

Hormone Circuits Lecture notes

Uri Alon (Spring 2021)

Lecture 8

Ovulation number

Review: we studied the HPA axis. It is one of several “HP” axes. They control major pillars of life: growth, stress, metabolism, and reproduction. Each axis has its own dedicated cells in the pituitary, and its own x_1 , x_2 and x_3 hormones. Reproduction is controlled by the H-P Ovary axis, which is the subject for our lecture.



Our goal is to understand how, out of many follicles that start a race every menstrual cycle, only one (or M in other species) gets to win and ovulate.

In humans $M=1$ (fraternal twins are rare $\sim 1/89$ unassisted pregnancies). In mice $M=6-8$. In deer typically $M=2$ (Fig 1.)

This is the “choose M” problem.



Figure 1

The ovulation number is important for evolutionary fitness.

This was shown nicely by a famous experiment by David Lack in the 1950s. Lack measured fitness using birds in a forest near Oxford (Fig. 2). He manipulated the egg number in the nest. The number of surviving offspring went up with the number of eggs. Then, at high egg numbers it began to drop. The reason for the drop is the burden on parents, as birds raced to feed so many hungry chicks. There is an optimum number of eggs, an optimal clutch size. The natural number for that bird species is at the optimum.

Mammals too have evolved different litter sizes. Mice live fast, die young and leave a good-looking batch of many babies. Bats, elephants, whales, and humans generally make only one baby at a time and spend time to teach it how to live in their environment

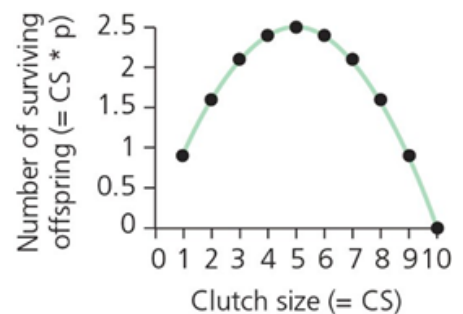


Figure 2

So, the ovulation number is important. How is it determined?

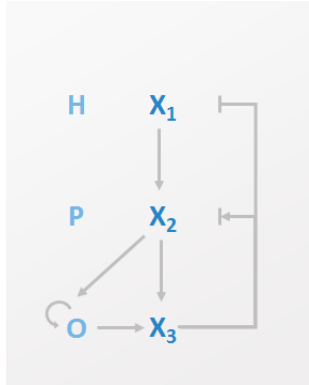


Figure 3

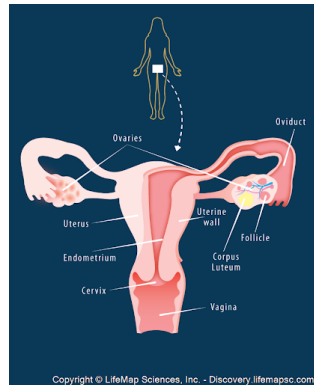
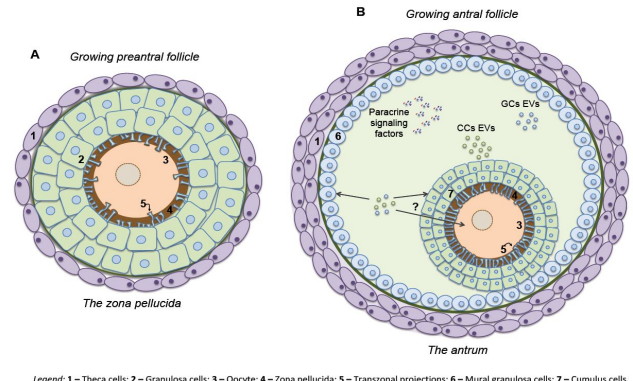


Figure 4



Legend: 1 – Theca cells; 2 – Granulosa cells; 3 – Oocyte; 4 – Zona pellucida; 5 – Transzonal projections; 6 – Mural granulosa cells; 7 – Cumulus cells.

Figure 5

Let's start with the HPO axis (Fig. 3). H secretes x_1 (GnRH) to make P secrete x_2 - the gonadotropins **LH** and **FSH**. FSH (follicle stimulating hormone) stimulates the follicles in the ovary. The two ovaries are walnut sized organs, on both sides of the uterus, connected to it by fallopian tubes (Fig. 4). They contain hundreds of thousands of egg cell (oocytes) surrounded by support cells. The spherical combination of an egg and its support cells is called a follicle (Fig. 5). Follicles include an egg- with a single copy of each chromosome, surrounded by granulosa and theca cells. FSH makes those support cells grow and divide.

At birth there are about 1 million follicles, which decay exponentially throughout life (Fig. 6), reaching almost zero at age 50, at menopause. Incidentally, humans are among the few species with menopause, raising theories about the fitness advantage of long life beyond the reproductive period, such as "grandmother" and "aunt" effects.

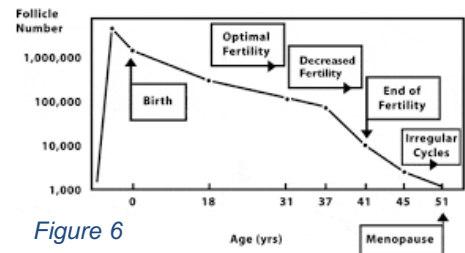


Figure 6

In each menstrual cycle, a batch of about 10-20 follicles start the race (Fig. 7). In women, these follicles are about 2-5mm in size and have about 2 million granulosa cells. They begin to grow under control of FSH. Granulosa and theca cells divide. The smaller follicles die, until only one (or only M, in other animals) is left. This **dominant follicle** is ~20mm large with ~60 million granulosa cells. It is the winning follicle, the one that will ovulate with a chance to meet sperm, fertilize, and make a baby.

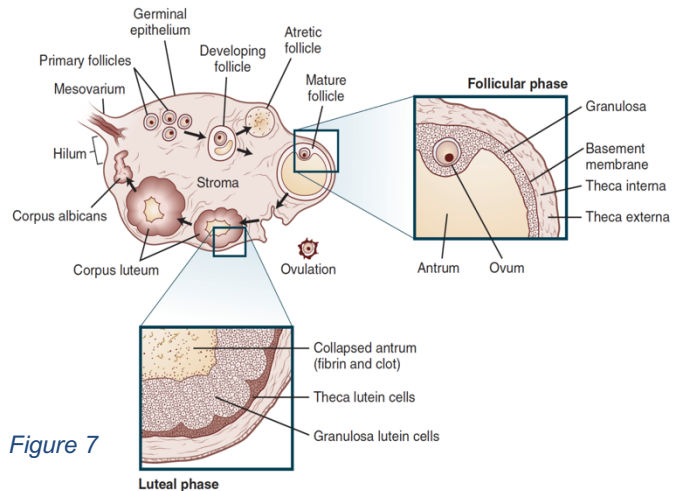


Figure 7

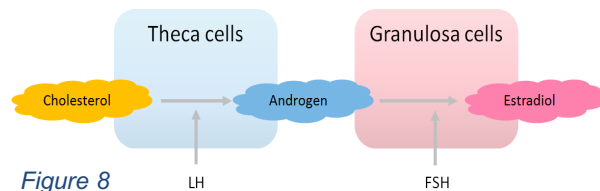


Figure 8

The race is regulated by hormones made by the follicles themselves (Fig. 8). Follicles are thus also hormone-producing glands. The

theca cells produce **androgen** (A) under the influence of LH. Some of the androgen goes to the circulation, the rest is converted by granulosa cells, under control of FSH, into the important hormone **estrogen** (E). Estrogen is the x_3 in this axis. Estrogen shuts off FSH production (production of x_1 and x_2), just like the long feedback in the HPA axis.

Thus, FSH drives the race. Estrogen is made by all participants and causes a reduction in FSH. This is a self-regulating race.

Unlike the HPA axis, where x_3 always inhibits x_2 , when estrogen is very high, its negative effect on x_2 becomes a positive effect. Negative feedback turns to positive feedback. This switch causes the LH surge at about day 14 of the menstrual cycle (Fig. 9). The LH surge triggers ovulation- the dominant follicle bursts the ovary in an inflammatory process. It goes down the fallopian tube to the uterus. The remaining G and T cells become a new hormone-secreting gland called the corpus luteum (LH is luteinizing hormone). The corpus luteum secretes progesterone, setting off the second half of the cycle. If there is no fertilization, the thickened lining of the uterus sloughs off causing the bleeding in the first 5 days or so of the next cycle.

By the way, the duration of the luteal phase is quite constant between individuals, about 14 days; in contrast, the first half of the cycle is quite variable so that total cycle length averages 28 days but can be as long as 35 days.

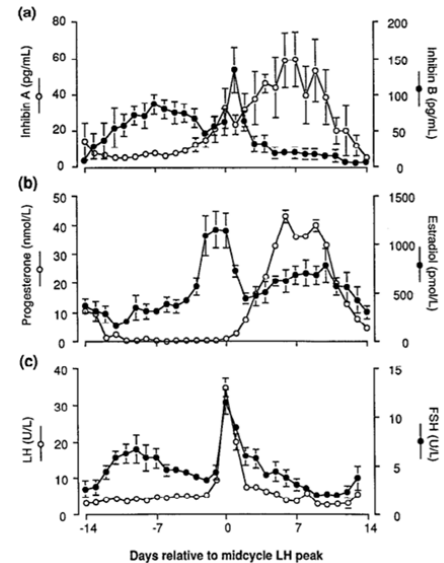


Figure 9

The granulosa and theca cells secrete hormones, and the granulosa cells also have a nurse role by tending the oocytes, providing nutrients.

The follicle competition is known to occur through the circulation and not primarily by contacts within each ovary. Evidence for this is that follicles start the race in both ovaries, but only one ovary ovulates. Which ovary ovulates appears to be random every cycle. If a female loses one ovary (ovariectomy), the remaining ovary ovulates every month - doubling its ovulation frequency.

Ultrasound measurements can track the sizes of follicles over the cycle. The sizes of the follicles, x_i , grow with time, and one by one the follicles die until only one is left. Interestingly, the dominant follicle size grows linearly with time,

$$x_i = v t$$

where v is the velocity. This is like in mechanics, a ball moving with constant velocity. It's unusual to see an organ size growing linearly, usually we expect exponential growth or decline. We will soon understand this linear rise.

The winner (follicle) takes it all/ABBA

G	Am/E
I don't want to talk	Nothing more to say
D/F#	D
About ovulation	No more FSH
Am/E	G
Follicles compete	The winner takes it all
D	B7
The cycle just repeats	The loser standing small
G	Em
I've played all my cards	Beside the victory
D/F#	D
And so have the other follicles	That's her destiny

Mathematical model of ovulation number control

To understand how M are chosen out of the n follicles that start the race, we need a mathematical model. The most elegant model is by Lacker and Peskin in the 1980s (Fig. 10). Lacker found that there must be a signal that makes follicles die if they are too small or too large compared to the rest. Their model had a biphasic function, but it did not correspond to known physiological factors.

Michal Shilo in her MSc followed the Lacker approach, and made an advance by finding a physiological factor that can choose M . *Figure 10*

We begin with an equation for the size of follicle i , denoted x_i . Since all cells come from cells, and granulosa and theca cells replicate to make more of themselves, we begin with the usual

$$dx_i/dt = x_i.$$

From now on we neglect parameters like a growth rate that multiplies this equation, to keep things intuitive and elegant. We will use the “~” symbol a lot. This is a step forward in our skill level compared to previous lectures where we carried constants around.

Next, we add the fact that FSH drives the growth of follicles (after all, it is called follicle stimulating hormone)

$$\frac{dx_i}{dt} = x_i FSH$$

To understand FSH as a function of time, recall that it is inhibited by estrogen E . Thus $FSH \sim 1/E$. Estrogen is made by each follicle, and the larger the follicle the more estrogen. Thus, estrogen goes as the sum of follicle sizes

REGULATION OF OVULATION NUMBER IN MAMMALS A FOLLICLE INTERACTION LAW THAT CONTROLS MATURATION

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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.

$$E \sim \sum x_i = x_T$$

Where x_T is the **total hormone producing mass** of the follicles.

Since FSH is inhibited by E, $FSH \sim \frac{1}{E} \sim \frac{1}{x_T}$, we conclude that

$$\frac{dx_i}{dt} = \frac{x_i}{x_T}$$

This is a nice result: the growth rate depends on the *relative size*, $\frac{x_i}{x_T}$.

If this was all, each follicle would keep growing and never die. To add the death term, we reasoned, after Lacker, that there is an additional factor that reduces growth at large and small sizes. To find such a factor our first clues came from analyzing a disease of ovulation called polycystic ovary syndrome, or PCOS. It is consoling that something as dire as a disease can offer clues to develop a mathematical model.

In PCOS, there are problems with the cycle and with fertility, with few or no ovulations and menses (Fig 11). Often, the ovary has multiple cysts, seen by ultrasound, which are many large follicles that do not ovulate (Fig 12). The defining feature of PCOS is high androgen level. Thus, androgen interferes with the race and with ovulation.

Table 21.1 Diagnostic Criteria for Polycystic Ovary Syndrome

1990 National Institutes of Health Criteria (Both 1 and 2)

1. Hyperandrogenism and/or hyperandrogenemia
2. Oligo-anovulation

Revised 2003 Rotterdam Criteria (At Least 2 Out of 3)

1. Clinical or biochemical hyperandrogenism
2. Oligo-anovulation
3. Polycystic ovaries (PCO)

Androgen Excess-polycystic ovary syndrome (AE-PCOS) Society Criteria (Both 1 and 2)

1. Hyperandrogenism (clinical and/or biochemical)
2. Ovarian dysfunction (oligo-anovulation and/or polycystic ovaries)

*Excluding other endocrinopathies, including congenital adrenal hyperplasia, Cushing syndrome, thyroid dysfunction, hyperprolactinemia, androgen-producing tumors, and drug-induced androgen excess.

Figure 11

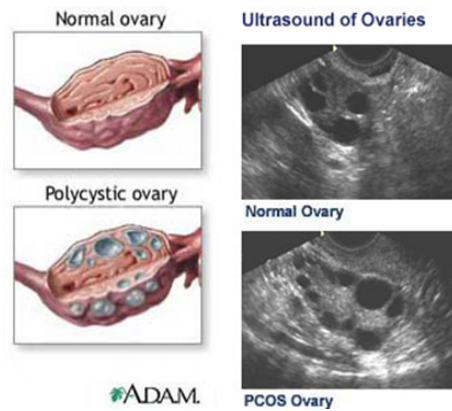


Figure 12

It was thus expected that lack of androgen would improve ovulation. This is not the case. When animals or follicles *in vitro* were treated with low levels of androgen, it enhanced follicle growth and increased the ovulation number. Thus, androgen has a **biphasic** effect on follicles (Fig 13).

We reasoned that since each follicle makes androgen, the defining factor must be the local androgen concentration in follicle i , denoted A_i . Thus

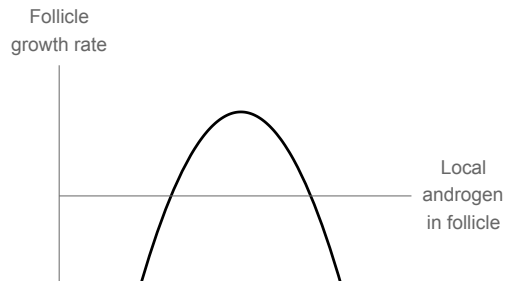


Figure 13.

$$\frac{dx_i}{dt} = \frac{x_i}{x_T} \phi(A_i)$$

Where ϕ is a biphasic function: it is negative at low and high levels of A_i , and positive in between. To understand the dynamics of local androgen A_i , we reasoned that each follicle makes androgen in proportion to its own size (its own number of theca cells),

$$A_i \sim \alpha x_i$$

Next, we noticed that total androgen, secreted by the sum of all follicles together into the circulation, is nearly constant across the 14 ovulation days. Thus

$$\text{Total androgen} = A_T = \alpha \sum x_i = \alpha x_T \sim \text{const}$$

The conclusion is that $\alpha \sim 1/x_T$. The local androgen level is thus, again, a function of the relative size of the follicle,

$$A_i \sim \frac{x_i}{x_T}$$

We end up with an elegant equation, in which the growth rate of a follicle depends in a biphasic way on its size relative to the sum of all the follicles in the race:

$$(1) \quad \frac{dx_i}{dt} = \frac{x_i}{x_T} \phi \left(\frac{x_i}{x_T} \right)$$

It turns out that one can easily solve this equation for the important case of M equal-sized follicles that grow while all others die. This is a **symmetric solution**, in which the relative size of the M growing follicles is $x_i/x_T = 1/M$. The other, losing, follicles have $x_i/x_T = 0$. For example, if we are interested in a symmetric solution with $M=3$, each of the follicles is $1/3$ of the total summed size. The other, losing, follicles have $x_i/x_T = 0$

Plugging this into Eq (1), we find that the growing follicles have a constant velocity

$$\frac{dx_i}{dt} = \frac{1}{M} \phi \left(\frac{1}{M} \right) = v$$

Thus

$$x_i(t) = v t \quad \text{with } v = \frac{1}{M} \phi \left(\frac{1}{M} \right)$$

For these follicles to grow in size, the velocity must be positive. This requires that $1/M$ fall between the two zero points of ϕ , so it can be in the region where it is positive. If we name the two zero points $1/M_1$ and $1/M_2$ (Fig. 14), we find the following condition for a growing solution:

$$M_1 < M < M_2 \quad \text{condition for growing solution}$$

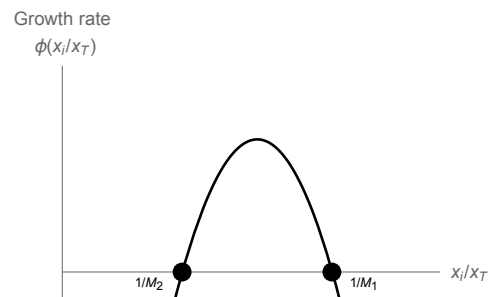


Figure 14

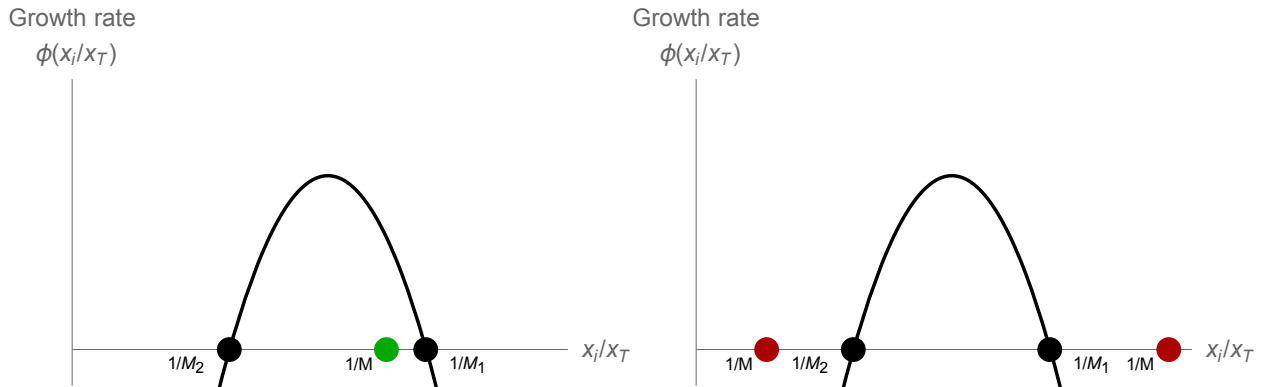


Figure 15

Since M_1 and M_2 are parameters determined by the levels of local androgen needed for growth or death, they are the same for all follicles, and depend on things like androgen receptor affinity. To get $M=3$, for example, we need $M_1 < 3$ and $M_2 > 3$ (Fig. 15).

But there is another condition, a bit more subtle. The symmetric solution needs not only to grow, but also to be stable. If we make one of the M follicles a bit bigger than the rest, we want it to shrink back to be equal. To see this graphically we can show that the region of stability occurs when $1/M$ is to the right of the maximum of ϕ . That is, $1/M$ must lie in the declining phase of the biphasic function.

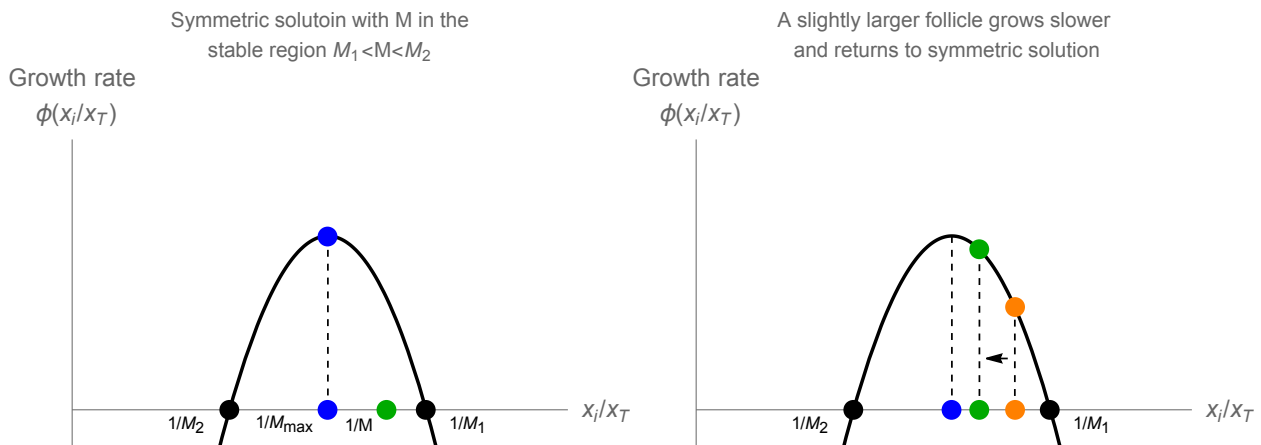


Figure 16

To see why, imagine that we make one follicle of the M a bit larger than the others (orange dot in Fig. 16). Since ϕ is declining, it grows a bit slower than the other follicles (green dot), and shrinks back. The solution is stable. Likewise, if one follicle is a bit smaller, it grows faster, and catches up, returning to the symmetric solution.

In contrast, in the rising part of ϕ , to the left of its maximum, the symmetric solution is unstable. A slightly larger follicle grows faster than the rest and keeps growing, breaking the symmetric solution.

Thus, the second criterion is that $M > 1/M_{max}$ or equivalently $M < 1/M_{max}$. If we assume for simplicity that ϕ is a parabola, the maximum point is midway between the zeros, $M_{max} =$

$\frac{1}{2} \left(\frac{1}{M_1} + \frac{1}{M_2} \right) = \frac{1}{2} \frac{M_1 + M_2}{M_1 M_2}$, and thus $\frac{1}{M_{max}} = 2 \frac{M_1 M_2}{M_1 + M_2}$. The criterion for stability and positive growth together is

$$M_1 < M < 2 \frac{M_1 M_2}{M_1 + M_2} \quad \text{condition for stable growing solution}$$

If we assume for simplicity that $M_2 \gg M_1$, we find

$$M_1 < M < 2M_1$$

This explains how setting a physiological parameter like M_1 , where $1/M_1$ is proportional to the the local androgen concentration that is toxic to the follicle, can determine the ovulation number M . For ovulation number $M=3$, for example, M_1 needs to be in the range between 3 and 6. For the human case $M=1$, M_1 needs to be between zero and one (in the $M=1$ case the stability criterion is unimportant).

Fig 17 shows the relation between the androgen toxicity parameter M_1 and the ovulation number. At $M_1=3.5$, for example one can have 4, 5 or 6 ovulating follicles. Fig 18 shows the follicle dynamics for a case with $M = 1$ ($M_1 = 0.9, M_2 = 3$) and a case with $M = 4$ ($M_1 = 3.9, M_2 = 7$).

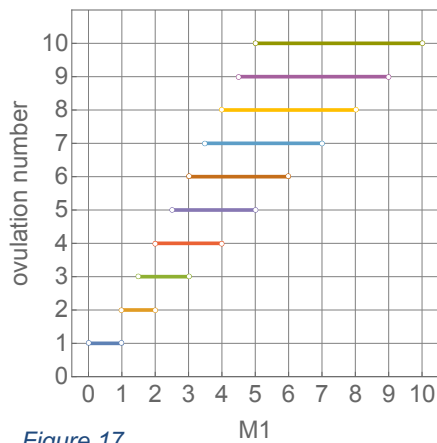


Figure 17

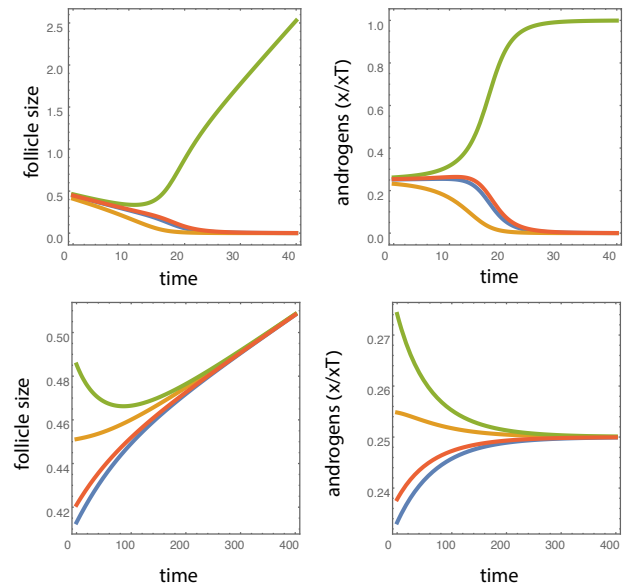


Figure 18

This model can also explain how high levels of circulating androgen might interfere with ovulation, as in PCOS. High levels of androgen are effectively like shifting the biphasic curve to the left (Fig. 19). This reduces $1/M_1$, or equivalently increases M_1 . When M_1 becomes larger than the initial number of follicles entering the race, about 10-20, there are no solutions, and no ovulations can occur.

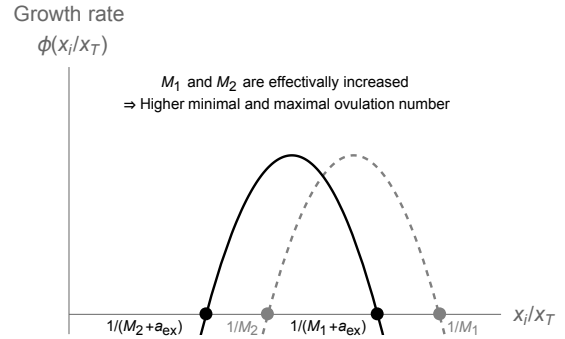


Figure 19

Fertility treatments for PCOS include estrogen receptor antagonists-agonists, which constantly provide only a low activation of the estrogen receptor and blocks additional signals. This increases FSH levels. Normalizing insulin resistance may also important, because many patients with PCOS have insulin resistance leading to high insulin levels that induces androgen production in follicles (theca cells have insulin receptors).

We can also understand how some fertility treatments work. These treatments use drugs that work on the estrogen receptor that increase FSH. This drives the race faster for a given x_T . Such treatments if given at high doses can even increase the chance for twins (Fig. 19).

So let's discuss twins. There are two types. Identical twins, also called monozygotic (MZ) twins, occur when a single egg cell is fertilized by a single sperm cell. The resulting zygote divides to a ball of cells that then breaks into two, with each twin originating from one half, and so have identical genomes. MZ **twins** occur in 3 to 4 per 1,000 births.

The other type are non-identical twins, with a frequency of $\sim 1/89$ pregnancies. They occur when two follicles ovulate. In the model twins can occur even if the only stable solution is $M=1$. If two follicles happen to start the race with very close initial sizes, they grow together (Fig. 20). Given

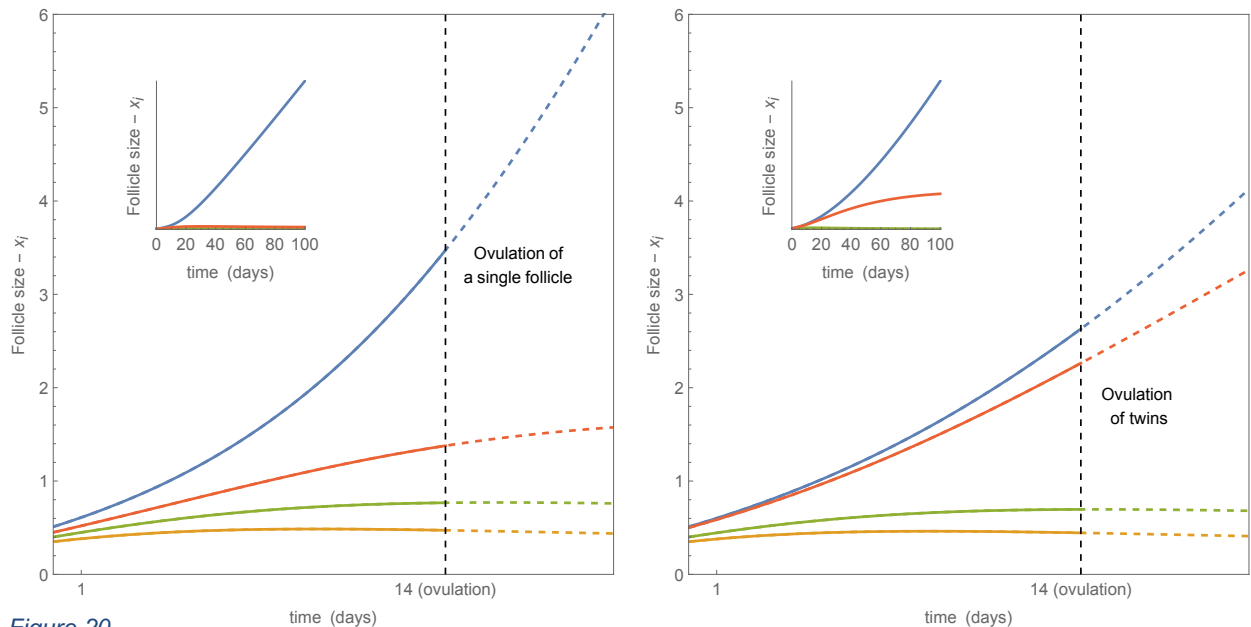


Figure 20

enough time, the smaller one will die in the model because $M=2$ is unstable. But in 14 days, it happens sometimes that the second follicle is so close in size to the dominant follicle that it also makes it to the LH surge and ovulation.

The probability for triplets and quadruplets in this picture drops exponentially because it is increasingly unlikely to have three or four such nearly identical initial sizes to keep together throughout the process. This is indeed similar to the observed frequencies

Naturally, twins occur in about one in 89 pregnancies, triplets in about one in 7910 pregnancies, and quadruplets in about one in 371126 pregnancies.

To summarize, follicles may solve the “choose M ” problem by a circuit in which in follicles race, measure their relative size by means of a global signal – estrogen as a shared circulating hormone that inhibits their growth - and an androgen as a local signal that kills them if their relative size is too large or too small.

References:

1. Chabbert-Buffet, N., Bouchard, P. The Normal Human Menstrual Cycle. *Rev Endocr Metab Disord* **3**, 173–183 (2002). <https://doi-org.ezproxy.weizmann.ac.il/10.1023/A:1020027124001>
2. Lacker HM. Regulation of ovulation number in mammals. A follicle interaction law that controls maturation. *Biophys J.* 1981;35(2):433-454. doi:10.1016/S0006-3495(81)84800-X
3. Goodarzi, M., Dumesic, D., Chazenbalk, G. *et al.* Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol* **7**, 219–231 (2011). <https://doi.org/10.1038/nrendo.2010.217>
4. Gervásio CG, Bernuci MP, Silva-de-Sá MF, Rosa-e-Silva ACJ de S. The Role of Androgen Hormones in Early Follicular Development. *ISRN Obstet Gynecol.* 2014;2014:1-11. doi:10.1155/2014/818010
5. Gougeon, Alain. (1996). Regulation of Ovarian Follicular Development in Primates: Facts and Hypotheses. *Endocrine reviews.* **17**. 121-55. 10.1210/edrv-17-2-121.
6. National Collaborating Centre for Women’s and Children’s Health (UK). Fertility: Assessment and Treatment for People with Fertility Problems. London: Royal College of Obstetricians & Gynaecologists; 2013 Feb. (NICE Clinical Guidelines, No. 156.) Available from: <https://www.ncbi.nlm.nih.gov/books/NBK247932/>