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Mathematical modeling of ovarian follicle development : A population dynamics viewpoint

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Abstract

Amongst endocrine organs, ovaries are unique because their function is supported by an ever-changing number of histological entities, the ovarian follicles, which are themselves subject to asynchronous development from their formation up to ovulation. Follicle development combines finely controlled processes of cell dynamics with original morphogenesis steps. Understanding the dynamics of follicle populations, on the whole ovary level, and the coupled dynamics of the oocyte and somatic cells, on the follicle level, is crucial to understand ovarian physiology and ovarian aging. Mathematical modeling studies combined with proper experimental approaches can be particularly helpful for gaining insight into follicle dynamics on different scales.

Keywords: population dynamics, regression models, structured populations, multiscale models ovarian follicles, cell fate, cell kinetics, morphogenesis

Introduction

The ovary is a unique instance of dynamic, permanently remodeling endocrine organ in adulthood. The ovarian function is supported by spheroid, multi-layered and multi-phasic structures, the ovarian follicles, which shelter the oocyte and secrete a variety of peptide and steroid hormones. Understanding the dynamics of ovarian follicle populations is critical to characterize the reproductive physiological status of females from birth (or even prenatal life) to reproductive senescence. Follicle development involves coordinated processes of cell kinetics and morphogenesis, which are deployed in the framework of the hypothalamo-pituitary-gonadal (HPG) axis. Getting mechanistic insight into follicle development requires investigating the spatio-temporal dynamics of somatic follicle cells, coupled with oocyte maturation, and subject to hormonal and mechanical controls.

In this review, we will focus on population dynamics issues raised by folliculogenesis in mammals, on the level of both the whole follicle population and the cell populations within developing follicles. We will highlight how historical and

recent modeling approaches have tackled these issues, what insights have been gained or can be expected in the future from the models, and which experimental data are or will be critical to ensure a mutual benefit from modeling and experimental studies. We refer the interested reader to [1, 2] for a broader overview on mathematical models dedicated to the HPG axis.

1. Follicle population dynamics

At any given time, the status of the ovaries can be assessed by the distribution of follicles into different maturity stages and by the total number of follicles (Figure 1). These observations contain condensed information on long-term interacting processes.

The total number is directly related to the remaining number of primordial follicles at a given age. In turn, the size of the primordial pool results from the combination of the initial (maximal) pool size and (possibly time-varying) rate of pool depletion by atresia and activation. The distribution of follicles is shaped by the asynchrony in the activation times and the relative rates of growth or atresia at different maturities, resulting from direct (paracrine) or indirect (endocrine, HPG-mediated) interactions between follicles (of similar or different maturities).

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Mathematical models based on statistical regression have dealt mainly with the depletion of the “non growing follicles” (including primordial, intermediate and primary follicles) with aging (see e.g. [3, 4] and [5] for a comparative review). A focus can also be put on antral follicle count – or rather on its hormonal counterpart, AMH (Anti-Müllerian Hormone) [6, 7], to restrict to the maturity stages targeted by ovarian stimulation treatments.

The main advantage of these models is that they only need the adjustment of a few parameters, and can be used to predict the age at menopause in a “demographic” manner, for groups of patients in physiological or pathological conditions. They can also detect changes in the depletion rate, and have especially emphasized an acceleration in the premenopausal period. Using a biphasic log-linear model has led to the notion of break slope at a threshold age, that should be interpreted with caution, given that polynomial models can fit the data as well [3, 5], and that the selected threshold age is subject to significant statistical uncertainty. Finer data fitting can be expected from adding covariables, or at best, using mixed-effect models [7], yet interindividual variability in the size of the initial pool and the (wasteful or thrifty) way of “consuming” the pool raises insurmountable obstacles for patient-specific prediction by regression models.

Compartment-based models can encompass both the depletion of the total number of follicles and their distribution into growing stages, generally sorted by the follicle diameter and considered up to the antral stages [8, 9]. Their main strength is that they fully exploit the available data on follicle numbers and introduce explicitly the dependence on time. They have also been used to obtain quantitative information on oogenesis before follicle formation [10], and assess the number of founder primordial germ cells [11].

Up to now, only linear models have been fitted to experimental data. The follicle trajectories are uncoupled, as such formulations do not account for interactions between follicles. As a consequence, they cannot be useful for understanding the link between the “static” reserve (primordial follicles) pool and the “dynamic” reserve (small antral follicles) [12], possibly involving buffering mechanisms [13], or the difference between the first wave of follicle initiation and the next ones [14]. They can neither help investigate mechanistically the main conditions perturbing these interactions (hypophysectomy [15], unilateral ovariectomy [16], AMH Knock-Out [17] or overexpression [18]). Another caveat is that the parameters critical for each compartment (death rate and

exit rate to the next stage) are hardly identifiable from a parameter estimation viewpoint. This may seem a technical detail, yet it explains why attempts to use such models to decipher whether the primordial pool is or not replenished cannot lead to a decisive result, since conclusions are drawn from divergent assessment of the atresia rate [10].

An extension of such models to a nonlinear framework is thus necessary. A comprehensive mathematical framework has been recently designed with this objective [19]. Before being fully exploited for modeling purposes, ongoing mathematical works need to be completed, since the nonlinear formulation raises open mathematical questions.

Finally, nonlinear models formulated in a continuous framework have tackled the specific issue of follicle selection. The follicle population corresponds to the cohort entering terminal development. The selection results from the dependency of follicles on FSH supply, whose levels are set in turn by the endocrine feedback of competing follicles. Nonlinearity is essential to widen the inequalities between follicles and separate the trajectories of ovulatory and atretic follicles. The first, phenomenological approaches have taken advantage of the mathematical properties of a specific class of functions (Abel functions) [20]. They can reproduce different combinations of ovulation rates and even cyst-like situations [21]. A more recent approach has considered selection as an emergent behavior emanating on the follicle level from the follicle cell level ([22, 23], see next section).

Whatever the granularity of the models, their common main output is the change in the number of follicles with time (hence age), and, as a corollary, the cumulative number of follicles lost by atresia. Follicle counts are thus critical data to fit the model parameters and control the quantitative behavior [24]. Unfortunately, there are many limits in follicle counts. First, except in the latest stages of development, counting follicles is an invasive measurement, so that there do not exist any true kinetic experimental data. Datasets retrieved from a given individual (animal or patient) provides only a snapshot of the follicle population at a given age, and time is somehow a statistical reconstruction obtained from different individuals observed at different ages. In addition, most studies are performed in 2D on sampled ovary slices, and the assessment of the whole population is based on stereological concepts, leading to a more or less reliable 2D/3D reconstruction [25]. Up-to-date protocols, such as those based on transparization (clearing the tissue to gain ob-

servation depth) [26, 27] can get rid of the 2D constraints, yet they still acquire static data and are limited to small species compatible with whole ovary imaging. Great efforts should be put on improving the time resolution and reliability of follicle counts, through novel or improved *in vivo* imaging techniques and associated image analysis algorithms, possibly involving deep learning [28].

2. Cell populations dynamics

Somatic cells, and especially granulosa cells, undergo a sequence of cell events marking out the different steps of folliculogenesis (Figure 2). A couple of cell-centered models have investigated these critical cell biology issues [29]:

- *Activation* [30] Is cell proliferation concomitant or posterior to the flattened-to-cuboidal transformation? Is the transformation rate amplified locally or globally, or do transformation events occur independently?
- *Preantral growth* [31, 32] How do granulosa cells organize into concentric layers, while sensing overcrowding? How does the molecular dialog between the oocyte and surrounding cells control the balance between oocyte enlargement and cell proliferation?
- *Terminal development* [23, 22] Which is the good timing to switch from proliferation to differentiation, and get a preovulatory follicle endowed with a proper number of mature cells? How will the cell distribution and vulnerability to apoptosis determine whether a follicle is selected for ovulation, in a fluctuating endocrine environment?

From the methodological viewpoint, these models belong to the so-called structured population models. In addition to evolving with time, cells are distributed according to one or several variables, such as for instance the cytologic age (progression along the cell cycle), or the location with respect to the oocyte. A stochastic formalism, in which cell events are probabilistic, is required in the first stages, due to the small number of cells. A deterministic formalism can be handled for later stages, when the cell number can be considered as a continuous variable. Both formalism types are amenable to rigorous mathematical analysis certifying the predictions on the model behavior. They tackle the cell biology issues using appropriate biomathematical concepts such as (i) extinction time for the duration of activation (transformation of all flattened cells), (ii) doubling times and leading layer for cell proliferation

in the preantral phase, and (iii) switching time and competition pressure for selection.

The concept of extinction time has also been deployed in a mean field model, where each primordial follicle is assimilated to a single average cell subject to irreversible stochastic switch in phenotype [33]. These models have been designed separately, yet, when put one after the other, they cover the whole folliculogenesis, from activation to ovulation; the final output of one model can be used as initial condition for the next, and it would be worth building a comprehensive simulation environment.

Reducing the structuring variable to a discrete type amounts to dealing with subpopulations in a compartmental setup, as that introduced for follicle population dynamics. For instance, a compartment model has been used recently to sort cell subpopulations according to predefined classes of telomere length [34].

The main difficulty faced to calibrate the model parameters is the absence of kinetic information; cell numbers are provided as a function of morphological variables such as oocyte or follicle diameter [35, 36]. Even worse, while there has been a “golden age” for cell kinetics in the 70s in many biological fields, including ovarian physiology (see e.g. [37]), studies dedicated to follicle cell populations have become scarcer and scarcer, compared to the ever increasing reports on molecular biology. In addition, despite of technical improvements (e.g. multiple fluorescent markers), there has been poor gain of information, especially on the quantitative ground. It would be all the more worth renewing the experimental approach, than the available models can be very helpful for interpreting cell kinetics experiments, or even analyzing the data. For instance, most studies assessing the cell cycle (and cell cycle phase) duration from mitotic and labeling indexes assumes implicitly that the population is fully proliferating, with no cell loss or cell cycle exit. Such an assumption is not valid for follicle development, while thorough *in silico* experiments have been performed in realistic situations [32, 38].

In the cell-centered models, the physical space may be explicitly accounted for, yet biophysical and mechanical processes are only considered implicitly in the rate for cell events such as division, death or migration. Fascinating open questions remain on how follicle cells are distributed within the follicle geometry, which is in turn shaped by cell dynamics. A key point would be to embed the orientation of cell division planes [39, 40] in relation to cell polarity, intercellular junctions [41], and cell cortical stress. Another would be to formulate follicle growth as a moving boundary prob-

lem, in which the follicle outer boundary is driven by an internal time-varying pressure due to oocyte growth (preantral stage) or antrum inflation. Appropriate coordinate setups (dealing with the loss of spheric symmetry and oocyte eccentricity) have been proposed to model antrum growth [42, 43] or diffusion of soluble factors up to the cumulus-enclosed oocyte [44], yet in static or quasi-steady state setups eluding the moving boundary difficulty. An interesting parallel could be drawn with the blastocyst to investigate the mechanisms underlying both antrum formation (concept of hydraulic fracturing [45]), or dynamic adaptation of the mural wall to antrum pressure (oscillations in the wall deepness around a steady-state [46]). Also, since antral growth is associated with a dramatic increase in vascularization, and expansion of the outer wall into the ovarian stroma, a notable advance would result from embedding the dynamics of theca cells [47].

Similar problems coupling cell dynamics with morphogenesis and mechanical constraints are also encountered during early oogenesis, with the combined migration and mitosis of primordial germ cells, and the formation and breakdown of germ cell cysts. An interesting phenomenological, cell-based modeling of oogenesis [48] has been developed within an IBM (individual-based model) simulation environment (CompuCell3D), that would deserve further analysis and quantitative validation.

Conclusion

A promising perspective is to couple cell population dynamics with follicle population dynamics in a multiscale approach. The marker of follicle maturity is then directly related to the status of all follicle cells (the simplest being the total cell number), possibly in a multi-valued (e.g. considering different cell status) or even infinite dimension framework (continuous distribution of cells). Such a methodologically difficult approach has already been set up to study the emergence of follicle selection from cellular bases [23, 22]. It would be worth extending it to earlier follicle stages, and ultimately designing a comprehensive, cell-biology based modeling framework of the whole folliculogenesis.

A parallel effort is expected on the experimental side to manage monitoring quantitatively and dynamically the ovarian follicle populations and follicle cell populations. A first step in this direction could come from exploiting *ex vivo/in vitro* devices such as cultures of ovarian cortex [49] or pooled follicles [50], organoid-like [51], and microfluid systems [52] to monitor follicle growth dy-

namically and quantitatively, even if culture systems depart from physiological situations.

Dealing with specific modeling issues devoted to the oocyte is beyond the scope of this review. We would yet like to mention interesting modeling works dedicated to different steps of meiosis – acentriolar spindle assembly during meiotic divisions [53], associated errors leading to aneuploidy [54], or resumption of meiosis II induced by calcium oscillations at fertilization [55] – as well as to the control of nucleus positioning [56] associated with oocyte fate.

Declarations of interest: none

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References of outstanding interest

- [18] This is a thorough study on the effect of transient AMH overexpression on the activation of primordial follicles. It includes particularly valuable kinetics on follicle counts (time to follicle “disappearance” after overexpression onset, time of “reappearance” of follicles in different stages after treatment).
- [14] This study has settled the question of the involvement of the first formed primordial follicles, originating from the ovary medulla in the early reproductive life in rodents, which was raised in the early 70s by J.A. Hirshfield. It also provides very interesting comparative studies on follicle growth rates between the first activated follicles and the next ones, which can be assimilated respectively to an AMH-free environment and AMH-regulated one.
- [45] A nice, very integrative study mixing live imaging, physical modeling, cell and developmental biology. The newly discovered mechanism of hydraulic fracturing underlying blastocyst formation is very inspiring for the investigation on follicle antrum formation.
- [52] An innovative and ingenious microfluidic device, integrating several components of the female reproductive system (of mixed human and mouse origin), and taking advantage of previous works that had led to optimized culture environments. The physiological relevance of this device is reinforced by dynamic perfusion of pituitary hormones and re-circulation of secreted factors emanating from cultured tissues. Combining in the future such a device with proper imaging sensors would offer a great potential for real-time monitoring of follicle dynamics.

References of special interest

- [28] This study applies cutting edge methodologies in deep learning to tackle the crucial question of ensuring the reliability of follicle counts.
- [41] This is one of the very few studies providing information on the types of intercellular junctions encountered along follicle development. Such cell biology-oriented studies, as others dedicated to the follicle cytoarchitecture, are essential to fill the gap between the molecular level and the tissular/organic level.
- [36] A wealth of quantitative information on cell numbers in relation to follicle and oocyte growth, covering both follicle activation and early growth. As other works of KP McNatty lab (see e.g. [35] for antral growth), such a study gives insight into the balance between cell proliferation and oocyte (or antrum) growth.
- [37] A seminal study on granulosa cell kinetics during follicle development, based on a former, still in use, classification of developmental stages established by the same author. The strong point in the cell cycle study is the achievement of labelled mitosis curves, which is a much more reliable approach than point MI (mitotic index) or LI (labeling index) measures.

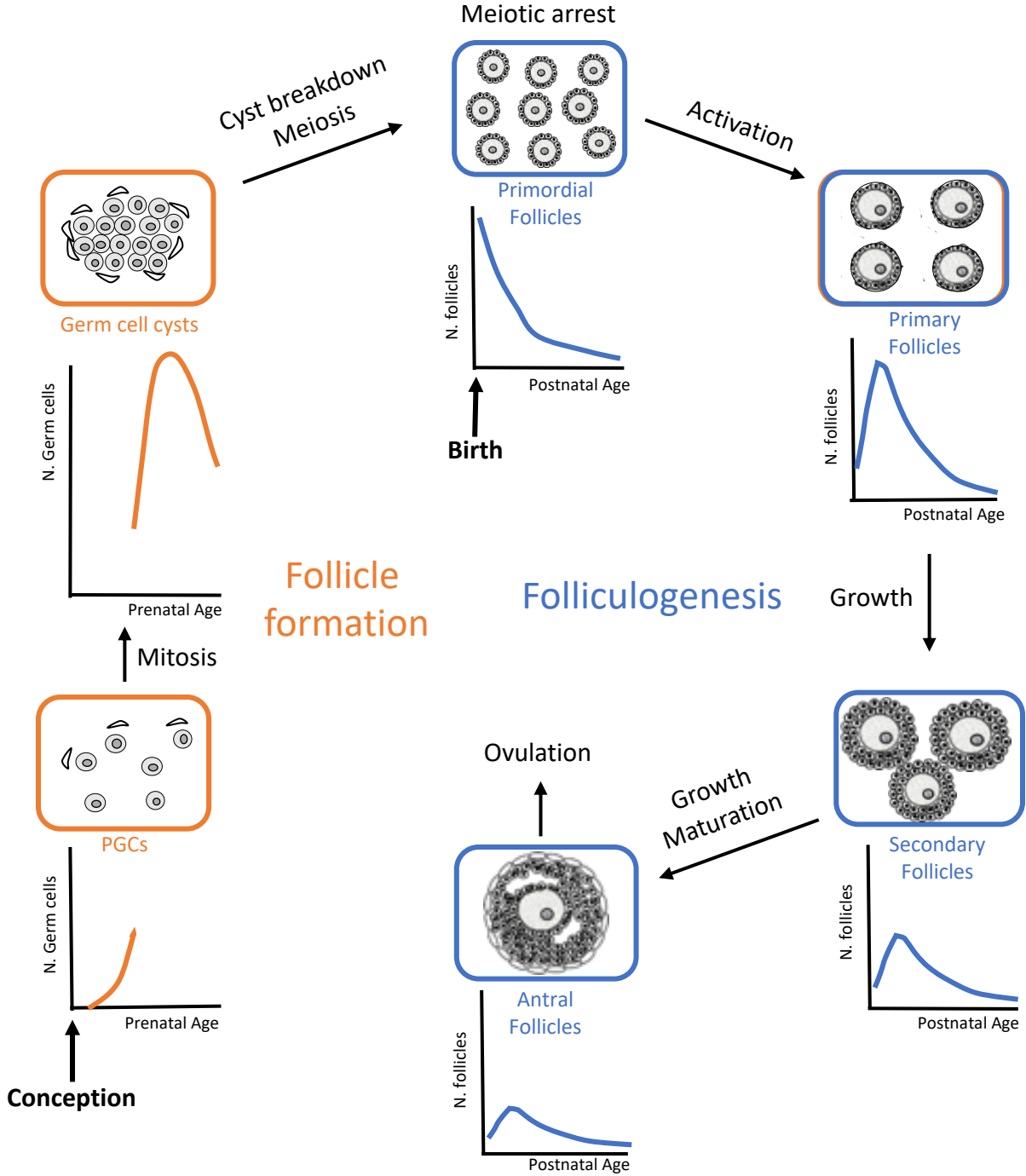
Figure 1: Main stages of follicle formation and follicle development, and changes in the follicle pool along lifespan

The different steps of extrafollicular oogenesis and follicle development are represented by rectangle boxes. The corresponding changes in the number of germ cells in the pre- or peri-natal (depending on the species) life, and in the number of ovarian follicles as a function of age are drawn schematically below each box. Left part: Follicle formation. A couple of primordial germ cells (PGCs) first arise from the embryo epiblast. They then migrate to the gonadal ridges, while undergoing a series of mitosis rounds. Clusters of germ cells get arranged in syncytium structures named germ cell cysts. The cyst fragmentation gives rise to individual ovarian follicles, in which the oocyte is surrounded by a single layer of flattened cells. Many oocytes die at this stage, which also coincides with entry into meiosis (up to the arrest in the diplotene stage of prophase I). Right part: Follicle development. After activation and transformation of flattened cells into cuboidal cells, the primordial ovarian follicles evolve into primary follicles. Follicle growth then results from the enlargement of the oocyte and proliferation of somatic cells, that organize into several concentric layers (secondary follicles). Finally, a fluid-filled cavity, the antrum, appears in the cell mass. The ovulatory follicles are selected from a cyclic cohort of antral follicles.

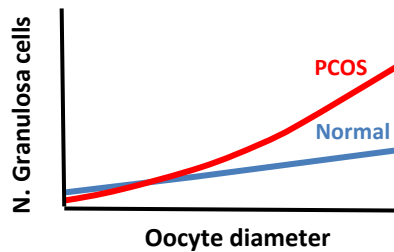
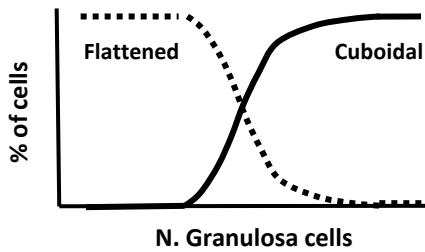
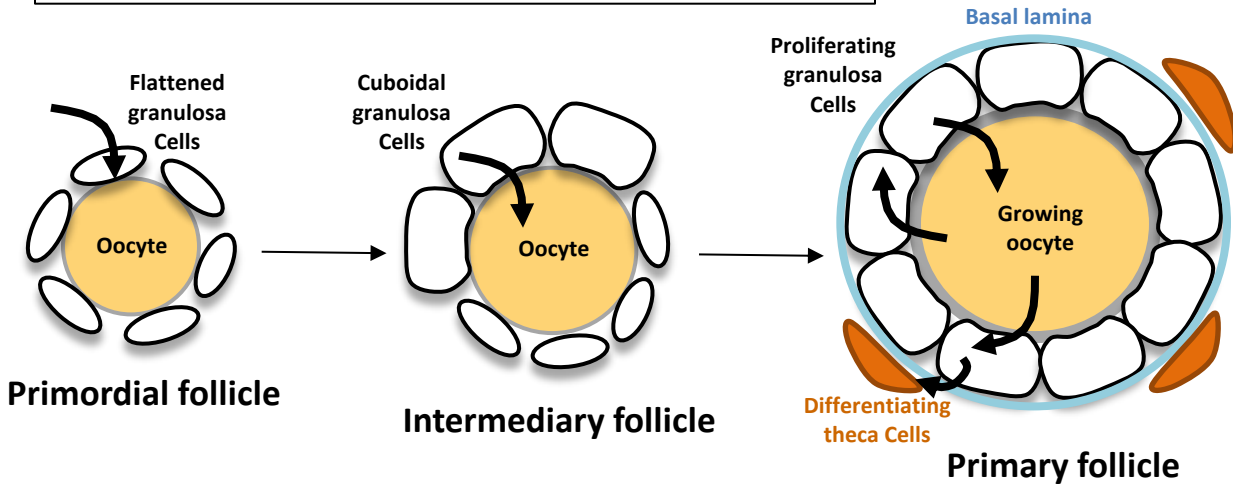
Figure 2: Cell fate and cell dynamics during early follicle development

Panel A : Activation of primordial follicles and initiation of follicle growth. Follicle development is triggered when somatic (granulosa) cells get stimulated by signals reaching the follicle environment in the ovarian cortex. Flattened cells then undergo a shape transition to transform into cuboidal cells. This transition is followed by the proliferation of cuboidal cells and initiation of the slow growth phase of the oocyte. On the cell population level, progression of activation can be monitored by the increase (resp. decrease) in the proportion of cuboidal (resp. flattened) cells in the whole cell population. On the follicle level, the more or less concomitant growth in oocyte diameter, with respect to the increase in the total cell number, preserves the unilaminar structuring of the follicle in these early stages. Subtle unbalance between proliferation and oocyte growth can yet be observed as soon as these very early stages in pathological conditions such as PCOS (PolyCystic Ovary Syndrome) [57].

Panel B : Follicle growth as a multi-layered structure. While the oocyte goes on enlarging, the increase in cell number associated with active cell proliferation exceeds the capacity of a single layer, and cells progressively occupy an increasing number of concentric layers. The balance between oocyte growth and granulosa cell proliferation is tuned by a tight molecular dialog. Somatic cells promote oocyte growth through the KIT signaling pathway, while growth factors of the BMP (Bone Morphogenetic Protein) family diffuse from the oocyte to the granulosa cells and stimulate proliferation. On the follicle level, this dynamic balance can be monitored by plotting the cell number against the oocyte diameter [31]. In sheep there exist natural mutations (FecII and FecBB affecting respectively BMP15 and BMP1B) disturbing this balance and leading to anormally large oocytes. In contrast, the KO of *Inha* (inhibin subunit α) in mouse leads to proliferation in excess with respect to oocyte growth.



A – Follicular activation and early growth



B – Follicular growth

