Multi-scale modeling of the follicle selection process in the ovary

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Abstract

The biological meaning of follicular development is to free fertilizable oocytes at the time of ovulation. The ovulation rate results from an FSH-dependent follicle selection process. In this paper, we designed a multi-scale model of follicular development, where selection arises from the endocrine feedback between the ovaries and pituitary gland and appeals to control theory concepts. Each ovarian follicle is described through a 2D density function giving an age and maturity-structured description of its cell population. The control intervenes in the velocity, gain and loss terms of the conservation law ruling the changes in the density. The model accounts for the changes in the total cell number, growth fraction and global maturity of both ovulatory and degenerating follicles for various intensities of the selection rate. The different selection process outputs (mono- or poly-ovulation, anovulation) predicted by the model are consistent with physiological knowledge regarding vascularization, pituitary sensitivity to ovarian feedback and treatment with exogenous FSH.

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1. Introduction

Ovarian follicles are spheroidal structures sheltering the maturing oocytes. Follicular development is the process of growth and functional maturation gone through by ovarian follicles, from the time they leave the pool of primordial (quiescent) follicles until ovulation, at which point they release a fertilizable oocyte. While the total duration of follicular development spans several months, its terminal part is achieved in a few days, within an ovarian cycle [1]. Actually, very few terminally developing follicles reach ovulatory size; most of them undergo a degeneration process, known as atresia [2]. The ovulation rate (number of ovulatory follicles) results from an FSH (follicle stimulating hormone)-dependent follicle selection process. FSH acts on the somatic cells surrounding the oocyte, making-up the granulosa cell layer, and controls their commitment towards either proliferation, differentiation or apoptosis. The cellular composition of the granulosa ultimately determines the follicular fate: a shift from a proliferative state to a differentiated one characterizes an ovulatory trajectory, while a trend towards apoptosis leads to atresia. FSH release by the pituitary gland is in turn modulated by granulosa cell products such as estradiol and inhibin [3]. The feedback is responsible for reducing FSH release, leading to the degeneration of all but those follicles selected for ovulation. In the selected follicles, LH (luteinizing hormone) can act as a surrogate for FSH, as granulosa cells become endowed with LH receptors [4–6]. Hence, according to their history and individual sensitivity to FSH, follicles do not respond in the same way to the same FSH environment.

The development of ovarian follicles is a crucial process for reproduction in mammals, as the biological meaning of folliculogenesis is to free fertilizable oocyte(s) at the time of ovulation. A better understanding of follicular development is both a clinical and zootechnical challenge; it is required to improve the control of anovulatory infertility in women, as well as ovulation rate and ovarian cycle chronology in domestic species.

The previous mathematical models dealing with follicular development can be split into two main approaches. The first one takes into account the mechanisms underlying follicular development on the cellular and molecular scales, considering separately ovulatory and atretic trajectories. It aims at characterizing and understanding FSH-induced changes in granulosa cells [7,8] and FSH signal transduction [9]. The other approach is concerned with the process of follicle selection per se from the viewpoint of follicular population dynamics [10–12]. A phenomenological and macroscopic evolution function distinguishes between either ovulatory or atretic follicular trajectories. Only the best-fitted follicles can survive in an unfavorable, FSH-poor environment.

In this paper, we aim at merging the molecular and cellular mechanistic description introduced by the former approach with the competition process dealt with in the latter. On the basis of individual granulosa cell changes, we intend to deeper characterize the individual behaviour of ovarian follicles exposed to a similar FSH environment defined by their mutual interactions. To build such a model, we use both multi-scale modeling and control theory concepts. For each follicle, the cell population dynamics is ruled by a first-order conservation law with variable coefficients which describes the changes in the granulosa cell age and maturity density. A control term representing FSH signal intervenes in the velocities, gain and loss terms of the conservation law. The multi-scale feature of the model operates through the zero- and first-order moments of the density, corresponding respectively to the total number of cells and global maturity in a follicle. Summing
those moments on the whole population of follicles gives further information on the ovarian scale. A schematic frame of our modeling approach is illustrated on Fig. 1.

In Section 2, the physiological mechanisms underlying the model assumptions as well as the model terms are described. Section 3 deals with the numerical simulation of the model equations. The final section discusses the physiological assumptions and model formulation.

2. Model design

2.1. Granulosa cell dynamics

2.1.1. Cellular phases

The granulosa cell population consists of proliferating cells (they are running along the cell cycle), differentiated cells (they have left the cell cycle) and apoptotic cells (they have engaged a dying program). We characterize the cells by their position within or outside the cell cycle and their sensitivity to FSH. This leads us to distinguish 3 cellular phases within the granulosa cell population.

- The G1 phase corresponds to cells going along the cell cycle but not yet committed to mitosis; they may respond to external (FSH) signal by either cell cycle exit or apoptosis entry.
• The SM phase aggregates the S, G2 and M phases of the cell cycle. It corresponds to cells committed to complete the whole cell cycle, whatever FSH signal may be, as they have crossed the G1/S restriction point [13].
• The D phase corresponds to differentiated cells that have exit the cell cycle and stop proliferating in an irreversible way. They may be subject to apoptosis in case of unfavorable FSH signal. The D phase is comparable to the G0 phase without quiescent cells.

The resulting cell flow chart is summarized on Fig. 2. The cell cycle consists of the cyclic G1–SM–G1 pathway. When it enters G1 from SM, a mother cell gives birth to two daughter cells. Cell differentiation corresponds to the one-way flow from G1 into D, according to the antagonism between proliferation and differentiation observed during terminal follicular development [14]. Entry into apoptosis arises from G1 and D phases; this assumption is substantiated by flow cytometry studies on granulosa cells showing that appearance of apoptotic cells occurs at the expense of G0/G1 cells [15].

2.1.2. Conservation law

For a given follicle, \( f \), and cellular phase, \( i \), we introduce the cell density function \( \phi_f^i(a, \gamma, t) \). \( t \) denotes time and \( a \) denotes the cell age, i.e. its position within the cell cycle, or, if the cell is already differentiated, the sum of the age reached at cell cycle exit and the time elapsed since. \( \gamma \) is a marker of cell maturity representing the cell responsiveness to FSH [3]. As the main transduction pathway of FSH leads to the activation of adenylyl cyclase and results in the synthesis of cAMP (cyclic adenosine monophosphate), the level of efficient adenylyl cyclase seems to be a good witness of the maturity [16]. Moreover, as cAMP synthesis intervenes relatively upstream in FSH signaling cascade, it can be related to the FSH-induced expression of aromatase (the enzyme transforming androgens into estradiol), inhibin \( \alpha \) subunit and LH receptor genes [17].

The generic form of the conservation law [18] for \( \phi_f^i \) is:

\[
\frac{\partial \phi_f^i}{\partial t} + \frac{\partial g_f(\gamma, t) \phi_f^i}{\partial a} + \frac{\partial h_f(\gamma, t) \phi_f^i}{\partial \gamma} = G_i - L_i
\]

Fig. 2. Cell flow chart. The cell cycle consists of the cyclic G1–SM–G1 pathway. When it enters G1 from SM, a mother cell gives birth to two daughter cells. Cell differentiation corresponds to the one-way flow from G1 into D. Entry into apoptosis arises from the G1 and D phases.
with initial conditions in each follicle and phase:

$$\phi^f_i (a, \gamma, 0) = \Gamma^f_i (a, \gamma)$$

The $g_f$ and $h_f$ functions, and $G_i$ and $L_i$ terms are respectively the aging and maturation velocities, and gain and loss terms. The $u_f$ argument is a local control term detailed below (see Section 2.2.2).

Let $\phi_f = \sum_i \phi^f_i,$ then $\phi_f da d\gamma$ is the number of granulosa cells whose age and maturity respectively lie in the $[a, a + \delta a]$ and $[\gamma, \gamma + \delta \gamma]$ intervals. The zero-order moment of $\phi_f$ corresponds to the number of granulosa cells, $\rho_f$, in a given follicle $f$:

$$\rho_f(t) = \int_{a_0}^{a_{\text{max}}} \int_{c_0}^{c_{\text{max}}} \phi_f(a, \gamma, t) \, da \, dc$$

We define as

$$M_f(t) = \int_{\gamma_0}^{\gamma_{\text{max}}} \int_{a_0}^{a_{\text{max}}} \phi_f(a, \gamma, t) \, da \, d\gamma$$

the follicular maturity related to FSH-induced cAMP synthesis in the whole granulosa, and

$$M^E_f(t) = \int_{\gamma_0}^{\gamma_{\text{max}}} \int_{a_0}^{a_{\text{max}}} H(\gamma) \phi_f(a, \gamma, t) \, da \, d\gamma$$

the maturity related to the follicle ability to secrete estradiol and inhibin. High levels of cAMP are correlated with high levels of aromatase [19], while inhibin secretion is predominant in the early phase of terminal follicular development [20], so that $H(\gamma)$ can be chosen as an increasing function of $\gamma$.

The ovarian maturity corresponds to the global estradiol and inhibin output from a population of $n$ follicles:

$$M(t) = \sum_f M^E_f(t) \quad \text{for } f = 1, \ldots, n$$

2.2. Control terms

2.2.1. Global control

The global control in the model corresponds to the plasmatic level of FSH, denoted by $U[M(t)]$. Its dynamics can be described classically as

$$\frac{dU[M(t)]}{dt} = S[M(t)] - kU[M(t)] + U_0(t) \quad (2)$$

$S[M(t)]$ is the FSH release from the pituitary gland. It is a decreasing sigmoid function and its dependence on $M(t)$ accounts in a compact way for the ovarian feedback exerted through estradiol and inhibin [21].

$kU[M(t)]$ is the clearance of plasmatic FSH and the rate $k$ is deduced from FSH half-life time. $U_0(t)$ represents a potential exogenous entry in FSH.
2.2.2. Local control

FSH bioavailability is subject to follicular modulation, so that we introduce a local control term \( u_f[M_f(t), U] \).

\[
uf = b_f[M_f(t)]U[M(t)]
\] (3)

\( b_f \) represents the local modulation of FSH, according to the follicular vascularization. As the size of the vascular network increases drastically with follicle growth and maturation [22], we chose \( b_f \) to be an exponential function of the global maturity in a follicle:

\[
b_f = b_1 + \frac{e^{b_2M_f(t)}}{b_3} \text{ for } b_f \leq 1 \text{ else } b_f = 1
\]

The upper bound of \( b_f \) comes from the multiplicative form of Eq. (3).

2.3. Aging and maturation velocities

The aging velocity is related to the transit time within a given phase. The transit time within the SM and D phases is not controlled by FSH, which amounts to the age changing like time:

\[
\frac{da}{dt} = 1
\]

In contrast, the transit time within the G1 phase shortens when the local control \( u_f \) increases, in consistency with the well-established enhancing effect of FSH on proliferation [23,24]. The aging velocity is thus an increasing function of \( u_f \). As we had no a priori on its shape, we used a simple linear formulation:

\[
\frac{da}{dt} = g_f(u_f) = g_1u_f + g_2
\]

The maturation velocity can be defined as the change in the cell responsiveness to FSH in terms of cAMP synthesis.

In the SM phase, cells are insensitive to FSH, so that their maturity level remains constant at the level reached at the end of the G1 phase:

\[
\frac{dc}{dt} = 0
\]

In the G1 and D phases, the maturity level can change as cells are sensitive to FSH. The expression for the maturation velocity was inspired from a previous modeling approach of the biochemical reactions leading to FSH-induced cAMP synthesis [9]. We retained the following simplified quasi steady-state equation ruling the changes in the level of efficient adenylyl cyclase:

\[
\frac{dc}{dt} = h_f(\gamma, u_f) = \beta \left( \frac{k_1}{1 + \frac{k_2}{\delta + \gamma} \left( \frac{1}{k_4u_f} \right)} - \gamma \right) \gamma
\]

where \( \beta \) is a time scale parameter; \( k_1 \) measures the degree of FSH signal amplification; \( \frac{k_2}{\delta + \gamma} \) is a Hill function of \( \gamma \), accounting for the desensitization of FSH bound receptors, with \( k_2 \) and \( \delta \) corresponding respectively to the saturation and half-saturation [cAMP] values; \( k_3 \) characterizes the renewal of free FSH receptors; \( k_4 \) is the FSH binding rate.
In case of a constant local control, the maturity level reaches steady-state after a transient period ruled by the value of the $\beta$ time scale parameter. Fig. 3 illustrates the increase in the steady-state level as a function of control. The sensitivity $\left(\frac{\partial \gamma}{\partial u_f}\right)$ decreases for increasing control values, until a maximal steady-state is reached, $\gamma_{\text{max}}$, how high the control may be.

2.4. Transition rates

2.4.1. Cell cycle exit rate

The cell cycle exit rate depends on cell maturity. Let $l(\gamma)$ be the probability distribution function for cell maturity at the time of cell cycle exit. The cell cycle exit rate $A(\gamma)$ can be expressed as

$$A(\gamma) = \frac{l(\gamma)}{1 - \int_0^\infty l(v) \, dv} h_f(\gamma, u_f)$$

$A(\gamma)$ is the instantaneous conditional probability to exit the cell cycle with maturity $\gamma$ knowing that the cell has not yet exit the cell cycle. The formulation of $A(\gamma)$ is similar to that of the hazard rate in the framework of survival models [25].

The support of $l(\gamma)$ corresponds to the maturity zone within which cells are likely to exit the cell cycle. If $l(\gamma)$ is a Dirac function centered on $\gamma = \gamma_s$, the cell cycle exit occurs as soon as the maturity $\gamma$ reaches the $\gamma_s$ threshold. Indeed, the accumulation of intracellular cAMP beyond a threshold appears to be a key point in cell cycle arrest [26], as it is believed to activate cyclin kinase inhibitors [27].

Fig. 3. Asymptotic cell maturity level as a function of control. How high the control may be there is a maximal reachable steady-state level.
2.4.2. Apoptosis entry rate

The apoptosis entry rate, denoted by $\lambda$, is assumed to depend both on the cell maturity level and the hormonal environment. Its formulation results from a balance between the cell vulnerability towards apoptosis and the level of FSH supply:

$$\lambda(\gamma, u_f, u_{f_{\text{max}}}) = \omega(\gamma) \left| \frac{u_f - u_{f_{\text{max}}}}{u_{f_{\text{max}}}} \right|$$

The $\omega(\gamma)$ frequency term assesses the intrinsic cell vulnerability towards apoptosis. The vulnerability window is roughly superimposed with that for cell cycle exit ($h(\gamma)$ support), so that maximum vulnerability coincides with the transition from a proliferating to a differentiated state [28]. Outside the window, the value of $\omega(\gamma)$ is set to zero, so that $\omega(\gamma)$ is shaped as a reversed bell. The upper bound of the vulnerability zone, $\gamma_r$, corresponds to the cell maturity level beyond which cells become endowed with LH receptors [29].

$$\left| \frac{u_f - u_{f_{\text{max}}}}{u_{f_{\text{max}}}} \right|$$

assesses the relative shortage in FSH supply. It compares the actual bioavailable FSH, $u_f = b_f U$, with the level that would be available if FSH were secreted at a maximal rate, $u_{f_{\text{max}}} = b_f U_{\text{max}}$.

Should the actual level of $U$ be upper than $U_{\text{max}}$, (which can occur when exogenous FSH is administered), the negative part insures $\lambda$ to be set to zero.

It is worth noticing that, after factorizing by $b_f$, $\left| \frac{u_f - u_{f_{\text{max}}}}{u_{f_{\text{max}}}} \right|$ amounts to $\frac{|U - U_{\text{max}}|}{U_{\text{max}}}$ so that we can also write:

$$\lambda(\gamma, U) = \omega(\gamma) \left| \frac{U - U_{\text{max}}}{U_{\text{max}}} \right|$$

2.5. Model summary

The whole model can now be summarized by the following conservation laws in the G1, SM and D cellular phases. The model variables and functions are listed in Table 1.

**G1 phase**

$$\frac{\partial \phi_{G1}}{\partial t} + \frac{\partial g_f(u_f) \phi_{G1}}{\partial a} + \frac{\partial h_f(\gamma, u_f) \phi_{G1}}{\partial \gamma} = -A(\gamma) \phi_{G1} - \lambda(\gamma, U) \phi_{G1}$$

(4)

**SM phase**

$$\frac{\partial \phi_{SM}}{\partial t} + \frac{\partial \phi_{SM}}{\partial a} = 0$$

(5)

**D phase**

$$\frac{\partial \phi_{D}}{\partial t} + \frac{\partial \phi_{D}}{\partial a} + \frac{\partial h_f(\gamma, u_f) \phi_{D}}{\partial \gamma} = A(\gamma) \phi_{G1} - \lambda(\gamma, U) \phi_{D}$$

(6)

---

1 The subscript ‘−’ stands for negative part. $|x|_- = \frac{1}{2}(|x| - x)$. 
Eq. (5) is a simple advection amounting to introduce a delay in the dynamics of $\phi_{G1}$. The right-hand side in Eqs. (4) and (6) includes a loss term, $\lambda$, due to apoptosis entry and a symmetrical term, $\Lambda$, (loss in Eq. (4) and gain in Eq. (6)) due to cell cycle exit.

These equations are completed by the initial conditions for each follicle and phase:

$$\phi_f(a, \gamma, 0) = \Gamma_f(a, \gamma)$$

and the boundary conditions:

$$\phi_{SM}(a_1, \gamma, t) = g_f(u_f)\phi_{G1}(a_1, \gamma, t)$$

(7)

$$g_f(u_f)\phi_{G1}(a_0, \gamma, t) = 2\phi_{SM}(a_2, \gamma, t)$$

(8)

Condition (7) means that G1 cells enter the SM phase once their age has reached the maximal age in the G1 phase, $a_1$. Condition (8) means that SM cells undergo mitosis once their age has reached the maximal age in the SM phase, $a_2$; they give birth to two daughter cells whose age is reset to $a_0$, the minimal age in the G1 phase.

In case the cell cycle exit occurs at the $\gamma_s$ threshold value, the symmetrical loss and gain terms in the G1 and D phases reduce to a unique boundary condition:

$$\phi_D(a, \gamma_s, t) = \phi_{G1}(a, \gamma_s, t)$$

(9)

Eqs. (4)–(6) are valid until ovulation is triggered. This happens after the conditions for the pituitary hormone LH (luteinizing hormone) surge have been fulfilled [30], which amounts to the global ovarian maturity $M(t)$ reaching a threshold value denoted $M_s$. The follicles are then sorted and distributed amongst ovulatory and anovulatory, according to the whole number of LH receptors in the granulosa:

$$M^{LH}_f(t) = \int_{\gamma_r}^{\gamma_{max}} \gamma \int_{a_0}^{a_{max}} \phi_f(a, \gamma, t) \, da \, d\gamma$$
For each follicle $f$, $M_f^{LH}$ is compared to a reference level $M_{fs}$, defining the ability to ovulate in response to the LH surge [31].

3. Numerical simulations

3.1. Domain definition

We have to define a geometrical domain to solve the equations numerically. As there are two space variables ($a$ and $c$), the domain is delimited by their ranges. The domain for the G1 phase is thus delimited by a rectangle, ranging horizontally from $a_0$ to $a_1$, and vertically from $c_0$ to $c_s$, where $c_s$ is the threshold for cell cycle exit. In the same way, the SM phase is delimited by a rectangle ranging from $a_1$ to $a_2$, and $c_0$ to $c_s$. In the D phase, cells keep on aging until the end of the simulation. The D phase is delimited by a rectangle ranging from $a_0$ to $a_{max}$, where $a_{max}$ is high enough to be beyond reach during a simulation, and from $c_s$ to $c_{max}$. To avoid numerical continuity between the cell distributions in the D and SM phases, we had to introduce a ‘notch’ in the domain to separate one phase from another. The three rectangles corresponding to each phase are merged in an L-shaped domain (see Fig. 4, top left).

3.2. Numerical solver

The model deals with conservation laws, and more precisely, with variable-coefficient with nonlinear source term equations. The optimal methods to solve numerically such equations are the finite volume methods. In this framework, we chose to work with BEARCLAW (Boundary Embedded Adaptive Refinement Conservation LAW package) software. It is a general purpose, free modular software package for solving conservation laws. It is based on an approximation of the integral of the solution over a ‘finite volume’, representing the subdivision of the spatial domain into $i = 1, \ldots, k$ intervals along the age axis, and $j = 1, \ldots, l$ along the maturity axis. At each time step, the values of the solution on the finite volumes are updated, using approximations to the flux through the endpoints of the volumes. The total mass within the computational domain is thus preserved, and the conservation law respected. Let $\phi_{ij}^n$ be the approximated average value of the granulosa cell density on the finite volume defined over the intersection between the $i$th and $j$th intervals at time $t_n$. Then $\phi_{ij}^{n+1}$ is computed at time $t_{n+1}$ by means of a fractional-step method with Godunov splitting [32], according to the following numerical scheme:

$$\phi_{ij}^* = \phi_{ij}^n - \frac{\Delta t}{\Delta a} \left[ (g_f \phi)_{i+\frac{1}{2},j}^n - (g_f \phi)_{i-\frac{1}{2},j}^n \right] - \frac{\Delta t}{\Delta c} \left[ (h_f \phi)_{i,j+\frac{1}{2}}^n - (h_f \phi)_{i,j-\frac{1}{2}}^n \right]$$

$$\phi_{ij}^{n+1} = \phi_{ij}^* \left[ 1 + \left( G_{ij}^n - L_{ij}^n \right) \Delta t \right]$$

where $G$ and $L$ are the gain and loss terms as defined in Eqs. 1 and (4–6).

\[\text{http://www.amath.unc.edu/Faculty/mitran/bearclaw.html.}\]
For sake of computing efficiency, we handled a single scalar equation on the whole domain. Eqs. (4)–(6) are distinguished by position-dependent differences in the velocities, gain and loss terms.

3.3. Numerical calibration

Even if the model is valid for most mammalian species with spontaneous ovulation, the numerical simulation is applied to a definite species, the ovine species, which is particularly interesting in the framework of follicle selection process. The ovulation rate in the ewe is indeed breed and strain specific [33] and may vary from 1 to more than 6 follicles. Each simulation is carried on for a cohort of follicles entering terminal development, moving on identical geometrical domains. The size of the domain, determined by the lengths of the G1 and SM phases (respectively the

Fig. 4. Cell density distribution on the L-shaped domain. Top left: initialization zones. G1 and SM: cell cycle phases ($a_1$ is the age at S entry, $a_2$ the age at mitosis), D: differentiation phase ($c_s$ is the maturity threshold for cell cycle exit, $c_r$ the maturity threshold for LH receptor endowment). Top right: initial distribution. Bottom: cell distribution at a given time for two follicles. Colors indicate the density values.
and growth fraction (proportion of proliferating cells amongst the total cell number and growth fraction), where \( \gamma_s \) is chosen so as to lead to values of the density zero-order moments compatible with available data on both total cell number and growth fraction (proportion of proliferating cells amongst the whole cell population: \( \rho_{G1} + \rho_{SM} \)). We chose \( a_0 = 0, a_1 = 0.5, a_2 = 1 \) and \( \gamma_0 = 0, \gamma_{\max} = 0.7, \gamma_s = 0.3, \gamma_r = 0.35 \). These dimensions are arbitrary and can be modified, but they are interdependent; for instance, the length of the G1 phase is linked with the position of the maturity threshold \( \gamma_s \). The \([a_0, a_2]\) length corresponds to the cell cycle duration that we further use as time reference. At the beginning of a simulation, only a limited area of the domain is occupied by follicular cells. The cell cycles are considered to be fully desynchronized, whereas the range of maturity values is rather narrow. The support of the initial cell density is illustrated on Fig. 4. It is divided into three zones within which the cell density is initialized homogeneously. The ranges of each zone are detailed in Table 2. A lower zone contains more cells than the upper. The initial growth fraction is set to one, and the most mature cells are on the point of being differentiated.

Within a cohort, follicles slightly differ from one another by their initial density distribution inside the initial domain, and their sensitivity to FSH, rendered by different parameters of the velocity functions. We assume that FSH plasmatic level reaches instantaneously steady-state as defined in Eq. (2) so that its dynamics is defined in the following way:

\[
U[M(t)] = U_{\min} + \frac{U_{\max} - U_{\min}}{1 + \exp[c(M(t) - m)]}
\]  

where the FSH half-life time parameter \( k \) is absorbed in \( U_{\max} \) and \( U_{\min} \).

In the ewe, FSH plasmatic level ranges from \( U_{\min} = 1.5 \text{ ng/ml} \) to \( U_{\max} = 3 \text{ ng/ml} \) along the ovarian cycle [36]. \( m \) is the ovarian maturity at the inflexion point (where \( U = \frac{U_{\max}}{2} \)) and roughly amounts to 90\% of \( M_s \) (for instance \( m = 4.5 \) with \( M_s = 5 \)). The slope parameter, \( c \), rules the steepness of the decrease in FSH. \( c = 2 \) makes the fall occur within the \([m - 2, m + 2]\) interval.

Without loss of generality, the \( H(\gamma) \) function is taken as the identity function: \( H(\gamma) = \gamma \). In the follicular fluid, bioavailable FSH level, \( u_f \), ranges from almost undetectable to FSH plasmatic level [22], so that \( b_f \) values are strictly positive and may reach unity. With \( b_1 = 8 \times 10^{-2}, b_2 = 2.25 \) and \( b_3 = 1.45 \times 10^3 \), \( u_f \) values range from 0.24 to 2.5 ng/ml.

The aging velocity, \( g_f \), rules the transit time across the G1 phase, as a function of \( u_f \). In the reference case \( g_f = 1 \) (age changes like time), FSH has no positive nor negative effects on the transit time which lasts half a cell cycle duration. \( g_f \) parameters are set so that the total duration of G1 varies of more or less 30\% from the duration in the reference case (0.7 < \( g_f < 1.3 \)): \( g_1 = 80 \) and \( g_2 = 0.7 \).

<table>
<thead>
<tr>
<th>Zone</th>
<th>Range in age</th>
<th>Range in maturity</th>
</tr>
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<tbody>
<tr>
<td>Zone 1</td>
<td>[0, 1]</td>
<td>[0.15, 0.2]</td>
</tr>
<tr>
<td>Zone 2</td>
<td>[0, 1]</td>
<td>[0.2, 0.25]</td>
</tr>
<tr>
<td>Zone 3</td>
<td>[0, 1]</td>
<td>[0.25, 0.3]</td>
</tr>
</tbody>
</table>
As far as the maturation velocity, $h_f$, is concerned, the parameter values were inspired from a previous model [9]. We chose the time scale parameter $\beta$ so that the maturity level in a cell increases from $0.15 \times 10^4$ to $0.4 \times 10^4$ molecules/cell in about 6 cell cycles.

### 3.4. Results

#### 3.4.1. Follicle selection within a cohort of two follicles

To explore the model behavior in a simple way, we began with a selection process within a cohort of two follicles. There are three possible situations at the end of a simulation: two anovulatory (situation 1), two ovulatory (situation 2), or one ovulatory and one anovulatory follicles (situation 3). We consider the latest as a reference. The common parameters are listed in Table 3, while the follicle-specific ones are listed in Table 4. The main difference between the follicles lies in their sensitivity to FSH and is rendered by a disparity in the aging and maturation velocity parameters.

Simulation outputs consist:

1. on the ovarian scale, in the changes in the global control and ovarian maturity;
2. on the follicular scale, in the changes in the local control, number of granulosa cells and follicular maturity;
3. on the cellular scale, in the cell density distribution on the geometrical domain.

Fig. 4 shows the cell density distribution for both follicles at a given time. The colorbar indicates the density value. Cells on the bottom side of the domain ($c < c_s$) are still proliferating cells, while those on the upper side are differentiated. On the snapshot displayed, follicle 1 is more mature than follicle 2 in consistency with its higher sensitivity to FSH.

Fig. 5 illustrates the outputs of the selection process for both follicles of the reference situation. Panel (a) shows the decrease in FSH plasmatic level, from 3 ng/ml to 2.3 ng/ml. FSH pattern is related with the changes in the ovarian maturity, as can be seen on Panel (b), which increases and reaches the ovarian threshold for ovulation triggering, $M_s = 5$. The bioavailable FSH for follicle 1 never ceases increasing and approaches the current plasmatic values (2 ng/ml) whereas that for follicle 2 slightly increases before decreasing to 0.2 ng/ml (see Panel (c)). Panel (d) shows the changes in the total number of granulosa cells in each follicle. Starting from a similar initial value ($10^6$ cells), the total cell number in follicle 1 increases up to $6.5 \times 10^6$ and remains at this steady value, while that in follicle 2, after reaching nearly $6 \times 10^6$ ends up by losing more than $2.5 \times 10^6$ cells. The global maturity in follicle 1 keeps on increasing even once the cell number is stabilized, as differentiated cells continue maturing, whereas it decreases in follicle 2, as displayed on Panel (b). At the time of ovulation triggering, follicle 1 has reached the follicular threshold for ovulation ($M_f = 3.5 > M_{fs}$) whereas follicle 2 has not ($M_{f2} = 1.5 < M_{fs}$). Hence, follicle 1 describes an ovulatory trajectory and follicle 2 an atretic one.

There is a delay corresponding to about 60% of a cell cycle duration between the fall in FSH and the decrease in the number of granulosa cells and global maturity of follicle 2. It is due to a kind of inertia in the follicle dynamics. Until plasmatic FSH falls, the bioavailable FSH in follicle 2 has been enhancing cell proliferation. As long as FSH plasmatic levels are not too low, the
apoptosis entry rate remains low and proliferation overrides apoptosis. The situation inverts when the fall in FSH becomes stronger.

The fate of a follicle depends on whether or not it can escape from the confinement in the maturity zone where cells are vulnerable to apoptosis. This zone is delimited by the support of the $\omega(\gamma)$ function, i.e. the $[0.2, 0.35]$ maturity interval. The escape is subject to the control of bioavailable FSH which may or not allow the cell maturity to cross the upper bound of the vulnerable zone.

### Table 3
Model parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definition</th>
<th>Nominal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U[M(t)]$</td>
<td>$U_{\text{max}}$ Maximal plasmatic FSH value</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>$U_{\text{min}}$ Minimal plasmatic FSH value</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>$c$ Slope parameter</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>$m$ Abscissa of the inflexion point</td>
<td>4.5</td>
</tr>
<tr>
<td>$b_{\text{f}}[M_{\text{f}}(t)]$</td>
<td>$b_1$ Basal level</td>
<td>$8 \times 10^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$b_2$ Exponential rate</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>$b_3$ Scaling parameter</td>
<td>1450</td>
</tr>
<tr>
<td>$g(\gamma)$</td>
<td>$g_1$ Slope parameter</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>$g_2$ Origin ordinate</td>
<td>0.7</td>
</tr>
<tr>
<td>$h(\gamma, u_f)$</td>
<td>$\beta$ Time scale parameter</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>$k_1$ Degree of FSH signal amplification</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>$k_2$ Hill function saturation parameter</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>$\delta$ Hill function half-saturation parameter</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>$k_3$ Recycling rate of FSH receptors</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>$k_4$ FSH binding rate</td>
<td>0.15</td>
</tr>
</tbody>
</table>

#### Domain length

| $a_0$ | Cellular age at the beginning of the G1 phase | 0 |
| $a_1$ | Cellular age at the beginning of the SM phase | 0.5 |
| $a_2$ | Cellular age at the end of the SM phase      | 1  |
| $a_{\text{max}}$ | Maximal cellular age in the D phase | 8  |

#### Domain height

| $\gamma_{\text{min}}$ | Minimal maturity level in the G1 and SM phases | 0  |
| $\gamma_s$ | Maturity threshold for cell cycle exit          | 0.3 |
| $\gamma_r$ | Maturity threshold for LH receptors acquisition | 0.35 |
| $\gamma_{\text{max}}$ | Maximal maturity level in the D phase            | 0.7 |

#### Ovulation thresholds

| $M_s$ | Ovarian threshold for ovulation triggering | 5  |
| $M_{\text{fs}}$ | Follicular threshold for ovulation ability | 2  |

### Table 4
Reference situation parameters

<table>
<thead>
<tr>
<th>$\beta$</th>
<th>$g_1$</th>
<th>$g_2$</th>
<th>Initial cell number ($10^6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone 1</td>
<td>Zone 2</td>
<td>Zone 3</td>
</tr>
<tr>
<td>Follicle 1</td>
<td>0.7</td>
<td>80</td>
<td>0.4</td>
</tr>
<tr>
<td>Follicle 2</td>
<td>0.6</td>
<td>68.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>
3.4.2. Sensitivity of the pituitary gland to the ovarian feedback

The effects of the sensitivity of the pituitary gland to the ovarian feedback can be investigated by modifying the parameters of the sigmoid function in Eq. (10). The higher \( m \) is, the later the fall in FSH occurs; the steeper the slope \( c \), the faster the fall. The values of the parameters tested are displayed in Table 5.

In case of a high pituitary sensitivity (situation 1) the selection process results in two anovulatory follicle trajectories (Fig. 6(a) and (b)). The ovarian maturity decreases before reaching the threshold for ovulation triggering, so that ovulation cannot occur. It is due to the fall in FSH occurring early, and the subsequent low FSH plasmatic level preventing follicles from maturing further. As bioavailable FSH for both follicles finally increases, the follicles end up by escaping from the apoptosis vulnerable zone. They reach a steady-state but the cell loss has been so high

<table>
<thead>
<tr>
<th>Situation</th>
<th>( m )</th>
<th>( c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Situation 1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Situation 2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Situation 3</td>
<td>4.5</td>
<td>6</td>
</tr>
</tbody>
</table>
that they cannot ovulate. Such cases may be encountered is some pathological situations of anovulation.

In case of a low pituitary sensitivity (situation 2) the selection process results in two ovulatory follicle trajectories (Fig. 6(c) and (d)). The fall in FSH occurs for a comparatively higher ovarian maturity. Both follicular trajectories seem similar, although follicle 1 (the more sensitive to FSH) ends up with a higher number of granulosa cells and global maturity. There is nearly 1 million cell loss for follicle 2 before it stabilizes and awaits ovulation, as it runs along the vulnerable zone slowly, and suffers more from apoptosis than follicle 1. Yet, despite a relatively low level of bio-available FSH, follicle 2 finally crosses the apoptosis vulnerable zone and ovulates.

Fig. 7(a) and (b) illustrates situation 3: one ovulatory and one anovulatory follicle trajectories in case of a high pituitary sensitivity to the changes in ovarian maturity. Compared with the reference parameter set (where \( c = 2 \), see Table 3), follicle 2 undergoes atresia more rapidly. Indeed, the steeper FSH fall speeds up apoptosis kinetics, as the \( \frac{|U-U_{\text{max}}|}{U_{\text{max}}} \) term in the apoptosis entry rate increases with \( c \), resulting in massive apoptosis.

### 3.4.3. Follicular sensitivity to FSH

We studied the effects of the follicular sensitivity to FSH, by altering the slope of the aging velocity and the time scale of the maturation velocity. The aging velocity differs from the reference
The time scale parameter of the maturation function is higher for follicle 1 than for follicle 2. Fig. 7(c) and (d) shows one anovulatory and one ovulatory follicular trajectory. FSH enhances proliferation in the same way for both follicles, but as granulosa cells exit the cell cycle more slowly in follicle 2 than in follicle 1, they proliferate longer. This proliferative resource can be exploited to raise the final number of differentiated cells, hence the follicle global maturity and contribution to ovarian feedback on FSH. Follicle 1 becomes unable to survive in the unfavorable FSH environment and undergoes atresia, whereas follicle 2 reaches the follicular threshold for ovulation.

Table 6

<table>
<thead>
<tr>
<th>Follicle</th>
<th>$\beta$</th>
<th>$g_1$</th>
<th>$g_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle 1</td>
<td>0.8</td>
<td>80</td>
<td>0.7</td>
</tr>
<tr>
<td>Follicle 2</td>
<td>0.6</td>
<td>80</td>
<td>0.7</td>
</tr>
</tbody>
</table>
3.4.4. Cohort of five follicles

In order to explore the model behavior facing a greater cohort, we simulated a cohort of five follicles. The initial distributions of the cell density, as well as follicle sensitivities to FSH slightly differ from one another, as summarized in Table 7. We adapted the pituitary sensitivity and ovulation threshold \((m = 9, M_s = 9)\) to the ovarian dynamics. Fig. 8(a) and (b) illustrates the selection process within the cohort: the ovulation rate is 2, follicles 1 and 3 are the ovulatory ones.

Table 7
Selection process within a cohort of five follicles

<table>
<thead>
<tr>
<th></th>
<th>(\beta)</th>
<th>(g_1)</th>
<th>(g_2)</th>
<th>Initial cell number ((10^6))</th>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle 1</td>
<td>0.7</td>
<td>80</td>
<td>0.7</td>
<td>0.4</td>
<td>0.35</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Follicle 2</td>
<td>0.6</td>
<td>68.6</td>
<td>0.6</td>
<td>0.3</td>
<td>0.3</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Follicle 3</td>
<td>0.7</td>
<td>80</td>
<td>0.7</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Follicle 4</td>
<td>0.65</td>
<td>74.2</td>
<td>0.65</td>
<td>0.2</td>
<td>0.15</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Follicle 5</td>
<td>0.55</td>
<td>62.8</td>
<td>0.55</td>
<td>0.45</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8. Panels a and b: selection process within a cohort of five follicles. Black lines correspond to ovarian outputs, blue lines to follicle 1, green lines to follicle 2, red lines to follicle 3, cyan lines to follicle 4, and pink lines to follicle 5. Panel (a): FSH plasmatic level (—) and ovarian maturity (---), Panel (b): number of granulosa cells. Two follicles are ovulatory. Panels c and d: effects of an exogenous entry in FSH. Panel (c): FSH plasmatic level (—) and ovarian maturity (---), Panel (d): number of granulosa cells. Follicle 5 is rescued from atresia by the administration of exogenous FSH.
The ovulation rate can be modified by means of exogenous FSH supply. It is administered after the cells have run along five cell cycles, in such a way as to keep the FSH plasmatic level at its maximal value, as showed on Fig. 8, Panel (c). Fig. 8, Panel (d) illustrates the rescue of follicle 5, which was atretic in the previous situation and now manages to ovulate. Ovulation rate thus rises from 2 to 3. The 2 other follicles (2 and 4) do not benefit sufficiently from additional FSH and still undergo atresia.

4. Discussion

We considered the follicle selection process as an FSH-dependent controlled process, from a mechanistic viewpoint. FSH acts on the molecular scale through a signal transduction pathway. Integration of FSH signaling on the cellular scale rules the dynamics of the transition rates between different cellular states, hence cell commitment towards either proliferation, differentiation or apoptosis. Further scaling determines the follicular fate based on its cell content and contribution to ovarian endocrine status. Summing-up each follicular contribution finally defines the ovarian feedback pressure on FSH release, closing the loop. Our approach thus managed to merge a detailed multi-scaled description of follicular development with population dynamics issues.

Actually, the control defined by the ovarian feedback is not directly the operating one. Indeed, this control is subject to local modulation on the follicular scale, which acts as some kind of input filter. On a physiological ground, such a filter can be mainly imputable to the degree of follicular vascularization [22], but also to intercellular communication via gap-junctions [37], and more globally to the so-called follicular micro-environment, in which the extra-cellular matrix [38] and paracrine interactions between the thecal and granulosa cells [1] participate. The local control term insures the model stability by preventing cells from proliferating or maturing in excess. For high local control values, for instance in case of exogenous FSH supply, proliferation enhancement is balanced by cell cycle exit. Follicle development is thus accelerated but still under control, as follicular maturity is also bounded. For low local control values, the cell cycle duration increases so that the proliferation rate slows down and compensates for the increase in growth fraction due to the lower cell cycle exit rate.

Depending on the scale considered, the notion of maturity may have several meanings. On the molecular scale, cAMP synthesis was retained, as it is a bottleneck for FSH signaling integrating cross-talks with other signaling pathways such as the IGF (insulin growth factor) and BMP (bone morphogenetic protein) systems [39], and can be connected with the cell status. On the follicular scale, maturity characterizes both the follicle endocrine contribution in terms of inhibin and estradiol secretion, and its ability to ovulate supported by the acquisition of LH receptors by granulosa cells. Finally, on the ovarian scale, the maturity defines the endocrine status of the ovary and sets the feedback pressure on the pituitary gland.

The timing of the granulosa cell cycles is described in a simple way: the S, G2 and M phases are aggregated into one single SM phase, and transitions between the G1 and SM phases occur when cells reach a given cellular age. We rather focused on the FSH-dependent aging dynamics. The transit time within the SM phase is fixed, but it may vary within the G1 phase according to FSH signal, so that each individual granulosa cell runs along its proper cell cycle. Within the differentiation phase, the age is never reset. Yet, the numerical implementation led us to introduce...
an age limit. Such a limit can be understood as a senescence limit, beyond which an old cell would undergo programmed cell death [28].

The maturity variable rules the transition from the G1 into the D phase. After mitosis, daughter cells inherit the maturity level reached by their mother cell at the end of the G1 phase. Even if the FSH receptor pool is roughly divided into two halves after mitosis, the maturation dynamics of the daughter cells can be considered as rapidly similar to the mother cell dynamics, as the pool is quickly reconstituted by receptor neosynthesis and the efficiency in cAMP is only moderately affected by the pool size [9]. In case of decreasing values of the local control, the maturation velocity can become negative, so that the model allows for cellular dedifferentiation. Yet, such dedifferentiation cannot lead to a return into the cell cycle as the cell has to cross-backwards the apoptosis vulnerable zone.

The apoptosis entry rate is an essential multi-scale term for the model. The selection process of the ovulatory follicles rests indeed on the removal of the other maturing follicles, due to their inability to survive in an unfavorable FSH environment [31]. The dependence of the apoptosis rate on cell maturity is a key point, as the escape from or confining within the vulnerability zone decides of the follicular fate. The beginning of FSH fall determines the time from which the selection process takes place, and the fall steepness the apoptosis intensity. Even the ovulatory follicles are affected by cellular apoptosis, yet at a lower degree than atretic ones. This comes in consistency with the observation of up to 10% apoptotic cells in healthy follicles [15].

The model results in terms of the selection process output (mono- or poly-ovulation, anovulation) are consistent with physiological knowledge regarding vascularization, pituitary sensitivity to ovarian feedback and treatment with exogenous FSH. It has been proposed that increased vascularity may be a primary determinant of follicular selection [40]; the selected follicles are more vascularized than the others and as a result they display a higher intake of serum gonadotropins, especially FSH [22]—just as it can be seen on Fig. 5(c). The model outputs in response to different levels of pituitary sensitivity suggest that a low pituitary sensitivity can result in a higher ovulation rate. This is in agreement with experimental investigations comparing the effect of exogenous estradiol (hence of increased ovarian feedback) in poly-ovulating (Finnish Landrace breed) or mono-ovulating (Scottish Blackface breed) ewes. The former tolerated higher levels of estradiol before FSH level was reduced [41,42]. Administration of exogenous FSH is well-known to increase ovulation rate as it is a basis for ovarian stimulation treatment in humans [43] as well as in domestic species [44]. In humans, the main drawback of such treatment may be overstimulation. Our very preliminary results suggest that fine tuning of FSH may be used to target a specific ovulation rate. Systematic exploration of the physiological and pathological situations represented by the model is a matter of current work.

Several computational efficiency constraints (simulation time, memory resource) imposed to adapt the model formulation to numerical implementation. The conservation laws in the SM and D phases could have been simplified; it is not compulsory to handle two space dimensions, as there is a redundancy between time and cell age. Yet, the 2D description allows to keep the same equation frame on the whole domain and to merge the 3 equations defined by Eqs. (4)–(6) into one single scalar equation, thus saving on computational cost. This merging led to simplify the dynamics of cell cycle exit. Compared to a random maturity level at cell cycle exit, a threshold one may modify the proliferative resources of follicles hence affect their final number of differentiated cells and global maturity, which may finally alter the hierarchy established within
a follicle cohort. Equation merging also led to introduce an artificial notch on the geometrical domain. At the end of the G1 phase, when the maturity level approaches $\gamma_s$, cells moving on the domain may enter the notch, leading to ‘numerical apoptosis’. Such a phenomenon seems not able to alter the selection issue.

Simulation cost also made us focus on the simulation of a pair of follicles rather than on a larger cohort. The pair configuration allowed to investigate in a simple way the model behavior for various pituitary and ovarian dynamics. Besides, the simulation of a few follicles may be not so far from that of a larger cohort amongst which follicles at comparable maturation stages including leading follicles can be distinguished. Moreover, the outputs obtained from simulations of a 5 follicle cohort in terms of ovulation rate allows to be confident in the model behavior facing a higher number of follicles. Anyway, the improvement of computational performances would allow to simulate the model as it is formulated, without any simplification and to deal with bigger cohorts.

Further work also remains to be done to analyze the model and define precise associated control problems. The dynamics of the zero- and first-order moments of the cell density could be written explicitly to obtain the changes in the granulosa cell number and global maturity directly for each follicle. It would help control the ovulation rate through the confinement of follicles within the apoptosis vulnerability zone or their escape from that zone, as well as the ovulation timing.

References


