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ORIGINAL ARTICLE

Modeling Endocrine Control of the Pituitary–Ovarian Axis: Androgenic Influence and Chaotic Dynamics

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Abstract Mathematical models of the hypothalamus-pituitary-ovarian axis in women were first developed by Schlosser and Selgrade in 1999, with subsequent models of Harris-Clark et al. (Bull. Math. Biol. 65(1):157–173, 2003) and Pasteur and Selgrade (Understanding the dynamics of biological systems: lessons learned from integrative systems biology, Springer, London, pp. 38–58, 2011). These models produce periodic in-silico representation of luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2), progesterone (P4), inhibin A (InhA), and inhibin B (InhB). Polycystic ovarian syndrome (PCOS), a leading cause of cycle irregularities, is seen as primarily a hyper-androgenic disorder. Therefore, including androgens into the model is necessary to produce simulations relevant to women with PCOS. Because testosterone (T) is the dominant female androgen, we focus our efforts on modeling pituitary feedback and inter-ovarian follicular growth properties as functions of circulating total T levels. Optimized parameters simultaneously simulate LH, FSH, E2, P4, InhA, and InhB levels of Welt et al. (J. Clin. Endocrinol. Metab. 84(1):105–111, 1999) and total T levels of Sinha-Hikim et al. (J. Clin. Endocrinol. Metab.

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83(4):1312–1318, 1998). The resulting model is a system of 16 ordinary differential equations, with at least one stable periodic solution. Maciel et al. (J. Clin. Endocrinol. Metab. 89(11):5321–5327, 2004) hypothesized that retarded early follicle growth resulting in "stockpiling" of preantral follicles contributes to PCOS etiology. We present our investigations of this hypothesis and show that varying a follicular growth parameter produces preantral stockpiling and a period-doubling cascade resulting in apparent chaotic menstrual cycle behavior. The new model may allow investigators to study possible interventions returning acyclic patients to regular cycles and guide developments of individualized treatments for PCOS patients.

Keywords Modeling · Androgens

1 Introduction

Periodic fluctuations in pituitary and ovarian hormones regulate the human female reproductive cycle. Gonadotropin-releasing hormone (GnRH) from the hypothalamus prompts the pituitary to produce follicle stimulating hormone (FSH) and luteinizing hormone (LH) which control ovarian follicular development and sex hormone secretion (Yen 1999; Hotchkiss and Knobil 1994). The ovaries secrete estradiol (E2), progesterone (P4), inhibin A (InhA), inhibin B (InhB) and androgens which in turn affect the synthesis and release of FSH and LH (Karch et al. 1973; Liu and Yen 1983) via positive and negative feedback relationships. Models for hormonal regulation of the menstrual cycle have been constructed using systems of ordinary differential equations where state variables represent serum hormone levels or different stages of monthly ovarian development, e.g., Harris-Clark et al. (2003); Reinecke and Deuflhard (2007), and Pasteur and Selgrade (2011). The model presented here expands on the models of Harris-Clark et al. (2003) and Pasteur and Selgrade (2011) by including the effects of the androgen testosterone (T) on the brain and on the ovaries.

Pulses of gonadotropin-releasing hormone (GnRH) produced by the hypothalamus on a time scale of minutes and hours cause pulses of FSH and LH to be produced by the pituitary. Because the ovaries respond to average daily blood levels (Odell 1979), our models track average daily concentrations of FSH and LH. Hence, we lump the effects of the hypothalamus and the pituitary together and just consider the synthesis and release of FSH and LH on the time scale of days. This simplification results in models, which give good fit to the daily serum data of McLachlan et al. (1990), and to the data of Welt et al. (1999), and which avoid the complication of multiple time scales. These models exhibit novel features, e.g., two stable periodic solutions—one ovulatory and the other non-ovulatory (Harris-Clark et al. 2003). The nonovulatory cycle lacks a surge in LH and has a contraceptive level of E2. Model simulations illustrate how exogenous P4 may be used to perturb the non-ovulatory cycle to the ovulatory cycle and how exogenous E2 may be used to perturb the ovulatory cycle to the nonovulatory cycle (see Harris-Clark et al. 2003). The latter is an example of how environmental estrogens may disrupt a woman's menstrual cycle. In addition, Selgrade (2010) and Margolskee and Selgrade (2011) explain the bistable behavior by analyzing bifurcation diagrams with respect to a parameter, which measures the LH response of the pituitary to E2 priming. The review article by Vetharaniam et al. (2010), describes many models of hormonal control of the female reproductive cycle and discusses their strengths and limitations.

The development of the follicle which releases its ovum in a specific menstrual cycle begins at least 60 days before that cycle (Nussey and Whitehead 2001). To model hormonal regulation of these early growing follicles, preantral, and early antral stages must be included. Abnormal development during this period of early growth may result in cycle irregularities such as polycystic ovarian syndrome (PCOS), a leading cause of female infertility (Alvarez-Blasco et al. 2006; Azziz et al. 2004; Yen 1999; Franks et al. 2008). In fact, Maciel et al. (2004) reported a "stockpiling" of preantral follicles in women with PCOS as compared to normally cycling women. The development of preantral follicles is gonadotropin independent but intraovarian factors (Skinner 2005; Reddy et al. 2010; Maciel et al. 2004) influence the early growth and the transition to the antral stage. Also, androgen receptors appear before FSH receptors (Rice et al. 2007), so in this study we consider the effects of testosterone (T) on preantral and early antral follicles. Understanding how variations in hormone levels and key ovarian growth parameters alter early follicular development may predict disordered ovulation 3 months later.

To this end, we describe a mathematical model for menstrual cycle regulation, which includes 12 distinct stages of ovarian development and 7 pituitary and ovarian hormones. Our model builds on previous models (e.g., see Harris-Clark et al. 2003; Pasteur 2008; Schlosser and Selgrade 2000; Selgrade and Schlosser 1999; Selgrade et al. 2009) for endocrine control of the cycle but adds three new stages of follicular development and includes the effects of T on the ovaries and on the pituitary. First, we discuss the biological background and the model equations. Second, parameters are identified using the data of Welt et al. (1999) and Sinha-Hikim et al. (1998). Third, model simulations are presented and compared to data. Finally, we illustrate how decreasing the transfer parameter between the preantral and early antral follicular stages results in the stockpiling of preantral follicles and a significant alteration in hormone levels. In fact, by varying this parameter we demonstrate a period-doubling cascade of bifurcations that results in chaotic hormone fluctuations.

2 Biological Background and Model Development

2.1 Follicular Growth and Ovarian Stages

The normal adult menstrual cycle is approximately 28 days in duration and can be divided into two phases: follicular (menstruation to ovulation) and luteal (ovulation to menstruation). A cohort of primordial follicles is activated at least 60 days before the LH surge promotes ovulation of the dominant follicle from that particular cohort (Gougeon 1986). Upon activation, this cohort migrates to the medullary region of the ovary, returning to the surface of the ovary in a process known as "emergence" as maturation proceeds (Fihri et al. 2004). During this time, follicles are classified into at least 4 categories: preantral, early antral, antral, and recruited. The preantral stage accounts for the majority of the gonadotropin independent follicular growth. Thereafter, follicles begin to develop an antrum and sensitivity to FSH increases rapidly (Gougeon 1986). A significant portion of these antral follicles degenerate through atresia while larger follicles with additional FSH receptors enter a recruitment phase. This development establishes the beginning the follicular phase of the monthly cycle. During this 14-day period, evidence suggests FSH must rise above a threshold level for 5 days during which a select subgroup of follicles experiences rapid growth. This span of time in the menstrual cycle is referred to as the FSH window (Fauser and Van Heusden 1997). The dominant follicle (the one destined for ovulation) is selected from this cohort by its acquisition of LH receptors. As serum FSH begins to decline, nondominant follicles begin to degenerate. The dominant follicle reaches a diameter of about 20 mm just prior to mid cycle and secretes large amounts of E2, which promotes pituitary LH production. Once LH reaches surge levels, the dominant follicle releases its ovum and is transformed into the corpus luteum (CL). CL tissue develops a yellow appearance, increases in mass until day nineteen of the cycle and produces high levels of E2, P4, and InhA. Ovarian steroid production peaks at day twenty-one and, in the absence of pregnancy, declines with the regression of the CL during the remainder of the luteal phase of the menstrual cycle.

2.2 Intraovarian Growth Regulation

Histological samples of ovarian tissue can reveal diverse numbers of follicles from 1 mm to 20 mm in diameter during most of the menstrual cycle (Gougeon 1986). As current ultrasound technology is best at identifying follicles greater than 2 mm (Jonard and Dewailly 2004), it is common to consider this as the point of primary follicle activation. A slight elevation in ovarian mass can be observed with the appearance of the dominant follicle (Lass 1999) with the peak in ovarian mass occurring at approximately day 19 of the cycle (Lass 1999) due to CL development. These findings suggest the existence of a signaling mechanism that maintains the total mass near steady state. Local factors identified in follicular fluid analysis are commonly accepted as major contributors in this regulation. It is hypothesized that follicles in a more advanced growth stage regulate activation of a new wave of follicles through the use of insulin like growth factors (Lucy 2011), transforming growth factor ($TGF\beta1$) (Kuo et al. 2011), granulosa-theca cell factors (Orisaka et al. 2009), antimüllerian hormone (Franks et al. 2008), and androgens (Rice et al. 2007). Follicular fluid concentrations of growth factors are significant and change dynamically, but it seems implausible that corresponding changes in serum levels will be detectable. We therefore focus our efforts on serum levels of T with the knowledge that androgen receptors have been found in granulosa cells of developing follicles as small as 0.2 mm, before FSH receptors appear (Rice et al. 2007). This allows us to capture fluctuations in follicular growth rates by integrating T levels into a subsystem of ovarian follicular development that self-regulates its total mass.

2.3 Ovarian Modeling Technique

In our attempt to model the ovarian mass of preantral and small antral follicles, we utilize mathematical theories of mass action kinetics. Often used to describe chemical

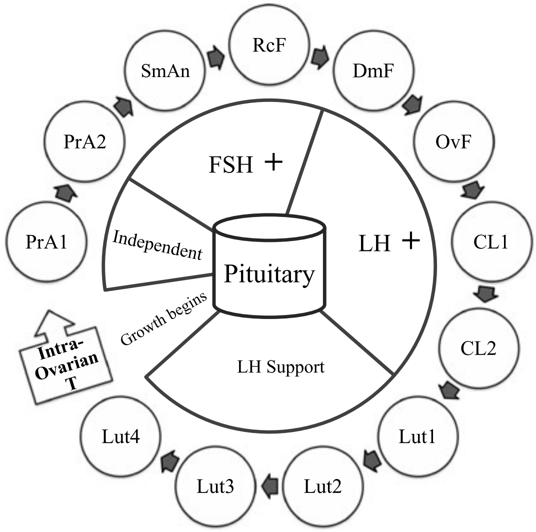

Fig. 1 State variables representing 12 stages of follicular development are shown. Follicle growth begins with the *PrA*1 stage and continues in a clockwise direction for a complete cycle. The pie chart indicates the timing of the regulatory effects of luteinizing hormone (LH) and follicle stimulating hormone (FSH) on follicular development

reactions where the total mass or volume of a system remains constant while the individual components change dynamically, the mass action approach allows us to reflect total mass steady state regulation. The model equations are built to reflect an interdependent shift of mass through three stages of follicular maturity, represented by the state variables *PrA*1 (preantral follicle 1), *PrA*2 (preantral follicle 2), and *SmAn* (small antral follicle). This approach captures the intraovarian effects on early follicular development reviewed in Sect. 2.2. Transitions through these stages depend on the masses of adjacent stages and available hormone levels (see Fig. 1). We assume that proximity between follicles determines the magnitude of interfollicular signaling with the most significant effects coming from the subsequent maturity levels as the follicles migrate toward the outer cortex of the ovary. This allows us to emulate intraovarian signaling when follicular fluid levels of growth factors cannot be quantified. Differential equations are then mechanistically constructed for each of our state variables.

The growth term in our first stage, *PrA*1, has a constant rate, m_1, of primordial follicle recruitment as suggested by Gougeon (1986). We introduce a T dependent transfer term that scales the product of the current mass of preantral follicles (*PrA*1) with the mass of the next stage of maturity (*PrA*2). This term reflects the appearance of androgen receptors before gonadotropin growth begins and contains an exponential, η, introduced to increase the rate of stimulatory response to T (Rice et al. 2007). The equation for *PrA*1 becomes:

$$\frac{d}{dt}PrA1 = m_1 - m_2 \cdot T^{\eta} \cdot PrA1 \cdot PrA2 \tag{1}$$

The product $PrA1 \cdot PrA2$ quantifies the intraovarian growth factors and becomes the growth term for *PrA*2. We then shift our mass from *PrA*2 utilizing an FSH dependent threshold term (e.g., see Zeleznik 2004)

$$m_3 \cdot \frac{FSH^{\nu}}{Km_{FSH}^{\nu} + FSH^{\nu}}.$$

This gradual acquisition of receptors and saturation behavior is translated mathematically through a Hill function whose product with the current stage, $PrA2$, and following stage, $SmAn$, defines our next transfer term.

$$\frac{d}{dt}PrA2 = m_2 \cdot T^{\eta} \cdot PrA1 \cdot PrA2 - m_3 \cdot \frac{FSH^{\nu}}{Km_{FSH}^{\nu} + FSH^{\nu}} \cdot PrA2 \cdot SmAn \tag{2}$$

As the mass of $PrA2$ is dependent on that of $SmAn$, there exists an indirect effect of $SmAn$ on our first stage, $PrA1$. This assumes that migration increases the distance from $SmAn$ follicles to the $PrA1$ follicles and, therefore, the interfollicular signaling between the two decreases. The last stage of mass action dependence, $SmAn$, provides the small antral follicle mass available for recruitment whose growth is partially regulated by follicles in $PrA2$, and indirectly by follicles in $PrA1$ through its inclusion in the equation for $PrA2$.

$$\frac{d}{dt}SmAn = m_3 \cdot \frac{FSH^{\nu}}{Km_{FSH}^{\nu} + FSH^{\nu}} \cdot PrA2 \cdot SmAn - b \cdot FSH^{\varrho} \cdot SmAn \cdot RcF \tag{3}$$

For the decay term, we assume that the rate of FSH receptor acquisition rapidly increases to a point directly proportional to the natural rise in follicular phase FSH at the beginning of the follicular phase (Gougeon 1986), rather than a threshold response used in the previous stage. Similarly, the transfer of mass from $SmAn$ is affected by the existing mass in the subsequent stage RcF whose growth is affected by $SmAn$ directly (see Eq. (4)) and by $PrA1$ and $PrA2$ indirectly (see Eqs. (2), (3)).

To reflect the increasing ovarian mass during the follicular and luteal phases of the cycle, linear growth and decay terms are employed in 9 different stages which represent ovarian development during the monthly cycle (Fig. 1). RcF represents recruited follicles with additional growth stimulated by FSH and transfer dependent on the appearance of LH receptors. Our equation for RcF marks transition from the mass action terms to linear compartmental terms as in Harris-Clark et al. (2003). These compartmental terms permit total ovarian mass to increase as the ovaries approach the time at which a dominant follicle is selected. DmF and OvF represent the dominant and ovulatory follicle. $CL1$ and $CL2$ portray the transition to the corpus luteum. The luteal phase consists of the four stages $Luti$, $i = 1, \dots, 4$ representing the regression of the CL and conclusion of the current monthly cycle (Fig. 1). These 9 stages correspond to the ovarian model of Harris-Clark et al. (2003). The growth and decay of these stages are influenced by the gonadotropins as indicated in the following differential equations:

$$\frac{d}{dt}RcF = b \cdot FSH^{\varrho} \cdot SmAn \cdot RcF + \left(c_1 \cdot FSH - c_2 \cdot LH^{\alpha}\right) \cdot RcF \tag{4}$$

$$\frac{d}{dt}DmF = c_2 \cdot LH^{\alpha} \cdot RcF + \left(c_3 \cdot LH^{\beta} - c_4 \cdot LH^{\xi}\right) \cdot DmF \tag{5}$$

$$\frac{d}{dt}OvF = c_4 \cdot LH^{\xi} \cdot DmF - c_5 \cdot LH^{\gamma} \cdot OvF \tag{6}$$

$$\frac{d}{dt}CL1 = c_5 \cdot LH^{\gamma} \cdot OvF - d_1 \cdot CL1 \tag{7}$$

$$\frac{d}{dt}CL2 = d_1 \cdot CL1 - d_2 \cdot CL2 \tag{8}$$

$$\frac{d}{dt}Lut1 = d_2 \cdot CL2 - k_1 \cdot Lut1 \tag{9}$$

$$\frac{d}{dt}Lut2 = k_1 \cdot Lut1 - k_2 \cdot Lut2 \tag{10}$$

$$\frac{d}{dt}Lut3 = k_2 \cdot Lut2 - k_3 \cdot Lut3 \tag{11}$$

$$\frac{d}{dt}Lut4 = k_3 \cdot Lut3 - k_4 \cdot Lut4 \tag{12}$$

Clearance from the blood for the ovarian hormones is on a fast time scale (Baird et al. 1969) as compared to ovarian development and clearance for the pituitary hormones. Hence, we assume that circulating levels of the ovarian hormones are maintained at a quasisteady state (Keener and Sneyd 2009) as did Bogumil et al. (1972). Implementation of this approach results in using linear combinations of the 12 ovarian stages to obtain serum levels of $E2$, $P4$, $InhA$, $InhB$, and T. The following five auxiliary equations result:

Auxiliary Equations (A)

$$E2 = e_0 + e_1 \cdot DmF + e_2 \cdot Lut4 \tag{A1}$$

$$P4 = p_1 \cdot Lut3 + p_2 \cdot Lut4 \tag{A2}$$

$$InhA = h_0 + h_1 \cdot OvF + h_2 \cdot Lut2 + h_3 \cdot Lut3 \tag{A3}$$

$$InhB = j_1 + j_2 \cdot PrA2 + j_3 \cdot SmAn + j_4 \cdot RcF + j_5 \cdot CL1^{j_6} \tag{A4}$$

$$\begin{aligned} T = {} & t_1 + t_2 \cdot PrA2 + t_3 \cdot SmAn + t_4 \cdot RcF + t_5 \cdot DmF \\ & + t_6 \cdot OvF + t_7 \cdot Lut1 + t_8 \cdot Lut3 \end{aligned} \tag{A5}$$

The equation for circulating T was originally constructed to be a linear combination of ovarian stages (1–12). However, we assume that hormones are not produced by newly activated follicles in $PrA1$ and parameter fitting to data revealed little to no synthesis of T from the ovarian stages $CL1$, $CL2$, $Lut2$, and $Lut4$.

2.4 Androgenic Feedback on the Pituitary

Recent studies of T feedback suggest that it is an important component in the modulation of LH secretion by the pituitary in women. In human females, elevated T is significantly correlated with elevated basal LH and dampened LH surge (Fauser and Van Heusden 1997; Taylor et al. 1997). Animal models have shown that T is a necessary component in priming the pituitary for GnRH induced LH synthesis, e.g., see Pielecka et al. (2006) and Yasin et al. (1996). Ovariectomized rats subjected to exogenous GnRH pulses showed significantly greater LHβ mRNA response when pretreated with T implants versus controls (Yasin et al. 1996). Treatment levels, similar to those documented at proestrous, demonstrate a 3-fold increase in the LHβ mRNA response to GnRH when compared to untreated controls. Yasin et al. (1996) noted that their results were independent of aromatization of T to estradiol. Hence, we suggest that T may directly stimulate LH synthesis and will include such an effect in our model.

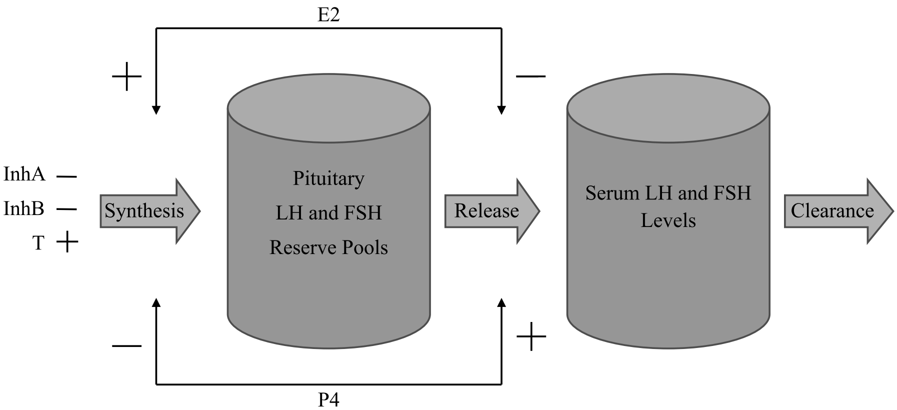

Fig. 2 Ovarian control of the GnRH modulated pituitary synthesis and release of LH and FSH. Stimulatory and inhibitory effects are denoted by + and − signs, respectively

2.5 Hypothalumus-Pituitary Modeling Technique

Schlosser and Selgrade (2000) proposed a pair of differential equations for each gonadotropin to model the hypothalamically controlled pituitary response to ovarian hormones. These equations described the synthesis in the pituitary, the release into the blood and clearance from the blood of *LH* and *FSH*; see Fig. 2. The coupling of hypothalamic and pituitary actions allowed the system to predict serum levels of gonadotropins on a daily time scale consistent with published clinical data (McLachlan et al. 1990 and Welt et al. 1999). In these differential equations, the state variables RP_{LH} and RP_{FSH} represent the amounts of gonadotropins synthesized in the pituitary via GnRH signaling and the state variables *LH* and *FSH* represent blood concentrations. The original model of Schlosser and Selgrade (2000) assumed a baseline LH synthesis rate v_0 independent of E2. Motivated by Yasin et al. (1996), for our model we assume that this baseline rate depends on T; see Eq. (13).

$$\frac{d}{dt}RP_{LH} = \frac{v_0 \cdot T(t-d_T)^{\kappa} + v_1 \cdot \frac{E2(t-d_E)^a}{(Km_{LH}^a + E2(t-d_E)^a)}}{(1 + \frac{P4(t-d_P)}{Ki_{LH}})} - k_{LH} \cdot \frac{(1 + c_{LHp} \cdot P4^{\delta})}{(1 + c_{LHe} \cdot E2)} \cdot RP_{LH} \tag{13}$$

$$\frac{d}{dt}LH = \frac{1}{v} \cdot k_{LH} \cdot \frac{(1 + c_{LHp} \cdot P4^{\delta})}{(1 + c_{LHe} \cdot E2)} \cdot RP_{LH} - r_{LH} \cdot LH \tag{14}$$

$$\frac{d}{dt}RP_{FSH} = \frac{V_{FSH}}{1 + (\frac{InhA(t-d_{InhA})}{Ki_{FSHa}}) + (\frac{InhB(t-d_{InhB})}{Ki_{FSHb}})} - k_{FSH} \cdot \frac{(1 + c_{FSHp} \cdot P4)}{(1 + c_{FSHe} \cdot E2^{\zeta})} \cdot RP_{FSH} \tag{15}$$

$$\frac{d}{dt}FSH = \frac{1}{v} \cdot k_{FSH} \cdot \frac{(1 + c_{FSHp} \cdot P4)}{(1 + c_{FSHe} \cdot E2^{\zeta})} \cdot RP_{FSH} - r_{FSH} \cdot FSH \tag{16}$$

Through changes in GnRH pulse frequency and amplitude, LH exhibits a biphasic response to E2 (Liu and Yen 1983), so to account for this the model assumes that the effect of E2 on LH synthesis is different than the effect on LH release. E2 inhibits release (see the denominator of the second term of Eq. (13)) but at high levels E2 promotes synthesis (see the numerator of the first term of (13)). On the other hand, P4 inhibits GnRH modulated LH synthesis but promotes its release from the pituitary. The release term in (13) appears as a growth term in (14), where it is divided by blood volume v. Equations (15) and (16) for FSH are similar except the synthesis term has constant growth and inhibition due to InhA and InhB. Since hormone synthesis is a biochemical process more complicated than hormone release, we allow for discrete time-delays, d_E, d_P, d_T, d_{InhA}, and d_{InhB}, in the synthesis terms.

2.6 Parameter Identification and Periodic Solution

Parameter estimation began with the best-fit parameters reported by Pasteur (2008). Nelder-Meade algorithm with least squares approximation optimized final parameters against data for normal mean serum levels for E2, P4, LH, FSH, InhA, and InhB as published by Welt et al. (1999) and data for normal serum Total T levels as reported in Sinha-Hikim et al. (1998). Supplementation of the Welt data set was necessary as a single data set for all seven serum hormones was not available at the time of this publication. The original data reported by Sinha-Hikim et al. (1998) included nine data points over 26 days. Therefore, linear extrapolation was used to generate 28 data points (see Fig. 5) for optimal parameter estimation. For comparison purposes we converted the Sinha-Hikim data from nmol/L to ng/dL. This conversion takes the range of values from $(0.7, 1.6)$ to $(20, 47)$. The best-fit parameter set is detailed in the Appendix, Tables 1, 2, 3 and 4.

The discrete time-delays present in the model (d_T, d_E, d_P, d_{InhA}, and d_{InhB}) necessitate the use of a delay differential equation solver *DDE23* by Shampine and Thompson available through MatLab 2010a (2012) to numerically approximate a solution. Day 1 values from Welt et al. (1999) served as initial conditions for LH and FSH equations. Numerical simulations with the best-fit parameter set exhibit an asymptotically stable periodic solution of period 29 days (Fig. 6) consistent with reports of average cycle lengths between 26 and 32 days (Gougeon 1986). Once this stable periodic solution was identified, the remainder of the initial conditions were determined by the values from the last day of simulation. Rounded to four significant digits, our initial condition vector is detailed in the Appendix, Table 5.

3 Results

3.1 Numerical Simulations

Given the parameters listed in Tables 1 through 4, Figs. 3–4 represent serum concentrations of LH, FSH, E2, P4, InhA, and InhB for 58 days as predicted by the model, with daily data for mean serum levels from Welt et al. (1999) for comparison (the 28 data values reported in Welt et al. 1999 are repeated here to obtain 58 data values).

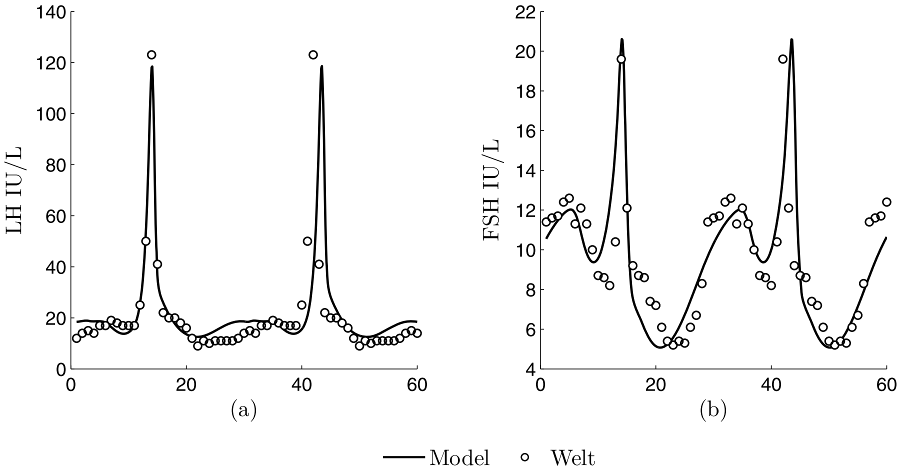

Fig. 3 Two 29 day cycles for (**a**) LH data from Welt et al. (○) and (**b**) FSH data from Welt et al. (○) are presented with current model simulations (*solid curves*)

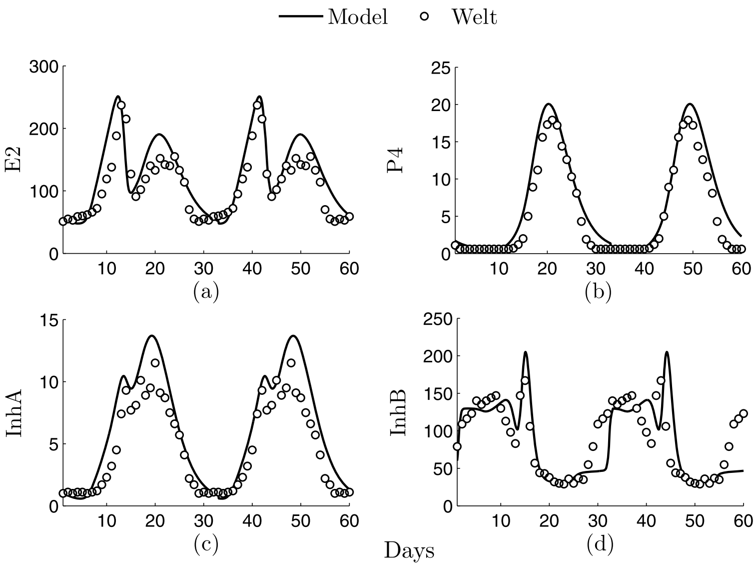

Fig. 4 Two 29 day cycles for (**a**) E2 data from Welt et al. (○) , (**b**) P4 data from Welt et al. (○), (**c**) InhA data from Welt et al. (○), (**d**) InhB data from Welt et al. (○) are presented with model simulations (*solid curves*)

Two complete 29 day cycles are presented for each hormone to support the stability conclusion.

One can observe the qualitative similarities to clinical observations. Serum T concentrations for a 29 day simulation follow in Fig. 5. Approximated T levels increase from a day 3 level of 30 ng/dL to a maximum level of 44 ng/dL on day 11 of the

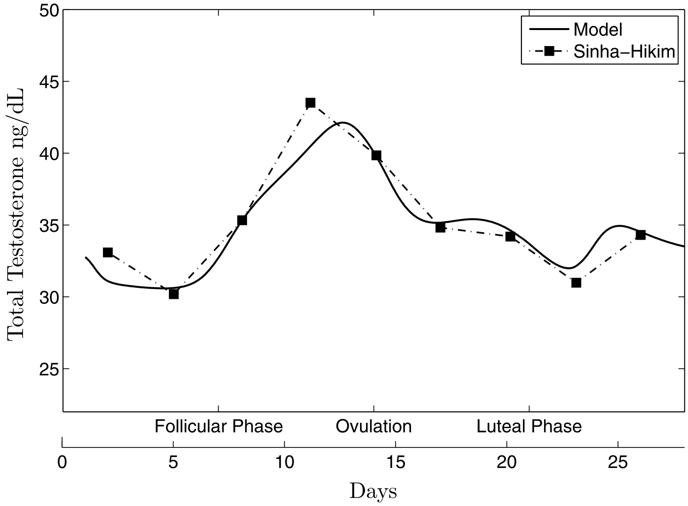

Fig. 5 The nine T data points (■) from Sinha-Hikim et al. (1998) are graphed with linear interpolation (— · —) and current model simulation (*solid curve*)

cycle. After ovulation, T slowly declines, plateauing from day 17 to 22 at approximately 34 ng/dL. At the end of the luteal phase, levels continue to decline before a slight rebound in circulating levels is observed during the luteal to follicular transition. Figure 5 presents data extracted from Sinha-Hikim et al. (1998) against model predictions for comparison.

3.2 Modeling Predictions Regarding Stockpiling of Follicles

Irregularities in cycle length are commonly seen in patients with PCOS. As a common cause of infertility, PCOS affects up to 10 % of reproductive age women and significantly correlates with increased risk of Type II diabetes and its associated morbidity (Alvarez-Blasco et al. 2006; Panidis et al. 2006; Azziz et al. 2004). Common phenotypes of PCOS also include elevated androgens and the appearance of polycystic ovaries on ultrasound (Azziz et al. 2009), both significant components in the etiology of PCOS (Ropelato et al. 2009; Medina and Nestler 1998; Hillier and Ross 1979). Histological studies of tissue samples taken from clinically diagnosed PCOS patients, recently reported by Maciel et al. (2004), suggests a "stockpiling" of preantral follicles when compared to controls. Their findings show a significantly ($P = 0.001$) increased number of follicles comprised of an oocyte and a single layer of cuboidal granulosa cells, i.e., primary follicles. It is hypothesized that the increase in primary follicle numbers is due to a longer growth pattern during this stage, represented by PrA1 in our model. We investigate m_2 as a possible model parameter to test this hypothesis. Because m_2 is measurement of mass transferred out of the primary stage, decreasing m_2 should delay primary follicle maturation, permitting additional growth of primary follicles.

For comparison, we begin by presenting LH concentrations from the stable periodic solution that best fits data from Welt and Sinha-Hikim in Fig. 6, i.e., the solution

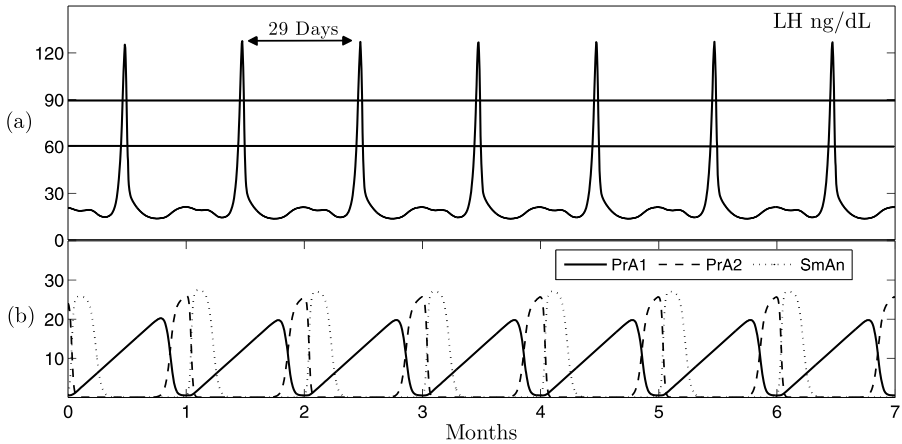

Fig. 6 (**a**) Simulated serum LH IU/L over 7 months with reference lines for 75 % and 50 % of mean Welt surge levels (**b**) First 3 stages of follicular development, PrA1, PrA2, and SmAn

with parameters listed in Tables 1 through 4. This simulation is graphed for 7 months showing a stable solution having an average cycle length of approximately 29 days, consistent with reports from Baerwald et al. (2003) and Gougeon (1986). We assume ovulation coincides with the LH surge given a follicle of sufficient size is available for rupture. Hence, we present Fig. 6 to depict 7 ovulations and the first 3 ovarian stages for comparison with Figs. 7, 8, and 9, where the parameter m_2 has decreased. As the exact surge level of LH necessary to induce ovulation is unique to each woman, we identify (by thick horizontal lines in Fig. 6) LH levels at 75 % and 50 % of the mean maximum LH level reported by Welt et al. (1999) for possible threshold references. While the ovarian mass values for PrA1 through SmAn (Fig. 6) are unitless, we note that the first preantral follicle mass peaks at approximately 20 units before transferring to the second androgen dependent preantral follicle mass. Follicular mass during this time is presented to demonstrate the interaction between the mass of developing follicles and ovulation assuming that surge levels of LH are indicative of the existence and timing of ovulation.

Figure 7 demonstrates the effect of reducing m_2 by approximately 50 % of the best fit value in Table 3. Analysis of the resulting behavior, over 7 months, reveals an LH surge exceeding 150 IU/L followed by a surge approximately 20 IU/L lower. While each peak surge occurs monthly, the pattern of alternating surge levels takes over 2 months to repeat. Similarly, the first preantral follicle mass begins to oscillate with a maximum mass of 30 units that results in lower mass transfer to the second preantral mass. In the normal case, our cycle length and the time for LH levels to return to the peak level of the previous cycle were the same. Reducing the transfer rate from PrA1 approximately doubles the time between peaks of the same magnitude, a phenomenon know as a period-doubling event (the period of the solution to our model equations is now $\sim$64 days). Identification of this behavior is mathematically significant for systems of this size and complexity. Although period-doubling often occurs in systems of nonlinear equations, it has rarely been demonstrated in a physiological model, which predicts data in the literature. As demonstrated in Fig. 8,

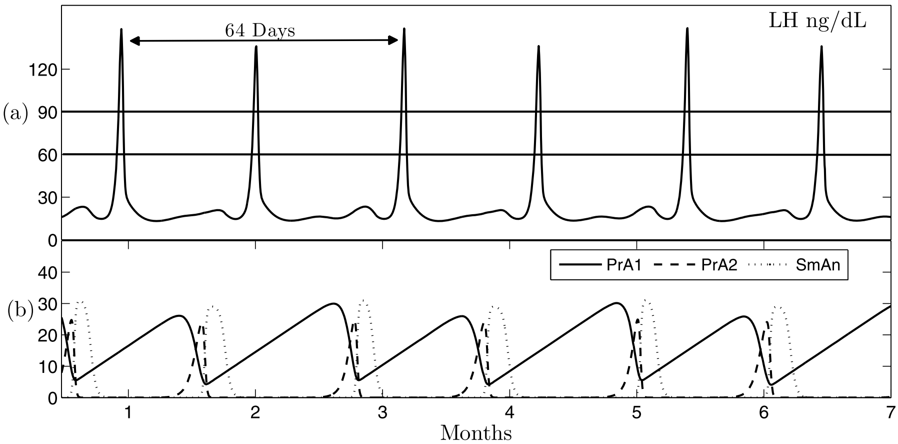

Fig. 7 (**a**) Serum LH level results after a 50 % reduction in parameter m_2 from best-fit value in Table 3 (**b**) Note the increase in PrA1 from Fig. 6

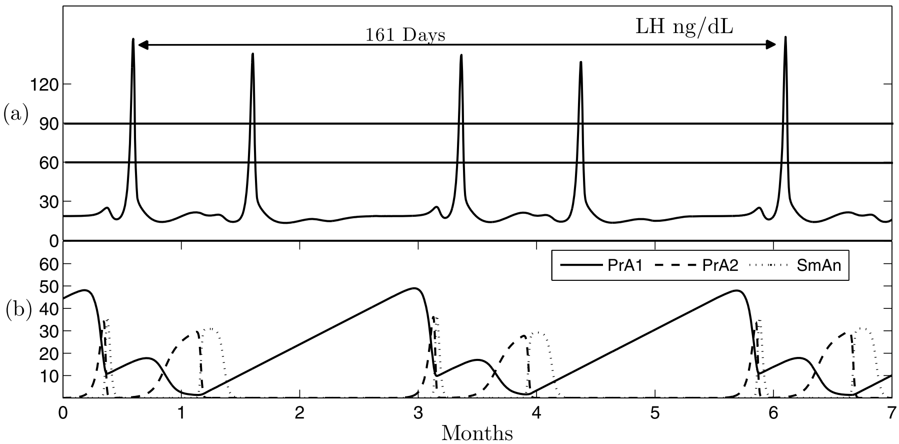

Fig. 8 (**a**) Serum LH level results after a 70 % reduction parameter m_2 best-fit value in Table 3 (**b**) First three stages of follicular development

reducing m_2 to 30 % of the best-fit value in Table 3, results in LH surges of four distinct values. In this simulation, the pattern of peak variation now repeats every 5.5 months with an average time between surges of approximately 40 days (another period-doubling has occurred). Examination of the resulting follicular mass reveals a distinct pattern of elevated PrA1, stockpiling of preantral follicles that completes its transfer to subsequent stages over a period of approximately 80 days. This suggests correlation between preantral growth and irregular menstrual cycles consistent with the “stockpiling” hypothesis of Maciel et al. (2004). Mathematically, this behavior indicates the existence of a period-doubling cascade of bifurcations as m_2 is decreased.

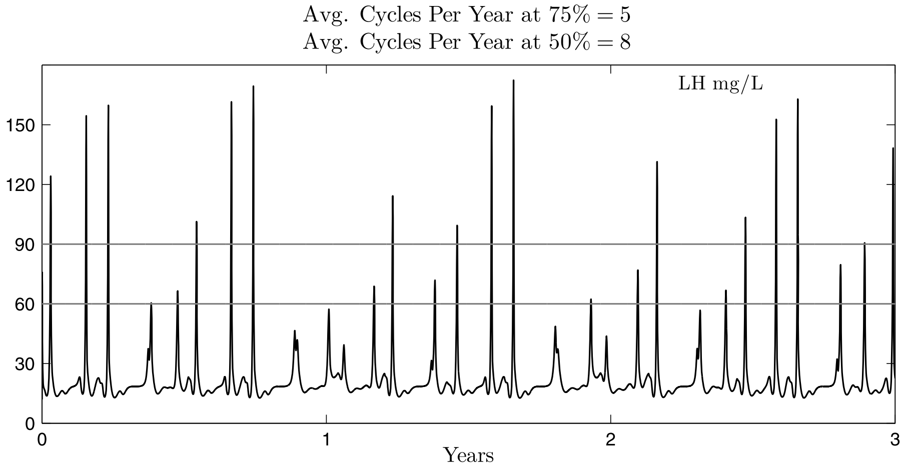

Fig. 9 Chaotic serum LH levels observed over 3 years as m_2 is reduced to 25 % of the best-fit value in Table 3. If ovulation occurs for an LH surge over 90 mg/L (75 % of the normal LH surge) then ~5 ovulations occur per year. If ovulation occurs for an LH surge over 60 mg/L, then ~8 occur per year

Complex dynamical systems with period-doubling cascades are often associated with chaotic behavior (Sander and Yorke 2012). Investigating this phenomenon motivated numerical experiments with additional decreases in m_2 to identify behavior consistent with chaotic attractors. Reducing m_2 by an additional 5 % causes the disappearance of the stable 4-cycle shown in Fig. 8 and the appearance of an apparent chaotic attractor as demonstrated in Fig. 9. Also, a common characteristic of chaotic behavior, i.e., the existence of a stable 3-cycle, appears in a very narrow range of m_2 values near 26 % of the value reported in Table 3. (Due to the lack of biological relevance of the 3-cycle solution, the results are not presented, merely noted.) If one assumes an LH threshold at 75 % of the reported mean, as demonstrated by the top horizontal line in Fig. 9, then the solution presented would ovulate approximately 5 times per year given the availability of a dominant follicle at the time of LH surge. Reducing the threshold assumption to 50 % increases the frequency of ovulation to an average 8 cycles annually over the 3-year window presented. These frequencies are consistent with a clinical diagnosis of oligomenorrhea, infrequent menstruation with 4 to 8 menstrual cycles per year, a primary phenotype of PCOS. The appearance of low amplitude peaks that do not exceed threshold values observed in Fig. 9 may be consistent with nonovulatory LH surges discussed in Baerwald et al. (2012).

4 Summary and Discussion

This study presents a nonlinear mechanistic model for the endocrine regulation of the human menstrual cycle, which consists of 16 delay differential equations and 5 auxiliary equations, with 71 parameters identified using data from the literature. Model simulations predict serum levels of LH, FSH, E2, P4, InhA, InhB, and T consistent with biological data (Welt et al. 1999; Sinha-Hikim et al. 1998). The asymptotically stable periodic solution resulting from the best-fit parameter set (Tables 1 through 4) has a period of 29 days, consistent with current literature. Moreover, increases in qualitative accuracy are obtained through the use of mass action kinetic theory to describe preantral follicular through early antral follicular stage growth. We believe this is the first model of its type to be able to predict serum T levels in a mathematical context that allows analysis of bifurcations and stable solutions of various periods.

A benefit of this approach is the identification of stable solutions that may resemble hormonal profiles consistent with some of the 16 PCOS phenotypes as defined in the Rotterdam consensus document (Consensus workshop group 2004). While it is generally agreed that T plays an important role in PCOS, only 8 of the 16 PCOS phenotypes present with elevated androgens (Consensus workshop group 2004). It is hypothesized that extended preantral follicular development could play an important role in explaining the appearance of polycystic ovaries (Maciel et al. 2004), a significant criterion in normal patients. Our approach of reflecting intraovarian follicular growth regulation systemically allows researchers to investigate effects of preantral growth abnormalities on menstrual cycle behavior. In our investigation, observations from increasing preantral follicle growth duration support the hypothesis presented by Maciel et al. (2004) that a "stockpiling" of immature follicles may be significant in the etiology of PCOS. Figures 6 through 9 reinforce this conclusion by demonstrating that the reduction of m_2 from its best-fit value in Table 3, which delays the transfer from preantral growth to androgen dependent growth, results in a visible "stockpiling" of preantral follicular mass (Figs. 7 and 8) and in irregular cyclicity (Fig. 9).

Furthermore, the identification of a period-doubling cascade of bifurcations leading to apparent chaotic behavior (Fig. 9) may actually increase accuracy in representing clinical findings of women with PCOS and/or oligomenorrhea over previous models. A recent paper by Derry and Derry (2010) presents a time series analysis of longitudinal menstrual cycle length data for 40 women over 20 to 40 years. They concluded that the human "menstrual cycle is the result of chaos in a nonlinear dynamical system" (Derry and Derry 2010) with only 5 degrees of freedom. They further referenced specifically the model of Harris-Clark et al. (2003) with the assertion that "any model producing only perfectly periodic menstrual cycles is, at best, incomplete" (Derry and Derry 2010). It is our belief that our current approach meets their criteria for a model of the human menstrual cycle that displays biologically relevant random behavior independent of external interference. It also shows that follicular dynamics cannot be abandoned in the quest for accuracy, rather it is the interplay between intraovarian mass and endocrine regulation that explains this behavior.

We anticipate the emerging collaborations with clinical endocrinologists and experimentalists will soon provide essential data necessary to refine additional parameter sets that can simulate additional PCOS phenotypes. Preliminary investigations include manipulating serum T levels, through altering t_1, as a reflection of excess adrenal androgen production, and t_3, as a reflection of increased insulin dependent theca cell T synthesis. These studies support the consideration of androgenic feedback on LH synthesis and encourage further examination of its effect on FSH synthesis. We believe these investigations will lend further understanding to PCOS phenotypes and adrenal disorders that present with elevated androgens.

The basic assumption that the hypothalamus–pituitary–ovarian axis is in itself a closed autonomous dynamical system presenting stable yet possibly chaotic behavior has led us to the development and presentation of our current model for endocrine regulation of female reproduction. Numerical approximations of stable solutions can be quickly computed as compactness was a major consideration in model development. These attributes make it a prime candidate for in silico investigations of cycle regularity. We have shown that modeling biological mechanisms, while considering clinical challenges and computational costs can lead to mathematically rich dynamics that present new model-derived insights that may guide future development of innovative individualized therapeutic interventions for women with PCOS.

Appendix: Parameters and Inital Conditions

Table 1 LH subsystem parameters

Parameter	Value	Unit
$v0_{LH}$	33.3	μ/day
$v1_{LH}$	160.75	μ/day
Ki_{LH}	13.6	L/nmol
Km_{LH}	47.33	ng/L
k_{LH}	19.99	1/day
c_{LH_P}	0.98	L/nmol
c_{LH_E}	0.9	L/ng
d_P	1	days
d_T	0	days
d_E	0	days
κ	0.92	dimensionless
δ	2	dimensionless
a	6.07	dimensionless
r_{LH}	14	1/day
v	2.5	liters

Table 2 FSH subsystem parameters

Parameter	Value	Unit
v_{FSH}	283.99	μ/day
Ki_{FSH_a}	6.38	μ/day
Ki_{FSH_b}	3000	L/nmol
k_{FSH}	3.41	1/day
c_{FSH_p}	1.26	L/nmol
c_{FSH_e}	0.16	L/ng
ζ	0.84	dimensionless
d_{InhA}	1	days
d_{InhB}	1	days
r_{FSH}	8.21	1/day

Table 3 Ovarian subsystem parameters

Parameter	Value	Unit
α	0.7100	dimensionless
β	0.6928	dimensionless
γ	0.0002	dimensionless
ξ	0.952	dimensionless
b	0.0189	L/day
c_1	0.0871	L/µg
c_2	0.1251	1/day
c_3	0.0533	1/day
c_4	0.0371	1/day
c_5	0.4813	1/day
d_1	0.7053	1/day
d_2	0.6488	1/day
k_1	0.7089	1/day
k_2	0.8712	1/day
k_3	1.038	1/day
k_4	1.052	1/day
m_1	0.9274	1/day
m_2	1.338×10^{-3}	1/day
η	1.162	1/day
m_3	0.15	1/day
Km_{FSH}	7.533	1/day
ν	8	1/day
ϱ	0.451	1/day

Table 4 Auxiliary equations parameter set

Parameter	Value	Unit
e_0	38.25	ng/L
e_1	2.3	1/kL
e_2	2.724	1/kL
h_0	0.0525	U/L
h_1	0.0251	(nmol/L)/µg
h_2	0.06315	U/L
h_3	0.1584	U/L
p_1	0.2548	(nmol/L)/µg
p_2	0.1276	(nmol/L)/µg
j_1	27.21	pg/L
j_2	1.885	1/L
j_3	3.738	1/L
j_4	3.411	1/L
j_5	3	1/L
j_6	0.0012	1/L
t_1	21.92	ng/dL
t_2	0.3721	1/dL
t_3	0.2961	1/dL
t_4	0.4538	1/dL
t_5	0.0411	1/dL
t_6	0.7029	1/dL
t_7	0.0459	1/dL
t_8	0.1998	1/dL
RP_{LH}	1071	

Table 5 Initial conditions

State variable	Initial condition
RP_{LH}	1071
LH	12.0
RP_{FSH}	155.6
FSH	11.40
PrA1	0.6112
PrA2	15.11

Table 5 (*Continued*)

State variable	Initial condition
SmAn	12.36
RcF	0.1511
DmF	1.337
OvF	1.168
CL1	1.003
CL2	1.865
Lut1	3.265
Lut2	4.071
Lut3	6.055
Lut4	9.029

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