A DELAY DIFFERENTIAL EQUATION MATHEMATICAL MODEL FOR THE CONTROL OF THE HORMONAL SYSTEM OF THE HYPOTHALAMUS, THE PITUITARY AND THE TESTIS IN MAN

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Abstract. In this paper we develop previously studied mathematical models of the regulation of testosterone by luteinizing hormone and luteinizing hormone release hormone in the human body. We propose a delay differential equation mathematical model which improves on earlier simpler models by taking into account observed experimental facts. We show that our model has four possible equilibria, but only one unique equilibrium where all three hormones are present. We perform stability and Hopf bifurcation analyses on the equilibrium where all three hormones are present. With no time delay this equilibrium is unstable, but as the time delay increases through an infinite sequence of positive values Hopf bifurcation occurs repeatedly. This is of practical interest as biological evidence shows that the levels of these hormones in the body oscillate periodically. We next discuss stability of the other equilibria heuristically using analytical methods. Then we describe simulations with realistic parameter values and show that our model can mimic the regular fluctuations of the three hormones in the body and explore numerically some of our heuristic conjectures. A brief discussion concludes the paper.

1. Introduction

As pointed out by Cartwright and Husain (1986) blood testosterone levels fluctuate over the short term (2-3 hours) in humans. Testosterone production in the testis is influenced by the level of pituitary hormone, luteinizing hormone (LH). LH is produced by the gonadotrophs, those pituitary cells which secrete it. The

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production of LH in turn is influenced by the level of the hypothalamic hormone luteinizing hormone release hormone (LHRH), sometimes also called gonadotropin releasing hormone (Liu and Deng, 1991). LHRH is secreted by the neurones in the medio-basal hypothalamus.

Several models have been postulated to try to explain the cyclic release of this set of hormones. The first type of model has no external pulse input. Instead the regular cyclic oscillations in the levels of the three hormones are a natural consequence of the dynamic interaction between them, or in other words "feedback oscillations". It is this first type of model which will concern us in this paper.

The second type of model hypothesizes a "neural clock", which forces pulsed secretion of LHRH in waves, hence driving the hormonal system consisting of the three hormones. For practical reasons LHRH release in the brain is virtually impossible to sample in humans. In general it is a lot easier to measure levels of hormone in the blood rather than hormone secretion levels. There is divided opinion in the literature on whether this type of model is valid. Cartwright and Husain (1986) state that this type of model does not describe the inhibitory effect of gonadal steroids on the LHRH pulse generator and leads to other inconsistencies with the observed data. On the other hand there is a body of biological opinion which believes that LHRH and LH release is pulsatile, released in irregular stochastic pulses. Dierschke et al. (1970) say that this was first discovered in the rhesus monkey, and then later in man and other species. For LH release Veldhuis et al. (1987) applied discrete devolution to LH time series which suggested that the majority of human hormone secretion could occur in discrete, self-limited bursts generated at random intervals.

One of the simplest models which attempted to explain the pulsatile release of these three hormones is due to Smith (1980). $R(t)$ denotes the density of LHRH, $L(t)$ the density of LH and $T(t)$ the density of testosterone in the bloodstream at time t. Smith's model is given by the following differential equations:

$$
\begin{aligned}
\frac{dR}{dt} &= f(T) - b_1(R), \\
\frac{dL}{dt} &= g_1(R) - b_2(L) \qquad (1) \\
\text{and} \qquad \frac{dT}{dt} &= g_2(L) - b_3(T).
\end{aligned}
$$

f is monotonic decreasing and the functions $b_1,\ b_2,\ b_3,\ g_1$ and g_2 are monotonic increasing. All of these functions are positive.

Although this model does explain the cyclic fluctuations in the levels of the three hormones there is a problem with it as it fails to explain the experimental observations that the concentrations of LH and LHRH in the blood after castration still oscillate.

Smith (1983) later tried to improve this model by introducing a time delay τ between the LH concentration and the production of testosterone. This time delay is due to delay between stimulation of the testis by LH and the ultimate release of testosterone into the blood stream and the delay from the transportation time due to the hormone travelling round the body from source to destination. Smith's improved model is the same as (1) above except that in the third equation the term $g_2(L(t))$ is replaced by $g_2(L(t-\tau))$:

$$
\begin{aligned}
\frac{dR}{dt} &= f(T) - b_1(R), \\
\frac{dL}{dt} &= g_1(R) - b_2(L) \qquad (2) \\
\text{and} \qquad \frac{dT}{dt} &= g_2(L(t-\tau)) - b_3(T).
\end{aligned}
$$

Murray (1989) analyzed this model with $b_1(R) = b_1 R,\ b_2(L) = b_2 L,\ b_3(T) = b_3 T,\ g_1(R) = g_1 R$ and $g_2(L) = g_2 L$, where $b_1,\ b_2,\ b_3,\ g_1$ and g_2 are positive constants. By using a linear analysis Murray showed that there is a critical time delay τ_c such that the positive steady state of Smith's model (2) is linearly unstable due to growing oscillations. For certain parameter values limit cycle periodic solutions could occur.

Ruan and Wei (2001) also considered the general model of Smith (1983) which included a time delay, where again $b_1,\ b_2,\ b_3,\ g_1$ and g_2 are general positive monotone increasing functions. They show that for certain parameter values the unique positive steady state of this model is locally asymptotically stable whatever the value of the time delay. However for other parameter values there is a critical time delay such that this unique positive steady state is locally asymptotically stable when the time delay is less than this critical value. As the time delay passes through the critical value there is a Hopf bifurcation and the steady state becomes unstable. So this model can explain the regular cyclic fluctuations of these three hormones for certain parameter values.

Cartwright and Husain (1986) postulated another model with differential equations as follows:

$$
\begin{aligned}
\frac{dR}{dt} &= -d_R R + r_R H\left[2 - \frac{L(t-\tau_A)}{L_0} - \frac{T(t-\tau_B)}{T_0}\right], \\
\frac{dL}{dt} &= -d_L L + r_L R(t-\tau_C) \\
\text{and} \qquad \frac{dT}{dt} &= -d_T T + r_T L(t-\tau_D-\tau_E).
\end{aligned}
$$

Here $d_R,\ d_L,\ d_T,\ r_R,\ r_L$ and r_T are all rate constants. $\tau_A,\ \tau_B,\ \tau_C,\ \tau_D$ and τ_E are all time delays. $H(x)$ is the Heaviside step function,

$$
H(x) = \begin{cases} 0, & x < 0, \\ \frac{1}{2}, & x = 0, \\ 1, & x > 0. \end{cases}
$$

This model accounts for the pulsatile release of the three hormones in men. It can also explain the cyclic behaviour of LHRH and LH after castration. Nevertheless there are some problems associated with it. These problems are also associated with Smith's (1983) model and are discussed in Liu and Yang (1990).

Liu and Yang (1990) proposed an improved version of this model. However there were still biological problems associated with the improved model. In particular it still contained a Heaviside step function which has no obvious biological meaning and it ignores the dynamic interaction of the number of Leydig cells with the three hormone system. Liu and Deng (1991) further improve on Liu and Yang's model

by formulating a four dimensional differential equation model taking account of the number of Leydig cells. This model does not contain any time delay as Liu and Yang considered the biological evidence for a time delay to be equivocal. However the number of Leydig cells is then assumed to be at a quasi-steady state and so the system of differential equations again reduces to a set of three rather complicated differential equations. The model is fitted to data and explains the regular pulsatile release of the three hormones, both before and after castration.

2. Mathematical Model

It is important to include as many experimental facts as possible to make the model more realistic. Further experimental evidence is contained in the works of Dluzen and Ramirez, 1983, Ellis and Desjardins, 1984 and Steiner et al. 1982 who observed the effects of testosterone on the hypothalamus and Motta et al. 1969 and Gay, 1974 who observed the effects of LH on the hypothalamus with the help of data.

Our differential equation model which describes the population dynamics of the hypothalamic hormone, R, the pituitary hormone, L and testosterone T is

$$\begin{aligned} \frac{dR}{dt} &= \frac{b_1 R}{(L+b_3T)^a} - b_2 R, \\ \frac{dL}{dt} &= c_1 \frac{R^a L}{R^a + b_5 T^a} - c_2 L \\ \text{and} \quad \frac{dT}{dt} &= b_6 L(t-\tau)T - b_4 T, \end{aligned} \tag{3}$$

with suitable initial conditions. Here $a, b_1, b_2, \ldots, b_6, c_1$ and c_2 are strictly positive constants. This model takes into account the fact that LHRH encourages the production of LH which in turn encourages the production of testosterone. There is also a time delay between stimulation of the testis by LH and the rise in testosterone level in the bloodstream. The rate of removal of all three hormones from the bloodstream is taken to be proportional to their concentration as in Murray (1989).

To explain the first differential equation in (3) note that the release of LHRH by the hypothalamus is influenced by the combined effect of the LH and testosterone hormones. If the concentrations of LH and testosterone are small then LHRH production will be high and vice-versa if the weighted combined concentration of LH and testosterone is high. The second equation in (3) is explained similarly. The secretion of LH will be high if the level of testosterone is small and the concentration of LHRH is high. On the other hand the LH secretion is low if testosterone is high and LHRH concentration is low. The parameter value $a = 1$ gives a simple mathematical model including these effects. The two properties above are amplified greatly for higher values of a. The assumption that the rate of production of LHRH is also proportional to LHRH has similarities with the model of Liu and Deng (1991) who assume production of R proportional to $a_1 + a_2 R + a_3 R^2$ where a_1, a_2 and a_3 are constants and we assume similar feedback in the L and T equations.

Note that the model is not well defined if $L = T = 0$ or $R = T = 0$. This is not a major problem as we are primarily interested in the biologically relevant situation where all three hormones are present and the dynamics of the feedback system in

this region, but it needs to be born in mind in our mathematical analysis of the system.

Khan et al. (1986) and Meredith et al. (1986) experimentally proved that secretion of testosterone is not only stimulated by the pituitary hormone LH, but it has an autonomous secretion which comes from the adrenal cortex and is independent of LH. However this value is small (only about 5% of the normal value, (Cartwright and Husain, 1986)) so we ignore it.

LH stimulates the secretion of androgen and produces a number of Leydig cells. However we have not included the number of Leydig cells as a separate class as Liu and Deng (1991) successfully argued that the proliferation of Leydig cells is small and that they could be regarded as being in a quasi-steady state.

Our model follows the classical models of Smith (1980, 1983) and Cartwright and Husain (1986) in that it assumes that testosterone concentration inhibits the production of LHRH. As in Cartwright and Husain (1986) we are including the experimental facts from our model that both testosterone and luteinizing hormone inhibit LHRH production, although we take a slightly different functional form for this mechanism (namely $b_1R/(L+b_3T)^a$, where b_1 and b_3 are positive constants) as the Heaviside step function which they use has no obvious biological justification. As in Cartwright and Husain's model low levels of testosterone and LH encourage LHRH secretion.

As in Smith's (1980, 1983) model we assume that the rate of production of LH is a monotone increasing function of the LHRH density. Cartwright and Husain (1986) and Murray (1989) assume that this is proportional to the LHRH density but many experiments (Nagayama, 1977 and Chase, 1983) showed that this was not necessarily so, and as the dose of injected LHRH increases, the rates of increase of LH may also saturate (see also Mendelson et al. 1975 and O'Connor et al. 1980). Moreover Nagayama, 1977, Caminos-Torres, 1977 and Scheckter et al. 1989 showed that testosterone directly inhibits the secretion of LH. We include the biological evidence in our model by taking the rate of LH production to be $c_1R^aL/(R^a+b_5T^a)$ where b_5 and c_1 are positive constants.

Exactly how concentrations of hypothalamic blood LH and testosterone levels combine to regulate LHRH production is open to question. Cartwright and Husain (1986) assume a linear combination for simplicity but suggest a multiplicative combination as another possibility.

As in the models of Smith (1983), Murray (1989) and Cartwright and Husain (1986) we assume that levels of LH affect the production of testosterone in the testis. This is supported by experimental evidence described by Sharpe (1986). As in the models of Smith, Murray, and Cartwright and Husain, as the levels of LH decline then the production of testosterone in the testis also decays. A time delay occurs between testis stimulation by LH and the release of testosterone into the bloodstream. This includes the delay corresponding to the time taken for the hormones to travel across the body.

The significance of our model is that it incorporates additional experimental facts over most previously studied models. Firstly we have postulated specific functional forms for the effect of LH and testosterone on LHRH production and LHRH and T on LH production. These qualitative effects on the production functions are supported by biological evidence as described below. This improves on the models of Smith (1980, 1983), Murray (1989) and Ruan and Wei (2001) who assumed that

these were functions of respectively testosterone and LHRH only. It also improves on the model of Cartwright and Husain (1986) who took the production of LH to be a function of LHRH only.

Our model has qualitative similarities with the models of Liu and Yang (1990) and also with the models of Liu and Deng (1991) (except that it does not contain a class of Leydig cells). However our model is simpler as there are many constants in their models which have no obvious biological interpretation. Also our model improves on theirs in that it incorporates a biologically realistic time delay in the testosterone production function corresponding to the delay between the stimulation of the testis by LH and the delay from the transportation time due to the hormone travelling around the body from source to destination. In brief summary the importance of our model is that it incorporates extra qualitatively observed biological mechanisms which previous models did not.

Theorem 1

The possible equilibria of the system (3) are:

(i) $E_1 : R = 0,\ T = 0$ *and* $L = 0$*;*

(ii) $E_2 : R = T = 0$ *and* $L = \overline{L}$ *where* $\overline{L} > 0$ *is any positive constant;*

(iii) $E_3 : R = \overline{R},\ T = 0$ *and* $L = \left(\dfrac{b_1}{b_2}\right)^{\frac{1}{a}}$*. Here* $\overline{R} > 0$ *is any positive constant.*

This equilibrium is possible only for the special parameter values $c_1 = c_2$*;*

and

(iv) $E_4 : R = R^* = \dfrac{b_5^{\frac{1}{a}}}{b_3} \dfrac{\left[\left(\dfrac{b_1}{b_2}\right)^{\frac{1}{a}} - \dfrac{b_4}{b_6}\right]}{\left(\dfrac{c_1}{c_2} - 1\right)^{\frac{1}{a}}},\quad T = T^* = \dfrac{1}{b_3}\left[\left(\dfrac{b_1}{b_2}\right)^{\frac{1}{a}} - \dfrac{b_4}{b_6}\right]$ *and* $L = L^* = \dfrac{b_4}{b_6}$*. This equilibrium is feasible only if* $c_1 > c_2$ *and* $b_1 b_6^a > b_2 b_4^a$*.*

Note that the term $R/(L + b_3 T)^a$ on the right hand side of (3) is not defined at E_1, also the term $R^a/(R^a + b_5 T^a)$ is not defined at E_1 or E_2, but if the former is interpreted as zero and the latter as any finite value at E_1 and (c_2/c_1) at E_2 these equilibria are possible.

Proof of Theorem 1

Setting the right hand side of equations (3) to zero at an equilibrium value $R^*,\ T^*,\ L^*$ we deduce that

(i) $R^* = 0$ or $(L^* + b_3 T^*)^a = \dfrac{b_1}{b_2}$,

(ii) $L^* = 0$ or $1 + b_5 \left(\dfrac{T^*}{R^*}\right)^a = \dfrac{c_1}{c_2}$,

and

(iii) $T^* = 0$ or $L^* = \dfrac{b_4}{b_6}$.

The remainder of the proof is straightforward.

We are primarily interested in the stability of the system about the equilibrium E_4 which has all three hormones present. As E_3 is possible only for special parameter values we do not examine it further. It is possible to regard E_3 as a special case of equilibrium E_4 if $c_1 \to c_2,\ b_1 b_6^a \to b_2 b_4^a$.

3. Stability Analysis of the Positive Equilibrium E_4.

It is straightforward to show that the stability matrix of the system linearized about the positive equilibrium E_4 is

$$\begin{bmatrix} -\omega & -p & -pb_3 \\ q & -\omega & -r \\ 0 & b_6 T^* e^{-\omega\tau} & -\omega \end{bmatrix}$$

where

$$p = \frac{ab_1 R^*}{(L^* + b_3 T^*)^{a+1}},\ q = \frac{c_1 b_5 L^* a R^{*a-1} T^{*a}}{(R^{*a} + b_5 T^{*a})^2} \text{ and } r = \frac{c_1 b_5 R^{*a} L^* a T^{*a-1}}{(R^{*a} + b_5 T^{*a})^2}.$$

This leads to the characteristic equation

$$\omega^3 + pqb_3 b_6 T^* e^{-\omega\tau} + pq\omega + r\omega b_6 T^* e^{-\omega\tau} = 0.$$

When $\tau = 0$ this reduces to

$$\omega^3 + (pq + rb_6 T^*)\omega + pqb_3 b_6 T^* = 0,$$

and it is straightforward to use the Routh-Hurwitz criteria to show that this equilibrium is unstable. In fact it has one strictly negative real root and two complex roots with strictly positive real parts.

For $\tau \geq 0$ write $\omega = \xi + i\eta$. The characteristic equation becomes

$$(\xi + i\eta)^3 + pq(\xi + i\eta) + (pqb_3 + r(\xi + i\eta))b_6 T^* e^{-\tau(\xi + i\eta)} = 0.$$

Expanding and equating real and imaginary parts of this equation we deduce that

$$\xi^3 - 3\xi\eta^2 + pq\xi + (pqb_3 + r\xi)b_6 T^* e^{-\tau\xi}\cos(\tau\eta) + \eta r b_6 T^* e^{-\tau\xi}\sin(\tau\eta) = 0 \tag{4}$$

and

$$3\xi^2\eta - \eta^3 + pq\eta + rb_6 T^* \eta e^{-\tau\xi}\cos(\tau\eta) - (pqb_3 + r\xi)b_6 T^* e^{-\tau\xi}\sin(\tau\eta) = 0. \tag{5}$$

If $\xi = 0,\ \eta = \eta^*$ is a purely imaginary root corresponding to $\tau = \tau^*$ then

$$pqb_3 b_6 T^* \cos(\tau^*\eta^*) + \eta^* r b_6 T^* \sin(\tau^*\eta^*) = 0 \tag{6}$$

and

$$pqb_3 b_6 T^* \sin(\tau^*\eta^*) - \eta^* r b_6 T^* \cos(\tau^*\eta^*) = -\eta^{*3} + pq\eta^*. \tag{7}$$

Squaring and adding

$$\eta^{*6} - 2pq\eta^{*4} + (pq)^2\eta^{*2} = (rb_6 T^*\eta^*)^2 + (pqb_3 b_6 T^*)^2. \tag{8}$$

Let $u = \eta^{*2}$, then

$$u^3 + d_1 u^2 + d_2 u + d_3 = 0, \tag{9}$$

where $d_1 = -2pq,\ d_2 = (pq)^2 - (rb_6 T^*)^2$ and $d_3 = -(pqb_3 b_6 T^*)^2$.

This equation (9) in u always has one positive real root and must have either exactly one or exactly three positive real roots. Let $f(u) = u^3 + d_1u^2 + d_2u + d_3$. The turning points of $f(u)$ satisfy

$$3u^2 + 2d_1u + d_2 = 0,$$

so are $$u_1 = \frac{2pq - \sqrt{p^2q^2 + 3r^2b_6^2T^{*2}}}{3}$$

and $$u_2 = \frac{2pq + \sqrt{p^2q^2 + 3r^2b_6^2T^{*2}}}{3}.$$

For three positive real roots we need $u_1, u_2 > 0$, i.e. $pq > rb_6T^*$ which is equivalent to $a^ab_2^{a+1} > b_1b_6^a$. We also need

$$\Delta = \frac{4}{27}d_2^3 - \frac{1}{27}d_1^2d_2^2 + \frac{4}{27}d_1^3d_3 - \frac{2}{3}d_1d_2d_3 + d_3^2 < 0.$$

Note that $\Delta = f(u_1)f(u_2)$, see Khan and Greenhalgh (1999).

Lemma 1

The equation $f(u) = 0$ cannot have three co-incident positive real roots.

Proof of Lemma 1

If it does and the roots are $\overline{u}_1$, $\overline{u}_2$ and $\overline{u}_3$ then $\overline{u}_1 = \overline{u}_2 = \overline{u}_3 = \frac{2pq}{3}$ so

$$d_2 = \overline{u}_1\overline{u}_2 + \overline{u}_2\overline{u}_3 + \overline{u}_1\overline{u}_3 = \frac{4p^2q^2}{3} > (pq)^2 - (rb_6T^*)^2 = d_2.$$

This is a contradiction proving the lemma.

Theorem 2

(i) If $a^ab_2^{a+1} > b_1b_6^a$ and $\Delta < 0$ then $f(u) = 0$ has three strictly positive distinct real roots;

(ii) If $a^ab_2^{a+1} > b_1b_6^a$ and $\Delta = 0$ then $f(u) = 0$ has three strictly positive real roots one of which is repeated

and

(iii) If $a^ab_2^{a+1} \leq b_1b_6^a$ or $\Delta > 0$ then $f(u) = 0$ has exactly one strictly positive real root.

Proof of Theorem 2

Note that $f(0) < 0$ and $f(u) \to \infty$ as $u \to \infty$.

(i) $u_1,\ u_2 > 0$ and $f(u_1)f(u_2) < 0$ hence $f(u) = 0$ has one root in each of the intervals $(0, u_1)$, (u_1, u_2) and (u_2, ∞).

(ii) Here $u_1,\ u_2 > 0$ and $f(u_1) = 0$ or $f(u_2) = 0$ so either u_1 or u_2 is a repeated root of $f(u) = 0$. If u_1 is the repeated root then the other root lies in (u_2, ∞). If u_2 is the repeated root then the other root lies in $(0, u_1)$.

(iii) If $a^ab_2^{a+1} \leq b_1$ then $u_1 \leq 0$. $f(u)$ is decreasing in (u_1, u_2) and increasing in (u_2, ∞) so has exactly one strictly positive root which must lie in (u_2, ∞). If $\Delta > 0$

then $f(u)$ does not change sign in (u_1, u_2) so has at most one strictly positive real root.

Corollary 1

The equation $f(u) = 0$ always has at least one simple real root.

We shall now show how the positive real roots u^* of $f(u) = 0$ correspond to critical time delays τ^* at which potential Hopf bifurcation may occur. If u^* is a strictly positive real root of $f(u) = 0$ and $\eta^* = \sqrt{u^*}$ then the equations

$$\sin\alpha = \frac{pqb_3}{\sqrt{(pqb_3)^2 + (\eta^* r)^2}}$$

and

$$\cos\alpha = \frac{\eta^* r}{\sqrt{(pqb_3)^2 + (\eta^* r)^2}}$$

determine $\alpha \in [0, \pi/2)$ uniquely. From (6) and (7)

$$\sin(\tau^*\eta^* + \alpha) = 0$$

and

$$\cos(\tau^*\eta^* + \alpha) = \frac{(\eta^{*3} - pq\eta^*)}{b_6 T^* \sqrt{(pqb_3)^2 + (\eta^* r)^2}}$$

determine $\alpha + \tau^*\eta^*$ uniquely in the range $[0, 2\pi^*)$. In fact $\tau^*\eta^* + \alpha = \beta_0^*(\eta^*)$ where $\beta_0^*(\eta^*) = 0$ or π, depending on whether or not $\eta^* > \sqrt{pq}$. Hence $\tau^*\eta^* + \alpha = \beta_0^*(\eta^*) + 2k\pi$, for some integer k, so $\tau^* = ((\beta_0^*(\eta^*) + 2k\pi - \alpha)/\eta^*)$ for $k \geq 1$ if $\beta_0^*(\eta^*) = 0$, and for $k \geq 0$ if $\beta_0^*(\eta^*) = \pi$. For $\beta_0^*(\eta^*) = 0,\ k \leq 0$ and for $\beta_0^*(\eta^*) = \pi,\ k < 0$, give negative values of τ which are infeasible.

The conditions for a Hopf bifurcation to occur as τ passes through τ^* are that a complex conjugate pair of eigenvalues cross the imaginary axis as τ passes through τ^* and the crossing is transversal

i.e.

$$\left.\frac{d\xi}{d\tau}\right|_{\tau=\tau^*} \neq 0.$$

We now show that provided that the corresponding value of u^* is a simple root of (9) this tranversality condition is satisfied. Differentiating equations (4) and (5) with respect to τ and then setting $\xi = 0,\ \eta = \eta^*$ and $\tau = \tau^*$ we deduce that

$$\left.\frac{d\xi}{d\tau}\right|_{\tau=\tau^*} P + \left.\frac{d\eta}{d\tau}\right|_{\tau=\tau^*} Q = R,$$

and

$$\left.\frac{d\xi}{d\tau}\right|_{\tau=\tau^*} (-Q) + \left.\frac{d\eta}{d\tau}\right|_{\tau=\tau^*} P = S,$$

where $P = -3\eta^{*2} + pq + rb_6T^*\cos(\tau^*\eta^*) - \tau^* pqb_3b_6T^*\cos(\tau^*\eta^*)$
$\qquad -\tau^*\eta^* rb_6T^*\sin(\tau^*\eta^*),$

$Q = -pqb_3b_6T^*\tau^*\sin(\tau^*\eta^*) + rb_6T^*\sin(\tau^*\eta^*) + \eta^* r\tau^* b_6T^*\cos(\tau^*\eta^*),$

$R = pqb_3b_6T^*\eta^*\sin(\tau\eta^*) - \eta^{*2} rb_6T^*\cos(\tau^*\eta^*)$

and $S = rb_6T^*\eta^{*2}\sin(\tau^*\eta^*) + pqb_3b_6T^*\eta^*\cos(\tau^*\eta^*).$

Solving for $\left.\frac{d\xi}{d\tau}\right|_{\tau=\tau^*}$ we deduce that

$$\left.\frac{d\xi}{d\tau}\right|_{\tau=\tau^*} = \frac{PR-QS}{P^2+Q^2}. \tag{10}$$

Note that $PR-QS =$

$$\begin{aligned}
&\Big(-3\eta^{*2}+pq+rb_6T^*\cos(\tau^*\eta^*)-\tau^*pqb_3b_6T^*\cos(\tau^*\eta^*)-\tau^*\eta^*rb_6T^*\sin(\tau^*\eta^*)\Big)\\
&\qquad\times\Big(pqb_3b_6T^*\eta^*\sin(\tau^*\eta^*)-\eta^{*2}rb_6T^*\cos(\tau^*\eta^*)\Big)\\
&-\Big(-pqb_3b_6T^*\tau^*\sin(\tau^*\eta^*)+rb_6T^*\sin(\tau^*\eta^*)+\eta^*rb_6T^*\tau^*\cos(\tau^*\eta^*)\Big)\\
&\qquad\times\Big(rb_6T^*\eta^{*2}\sin(\tau^*\eta^*)+pqb_3b_6T^*\eta^*\cos(\tau^*\eta^*)\Big),\\
&=(-3\eta^{*2}+pq)\eta^*(-\eta^{*3}+pq\eta^*)\\
&+rb_6T^*\cos(\tau^*\eta^*)\Big(pqb_3b_6T^*\eta^*\sin(\tau^*\eta^*)-\eta^{*2}rb_6T^*\cos(\tau^*\eta^*)\Big)\\
&-rb_6T^*\sin(\tau^*\eta^*)\Big(rb_6T^*\eta^{*2}\sin(\tau^*\eta^*)+pqb_3b_6T^*\eta^*\cos(\tau^*\eta^*)\Big),\\
&\qquad\qquad\text{using (6) and (7),}\\
&=\quad\eta^{*2}\Big[3\eta^{*4}-4pq\eta^{*2}+\Big((pq)^2-(rb_6T^*)^2\Big)\Big],\\
&=\quad\eta^{*2}\left.\frac{df}{du}\right|_{\eta^{*2}}\neq 0, \qquad (11)
\end{aligned}$$

as u^* is a simple root of (9).

Moreover if $P=Q=0$ then $R=S=0$, so $-\eta^{*3}+pq\eta^*=0$. Therefore from (8) $(rb_6T^*\eta^*)^2+(pqb_3b_6T^*)^2=0$, a contradiction. Hence from (10),

$$\left.\frac{d\xi}{d\tau}\right|_{\tau=\tau^*}\neq 0.$$

We deduce that if u^* corresponds to a simple root of (9) Hopf bifurcation occurs as τ passes through τ^*.

Hence for $\tau=0$ the model (3) is unstable and whenever τ passes through a value $\tau^*=(\beta_0^*(\eta^*)+2k\pi-\alpha)/\eta^*$ corresponding to $\eta^*=\sqrt{u^*}$, where u^* is a simple root of $f(u)=0$, Hopf bifurcation occurs. As $f(u)=0$ has either one or three positive simple roots there are an infinite number of such values of τ^* separated by $2\pi/\eta^*$. Hence we expect that as τ increases the model starts off unstable and as τ passes through progressively increasing values Hopf bifurcation occurs repeatedly. In the regions where the equilibrium with all three hormones present is unstable we might expect limit cycle behaviour as observed biologically and found in simpler simulation models (Murray, 1989), but chaotic behaviour is another possibility. We shall explore this by simulation in Section 8.

4. Stability of the Zero Equilibrium

If $R,\ L$ and T are all small and strictly positive for $t \geq 0$ then $R \to \infty$ as $t \to \infty$ so the equilibrium E_1 is never locally asymptotically stable.

5. Heuristic Discussion of Behaviour when $c_2 > c_1$ or $b_2 b_4^a > b_1 b_6^a$.

If $c_2 > c_1$ or $b_2 b_4^a > b_1 b_6^a$ then the equilibrium with all three hormones present is not possible. If $c_2 > c_1$ then it is straightforward to show that L and T tend to zero and R tends to infinity as t becomes large.

If $c_1 > c_2$ and $b_2 b_4^a > b_1 b_6^a$ then the potential behaviour is more interesting. Define $\hat{L}$ to be the unique positive root of

$$\frac{-b_1}{L^a} + b_2 = -b_6 L + b_4.$$

Note that $\hat{L} < (b_4/b_6)$.

For $L \leq (b_1/b_2)^{\frac{1}{a}}$ indefinitely, $0 \leq T \leq T_0 e^{-pt} \to 0$ as $t \to \infty$, where $p = (b_4 - b_6(b_1/b_2)^{\frac{1}{a}}) > 0$. Hence

$$\frac{1}{R}\frac{dR}{dt} = \frac{b_1}{(L + b_3 T)^a} - b_2 \to \frac{b_1}{L^a} - b_2 \geq 0, \qquad \text{as } t \to \infty.$$

So given ϵ_1 with $p > \epsilon_1 > 0$ there exists t_0 such that for $t \geq t_0$,

$$\begin{aligned} R(t) &\geq R(t_0) e^{-\epsilon_1 (t - t_0)} \\ \text{and} \qquad T(t) &\leq T(t_0) e^{-p(t-t_0)}. \end{aligned}$$

Therefore for $t \geq t_0$, $\dfrac{R^a}{R^a + b_5 T^a} \geq \dfrac{R(t_0)^a}{R(t_0)^a + b_5 T(t_0)^a e^{-(p-\epsilon_1)(t-t_0)}} \to 1 \quad \text{as } t \to \infty.$

So $L \to \infty$ as $t \to \infty$. This is a contradiction.

Similarly for $L \geq (b_4/b_6)$ indefinitely $T \geq \tilde{T}$, where $\tilde{T} = T(0) > 0$ is a strictly positive constant, $R \to 0$ and $L \to 0$. Again this is a contradiction. Hence L enters the region $((b_1/b_2)^{\frac{1}{a}}, (b_4/b_6))$ infinitely often.

If $L \approx \hat{L}$ indefinitely:

$$T(t) \approx T_0 e^{\left(b_6 \hat{L} - b_4\right) t} \to 0 \text{ as } t \to \infty.$$

After a sufficiently large time t_1,

$$\begin{aligned} R(t) &\approx R(t_1) \exp\left[\left(\frac{b_1}{\hat{L}^a} - b_2\right)(t - t_1)\right] \to 0 \text{ as } t \to \infty, \\ T(t) &\approx T(t_1) e^{\left(b_6 \hat{L} - b_4\right)(t - t_1)}, \\ \text{and} \qquad \frac{dL}{dt} &\approx \left(\frac{c_1 R(t_1)^a}{R(t_1)^a + b_5 T(t_1)^a} - c_2\right) L. \end{aligned}$$

Moreover if $L \geq \hat{L} + \epsilon_2$ indefinitely for some $\epsilon_2 > 0$:

$$T \geq T_0 e^{\left(b_6(\hat{L}+\epsilon_2)-b_4\right)t}$$

and
$$R \leq R_0 \exp\left[\left(\frac{b_1}{(\hat{L}+\epsilon_2)^a}-b_2\right)t\right].$$

Hence
$$\frac{T^a}{R^a} \geq \frac{T_0^a}{R_0^a}\, e^{aft}, \qquad \text{for some } f>0.$$

Therefore
$$\frac{1}{1+b_5\left(\frac{T^a}{R^a}\right)} \leq \frac{1}{1+b_5\frac{T_0^a}{R_0^a}\, e^{aft}} \to 0, \qquad \text{as } t\to\infty.$$

So L tends to zero as $t\to\infty$. This is a contradiction. We deduce that $L_\infty \leq \hat{L}$.

Similarly if $L \leq \hat{L}-\epsilon_3$ indefinitely for some $\epsilon_3>0$,

$$T \leq T_0 e^{\left(b_6(\hat{L}-\epsilon_3)-b_4\right)t}.$$

Hence there exists t_2 such that for $t\geq t_2,\ T\leq(\epsilon_3/2b_3)$. For $t\geq t_2$,

$$T \leq T(t_2) e^{\left(b_6(\hat{L}-\epsilon_3)-b_4\right)(t-t_2)},$$

and
$$R \geq R(t_2)\exp\left[\left(\frac{b_1}{\left(\hat{L}-\frac{1}{2}\epsilon_3\right)^a}-b_2\right)(t-t_2)\right].$$

We deduce that
$$\frac{T(t)^a}{R(t)^a} \leq \frac{T(t_2)^a}{R(t_2)^a}\, e^{-ga(t-t_2)}, \qquad \text{for some } g>0.$$

Therefore
$$\frac{1}{1+b_5\frac{T(t)^a}{R(t)^a}} \geq \frac{1}{1+b_5\frac{T(t_2)^a}{R(t_2)^a}\, e^{-ga(t-t_2)}} \to 1, \qquad \text{as } t\to\infty.$$

So L tends to infinity. This is a contradiction so $L^\infty \geq \hat{L}$. It is tempting to conjecture that $L\to\hat{L}$ as $t\to\infty$.

Note that if $L\approx\hat{L}$ and $R^a/(R^a+b_5T^a)<(c_2/c_1)$ then L decreases, so after a delay the rate of exponential decrease of T becomes larger. Also the rate of exponential decrease of R becomes smaller. Hence $R^a/(R^a+b_5T^a)$ increases. Conversely if $L\approx\hat{L}$ and $R^a/(R^a+b_5T^a)>(c_2/c_1)$ then L increases, so after a delay the rate of exponential decrease of T becomes smaller. Also the rate of exponential decrease of R becomes larger. Hence $R^a/(R^a+b_5T^a)$ decreases. Therefore although this is only a heuristic argument it suggests that $R^a/(R^a+b_5T^a)$ may approach (c_2/c_1) as t becomes large. In this situation it is plausible that L remains constant and T and R exponentially decrease at the same rate, which in some sense corresponds to the possible equilibrium E_2 of Theorem 1 being globally stable. This is a topic which we explore further by simulation in the next section.

6. Simulations

We aim to conduct simulations using realistic parameter values to see whether our model can predict the regular oscillations observed in levels of LHRH, LH and testosterone in the bloodstream. The clearance rates of the three hormones are estimated by Keenan and Veldhuis (1998) as $b_2 = 0.23$ - 0.69/min, $c_2 = 0.0087$ - 0.014/min and $b_4 = 0.046$/min so we take $b_2 = 0.5$/min, $c_2 = 0.01$/min and $b_4 = 0.046$/min. Keenan et al. (1998) suggest from data that the elimination of LH may follow a biexponential form, but also discuss a single exponential rate. We assume a simple exponential rate for simplicity. We take $b_3 = 0.001$ and $b_5 = 0.001$ as trial and error shows that these give sensible results. We took $a = 1$ for simplicity as this is the simplest case illustrating the qualitative features of our model.

Plausible equilibrium levels of LH in the bloodstream after evening out the pulsatile variations are approximately 5 IU/l (Urban et al. 1988, Berne and Levy, 1993 and Keenan et al. 1998). Direct measurement of LHRH levels in the bloodstream are difficult to find but simulations by Keenan et al. (2000) suggest a plausible equilibrium level of 1 pg/ml. These simulations together with data in Berne and Levy (1993) suggest plausible equilibrium levels of testosterone of 600 ng/dl. Assuming these equilibrium levels we use the equilibrium equations to give $b_1 = 2.8$/min, $b_6 = 0.0092$/min and $c_1 = 0.016$/min.

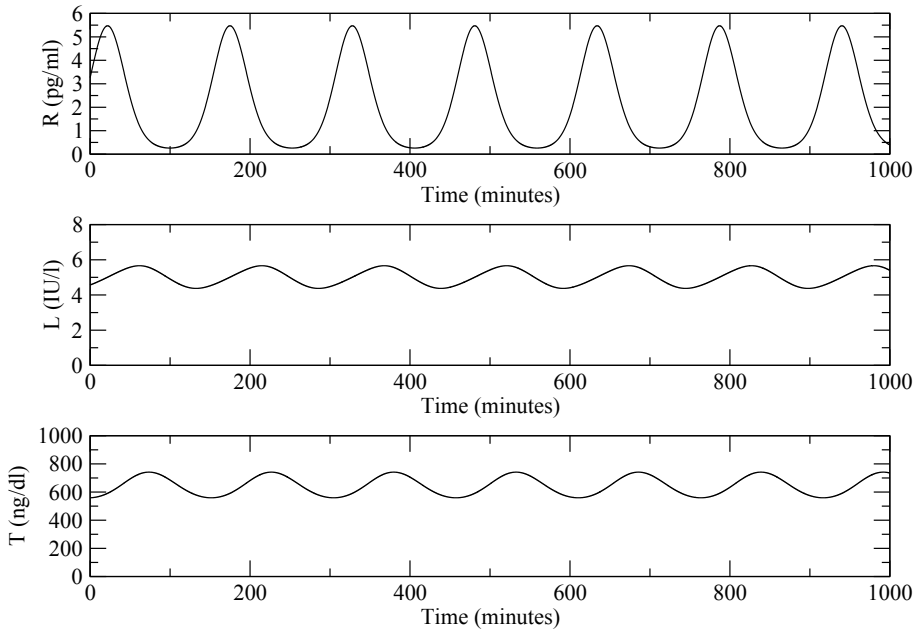

Figure 1. Regular oscillations of LHRH, LH and T in the bloodstream.

Numerical calculation, using a program written in FORTRAN and comprehensively verified using detailed output from a large number of runs shows that we expect the first three bifurcation points to be at $\tau_1 = 58.76$ minutes, $\tau_2 = 122.45$ minutes and $\tau_3 = 228.47$ minutes. In the region where $\tau \in [0, \tau_1)$ the positive equilibrium was locally unstable and L, R and T exhibited oscillations of increasing amplitude. In the region $\tau \in (\tau_1, \tau_2)$ the system converged to a stable equilibrium and in the region $\tau \in (\tau_2, \tau_3)$ convergence to stable limit cycle behaviour occurred.

We used the computer package SOLVER to numerically integrate the differential equations. Figure 1 shows simulated cycles of LHRH, LH and T in the bloodstream, in the stable limit cycle region where $\tau = 130$ minutes. The initial conditions for these simulations were $R(0) = 1$ pg/ml, $T(0) = 500$ ng/dl and $L(u) = 5$ IU/l for $-\tau \leq u \leq 0$. The oscillations are qualitatively similiar to observed and other simulated hormone fluctuation levels (Berne and Levy, 1993, Keenan and Veldhuis, 1998). In particular the LHRH oscillations are much sharper than the oscillations in the levels of the other hormones. The frequency of testosterone fluctuations at 10 pulses/day also agrees well with observed data (Keener and Sneyd, 1998).

We also investigated computationally our heuristic discussion of the behaviour when $c_1 > c_2$ and $b_2 b_4^a > b_1 b_6^a$. We observed three sorts of behaviour which seemed to correspond to (i) L converges to $\hat{L}$ and R and T decrease exponentially to zero, (ii) L exhibits stable limit cycle behaviour and R and T decrease to zero and (iii) L, R and T exhibit increasing oscillations and the solutions terminate because R becomes extremely large. For simulations where the first type of behaviour occurred our conjectures in Section 7 were clearly true in the sense that L converged to $\hat{L}$, $R^a/(R^a + b_5 T^a)$ approached (c_2/c_1) and L and T both decreased exponentially at rate $b_6\hat{L} - b_4 < 0$. For simulations where the second type of behaviour occurred the hormone L showed limit cycle behaviour, $R^a/(R^a + b_5 T^a)$ approached (c_2/c_1) in an oscillatory fashion and both R and T decreased exponentially (although still with oscillations) at long term exponential rate approximately $b_6\hat{L} - b_4$.

The convergence of $R^a/(R^a + b_5 T^a)$ and exponential decrease of R and T were sharper and clearer in the situations where L converged to a stable value, but occurred in both the cases (i) and (ii) above. Simulations were performed with a wide range of parameter values and initial conditions and in each case one of these three types of behaviour occurred, although cases (i) and (ii) appeared more common than case (iii).

As an illustrative example where L converged to a stable value we consider simulations with the same parameters as in Figure 1 but with b_3 reduced to 0.0 and b_6 reduced to 0.0046/min. The conditions $c_1 > c_2$ and $b_2 b_4^a > b_1 b_6^a$ are clearly satisfied. The initial values were $R(0) = 1$ pg/ml, $T(0) = 600.0$ ng/dl and $L(u) = 5.7$ IU/l for $-\tau \leq u \leq 0$. The results are shown in Figure 2. LH ultimately tends to $\hat{L} = 5.8238$ IU/l and R and T ultimately decrease exponentially at rate $b_6\hat{L} - b_4 = -0.01921$/min.

7. Summary and Discussion

In this paper we have discussed a delay-differential equation model to explain the cyclic release of three hormones in the body: the hypothalamic hormone LHRH, pituitary hormone LH and testosterone. We postulated a new delay-differential equation model which improves on previous modelling efforts by taking into account the fact that LHRH encourages the production of LH which in turn encourages the

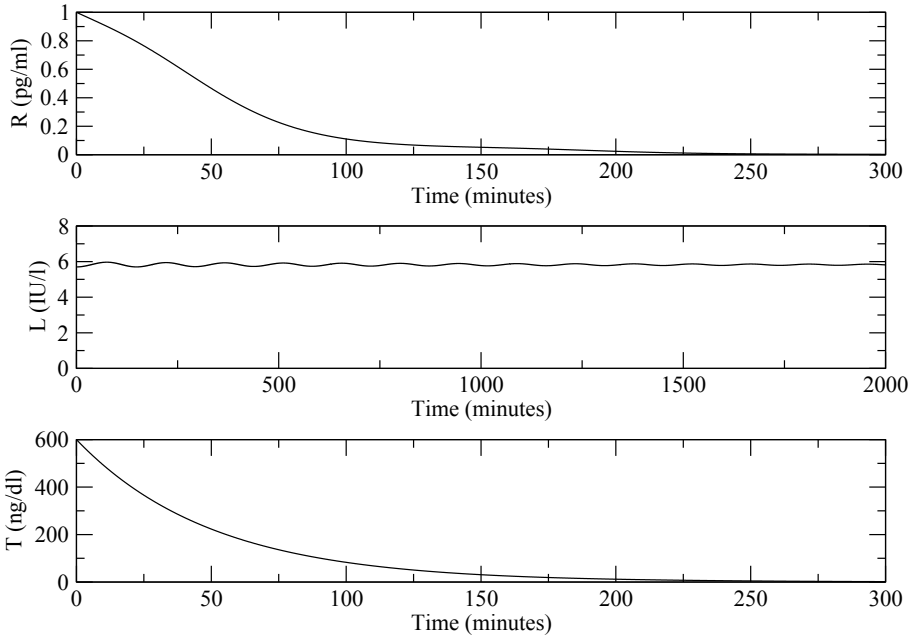

Figure 2. LH tends to a constant level, exponential decline of LHRH and T.

production of testosterone. We also took into account a time delay between stimulation of the testis by LH and the rise in testosterone level in the bloodstream. In our model we found that there was a unique equilibrium with all three hormones present. This was unstable when the time delay was zero and as the time delay τ increases passing through a progressively increasing infinite series of values Hopf bifurcation occurs repeatedly. We then briefly discussed the likely stability properties of the other equilibria. Simulations with realistic parameter values illustrated qualitatively similar patterns of cyclic hormone fluctuations as reported in the literature from biological observations and other simulations. In regions of instability the hormone levels either exhibited limit cycles or increasing oscillatory patterns about the unique equilibrium with all three hormones present. For other parameter values LH either tended to a constant level or oscillated about a constant level, whilst LHRH and testosterone ultimately decreased exponentially to zero.

As with any other mathematical model our results are dependent on the modelling assumptions and it is difficult to be sure about all of these. There is clearly a lot of stochasticity in the observed levels of hormones in the bloodstream which our model does not capture. Also other models have proposed stochastic pulsatile secretion of LHRH and LH (but not testosterone) and used stochastic differential equations to model this. The model incorporated a stochastic pulsing mechanism with a 24 hour circadian rythm (Keenan and Veldhuis, 1998, Keenan et al. 1998). Most of the evidence for pulsatile secretion is indirect as it is difficult to directly measure secretion of these hormones in man. Also these models use different functional forms for the differential equations (involving combinations of univariate and

bivariate logistic functions) and are much more complex than our relatively simple models. Models of this type can explain some data well (Keenan and Veldhuis, 1998, Keenan et al. 1998) but have also been criticised by Cartwright and Husain (1986) who claim that this type of model does not describe the inhibitory effect of gonadal steroids on the LHRH pulse generator and leads to other inconsistencies with the observed biological data.

Our results complement these more complex simulations. Even if the release of LHRH and LH is actually pulsatile, the fact that simulations of relatively simple approximate deterministic models also closely qualitatively resemble observed fluctuations in hormone data driven via limit cycle fluctuations provides an alternative underlying mechanism re-inforcing the cyclic behaviour, in parallel with the pulsatile release.

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REFERENCES

1. R. M. Berne and M. N. Levy, Physiology, Mosby Year Book, St. Louis (1993).

2. R. Caminos-Torres, L. Ma and P. J. Snyder, Testosterone-induced inhibition of the LH and FSH responses to gonadotropin-releasing hormone occurs slowly, *Journal of Clinical Endocrinology and Metabolism*, **44** (1977) 1142-1153.

3. M. Cartwright and M. Husain, A model for the control of testosterone secretion, *Journal of Theoretical Biology*, **123**, (1986) 239-250.

4. D. J. Chase, Modification of acute testosterone responsiveness to luteinizing hormone by follicle-stimulating hormone and luteinizing hormone in the domestic cockerel, *Biology of Reproduction*, **29**, (1983) 143-150.

5. D. J. Dierschke, A. N. Bhattacharya, L. E. Atkinson and E. Knobil, Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey, *Endocrinology*, **87**, (1970) 850-853.

6. D. E. Dluzen and V. D. Ramirez, In vivo LHRH release from the median eminence of conscious unrestrained intact, acute castrate and long term castrate male rats as determined with push-pull perfusion, Program, 65th Annual Meeting of the Endocrine Society, (1983) p.156.

7. G. B. Ellis and C. Desjardines, Orchidectomy unleases pulsatile leutinizing hormone secretion in the rat, *Biology of Reproduction*, **30**, (1984) 619-627.

8. V. L. Gay, Decreased metabolism and increased serum concentrations of LH and FSH following nephrectomy of the rat: absence of short-loop regulatory mechanisms, *Endocrinology*, **95**, (1974) 1582-1588.

9. D. M. Keenan, W. Sun and J. D . Veldhuis, A stochastic biomathematical model of the male reproductive hormone system, *SIAM Journal of Applied Mathematics*, **61**, (2000) 934-965.

10. D. M. Keenan and J. D. Veldhuis, A biomathematical model of time-delayed feedback in the human male hypothalamic-pituitary-Leydig cell axis, *American Journal of Physiology: Endocrinology and Metabolism*, **275**, (1998) E157-E176.

11. D. M. Keenan, J. D. Veldhuis and R. Yang, Joint recovery of pulsatile and basal hormone secretion by stochastic nonlinear random effects analysis, *American Journal of Physiology: Regulatory Integrative Comparative Physiology*, **275**, (1998) R1939-R1949.

12. J. Keener and J. Sneyd, Mathematical Physiology, Springer-Verlag, New York, (1998).

13. I. A. Khan, E. Lopez and J. Leloup-Hatey, Effects of hypophysectomy on the testis of the European eel (Anguilla anguilla L.), *General and Comparative Endocrinology*, **62**, (1986) 411-418.

14. Q. J. A. Khan and D. Greenhalgh, Hopf bifurcation in epidemic models with a time delay in vaccination, *IMA Journal of Mathematics Applied in Medicine and Biology*, **16**, (1999) 113-142.

15. B.-Z. Liu and G.-M. Deng, An improved mathematical model of hormone secretion in the hypothalamo-pituitary-gonad axis in man, *Journal of Theoretical Biology*, **150**, (1991) 51-58.

16. B.-Z. Liu and L. Yang, (In Chinese), *Journal of Biomathematics*, **5**, (1990) 148-.

17. C. Mendelson, M. Dufau and K. Catt, Gonadotropin binding and stimulation of cyclic adenosine 3':5'-monophosphate and testosterone production in isolated Leydig cells, *Journal of Biological Chemistry*, **250**(22), (1975) 8818-8823.

18. S. Meredith, D. Kirkpatrick-Keller and R. L. Butcher, The effects of food restriction and hypophysectomy on numbers of primordial follicles and concentrations of hormones in rats, *Biology of Reproduction*, **35**, (1986) 68-73.

19. M. Motta, F. Fraschini and L. Martini, Short feedback mechanisms in the control of anterior pituitary function, In: Frontiers in Neuroendocrinology, eds. W. F. Ganong and L. Martini, Oxford University Press, New York (1969) 221-253.

20. J. D. Murray, Mathematical Biology, Springer-Verlag, New York, 1989.

21. Y. Nagayama, Direct feedback inhibition by endogenous testosterone on the pituitary in man, *Journal of Clinical Endocrinology and Metabolism*, **45**, (1977) 215-220.

22. J. L. O'Connor, M. B. Allen and V. B. Mahesh, Castration effects on the response of rat pituitary cells to luteinizing hormone-releasing hormone: retention in dispersed cell culture, *Endocrinology*, **106**, (1980) 1706-1714.

23. S. Ruan and J. Wei, On the zeros of a third degree exponential polynomial with applications to a delayed model for the control of testosterone secretion, *IMA Journal of Mathematics Applied in Medicine and Biology*, **18**, (2001) 41-52.

24. C. B. Scheckter, A. M. Matsumoto and W. J. Bremner, Testosterone administration inhibits gonadotropin secretion by an effect directly on the human pituitary, *Journal of Clinical Endocrinology and Metabolism*, **68**, (1989) 397-401.

25. R. M. Sharpe, Paracrine control of the testis, *Clinics in Endocrinology and Metabolism*, **15**(1), (1986) 185-207.

26. W. R. Smith, Hypothalamic regulation of pituitary secretion of luteinizing hormone II. Feedback and control of gonadotropin secretion, *Bulletin of Mathematical Biology*, **42**, (1980) 57-78.

27. W. R. Smith, Qualitative mathematical models of endocrine systems. *American Journal of Physiology: Regulatory Integrative Comparative Physiology*, **245**, (1983) R473-R477.

28. R. A. Steiner, W. J. Bremner and D. K. Clifton, Regulation of luteinizing hormone pulse frequency and amplitude by testosterone in the adult male rat, *Endocrinology*, **111**, (1982) 2055-2061.

29. R. J. Urban, W. S. Evans, A. D. Rogol, D. L. Kaiser, M. L. Johnson and J. D. Veldhuis, Contemporary aspects of discrete peak-detection algorithms. I. The paradigm of the luteinizing hormone pulse signal in men, *Endocrine Reviews*, **9**, (1988) 3-37.

30. J. D. Veldhuis, Issues in quantifying pulsatile neurohormone release, Chapter 11 in: Methods in Neuroendocrinology, ed. Louis D. van de Kar, CRC Press: Cellular and Molecular Neuropharmacology, Boca Raton, Florida (1998).

31. J. D. Veldhuis, V. Guardabasso, A. D. Rogol, W. S. Evans, K. Oerter, M. L. Johnson and D. Rodbard, Appraising the nature of luteinizing hormone secretory events in man, *American Journal of Physiology: Endocrinology and Metabolism*, **252**, (1987) E599-E605.