Morphology and Biochemistry of Ovulation Morfologia e bioquímica da ovulação

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Abstract

The process of ovulation involves multiple and iterrelated genetic, biochemical, and morphological events: cessation of the proliferation of granulosa cells, resumption of oocyte meiosis, expansion of cumulus cell-oocyte complexes, digestion of the follicle wall, and extrusion of the metaphase-II oocyte. The present narrative review examines these interrelated steps in detail. The combined or isolated roles of the folliclestimulating hormone (FSH) and luteinizing hormone (LH) are highlighted. Genes indiced by the FSH genes are relevant in the cumulus expansion, and LH-induced genes are critical for the resumption ofmeiosis and digestion of the follicle wall. A nonhuman model for follicle-wall digestion and oocyte release was provided.

Full Text

Introduction

Ovulation is the term used to define the ovarian release of the female mature gamete that is ready to be fertilized. The process of ovulation includes a series of morphological and biochemical events within the preovulatory follicle. Several genes are activated in the ovarian environment, leading to enzymatic and structural transformations under the influence of gonadotropins and sex steroids that are modulated by several growth factors. All of these events ensure that the oocyte becomes likely to be fertilized and extruded on the ovarian surface to form the corpus luteum.[1] The clinical marker of the beginning of the reproductive cycle responsible for the maturation and extrusion of the oocyte is menstruation. In regular cycles, at intervals of 24 to 38 days,[2] ovulation occurs mid-cycle, at around the 14th day. In this scenario, in an orchestrated way, the follicle-stimulating hormone (FSH), and the luteinizing hormone (LH) actively participate in the events that ensure ovulation, mostly through activation of multiple genes in theca and granulosa cells. The present review aims to examine the basic mechanisms of ovulation and describe the morphological and molecular events interconnected during the ovulatory process.

Methods

We searched for articles published in English in the PubMed and Google Scholar databases. The keywords were as follows: *menstrual cycle*, *menstrual cycle physiology*, *folliculogenesis*, *theca cells*, *granulosa cells*, *oocyte*, *oocyte-cumulus complex*, *follicular wall digestion*, *cumulus-oocyte-complex expansion*, *oocyte maturation*, *gene expression*, *FSH*, *LH*, and *progesterone receptor*. We expanded the search to the references of the retrieved articles.

Follicular Dynamics and Folliculogenesis

The more advanced stages of follicle development are characterized by the appearance of intercellular space filled by antral fluid. At this stage, the granulosa cells are differentiated into two distinct populations: cumulus cells, which are those closely linked to the oocyte, and wall or mural granulosa cells, which internally line the follicular wall. Although these two cell types share a common origin, there are differences in the production of transcribers and proteins.[3] At the end of follicular development, the FSH and estradiol promote the expression of the LH receptor (LHR) in granulosa cells. Most LH molecules bind to mural granulosa cells rather than to cumulus cells.[4] Cumulus cells provide energy input to the oocyte, controlling its growth and metabolism.[5] On the other hand, mural granulosa cells are responsible for steroid synthesis and differentiation in luteum cells after ovulation.[6] Cumulus granulosa cells play a smaller role regarding the function of the corpus luteum. Follicular architecture is provided by the inner and outer theca-cell layers ([Fig. 1]). The theca cells, provided with LHR, are responsible for the capture of the substrate cholesterol and its enzymatic conversion into androgens, mainly testosterone (T) and androstenedione (A4). In turn, granulosa cells, which are adjacent to the theca cells, capture A4 and T and, by the action of the aromatase enzyme, convert them into estrone and estradiol respectively ([Fig. 2]).[7]
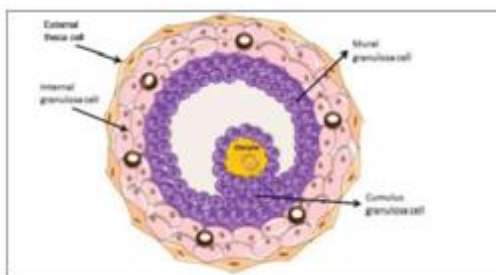

Fig. 1
Structure of the periovulatory follicle showing internal and external theca cell layers, granulosa cells, and the oocyte.
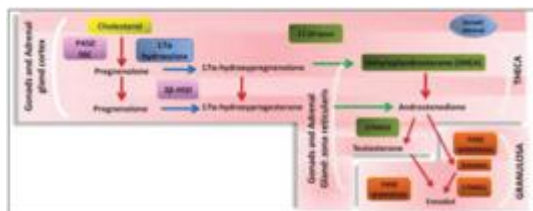
Fig. 2
Scheme showing the steroidogenesis of theca and granulosa ovarian cells. Abbreviations: P450ssc, cytochrome P450 for cleavage of cholesterol side chains; 3-βSHD, 3 β hydroxysteroid dehydrogenase; 17-HSD3, 17-hydroxysteroid dehydrogenase.

Folliculogenesis begins with the formation of the primordial follicle, and ends with the preovulatory follicle.8 The FSH, released by the anterior pituitary gland, promotes the recruitment of follicular waves that, in response, secrete estradiol and inhibin. When synthesized, these hormones modulate the release of pituitary FSH and LH in a pulsatile way. At the end of folliculogenesis, the preovulatory peaks of FSH and LH induce a complex sequence (or even a concurrence) of events: oocyte maturation, cumulus cell expansion, follicular wall digestion, and release of the cumulus-oocyte complex.9

Ovulation Process

Genetic Aspects Determining Ovulation

The ovulation process occurs in a coordinated and interrelated way in five complex steps: interruption of granulosa cell proliferation, resumption of meiosis, expansion of the cumulus with oocyte release inside the antrum, lysis of the follicular wall, and oocyte extrusion at the metaphase II (MII) stage. In mammals, oocytes are stationed in meiosis I at prophase I. The resumption of meiosis I occurs during puberty as a result of the gonadotropic stimulus in follicles in the preovulatory stage, culminating in the rupture of the germ vesicle.10 The increase in the concentrations of LH and FSH in the mid-cycle in the presence of the preovulatory follicle, now provided with LHR in granulosa cells, promotes the activation of several genes that encode the synthesis of various proteins. This process is similar to inflammatory processes.11 The LH activates cyclase, resulting in intracellular increases in cyclic adenosine monophosphate (cAMP) that activate cAMP-dependent kinases and the expression of the hyaluronic synthase 2 (HAS-2) and cyclooxygenase 2 (COX-2) enzymes, the tumor necrosis factor-inducible gene 6 protein (TSG-6), pentraxin 3 (PTX-3), and genes of the epidermal growth factor (EGF)-like family, such as amphiregulin (AREG), epiregulin (EREG), and betacellulin (BTC).12 13 14 Tissue rearrangement occurs as a result of the activation of these genes participating in the cascade of ovulation events.

The Role of the Follicle-stimulating Hormone

Periovulatory gene expression induced by the FSH in cumulus cells plays a minor but necessary role in the mediation of ovulation (Fig. 3). The occurrence of the FSH peak activates its own receptor (FSHR), stimulates the expression of steroidogenic factors, and induces LHR synthesis in granulosa cells. Such functions of the FSHR are related to the FSH activation of cAMP synthesis, and are triggered mainly through the expression of protein kinases A (PKA) and C (PKC) enzymes in granulosa cells.15 The FSH activates the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway to mediate cell survival and granulosa proliferation, including the expression of the vascular endothelial growth factor (VEGF) gene, and it activates extracellular-regulated kinase (ERK) signaling in mural

granulosa and cumulus cells, facilitating cumulus expansion.[16] The FSH may also induce COX-2 and other prostaglandin synthases through cAMP/PKA activation.[17] Activation of the COX-2 gene results mainly in prostaglandin F-α (PGF2α) that induces changes in the gene expression of the cumulus-oocyte-complex, which is critical for cumulus-oocyte-complex expansion.[18] Additionally, the FSH induces the expression of genes belonging to the family of disintegrin and metalloproteinases (A disintegrin and metalloproteinase with thrombospondin motifs, ADAMTS), molecules relevant in the process of cleavage of the extracellular matrix (Fig. 3). It seems that these proteins are the main regulators of the release of EGF-like proteolytic factors in a soluble form (AREG, EREG, and BTC),[19] which activate the EGF receptor tyrosine kinase and the extracellular signal-regulated kinase (ERK) involved in cumulus expansion.[13] Metalloproteinases ADAMTS-1, ADAMTS-4, ADAMTS-5, and ADAMS-16, genes expressed in granulosa cells, are involved in the dissociation of the cumulus-oocyte complex and in the formation of the corpus luteum.[20][21] Then, the FSH, in the same way as in the mucification of the cumulus, plays a role with the LH in the synthesis of enzymes responsible for the digestion of the follicle wall.
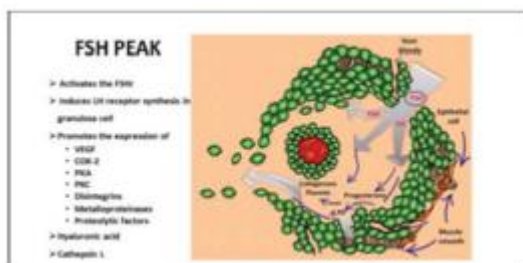

Fig. 3
Biochemical events initiated by the follicle stimulating hormone (FSH) in the preovulatory follicle. Abbreviations: VEGF, vascular endothelial growth factor; COX-2, cyclaoxygenase-2; PKA, protein kinase A; PKC, protein kinase C.

The Role of the Luteinizing Hormone

The role the LH in the ovulation process is complex and fundamental for the resumption of meiosis, loosening of the cumulus cells, and rupture of the follicle.[22] With the peak of the LH, the messenger ribonucleic acid (mRNA) for the progesterone receptor (PR) as well as other genes is now transcribed into the granulosa cells of preovulatory follicles (Fig. 4).[23] The PR has an indirect influence on the synthesis of proteolytic enzymes cathepsin L and ADAMTS-1, which together play a role in tissue degradation and the remodeling of the extracellular matrix at the apex of the preovulatory follicle until ovulation occurs.[24] The LH peak, modulated by AMP, participates in the process of suppression of the proliferation of granulosa cells, and restarts meiosis, dissociation of the granulosa, digestion of the follicle wall, and luteinization.
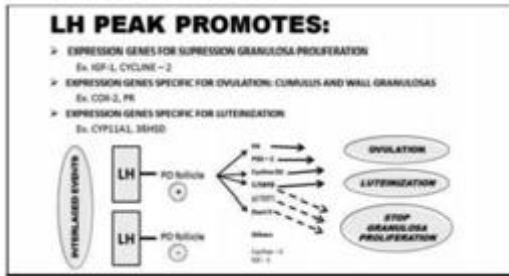
Fig. 4
Expression of several genes induced by the luteinizing hormone (LH) peak.

Biochemical Aspects Determining Ovulation

Mucification and Cumulus Expansion

The genetic and biochemical events responsible for cumulus mucification are summarized in Fig. 5.23 The matrix on which the cumulus cells move has three major components: hyaluronic acid (HA) and two HA binding proteins, TSG-6, and inter-α-trypsin inhibitor (ITI).9 25 Induced by the peaks of FSH and LH, HAS-2 is the main enzyme responsible for the synthesis of arachidonic acids and HAs in the cumulus-oocyte complex, and, in synergy with COX-2, causes the synthesis of prostaglandins (PGs) from arachidonic acid in the granulosa cells of the cumulus. Thus, the expression of COX-2 in the cumulus cells promotes the synthesis of PGs, mainly prostaglandin E (PGE), and ensures the expansion of the cumulus.18 25 26 However, cumulus expansion occurs only when the ITI enters the follicle. The TSG-6 and the proteoglycans brevican and versican, induced by high concentrations of LH and HA stabilization, are rapidly expressed in the cumulus granulosa cells of preovulatory follicles.16 In the context of deficiency of the TSG-6 enzyme, the extracellular matrix is not structured, compromising cumulus expansion.27 The PTX-3 protein, with an affinity for TSG6, is also responsible for the stability of the cumulus matrix. The interaction between these enzymes appears to be crucial for the structuring and expansion of the cumulus matrix, enabling the dispersion of the cumulus cells away from the oocyte.20 Collectively, these observations indicate that HA, ITI, and COX-2, induced by the TSG-6 gene, are critical for cumulus matrix formation, cumulus cell differentiation, and, ultimately, cumulus expansion.
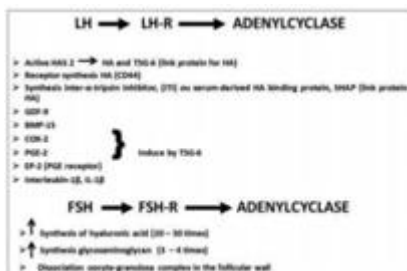

Fig. 5
Combined actions of the FSH and LH in the expansion of oocyte-cumulus cells.

Oocyte Maturation

The oocyte maturation process aims to empower the female gamete and ensure its subsequent development until the activation of the embryonic genome occurs. Therefore, chromatin condensation is relevant in the continuity of meiosis, redistribution of organelles in the cytoplasm, and alterations in the cytoskeleton; all of these modifications are precisely regulated and coordinated (Fig. 6).[28] For this to happen, there is paracrine cross-talk between the oocyte and cumulus cells. Cumulus cells penetrate the zona pellucida and limit the ooelema gap junction between the cumulus and the oocyte transfer of small molecules.[28] Biochemically, the oocyte regulates the metabolism of cumulus cells, which in turn provide ions, metabolites, amino acids, and small oocyte regulatory molecules (Fig. 7).[29] Paracrine oocyte factors are soluble, and are generically referred to as oocyte-secreted factors (OSFs).[30] The growth differentiation factor 9 (GDF-9), the bone morphogenetic protein 15 (BMP15), and, to a lesser extent, the BMP6 are considered OSFs; all belong to the family of transforming growth factors β (TGFβ).[30][31] These factors coordinate the differentiation lineage and function of granulosa cells.

Fig. 6
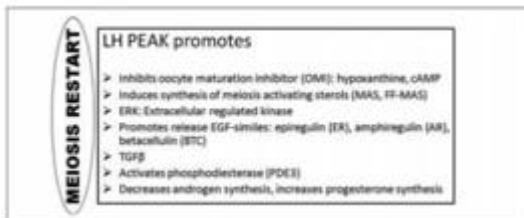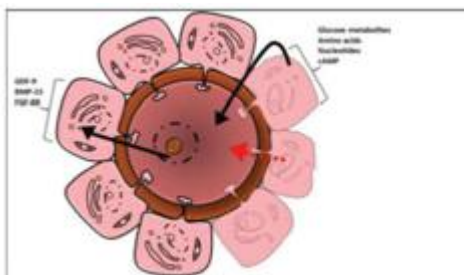The role of the LH in meiosis resumption.

Fig. 7
Cell-cell signaling between the oocyte and granulosa cells in the final stage of follicle development.

The functions of the OSFs include growth stimulation, prevention of apoptosis, inhibition of luteinization, regulation of energy metabolism, cholesterol biosynthesis, and regulation of cumulus expansion.[32][33][34] The factors that regulate the relationship between cumulus granulosa cells and the oocyte include ions, metabolites, amino acids, and small intracellular signaling molecules such as

cAMP, cyclic guanosine monophosphate (cGMP), and inositol triphosphate-3 (IP3).6 32 In the regulation of meiosis, cAMP synthesized by the oocyte itself and by cells of the mural granulosa and cumulus reaches the oocyte through the junctions of the hexameric lacunar canal composed of connectin proteins.35 36

In general, the properties of lacunar junctions enable the direct and bidirectional transport of small molecules between the oocyte and the granulosa cells. High intraoocyte levels of cAMP maintain the oocyte in the stage of germ vesicle, through suppression of the activity of the maturation-proimoting factor (MPF).37 38 39 Follicle somatic cells also provide cGMP to the oocyte, inhibiting the phosphodiesterase enzyme type 3A (PDE3A), thereby preventing the degradation of cAMP with the accumulation of this factor and inhibition of the resumption of meiosis.38 40 With the LH stimulus at high concentrations, the connectins close, decreasing the contribution of cAMP and cGMP from the cumulus cells to the oocyte. Therefore, the decrease in cAMP levels leads to the phosphorylation of PDE3A that degrades the cAMP. The degradation of cAMP enables the synthesis of the MPF, which promotes the resumption of meiosis I.41

In a recent study42 in mice, the expression of natriuretic peptide type C (NPPC) was found in the mural granulosa cells, and natriuretic peptide receptor 2 (NPR2) was found in cumulus cells. With the communication between these two cell types the NPPC ligand and NPR2 stimulate the secretion of cGMP and cAMP. By adding NPPC to the culture media, an increase in the rates of oocytes that did not resume meiosis was observed, favoring the synchrony between nuclear maturation and cytoplasmic maturation.42 During cytoplasmic maturation, there is a physical rearrangement of mitochondrial groups and endoplasmic reticulum, following the maturation time and energy dependence of the meiotic spindles so that chromatin is divided.

The meiotic spindles are responsible for the continuity of the meiotic division and extrusion of the two polar corpuscles. Initially, the mitochondrial groups are in a central position in the oocyte. As the maturation progresses, they migrate to the edges of the oocyte, close to the extruding regions of the polar body.43 44 The MPF is the factor directly involved in cytoplasmic maturation, because, in addition to inducing the breakdown of the germ vesicle, it promotes the condensation of chromosomes, moving them from prophase I to metaphase I (MI), in which there is the formation of the meiotic spindle and the alignment of chromosomes in the center of the spindle. Then, anaphase I occurs, which consists of the separation of homologous chromosomes. Sequentially, telophase I begins with the extrusion of the first polar body, and the oocyte is in the metastasis II stage. At this stage, there is the formation of the second meiotic spindle and alignment of chromosomes, following anaphase II and telophase II and, finally, the extrusion of the second polar body.45 46 The oocyte remains in this stage until ovulation occurs and there is the penetration of the sperm.

Follicular Wall Digestion

Morphological and biochemical changes that result in rupture of the follicular wall and oocyte extrusion occur basically by the action of the LH, because it induces the synthesis and secretion of various enzymes (Fig. 8). The role of the FSH is smaller in this process, when the oocyte and cumulus cells are still fixed in the extracellular matrix (ECM). With the LH peak, LHR on the surface of the granulosa cells activates the digestion of the ECM within the theca layers and tunica albuginea at the ovarian surface via adenyl cyclase. The effectiveness of ECM digestion occurs through the balance between matrix components and proteases in the cumulus, oocyte, and endothelium cells that form the corpus luteum.21 Theca cells express a variety of matrix metalloproteinases (MMPs), including MMP2 (gelatinase A), MMP9 (gelatinase B), MMP13 (collagenase), MMP14, MMP16, MMP19, and tissue inhibitor of MMPs-1 (TIMP-1).9
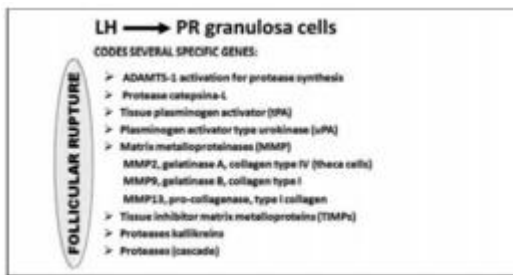
Fig. 8
Role of LH-induced genes in the digestion of the follicular wall.

The ADAMTS 16, present in luteinized granulosa cells, responds to FSH stimulation and actively participates in the process of structural follicle remodeling at the time of ovulation. The role of the LH on PR is mimicked by cAMP-inducing agonists (FSH, forskolin). Targets of PR appear to control the rupture of the follicle, mainly ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin) and cathepsin L. Among the proteases involved, thrombospondins 1 and 4 (ADAMTS1/4) promote the breakdown of the proteoglycan family structures, such as versican, through granulosa activation by PRs,47 thereby contributing to the follicular rupture. Through its receptor in granulosa cells, the LH induces the transcription of early growth regulatory factor-1 (EGR-1), *CAAT* enhancer-binding protein β (C/EBPβ), PR, and other activator protein-1 family members (proto-oncogenes, c-Fos, c-Jun, Fra2, JunD), all involved in the functional activity of the granulosa cells of the ovulating follicle.

The proteoglycan (versican, brevican) components of the ECM induced by the LH peak, on either granulosa or theca cells, serve as substrates preferably for ADAMTS 1, culminating in follicular rupture.47 Metalloproteinases such as plasminogen and collagenase are part of the follicular digestion process, and their control is mediated by metalloproteinase inhibitors, ensuring local homeostasis and completion of the ovulation process.48 To illustrate, the model proposed by Ogiwara et al.49 in the Japanese rice fish, also known as medaka, shows the involvement of proteinases in the lysis of the follicular wall (Fig. 9).
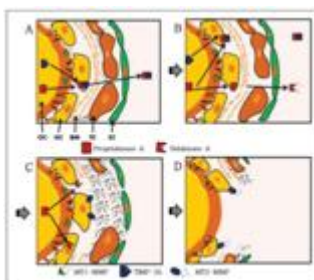

Fig. 9
A model of follicle rupture during ovulation in the Japanese rice fish, also known as medaka. (**A**) In the follicle, a few hours before ovulation, progelatinase A is activated by membrane type 1-matrix metalloproteinase (MT1-MMP) on the surface of the oocyte, whereas gelatinase A is immediately

inactivated by the tissue inhibitor of matrix metalloproteinases-2b (TIMP-2b). (**B**) At the time of ovulation, the hydrolysis of basement membrane type-IV collagen is initiated by active gelatinase A at the follicle–ovarian surface contact site. (**C**) membrane type 2-matrix metalloproteinase (MT2-MMP), which is now expressed on the surface of the granulosa cells, can degrade the type-I collagen that is present in the theca cell layer. (**D**) As a result, the oocyte is exposed at the contact site, leading to ovulation. Abbreviations: BM, basement membrane; EC, epithelial cell; GC, granulosa cell; OC, oocyte; TC, theca cell. Source: Ogiwara et al.49 We would like to thank the National Academy of Sciences of the United States by permission

After the rupture of the follicular wall, there is tissue reorganization by the activation of promatrix factors, which, in an organized and vascularized way, causes granulosa cell differentiation into luteal cells, thereby originating the corpus luteum. The corpus luteum is composed of functional cells for the synthesis of progesterone, the main regulator of the pituitary secretion of gonadotropins, the principal factors involved in the maintenance of the corpus luteum until initial gestation.50 In the absence of maternal recognition of pregnancy, the corpus luteum regresses rapidly, and the ovarian cycle is resumed.51

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