]$$

$$\text{rel}_{FSH}(E_2, P_4, RP_{FSH}) = \frac{k_{FSH-rel}[1 + C_{FSH-P} \cdot P_4(t)]RP_{FSH}}{1 + C_{FSH-E} \cdot E_2(t)^2} \quad [2d]$$

and

$$\text{clear}_{FSH}(FSH) = k_{FSH-d} \cdot FSH. \quad [2e]$$

The syn_{FSH} term, Equation 2c, reflects the negative effect of inhibin $Ih(t)$ on FSH synthesis, which has a time-delay d_{Ih} . The release term, Equation 2d, is similar to that in the LH system except that the negative effect of E_2 on FSH release is second order instead of first order.

The input functions used for the concentrations of the ovarian hormones are continuous functions of time, chosen to approximate the 30-day data for normally cycling women in McLachlan et al. (29). [These functions are meant to be entirely descriptive (empirical) to test the behavior of our gonadotropin model. A mechanistic model for ovarian hormones was described by Selgrade and Schlosser (7).] The data for E_2 are depicted by a 2-humped graph with a sharper and higher peak right before the mid-cycle LH surge (day 0) centered at day -1 and a broader and shorter peak during the luteal phase centered at day 8 (Figure 4). To obtain this profile, we superposed a quadratic rational function and

a negative exponential function. Thus the E_2 concentration is given by:

$$E_2(t) = 300 - \frac{240 \cdot (t+1)^2}{3 + (t+1)^2} + 90 \cdot \exp\left(-\frac{(t-8)^2}{10}\right) \quad [3]$$

To portray the P_4 and I_h data, we use 1-humped graphs of negative exponentials centered at day 7 (Figure 4). These functions are:

$$P_4(t) = 52 \cdot \exp\left(-\frac{(t-7)^2}{18}\right) \quad [4]$$

and

$$I_h(t) = 300 + 1,330 \cdot \exp\left(-\frac{(t-7)^2}{19}\right) \quad [5]$$

These three functions are plotted against the data of McLachlan et al. (29) in Figure 4.

Input functions in this form are handled easily by XPP, the UNIX version of PhasePlane (33), the numerical software package we use. In fact, if $E_2(t)$, $P_4(t)$, and $I_h(t)$ are extended as periodic functions of periods of 30 days, then Equations 1a–e and 2a–e may be solved explicitly for unique solutions of 30-day periods, and these solutions are globally asymptotically stable (7). Because of the complicated form of these exact solutions, they are difficult to implement for parameter estimation and comparison of the dynamic behavior to data. Therefore our simulation runs are performed using various numerical methods for approximating solutions (usually Runge-Kutta).

The LH system has 12 parameters, and the FSH system has 8 parameters. The values for the clearance rates of LH and FSH are taken from the steady-state infusion studies of Kohler et al. (34) and Coble et al. (32), respectively, scaled to a 2.5-L volume of distribution. The E_2 , P_4 , and I_h functional parameters are selected to approximate data for these hormones in McLachlan et al. (29) for normally cycling women. The remaining parameters for the LH and FSH models are initial fits to the LH and FSH profiles in McLachlan et al. (29). (The parameter choices have not been optimized because the functions used for the ovarian hormones are also not optimal and because our current goal is to determine if the model structure is correct.) All parameter values are listed in Tables 1 and 2. These include the volume of distribution v_{dis} ; the clearance rates k_{LH-cl} and k_{FSH-cl} ; the time-delays d_E , d_P , and d_{Ih} ; the noninduced V_{max} , V_{0-LH} ; the maximal induction V_{1-LH} ; and the Hill coefficient h .

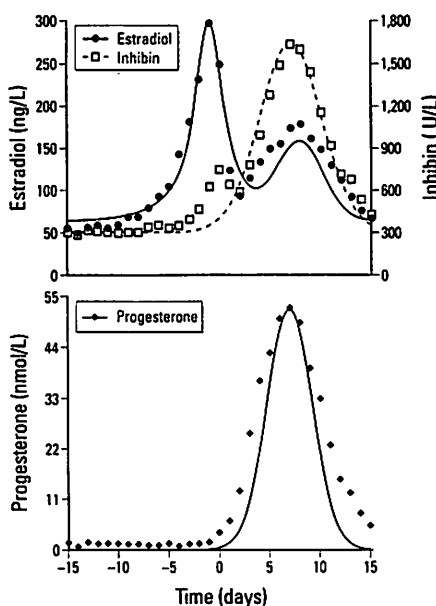


Figure 4. Graphs of functions used to simulate blood levels of estradiol (solid curve), progesterone (dashed curve), and inhibin during the menstrual cycle (solid curve in lower panel). Data from McLachlan et al. (29) for estradiol, inhibin, and progesterone normalized around the day of the LH surge (day 0) are shown for comparison.

The parameter values in Tables 1 and 2 were used for simulations of the LH system (Equation 1a–e) and the FSH system (Equation 2a–e). Initial conditions of 24.2 and 130 $\mu\text{g/L}$ were taken for LH and FSH, respectively, to correspond to concentrations in McLachlan et al. (29) at the beginning of the cycle. Initial amounts of RP_{LH} and RP_{FSH} were 417.4 and 1,300 μg , respectively, which produced the slopes of the LH and FSH profiles observed at the beginning of the cycle.

In addition to simulating the menstrual cycle using the normal ovarian profiles (described above) as inputs, the model was also tested by simulating the effect of clinical challenges with estrogen and progesterone. Briefly, the response of LH to a bolus challenge with estrogen was first simulated with a modified version of the model in which there was no positive feedback (the positive effect of E_2 on LH synthesis was suppressed) to determine if a biphasic response could be predicted with only negative feedback. Second, the response to estrogen infusion during the follicular phase, such that elevated estrogen levels were maintained for a significant length of time, were simulated using the full model. Finally, the effect of the same estrogen infusion followed by a bolus progesterone injection was simulated. The model predictions from these three scenarios are depicted, and their similarity to data reported in the literature is discussed.

Table 1. Parameter values for the LH system, Equation 1a–e.

Parameter	Value
k_{LH-rel}	3 day ⁻¹
k_{LH-cl}	14 day ⁻¹
h	8
V_{0-LH}	1,400 $\mu\text{g/day}$
V_{1-LH}	95,900 $\mu\text{g/day}$
K_{M-LH}	360 ng/L
K_{I-LH-P}	26 nmol/L
C_{LH-E}	0.008 L/ng
C_{LH-P}	0.024 L/ng
d_E	0.42 day
d_P	2.9 day
v_{dis}	2.5 L

Table 2. Parameter values for the FSH system, Equation 2a–e.

Parameter	Value
V_{FSH}	4,400 $\mu\text{g/day}$
k_{FSH-cl}	8.21 day ⁻¹
$k_{FSH-rel}$	45 day ⁻¹
d_{Ih}	2 day
$K_{I-FSH-Ih}$	1,176.5 U/L
C_{FSH-E}	0.005 L/(ng) ²
C_{FSH-P}	3 L/ng
v_{dis}	2.5 L

Results

The 30-day concentrations of LH and FSH from XPP simulations of Equations 1a–e and 2a–e, respectively, are depicted in Figure 5. Our graphs are qualitatively similar to Figure 1 in McLachlan et al. (29). The LH and FSH surges commenced 1–2 days before mid-cycle, peaked at mid-cycle (day 0), and subsided by 2 days after mid-cycle.

A biphasic response by serum LH to administration of E_2 has been observed in many experiments with women and animals, e.g., Tsai and Yen (17), Odell and Molitch (35), and Clarke and Cummins (36). This behavior is usually attributed to negative and then positive feedback of E_2 on LH secretion. Here we used a restricted version of the LH system, Equation 1a–e, to illustrate that only E_2 negative feedback was needed to cause a biphasic LH response, although the spike in LH was not as high as the midcycle LH surge during the normal cycle. The administration of E_2 consisted of a gradual increase in E_2 from low levels (~ 5 ng/L) to a maximum of 300 ng/L after 2 days and then a gradual decline to the initial level by day 4 (profile A, Figure 6). Equation system 1a–e was modified to reflect only the effect of E_2 on LH release by fixing the synthesis term, Equation 1c, at the constant V_{0-LH} and by setting the P_4 coefficient C_{LH-P} in Equation 1d to zero. The remaining parameters were held at the values for normally cycling women given in Table 1.

For our initial concentrations, we took $LH = 40 \text{ ng/L}$ and $RP_{LH} = 467 \text{ ng}$, which were steady-state values for our modified Equation 1a–e, with $E_2 = 0$. The biphasic LH response to an E_2 challenge (time-course A in Figure 6) is depicted in Figure 7. LH concentration decreased to 65% ($\sim 26 \text{ ng/L}$) of its steady-state level after 1.67 days and then gradually rebounded to 150% ($\sim 60 \text{ ng/L}$) of its steady-state level after 3.5 days before returning to steady state between days 5 and 6. The rebound commenced slightly before the E_2 peak because the releasable pool had continually increased since the

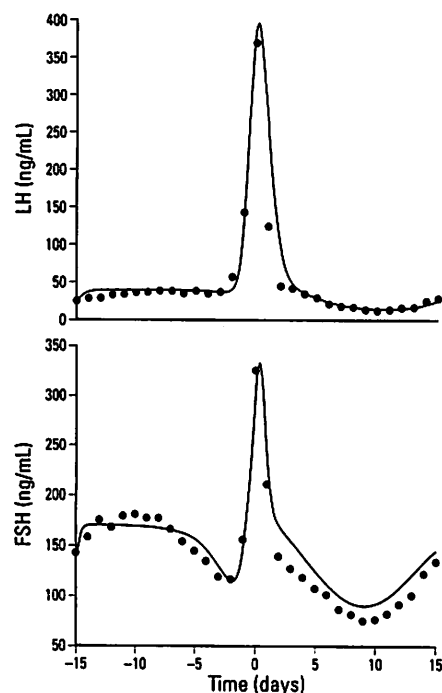


Figure 5. Graphs of LH and FSH dynamics (solid curves) predicted by Equations 1a–e and 2a–e, respectively, with ovarian hormone levels as shown in Figure 4. Data from McLachlan et al. (29) (•) are shown for comparison.

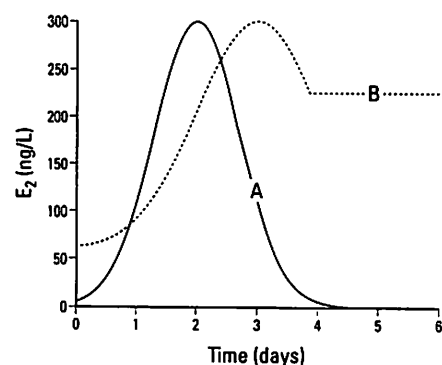


Figure 6. Time courses for E_2 blood levels used as inputs to the model to simulate experimental challenges. A is the input time course for biphasic response simulation shown in Figure 7. B is the input time course for induced surge responses shown in Figures 8 and 9, with maximum E_2 of 300 ng/L. A similar profile was used for maximum E_2 of 200 ng/L.

beginning of E_2 administration. This rebound was augmented by the E_2 decline starting at day 2, which decreased the inhibition on release. The LH profile in Figure 7 is similar to the LH response observed by Clarke and Cummins (36) in experiments with ovariectomized ewes given injections of estradiol benzoate.

Additional evidence for biphasic behavior via the negative feedback of E_2 is the mechanism by which the FSH surge is produced in our model. An essential component of the FSH surge profile (Figure 5) is the quadratic inhibition of E_2 on FSH release. The normal pre-surge increase in E_2 causes the pre-surge dip in serum FSH. Then, as serum E_2 levels fall, the FSH surge commences. At present, our model contains no effect of E_2 on FSH synthesis. The height and sharpness of the FSH surge is augmented by the strong positive effect of P_4 on FSH release.

Next, we ran model simulations in an attempt to mimic the experiments of Liu and Yen (19) on normal women in mid-follicular phase and hypogonadal women and similar experiments on castrate female rats (35). These experiments administered various levels of estradiol and estradiol with progesterone and observed serum LH and FSH fluctuations and surges.

In our first set of simulations, E_2 gradually increased from a basal level of $\sim 60 \text{ ng/L}$ to a maximum level at day 3 of either 200 or 300 ng/L and then decreased 25% before leveling off at 150 or 225 ng/L on day 3.86 (profile B, Figure 6). The model parameters for these simulations were those in Tables 1 and 2 for normally cycling women. Because of the E_2 inhibition on release, initially serum

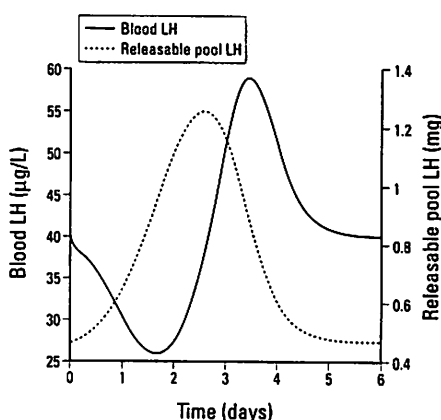


Figure 7. LH response to an E_2 challenge (time-course A in Figure 6) predicted by Equation 1a–e with the positive feedback term suppressed; i.e., the rate of LH synthesis was held constant and the only effect of E_2 was via negative feedback on release as described by Equation 1d. Solid curve is LH level in blood (scale on left) and dashed curve is the LH level in the releasable pool (scale on right). This demonstrates that a biphasic response in LH can be described by a model without positive feedback.

LH decreased slightly from steady state until an increase began between 2.5 and 3 days (Figure 8). An E_2 concentration of 200 ng/L was too low to significantly affect LH synthesis, so the slight rise in LH in this case was due to an LH increase in the pituitary (subsequent to inhibition of release), which then was released into the blood, as discussed in the Methods section. This constant or slightly increased level in serum LH was observed by Liu and Yen (19) and Odell and Molitch (35) after incremental infusion of low concentrations of estradiol. However, for an E_2 concentration of 300 ng/L, increased LH synthesis caused a dramatic increase in RP_{LH} , which produced an LH surge (Figure 8). Odell and Molitch (35) reported an abrupt increase in LH in castrate female rats 4 days after beginning administration of high doses of ethinyl estradiol. Liu and Yen (19) observed an LH surge within 12 hr of the time when E_2 reached a high concentration in the blood for both normal and hypogonadal women. Just before 4 days, serum LH began to decrease because falling E_2 results in less LH synthesis, which is consistent with Liu and Yen (19), who observed fluctuations in serum LH levels corresponding to fluctuations in their measurements of serum E_2 .

The FSH serum concentration in our two simulations decreased gradually as E_2 built up to a maximum as expected because of the quadratic inhibition of E_2 on release in our FSH system. The maximum E_2 concentration of 300 ng/L caused a somewhat more rapid FSH decrease than the 200-ng/L concentration (Figure 8). This same behavior was reported by Odell and Molitch (35) in experiments with rats. After day 3, the simulations

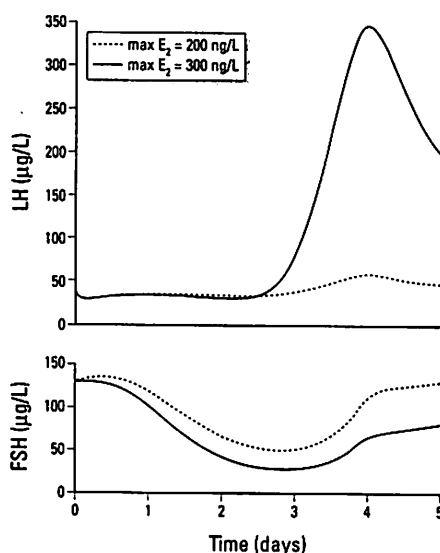


Figure 8. Predicted LH and FSH responses to E_2 challenge with maximum levels of 200 ng/L E_2 (dashed curves) or 300 ng/L E_2 (solid curves) and zero progesterone. (The time course for E_2 is depicted in Figure 6B.)

showed a steady increase in FSH due to the decrease in E_2 and the high level of FSH in the pituitary. The experiments of Liu and Yen (19) showed a similar increase in FSH that was concurrent with a 25% drop in serum E_2 from its peak.

Our final set of simulations corresponds to the experiment of Liu and Yen (19), where E_2 was gradually infused and after serum E_2 reached a significant level, a small amount of P_4 was infused. Odell and Molitch (35) reported similar experiments in which a single dose of progesterone was administered to rats after 4 days' administration of ethinyl estradiol. The E_2 concentration for our simulations increased to a maximum of 300 ng/L before decreasing as in the previous simulation (Figure 6, profile B). Liu and Yen (19) measured serum P_4 in their experiment, so we based our P_4 concentration profile on their data. Hence, we assumed that P_4 increased from a level of 1 pmol/mL beginning at day 2 to 8 pmol/mL at day 5 according to the function:

$$1 + 7 \cdot \exp[-0.5 \cdot (t - 5)^2]. \quad [6]$$

We compared LH and FSH serum levels resulting from this P_4 concentration with levels resulting from a constant P_4 concentration of 1 pmol/mL. Since P_4 promotes LH and FSH release, the higher P_4 concentration resulted in higher LH and FSH levels (Figure 9). This is consistent with observations of Liu and Yen (19) and Odell and Molitch (35). The difference in concentrations is much more pronounced with FSH because the P_4 release constant for our FSH model is much larger than that for our LH model (Table 1). P_4 does have a small negative effect on LH synthesis, but this was inconsequential for these simulations because of the time delay of 2.9 days.

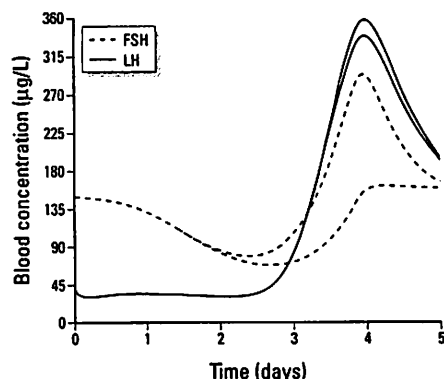


Figure 9. Predicted LH (solid curves) and FSH (dashed curves) responses to E_2 challenge (shown in Figure 6B) and P_4 infusion of either 1 pmol/mL (lower curve of each pair) or that described by Equation 6 (upper curve of each pair).

Discussion

In this study, we developed a mathematical model of gonadotropin synthesis and release that, despite its simplicity, reflects the current state of knowledge on the underlying mechanisms. Unlike many previous models, our model contains no absolute thresholds or switches; i.e., the system variables are continuous functions of the circulatory levels of ovarian steroids and peptides, although some of these functions exhibit strong nonlinearity. The model correctly describes the dynamics of the menstrual cycle. Since the parameters were selected to match this behavior, this only shows that the model structure is consistent with the data. However, the model was also shown to correctly reproduce responses observed in clinical studies in which estrogen and progesterone were given exogenously. In this case, the data were not used in setting the model parameters, so these results indicate that the model can be used to predict the response to exogenous chemicals that can activate estrogen or progesterone receptors.

Using a modified version of the model in which the positive effect of E_2 on LH synthesis was turned off, we also showed that the classic biphasic response in LH levels observed after bolus exposures to E_2 can be explained with a model that does not contain positive feedback. This demonstrates that this biphasic response, in and of itself, is not sufficient evidence that positive feedback occurs. In fact, more recent studies have clearly demonstrated the positive effect of E_2 on LH levels, and that simply blocking the inhibitory effect of E_2 on LH release is not sufficient to induce an LH surge of the magnitude observed at mid-cycle (37).

With regard to positive and negative feedback, the observation that low levels of E_2 inhibit or suppress LH serum levels, whereas high levels induce a surge in LH, has led to the conjecture that the underlying mechanism involves a switch from negative to positive feedback. The mechanism by which this switch might occur has not been elucidated, however. Further, most of the experimental evidence derives from observations of LH levels in serum, which is only an indirect measure of what is occurring in the pituitary. There are two ways in which such nonmonotonic behavior can arise: through a switch or nonmonotonic behavior in a single, regulated process (such as LH release) or as the result of two competing processes, one of which dominates at low concentrations, the other of which dominates at high concentrations. Our model is a quantitative implementation of the latter hypothesis. In particular, we presume that E_2 has a monotonic inhibitory effect on LH release and a monotonic positive effect on LH synthesis, with the inhibitory effect being dominant at low E_2

levels and the positive effect being dominant at high levels. Although we have not rigorously tested our model against all of the experimental data available in the literature, it appears thus far to be qualitatively consistent with that data.

Testing our hypothesis of competing positive and negative regulation would best be accomplished by careful measurement of both LH levels in the pituitary and serum LH levels in response to varying concentrations of E_2 . But measuring the timecourse of pituitary LH levels in humans or nonhuman primates would be at the least technically challenging and probably not ethically feasible. A direct experiment in rodents could be conducted. Our model also suggests that if lower levels of E_2 were maintained at a steady concentration (e.g., by an implant in an ovariectomized animal or individual at levels below those that induce surges), a steady LH serum level should be achieved (possibly requiring several days to reach that point) and this serum level should increase as a monotonic function of E_2 concentration. The key in conducting such experiments is that it is only with long-term observation (on the order of days) that one can truly determine the effect on synthesis compared to release, as at short periods of time synthesis may be faster or slower than release, but over long periods of time the two must match by virtue of conservation of mass.

Previously, the stability of model behavior was checked when repeated cyclic input from ovarian hormones was applied. Model behavior was found to be stable with time (7). Therefore the cycle it exhibits will neither collapse nor become wildly unpredictable if the model is run for long periods of time, which would be biologically realistic. Finally, this initial model can be readily expanded to include additional mechanisms and hence more complexity. The remainder of this discussion focuses primarily on ways in which the model can be expanded to include additional mechanistic detail.

The types of mechanisms one could consider adding to the model fall into three general categories: mechanisms of ovarian hormone regulation, mechanisms of gonadotropin clearance, and more detailed mechanisms within the hypothalamus and pituitary (including regulation by factors not included in the current model). A model for the regulation of synthesis, release, and circulatory levels of ovarian hormones was described previously (7). Empirical equations were used here to describe the concentrations of E_2 , P_4 , and Ih to keep this article to a reasonable length.

In the current model, clearance from the blood is presumed to be directly proportional to the blood concentration, with the proportionality constant fixed to a value reported in the open literature. Further, clearance is

presumed to occur from a single blood compartment whose volume is presumed to be the volume of distribution for the gonadotropins. Since LH and FSH are peptides, distribution outside the blood is likely to be limited. However, additional compartments, ancillary to the blood compartment, can be added either via traditional pharmacokinetics approaches or physiologically based pharmacokinetic modeling (38). The equation for elimination can be linear, as used here, or nonlinear if appropriate. Finally, the binding of both gonadotropins and steroid hormones to macromolecules (e.g., serum-binding hormones), which may serve to sequester them from clearance, can also be included as well as submodels describing the regulation of those macromolecules. Such an addition would require changing the current parameter values to reflect dependence on free rather than total blood concentrations. For example, if only 5% of E_2 is free because of serum binding, the concentration of E_2 used in the LH and FSH equations would be 0.05 times the total, but if we multiply by 20 the parameters that multiply E_2 in the LH and FSH models, the model predictions will be identical to those we have now. Thus, we do not expect inclusion of serum binding to have a significant impact on the structure or behavior of the model for LH and FSH once the parameters have been adjusted accordingly.

The place where additional mechanistic detail is likely to be most important is in the description of gonadotropin synthesis and release itself. Here we believe that the simplicity of the model is also a great strength. The model structure basically splits the process into the two primary steps, synthesis and release. By using relatively simple equations to describe these two steps, we are able to demonstrate the utility and significance of this model structure. Synthesis and release are clearly distinct processes, each with its own mechanism and control cascade (though some of the mechanisms may be shared). In each case, the current single step can be subdivided into multiple steps. For synthesis one could describe the transcription and degradation of mRNA, the rate of transcription to initial protein products, rates of post-translational modification for both the alpha and the beta protein subunits, the rate of dimerization of those subunits, and any rate of degradation of the mature protein. Similarly, one could explicitly build in rates of receptor binding and gene activation. One could separate out the control at the level of the hypothalamus, introducing a model of GnRH. Finally, one could also introduce models of peptide hormone binding to surface receptors and the subsequent signal transduction. All these details would then feed into the current model by replacing the current single equation for

synthesis. In short, the model is almost infinitely flexible. However, in the spirit of parsimony, we believe that the model should only be elaborated as needed to predict effects and processes of concern.

The delays for effects on synthesis (in particular, the delays of 2 days for I_h and 3 days for P_4) may well be unrealistically large. Because the term includes effects via the hypothalamic pulse generator, and possibly suprahypothalamic regions of the brain for which mechanisms are not well understood, it is possible that such delays occur. However, there is also the possibility that by raising the P_4 and I_h terms in the synthesis equations to powers higher than 1, a smaller delay can be used. This possibility will be explored during future model optimization.

It should be clear that the current model describes the behavior of an average individual and does not account for interindividual variability. The model does allow for intra-individual variability in that variations in E_2 , P_4 , and/or I_h levels will result in variations in LH and FSH levels. But both inter- and intraindividual variability is known to occur (39). The sources of variability are beyond the scope of this paper. However, interindividual variability could be accounted for by variations in the model parameters. We have not investigated the sensitivity of model responses to particular parameters, but it seems likely that this should be sufficient to account for observed variability. Intraindividual (i.e., month-to-month) variability in the current model would only be predicted as a result in variability in ovarian hormone levels (or exogenous compounds with hormone activity). For example, if the length of the ovarian hormone cycle used as input were changed, also changed would be the length of the predicted LH and FSH cycles. However, factors such as aging, stress, and diet are likely to change the responsiveness of the hypothalamus and/or pituitary to hormone signaling over time. This form of variability would have to be explained by variation in the model parameters just as for interindividual variability.

Finally, we recognize that nonsteroidal mechanisms of action apply for many exogenous compounds, but we believe that the current model will still be useful in health effects research. For example, the model can be used to test the hypothesis that the action of a xenobiotic can be explained as being due solely to its steroidal activity, and there are a number of compounds for which this is believed to be the principle mode of action. Extension of the model to describe a compound with nonsteroidal action would depend on the mechanism of the compound of interest, which is beyond the scope of this article.

In summary, we have developed a simple yet flexible model of gonadotropin synthesis and release for adult women. The model correctly predicts the time course of LH and FSH in the blood during the menstrual cycle. The model also reproduces several types of responses to exogenous hormone administration observed in clinical studies. The model should be useful in predicting the response of LH and FSH to compounds with a steroidal mechanism of action both in the pharmaceutical sciences and in toxicology. Mathematical models such as this can be used to simulate the effects of exogenous compounds on the sexual endocrine system of adult women. Simulations of this type may be helpful in evaluating hypotheses about the role of xenoestrogens in breast and ovarian cancers and may be useful for testing hormonal methods of birth control, which function by suppressing the mid-cycle surge in LH. Finally, simulations and experimentation with versions of these models coupled appropriately should help in understanding the phenomenon of menstrual cycle synchronization (40).

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