

REGULATION OF OVULATION NUMBER IN MAMMALS

A FOLLICLE INTERACTION LAW THAT CONTROLS MATURATION

H. MICHAEL LACKER, *Department of Pathology, New York University Medical Center, New York 10016 and Courant Institute of Mathematical Sciences, New York University, New York 10012*

ABSTRACT The assumption that developing follicles communicate through circulating hormones has been used to obtain a class of interaction laws that describe follicle growth. A specific member of this class has been shown to control ovulation number. Although all interacting follicles obey the same growth law and are given initial maturities that are chosen at random from a uniform distribution, ovulatory and atretic follicles emerge. Changing the parameters in the growth law can alter the most probable ovulation number and the shape of the frequency distribution of ovulation numbers. For certain parameter values, anovulatory states are also admitted as possible solutions of the growth law. The behavior of the model is examined for interacting follicle populations of different size. Methods are suggested for identifying growth laws in particular mammals. These can be used to test the model from experimental data.

1. INTRODUCTION

In many forms of plant and animal life, nature has selected reproductive systems which deliver large numbers of germ cells (often on the order of 10^5 – 10^6) to the external environment at times propitious for fertilization and growth. Most female mammals, in contrast, release a small (10^0 – 10^1) and relatively characteristic number of eggs at the time of ovulation. This often results in a litter size that is typical of the species or breed (3).

A large reserve pool of follicles is formed before birth in many mammals. Each follicle in the reserve pool consists of an oocyte and a few surrounding supporting cells. Follicles continually initiate growth from this reserve pool so that, at any time, the ovary contains many follicles in different stages of maturation. During each estrous (menstrual) cycle only a small number of the growing follicle population reach ovulatory maturity. The remainder atrophy and die at various times and stages of development. The developing follicles interact through circulating hormones, including estradiol and the gonadotropins luteinizing hormone and follicle-stimulating hormone. This paper explores the hypothesis that this interaction regulates the number of follicles that eventually mature and release ova.

2. FORMULATION OF MODEL

In this section, a model is proposed to describe the interaction between developing follicles by means of circulating hormones. It should be emphasized that the aim is not to provide a

Address reprint requests to Dr. Lacker at the Courant Institute of Mathematical Sciences, New York, 10012.

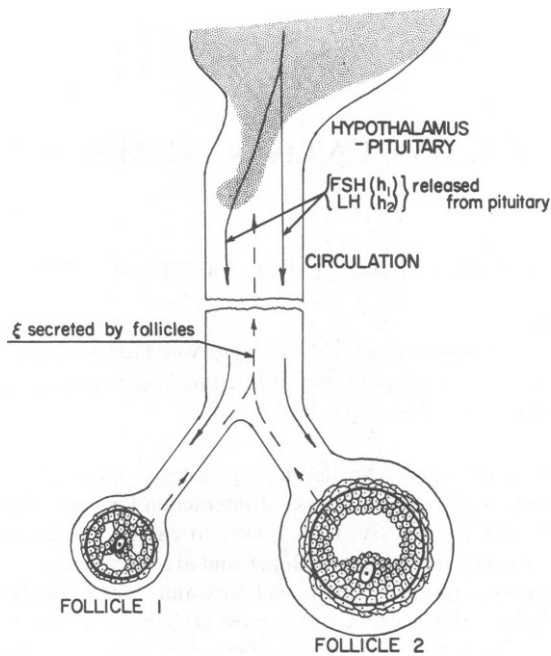


FIGURE 1 Schematic representation of the interaction between two developing follicles. Follicle estradiol secretory rate is used as a measure of follicle maturity. The circulating concentration of estradiol, ξ , is assumed to control the release of the pituitary gonadotropins FSH and LH. These pituitary hormones regulate the rate of follicle maturation. The response of a follicle to the circulating concentrations of FSH (h_1) and LH (h_2) at any particular time is assumed to depend on follicle maturity.

detailed description of follicle development, which is, of course, a very complicated process. The intent is rather to formulate a simple scheme that correctly represents the qualitative behavior and at the same time provides a conceptual framework for more detailed models.

The following idealizations will be formalized and explored as a mechanism for the regulation of ovulation number¹ (Fig. 1).

I) Follicle estradiol secretion rate is a marker of follicle maturity.

II) The serum concentration of estradiol controls the release of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

III) The serum concentrations of FSH and LH, in turn, regulate the rate of follicle maturation. At a given instant, the response of each follicle to FSH and LH depends on the follicle's maturity.

We formalize these assumptions with the following definitions:

- h_1, h_2 serum concentrations of FSH and LH, respectively
- ξ serum estradiol concentration
- γ serum estradiol clearance constant

¹See references 35, 45, 56, 59, and 67 for a critical review of the extensive experimental literature supporting these assumptions.

| | |
|---------|--|
| V | plasma volume |
| s_i | estradiol secretion rate of the i th follicle |
| ξ_i | contribution that the i th follicle makes to the serum estradiol concentration |
| N | number of interacting follicles |
| t | time |

Consider a system of N interacting follicles. Each follicle is characterized by its estradiol secretion rate $s_i(t)$, $i = 1, \dots, N$. Assume that estradiol is distributed in the plasma volume V at concentration $\xi(t)$ and that it is removed at a rate proportional to its concentration, $\gamma\xi$ (7, 64). Inasmuch as the rate of change of serum estradiol must be equal to the difference between its production rate and removal rate, it follows that

$$V d\xi/dt = \sum_{i=1}^N s_i(t) - \gamma\xi. \quad (1)$$

If estradiol is removed from the plasma at rates that are fast on the time scale of follicle maturation (7, 48, 64), then $\xi(t)$ is always near its equilibrium value. More precisely, if $s_i(t)$ slowly varies on the time scale given by $\tau = V/\gamma$, then

$$\xi(t) = (1/\gamma) \sum_{i=1}^N s_i(t). \quad (1a)$$

If h_1 and h_2 represent the serum hormone concentrations of FSH and LH, respectively, then assumption III can be written as

$$ds_i/dt = \bar{f}(s_i, h_1, h_2), \quad i = 1, \dots, N. \quad (2)$$

The function \bar{f} represents the intrafollicular mechanism by which FSH and LH regulate the rate of follicle maturation. Ultimately, this is expressed at the cellular level by the way these receptor-bound hormones effect cell division, protein synthesis, and the further production of steroids and receptors (see, for example, references 28, 46, and 54). \bar{f} , therefore, implicitly represents a complex, interrelated sequence of events involving many chemical mediators that act within and between the different cellular compartments that form a follicle.

Eq. 1a can be rewritten in the form

$$\xi(t) = \sum_{i=1}^N \xi_i(t), \quad (3)$$

where $\xi_i(t) = s_i(t)/\gamma$ is the contribution that each follicle makes to $\xi(t)$. Since ξ_i is proportional to s_i , it is an equally valid measure of follicle maturity (assumption I). As above, we assume that the equilibrium rates for FSH and LH are fast on the time scale of follicle growth (14, 62, 66). Then assumption II implies $h_1 = h_1(\xi)$, $h_2 = h_2(\xi)$. We can therefore write Eq. 2 in the form

$$d\xi_i/dt = f(\xi_i, \xi), \quad i = 1, \dots, N, \quad (4)$$

where $f(\xi_i, \xi) = (1/\gamma) \bar{f}[\gamma\xi_i, h_1(\xi), h_2(\xi)]$.

Our model consists of Eqs. 3 and 4, which we write together as a system for future reference:

$$\left. \begin{aligned} d\xi_i/dt &= f(\xi_i, \xi), \quad i = 1, \dots, N \\ \xi &= \sum_{j=1}^N \xi_j. \end{aligned} \right\} \quad (5)$$

System 5 summarizes assumptions I–III. It is important to recognize that the effects of FSH and LH are still present in system 5, although their explicit representation has been removed. System 5, of course, represents a class of models that becomes a particular model when the function f is specified, as we shall do.

There are two important symmetries in system 5. First, the form of f is the same for all i . This means that all follicles are assumed to obey the same law of growth. The second symmetry in system 5 is that interactions between different follicles occur only through ξ , which is a symmetrical function of ξ_i . This expresses the idea that follicles communicate only by means of circulating hormones and that all follicles are exposed, at any instant, to only one circulating concentration of each of these hormones. Follicles can, of course, react differently to the same circulating milieu, since f depends on maturity, ξ_i , and ξ .

3. SUPPORT FOR THE ASSUMPTION THAT GLOBAL INTERACTIONS REGULATE OVULATION NUMBER

We therefore propose a mechanism of interaction between follicles that is independent of the distance separating them. The assumption that ovulation number is regulated by a spatially independent mechanism is consistent with the finding in mice that the distribution of eggs shed from left and right ovaries, conditioned on a given ovulation number, satisfies the binomial law (25, 44). The hypothesis that ovulation number is regulated by a mechanism that involves significant follicle-follicle interaction through direct diffusion of locally produced growth mediators such as estradiol is not, in general, consistent with the absence of significant deviation from binomial statistics. Results similar to those of references 25 and 44 are suggested by earlier studies on other species (11–13, 21). A spatially independent mechanism is also supported by Lipschütz's law of follicular constancy (40), which expresses a common observation that, in many mammals studied, removal of one ovary does not change the total number of ova released per cycle (1, 9, 19, 20, 26, 29, 32, 33, 41, 42, 49, 50, 51, 53, 57, 61).

It is important to note that both Lipschütz's law and the statistical evidence referred to above are also consistent with the hypothesis that ovulation number is regulated by mechanisms that do not involve follicle interaction at all. However, the assumption of independent follicle growth is rejected because it cannot explain the degree of control that mammals achieve.² It is for this reason that follicle-independent factors that influence

²The assumption of independent follicle growth and a large number of developing follicles when compared with the mean ovulation rate implies that the distribution of ovulation number frequencies should obey Poisson statistics. In women, for example, this means that the frequency of anovulatory cycles should be close to 40% and that the frequency of double ovulations should be near 20%. Since the actual estimated frequencies are much lower than those numbers (23, 47), the assumption that regulation of ovulation number occurs by independent follicle growth has been rejected.

gonadotropin release have not been included in the simple formalism. These influences are believed to modulate the proposed mechanism for regulation of ovulation number, and, therefore, would be considered in a more detailed scheme. Some of these factors are seasonal variation, diurnal lighting patterns, vaginal wall stimulation, and steroid production by sources other than growing follicles, including the corpus luteum (see reference 58 for a review).

4. QUALITATIVE FEATURES OF A FOLLICLE MATURATION LAW

Can a single developmental scheme in which follicles influence each other only through circulatory hormones

- 1) allow a few follicles to emerge from the interacting population with ovulatory maturity while the remainder atrophy and die at different times and stages of growth;
- 2) keep the ovulation number nearly independent of the initial distribution of follicle maturities; and
- 3) account for the fact that mammalian species and breeds have different characteristic litter sizes?

In this section, we will begin to study a particular maturation law that exhibits these features. Although the details of this maturation law are too simple to be realistic, we believe that it exhibits the qualitative behavior that is needed for control of ovulation number. Experiments for identifying such maturation laws in mammals and for testing this belief are suggested in section 9.

The specific example that we will analyze is

$$\left. \begin{aligned} d\xi_i/dt &= \xi_i \phi(\xi_i, \xi), \quad i = 1, \dots, N \\ \xi &= \sum_{j=1}^N \xi_j \\ \phi(\xi_i, \xi) &= 1 - (\xi - M_1 \xi_i)(\xi - M_2 \xi_i), \end{aligned} \right\} \quad (6)$$

where M_1 and M_2 are parameters. Note that M_1 and M_2 do not change for different follicles. All N follicles obey the same developmental plan. The function f , developed in section 2, has been written in the equivalent form $f(\xi_i, \xi) = \xi_i \phi(\xi_i, \xi)$, because ϕ can be given a simple physiologic interpretation. If s_i is assumed to be proportional to the number of estradiol-secreting cells within a developing follicle, then ϕ will be proportional to the net growth rate per cell (see section 9).³ It should be emphasized that this assumption is not necessary for the validity of the model, because ϕ can be defined more generally as $\phi = (1/\gamma)(d/dt) \ln s_i$.

The particular model represented by the system 6 is motivated by the stability of its equilibria and the properties of its symmetric solutions. Before discussing these features, however, a rough idea of how the model works is obtained by considering the behavior of the function ϕ when ξ is fixed and ξ_i varies. This corresponds to the situation in the ovary at a particular time, since there is a distribution of follicle maturities but only one concentration of

³For any given follicle, the net growth rate is defined as the net difference between the rate of cell division and the rate of cell death.

each circulating gonadotropin. As a function of the maturity ξ_i the growth rate ϕ has a parabolic form with a maximum at a particular value of maturity. This value is proportional to the instantaneous value of ξ . That is,

$$\xi_{i_{\max}} = \xi \frac{1}{2} \left(\frac{1}{M_1} + \frac{1}{M_2} \right). \quad (7)$$

When ξ_i varies too much in either direction from this optimal value, the growth rate is negative. Thus, the model promotes the growth of follicles whose individual maturities lie in a certain range. Since the optimal maturity is proportional to ξ , which increases with time, a group of follicles is selected for growth. It should be emphasized, however, that it is difficult to predict the outcome of the interaction without further analysis (see section 5). At any instant, every developing follicle exerts both stimulatory and inhibitory effects on all other developing follicles, and the net result of these simultaneous interactions determines the serum estradiol concentration ξ .

A representative numerical solution of system 6 is illustrated in Fig. 2. Although all five follicles satisfy the same growth law, two follicles emerge with ovulatory maturity at nearly the same time. The remaining follicles atrophy and die after reaching different peak maturities at various times.

Ovulatory maturation curves are assumed to increase in slope near the time of ovulation for the following reason: in women and primates, the serum estradiol concentration during the

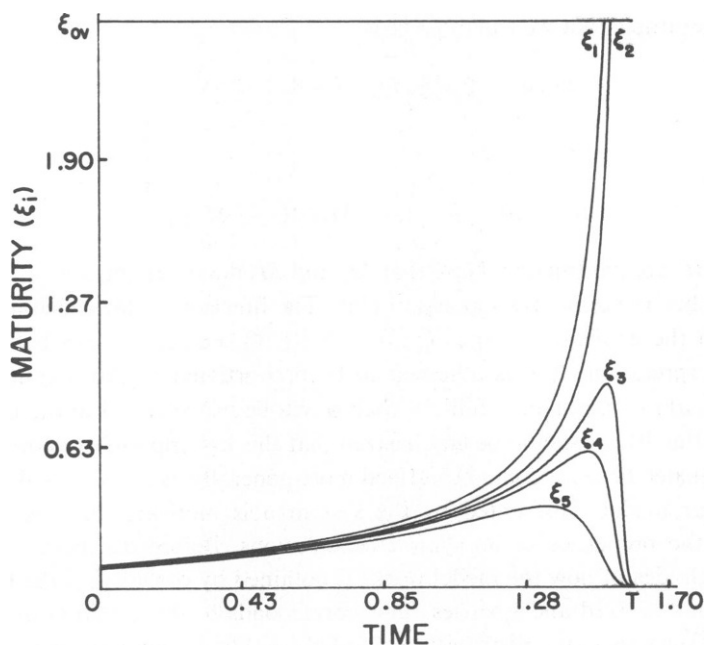


FIGURE 2 Ovulatory and atretic solutions for five follicles with slightly different initial maturities. All follicles obey Eq. 6, with $M_1 = 1.95$ and $M_2 = 6.5$. Two follicles emerge with ovulatory maturity, ξ_{ov} , at nearly the same time. All other follicles atrophy and die, although they reach different peak maturities at different times.

midfollicular to late follicular phase of the cycle is almost entirely due to a single ovulatory follicle (4–7). During this time, the serum estradiol curve does not approach an equilibrium but continues to increase in slope (6, 62). High, fast-rising serum estradiol levels appear to be important in triggering the preovulatory gonadotropin surge (38). On the time scale of follicle development, the surge is essentially an instantaneous event that causes egg release from those follicles that have emerged with ovulatory maturity.

On the basis of the very steep slope of the ovulatory curve and the assumption of converging ovulatory solutions near the midcycle surge, the following natural idealization is made. We consider the curves $\xi_1(t)$ and $\xi_2(t)$ of Fig. 2 to arise from solutions to system 6 that “blow up” in finite time T with ratio 1. In this idealized model, ovulation is interpreted as a simultaneous event, where M follicles reach the same (infinite) maturity in finite time T . In this way, the proposed interaction mechanism will not only control ovulation number but also ovulation time. It should be emphasized, however, that factors independent of the proposed interaction mechanism are also important in the timing of ovulation in many species (58). For example, in the rat, diurnal surges in gonadotropin appear to drive the estrous cycle at higher frequencies than the period of follicle maturation.

5. SYMMETRIC SOLUTIONS AND STABILITY OF SYMMETRIC EQUILIBRIA

We begin to understand how the dynamical system 6 regulates ovulation number by studying the special case in which M follicles are identical and the rest are dormant. Since ξ is the sum of the contributions made by M identical follicles, it follows that

$$\xi_i(t) = \begin{cases} \xi(t)/M, & i = 1, \dots, M \\ 0, & i = M + 1, \dots, N. \end{cases} \quad (8)$$

Substituting Eq. 8 into system 6 leads to

$$d\xi/dt = \xi + \mu\xi^3, \quad (9)$$

where $\mu = -(1 - M_1/M)(1 - M_2/M)$. Without loss of generality, we will assume that $M_2 > M_1$.

When the number of identical follicles M is between M_1 and M_2 , the stimulatory interaction term dominates ($\mu > 0$), and the maturation trajectories $\xi_i(t)$ are ovulatory (Fig. 3 b). The idealized ovulation time T is given by

$$T = \ln [(1 + \mu\xi_0^2)/\mu\xi_0^2]^{1/2} \quad (10)$$

where ξ_0 is the initial serum estradiol concentration. If the number of follicles M lies outside the interval (M_1, M_2) , then $\mu < 0$. The cubic term in Eq. 9 will now inhibit growth, and an equilibrium maturity, $\xi_M = 1/\sqrt{(M - M_1)(M - M_2)}$, and equilibrium serum estradiol concentration, $M\xi_M$, will be approached (Fig. 3 a). This will be true for arbitrary initial values of ξ . Thus, ovulation numbers are restricted to the interval (M_1, M_2) .

These special solutions can be represented in a different way, which will be helpful in understanding the more general case in which N follicles with different maturities interact. If we assign to each follicle a coordinate axis in N -dimensional space, then the special solutions

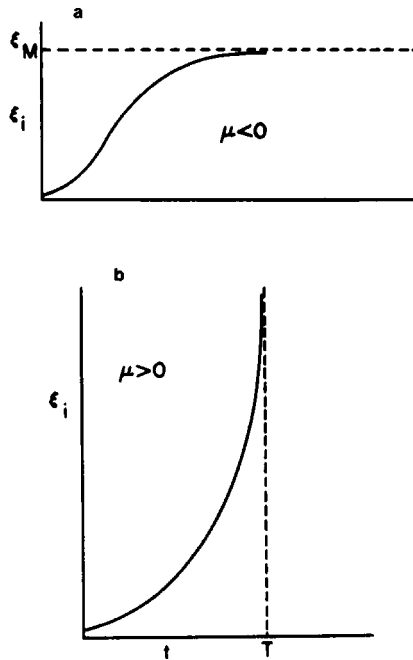


FIGURE 3 Qualitative behavior of the symmetric solutions of Eq. 6. These correspond to the special case in which M developing follicles are imagined to interact with *exactly* the same maturity and all other follicles are assumed dormant. If the number of follicles, M , is between M_1 and M_2 ($\mu > 0$), then an ovulatory solution develops (b). However, if M lies outside the interval (M_1, M_2) then the M follicles approach an equilibrium $\xi_M = 1/\sqrt{(M - M_1)(M - M_2)}$ maturity (a). Note that the idealized ovulation time, T , (b) depends on M and initial maturity (see Eq. 10).

represented by Eq. 8 will lie along the lines of symmetry l_M of the M -dimensional coordinate hyperplanes (see Fig. 4 a). When the number of identical follicles M is outside (M_1, M_2) , l_M will contain an equilibrium point P_M that blocks ovulation. However, if M is in the interval (M_1, M_2) , then there will be no blocks along l_M , and the trajectories will escape to ∞ in a finite time T given by Eq. 10.

Any arrangement of maturities of the N follicles can be represented by a point P in N -space with coordinates $(\xi_1, \xi_2, \dots, \xi_N)$. Given an initial arrangement $P(t_0)$, Eq. 6 will determine a unique phase-space trajectory $P(t)$. The projection of $P(t)$ onto the i th coordinate axis will represent the development of the i th follicle $\xi_i(t)$.

We can get an idea of how these trajectories will behave by examining the stability of the equilibria P_M to small perturbations in any direction. Let $\tilde{P} = (\tilde{\xi}_1, \dots, \tilde{\xi}_N)$ be an arbitrarily small perturbation from P_M (defined by M of its coordinates equal to $\xi_M = 1/\sqrt{(M - M_1)(M - M_2)}$ and the remainder zero). Substituting $P_M + \tilde{P}$ into Eq. 6 leads to the linear system,

$$d\tilde{P}/dt = A\tilde{P}, \quad (11)$$

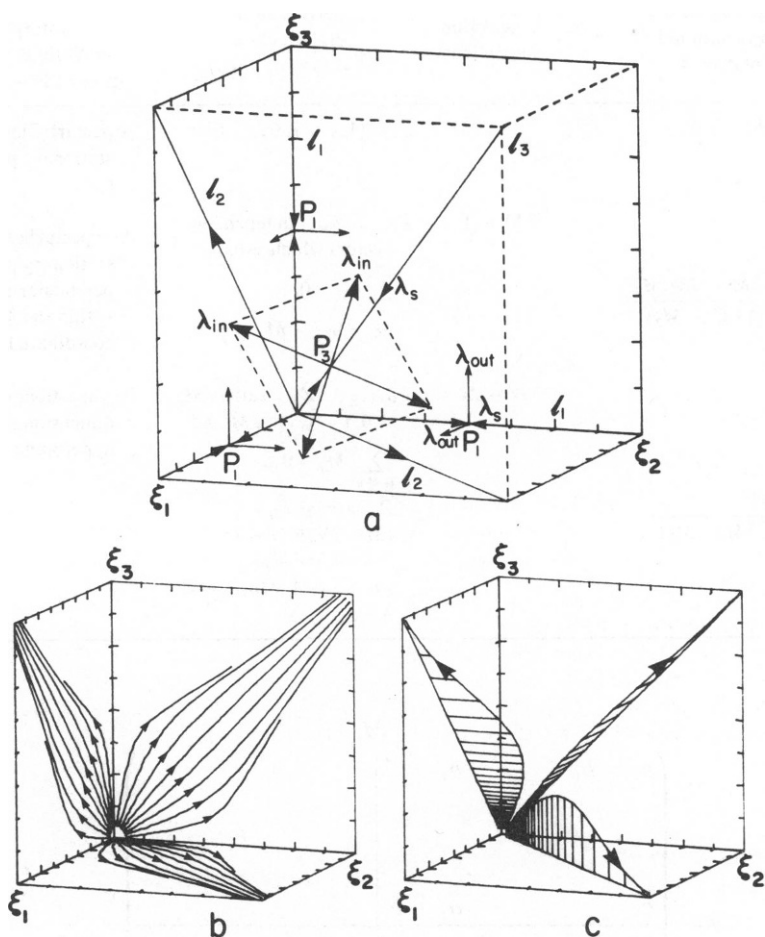


FIGURE 4 N space trajectories for a system of three interacting follicles. The dashed lines in (a) are out of the plane of the paper. The coordinates of each point represent the maturities of the three follicles. All three follicles obey Eq. 6, with $M_1 = 1.9$ and $M_2 = 2.9$. These parameters are chosen so that the number of follicles that ovulate will be regulated at 2. (a) Symmetric solutions. Since 1 and 3 are outside (M_1, M_2) , equilibrium states P_1 and P_3 occur on the lines of symmetry l_1 and l_3 , respectively. P_1 prevents one follicle from ovulating when the other two are dormant (solutions along l_1), and P_3 prevents all three follicles from ovulating when they start with identical maturities (solutions along l_3). In contrast, 2 is in the interval (M_1, M_2) , and therefore there is no equilibrium on l_2 to prevent two identical follicles from ovulating when the third is dormant. (Trajectories on l_2 escape to ∞ in finite time given by Eq. 10). P_1 and P_3 are unstable saddle point equilibria that direct solutions toward l_2 . This is demonstrated in b for solutions in the coordinate planes and in c for trajectories in which all three follicles are nondormant. Since the trajectories along l_1 and l_3 are unstable, two follicles will ovulate and one will die independent of the initial maturities of the follicles.

TABLE I
SUMMARY OF THE STABILITY ANALYSIS FOR EQUILIBRIA P_M

| Eigenvalues of the variational matrix A | Eigenvalue multiplicity | Perturbation eigenvectors $\bar{Z} = (\delta\xi_1, \dots, \delta\xi_N)$ | Geometric interpretation in N -dimensional phase space of $\bar{Z} = (\delta\xi_1, \dots, \delta\xi_N)$ |
|--|-------------------------|--|--|
| $\lambda_1 = a_1 + Mb_1 = -2$ | 1 | $Z_1 = (1, \dots, 1, 0, \dots, 0)$ | A perturbation from the stationary point P_M along ℓ_M |
| $\lambda_{in} = a_1 = \frac{(M_1 + M_2)M - 2M_1M_2}{(M - M_1)(M - M_2)}$ | $M - 1$ | Z_1, \dots, Z_{M-1} independent vectors which satisfy $\sum_{i=1}^M \delta\xi_i = 0$, $\delta\xi_i = 0, i = M + 1, \dots, N$ | Any perturbation from the stationary point P_M perpendicular to ℓ_M but within the M -dimensional coordinate hyperplane |
| $\lambda_{out} = a_2 = 1 - \frac{M^2}{(M - M_1)(M - M_2)}$ | $N - M$ | $\bar{Z}_{M+1}, \dots, \bar{Z}_{N-1}$ satisfy $\delta\xi_i = 0, i = 1, \dots, M$ and $\sum_{i=M+1}^N \delta\xi_i = 0$ \bar{Z}_N satisfies $\delta\xi_i = (M - N)b_1, i = 1, \dots, M$ and $\delta\xi_i = (a_1 - a_2) + Mb_1, i = M + 1, \dots, N$ | Perturbations out of the M -dimensional coordinate hyperplane |

where

$$A = \begin{pmatrix} a_1 + b_1 & & & b_1 & & & \\ & \ddots & & & & & \\ & & \ddots & & & & \\ & & & \ddots & & & \\ & & & & \ddots & & \\ b_1 & & & a_1 + b_1 & & & \\ & & & & & a_2 & 0 \\ & & 0 & & & & \ddots \\ & & & & & 0 & a_2 \end{pmatrix} \quad M.$$

$$a_1 = \xi_M \frac{\partial \phi}{\partial \xi_i} \bigg|_{(\xi_M, M\xi_M)}; a_2 = \phi(0, M\xi_M); b_1 = \xi_M \frac{\partial \phi}{\partial \xi} \bigg|_{(\xi_M, M\xi_M)}.$$

The eigenvalues and eigenvectors of A are summarized in Table I and Figs. 5 and 6. (A more complete stability analysis of the model can be found in Lacker and Peskin [1981]).⁴

Consider the case illustrated in Fig. 4. The number of interacting follicles, N , is 3. The parameters M_1 and M_2 in the maturation law have been chosen so that the lines of symmetry

⁴Lacker, H. M., and C. S. Peskin. 1981. Control of ovulation number in a model of ovarian follicular maturation. *Lect. Math. Life Sci. Vol. 14*. In press.

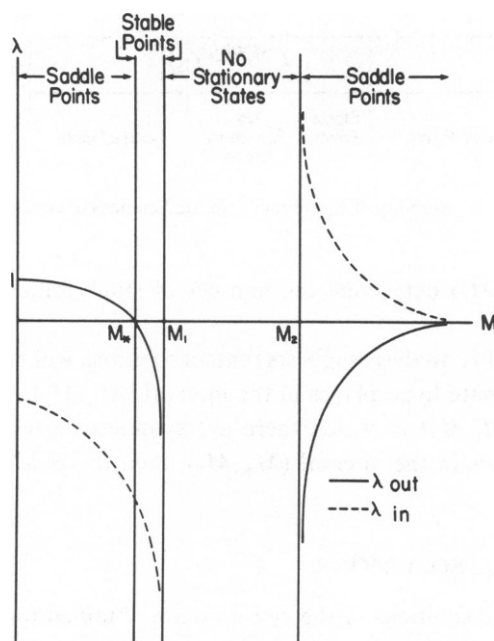


FIGURE 5 Sketch of the eigenvalues of A as a function of M (see Table I). λ_{out} is associated with those eigenvectors that are orthogonal to the M -dimensional coordinate hyperplane. λ_{in} is associated with perturbations that are orthogonal to I_M but within the M -dimensional coordinate hyperplane. The eigenvalue λ_s associated with the eigenvector along the line of symmetry I_M is always stable and is not indicated in the diagram. Only integer values of M have physical meaning.

I_1 and I_3 have equilibria P_1 and P_3 , respectively, but so that I_2 does not (Fig. 4 a). This is accomplished by selecting M_1 and M_2 so that 2 is the only integer in the interval (M_1, M_2) . The stability analysis of Eq. 11 shows that the equilibria P_1 and P_3 are saddle points (unstable).⁵ The eigenvectors direct solutions toward the ovulatory line of symmetry I_2 (Fig. 4 b and c). This results in two follicles ovulating independent of the initial maturity distribution of the three follicles.⁶

In most cases, the stability analysis shows that the equilibria of Eq. 6 are saddle points that direct solutions toward the coordinate hyperplanes that contain ovulatory solutions, that is, toward coordinate hyperplanes in the interval (M_1, M_2) . Figs. 5 and 6, however, show that this need not always be the case. If M_1 and M_2 are chosen so that there are integers in the interval $(M_* = M_1 M_2 / (M_1 + M_2), M_1)$, then the equilibria P_M associated with these integers are stable (all eigenvalues are negative). Unlike the saddle point equilibria, these states have a finite probability of occurring that will be related to the size of their capturing regions in N -space. Physiologically, these equilibria are interpreted as anovulatory states because a certain number of follicles become "stuck" at an equilibrium maturity (Fig. 10). The integers

⁵The complete stability analysis of Eq. 6 shows that when additional equilibria exist off the lines of symmetry, then they are also unstable.

⁶Of course any solution which starts *exactly* on I_1 or I_3 does not result in two ovulations, since these end at the stationary points P_1 or P_3 , respectively. These solutions are, however, unstable. Any perturbation, no matter how slight, will result in the solution approaching I_2 in finite time.

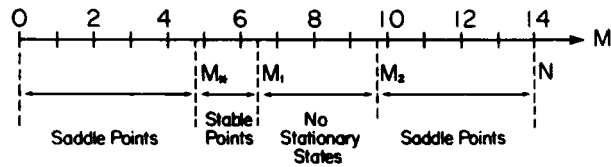


FIGURE 6 Stability of equilibria P_M located on lines of symmetry l_M .

M in the interval (M_*, M_1) determine the number of stuck follicles and their equilibrium maturity ξ_M .

In summary, the stability analysis suggests that all solutions will be directed by saddle point equilibria toward coordinate hyperplanes in the interval (M_*, M_2) . This interval includes the hyperplanes between (M_1, M_2) in which there are symmetric ovulatory solutions. Furthermore, if there are integers in the interval (M_*, M_1) , then the possibility of anovulation also exists.

6. NUMERICAL SOLUTIONS

In this section, numerical solutions of the system 6 are obtained for N follicles whose initial maturities are chosen independently from a given probability distribution. This gives the

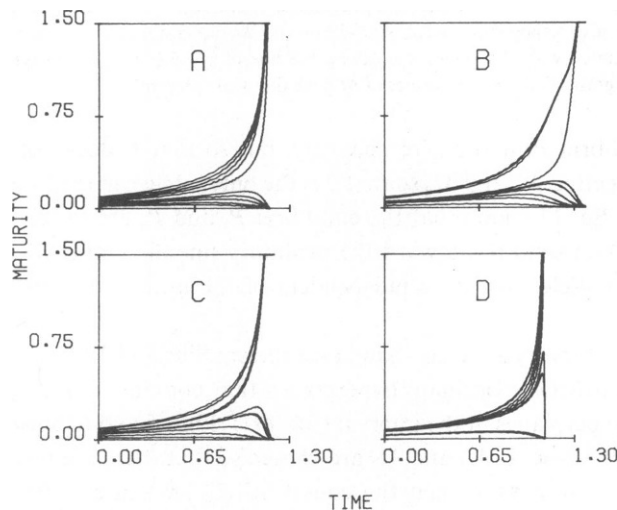


FIGURE 7 Follicle maturation curves in four cycles. Each curve represents the development of a follicle whose initial maturity is chosen at random from a uniform distribution of maturities between 0 and 0.1. Although every follicle obeys the same law of growth, some follicles are selected for continued development while others become atretic. The growth law parameters M_1 and M_2 of Eq. 6 are the same for each follicle ($M_1 = 3.85$, $M_2 = 15.15$). In cycles *A* and *D* five ovulatory follicles emerge. In *B* and *C* the ovulation number is four. In each cycle 10 follicles interact. Note that it is possible for an ovulatory follicle and an atretic follicle to have almost the same maturation curve for most of the length of the cycle (see cycle *D*). On the other hand, a significantly smaller follicle can occasionally "catch up" and ovulate (see cycle *B*). The ovulation time is slightly different in each cycle. The distribution of ovulation times and numbers for 1,000 cycles is illustrated in Fig. 8 *A*.

model a probabilistic aspect, as suggested by the observation that the reserve pool decays exponentially with age (34, 36, 52). A different way to model entry of follicles into the growing population from the reserve pool would be to assume that these events occur at random times given by a Poisson process.

Fig. 7 illustrates the numerical solutions of four representative cycles in which 10 follicles interact, starting with maturities that are chosen independently from a uniform distribution in the interval (0, 0.10). Ovulatory and atretic follicles emerge even though all follicles obey the same maturation law and influence each other only through the circulation. Atretic follicles are characterized by initial growth followed by regression at different times and stages of maturation. In contrast, ovulatory follicles undergo continuous and accelerating growth. The ratio of maturities of any two ovulatory follicles equals 1 as the idealized ovulation time T is approached.

For each trial in Fig. 7, there appears to be a certain threshold of initial maturity that separates ovulatory and atretic follicles. The results of many cycles (Fig. 8 A), however, strongly suggest that the interaction law, in effect, automatically adjusts the threshold so that the number of emerging ovulatory follicles is relatively insensitive to the initial arrangement of maturities.

Although all ovulation numbers fall within the predicted interval (M_1, M_2), these numbers do not occur with equal probability. In fact, Fig. 8 shows that many ovulation numbers in the interval (M_1, M_2) are not observed at all, even though symmetric solutions corresponding to these numbers have been shown to exist! (This surprising result will be explained in the following section.) The most probable ovulation number and range of possible ovulation numbers and times can be adjusted by changing the growth law parameters M_1 and M_2 . In general, these distributions appear to be unimodal and asymmetric. (Figs. 8 and 9)

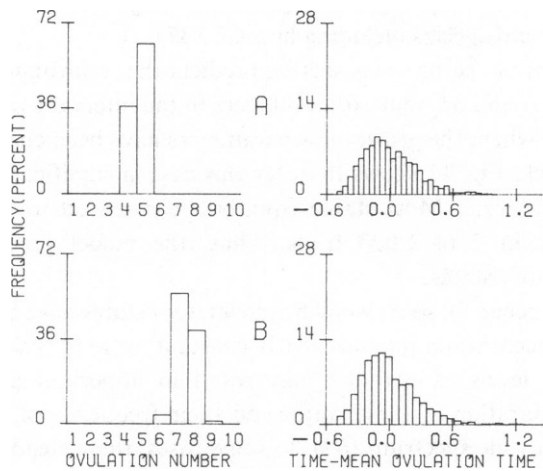


FIGURE 8 Distribution of ovulation numbers and ovulation times. Each graph is the result of 1,000 cycles. The number of interacting follicles in each cycle is 10. The initial maturity of a follicle is chosen independently from a uniform distribution of maturities in the interval (0,0.05). In *A* the growth law parameters are the same as for Fig. 7 ($M_1 = 3.85$, $M_2 = 15.15$). In *B* the parameters have been changed to $M_1 = 5.5$, $M_2 = 61.7$. Statistics (mean \pm SD): (*A*) ovulation number = 4.63 ± 0.48 , ovulation time = 1.98 ± 0.25 ; (*B*) ovulation number = 7.38 ± 0.58 , ovulation time = 1.25 ± 0.26 .

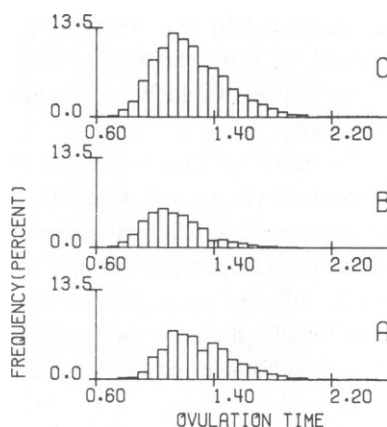


FIGURE 9 The distribution of ovulation times conditioned on ovulation number. The initial maturity of each follicle is chosen independently from a uniform distribution in the interval (0,0.05). The growth law parameters ($M_1 = 5.5$, $M_2 = 61.7$) and number of interacting follicles per cycle ($N = 10$) are the same as for Fig. 8 B. A represents the ovulation time frequencies for those cycles in which seven follicles ovulate (mean \pm SD = 1.31 ± 0.25). B represents the distribution of ovulation times for those cycles in which eight follicles ovulate (mean \pm SD = 1.13 ± 0.20). The area under each graph is equal to the probability of achieving that ovulation number. The results are obtained from a total of 1,500 cycles. The distribution of ovulation times for all cycles is represented in C (mean \pm SD = 1.25 ± 0.25).

The distribution of ovulation numbers is insensitive to the maximum allowed initial maturity ξ_{\max}^0 . The shape of the distribution of ovulation times is also unaffected by ξ_{\max}^0 as long as the initial phase of development is still dominated by the uncoupled exponential growth term. The ovulation time distribution does, however, translate along the time axis when ξ_{\max}^0 is changed within this range. The qualitative shape of the distribution of ovulation times is observed in several species including humans (37).

The stability analysis of the previous section predicts that anovulatory states should exist for special choices of M_1 and M_2 that admit integers in the interval (M_*, M_1) . This is verified numerically in Fig. 10, where the growth law parameters have been chosen to allow five or six follicles to become stuck. Fig. 11 shows that, for this case, a significant number of trials are captured by stable equilibria. Most stable equilibria correspond to $M = 6$, although five follicles become stuck in 2 of 1,000 trials. Thus, the model predicts the existence of (pathological) anovulatory states.

Such states actually occur. In some women, anovulatory states have been observed in which the serum estradiol concentration remains nearly constant for long periods (63). Long periods of exposure to steady levels of estradiol may result in important pathology in estrogen-sensitive tissues. The duration of these states and their frequency of occurrence in a given individual varies over a wide spectrum (69). In some cases, these steady states are associated with the presence of persistent functioning follicles or follicular cysts within the ovary (63). We postulate that these follicles are stuck at an equilibrium distribution of maturity. Spontaneous escape from these anovulatory states does occur and may be the result of random perturbations. Such perturbations could occur naturally, for example, by the continual entry of follicles into the interacting population from the reserve pool at random times. In the model examined in this paper, escape from anovulation is not possible, because follicle activation

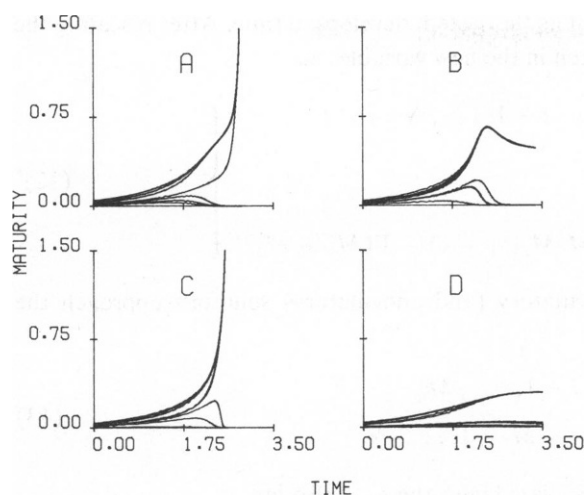


FIGURE 10

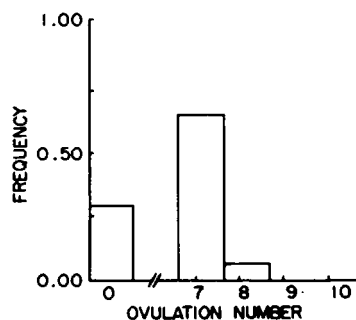


FIGURE 11

FIGURE 10 Follicle maturation curves for parameters that admit both ovulatory solutions and anovulatory states. Every follicle satisfies Eq. 6, with the same parameter values $M_1 = 6.5$, $M_2 = 15.5$. The initial maturity of each follicle is chosen at random from a uniform distribution in the interval $(0, 0.05)$. In A and C, seven follicles ovulate. In B, an anovulatory state occurs in which six follicles approach an equilibrium maturity of 0.46. In D, five follicles approach a maturity of 0.25. Note that the approach to equilibrium need not be monotonic (B). The statistics of 1,000 trials are illustrated in Fig. 11.

FIGURE 11 The statistics of 1,000 trials similar to the four illustrated in Fig. 10. The growth law parameters M_1 and M_2 are 6.5 and 15.5, respectively. 10 follicles interact in each trial, and their starting maturities are chosen independently from a uniform distribution in the interval $(0, 0.05)$. Anovulatory states occurred in a significant proportion of trials. These correspond to either five or six "stuck" follicles. Five stuck follicles occurred in only two of 1,000 trials.

occurs only at $t = 0$. However, in another paper,⁴ we will begin to explore the behavior of the interaction law under an alternative hypothesis in which follicles are activated from the reserve pool at random times throughout the cycle. It is emphasized that only special choices for M_1 and M_2 admit the existence of both anovulatory and ovulatory solutions. Therefore, the model is consistent with the observation that some species (and individuals within species) do not exhibit anovulatory states.

7. STABILITY ANALYSIS OF N -SPACE TRAJECTORIES

The numerical results presented in the previous section agree with the theoretical predictions based on the stability analysis of section 5. However, some ovulation numbers have not been observed numerically (see Fig. 8), even though the symmetric analysis showed that there were solutions for these ovulation numbers (see Fig. 3). One might think that the unobserved ovulation numbers simply have a low probability of occurring. In fact, however, their probability is zero, as we now show.

The first step is to rewrite system 6 with the following change of variables.⁷ Let $\gamma_i = \xi_i/\xi_1$, where ξ_1 is the most mature of N interacting follicles. Since solutions $\xi_i(t)$ do not cross, the

⁷This change of variables was suggested by J. Moser (personal communication).

most mature follicle will remain the largest as the system develops in time. After rescaling the time $\tau = \int_0^t \xi^2(t') dt'$, system 6 can be written in the new variables as

$$\left. \begin{aligned} d\gamma_i/d\tau &= \gamma_i \psi(\gamma_i, \Gamma), \quad i = 1, \dots, N \\ \Gamma &= \sum_{j=1}^N \gamma_j \\ \psi(\gamma_i, \Gamma) &= (1 - \gamma_i)[M_1 M_2 (\gamma_i + 1) - \Gamma(M_1 + M_2)]. \end{aligned} \right\} \quad (12)^8$$

In the transformed phase space all ovulatory (and anovulatory) solutions approach the equilibria specified by

$$\gamma_i = \begin{cases} 1, & i = 1, \dots, M, \\ 0, & i = M + 1, \dots, N. \end{cases} \quad (13)$$

There are only two distinct eigenvalues associated with these equilibria:

$$\begin{aligned} \lambda_1 &= (M_1 + M_2)M - 2M_1 M_2 \\ \lambda_2 &= -(M_1 + M_2)M + M_1 M_2. \end{aligned} \quad (14)$$

Fig. 12 shows that there is a region of stability in the interval $(M_*, 2M_*)$. Since $2M_*$ is the harmonic mean of M_1 and M_2 , it breaks the interval (M_1, M_2) into a stable $(M_1, 2M_*)$ and an unstable range $(2M_*, M_2)$. Ovulation numbers in the interval $(2M_*, M_2)$ have zero probability of being achieved because they correspond to unstable solutions. Any perturbation, however slight, from these solutions will result in the trajectory moving further away toward lower dimensional coordinate hyperplanes. Eventually, these solutions will converge toward the line of symmetry associated with a coordinate hyperplane in the stable interval $(M_1, 2M_*)$. The dimension of the coordinate hyperplane corresponds to the number of follicles that will ovulate. Another possibility is that solutions will end at stable, anovulatory, stationary points located in coordinate hyperplanes between M_* and M_1 (if integers exist in this interval).

These predictions are consistent with the numerical results reported in the previous section. For example, consider the case represented by Fig. 8 A, where $M_1 = 3.85$ and $M_2 = 15.15$. Although there are symmetric solutions corresponding to ovulation numbers between 4 and 15, the stable ovulation number range, $(M_1, 2M_*) = (3.85, 6.14)$, indicates that ovulation numbers >6 should not occur. Ovulation numbers <4 or >6 were never observed numerically, even when the number of interacting follicles per cycle was increased to 1,000.

8. EFFECTS OF INTERACTING POPULATION SIZE ON MODEL BEHAVIOR

The distribution of ovulation numbers and times is not only effected by the growth law parameters M_1 and M_2 but also by the number of follicles N in the nondormant interacting

⁸Grossberg (27) has studied some of the properties of a class of systems that includes Eq. 12 and has shown that $\lim_{t \rightarrow \infty} \gamma_i(t)$ exists for all $\gamma_i(0) \geq 0$, $i = 1, \dots, N$.

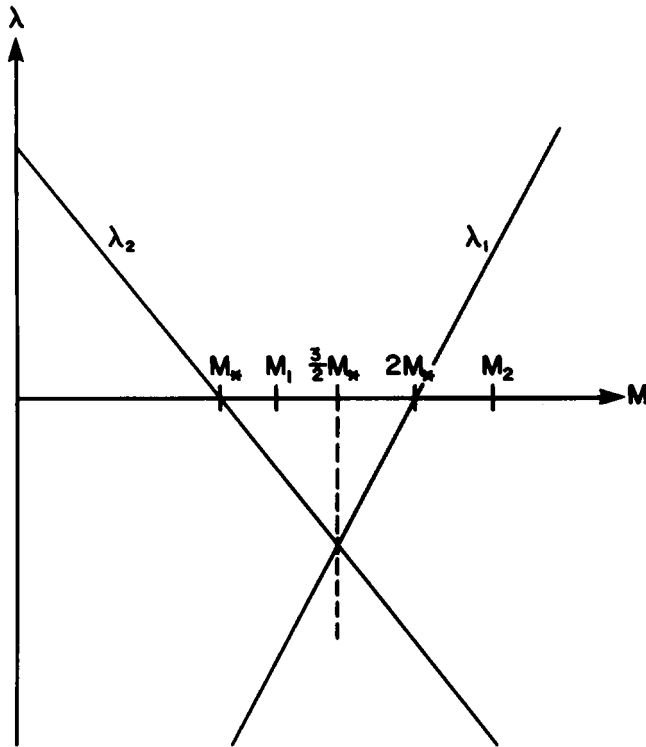


FIGURE 12 Stability analysis of the equilibria represented by Eq. 13. Each equilibrium is characterized by M , the number of follicles with relative maturity $\gamma_i = 1$. λ_1 and λ_2 are the distinct eigenvalues associated with each equilibrium. These eigenvalues are linear in M (see Eq. 14) and divide the range of ovulation numbers in the interval (M_1, M_2) into a stable range $(M_1, 2M_*)$ and an unstable range $(2M_*, M_2)$. This explains why larger ovulation numbers in the interval (M_1, M_2) are not observed, even though solutions for these ovulation numbers have been shown to exist (see section 4). The figure also shows a stable region between M_* and M_1 . If integers exist in this interval they correspond to the presence of stable anovulatory states. (See text for a more complete explanation.)

population. This is illustrated in Fig. 13, which shows both striking improvement in the control of ovulation time and a shift favoring lower ovulations as the number of interacting follicles increases. Inasmuch as N decreases as women age, perhaps these effects contribute to the increased dizygotic twinning rate in older women (23, 43, 47) and the increased variance in the follicular phase of the cycle during the perimenopausal period (39, 65, 68).

In mammals, most follicles undergo atresia. Only a few of the follicles that initiate growth reach ovulatory maturity. In contrast, many simpler forms of life have reproductive systems that deliver large numbers of germ cells to the external environment. Presumably, the developmental constraint of intrauterine growth has led to the selection of reproductive systems that prevent the majority of activated follicles from completing maturation. The results presented in this section suggest that a superimposed regulatory scheme has evolved that does not discard the older mechanism, but, instead, uses it to improve control.

Fig. 13 shows that the ovulation time distribution approaches a singular limit with increasing N . This does not seem to be true for the distribution of ovulation numbers. It should

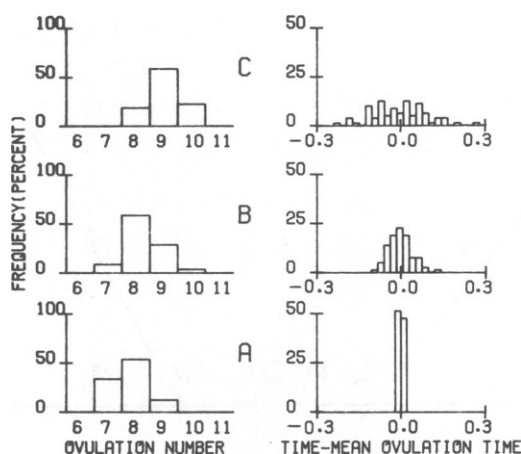


FIGURE 13 The effect of interacting follicle population size on the distribution of ovulation times and numbers. Larger numbers of interacting follicles improve control of ovulation time and favor smaller ovulation numbers. In *A*, 1,000 follicles interact in each cycle; in *B*, 100 follicles interact per cycle; and, in *C*, 30 follicles interact. Each graph represents the results of 80 cycles. Every follicle obeys Eq. 6, with $M_1 = 6.1$, $M_2 = 5,000.0$. Initial maturities are chosen at random to be a number from a uniform distribution between 0 and 10^{-5} . Statistics (mean \pm SD): *A* ovulation number = 7.79 ± 0.65 , ovulation time = 4.37 ± 0.01 ; *B* ovulation number = 8.28 ± 0.67 , ovulation time = 5.55 ± 0.04 ; *C* ovulation number = 9.04 ± 0.65 , ovulation time = 6.33 ± 0.10 .

be noted that the range of stable ovulation numbers ($M_1, 2M_*$) is not effected by the number of interacting follicles, since the eigenvalues λ_1 and λ_2 in Eq. 14 are independent of N . Changing N , however, alters the dimension of the phase space and the geometry of the capturing region associated with a given stable ovulation number.

9. EXPERIMENTAL SUGGESTIONS FOR IDENTIFYING FOLLICLE MATURATION LAWS IN MAMMALS

Although the particular follicle maturation law examined in this paper exhibits many interesting physiologic features, it is, of course, unlikely to be found in nature. Nevertheless, its qualitative behavior is probably similar to many models that regulate ovulation number and satisfy assumptions I–III. These physiological assumptions were used to develop the general form 5. The properties of the particularly simple example studied in this paper suggest that other members of 5 may closely approximate ovarian follicle development in some mammals. In this section, experiments for identifying such maturation laws and for testing the theory are proposed.

One approach would be to construct the function $\tilde{f}(s_i, h_1, h_2) = ds_i/dt$ directly by perfusing isolated follicles with constant concentrations of FSH (h_1) and LH (h_2) (60) and measuring the follicle estradiol secretory rate, $s_i(t)$. This technique has the advantage that the explicit LH and FSH dependence in the maturation law is not lost. It also does not depend on any explicit assumptions regarding the intrafollicular and cellular mechanisms that control estradiol production. Transformation of $\tilde{f}(s_i, h_1, h_2)$ to $f(\xi, \xi)$ is obtained by determining the functions $h_1(\xi)$ and $h_2(\xi)$ and noting that $s_i = \gamma \xi_i$. Experimental methods for finding these functions have appeared in the literature (10).

The in vitro disadvantage of this technique might be avoided if an appropriate quantitative, microscopic label for s_i could be found. Measuring the number of follicles which secrete near rate s_i for different times of the cycle, $p(s_i, t)$, could be used to reconstruct $f(\xi_i, \xi)$.

The following idealizations lead to a simple physiologic interpretation of ϕ in Eq. 6 and, in addition, suggest an experimental method for identifying $f(\xi_i, \xi)$. Let T_D represent the doubling time of an exponentially growing cell population. If X_i is the number of estradiol-secreting cells in the i th follicle and if T_D for these cells is assumed to depend on FSH, LH, and follicle maturity (48), then

$$dX_i/dt = X_i [\ln 2/T_D(s_i, h_1, h_2)]. \quad (15)$$

Assume, as has been observed for other steroid secreting cells, that constant saturating rates of estradiol production per cell, σ , are reached at gonadotropin concentrations that are at the lower limit of the physiological range and at times that are fast when compared with cell doubling (8, 15–17, 22, 55). Eq. 15 can now be written in the form

$$\frac{d\xi_i}{dt} = \xi_i \phi(\xi_i, \xi), \quad (15a)$$

where $\phi(\xi_i, \xi) = \ln 2/T_D[\gamma\xi_i, h_1(\xi), h_2(\xi)]$ and $X_i = s_i/\sigma = \gamma\xi_i/\sigma$. Since this is in the same form as Eq. 6, ϕ is, under these additional assumptions, inversely proportional to the cell doubling time of the estradiol-producing cell population [$T_D = \ln 2/\phi(\xi_i, \xi)$]. ϕ , of course, need not have the simple explicit form given by Eq. 6.

A strict interpretation of the two cell hypotheses (2, 24) would identify X_i as the number of granulosa cells in the i th follicle. According to this hypothesis, FSH-stimulated granulosa cells convert androgens, produced by LH-stimulated theca cells, to estradiol. Determining T_D for the granulosa cell population in different follicles at different times in the cycle (48) or measuring the distribution $\bar{p}(X_i, t)$ (30, 31) can be used to obtain $f(\xi_i, \xi)$. However, a more careful interpretation of X_i is required for species in which both granulosa and theca cells secrete estradiol in significant amounts (see reference 18 for a critical review). Once $f(\xi_i, \xi)$ has been obtained from experimental data, the methods developed in this paper can be used to make detailed predictions about the statistics of ovulation number and time.

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