Modeling Endocrine Regulation of the Menstrual Cycle Using Delay Differential Equations

Leona A. Harris
Department of Mathematics and Statistics
The College of New Jersey, Ewing, NJ 08628
e-mail: harrisl@tcnj.edu
and
James F. Selgrade [1]
Department of Mathematics and Biomathematics Program
North Carolina State University, Raleigh, NC 27695-8205
e-mail: selgrade@math.ncsu.edu

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Abstract

This chapter develops a mathematical model describing blood levels of five hormones important for regulating the menstrual cycle of adult women. The resulting system of 13 nonlinear, delay, differential equations with 44 parameters correctly predicts the serum concentrations of ovarian and pituitary hormones found in the biological literature for normally cycling women. In addition to this normal cycle, the model exhibits another stable cycle which may describe a biologically feasible "abnormal" condition such as polycystic ovarian syndrome. Model simulations illustrate how one cycle can be perturbed to the other cycle. This model may be used to test the effects of external hormone therapies on abnormally cycling women as well as the effects of exogenous compounds on normally cycling women. Sensitive parameters are identified and bifurcations in model behavior with respect to changes in these parameters are examined. Modeling various aspects of menstrual cycle regulation should be helpful in predicting successful hormone therapies, in studying the phenomenon of cycle synchronization and in understanding many factors affecting the aging of the female reproductive endocrine system.

Chapter 1

1.1 Introduction

Complex endocrine signaling between the ovaries and the hypothalamus and pituitary glands is crucial for regulating and maintaining the female reproductive system of many mammals and birds. Abnormal levels of reproductive hormones often result in cycle irregularities. For instance, polycystic ovarian syndrome (PCOS), a leading cause of infertility in women [1, 2, 61], is usually associated with hormonal imbalances. Many PCOS women exhibit high androgen and low progesterone levels and their estrogen fluctuates very little during the month at levels which may be contraceptive [61]. Another example pertains to the observation that the breeding of dairy cows to maximize milk production is concurrent with a decrease in bovine fertility [39, 42, 43, 54]. There is evidence that high milk yield cows have lower amounts of progesterone and luteinizing hormone than cows which were not genetically engineered. Also, there is concern [10, 31, 44, 58] that environmental substances with estrogenic activity may disrupt the sexual endocrine system and, hence, may contribute to the increased incidence of breast cancer [11], to declines in sperm counts [52], and to developmental abnormalities [30]. Mathematical models may be used to simulate the effects of exogenous compounds and hormonal treatments on the reproductive endocrine system.

That the hypothalamus and pituitary glands are essential to the control of the female reproductive cycle was not known until the twentieth century (see Greep [16]). Much research (e.g., see [24, 33, 62, 63]) has been done to understand the physiological mechanisms involved in the regulation of the menstrual and estrous cycles. However, many aspects are not completely understood because of experimental difficulties in determining these mechanisms especially at the level of the hypothalamus and pituitary. Modeling various aspects of menstrual and estrous cycle regulation may be helpful in understanding the roles of the many components of the reproductive endocrine system and may assist the experimentalist by indicating directions of investigation.

Most mathematical models of cycle regulation track blood levels of hormones produced by the brain and the ovaries. Follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are produced by the pituitary gland responding to signaling from the hypothalamus, initiate the development of ovarian follicles and promote ovulation and the formation of the corpus luteum (see [24, 62, 63]). Simultaneously, at least three ovarian hormones, estradiol (E_2), progesterone (P_4), and inhibin (Inh), affect the synthesis and release of LH and FSH (see [25, 27, 56]). One of the early models of the female reproductive cycle was developed by N. Schwartz, 1970 [46], to describe the rat estrous cycle. Similar to humans, a surge in LH leads to ovulation but rats ovulate at night so Schwartz's model contains a 24 hour clock to force the right timing of ovulation. Another early model was published by Bogumil *et al.*, 1972 [4, 5], which consists of 34 algebraic and ordinary differential equations. In order to produce the LH surge, their model assumed that the pituitary produced 'tonic' and 'surge' amounts of LH. They also expressed a LH surge threshold in terms of convolution integrals to weight more heavily recent concentrations of E_2 and P_4. Subsequent models of cycle regulation include McIntosh and McIntosh, 1980 [29] , and Plouffe and Luxenberg, 1992 [38]. For articles which review the literature on mathematical models of the menstrual cycle and the estrus cycle, see Chávez-Ross [7] and Vetharaniam *et al.* [55]. All of these models describe some biological mechanisms but also many contain artificial features such as clocks or convolution integrals.

Over the last decade, we have developed and analyzed a mechanistic, deterministic, mathematical model ([19, 20, 37, 45, 49, 50, 51]) which predicts average serum concentrations of FSH, LH, E_2, P_4 and Inh that agree with data in the biological literature for normally cycling adult women (McLachlan *et al.* [32]). Because of the interplay between the brain and ovaries, this system may be described as dual control. Hence the modeling procedure is divided into three distinct steps. First we derive a linear system of ordinary differential equations for the synthesis and release of FSH and LH in the pituitary which respond to the signaling of the ovarian hormones E_2, P_4 and Inh. The McLachlan data [32] are used to obtain explicit time-periodic input functions for serum levels of E_2, P_4, and Inh and the unknown state variables in the system of differential equations are FSH and LH. Then the parameters of this system are estimated from the McLachlan data for FSH and LH using a numerical optimization routine such as Nelder-Meade [34, 59] with least squares. The second step reverses this process by developing a model for the monthly cyclic changes in the ovarian hormones E_2, P_4 and Inh under the influence of the pituitary hormones FSH and LH. This linear system of differential equations for the ovarian hormones contains parameters and time-periodic input functions for FSH and LH. Parameter identification is performed on this system using the data from McLachlan *et al.* [32] for E_2, P_4 and Inh. With a complete set of parameters determined, the final step

is to merge these two linear systems into one system which is highly nonlinear because all the variables are considered as state variables.

As an illustration of this process, suppose that experimental data over a span of time, t, are available for two state variables x and y, where $x = x(t)$ and $y = y(t)$. First we derive a single differential equation describing the rate at which the state variable x is changing with respect to t based on known biological interactions between x, y, and $\frac{dx}{dt}$. The resulting equation contains an explicit input function $y(t)$, derived empirically from the data set, and contains unknown parameters values, $p_1, p_2, \ldots, p_n$. Equation (1.1.1) gives an example of such a differential equation with two parameters p_1 and p_2:

$$\frac{dx}{dt} = \frac{p_1\, y(t)}{p_2 + y(t)}\, x. \tag{1.1.1}$$

Notice that the differential equation in (1.1.1) is linear in the state variable x and has a time-dependent coefficient function $\frac{p_1\, y(t)}{p_2+y(t)}$. The existing experimental data for x are used to estimate the parameters p_1 and p_2 by applying a parameter identification numerical routine in conjunction with numerically solving the differential equation for $x = x(t)$.

The procedure is then reversed by introducing a differential equation for the state variable y which contains an explicit empirical approximation for $x(t)$ derived from the data and which contains unknown parameters, say p_3 and p_4. An example of such a differential equation is given by (1.1.2):

$$\frac{dy}{dt} = p_3\, x(t)\, y + p_4\, x^2(t). \tag{1.1.2}$$

Here experimental data for y and a parameter identification routine are used to estimate p_3 and p_4 and numerically solve for $y = y(t)$. With these estimates for the four model parameters, p_1, p_2, p_3, and p_4, Equations (1.1.1) and (1.1.2) are then merged together to create the following system of differential equations in (1.1.3) which describes the rates at which the state variables x and y are changing with respect to time:

$$\begin{aligned} \frac{dx}{dt} &= \frac{p_1\, x\, y}{p_2 + y} \\ \frac{dy}{dt} &= p_3\, x\, y + p_4\, x^2 \end{aligned} \tag{1.1.3}$$

Notice that the system of differential equations in (1.1.3) is nonlinear in two state variables x and y as compared to the single, linear differential equations in (1.1.1) and (1.1.2) . It is important to note here that in order to fit this system of differential equations to the existing data for x and y simultaneously, it may be necessary to re-estimate all four parameters in (1.1.3), but the estimates already obtained serve as a good starting place.

After discussing biological background, we present the three components of our menstrual cycle model in detail.

1.2 Biological Preliminaries

Typically, a woman is born with from 500,000 to 700,000 primordial follicles and this number decreases due to atresia with an increasing decay rate as the woman ages (e.g., see Hansen et al. 2008 [18]). During her reproductive life only a small number of these follicles develop to ovulatory status before the onset of menopause, which occurs at an average age of 51. The length of a normal menstrual cycle (Figure 1.2.1) for an adult woman is 28 days on average but may range from 25 to 35 days (Ojeda [36]). The cycle is divided into the follicular phase (roughly 14 days), ovulation and the luteal phase (roughly 14 days). The brain regulates ovarian cycling via the hypothalamus and the pituitary glands. The hypothalamus produces gonadotropin-releasing hormone (GnRH) which modulates the pituitary's secretion of the gonadotropin hormones FSH and LH (see Clayton *et al.* [9]). To simplify our model we lump the effects of the hypothalamus and the pituitary together and just consider the synthesis and release of FSH and LH. These hormones are secreted in a pulsatile pattern on the time scale of minutes but, because the ovaries respond to average daily blood levels (Odell [35]), our model tracks average daily gonadotropin concentrations in the blood. As part of its normal function, the ovary produces E_2, P_4, and Inh, which control the pituitary's synthesis and release of the gonadotropin hormones during the various stages of the cycle (Figure 1.2.1).

The follicular phase of the cycle begins with the first day of menstrual flow, when blood levels of FSH rise and promote the recruitment and growth of 6 to 12 immature follicles. As these follicles develop by adding layers of granulosa cells (Odell [35]), the production of E_2 increases. During the second third of the follicular phase, typically a single dominant follicle is selected to continue its development and ultimately to release its ovum and the remaining follicles begin to atrophy. We do not model the process of follicle selection because the biological mechanism is not understood. As the ovaries pass into the primary follicular stage, the dominant follicle grows more rapidly and produces E_2 in large amounts. During the first two-thirds of the follicular phase, LH levels are roughly constant. But E_2 primes the pituitary for gonadotropin synthesis and, one day after E_2 reaches its maximum, LH peaks at approximately 10 times its early follicular concentration. This rapid rise and fall of LH over a period of 5 days is referred to as the LH surge and is necessary for ovulation. The day of the LH peak is considered the midpoint of the menstrual cycle and hormone data are usually centered at the day of LH

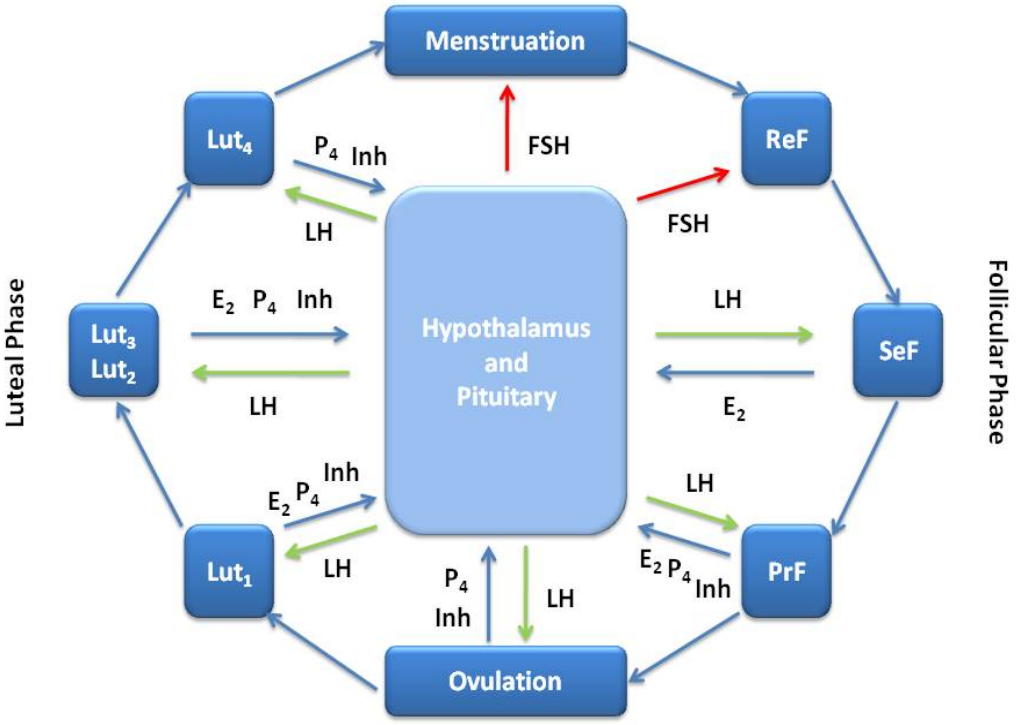

Figure 1.2.1: The Follicular and Luteal Phases of the Menstrual Cycle. The outer ring depicts various stages of the ovary during a monthly cycle. ReF, SeF and PrF represent the recruited, secondary and primary follicle and Lut_i, i=1...4, represent the corpus luteum. Directed arrows indicate hormonal actions.

surge before averaging is done or comparisons made. After a significant decrease during the primary follicular stage, FSH also surges concurrently with LH.

Ovulation occurs within a day after the LH surge [36] and, hence, the dominant follicle is transformed into the corpus luteum. The corpus luteum ("yellow body") secretes hormones in preparation for pregnancy and is characterized by increased fat storage in the theca and granulosa cells. P_4, which is low during the follicular phase, begins to rise several days before ovulation and continues to increase to a maximum midway through the luteal phase. The Inh profile is similar to that of P_4. During the luteal phase P_4 and Inh inhibit the synthesis of LH and FSH, respectively, so that no immature follicles begin to grow [8, 32]. If fertilization does not occur then the corpus luteum decreases in size and hormone secretion and becomes inactive by the end of the month. The decline of the

corpus luteum results in a decrease in P_4 and Inh and, consequently, the removal of the inhibition on LH and FSH synthesis. The resulting gradual rise in FSH at the end of the month promotes the growth of a new cohort of immature follicles and initiates the next cycle.

1.3 Model Development

Our modeling approach is divided into three components: the pituitary model, the ovarian model and the merged model. The pituitary model describes the production of pituitary hormones LH and FSH during the menstrual cycle in response to circulating ovarian hormones E_2, P_4 and Inh (inputs to the model). The ovarian model describes follicular and luteal development during the menstrual cycle and the production of the ovarian hormones in response to the pituitary hormones LH and FSH (inputs to the model). Each of these models are linear systems of differential equations with time-dependent coefficients (inputs) that are derived empirically from existing clinical data. The third component of the modeling process involves merging the pituitary and ovarian models together, creating a 13-dimensional, highly-nonlinear, autonomous (time-independent) system of differential equations that describes the stages of the menstrual cycle and the interactions of all five hormones during the menstrual cycle while eliminating the use of input functions derived from clinical data.

1.3.1 The Pituitary Model: Systems of Differential Equations

The pituitary model, first developed by Schlosser and Selgrade [45], describes the synthesis, release, and clearance of LH and FSH based on the pituitary's response to circulating levels of the ovarian hormones E_2, P_4 and Inh. The model consists of two systems, the LH system and the FSH system, of ordinary differential equations with time-dependent coefficients. Each system is linear in its state variables, however the time-dependent coefficients are nonlinear functions of the ovarian hormones. Functions that approximate clinical study data (McLachlan *et al.* [32]) for the daily mean serum levels of E_2, P_4 and Inh during the menstrual cycle of 33 normally cycling women are used as inputs to the pituitary systems in order to predict the serum levels of LH and FSH during that cycle. Because the McLachlan data contain hormone values for 31 consecutive days, we assume a menstrual cycle of period 31 days and use the following input functions to approximate the ovarian hormone profiles over two menstrual periods:

$$E_2(t) = 62.5 + 230e^{-\frac{(t-14)^2}{5}} + 115e^{-\frac{(t-23)^2}{20}} + 230e^{-\frac{(t-45)^2}{5}} + 115e^{-\frac{(t-54)^2}{20}} \quad (1.3.1)$$

$$P_4(t) = 0.8 + 52.24e^{-\frac{(t-22)^2}{19.15}} + 52.24e^{-\frac{(t-53)^2}{19.15}} \quad (1.3.2)$$

$$Inh(t) = 290 + 1401.5e^{-\frac{(t-22)^2}{15}} + 1401.5e^{-\frac{(t-53)^2}{15}} \quad (1.3.3)$$

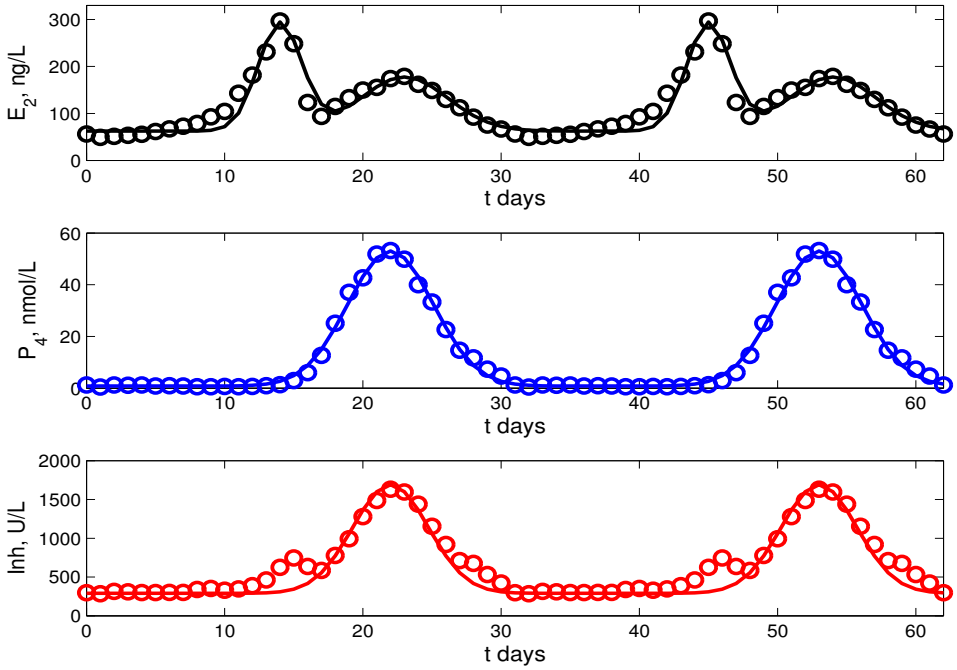

Figure 1.3.1: Open circles are daily mean serum levels of estradiol, progesterone and inhibin of 33 normally cycling women as measured by McLachlan *et al.* [32]. Time-dependent functions (solid curves) approximating these values over two menstrual cycles are used as inputs to the LH and FSH systems.

The ovarian input functions are graphed against the ovarian hormone data in McLachlan *et al.* [32] over two menstrual cycles in Figure 1.3.1. In the McLachlan data, the follicular phase E_2 peak occurred at day 14 and the luteal peak occurred at day 23. To produce these elevations in $E_2(t)$, we use negative exponential functions where the exponents are translated to days 14 and 45 for the follicular phases and translated to days 23 and 54 for the luteal phases of two cycles. The input functions $P_4(t)$ and $Inh(t)$ are constructed in a similar manner.

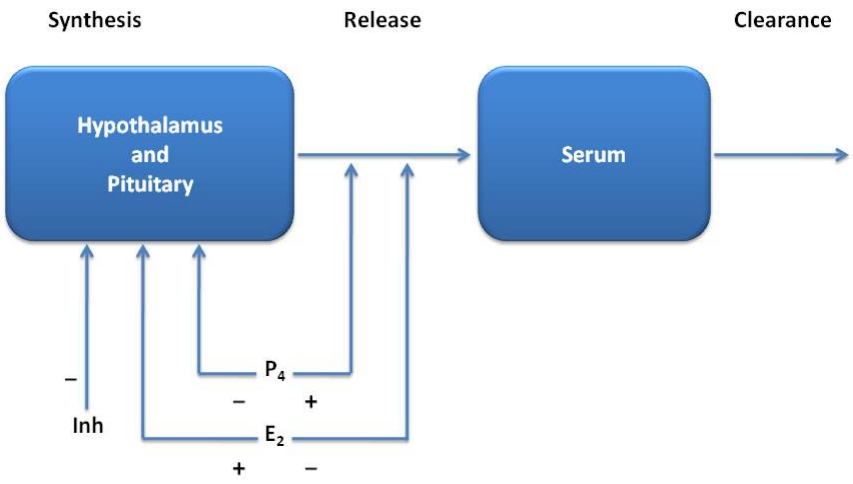

Figure 1.3.2: The ovarian hormones control synthesis and release of LH and FSH in the brain. Plus arrows indicate stimulation and minus arrows indicate inhibition.

The pituitary systems of differential equations model the synthesis, release, and clearance of LH and FSH, in response to stimulatory and inhibitory effects of the ovarian hormones. The schematic diagram in Figure 1.3.2 illustrates the effects of circulating levels of E_2, P_4 and Inh and outlines two major modeling assumptions: (1) LH and FSH synthesis occurs in the pituitary and (2) LH and FSH are held on reserve in the pituitary in what we call the "reserve pool" awaiting release into the bloodstream.

The LH system of differential equations has two state variables, RP_{LH}, representing the amount of LH in the reserve pool awaiting release into the bloodstream, and LH, representing the concentration of LH in the blood. In the model, the synthesis and release rates of LH are described as rational functions of ovarian hormones in which stimulatory effects appear in the numerators and inhibitory effects appear in the denominators.

It has been shown that high blood levels of estradiol promote rapid LH synthesis, therefore the numerator of the LH synthesis term contains a Hill function (see Equation (1.3.6)) to reflect estradiol's stimulatory effect on LH. This effect is most evident in the late follicular phase of the menstrual cycle when large amounts of estradiol are secreted

by the dominant follicle, inducing the LH surge. This Hill function was selected because it increases rapidly as estradiol concentrations vary within a range of 200 and 600 pg/mL during the late follicular phase. This range includes normal and elevated levels of estradiol [45] and therefore the model can be used to monitor the effects of administering exogenous estrogens to existing estradiol levels. The exponent in the Hill function, called the Hill coefficient, was chosen to be $h = 8$ so that the Hill function begins increasing around 200 pg/mL and reaches its maximum around 600 pg/mL. It can easily be shown that if the Hill coefficient is $h = 9$, the synthesis rate increases too rapidly and if $h = 7$ the increase is not rapid enough. During the luteal phase of the cycle estradiol blood levels peak for a second time, however, this peak is not as substantial as the late follicular phase peak. It is believed that during this time progesterone blood levels inhibit LH synthesis [48]. The period of time between changes in estradiol and progesterone blood levels and changes in the synthesis rate of LH is captured by incorporating time delays, δ_E and δ_P, into the input functions $E_2(t)$ and $P_4(t)$ which appear in the LH synthesis term.

It has also been shown that estradiol and progesterone have similar effects on the release of LH and FSH into the bloodstream. A study by Chang and Jaffe [6] showed that progesterone stimulates the release of LH and FSH when estradiol blood levels are in a normal range during the late follicular phase. Tsai and Yen [53] demonstrated that blood levels of LH and FSH decline after the administration of ethinyl estradiol. This suggests that estradiol inhibits the release of LH and FSH into circulation. Finally, the clearance rate of LH is assumed to be proportional to LH blood levels. Therefore the equations that govern the synthesis, release, and clearance of LH have the form:

$$\frac{d}{dt}RP_{LH} = syn_{LH}(E_2, P_4) - rel_{LH}(E_2, P_4, RP_{LH}) \tag{1.3.4}$$

$$\frac{d}{dt}LH = \frac{1}{v}\, rel_{LH}(E_2, P_4, RP_{LH}) - clear_{LH}(LH) \tag{1.3.5}$$

where

$$syn_{LH}(E_2, P_4) = \frac{V_{0,LH} + \dfrac{V_{1,LH}\, E_2(t-d_E)^8}{Km_{LH}^8 + E_2(t-d_E)^8}}{1 + P_4(t-d_P)/Ki_{LH,P}}, \tag{1.3.6}$$

$$rel_{LH}(E_2, P_4, RP_{LH}) = \frac{k_{LH}\ [1 + c_{LH,P}\, P_4(t)]\ RP_{LH}}{1 + c_{LH,E}\, E_2(t)}, \tag{1.3.7}$$

$$clear_{LH}(LH) = a_{LH}\, LH. \tag{1.3.8}$$

The compartmental structure of the FSH system of differential equations is identical to that of the LH system of differential equations with state variables, RP_{FSH}, representing the amount of FSH in the reserve pool, and FSH, representing the concentration of FSH in the blood. However there are variations in the synthesis and release terms because FSH responds differently to the ovarian hormones. There is evidence that inhibin has an inhibitory effect on FSH synthesis [17, 21, 32, 47] and, as with $E_2(t)$ and $P_4(t)$ in the LH synthesis term, a time delay δ_{Inh} is used in the input function $Inh(t)$ which appears in the denominator of the FSH synthesis term (see Equation (1.3.11)).

Recall that estradiol and progesterone have similar effects on the release of LH and FSH into the bloodstream: estradiol inhibits the release of LH and FSH and progesterone stimulates the release of LH and FSH. Tsai and Yen [53] also showed that estradiol has a greater inhibitory effect on FSH release. In addition, the preovulatory decline in FSH blood levels, not present in the LH profile, provides further evidence of the greater inhibitory effect of rising estradiol levels in the late follicular phase of the cycle. Therefore a second order inhibitory effect of estradiol on FSH release is used in the FSH system of differential equations instead of the first order effect used in the LH equations [45, 51]. Finally, the clearance rate of FSH is assumed to be proportional to FSH blood levels. Therefore the equations that govern the synthesis, release, and clearance of FSH are given by:

$$\frac{d}{dt}RP_{FSH} = syn_{FSH}(Inh) - rel_{FSH}(E_2, P_4, RP_{FSH}) \tag{1.3.9}$$

$$\frac{d}{dt}FSH = \frac{1}{v} rel_{FSH}(E_2, P_4, RP_{FSH}) - clear_{FSH}(FSH) \tag{1.3.10}$$

where

$$syn_{FSH}(Inh) = \frac{V_{FSH}}{1 + Inh(t - d_{Inh})/Ki_{FSH,Inh}} \tag{1.3.11}$$

$$rel_{FSH}(E_2, P_4, RP_{FSH}) = \frac{k_{FSH}\ [1 + c_{FSH,P}\, P_4(t)]\ RP_{FSH}}{1 + c_{FSH,E}\, (E_2(t))^2} \tag{1.3.12}$$

$$clear_{FSH}(FSH) = a_{FSH}\, FSH \tag{1.3.13}$$

1.3.2 The Ovarian Model: System of Differential Equations and Auxiliary Equations

The ovarian model, first developed by Selgrade and Schlosser [51], describes nine stages in the monthly development of the ovary and the production of the ovarian hormones E_2, P_4 and Inh. The model consists of a linear, time-dependent system of nine ordinary differential equations that represent the active capacities of follicular and luteal tissue to produce hormones under the influence of the pituitary hormones. Here "active" means actively growing and secreting hormones. The follicular phase of the menstrual cycle is divided into three stages: the recruited follicular stage ReF, the secondary follicular stage SeF, and the primary follicular stage PrF. Ovulation and luteinization are represented by two ovulatory follicular stages: Ov_1 and Ov_2. The luteal phase of the cycle is represented by four stages of luteal development: Lut_i where $i = 1 \ldots 4$.

The pituitary hormones stimulate the growth of follicular tissue within a stage and the transfer of follicular tissue from one stage to the next as indicated in Figure 1.3.3. The capacity to produce hormones at each stage of the cycle is assumed to be proportional to the mass of the ovarian follicles or corpus lutea at that stage and therefore, the schematic diagram of the ovarian model in Figure 1.3.3 also illustrates the stages of luteal tissue development and the production of E_2, P_4 and Inh by the secondary follicles, primary follicle and the corpus luteum.

Functions that approximate the date in McLachlan *et al.* [32] for the daily mean serum levels of LH and FSH are used as inputs to the ovarian system in Equations (1.3.16) - (1.3.24) in order to predict the serum levels of E_2, P_4 and Inh during that cycle. Using the McLachlan data, we assume a menstrual cycle of period 31 days and use the following input functions to approximate the pituitary hormone profiles over two menstrual periods:

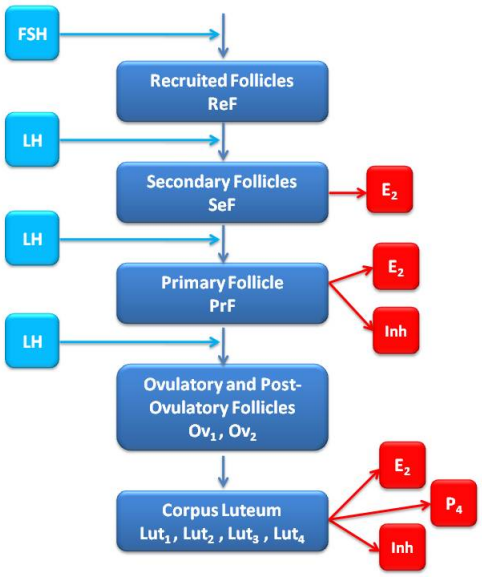

Figure 1.3.3: The compartments represent stages of follicular and luteal development during one menstrual cycle. FSH and LH promote growth and transition between stages. The ovarian stages secrete hormones as indicated on the right.

$$\begin{aligned} FSH(t) &= 175e^{\frac{-(t-5)^2}{110}} + 210e^{-(t-15)^2} + 65e^{\frac{-(t-18)^2}{20}} + 174.85e^{\frac{-(t-36)^2}{121}} \\ &\quad 205e^{-(t-46)^2} + 65e^{\frac{-(t-49)^2}{20}} + 175e^{\frac{-(t-67)^2}{130}}, \end{aligned} \tag{1.3.14}$$

$$\begin{aligned} LH(t) &= 0.156 + 24.38e^{\frac{-(t-7)^2}{230}} + 332.12e^{-(t-15)^2} + 17.24e^{\frac{-(t-18)^2}{35.16}} + \\ &\quad 24.38e^{\frac{-(t-38)^2}{230}} + 332.12e^{-(t-46)^2} + 17.24e^{\frac{-(t-49)^2}{35.16}} + 24.38e^{\frac{-(t-69)^2}{230}} \end{aligned} \tag{1.3.15}$$

These functions are used to approximate the pituitary hormone profiles in McLachlan *et al.* [32] over two menstrual periods as seen in Figure 1.3.4 and are used as inputs to the

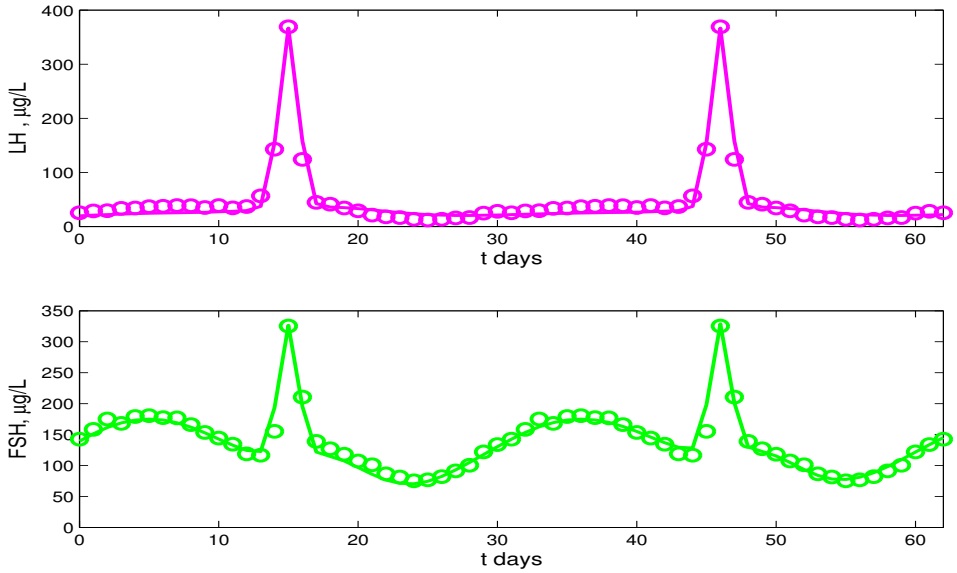

Figure 1.3.4: Daily mean serum levels of LH and FSH for 33 normally cycling women as measured by McLachlan *et al.* [32]. Time dependent functions approximating these values over two menstrual cycles are as used as inputs to the ovarian model.

following system of ordinary differential equations representing the ovarian model:

$$\frac{d}{dt}\,ReF = b\,FSH(t) + \left[c_1\,FSH(t) - c_2\,(LH(t))^{\alpha}\right]\,ReF \tag{1.3.16}$$

$$\frac{d}{dt}\,SeF = c_2\,(LH(t))^{\alpha}\,ReF + \left[c_3\,(LH(t))^{\beta} - c_4\,LH(t)\right]\,SeF \tag{1.3.17}$$

$$\frac{d}{dt}\,PrF = c_4\,LH(t)\,SeF - c_5\,(LH(t))^{\gamma}\,PrF \tag{1.3.18}$$

$$\frac{d}{dt}\,Ov_1 = c_5\,(LH(t))^{\gamma}\,PrF - d_1\,Ov_1 \tag{1.3.19}$$

$$\frac{d}{dt}\,Ov_2 = d_1\,Ov_1 - d_2\,Ov_2 \tag{1.3.20}$$

$$\frac{d}{dt}\,Lut_1 = d_2\,Ov_2 - k_1\,Lut_1 \tag{1.3.21}$$

$$\frac{d}{dt}\,Lut_2 = k_1\,Lut_1 - k_2\,Lut_2 \tag{1.3.22}$$

$$\frac{d}{dt}\,Lut_3 = k_2\,Lut_2 - k_3\,Lut_3 \tag{1.3.23}$$

$$\frac{d}{dt}\,Lut_4 = k_3\,Lut_3 - k_4\,Lut_4\ . \tag{1.3.24}$$

The first term $b\,FSH(t)$ in Equation (1.3.16) initiates the recruitment and growth of

inactive antral follicles. During the follicular phase of the cycle, follicular growth rates and transfer rates are assumed to be proportional to $FSH(t)$ and powers of $LH(t)$ as indicated by Equations (1.3.16) - (1.3.19). The transition from the secondary follicular stage to the primary follicular stage depends on LH serum levels as indicated in Equations (1.3.17) - (1.3.18) and corresponds to the selection of the dominant follicle. Since ovulation and luteinization of the primary follicle are processes that are not instantaneous events [35] they are represented by two stages of ovulatory follicular development, Ov_1 and Ov_2. Little hormone production is assumed during this time. Finally, the model divides the luteal phase of the cycle into four stages represented by Equations (1.3.21) - (1.3.24), and reflects the corpus luteum as the primary source of P_4 and Inh production.

Because the clearance of the ovarian hormones from the blood is rapid compared to the clearance of the pituitary hormones, we assume that the blood levels of the ovarian hormones are at quasi-steady state [26] and their concentrations are modeled as linear combinations of the appropriate ovarian stages of the cycle. The following three auxiliary equations represent the serum levels of the ovarian hormones:

$$E_2 = e_0 + e_1\, SeF + e_2\, PrF + e_3\, Lut_4 \tag{1.3.25}$$

$$P_4 = p_0 + p_1\, Lut_3 + p_2\, Lut_4 \tag{1.3.26}$$

$$Inh = h_0 + h_1\, PrF + h_2\, Lut_3 + h_3\, Lut_4 \tag{1.3.27}$$

Because of the form of Equations (1.3.25) - (1.3.27), the effect of an exogenous ovarian hormone on the menstrual cycle may be simulated by adding a function representing an amount of that hormone to the appropriate equation.

1.3.3 The Merged Model

The third and final step of the modeling process, as developed by Harris-Clark et al. [20], is to merge the pituitary model and ovarian model together to create a single 13-dimensional system of nonlinear, delay differential equations (1.3.28)-(1.3.40) with three auxiliary equations (1.3.25) - (1.3.27). The merged system has the form:

$$\frac{d}{dt}RP_{LH} = \frac{V_{0,LH} + \dfrac{V_{1,LH}\,E_2(t-d_E)^8}{Km_{LH}^8 + E_2(t-d_E)^8}}{1 + P_4(t-d_P)/Ki_{LH,P}} - \frac{k_{LH}\,[1 + c_{LH,P}\,P_4]\,RP_{LH}}{1 + c_{LH,E}\,E_2} \tag{1.3.28}$$

$$\frac{d}{dt}LH = \frac{1}{v}\frac{k_{LH}\,[1 + c_{LH,P}\,P_4]\,RP_{LH}}{1 + c_{LH,E}\,E_2} - a_{LH}\,LH \tag{1.3.29}$$

$$\frac{d}{dt}RP_{FSH} = \frac{V_{FSH}}{1 + Inh(t-d_{Inh})/Ki_{FSH,Inh}} - \frac{k_{FSH}\,[1 + c_{FSH,P}\,P_4]\,RP_{FSH}}{1 + c_{FSH,E}\,E_2^2} \tag{1.3.30}$$

$$\frac{d}{dt}FSH = \frac{1}{v}\frac{k_{FSH}\,[1 + c_{FSH,P}\,P_4]\,RP_{FSH}}{1 + c_{FSH,E}\,E_2^2} - a_{FSH}\,FSH \tag{1.3.31}$$

$$\frac{d}{dt}ReF = b\,FSH + [c_1\,FSH - c_2\,LH^{\alpha}]\;ReF \tag{1.3.32}$$

$$\frac{d}{dt}SeF = c_2\,LH^{\alpha}\,ReF + \left[c_3\,LH^{\beta} - c_4\,LH\right]\;SeF \tag{1.3.33}$$

$$\frac{d}{dt}PrF = c_4\,LH\,SeF - c_5\,LH^{\gamma}\,PrF \tag{1.3.34}$$

$$\frac{d}{dt}Ov_1 = c_5\,LH^{\gamma}\,PrF - d_1\,Ov_1 \tag{1.3.35}$$

$$\frac{d}{dt}Ov_2 = d_1\,Ov_1 - d_2\,Ov_2 \tag{1.3.36}$$

$$\frac{d}{dt}Lut_1 = d_2\,Ov_2 - k_1\,Lut_1 \tag{1.3.37}$$

$$\frac{d}{dt}Lut_2 = k_1\,Lut_1 - k_2\,Lut_2 \tag{1.3.38}$$

$$\frac{d}{dt}Lut_3 = k_2\,Lut_2 - k_3\,Lut_3 \tag{1.3.39}$$

$$\frac{d}{dt}Lut_4 = k_3\,Lut_3 - k_4\,Lut_4\;. \tag{1.3.40}$$

where the ovarian hormone functions E_2, P_4, and Inh in Equations (1.3.28)-(1.3.31) are linear combinations of the ovarian state variables, as defined by the auxiliary equations (1.3.25) - (1.3.27). The pituitary hormone functions LH and FSH in Equations (1.3.32)-(1.3.35) are the pituitary state variables represented by Equations (1.3.29) and (1.3.31). Therefore the merged system is an autonomous system of differential equations since there are no time-dependent inputs to the system of differential equations as there were in the unmerged, pituitary and ovarian models. In addition, the merged system is nonlinear because many of the equations involve nonlinear functions of the state variables as opposed to the unmerged models where the nonlinearities appeared in the time-dependent

coefficients of the linear differential equations. Finally, the merged system involves delay differential equations as the ovarian state variables are delayed in the LH and FSH synthesis terms (see Equations (1.3.28) and (1.3.30)).

1.4 Parameter Estimation and Model Simulations

In order to study the dynamical behavior of the merged model, estimates of the 44 model parameters are obtained either from the literature or through a parameter estimation scheme. The only known model parameters are the clearance rates for LH and FSH and the blood volume v, and the remaining 41 model parameters were estimated using daily mean serum levels of LH, FSH, estradiol, progesterone, and inhibin of 33 normally cycling women as measured by McLachlan *et al.* [32]. To estimate the 15 unknown pituitary parameters in Equations (1.3.4)-(1.3.12), Harris-Clark *et al.* [20] applied the Nelder-Mead Method in MATLAB to a least square cost function in order to fit the pituitary model to the LH and FSH data in McLachlan *et al.* [32]. To estimate the ovarian model parameters, Harris-Clark *et al.* [20] estimated the 15 ovarian system parameters in Equations (1.3.16)-(1.3.24) and the 4 estradiol parameters in Equations (1.3.25) using the Nelder-Mead Method and a least squares cost function that fit the ovarian system and estradiol auxiliary equation to the E_2 data in McLachlan *et al.* [32]. Then the remaining 7 parameters in the auxiliary equation for progesterone and inhibin, Equations (1.3.26)-(1.3.27), were estimated using separate least squares cost functions for P_4 and Inh. For a complete description of the parameter estimation process, refer to Harris-Clark *et al.* [20] and Harris [19].

Once the pituitary and ovarian models are merged together, the 44 parameters obtained in the preceding two steps are then used as estimates of the merged model parameters. Numerical simulations of the merged model were run in MATLAB using the delay differential equation solver *dde23* to analyze the model output. These simulations are discussed in detail in [20, 50] and will be described briefly here. Using appropriate initial conditions, we observe the existence of two locally asymptotically stable periodic solutions for the same set of parameter values. One is a large amplitude solution with a period of 29.5 days that approximates the McLachlan data for normally cycling women. See Figure 1.4.1 for graphs of the model simulations of E_2 and LH as compared to the data. We refer to this solution as the normal cycle. The second is a smaller amplitude solution that has a period of 24 days and represents an abnormal menstrual cycle. Because there is no LH surge, the abnormal cycle is anovulatory and its acyclic E_2 profile suggests the possibility of PCOS [61] (see the dashed LH and E_2 curves in Figures 1.4.2 and 1.4.3 which compare

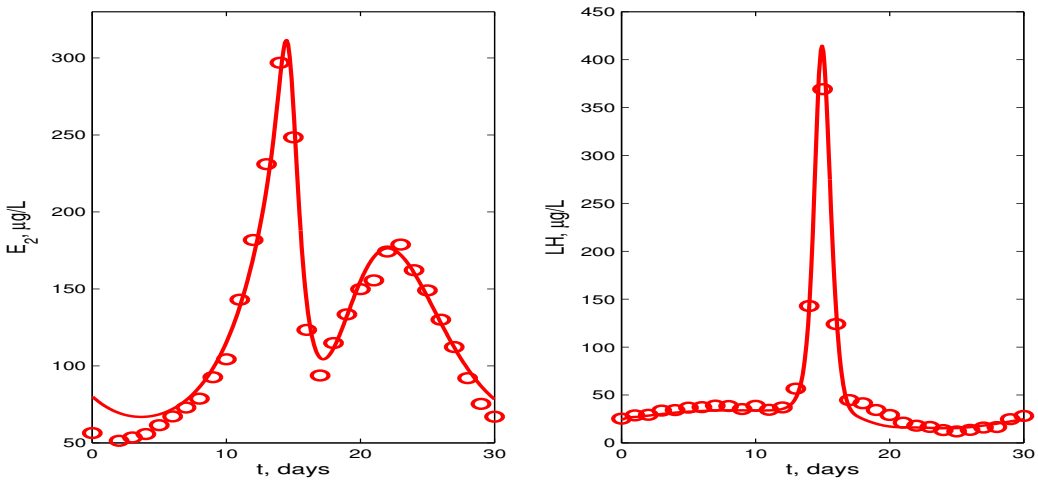

Figure 1.4.1: Simulations (solid curves) of the merged model, Equations (1.3.28)-(1.3.40), giving the normal cycle compared to clinical data (open circles) in McLachlan *et al.* [32].

the hormone profiles of the normal and abnormal cycles over 120 days).

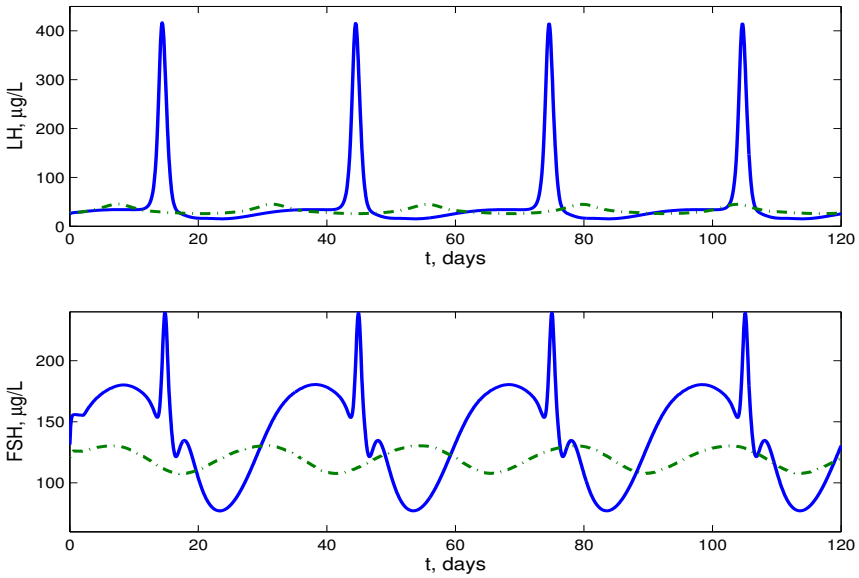

Figure 1.4.2: Profiles of pituitary hormones for the normal (solid curves) and abnormal (dashed curves) cycles. Notice that the abnormal cycle has no LH surge and therefore is anovulatory.

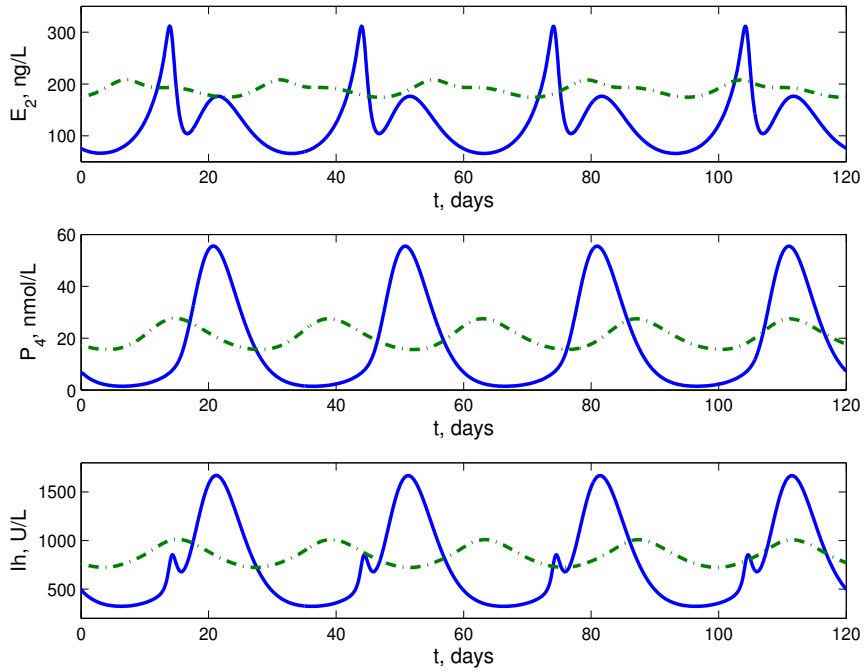

Figure 1.4.3: Profiles of ovarian hormones for the normal (solid curves) and abnormal (dashed curves) cycles.

1.5 Sensitivity Analysis and Bifurcation Analysis: Perturbing the Model Parameters

Since the Nelder Mead Method was used to search for a parameter set that minimized the least square cost functions locally, it is quite possible that other parameter sets exist that fit the data well. As such it is important to determine how sensitive the model is to changes in the model parameters. A local sensitivity analysis of the model parameters was performed by Selgrade *et al.* [50] to determine the effects of small variations in the model parameters on model outputs. In this analysis, normalized sensitivity coefficients were measured by discrete changes in a model output relative to the output value divided by changes in a model parameter relative to the parameter value. For example, if the original value of the parameter p is increased by 1% and a model output is denoted by a function of p, $MO(p)$, then the normalized sensitivity coefficient is computed according to the formula:

$$S(p) = \frac{\triangle MO}{MO}\,\frac{p}{\triangle p} = \frac{MO(1.01p) - MO(p)}{MO(p)}\,\frac{p}{0.01p} = 100\,\frac{MO(1.01p) - MO(p)}{MO(p)}\,. \quad (1.5.1)$$

This coefficient approximates the partial derivative of some model output, a function of the model state variables, with respect to a model parameter that is normalized so that comparisons may be made across model outputs and across model parameters.

Selgrade *et al.* [50] decided to use the height of the E_2 mid-cycle peak along the normal cycle as the model output in this analysis because a significant follicular phase rise in E_2 stimulates the secretion of LH and causes the LH surge, which is necessary for ovulation and normal ovarian function. After computing normalized sensitivity coefficients for the 44 model parameters, Selgrade *et al.* [50] found that with respect to the E_2 mid-cycle peak there were six parameters most sensitive to small variations: $\alpha, Km_{LH}, c_2, V_{FSH}, c_1$, and $V_{0,LH}$. To further study the impact of perturbing sensitive model parameters, a bifurcation analysis was performed to determine the effects that variations of the parameter values have on the existence of the two locally asymptotically stable periodic solutions observed in Figures 1.4.2 and 1.4.3. Selgrade *et al.* [50] chose the parameter Km_{LH} for this analysis because of its physiological significance.

The bifurcation diagram for the merged model is shown in Figure 1.5.1, where the vertical axis denotes the difference between the maximum and the minimum of the first state variable, RP_{LH}, along a periodic solution or at an equilibrium (a solution where all state variable are constant in time). Hence, this difference is a measure of the amplitude of the periodic solution or is zero at an equilibrium. For a detailed description of the tedious method that was used to track the positions of stable and unstable periodic solutions as the parameter Km_{LH} is varied refer to Selgrade *et al.* [50] The bifurcation diagram has a closed loop of stable and unstable cycles (periodic solutions) where the upper half of the loop (solid curve) represents stable large amplitude cycles and the lower half (dashed curve) represents unstable cycles. Saddle-node bifurcations occur at $Km_{LH} \approx 270$ and $Km_{LH} \approx 770$ where the stable and unstable cycles coalesce. The horizontal axis in Figure 1.5.1 represents a curve of equilibria. Along this axis a supercritical Hopf bifurcation occurs at $Km_{LH} \approx 265$ resulting in a stable small amplitude periodic solution and an unstable equilibrium solution. The branch of stable small amplitude cycles continues through $Km_{LH} \approx 1500$ and then disappears (not shown on graph). The value $Km_{LH} = 360$ is the parameter value that fits the McLachlan data best. At $Km_{LH} = 360$, the normal cycle is indicated by a $*$ in Figure 1.5.1 but there also exists a stable, small amplitude abnormal cycle. Hence, at $Km_{LH} = 360$, a woman has the possibility of having a normal menstrual cycle or an abnormal menstrual cycle depending on her initial hormone levels. In fact, there are two stable periodic solutions that exist for every Km_{LH} value between 270 and 770 (as seen in Figure 1.5.1). Therefore the initial hormone levels of a woman with a Km_{LH} in this range will determine whether she will cycle normally or abnormally. When Km_{LH} has a value outside this range, the amount of RP_{LH} is too low to produce

an LH surge and, therefore, the woman will have only an anovulatory cycle.

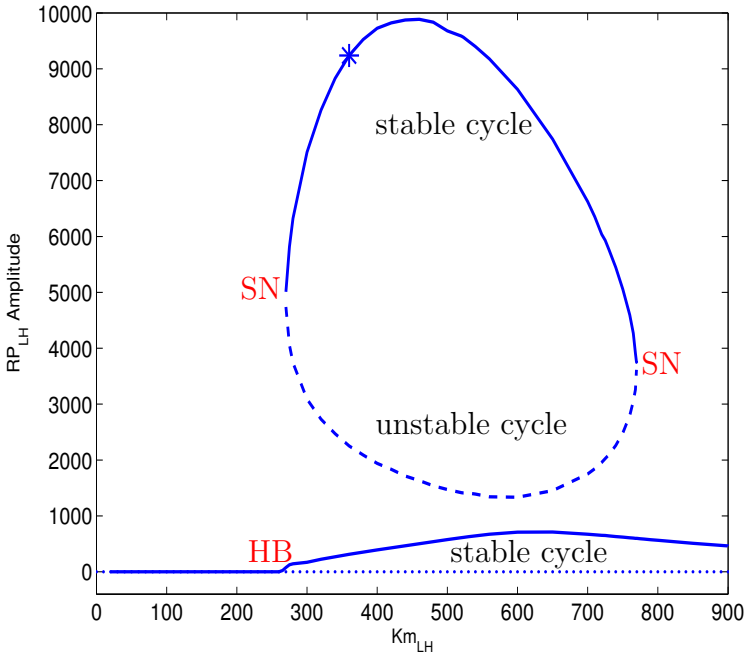

Figure 1.5.1: Bifurcation diagram plots cycle amplitude against the parameter Km_{LH}. The horizontal axis represents equilibria. A solid curve indicates stable cycles or equilibria and a dashed curve, unstable cycles or equilibria. HB and SN denote Hopf and saddle-node bifurcations. $*$ indicates the normal cycle where $Km_{LH} = 360$.

1.6 Exogenous Exposure of Ovarian Hormones

In Section 1.4, we observe that the merged model produces two asymptotically stable period solutions for the same set of model parameters, a large amplitude cycle (normal cycle) fitting the McLachlan data for normally cycling women and a small amplitude cycle (abnormal cycle) that resembles the hormone profiles of women with menstrual cycle irregularities, possibly polycystic ovarian syndrome (PCOS). Since a specific set of parameters represents the behavior of an individual woman, Figures 1.4.2, 1.4.3, and 1.5.1 indicate that a woman's initial hormone levels will determine whether she will cycle normally or abnormally. By perturbing one parameter and keeping all of the other parameters fixed, we also observe that women with similar hormone profiles can also have a normal or an abnormal cycle (see Figure 1.5.1). These results lead us to the following questions: Can

the abnormal cycle be perturbed into the normal cycle by applying some exogenous exposure of ovarian hormones (while keeping the parameter values fixed)? Similarly, can the normal cycle be perturbed into the abnormal cycle?

1.6.1 PCOS and Progesterone Treatment

Polycystic ovarian syndrome (PCOS), a menstrual cycle abnormality that is a leading cause of infertility in women [1, 2, 61], is usually associated with abnormal hormone profiles. Many PCOS women exhibit high androgen levels and low progesterone levels [61]. For example, low progesterone during the luteal phase permits more LH secretion at the expense of FSH secretion because of too rapid pulsing of gonadotropin releasing hormone which affects the pituitary's synthesis and release of the gonadotropins, see Marshall *et al.* [28]. Assuming that the abnormal cycle (dashed curves in Figure 1.4.3) of our model represents PCOS, a progesterone treatment may be tested in the setting of this model by trying to perturb the abnormal solution to the normal cycle (solid curves in Figure 1.4.3) with exogenous P_4. In fact, the administration of exogenous P_4 was implemented by Harris-Clark *et al.* [20] by adding a constant term to the progesterone auxiliary equation (1.3.26). The progesterone therapy shown in Figure 1.6.1 adds 80 nmol/L of P_4 to (1.3.26) for 5 days at the beginning of the luteal phase of the abnormal cycle (from day 8 to day 13 of the cycle) and results in normal serum levels of all 5 hormones by the next cycle.

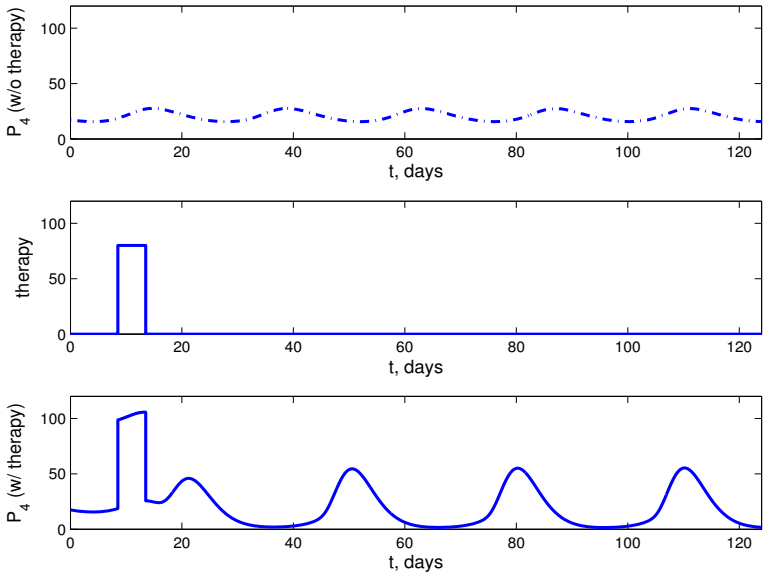

Figure 1.6.1: The upper graph is P_4 for the abnormal cycle. The middle graph is a P_4 treatment of 80 nmol/L for the first 5 days of the luteal phase of the first cycle. The lower curve graphs P_4 with this treatment to show that normal P_4 levels are restored after one cycle.

1.6.2 Endocrine Disruption

The model described in this chapter can also be used to simulate the effects of exogenous substances on normal menstrual cycle behavior. There are concerns that environmental chemicals with estrogenic activity can disrupt the reproductive endocrine system and may contribute to the increased incidence of breast cancer [11], declines in sperm counts [52], and developmental abnormalities [30]. To test whether the normal cycle of our model can be perturbed (disrupted) into the abnormal cycle due to exposure to exogenous estrogen, a constant term can be added to the estradiol auxiliary equation (1.3.25). Figure 1.6.2 shows that the administration of 50 ng/L of E_2 for one complete cycle ($\sim$30 days) starting at day 6 of the follicular phase of the normal cycle results in E_2 hormone levels that are too low to produce an LH surge, and hence, disrupts the normal menstrual cycle. A more complicated estrogen disruption was carried out by Harris-Clark *et al.* [20] which perturbed the normal cycle to the abnormal cycle.

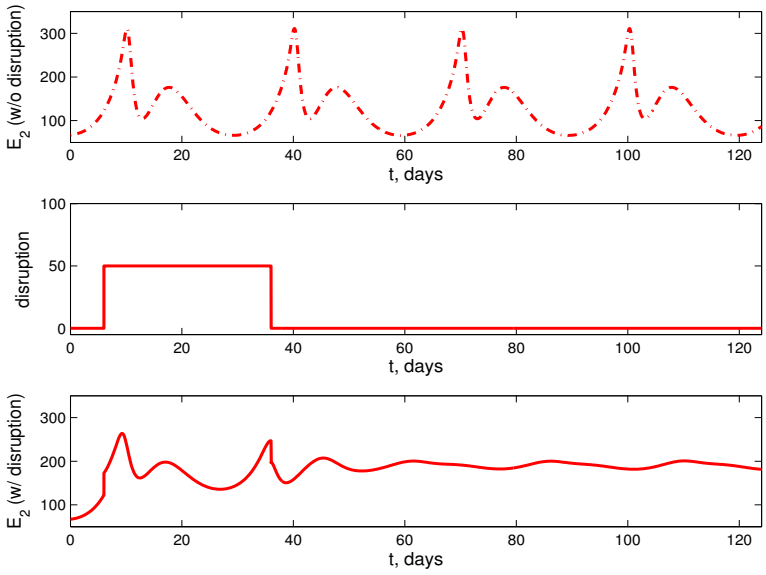

Figure 1.6.2: The upper graph shows the normal E_2 profile. The middle graph depicts a 30 days exposure to 50 ng/L of exogeneous E_2 starting on day 6 of the normal cycle. The lower graph shows that this exposure perturbs the normal cycle into the abnormal cycle.

1.7 Summary and Discussion

The mathematical model presented here describes the biological mechanisms pertinent to hormonal control of the menstrual cycle of adult women. Average daily blood levels of five essential hormones are tracked. Because the biological system is dual control, the model may be decomposed into two submodels — one submodel for the pituitary hormones LH and FSH under the control of only ovarian hormones and the other submodel for the ovarian hormones E_2, P_4 and Inh under the control of only pituitary hormones. Each submodel is linear in its state variables with time-dependent input functions for the control variables estimated from data in the literature (e.g., McLachlan *et al.* [32]). Parameter identification is performed on each submodel separately. These parameter estimates are good starting values for parameter identification for the merged model. The final merged model is a system of 13 nonlinear ordinary differential equations with three discrete time-delays representing time lags in the pituitary's synthesis response to changes in ovarian hormone levels. Simulations of the merged model provide an excellent approximation to the hormone data in McLachlan *et al.* [32] for normally cycling women, see Figure 1.4.1.

Surprisingly, this model with the parameters which fit the McLachlan data best also has another stable periodic solution, which we refer to as the abnormal cycle. Because of a lack of an LH surge, the abnormal cycle is anovulatory and hormone profiles are reminiscent of PCOS. In fact, the acyclic E_2 level of about 200 ng/L may be contraceptive, see Figure 1.4.3. We illustrate how exogenous ovarian hormones can be used to perturb one stable cycle to the other. Although the model with this parameter set is bistable, multiple simulations indicate that the state space region of initial conditions giving solutions which approach the normal cycle is much larger than the region of initial conditions approaching the abnormal cycle.

Biological data are inherently variable. Data collected by Welt *et al.* [57] for the same five hormones in McLachlan *et al.* [32] has been used by Pasteur [37] to estimate parameters for the model described above. The McLachlan and Welt data sets are somewhat different so the resulting parameter sets are different. Pasteur's simulations of the model using the Welt data exhibit only one stable periodic solution and it fits the Welt data for normally cycling women. Selgrade *et al.* [50] explained this apparent inconsistency by showing that changing the value of Km_{LH} in the Welt system resulted in the Welt model exhibiting two stable cycles like the McLachlan model. As discussed in Section 1.5, model output is sensitive to changes in the parameter Km_{LH}. Small changes in a sensitive parameter may result in changes in the possible asymptotic behavior of model solutions because of the occurrence of bifurcations. Hence, sensitivity analysis and bifurcation analysis are essential to understanding and using a mathematical model.

What does this model say about the menstrual cycle of individual women? Depending on an individual's parameters, she may cycle normally after a length of time regardless of her initial hormone levels. Her model corresponds to the Welt parameter set. On the other hand, another woman may have two possible menstrual cycles depending on initial hormone levels and one of these cycles is anovulatory. Her model is like the McLachlan model. If her cycle is anovulatory then we demonstrate how the administration of exogenous hormones may perturb it to the ovulatory cycle.

Finally, this model may be refined by including additional important reproductive hormones. Welt *et al.* [57] collected data for two types of inhibin, Inh A and Inh B. Both inhibit FSH synthesis but Inh B is a good indicator of ovarian aging and would be useful for extending the model to older reproductive women in the age range 35-45 years. Another improvement would be to separate the functions of the pituitary and hypothalamus in order to describe the role of gonadotropin releasing hormone (GnRH), because many cycle abnormalities involve irregular GnRH pulsing. However, the time scale for GnRH pulsing is that of minutes and hours. The present model is on a time scale of days and months. Handling multiple time scales will complicate the model significantly.

Acknowledgements

Figures 1.3.2, 1.4.1, 1.4.2, 1.4.3 and 1.6.1 are reproduced here with kind permission from Springer Science + Business Media: Bulletin of Mathematical Biology, "Multiple Stable Periodic Solutions in a Model for Hormonal Control of the Menstrual Cycle," volume 65, 2003, pages 157-173, Leona Harris Clark, Paul M. Schlosser and James F. Selgrade, Figure 1, 3, 4, 5 and 6. Figure 1.5.1 is reproduced here from Figure 8 in the Journal of Theoretical Biology, volume 260, J.F. Selgrade, L.A. Harris and R.D. Pasteur, " A model for hormonal control of the menstrual cycle: Structural consistency but sensitivity with regard to data," pages 572-580, 2009, with kind permission from Elsevier.

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