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Folliculogenesis and Abnormalities

Giuseppe Loverro[1], Maria Bardi[1], Antonio Maurizio Pellegri[2], Matteo Loverro[3], Edoardo Di Naro[1] and Gianluca Raffaello Damiani[1]*

[1]Department of Biomedical and Human Oncological Science (DIMO), University of Bari, Polyclinic of Bari, Italy

[2]San Raffaele Institute, University of Milan, Italy

[3]Department of Woman, Child and Public Health, A. Gemelli University Hospital Foundation IRCCS, Rome, Italy

ABSTRACT

Research on oocytes and their associated follicles has advanced significantly over the past two decades, shedding light on the fragility of the processes governing their active life. The ovarian follicles and the germ cells within them are crucial to understand conception, growth, and survival mechanisms. Folliculogenesis, the process by which ovarian follicles develop, starts with the primordial follicle, which contains oocytes arrested in meiotic division. These follicles are crucial for the ovarian reserve and serve as the sole source of oocytes throughout a woman's life. Follicle maturation is regulated by a series of biochemical signals involving granulosa and theca cells, as well as hormones like FSH and AMH. This process begins during fetal development and continues until menopause, with primordial follicles being activated and transitioning into primary follicles. Each stage of follicular maturation is linked to meiotic events that drive the oocyte's progressive development. Despite the continuous loss of primordial follicles, these cells retain autocrine and paracrine factors that regulate their activation, promoting oocyte growth and supporting both spontaneous and assisted reproduction. The early stages of folliculogenesis involve critical signaling pathways and growth factors such as mTOR, PI3K, and Hippo, which regulate follicle development even before gonadotropin involvement. Initially, follicular growth is influenced by local growth factors like activins and AMH, with gonadotropins playing a larger role as the follicle progresses. Follicles develop through four stages—primary, secondary, tertiary, and Graafian—each regulated by intra-ovarian mechanisms. Oocytes also actively contribute to folliculogenesis by secreting growth factors that interact with granulosa and theca cells. As follicles grow, the oocyte is suspended in follicular fluid and surrounded by granulosa cells forming a structure called the corona radiata. Meiosis begins in fetal life but is arrested until puberty when it resumes just before ovulation. However, not all oocytes will be ovulated; many undergo atresia through apoptosis. Abnormalities in signaling pathways or follicle development can lead to reproductive disorders, highlighting the importance of understanding the molecular mechanisms involved in ovarian function.

***Corresponding author**

Gianluca Raffaello Damiani, Department of Biomedical and Human Oncological Science (DIMO), University of Bari, Polyclinic of Bari, Piazza Giulio Cesare 11, 70124, Italy. Tel: 39-080-5612443.

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Introduction

Discussing scientifically about follicles and their associated oocytes is no longer as abstract as it was two decades ago. Research has indeed taught us how fragile the active life of an oocyte and its envelope, the follicle, can be, and how complex the relationships can be between its activation factors, its biological signaling pathways, and, above all, how little we know about the mechanisms of conception, growth, and survival of follicles, even though these processes are aimed at directing the formation and intra-ovarian growth of oocytes, as tiny cells able to generate a new individual with its existential and social expectations. For these reasons, and to integrate what we already know today with the results that recent research has highlighted during the last years, we found it useful to delve into this article about the new knowledge regarding the nature of the founder cells of the follicular structure, where they come from, how the follicular structures evolve during folliculogenesis, and what limits the use, in adulthood, of the large numerical availability of new primordial follicles, a choice that still today seems to be the only possibly feasible therapeutic strategy to treat the reduction of ovarian reserve, despite the ample availability of founder follicles, as primordial follicles (PF) are considered. The oocyte contained in primordial follicles, called Primordial Germ Cell (PGC), is nothing but a totipotent germinal stem cell that, in order to continue transfering the essential components for the transmission of its perpetually inheritable traits to other germ cells, enters the chain of life, even though it must be degraded from a totipotent cell to pluripotent cells, as long as this can be useful for the perpetuation of the human species.

Folliculogenesis

Folliculogenesis aims to study the possibility and ability of each ovary to effectively transmit the regenerative capacities that these cells are capable of.

Much of the current literature refers to a few sequential steps of folliculogenesis, which we summarize here for the sake of completeness. The follicle is a composite structure destined to

produce mature oocytes. The primordial follicle is made up of germ cells with a single layer of mesodermal cells.

As the follicle develops, the oocyte will acquire a halo of cells and membranes destined to form, nourish, and protect the complex follicular structure, such as:

- Zona pellucida (ZP)
- Granulosa cells
- Inner and outer theca cells

Figure 1 can help understand the correlations between meiotic events and follicular development in the ovary, emphasizing that both events are not distinct and independent, but clearly intertwined and aimed at mutual enhancement.

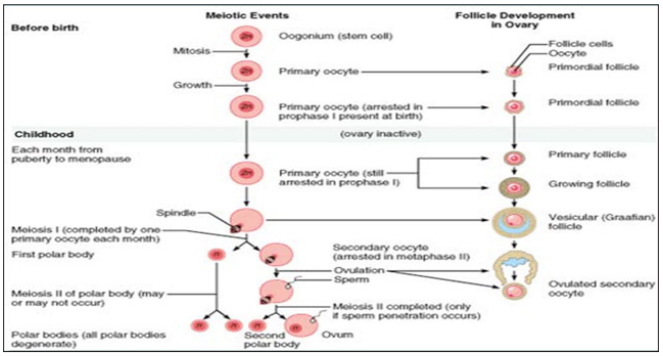

Figure 1: Shows that there can be no evolutionary autonomy between folliculogenesis and gametogenesis, but especially that as the structural and functional complexity of the various types of follicles increases, there is a different maturation of the entire follicle, which cannot evolve independently of the changes undergone by oogenesis

The starting cell, during intrauterine life, is the oogonium, which, as a stem cell, undergoes mitosis, giving rise to millions of primary oocytes. On the follicular development side and respecting the symmetry between folliculogenesis and oogenesis, there are the follicular cells and the oogonia that come together to generate the primordial follicle (PF).

The primary oocyte is the cornerstone of folliculogenesis and is contained in primordial follicles, remaining stationary and arrested in prophase I.

In the following stages, the primordial follicle is transformed into a primary follicle. Therefore, the ovaries, at birth, are inactive from the point of view of folliculogenesis until puberty, remaining in a dictiato stage of the first meiotic division. The oocyte, in the post-natal phase, switches from a primary follicle to a growing follicle. While these modifications occur, primary follicles are transformed into growing follicles.

After puberty and every month from puberty to menopause, primary follicles and then growing follicles house primary oocytes still arrested in prophase I. At the end of the primordial follicle's growth, the germinal vesicle is formed.

On the meiotic events level, meiosis I is completed with the emission of the first polar body. This sequence of events is critical for the integrity of the chromosomal complement and is carried out by primary oocytes every month.

Regarding folliculogenesis, we observe the maturation of the secondary oocyte, which is arrested in metaphase II.

Upon sperm penetration, the secondary follicle expels the second polar body, and meiosis II is completed only if sperm penetrates the oocyte.

Formation and Role of PGCs

In the early stage of fetal development, oocytes, called primordial germ cells (PGCs), group together in small sacs called nests, in order to achieve a uniform conditioning of the germ cells that flow into them. When these biological niches (nests) break down, most oocytes enter meiosis, and primordial follicles begin to form, each containing a PGC [1].

The activation and maturation of PGCs are at the core of oogenesis and are closely associated with the development of follicles, due to factors produced by the oocytes themselves, as well as by the somatic cell layers of the granulosa and theca. Folliculogenesis always begins in the innermost part of the ovarian cortex and allows the formation and growth of primordial follicles, which consist of primary oocytes surrounded by a layer of flattened squamous cells called pre-granulosa cells of somatic origin, with a diameter of about 25 μm.

Role of Primordial Follicles

Primordial follicles are the only and therefore precious available source of oocytes throughout a woman's life, and they are delimited by a basal membrane, outside of which there is a layer of flat granulosa cells.

Thanks to the basal lamina, the granulosa cells and the oocyte live in an isolated microenvironment where direct contact with other cells cannot occur, as they are blocked in a dictiato stage, that can occur even decades before the onset of the first meiotic division.

Therefore, the number of primordial follicles present in the ovary during intrauterine life represents the ovarian reserve that each woman can rely on after birth and throughout her intrauterine life, and likely, even post-natally.

This condition must be kept in mind for both spontaneous and assisted reproduction, as from the sixth to the ninth month of pregnancy, there is a persistent loss of primordial follicles, due to apoptosis and neoformation of primordial follicles.

Role of Gametogenesis in the Formation of Primordial Follicles

In humans, primordial germ cells (PGCs) are undifferentiated stem cells that, to ensure the continuation of the species, will differentiate towards the formation of gametes, namely sperm and oocytes. This important role is called gametogenesis.

During the early stages of development, the cells destined to form female gametes migrate to the dorsal part of the developing intestine, eventually reaching the site where the gonad will originate.

Simultaneously, primordial oocytes secrete platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), which stimulate granulosa cells to secrete Kit-ligand, keratinocyte growth factor, BMP-4, and BMP-7, which are essential for the activation and proliferation of primordial germ cells.

Finally, primordial follicles are activated by bFGF to stimulate granulosa cells and produce KL, which binds with Kit, forming the Kit-KitL dimer, which stimulates Phosphatidylinositol 3 Kinase (PI3K) signaling, leading to the transition to a primary follicle.

Gametogenesis

Gametogenesis is the process that coordinates the development from primordial germ cells to mature gametes: oogenesis in females and spermatogenesis in males. Both in females and males, gametogenesis begins with diploid germ cells, which undergo mitosis, meiosis, and cytodifferentiation into haploid gametes.

Gametogenesis occurs in the gonads (testes or ovaries), and in females, it is referred to as oogenesis.Gametogenesis occurs when the haploid cell (n) is formed from a diploid cell (2n) through meiosis.Germ cells undergo different stages of development. In the first phase, the primordial germ cells (PGCs) actively migrate towards the gonadal ridge.

In the second phase, the germ cells get specific signals from their microenvironment and begin one or two different programs of controlled cell divisions, meiosis, and differentiation towards oogenesis to form the gametes. The molecular basis of both processes and the development of early germ cells are adequately known only in two species, Drosophila and Caenorhabditis elegans, where systematic genetics has identified many genes necessary for this process. PGCs in humans have not been sufficiently studied due to ethical issues. Much of our knowledge on the specification of mammalian PGCs were obtained from studies in early mouse embryos, in which the fate of germ cells is induced in the proximal pluripotent cells of the epiblast shortly after implantation in the uterine wall [2,3].

Primordial Follicles

Folliculogenesis consists of a series of events that occur sequentially during follicular development and are regulated by a significant number of genes, transcription factors, and hormones secreted by the hypothalamic-pituitary-ovarian axis. During the early phase of fetal development, the first oocytes found in the fetal ovary (also called primordial germ cells, PGCs) put together into structures known as nests, in order to ensure uniform conditioning of the germ cells within the context of the nests. When these nests break down, most of the oocytes enter meiosis and begin to form primordial follicles, so that each follicle contains a single PGC.

The maturation of PGCs is fundamental to oogenesis, which is closely associated with follicular development, thanks to factors produced by the oocytes themselves, which significantly impact the development of the granulosa and theca cell layers and vice versa. Folliculogenesis always begins in the innermost part of the ovarian cortex, and primordial follicles are made up of primary oocytes surrounded by a flattened layer of squamous cells called pre-granulosa cells. Primordial follicles are the only source of oocytes available during a woman's reproductive years and are not interchangeable with any type of ovarian stem cells (OSCs).

These primordial follicles have a diameter of about 25 μm and are surrounded by a layer of flattened granulosa cells and a basal membrane. The oocyte inside these follicles is arrested in the dictiato stage of the first meiotic division. The number of primordial follicles in the ovary during intrauterine life reflects the ovarian reserve available for both spontaneous and assisted reproduction, keeping in mind that from the 6th to the 9th month of pregnancy there is a significant loss of primordial follicles due to apoptotic events.

Nevertheless, primordial follicles have intrinsic autocrine and paracrine factors that enable these follicles to cope with the depletion of ovarian reserve, which at this stage is due to apoptosis. These factors, in fact, induce a negative regulation of primordial follicle activation through the local action of PTEN, Foxo3a, and SDF-1 (stromal cell-derived factor-1), which act by limiting the previously described follicular activation.

At the same time, primordial oocytes secrete platelet-derived growth factor (PDGF) and bFGF, which stimulate granulosa cells to secrete Kit-ligand, keratinocyte growth factor, BMP-4, and BMP-7, which positively influence the activation of primordial follicles, while granulosa cells produce GDF-9 and BMP-15, which promote the proliferation of granulosa cells.

Primary Follicles

Primordial germ cells proliferate and migrate from the yolk sac to the gonadal ridge where they settle in the developing ovary. The primordial germ cell lineage early settles down in the proximal region of the epiblast, in close proximity to the extra-embryonic endoderm. Primordial germ cells (PGCs) are the first population of germ cells to be identified and stabilized during development, and they represent the common origin of both oocytes and sperm.

A determining role is played by PI3K (Phosphoinositide 3-kinases, also known as Phosphatidylinositol 3-kinases), a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, and survival, just to give an idea of the biological power that PGCs use to ensure the good survival of the progeny. PI3K is responsible for the transition from primordial follicle to primary follicle. However, primary follicles are prevented from further development by the action of AMH/MIS and SDF-1, although the factors that promote the transition to primary follicle are BMP, PDGF, bFGF, c-Kit/lig, LIF, and GDF9.

Ovarian follicles begin their maturation with the development of primordial follicles and get through several stages before reaching reproductive maturity. Nevertheless, not all follicles go through the existing stages, even though they are crucial for reaching full maturity, suggesting an integrative movement.

A primary follicle is defined by the presence of one or more layers of cuboidal granulosa cells, which are organized into a single layer around the oocyte. The main event that occurs in the primary follicle is, however, the appearance of FSH receptors, which stimulate the growth and differentiation of the oocyte.

It can be considered that in primary follicles, the primary oocyte develops, arrested in meiotic prophase, surrounded by a single layer of flat cells called pre-granulosa cells, known as the lamina or basal membrane. Primary follicles are first found in the ovarian stromal cortex and are only visible after the first menstruation, although they can remain in this (dictyate) state for periods that can last up to 40 years.

For these reasons, primary follicles are defined as "quiescent," even though they are metabolically active. Some undergo a very slow transformation into an intermediate stage before becoming primary follicles and are structurally characterized by the presence of a set of squamous and cuboidal granulosa cells, without any change in the size of the oocyte or the follicle. Actually, primary follicles represent a later stage of functional maturation, although few of these primordial follicles progress to primary follicles.
The process begins, as previously mentioned, at puberty and continues every day, regardless of other modulating factors and independently of the presence or absence of pregnancy or ovulation, stopping only at menopause.

As primordial follicles develop into primary follicles, changes occur also in the oocytes, which increase in diameter and further develop the basal membrane.

When the primary follicle is stimulated, it expands and the number of granulosa cell layers increases, along with the development of a glycoprotein layer called the zona pellucida between the oocyte and the granulosa cells. Primary follicles consist of a primary oocyte arrested in meiotic prophase and are surrounded by a single layer of pre-granulosa cells, without a basal membrane or zona pellucida. These follicles can remain in this stage for long periods, even up to 40 years, and are located in the cortical stroma. As primordial follicles develop into primary follicles, changes also occur in the oocytes.

In fact, only a few primordial follicles progress to primary follicles each day. This process begins after birth and increases at puberty, regardless of other modulating factors, independently of pregnancy or ovulation, until menopause. The oocyte increases in diameter and then develops the zona pellucida. Reactivation of the primordial oocyte genome (which should have occurred during intrauterine life) induces the oocyte to secrete growth factors that play a crucial role in follicular growth. Primary follicles consist of two layers of mitotically active granulosa cells, which change from flat to cuboidal forms and reach a size of about 0.1 mm. When the primordial follicle is stimulated, it expands and the number of granulosa cells increases, transforming into a primary follicle. Finally, this type of follicle develops a glycoprotein layer called the zona pellucida between the oocyte and the granulosa cells.

Secondary Follicles

As the follicle grows, the granulosa cells (of somatic and not germinal origin) split by mitosis, transforming the follicle into secondary follicles. They have two to six layers of cuboidal granulosa cells. They also acquire additional somatic layers, called the theca cells. The formation of the theca layer depends on differentiation factors like GDF-9 (Growth Differentiation Factor-9), produced by the oocyte. The theca layer forms around the basal membrane in secondary follicles and eventually differentiates into the inner theca and outer theca. The growth of follicles from primordial to secondary is gonadotropin-independent.

Secondary follicles represent the most advanced stage in the intermediate stage of folliculogenesis, where the outermost layer of the follicle becomes prominent and contributes to the production of estrogens.

Secondary follicles are very similar to primary follicles, except they are larger, contain more follicular cells, and allow fluids accumulation in the intercellular spaces, which play a role in feeding the oocyte. These fluids gradually accumulate near the oocytes, forming the antrum, while the theca cells contribute to estrogen production. Preantral follicle sizes grow from 0.1 to 0.5 mm. Their increasing size expands until the follicle forms an antral follicle filled with fluid, also known as a tertiary or Graafian follicle. These early stages of folliculogenesis occur in the absence of gonadotropin support and are likely controlled by intraovarian growth factors such as insulin-like growth factors (IGF), members of the transforming growth factor beta (TGF-β) superfamily, and vascular endothelial growth factor (VEGF).

The granulosa cells surrounding the oocyte are called the cumulus oophorus, meaning “cluster of eggs” in Greek, within which two layers differentiate: the inner theca (composed of round cells that secrete androgens and follicular fluid) and a more fibrous layer known as the outer theca, where the cells appear fusiform. The theca layer forms around the basal membrane in secondary follicles and eventually differentiates into the inner and outer theca layers. The androgens produced by the theca are converted into estrogens by the granulosa cells. The growth of follicles from primordial to secondary remains gonadotropin-independent.

Thus, secondary follicles are very similar to primary follicles, except they are larger, contain more follicular cells, and have various fluid accumulations in the intercellular spaces, which are needed to nourish the oocyte.

Tertiary Follicle

Tertiary follicles are associated with continuous proliferation of granulosa and theca cells, as well as with further vascularization of the theca and noticeable enlargement of the oocyte. Thanks to the transition from secondary to tertiary follicle, the ovary functions as a perfect endocrine organ.

The tertiary follicle (also known as Graaf’s follicle) is larger than the secondary follicle. It is also referred to as an antral follicle, as it contains a cavity known as the “antrum”, which is filled with fluid. When follicles reach this stage of development, they are much larger and can be observed via ultrasound, continuing to grow in size. As they progress from secondary follicles to antral follicles, granulosa cells secrete fluid that accumulates between the cells. A large amount of additional fluid spreads from the thecal blood vessels, and this fluid forms the follicular fluid, which contains steroids, protein hormones, anticoagulants, and enzymes.

The formation of the antrum in the tertiary follicle corresponds to the final phase of folliculogenesis and is based on the transition from intra-ovarian regulation to extra-ovarian regulation, coordinated by the hypothalamic-pituitary-ovarian axis.

From a functional standpoint, the mechanistic role of growth factors and signaling pathways in the early stages of folliculogenesis involves several important molecules and signaling pathways, such as the mechanistic target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K), and components of the mammalian Hippo signaling pathway [4].

The early stages of folliculogenesis are not dependent on gonadotropins. After initial activation, synthesis of growth factors, activins, and anti-Müllerian hormone (AMH) begins in the follicles and can act on them both locally and through the hypothalamic-pituitary system. These components are secreted by the granulosa cells. As the antral cavity forms, follicular growth becomes increasingly dependent on gonadotropins.

After initial activation, growth factors, activins, and anti-Müllerian hormone (AMH) start to appear in the follicles and can already act on them either locally or through the hypothalamic-pituitary system. As the antral cavity forms and is completed, follicular growth becomes increasingly dependent on gonadotropins. There are two types of follicles: non-growing follicles, which make up 90-95% of the follicles, and growing follicles, which are divided into 4 stages (primary, secondary, tertiary, and Graafian follicles). The first three stages are regulated by intra-ovarian mechanisms, and for this reason, they need to be investigated further, as they could provide valuable tools for the treatment of many folliculogenesis anomalies. When follicles reach this stage of development, they become larger and can be observed via ultrasound.

As they progress from secondary to antral follicles, granulosa cells secrete fluid that accumulates between the cells, spreading from the thecal blood vessels. This fluid contains steroids, protein hormones, anticoagulants, enzymes, and electrolytes, and is similar in composition to blood serum.

Follicles filled with follicular fluid are tertiary follicles, characterized by the presence of a mural granulosa cell layer, with an additional thecal layer differentiating into an intermediate position between the inner and outer layers of the theca.

An emerging idea is that the oocyte plays an active role in regulating folliculogenesis, as there is significant exchange between oocytes secreting growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), granulosa cells, and theca cells. Oocytes in tertiary follicles are suspended in follicular fluid by a bridge of granulosa cells, but are connected to the cumulus oophorus, which holds them attached to the layers of the thecal cells that will form the future placenta. To maintain the efficiency of the suspended oocyte, there is also a thin mantle of granulosa cells, known as the corona radiata. At this stage, the follicle is called the "Graafian follicle" and appears as a transparent vesicle protruding from the surface of the ovary.

Although the function of the ovary is to produce oocytes, many oocytes are never ovulated. It is worth noting that the number of oocytes reaches its peak after the ovaries are formed. After this phase, the number of oocytes decreases irreversibly.

At birth, a woman already has all the oocytes she will have throughout her reproductive life, in the absence of evidence of neo-oogenesis. However, most of the oocytes, enclosed in follicles, are eliminated before ovulation through a process called atresia, which is induced by the activation of apoptosis, a phenomenon that occurs both in oocytes and granulosa cells. Atresia is present, though with varying intensity, at all stages of follicular development.

Primordial Germ Cells

Primordial germ cells are established in extraembryonic tissues at around 3 weeks after fertilization in response to transforming growth factor-β (TGF-β) family signals secreted by surrounding tissues.

Transcription factors, pluripotency factors, ligand/receptor signaling pairs, and microRNAs participate in regulating the development and migration of primordial germ cells. Primordial germ cells proliferate as they migrate from the yolk sac to the gonadal ridge where they enter the developing ovary.

The primordial germ cell lineage is established early in development, originating in the proximal region of the epiblast, close to the extraembryonic endoderm. A small number of cells emerge under the influence of inductive signals from the extraembryonic ectoderm delivered by members of the TGF-β superfamily, including bone morphogenetic proteins (BMP)-2, BMP-4, and BMP-8B.

The WNT signaling pathway ligand, WNT3, must be expressed in the proximal epiblast cells to promote their competence to respond to BMP signals. WNT3 signaling induces β-catenin-mediated transcription of the mesodermal transcription factor T (also known as brachyury), which then activates expression of downstream genes, including Prdm1 (previously known as Blimp1), Prdm14, and Tfap2c, which are required for primordial germ cell specification.

Signaling Pathways and Growth Factors Involved in Folliculogenesis

About follicular functionality, the mechanistic role of growth factors and signaling pathways in the early stages of folliculogenesis involves several important molecules, such as the mechanistic target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K), and molecules of the Hippo signaling pathway in mammals, a highly conserved evolutionary pathway present in all animals, from lower to higher species [5].

The early stages of folliculogenesis are not dependent on gonadotropins, and the early activation is the result of the action of growth factors, in particular paracrine and endocrine growth factors such as activins and AMH within the follicle. These factors start acting on the follicles in their intermediate growth stage, either locally or via the hypothalamic-pituitary system. As the antral cavity forms, follicular growth becomes increasingly dependent on gonadotropins [6].

Preantral (or secondary) follicles increase in size and, reaching a diameter of 0.1 - 0.5 mm, form an antral follicle filled with fluid, thus becoming known as a tertiary or Graafian follicle, which is sensitive to gonadotropins.

These early stages of folliculogenesis, however, occur even in the absence of gonadotropin support and are most likely regulated by intra-ovarian growth factors such as insulin-like growth factors (IGF), members of the transforming growth factor-beta (TGF-β) superfamily, and vascular endothelial growth factor (VEGF) [7].

Time of Conclusion of Meiosis I in The Ovary

Secondary follicles, and even more tertiary ones, evolve into the Graafian follicle. During this process, meiosis is completed, and the oocytes acquire the features of secondary oocytes, which then begin the second meiotic division.

During meiosis, cytokinesis is asymmetric, so, along with the oocyte. A small polar body is generated, consisting of very little cytoplasm, which is instead abundantly retained in the single normal oocyte of that same Meiosis I.

The follicular fluid fills all available spaces in the antrum, inducing the formation and enlargement of the antrum.

The granulosa cells surrounding the oocyte and extending into the antrum are called cumulus oophorus and are delimited externally by a basal membrane formed between the granulosa cells and the internal theca, while the fibrous theca remains more external and emerges from the surrounding stroma.

Timing and Sites of Oocyte Meiosis I and Meiosis II in Humans

Meiosis I begins and is interrupted during prophase in fetal life. It is completed just before ovulation, from puberty until menopause. Immediately after the completion of Meiosis I, Meiosis II begins but is arrested at metaphase II.

After birth, the oocyte, zona pellucida, and the surrounding follicular cells (also known as the corona radiata) are expelled during ovulation and are aspirated into the fallopian tube. Following the LH peak and once the oocyte is released, the second meiotic division is reactivated, progressing only to metaphase II. The formation of embryonic cells occurs, of course, only when the oocyte is fertilized. The development of follicles includes a series of sequential stages, which include primordial, primary, secondary, preantral, antral, and pre-ovulatory follicles in their

various stages of development [8].

Oocyte maturation differs from spermatozoa maturation in several aspects. First, female germ cells begin meiosis I (MI) during embryonic development, which arrests at the diplotene stage of prophase I (MI). Second, as the woman matures sexually, some previously dormant oocytes resume MI following a rapid increase in LH secreted by the pituitary gland during estrus in animals or the menstrual cycle in women. Under normal circumstances, 10–20 oocytes in meiotic arrest resume meiosis in each menstrual cycle, while only one oocyte fully matures and is ovulated from the ovary. (Transduction mechanisms refer to all those intracellular enzymatic systems that allow the conversion of the extracellular signal produced by the drug or endogenous substance into an intracellular signal through the activation of an effector.) Once the effector is activated and the signal is converted, the cell will give its biological response.

The signal transduction mechanism normally exists in all cells and is necessary for all endogenous mediators, including drugs, because, as mentioned, medicines mimic or antagonize endogenous substances, generating or not generating a cellular response. FSH upregulates cAMP levels in GCs and stimulates cumulus expansion. LH inhibits the NPPC-NPR2 signaling pathway by activating the EGFR-Ca2+ signaling pathway to downregulate cGMP levels in GCs, thereby reducing cGMP levels in oocytes and activating PDE3A-mediated hydrolysis of cAMP. Low cAMP levels activate MPF, which consists of CDK1 and cyclin B1, thereby promoting oocyte MI resumption. MPF not only phosphorylates and inactivates protein phosphatase 1 (PP1) but also phosphorylates APC/C to help maintain phosphorylation of other CDK1 substrates. Phosphorylation of lamin A/C leads to nuclear membrane rupture, which in turn promotes oocyte MI resumption.

Role of the Germinal Vesicle

Each oocyte in the antral follicle contains a nucleus enclosed by the nuclear membrane, called the germinal vesicle (GV). In case of an LH surge during the menstrual cycle, oocytes of antral follicles undergo a series of processes related to nuclear maturation, which involves chromatin condensation and the breakdown of the germinal vesicle (GVBD). After germinal vesicle breakdown (GVBD), oocytes progress to metaphase I (MI). Subsequently, the first polar body (PB1) is eliminated while the oocytes complete MI. Then, the oocytes begin meiosis II (MII) and arrest at metaphase, awaiting fertilization. Meiosis is completed once the oocyte is fertilized. The precise regulation of oocyte meiosis is an important guarantee for the nuclear maturation of the oocyte, marked by the extrusion of PB1. The distribution and quantity of organelles, such as mitochondria, ribosomes, cortical granules, and the endoplasmic reticulum, differ during the transition from GV to metaphase MII, with their distribution and function influencing cytoplasmic maturation.

The maturation of the oocyte from GV to MII involves a series of complex cytoplasmic events that are prerequisites for oocyte development and maturation. In mammals, including humans, several signaling pathways are considered critical for meiotic maturation. These pathways play a crucial role in the regulation of oocyte maturation, and their interplay is essential for the production of high-quality oocytes. For this reason, any abnormalities in the various stages of this process can result in ovulatory dysfunction, including conditions such as polycystic ovary syndrome (PCOS), which lead to infertility. (Figure 2)

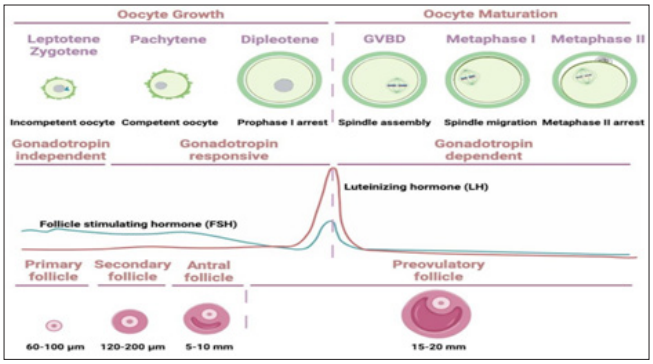

Figure 2: Diagram of Key Stages of Oocyte Maturation and Folliculogenesis. Upper Panel: Oocyte growth, entry into meiosis, and meiotic arrest. Meiotic maturation begins with GVBD (germinal vesicle breakdown), followed by the first meiotic division, homologous chromosome separation, extrusion of the first polar body, and arrest at metaphase II. Oocytes ovulate at the MII stage. The sister chromatids will separate after fertilization. **Middle Panel:** Hormonal levels during corresponding stages of oocyte maturation and follicular development. Oocyte and follicle growth can be classified into distinct stages. From primordial follicle to secondary follicle, gonadotropins may not be necessary. At this stage, follicular development can be defined as gonadotropin-independent. The transition from preantral to early antral stage is primarily controlled by intraovarian regulators, with gonadotropins potentially not being required, termed gonadotropin-responsive. Subsequent growth from the antral to preovulatory stage may be gonadotropin-dependent. **Lower Panel:** Follicular development stages and follicle sizes are indicated, with a possible re-entry of the oocyte into meiosis and its release from the follicles.

Disorders of Folliculogenesis

The intrauterine development of primordial follicles begins in rodents on day 5 of pregnancy (E5) and ends two days after birth (P2). This process is driven by a series of signaling pathways, which, in turn, guide the activation of complex and sub-microscopic processes. As a result, the disruption of these signaling pathways can lead to negative reproductive outcomes, such as smaller primordial follicles, incomplete follicle development, and failure of sexual differentiation, although these same consequences are difficult to identify before adulthood. Therefore, it is essential to implement more suitable methods to identify, if possible at an early stage, the outcomes of molecular abnormalities related to abnormal signaling mechanisms, such as pathological hormonal homeostasis and reproductive failure [9-12].

The development of a normal ovary during fetal life is crucial for the production and ovulation of a high-quality oocyte in adult life. At the beginning of embryogenesis, primordial germ cells (PGCs) migrate and colonize the genital ridges. Once the PGCs reach the bipotential gonad, the absence of the sex-determining region on the Y chromosome (SRY) and the presence of female-specific genes ensure that the indifferent gonad takes the female pathway and forms an ovary.

PGCs enter into meiosis, transform into oogonia and ultimately give rise to oocytes that are later surrounded by granulosa cells to form primordial follicles. Various genes and signals are implicated in germ and somatic cell development, leading to successful follicle formation and normal ovarian development. This review focuses on the differentiation events, cellular processes and molecular mechanisms essential for foetal ovarian development in the mice and humans.

Normal ovarian development during embryogenesis is the key to fertility and reproductive success later in life. In mammals, reproductive capacity is limited by the size of the non-renewable pool of oocytes, which is established during foetal life. Follicles, the basic unit of the ovary, house the oocytes and are essential for their development and survival. In the early stages of development, follicles consist of an oocyte surrounded by somatic granulosa cells and the extracellular matrix (ECM). As the follicle grows and differentiates, theca cells are recruited; these cells are the source of the oestrogen substrate, androstenedione. Each month throughout the course of a female's reproductive life, primordial follicles are activated to begin follicle development. This process culminates in a follicle that contains a mature fertilisable oocyte ready for release at ovulation. Disturbances in the initial steps or processes that facilitate foetal ovarian differentiation result in incomplete sexual development and may contribute to childhood and adult diseases such as gonadal dysgenesis, infertility or ovarian cancer.

Beginnings of the Ovary

One of the earliest events in embryonic development is X chromosome inactivation, a process that occurs at the two-cell stage of the zygote and enables males and females to have equal transcript levels by genetic inactivation of one of the two X chromosomes in females. In mice, between the four- and eight-cell stage, inactivation of the paternally inherited X chromosome occurs in all female somatic cells. In the developing germ line, X inactivation is reactivated in primordial germ cells (PGCs) such that both X chromosomes are active in oogenesis. Epigenetic regulation of gene expression is an essential part of organogenesis and leads to heritable changes in gene function without inducing a change in the DNA sequence.

DNA methylation or histone acetylation can affect such changes. The study of epigenetic events in the oocyte is an expanding field of research and involves studying the biology behind developmental processes such as genomic imprinting, X inactivation and transcriptional repression.

In the mouse embryo, oocytes develop from PGCs, which migrate into the extraembryonic mesoderm from about 6 days post coitus. By 7.5 dpc, the germ cell pool is located in the allantois (yolk sac). Various genes and factors are involved in germ cell specification such as the pluripotency marker POU class 5 homeobox 1 Between 9 and 10.5 dpc in the mouse, the PGCs migrate from the yolk sac to colonise the gonadal (genital) ridge. The genital ridge is identical in both males and females and remains 'bipotential' until 11.5 dpc in the mouse. In humans, PGCs proliferate and migrate into the mesoderm colonising the genital ridges until the 6th week of pregnancy. The gonads are indifferent but bipotential.

Processes Involved in Mouse and Human Ovarian Development
Organisation of follicular structures in the ovary

In humans, two processes are fulfilled during foetal ovarian development and before birth:

1. The ovarian endowment of primordial follicles and the recruitment of resting primordial follicles into the growing follicle population (Figure 3).
2. contrast, in rodents, most follicles are formed and recruited to grow into primary/more advanced follicles after birth, although emerging evidence suggests that follicles begin to form at 17.5 dpc. In both human and rodent species, follicle growth continues until the depletion of the primordial follicle population. (figure 4).

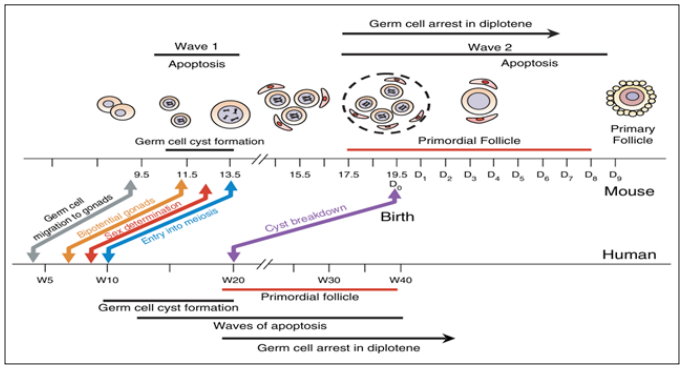

Figure 3: Chronology of mouse and human for the main processes occurring during fetal development. This diagram is a schematic representation of the various processes that occur during oogenesis in mice and humans, starting from the migration of PGCs to the gonadal ridges until the onset of folliculogenesis. The mouse timeline is represented in dpc (days post coitum), while the human timeline is represented in W (weeks post coitum). Do is the day of birth in mice

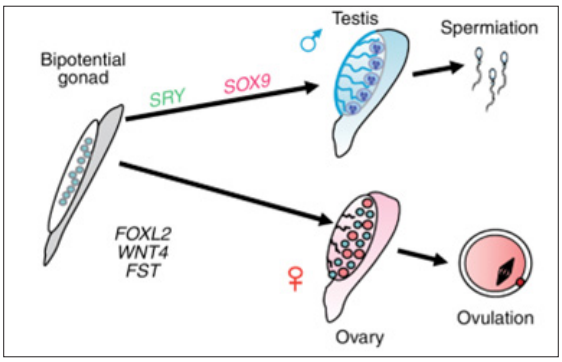

Figure 4: Differentiation of the ovary and testis from the bipotential gonad. The ovary and testis arise from the bipotential gonad. In the presence of the sex-determining region of the Y chromosome gene (SRY) and various male-specific genes such as SOX9, the gonad differentiates into a testis. In the absence of Sry and in the presence of female-specific genes such as FOXL2, WNT4, and FST, the bipotential gonad differentiates into an ovary

Breakdown of Oocyte Cysts, Oocyte Loss, and Primordial Follicle Formation

Oocytes that enter meiosis are grouped into cysts and lack a close association with pre-granulosa cells. Not all oocytes within these cysts survive to form follicles; some die, leaving only one or two oocytes. The regulation of oocyte number is an important process that occurs through the disintegration of cysts. Around the time of birth in mice and after 20 weeks of gestation in humans, cysts disintegrate so that primordial follicles can form. Primordial follicles are composed of oocytes surrounded by a single layer of squamous granulosa cells.

Only one-third of the oocyte pool forms primordial follicles, while the remaining oocytes undergo perinatal apoptosis in mice or prenatal apoptosis in humans. The fate of the oocytes incorporated into primordial follicles is controlled by the oocyte itself; this contrasts with follicle growth, which is controlled by the apoptosis of somatic cells. The loss of germ cells during oogenesis occurs through programmed cell death (PCD).

Three Types of PCD have been Described in the Ovary:

- **Type I:** Nuclear apoptosis characterized by condensation, fragmentation, cleavage of chromosomal DNA, shrinkage,

and packaging of the dying cell into apoptotic bodies without rupture of the plasma membrane;

- **Type II:** Autophagy or lysosomal degradation involving vacuoles;
- **Type III:** Non-lysosomal degradation involving necrosis, characterized by rupture of the plasma membrane, alteration of membrane permeability, destruction of cytoplasmic structures, and nuclear degeneration.

In humans, germ cell depletion occurs in the fetal ovary during mid-gestation, with the most intense apoptosis between 14 and 28 weeks and regression towards birth. At birth, approximately two million oocytes are present, 400,000 are lost by puberty, and 400 oocytes reach the ovulatory stage. Non-ovulatory follicles undergo apoptosis and become atretic. Although the process of apoptosis is crucial for regulating oocyte number, its genetic control requires further study.

Two apoptotic pathways have been identified: the extrinsic and intrinsic pathways, or mitochondria-associated pathways. Some of the extrinsic growth pathways are soluble or membrane-bound ligands such as FAS ligand, tumor necrosis factor-alpha (TNFα), and TNF-related apoptosis-inducing ligand (TRAIL/TNFSF10). Intrinsic pathways are mediated by the B cell lymphoma-2 (Bcl-2) family of cytoplasmic and mitochondrial proteins, which consist of anti-apoptotic genes (Bcl2l1 (Bcl-XL), Bcl2, and Mcl1) and pro-apoptotic genes (Bid, Bax, Bok, and Bad). The extent of apoptosis depends on the balance between these competing family members. Analysis of transgenic mouse models has identified various genes that regulate oocyte survival, apoptosis, or cyst breakdown.

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