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ISBN 978-94-007-1133-4 e-ISBN 978-94-007-1134-1
DOI 10.1007/978-94-007-1134-1
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2011929630

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Part I
Introduction

Chapter 1
Brief Description of the Histological, Cytological and Functional Aspects of the Ovary

María Luisa Escobar, Gerardo H. Vázquez-Nin, and Olga M. Echeverría

Abstract The ovary is formed by three main compartments: superficial epithelium, cortex and medulla. The superficial epithelium is constituted by one layer of cubic cells. The cortex is a wide peripheral zone containing the follicles, the functional and structural unit of the ovary, and a stroma formed by compact connective tissue. Every follicle is formed by one oocyte surrounded by follicular cells, also called granulosa cells, and a basal lamina surrounding them. The medulla is the central region of the ovary formed by connective tissue with numerous blood vessels. As the follicles develop they change their size, morphology and physiology. Primordial follicles are formed by the oocyte surrounded by flat follicular cells. Primary follicles are characterized by the initiation of follicular growth. Secondary follicles are characterized by two or more layers of granulosa cells and no antrum. The early antral follicles are characterized by the formation and progressive growth of a cavity, due to the accumulation of a fluid. Once the antrum is formed the follicle goes through several stages: (a) basal growth, (b) selection and (c) dominance. The process of follicular growth is controlled by extra-ovarian and intra-ovarian factors and the importance of each of these factors depends on the stage of follicle development. Extra-ovarian factors regulate growth of antral and preovulatory follicles, while intra-ovarian factors regulate growth of preantral and early antral follicles. The ovary is not only involved in sexual reproduction, but also has great influence on the entire hormonal functioning during development of the organism. The ovary is the site of the highest synthesis and secretion of progesterone and estrogen in mammals and gives rise to cyclical fluctuations in the levels of these hormones in the blood. Before ovulation, granulosa cells mature to form the corpus luteum, which is responsible for the secretion of progesterone and estrogen.

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G.H. Vázquez-Nin et al., *Cell Death in Mammalian Ovary*,
DOI 10.1007/978-94-007-1134-1_1,

List of Abbreviations

LH	Luteinizing hormone
FSH	Follicle stimulant hormone
TGFβ	Transforming growth factor beta
EGF	Epidermal growth factor

1.1 Morphological Characteristics of the Ovary

The primary reproductive organs of the female are the ovaries. Their main functions are the production of fertilizable oocytes and the secretion of steroid hormones (estrogen and progesterone), which are required for the correct function of the reproductive organs such as the Fallopian tubes, uterus and vagina.

The ovary is formed by three main compartments: superficial epithelium, cortex and medulla (Fig. 1.1). The superficial epithelium is constituted by one layer of cubic cells, which are continuous with peritoneal epithelium at the periphery of the ovary. The cortex is a wide peripheral zone containing the follicles, the functional and structural unit of the ovary, and a stroma formed by compact connective tissue.

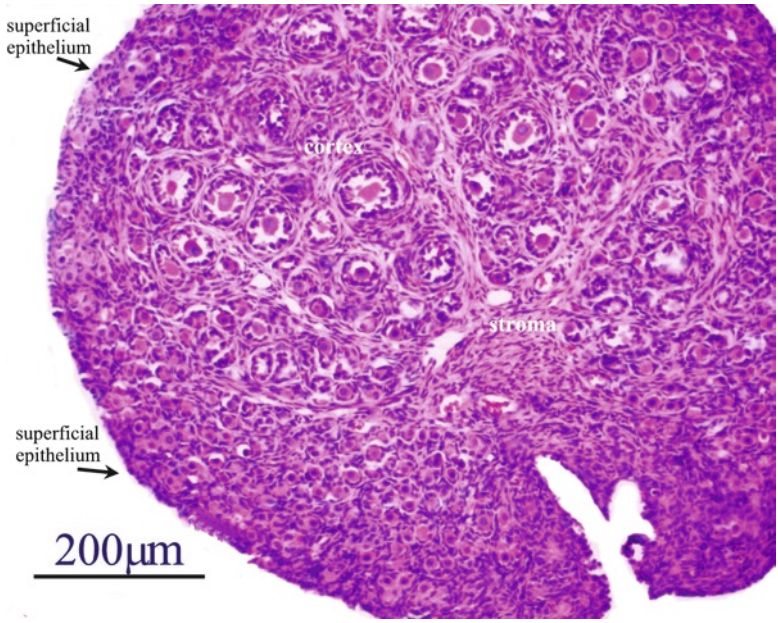

Fig. 1.1 Rat ovary 5 days old. The ovary is constituted by three regions: superficial epithelium, cortex and stroma. In the cortex zone diverse follicles are observed. Haematoxylin-eosin stain technique

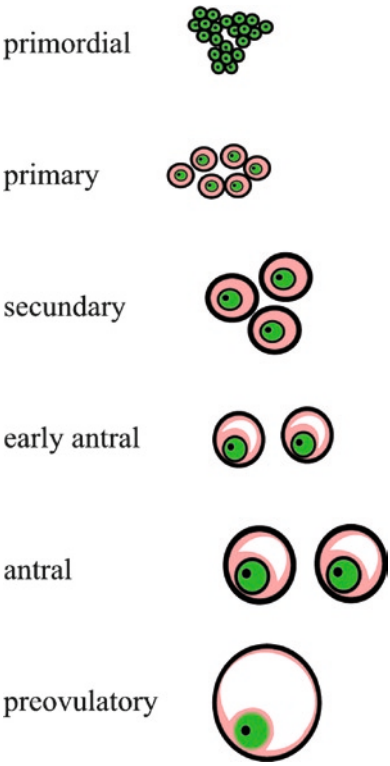

Fig. 1.2 Follicular classification according to McGee and Hsueh (2000). The classification of the authors includes six different development follicular phases

Every follicle is formed by one oocyte surrounded by follicular cells, also called granulosa cells, and a basal lamina surrounding them. The medulla is the central region of the ovary formed by connective tissue with numerous blood vessels. In some individuals there is a net of epithelial cords or tubules near the hilum, the *rete ovarii*.

As the follicles develop they change their size, morphology and physiology. This development has been traditionally classified in several stages. However, there are differences in the name and the characterization of the stages. McGee and Hsueh (2000) described the following phases of development of the follicles as: primordial, primary, secondary, early antral, antral and pre-ovulatory stages (Fig. 1.2). These authors also characterized the hormonal factors involved in the survival and development of rodent follicles.

Primordial follicles are formed by the oocyte surrounded by flat follicular cells (Fig. 1.3). The oocytes in these follicles are called quiescent because they remain unchanged for months in rodents or even years in ruminants and primates.

There is a system of communication between the oocyte and the granulosa cells which is important in the coordination of the initial follicular development. Several studies carried out on various mammals demonstrate that primordial follicles may express leukemia inhibitory factor (LIF), which promotes follicular growth and

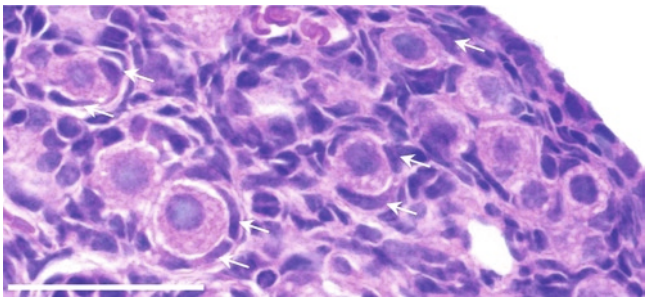

Fig. 1.3 Primordial follicles. The oocytes are surrounded by a few flattened cells (*arrows*). Scale bar 50 μm. Haematoxylin-eosin stain technique

stimulates oocyte growth, proliferation of thecal cells and transition from primordial follicle to primary stage. LIF is mainly produced by granulosa and somatic cells (Nilsson et al. 2002). In this system of communication the Kit ligand (KitL) is also involved as an essential factor for the proliferation of the granulosa cells (Huang et al. 1993). KitL is segregated to intercellular space before reaching its receptor Kit. KitL stimulates oocyte growth via Kit (Montro and Bernstein 1993; Tisdall et al. 1999; Klinger and De Felici 2002). LIF interacts with KitL in the activation of primordial follicles (Nilsson et al. 2002). Mouse oocytes express the tyrosine kinase Kit receptor in all stages of follicular growth, including primordial follicles (Manova et al. 1990). Natural mutations in KitL or in Kit induce alterations of follicular development causing infertility (Manova et al. 1990; Driancourt et al. 2000). The gene *nobox* (newborn ovary homeobox) is also expressed in primordial follicles and helps in the transition to primary stage (Rajkovic et al. 2004). The pool of primordial follicles is now known to be maintained in a dormant state by various forms of inhibitory machinery, which are provided by several inhibitory signals and molecules. Several recently reported mutant mouse models have shown that a synergistic and coordinated suppression of follicular activation provided by multiple inhibitory molecules is necessary to preserve the dormant follicular pool. Loss of function of any of the inhibitory molecules for follicular activation, including PTEN (phosphatase and tensin homolog deleted on chromosome 10), Foxo3a, p27, and Foxl2, leads to premature and irreversible activation of the primordial follicle pool. Such global activation of the primordial follicle pool leads to the exhaustion of the resting follicle reserve, resulting in premature ovarian failure (POF) in mice (for a review, see Adhikari and Liu 2009).

Primary follicles are characterized by the initiation of follicular growth, by a change in the shape of granulosa cells, from flat cells they become cubic, the increase in the size of the oocyte and the formation of the zona pellucida (Rankin et al. 1996). The image of primary follicles is characterized by an oocyte surrounded by one layer of cubic granulosa cells and the basal lamina. Blood vessels are present only in the surrounding connective tissue and do not penetrate through the basal lamina (Fig. 1.4). Granulosa cells are related by gap junctions, allowing the passage of some molecules between cells and thus forming a metabolic syncytium, which compensate the

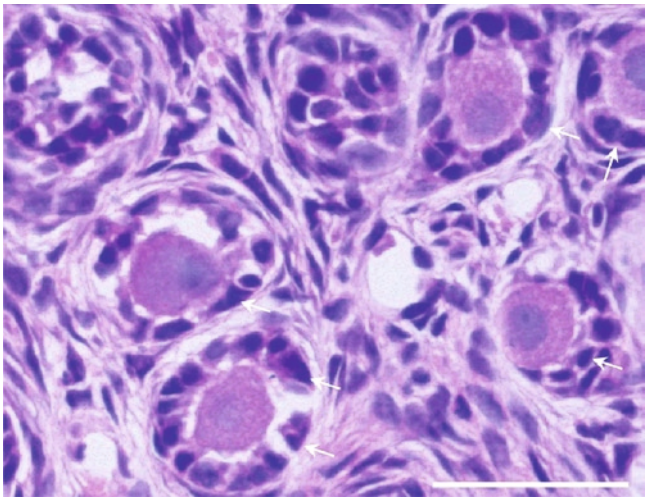

Fig. 1.4 Primary follicles. Oocytes surrounded by a single line of granulosa cells (*arrows*). The granulosa cells have acquired the cubic shape. Scale bar 50 μm. Haematoxylin-eosin stain technique

remoteness of the blood vessels for themselves as well as for the oocyte. The gap junctions allow for communication among granulosa cells and also between granulosa cells and the oocyte. The gap junctions allow the transference of nutrients and metabolic precursors such as amino acids and nucleotides, hormones, neutropins and growth factors, as well as regulatory signals of meiosis. As such, there are interchanges that may promote the growth and differentiation of the oocyte. These gap junctions contain different connexins as: −32, −43, −45 (Rankin et al. 1996).

Secondary follicles are characterized by two or more layers of granulosa cells and no antrum. This is a period of intense oocyte growth and rapid proliferation of granulosa cells. During this stage the differentiation of thecal cells takes place. Some of the cells of the connective tissue become arranged parallel to the basal lamina. The cells located closer to the basal lamina give rise to the internal theca and the remaining cells form the external theca (Fig. 1.5). This differentiation begins when the follicle has three layers of granulosa cells. During development, these cells acquire important functions such as synthesis of androgens and paracrine secretions for granulosa cells, fundamental for follicular development and maturation (Magoffin 2005).

The secondary follicles are close to a net of anastomosed capillaries originating in one or two arterioles, thus they are much better irrigated than smaller follicles (Bassett 1943).

The oocytes in secondary follicles increase their volume and their cytoplasmic organization becomes more complex, due to the synthesis of new RNAs, proteins, glycogen and lipids, as swell as increase in the number of ribosomes, mitochondria and other organelles (Picton et al. 1998). One of the main changes during oocyte growth is the secretion of glycoproteins, mainly the proteins ZP1, ZP2 and ZP3, the

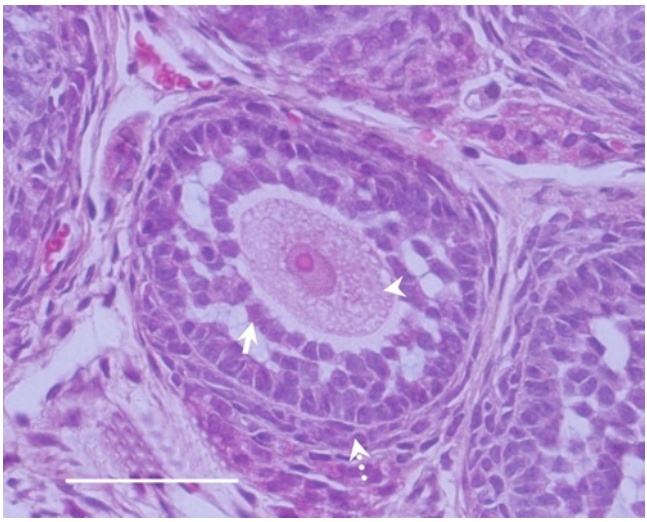

Fig. 1.5 Secondary follicles. Oocyte (*arrow head*) surrounded by various lines of granulosa cells (*arrows*). The follicles in this phase is surrounded by flattened cells of the theca (*dotted arrow*). Scale bar 50 μm. Haematoxylin-eosin stain technique

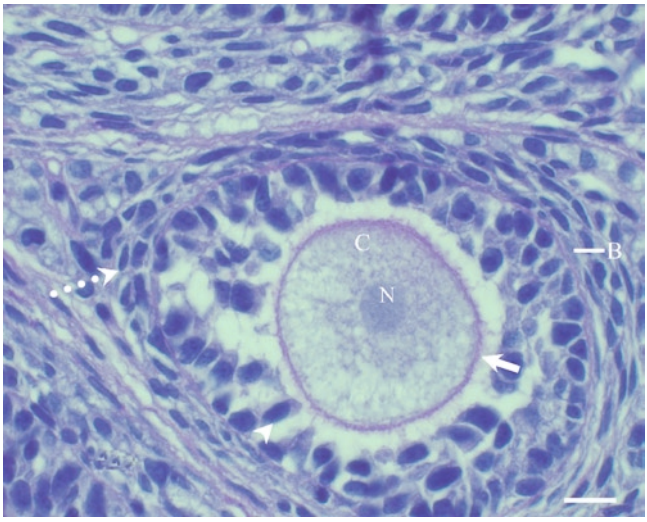

Fig. 1.6 Secondary follicle stained with Periodic Acid Schiff (PAS) method for polysaccharides (*red*). The oocyte is surrounded by the PAS positive zona pellucida (*arrow*). Several granulosa cells (*arrow head*) are disposed around the oocyte. Basal lamina (*B*) is also PAS positive. The dotted arrow points to theca cells. *N* oocyte nucleus, *C* oocyte cytoplasm. Scale bar 10 μm

principal constituents of the zona pellucida (Fig. 1.6). The formation of the zona pellucida is essential for follicular development. The transcription factor germline alpha (Fig. α) is required for the expression of these three proteins (Soyal et al. 2000). Fig. α is expressed specifically in oocytes and is required for the formation of

primordial follicles (Soyal et al. 2000). In Fig. α null mice the formation of the gonad takes place but there are no primordial follicles. Fig. α is required for the expression of the gene coding for ZP proteins. The protein Dazla, a germ cell specific RNA binding protein is also essential for differentiation and development of germ cells (Rugglu et al. 1997). The germ cell nuclear factor (GCNFd) is another specific factor needed for the development of germinal cells (Katz et al. 1997).

The cytoplasmic prolongations of the granulosa cells surrounding the oocyte go through the zona pellucida and contact the cell membrane of the oocyte, as classical electron microscopical studies have demonstrated (Sotelo and Porter 1959; Franchi 1960; Albertini et al. 2001; Motta et al. 1994). These contacts are numerous in the preantral stage and during rapid growth of the oocyte (Fig. 1.7). These contacts are vital for metabolic interchange between the oocyte and the granulosa cells.

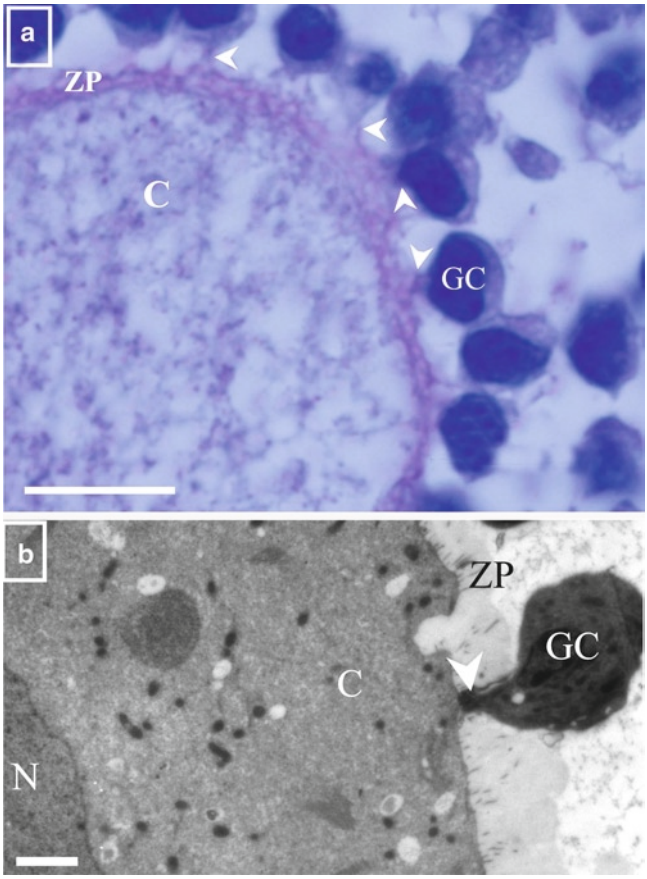

Fig. 1.7 Oocytes showing the zona pellucida (*ZP*), the granulosa cells (*GC*) are in contact with the cytoplasm of the oocyte (*C*) via cytoplasmatic prolongations (*arrow heads*). (**a**) Periodic Acid Schiff (PAS) method. (**b**) Electron micrograph contrasted with uranyl acetate-lead citrate staining method. Scale bars: a 10 μm; b 2 μm

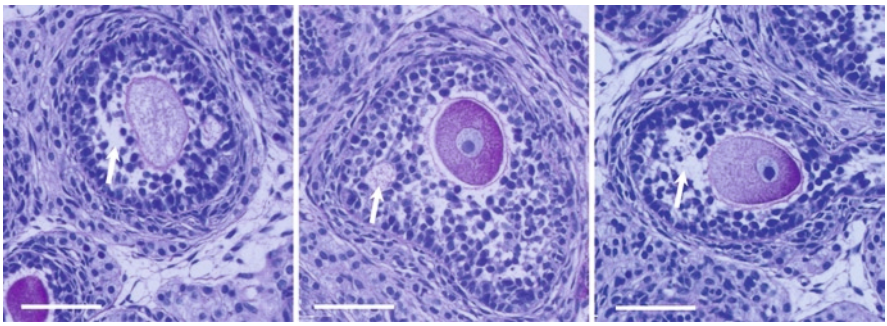

Fig. 1.8 Early antral follicles. In this phase the follicle begins to form the cavity that will constitute the antrum (*arrows*). Periodic Acid Schiff (PAS). Scale bar 50 μm

In spite of the limited vasculature of the secondary follicles, they develop to antral follicles under the stimulation of gonadotropic hormones as Follicle Stimulating Hormone (FSH) (van den Hurk et al. 2000; McGee and Hsueh 2000). The previous action of the Luteinizing Hormone (LH) seems to be important for the action of FSH because it acts through the LH receptors of the thecal cells initiating androgen biosynthesis. The androgens stimulate the formation of FSH receptors in granulosa cells allowing the action of FSH in the development of secondary follicles (van den Hurk et al. 2000; van den Hurk et al. 1999).

The early antral follicles are characterized by the formation and progressive growth of a cavity, due to the accumulation of a fluid (Fig. 1.8). This antral fluid contains different substances derived from the blood and secretion of follicular cells as regulatory proteins, gonadotropins, steroids, growth factors, proteoglycans, lipoproteins and numerous small molecules. During follicular development the size of the follicle rises rapidly due to an increment in the production of the antral fluid which is caused by an increase in the vascularization of the theca interna and in the permeability of the capillaries surrounding the follicle. In this stage there are two types of granulosa cells, those which form the wall of the follicle and those surrounding the oocyte, forming the *cumulus oophorus*. During this phase of follicle development, there is an increase in the size of the oocyte and an increase in the number of the granulosa cells forming the *cumulus oophorus*.

Once the antrum is formed the follicle goes through several stages: (a) basal growth; (b) selection and (c) dominance (Fig. 1.9).

(a) Basal growth. The thecal cells increase the expression of enzymes involved in the synthesis of steroids and granulosa cells shut down the expression of aromatases. These changes probably mean that progesterone and androgens are the main steroid hormones produced by the growing follicle.
(b) Selection. The selection of growing follicles begins in the presence of low levels of FSH and high LH secretion. In this process a number of dominant follicles are selected to continue their development, according to the size of the litter. During the selection phase, the follicles become more dependent on FSH

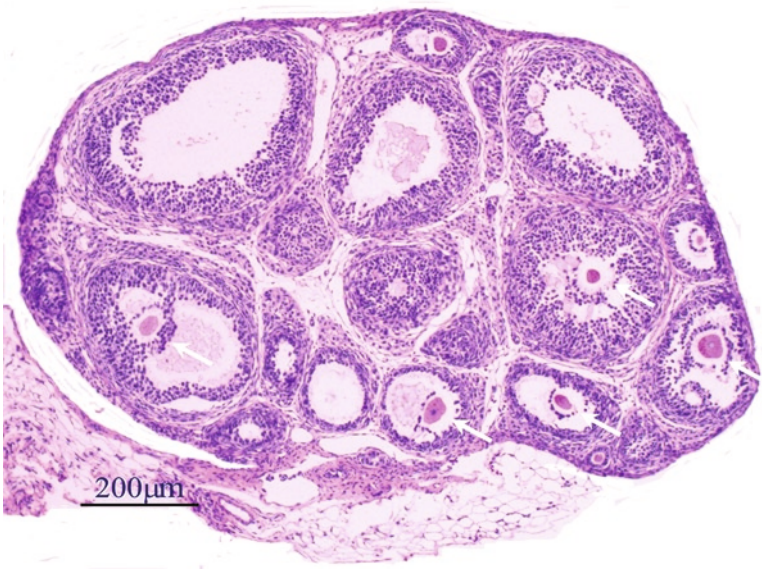

Fig. 1.9 Low magnification image of rat ovary stained with haematoxylin-eosin technique. Antral follicles are in different phases of development (*arrows*)

and the proportion of atretic oocytes decreases if FSH is experimentally increased (Gougeon 1984). The non-selected oocytes undergo a process of atresia. The mechanism of selection of the dominant follicles involves intraovarian factors and endocrine signals. The dominant follicles have a higher expression of Luteinizing Hormone Receptor mRNA and of 3 β–hydroxysteroid dehydrogenase (3β HSD) than the follicles heading for atresia (Webb et al. 1999). During selection the ovulation bound oocytes change androgen production into estrogen production, expressing aromatase activity induced by FSH.

(c) Dominancy. The EGF-like growth factors induced by LH causes the expansion of the *cumulus oophorus* and the maturation of the oocyte. The EGF-like growth factors are also paracrine signals mediating the action of LH during ovulation (Park et al. 2004).

The differentiation of granulosa cells to *cumulus* cells (Fig. 1.10) involves the acquisition of additional regulatory mechanisms such as higher sensibility to cumulus expansion factors (CEEFs), and increased levels of transcripts related to cell proliferation, caused by the activation of MAPK3/1 and MAPK14. One of these transcripts is the product of the Tnfaip 6 gene. During the pre-ovulatory peak of gonadotrophins, the cells of the *cumulus oophorus* of the antral follicles begin to expand. This process is not present in the granulosa cells of the preantral follicles due to the lack of active CEEFs secreted by the oocytes, thus there is practically no activation of MAPKs and there is no Tnfaip6 mRNA (Park et al. 2004).

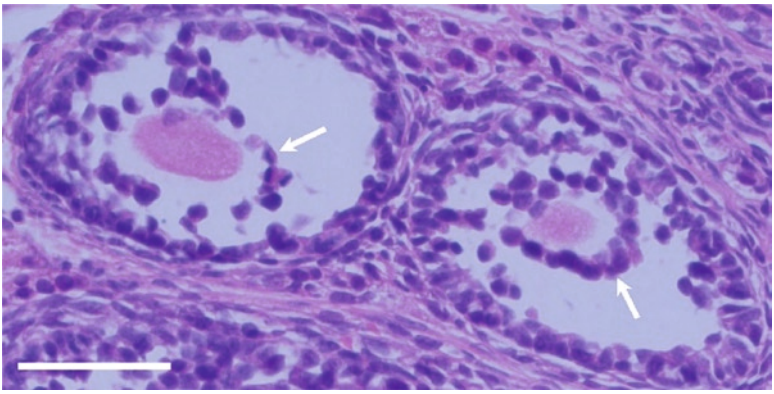

Fig. 1.10 Antral follicles the oocyte is surrounded by cumulus cells (*arrows*). Haematoxylin-eosin technique. Scale bar 50 μm

Another characteristic of dominant follicles is the presence of at least two factors, the insulin-like growth factor I (IGF-I) and the vascular endothelial growth factor (VEGF). Factor VEGF is a potent promoter of angiogenesis derived from thecal cells whose production is stimulated by LH (Garrido et al. 1993).

Increased blood supply to the dominant follicle maximizes the influx of LH and FSH to the follicle. In addition to gonadotropins and the above mentioned intra-follicular factors, other factors may influence cell proliferation in the granulosa and the theca, as well as the differentiation and fate of an early antral follicle. Some of these factors are growth hormone (GH), IGF-I, insulin, metabolic factors and local factors such as fibroblast growth factors (FGFs) and BMPs (Knight and Glister 2003).

At this stage the oocyte actively leads the somatic cell function, as in the antral stage, since the oocytes continue to form the zona pellucida proteins and connexin-37, which promotes the proliferation and differentiation of granulosa cells through the secretion of paracrine factors which may differentially affect cumulus cells and the mural granulosa cells.

The dominant follicles exert the effect of dominance on subordinate follicles, limiting the development of gonadotropin-dependent follicles suppressing the FSH and inducing their atresia (Campbell et al. 1995). It has been shown that in the antral phase the viability of antral granulosa cells becomes a factor in the fate of the follicle, as are those which provide support to germ cells (Morita and Tilly 1999; Tilly 2001). At this stage of development apoptosis in a large number of granulosa cells is indicative of a follicle in the process of elimination.

The pre-ovulatory follicles are characterized by high dynamics of growth and they develop a great capacity to respond to stimulation with FSH, they secrete large amounts of estradiol and inhibin and they are potentially capable of being ovulated.

All follicle cells and especially pre-ovulatory granulosa cells begin to assume their endocrine function and to complete a highly regulated endocrine process that leads to a state of proliferation and differentiation. This process is the primary control of two pituitary hormones: FSH and LH.

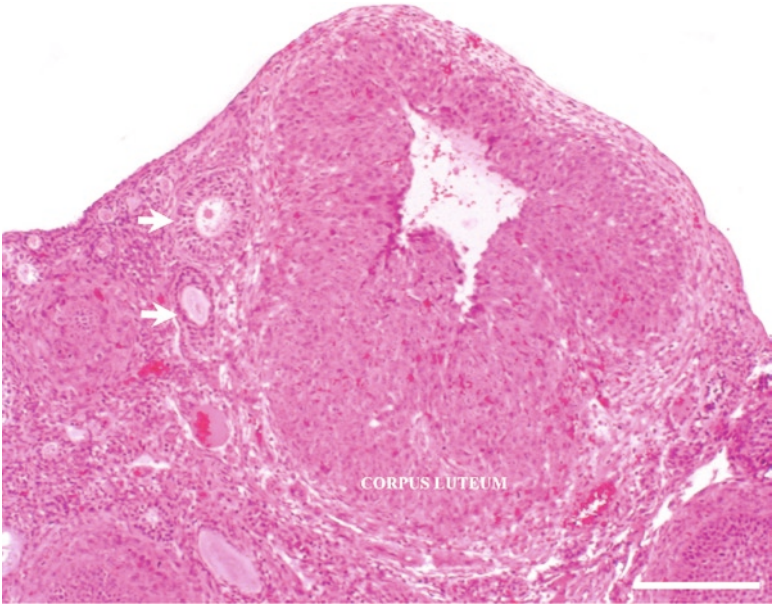

Fig. 1.11 *Corpus luteum*. Arrows show secondary follicles. Haematoxylin-eosin technique. Scale bar 50 μm

During the process of maturation of ovulatory follicles, the following changes take place at the same time: increase in follicular size; rapid proliferation of the granulosa cells and morphological changes in cells caused by marked esteroidogenic activity. The aromatase activity increases progressively during the preovulatory phase. Just before ovulation, granulosa cells are fully differentiated, proliferation stops and high levels of steroids are produced. The ability of follicles to respond to gonadotropins increases progressively according to the development of preantral follicle stage to preovulatory stage.

The basic function of an ovulatory follicle is to produce an oocyte the can be fertilized, and function as endocrine gland during maturation, and after ovulation through the transformation of the ovulated follicle in a functional *corpus luteum* (Fig. 1.11). Once the luteal phase starts, after ovulation, the granulosa cells increase in size and initiate an accumulation of a yellow pigment called lutein (hence the name corpus luteum). Corpus luteum has the ability to produce estrogen and progesterone. The regulation of the secretion of these compounds is defined by the estral or menstrual cycle. When fertilization does not occur, the corpus luteum begins to degenerate and the levels of estrogen and progesterone diminish, stimulating the growth of new follicles. However, when fertilized, the corpus luteum increases progesterone production to inhibit the growth of new follicles. The continuity of the corpus luteum is maintained by human chorionic gonadotropin (hCG) at the start of pregnancy, then the placenta takes the function of producing the necessary estrogen and progesterone.

In the early stages of follicular growth there is no significant increase in the size of the oocyte. Evidence in bovine oocytes indicates that this may be due to the association of growth with the relocation of cytoplasmic organelles and to the development of specific structures of the oocyte, such as the zona pellucida and cortical granules (Fair et al. 1997a). Fair et al. (1997b) showed that the nucleolar functions are activated gradually and that the transcription in the oocyte begins when the follicles reach secondary stage. On the other hand, the granulosa cells are in active proliferation when the oocyte is at a relatively low transcriptional activity. In mice, it was also observed that the synthetic activity is low in oocytes in primordial follicles and increases in the oocytes in primary follicles (Moore et al. 1974).

1.2 Molecular Factors Regulate the Function of the Ovary

The process of follicular growth is controlled by extra-ovarian and intra-ovarian factors and the importance of each of these factors depends on the stage of follicle development. Extra-ovarian factors regulate growth of antral and preovulatory follicles, while intra-ovarian factors regulate growth of preantral and early antral follicles (Hirshfield 1991).

The transforming growth factor beta family (TGFβ) has been implicated in various aspects of follicular development (Chang et al. 2002). This family consists of more than 35 members in vertebrates. The receptor consists of a TGFβ receptor type I and a type II. TGFβ receptor is coupled to a protein complex on the surface of the cell membrane (Massague et al. 1994; Brand and Schneider 1996). Both receptors contain an amino terminal signal sequence, an extracellular domain rich in cysteine ligand with sites of N-linked glycosylation, a single hydrophobic transmembrane domain and a cytoplasmic kinase domain (Massague 1992).

The route of the TGFβ signaling is mediated by Smad proteins through a cascade of phosphorylation induced by the ligand (reviewed in Zimmerman and Padgett 2000). The ligand-specific type II receptor phosphorylates the type I receptor, which subsequently activates the downstream cascade of Smad signaling proteins (Attisano and Wrana 1998). The role of each of the TGFβ is regulated by the Smad, and the Downstream Smad signaling cascade is defined by the type of effect of the factor (Fig. 1.12).

The TGFβ exerts different stimuli on cells either in growth, differentiation, mobility, organization or cell death, depending on the environment and/or cell type (Massague 1992; Massague et al. 1994). In rat ovary the expression of TGFβ receptor II mRNA has been found in cultured porcine cells and the expression of TGFβ receptor-I and II mRNAs was also observed (Goddard et al. 1995).

The ovary expresses several members of this family**:** in oocytes GDF-9, BMP-6 and BMP-15 occur**;** in granulosa cells: inhibins, activins, TGFb1, TGFb2 and TGFb3 are formed; and the thecal cells synthesize BMP-4 and BMP-7 (Chang et al. 2002; Drummond et al. 2003) (Fig. 1.13).

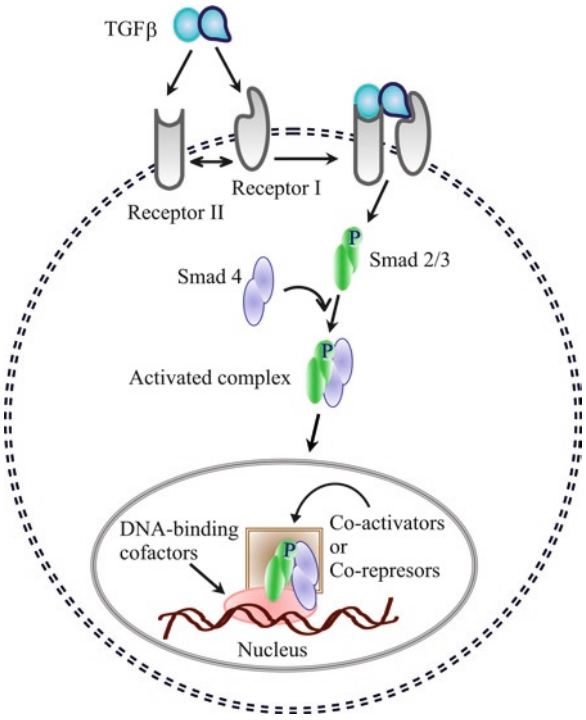

Fig. 1.12 The TGFβ signaling pathway

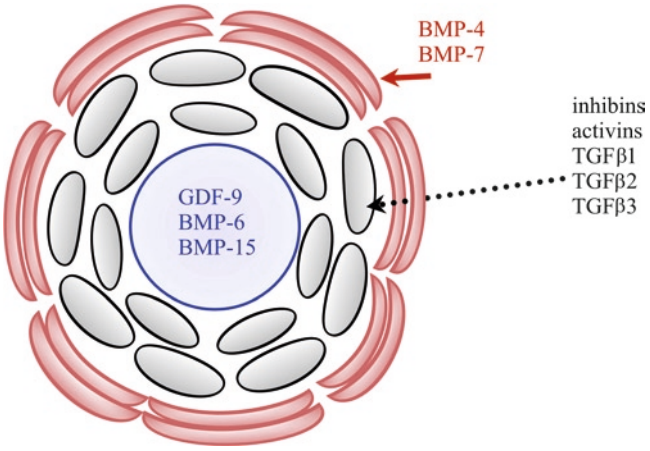

Fig. 1.13 TGFβ members family expressed in the ovary. Specific factors are expressed in each cell type

There are two growth factors produced by the oocyte that can impact initial follicle growth: the growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15 or GDF-9B). Both factors are expressed exclusively in the oocyte (McGrath et al. 1995; Dube et al. 1998). GDF-9 is expressed in several species, including humans and mice, in all follicular stages except in primordial follicles of newborn and adult mice (McGrath et al. 1995). GDF-9 acts as a paracrine regulator in the proliferation and differentiation of granulosa cells in primordial follicles. GDF-9 and BMP15 promote proliferation of granulosa cells of early antral follicles (Hayashi et al. 1999; Otsuka et al. 2000; Vitt et al. 2000), so the GDF-9 allows primary follicles pass to the following stages of development. BMP15 is considered a luteinization inhibitor as it inhibits the FSH production stimulated by progesterone in granulosa cells of the rat (reviewed in Shimasaki et al. 2004).

The Smad3 receptor of TGFβ is highly expressed in the ovarian surface epithelium, in granulosa cells and in oocytes of various animal models (Symonds et al. 2003; Xu et al. 2002; Tomic et al. 2002). In Smad3-deficient mice, the genes that control cell cycle progression are affected, causing failure in folliculogenesis (Tomic et al. 2004).

The inhibins are protein hormones that are produced primarily in the gonads. They down regulate the synthesis and secretion of FSH from the pituitary. The inhibins are composed of an α subunit and one or two β subunits (βA or βB), the β-dimers with α-βA and inhibin βB form the A and B respectively. Besides sharing the β subunits, the activins and inhibins are functional antagonists in many physiological contexts (Wang et al. 1996). These proteins, in addition to their role in modulating the release of FSH, may also serve as local regulators of folliculogenesis (Findlay 1993). Type I receptors for activins (ACVR1 and ACVR2B) are expressed in granulosa cells, meanwhile the ligands are expressed in the oocyte and in somatic cells.

Once the primordial follicles begin folliculogenesis to form preantrales and antral follicles they begin to be sensitive to gonadotropins and the GCs surrounding the oocyte begin to synthesize predominantly B and also inhibin A (Jaatinen et al. 1994). In the late luteal phase and in early follicular phase of the menstrual cycle, FSH levels rise. Under the stimulation of FSH, inhibin levels in serum increase until a negative feedback occurs and FSH levels fall before ovulation. The dominant follicle which is now responsive to LH produces more inhibin A than B. After the LH peak, the corpus luteum produces inhibin βA. This shows that there is differential expression of inhibins during the menstrual cycle.

The activins are homo or heterodimers of the subunits βA or βB. They are regulators of FSH and bind to the receptor type II. When activin binds to its receptor, signalling events leading to a specific biological response to activins are initiated. The activin response is blocked when the inhibins bind to the same receptor as the activin or to betaglycans, generating a nonfunctional receptor complex. The inhibins bind to inhibin binding protein (InhBP/p120), which is also known as the product of immunoglobulin superfamily gene 1 [IGSF1], which is expressed in the pituitary and testis. This binding activates a transduction pathway of inhibins,

which causes a specific response for inhibin ligands (Massague and Chen 2000). In undifferentiated GCs, the activin increases response to FSH, but in differentiated cells it is an inhibitor (Miro et al. 1995).

The inhibin regulates FSH at high concentrations, antagonizes the activin and is a potent stimulator of FSH secretion. In the ovary, FSH combined with activin causes a dose-dependent increase of DNA synthesis, suggesting that FSH in the presence of activin is mitogenic. It is believed that inhibin is a hormone that plays a key role in the regulation of FSH and it has been shown that it can have an important physiological role for embryonic development and survival in mink.

The neutralization of inhibin increases the ovulation rate in many species, and probably increases the concentration of serum FSH; however, it has been shown that the inmunoneutralization of inhibin suppresses the embryonic development in mink (Ireland et al. 1992).

It appears that the activin is an intrafollicular protein that controls the activation of primordial follicles and their effect depend on factors such as age and availability of follistatin. The follistatin is synthesized by granulosa cells and can bind to activin, contributing to a complex pattern of regulation of secretion of progesterone.

Follistatin is a single chain polypeptide that was initially identified by its activity to suppress FSH (Robertson et al. 1987), but later it was discovered that it binds to activin and inhibin through the common β subunit and neutralizes the bioactivity of the activin (Shimonaka et al. 1991). Activin regulates progesterone production stimulated by FSH in granulosa cells of the rat (Miro et al. 1995).

The connective tissue growth factor (CTGF) is a member of CTGF / cysteine-rich over-express 61/nephoblastoma gene family that mediates the regulation of connective tissue synthesis induced by TGFβ in various cell types. An abundant expression of CTGF mRNA was also detected in granulosa and theca cells of the ovaries of pigs (Wandji et al. 2000). CTGF gene expression in granulosa cells is inversely related to the state of granulosa cell differentiation, being directly inhibited by FSH signaling pathway mediated by AMP. The abundance of CTGF mRNA in undifferentiated granulosa cells in vitro is regulated by TGFb1, GDF-9 and activin, which may indicate paracrine functions of these growth and differentiation factors in the regulation of CTGF synthesis in ovaries of mammals (Harlow et al. 2002).

The Müllerian inhibitory substance (MIS) is another member of the TGFβ family of glycoprotein hormones that presents certain patterns of expression in follicles at various stages of development. This hormone was identified as a testicular product that induces regression of Müllerian ducts in males (Cate et al. 1986). Also known as anti-Müllerian Hormone (MIS), it is expressed in granulosa cells of small and early growing follicles (Ueno et al. 1989). Granulosa cells of pre-antral follicles and small antral express MIS type II receptor (Teixeira et al. 1996). The theca cells also have a marked expression of MIS in small antral and preantral follicles, but unlike the granulosa cells, theca cells continue to express the receptor in antral follicles and in early atresia (Ingraham et al. 2000). It was also noted that MIS inhibits the growth of follicles, as it is expressed in the granulosa cells of primary and early antral follicles (Durlinger et al. 1999). The transcription of MIS genes is

regulated by nuclear transcription steroidogenic factor-1 (SF-1), originally identified as a factor that regulates steroid hydroxylase genes (Ingraham et al. 2000). SF-1 also regulates LHB (Keri and Nilson 1996) and FSH receptor (Levallet et al. 2001). The transcription of MIS and SF-1 is also regulated by members of the family of GATA transcription factors. GATA-4 and GATA-6 are expressed in granulosa cells of developing follicles (Tremblay and Viger 2001).

The previous results highlight the broad impact of the members of the TGFβ superfamily in various functions and stages of ovarian development.

1.3 Hormonal Aspects of the Ovarian Functions

The ovary is not only involved in sexual reproduction, but also has great influence on the entire hormonal functioning during development of the organism.

The ovary is the site of the highest synthesis and secretion of progesterone and estrogen in mammals and gives rise to cyclical fluctuations in the levels of these hormones in the blood (Norman and Litwack 1987). The follicles are responsible for the secretion of both hormones and the release of eggs during the normal cycle.

According to the theory 'two cell, two gonadotropin' (Armstrong et al. 1979), the theca interna cells are stimulated by LH to produce androgens which are transported to the granulosa cells (Fig. 1.14). There they are converted to estrogen by

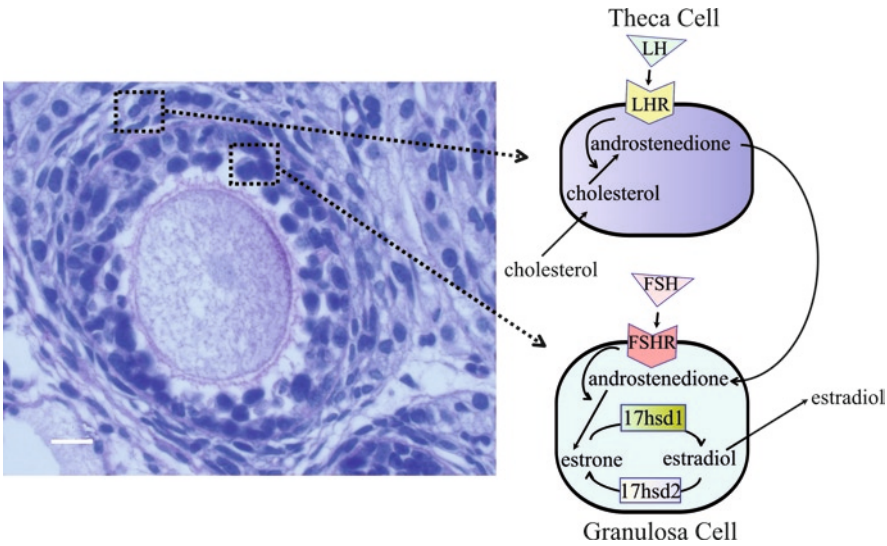

Fig. 1.14 Two cell-two gonadtrophin theory. LH induces androstenedione synthesis in theca cells. The FSH stimulus provokes granulose cells process androstenedione into estrone which is further converted into estradiol via 17hsd enzyme