CHAPTER EIGHT

Luteinizing hormone-induced changes in the structure of mammalian preovulatory follicles

Corie M. Owen* **and Laurinda A. Jaffe***
Department of Cell Biology, University of Connecticut Health Center, Farmington, CT, United States
*Corresponding authors. e-mail address: coowen@uchc.edu; ljaffe@uchc.edu

Contents

Abstract

Ovulation of a mammalian oocyte from its follicle, which occurs in response to luteinizing hormone (LH), requires complex restructuring of the ~20 layers of surrounding somatic cells. This chapter describes the cellular architecture of preovulatory follicles, the localization of the receptors for LH, and the LH-induced changes in follicular structure, focusing on mice and other small mammals. The multiple interrelated processes that result in ovulation include breakdown of existing extracellular matrix, generation of new extracellular matrix, thinning of the follicular apex where the oocyte will be released, invagination of the follicular surface, and responses of the vascular system to support these dynamic changes. However, much remains unknown about how these events function together to release a fertilizable egg.

1. Introduction

Ovulation is a universal process by which animal eggs are released from the ovary such that they can participate in fertilization. The events of

Current Topics in Developmental Biology, Volume 162
ISSN 0070-2153, https://doi.org/10.1016/bs.ctdb.2024.10.011

mammalian ovulation are similar in many ways to those in other vertebrates, starfish, and even jellyfish. In each of these species, the ovulatory signal acts on a G-protein coupled receptor in the somatic cells of the follicle that surrounds the oocyte. In mammals and other vertebrates, the signal is luteinizing hormone (LH) from the pituitary (Hunzicker-Dunn & Mayo, 2015; Omony et al., 2022), named "luteinizing" because in addition to triggering ovulation, LH causes the follicle to transform into the corpus luteum that supports pregnancy in mammals. In starfish, the stimulus for ovulation is a relaxin-like peptide from nerves in the gonoduct (Feng et al., 2023; Mita, 2023), and in jellyfish, it is light (Quiroga Artigas et al., 2020). In all of these animals, activation of the receptor in the somatic cells of the follicle results in resumption of meiosis and cytoplasmic changes in the oocyte that prepare it for fertilization. Receptor activation also results in rupture of the follicle, releasing the oocyte.

This chapter reviews structural changes in mammalian follicles that are stimulated by luteinizing hormone, and discusses some of the unanswered questions about how these structural changes in the somatic cells act together to enable oocyte release. The ovulatory process in mammalian ovaries is especially complex because the follicular structure has ~20 cellular layers, in contrast to starfish where there is just a single layer (Feng et al., 2023), or frogs and fish where there are ~3 layers (Dumont & Brummett, 1978; Takahashi, Hagiwara, & Ogiwara, 2018). We focus on studies of mice, rats, hamsters, and rabbits – small mammals that have been studied extensively because they are accessible to experimental manipulation. We review some of the classic and current studies about the changes in cellular and tissue structure that are induced by LH in the follicle, and discuss how these structural changes may act together to release the oocyte from the follicle at ovulation. Other aspects of ovulation including gene regulation in the granulosa cells, oocyte maturation, and mechanisms of ovulation in humans and other large mammals, are reviewed elsewhere (Duffy, Ko, Jo, Brannstrom, & Curry, 2019; Hughes & Murphy, 2021; Hunzicker-Dunn & Mayo, 2015; Jaffe & Egbert, 2017; Mogessie, Scheffler, & Schuh, 2018; Richards & Ascoli, 2018; Robker, Hennebold, & Russell, 2018).

2. Structure of mammalian preovulatory follicles

In small mammals such as mice, an ovary at the preovulatory stage has multiple fully grown follicles located around its surface. The ovary also

contains thousands of smaller follicles that will grow in subsequent reproductive cycles. At ovulation, oocytes will be released from the "apical" side of each preovulatory follicle, the side that faces the ovary surface and is adjacent to the ovarian surface epithelium. The non-apical side of each follicle is referred to as "basolateral".

The elaborate complex of somatic cells that comprises each preovulatory follicle includes 2 concentric regions separated by a basal lamina: granulosa cells and theca cells (Fig. 1A). The multiple layers of granulosa cells are connected to each other and to the oocyte by gap junctions (see Norris et al., 2008). The ~2–3 layers of granulosa cells closest to the oocyte are called cumulus cells; these send fine processes through the extracellular matrix around the oocyte (zona pellucida) to form contacts with oocyte

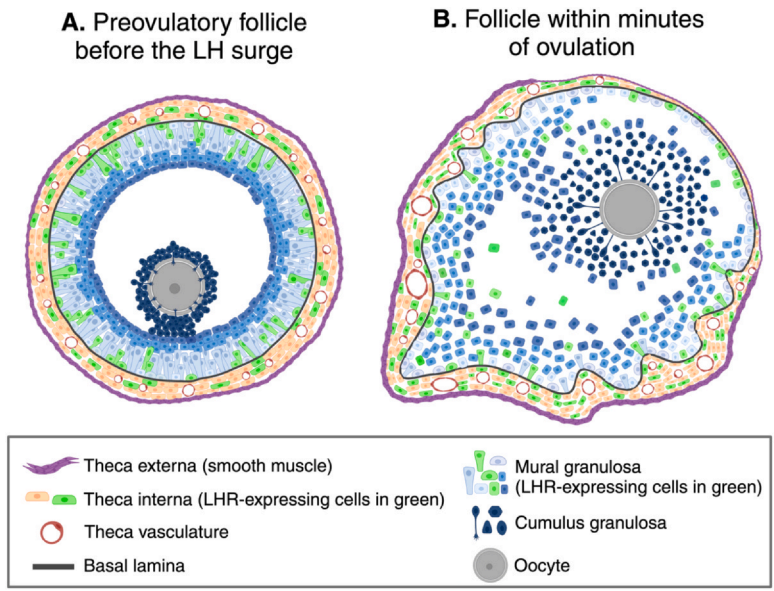

Fig. 1 Cellular structure of preovulatory follicles before and after the LH surge. (A) Before LH exposure. The oocyte is surrounded by several concentric layers of cells: cumulus granulosa, mural granulosa, theca interna (steroidogenic and non-steroidogenic cells with interspersed vasculature), and theca externa (smooth muscle). The graded shades of blue in the granulosa cells represent the gradient of signaling molecules emanating from the oocyte. Green indicates cells that express the receptor for LH (LHR) (see Fig. 3A). (B) After LH receptor activation, within minutes of ovulation. The mass of cumulus cells has expanded, and the follicular volume has increased. The apical region where ovulation will occur has thinned. Basolateral regions have invaginated. Blood vessels within the theca have dilated. Figure made in Biorender.com.

microvilli (Baena & Terasaki, 2019; Clarke, 2022). The outer ~5–15 granulosa layers are called mural granulosa. The ~3 outermost mural cell layers form a pseudostratified epithelium with cell bodies extending projections that contact the basal lamina (Baena & Terasaki, 2019; Baena et al., 2020; Lipner & Cross, 1968). The cumulus and mural cells are separated by a fluid-filled antral space, except at one region where the cumulus-oocyte complex and mural cells are connected, until they are separated during ovulation. The position of the cumulus-oocyte complex with respect to the apical side of the follicle is variable (for examples of the variability, see Owen & Jaffe, 2024, supplementary material).

Cells at different radial positions within the granulosa cell compartment show different patterns of gene expression, as a consequence of opposing signaling gradients within the follicle. The oocyte in the center of the follicle releases transforming growth factor beta signaling proteins that suppress mural transcripts, such as the LH receptor, and promote cumulus transcripts in the cells closest to the oocyte – in the cumulus cells as well as some of the mural cells closest to the antrum (Diaz, Wigglesworth, & Eppig, 2007; Eppig, Wigglesworth, Pendola, & Hirao, 1997; Salustri, Yanagishita, Underhill, Laurent, & Hascall, 1992; Su et al., 2008). Conversely, follicle stimulating hormone promotes mural transcripts and suppresses cumulus transcripts, and it has been proposed that due to its delivery to the follicle by the blood vessels in the theca, FSH may be present at a higher concentration in the outer mural granulosa cells vs the follicle interior (Diaz et al., 2007). In addition, a signal from the basal lamina acts on the outer granulosa cells that contact it to localize LH receptor expression to these cells (Eppig et al., 1997; Furman, Rotmensch, Kohen, Mashiach, & Amsterdam, 1986).

Outside of the basal lamina is a multi-layered theca region that is comprised of multiple types of cells (Fig. 1A). The theca interna contains interspersed steroidogenic and non-steroidogenic cells (Nicol, Rodriguez, & Yao, 2020) as well as endothelial cells that form a dense network of blood capillaries that support the avascular granulosa region and deliver hormones including LH (Macchiarelli, Jiang, Nottola, & Sato, 2006; McKey, Cameron, Lewis, Batchvarov, & Capel, 2020). Surrounding the theca interna is a layer of smooth muscle cells (theca externa) (Nicol et al., 2020). The theca on the apical side of each preovulatory follicle abuts against the ovarian surface epithelium, which surrounds the ovary.

The structure of the preovulatory follicle is similar in all mammals, except that its diameter is larger in larger species – ~0.4 mm in a mouse,

~2 cm in a woman, ~12 cm in an Indian rhinoceros (Bächler, Menshykau, De Geyter, & Iber, 2014). Oocyte diameters range from ~60 μm to ~130 μm, with a slight correlation with body weight (Gosden & Telfer, 1987). The numbers of mural cell layers and cumulus cell layers in the preovulatory follicle show only small differences among different species, but the antral space and total number of granulosa cells are much larger in the follicles of larger animals (Bächler et al., 2014; Gosden & Telfer, 1987). The larger number of granulosa cells is essential in larger animals because in addition to providing a nest for the oocyte, the granulosa cells of the follicle produce estrogen. Larger animals need the greater mass of granulosa cells to produce more estrogen.

3. Luteinizing hormone receptor localization and signaling

In preovulatory follicles of mice, rats, and hamsters, LH receptors are expressed in some of the mural granulosa cells and in some of the theca cells (Fig. 1A). Before the preovulatory stage, only the theca cells, not the granulosa cells, express receptors for LH. The localization of these receptors was originally determined by in situ hybridization of probes for mRNA and by binding of a radioactively labeled agonist (Bortolussi, Marini, & Dal Lago, 1977; Camp, Rahal, & Mayo, 1991; Oxberry & Greenwald, 1982; Richards et al., 1976). More recently, higher resolution localization was accomplished by use of mice with an HA epitope tag at the N-terminus of the endogenous receptor, such that HA antibodies could be used for immunolocalization (Baena et al., 2020; Owen & Jaffe, 2024) (see Fig. 3A below). The epitope tag was needed because native levels of the LH receptor have not been conclusively detectable with antibodies against the receptor protein itself. Within the mural granulosa cells, the LH receptors are present only in the outer few layers of cells that extend processes that contact the basal lamina (Baena et al., 2020). Among these cells, only some express the LH receptor (Baena et al., 2020). In the theca, LH receptor expression is also heterogeneous. Apical and basolateral regions show no differences in the distribution of LH receptors (see examples in the supplementary material of Owen & Jaffe, 2024).

LH receptors in the mural granulosa cells are well established to transduce the signal from the LH surge to cause ovulation, but while LH receptors in theca cells are essential for follicle growth (Pakarainen, Zhang,

Nurmi, Poutanen, & Huhtaniemi, 2005), the role of theca LH receptors in responding to the LH surge is uncertain. Which cell types in the theca express the LH receptor has also not been determined. Single cell RNA sequencing databases (Fan et al., 2019; Morris et al., 2022) indicate that some cells express both LH receptors and steroidogenic enzymes, but have not conclusively defined whether only the steroidogenic cells express LH receptors. Analysis of spatial transcriptomics databases (Huang et al., 2024; Mantri, Zhang, Spanos, Ren, & De Vlaminck, 2024) could be informative. Studies of primate endothelial cell lines have raised the question of whether LH receptors may also be expressed in cells of the theca vasculature (Lund, Pearson, Sage, & Duffy, 2023). mRNA encoding the LH receptor has been detected in these cell lines, and LH elevates their cAMP levels and elicits other responses. However, the lack of well validated LH receptor antibodies has limited conclusions regarding the presence of LH receptors in theca vasculature of intact follicles. Immunolocalization using thin sections of mouse ovaries expressing the HA-tagged LH receptor, together with immunolocalization of proteins marking other cell types in the theca, could address these questions.

Following LH release from the pituitary, plasma LH levels remain above baseline for several hours, in mice (Czieselsky et al., 2016; Kovacic & Parlow, 1972; Owen, Zhou, Bernard, & Jaffe, 2021), rats (Daane & Parlow, 1971), and hamsters (Norman & Greenwald, 1972). The ~30 kDa LH protein is small enough to permeate through the capillaries in the theca layers of the follicle (Hess, Chen, & Larsen, 1998). LH then crosses the basal lamina and activates its receptors in the mural granulosa cells. In mice (Kovacic & Parlow, 1972), rats (Daane & Parlow, 1971), and hamsters (Norman & Greenwald, 1972), ovulation occurs approximately 11–14 h after the start of the LH surge.

The LH receptor is coupled to multiple G-proteins, including G_s and $G_{q/11}$. Most of the granulosa cell responses are mediated by G_s activation of adenylyl cyclase and the resulting elevation of cAMP and activation of protein kinase A (PKA) (Hunzicker-Dunn & Mayo, 2015). cAMP diffuses through gap junctions to all of the granulosa cells (Lyga et al., 2016), such that the signal initiated in the outer mural granulosa cells results in rapid protein phosphorylation and ensuing signaling cascades throughout the entire granulosa compartment. Signals also spread inwards from the outer to the inner mural granulosa cells and to the cumulus cells by extracellular diffusion of EGF receptor ligands that are generated by LH receptor activation (Conti, Hsieh, Zamah, & Oh, 2012).

Elevating cAMP by applying inhibitors of cAMP phosphodiesterases is sufficient to trigger ovulation, and to increase synthesis of progesterone and progesterone receptors to levels comparable to those in response to LH (Vigone et al., 2018). Progesterone signaling is essential for ovulation (Kim, Bagchi, & Bagchi, 2009; Lydon et al., 1995). Many other transcriptional events that are also essential for ovulation, including synthesis of prostaglandin synthase (Sirois et al., 2004), are triggered at least in part by LH activation of PKA (Hunzicker-Dunn & Mayo, 2015).

However, mice with granulosa cell-specific deletion of $G\alpha_q$ and $G\alpha_{11}$, G-proteins that act to elevate Ca^{2+} rather than cAMP, ovulate only about half the number of oocytes as controls (Breen et al., 2013). Correspondingly, LH-induced increases in mRNA encoding several proteins required for ovulation (including the progesterone receptor, endothelin, and the protease ADAMTS1) are reduced in ovaries from these mice (Breen et al., 2013). Pharmacological inhibition of $G_{q/11}$ also delays ovulation (Egbert et al., 2019). These results indicate that signaling by both G_s and $G_{q/11}$ is required for optimal ovulation. LH induces elevation of Ca^{2+} in both mural granulosa and theca cells (Egbert et al., 2019), but the spatiotemporal dynamics of the Ca^{2+} elevation in the follicle during the later phases of ovulation have not been investigated.

4. Methods for inducing and observing ovulation

Studies of follicular structure during ovulation were initially conducted using ovaries from naturally cycling animals on the day of proestrous (for example, Norman & Greenwald, 1972). For mice (Czieselsky et al., 2016; Kovacic & Parlow, 1972), rats (Daane & Parlow, 1971), and hamsters (Norman & Greenwald, 1972), the LH surge begins a few hours before the circadian lights go off in the animal room, with some variability in timing for individual animals (Czieselsky et al., 2016). Because of this variability, most studies of ovulation are now performed using ovaries from animals stimulated by injection of LH or an LH analog, or by isolating follicles and exposing them to LH or an LH analog in vitro (Tsafriri, Lindner, Zor, & Lamprecht, 1972; Vigone et al., 2018; Thomas, Marx, Penir, & Schuh, 2024).

Many studies have used a different LH receptor agonist, human chorionic gonadotropin, (hCG) in place of LH. hCG is produced by trophoblast cells of the human placenta, and is readily available from urine of

pregnant women. hCG acts on the corpus luteum to maintain early pregnancy (Stouffer & Hennebold, 2015). In women and other primates, LH and chorionic gonadotropin are both natural agonists of the same receptor, produced by the *LHCGR* gene. Mice, rats, hamsters, and rabbits do not produce chorionic gonadotropin, but hCG is an effective agonist for their LH receptors. The use of hCG in place of LH originated with early bioassays for human pregnancy, in which mice or rabbits were injected with urine from women; ovulation of the mouse or rabbit indicated pregnancy. Follicular growth and LH receptor expression are usually stimulated by injection of animals on the first day of their 4-day estrous cycle with equine chorionic gonadotropin (eCG, also known as PMSG), which mimics the action of follicle stimulating hormone. Subsequent hCG injection ~2 days later causes ovulation with a time course close to that following an endogenous LH surge. However, some aspects of LH receptor signaling, such as the kinetics of cAMP production, are not identical for LH and hCG (Casarini et al., 2012).

By 10 min after intraperitoneal injection, hCG reaches the ovarian follicles, as indicated by its rapid stimulation of an increase in permeability of theca blood vessels (Powers, Chen, Russell, & Larsen, 1995) (see below). However, the concentration of hCG at the follicle, and the kinetics of its concentration, are not known exactly, and some aspects of LH receptor signaling depend on agonist concentration (for example, Vigone et al., 2018). Alternatively, ovulation can be stimulated by injection of neuropeptides that induce an endogenous LH surge by signaling in the hypothalamus. During a natural LH surge, kisspeptin release stimulates gonadotropin releasing hormone release, which then stimulates LH release from the pituitary (Skorupskaite, George, & Anderson, 2014). Injection of kisspeptin results in a transient increase in serum LH with concentration and kinetics close to that in cycling mice (Owen et al., 2021) and also induces ovulation in mice (Owen & Jaffe, 2024; Owen et al., 2021) and women (Abbara et al., 2020). Likewise, GnRH injection stimulates ovulation in rats (Dekel, Sherizly, Tsafriri, & Naor, 1983). Injection of kisspeptin or GnRH to elicit an endogenous LH surge provides a more physiological method for stimulation of ovulation compared with injection of hCG or LH.

Many studies of ovulation use 3–4-week old prepubertal mice or rats. Injection of these animals with eCG causes a set of their follicles to grow to the preovulatory stage and induces expression of the LH receptor. One advantage of using prepubertal instead of adult mice is the ability to obtain ovaries at a precisely defined stage of development. When subsequently

injected with hCG, LH, or kisspeptin, these hormonally "primed" prepubertal mice ovulate with normal kinetics (for example, see Owen & Jaffe, 2024).

Follicles isolated from prepubertal mice and incubated with follicle stimulating hormone to allow progression to the preovulatory stage have been another useful system for studies of ovulation (Egbert et al., 2019; Vigone et al., 2018; Thomas, Marx, Penir, & Schuh, 2024). These follicles are optically clearer than follicles obtained from eCG-primed mice, and when cultured on optically clear organotypic membranes (Millicell cell culture inserts) they flatten somewhat, facilitating live imaging of the ovulatory process. Ovulation in these isolated follicles occurs with normal or close to normal kinetics (Egbert et al., 2019; Thomas, Marx, Penir, & Schuh, 2024). Isolated follicles that have been cultured in a 3-dimensional alginate matrix can also ovulate, following removal from the alginate and transfer to a culture plate (Skory, Xu, Shea, & Woodruff, 2015). Use of isolated follicles has many advantages, particularly for imaging, but blood circulation to the follicle is not preserved, and the amount of theca retained after isolation is variable. A more physiological but more challenging approach is to image ovulation in ovaries that are still connected to the vasculature (Blandau, 1955; Migone et al., 2016).

Overall, these various approaches have provided consistent and complementary information about the ovulatory process. In the sections below, we discuss conclusions and questions that have emerged from these studies about several interrelated structural changes in preovulatory follicles that occur in response to LH (Fig. 1B).

5. Cumulus expansion and follicular enlargement, interrelated events

By 5 h after LH receptor stimulation, the extracellular spaces between the cumulus cells, and between the mural cells adjacent to the antrum increase (see Norman & Greenwald, 1972; Parr, 1974) (Fig. 1B). The spaces arise as a consequence of a dramatic increase in amount of extracellular matrix, due to the transcription of hyaluronic acid synthase (HAS2), the secretion of hyaluronic acid, and the association of several proteins with the hyaluronic acid (Salustri, Campagnolo, Klinger, & Camaioni, 2019). This process, known as "cumulus expansion" is accompanied by loss of gap junctional communication between the cumulus cells and the oocyte at 6–9 h after LH receptor stimulation (Eppig, 1982).

In mice lacking the hyaluronic acid-linking protein inter-α-trypsin inhibitor protein, cumulus expansion fails to occur, but oocytes are still ovulated, as assayed by their presence in the oviduct (Zhuo et al., 2001). However, the number of oocytes present in the oviduct is only 40 % of control (Zhuo et al., 2001). It is unknown if this reduction is due to fewer oocytes being ovulated or to less efficient capture of the oocytes by the ciliary action of the oviduct.

Accompanying cumulus expansion, the volume of the antral space in the follicle increases (Martin & Talbot, 1987; Norman & Greenwald, 1972; Thomas, Marx, Penir, & Schuh, 2024). Based on measurements of cross-sectional area, the antral volume of hamster follicles increases by almost 4-fold during the 11 h after hCG injection; it then decreases during the 3 h preceding ovulation, to about half of the peak value (Martin & Talbot, 1987). Follicular enlargement followed by contraction is also seen in isolated mouse follicles, although the magnitude of the changes is smaller (Thomas, Marx, Penir, & Schuh, 2024). The increase in antral volume has been proposed to be due to hyaluronic acid generating an osmotic gradient that draws fluid into the antrum (Martin & Talbot, 1987). This hypothesis was recently tested by applying an inhibitor of HAS2 (4-MU) to isolated mouse follicles (Thomas, Marx, Penir, & Schuh, 2024). 4-MU partially inhibits the increase in follicular volume, although 4-MU can have effects other than inhibiting HAS2 (Pibuel et al., 2023). The concept that an osmotic gradient causes the increase in follicular volume is also supported by inhibition of the volume increase in response to applying dextran outside of the follicle (Thomas, Marx, Penir, & Schuh, 2024).

6. Apical thinning, a multistep process

Release of the oocyte requires thinning of the cellular layers of the mural granulosa, theca and surface epithelium on the apical side of the follicle (Figs. 1B and 2A). The thinning of the apical mural granulosa layer begins within 6 h of the ovulatory stimulus (Fig. 2B); by 8 h, it decreases to about 30–70 % of its original thickness, reflecting a decrease in the number of cell layers (Martin & Talbot, 1987; Owen & Jaffe, 2024). The basolateral mural granulosa layer also thins in hamster, but not in mouse follicles. There is little or no change in the thickness of the theca interna during the initial 8 h (Martin & Talbot, 1987).

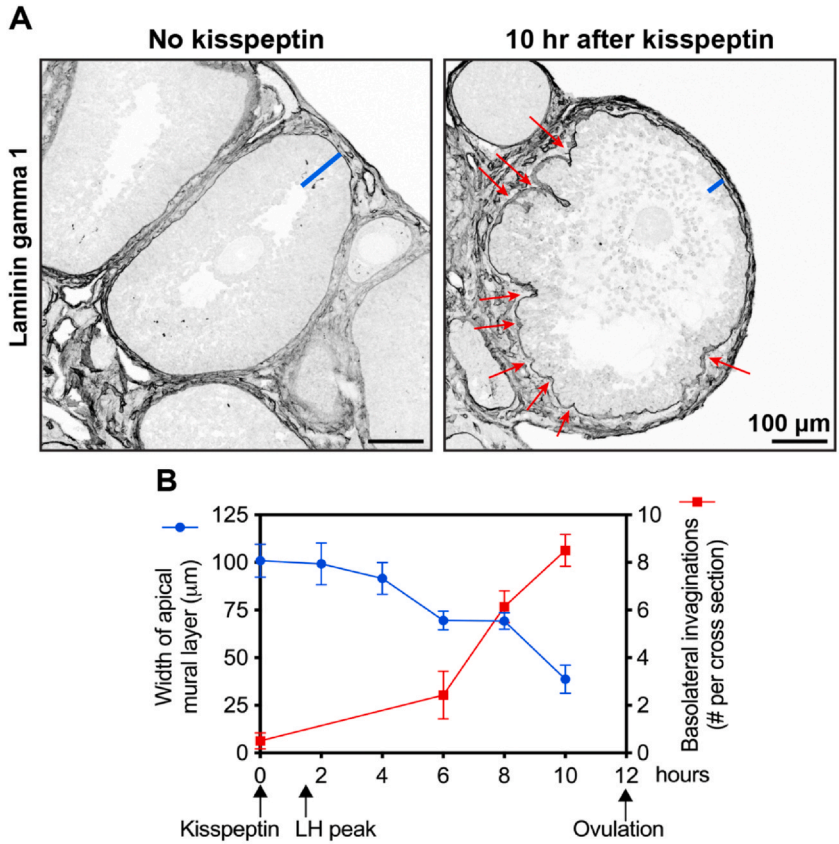

Fig. 2 Apical thinning and basolateral invaginations induced by LH in mouse preovulatory follicles. (A) Preovulatory follicles within ovaries from eCG-primed ~24-day-old mice, before or 10 h after kisspeptin injection to induce an LH surge. Cryosections were labeled with an antibody against laminin gamma 1 (Santa Cruz Biotechnology, sc-65643), to mark the basal lamina. Images are aligned with the apical surface on the right. The blue lines indicate thinning of the mural granulosa layer at the follicle apex, and the red arrows indicate basolateral invaginations. (B) Kinetics of apical thinning of the mural granulosa and formation of basolateral invaginations after the LH surge. *B is modified from Owen and Jaffe (2024), with permission.*

It is not well understood how LH signaling causes the initial thinning of the mural granulosa layer, although one factor appears to be the secretion of hyaluronic acid by the inner layers of the mural granulosa, resulting in these cells separating from the mural layer and adhering to the cumulus mass (Salustri et al., 1992) (Fig. 1B). These "mucified" mural cells are recovered with cumulus masses when follicles are punctured at 9 h after hCG injection. Correspondingly, the number of cells in the cumulus mass increases from

~1000 in an eCG-primed mouse before hCG injection to ~3000 at 15 h after hCG injection (Salustri et al., 1992). Cumulus cell proliferation is a possible alternative explanation of the increase in cell number (Salustri et al., 1992), although mitosis is infrequent after the LH surge, and the few cells that were classified as mitotic might be difficult to distinguish from atretic cells (Norman & Greenwald, 1972). The concept that the mural granulosa layer thins because some of its cells separate and join the cumulus mass is supported by images of hamster and mouse follicles that show mural granulosa cells sloughing off into the antrum during this time frame (Martin & Talbot, 1987; Norman & Greenwald, 1972; Zhuo et al., 2001). Loss of the gap junctions that hold the mural granulosa cells together, due to their internalization (Larsen, Wert, & Brunner, 1987), and possibly loss of other adhesion proteins due to internalization or proteolysis (see more about proteolysis below) might facilitate cells leaving the mural epithelium.

Additionally, within the first 8 h after the ovulatory stimulus, some of the LH receptor-expressing mural granulosa cells in the outer half of the mural layer migrate into the inner half of the mural layer, and some of these cells come into contact with the cumulus mass (Owen & Jaffe, 2024) (Fig. 3). Whether cells that do not express the LH receptor also migrate inwards, and whether cellular ingression contributes to apical thinning, is unknown. Possibly the cellular migration acts together with the several factors discussed above to cause relocation of cells from the mural layer to the cumulus-oocyte complex, resulting in the initial thinning of the apical mural granulosa layer. Another factor that might contribute to the thinning of the mural granulosa region is the enlargement of the antral volume, causing the cells of the mural epithelium to redistribute into a thinner layer with a larger surface area (Martin & Talbot, 1987).

In the final ~3 h preceding follicle rupture, the number of cell layers in the the apical mural granulosa layer, and in the apical theca layer, drops precipitously (Martin & Talbot, 1987). Proteolysis of collagen is required for ovulation (Curry & Smith, 2006; Duffy et al., 2019; Ichikawa, Ohta, Morioka, & Murao, 1983; Robker et al., 2018), suggesting that proteolysis of extracellular matrix could be a factor in apical thinning. In support of this concept, local protease activity in the apical region of the mural granulosa layer was detected by placing frozen sections of rat ovaries, 12 h after hCG injection, on a fluorescent gelatin substrate (Curry, Song, & Wheeler, 2001). The protease activity is metal-dependent, based on inhibition by EDTA. The time course of this local increase in metalloprotease activity has not been reported.

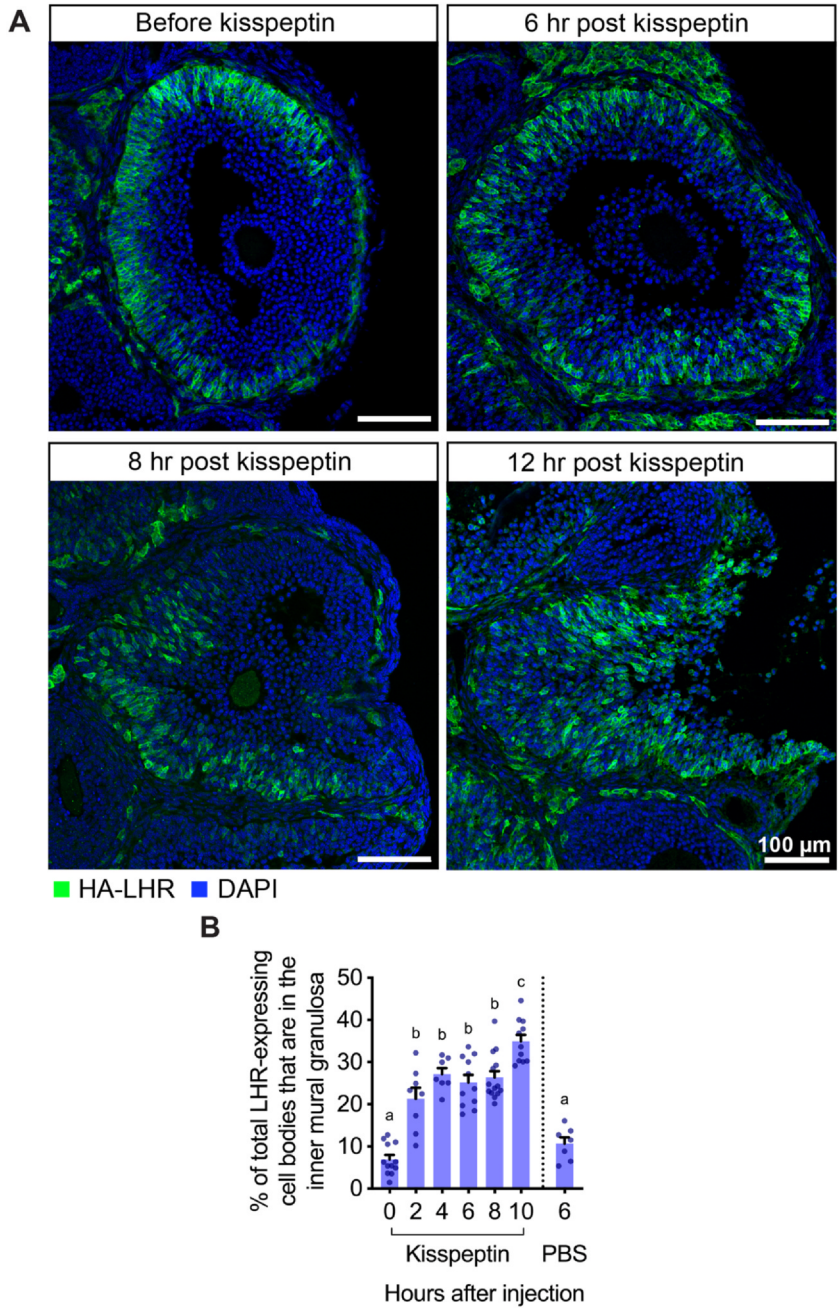

Fig. 3 Migration of LH receptor-expressing mural granulosa cells into the ovarian follicle interior in response to LH. (A) Confocal images of cryosections of follicles in ovaries before and at 6, 8, and 12 h after injection of HA-LHR-expressing

(Continued)

Several different extracellular matrix-targeting proteases are synthesized in response to an ovulatory stimulus, in both granulosa and theca cells (Ohnishi, Ohnishi, Shibuya, & Takahashi, 2005). ADAMTS1 is of particular interest because the mRNA encoding it increases > 100-fold fold by 8 h after injection of rats with hCG; the mRNA is localized in the mural granulosa cells (Espey et al., 2000). Mice lacking *Adamts1* show reduced ovulation, with ~20–30 % of the control number of oocytes ovulated (Brown et al., 2010; Mittaz et al., 2004). In the $Adamts1^{-/-}$ mice, oocytes are seen trapped in unruptured follicles. However, the initial thinning of the apical mural granulosa layer still occurs, with only 1–2 layers of mural cells present at the apex in images at 12 h after hCG (Brown et al., 2010; Mittaz et al., 2004). In contrast, images of the theca in the $Adamts1^{-/-}$ mice did not show thinning, suggesting that the inhibition of ovulation in these mice might be primarily due to the theca remaining intact. Possibly the burst of ADAMTS1 synthesis in the mural granulosa cells acts to degrade the extracellular matrix of the basal lamina separating the granulosa and theca cells, and the matrix between the theca cells, causing the final thinning of the mural granulosa and theca layer that allows the follicle to rupture. Mice lacking various other individual proteases have shown little or no effect on ovulation, possibly due their redundant functions (Ohnishi et al., 2005).

7. Invagination and contraction of the follicular surface

While the follicular apex is thinning in preparation for rupture, invaginations appear in the basolateral surface, as seen in ovarian histological sections, (Brown et al., 2010; Szołtys, Galas, Jabłonka, & Tabarowski, 1994; Zhuo et al., 2001) (Figs. 1B and 2A). These invaginations start as early as 6 h before ovulation (Owen & Jaffe, 2024) (Fig. 2B) and become progressively larger, often forming a V-shape at the base of the follicle (Gothié, 1967; Martin & Talbot, 1981; Owen & Jaffe, 2024) (Figs. 1B and 3A). The apical

Fig. 3—Cont'd mice with kisspeptin to induce an endogenous LH surge. At 6 and 8 h, HA-LHR-expressing cells are migrating inwards. At 12 h, the follicle wall has ruptured, releasing the cumulus-oocyte complex. Sections were labeled for HA-LHR immunofluorescence and nuclei (DAPI). Images were obtained from ~24-day-old eCG-primed mice. Similar inward migration of mural granulosa cells occurs in naturally cycling adult mice (Owen & Jaffe, 2024). (B) Time course of the inward migration. *Modified from Owen and Jaffe (2024), with permission.*

side of the follicle sometimes collapses inwards as well (Owen & Jaffe, 2024; Zhuo et al., 2001) (Fig. 3A). Correlating with these indications of contractile activity, follicular volume decreases in the few hours preceding ovulation (Martin & Talbot, 1981; Thomas, Marx, Penir, & Schuh, 2024).

Contraction of smooth muscle cells in the outer theca has been proposed to be a factor in causing contraction of the follicular surface, based on electron micrographs of ovaries just before ovulation showing smooth muscle cells with a contracted shape, relative to what is seen 3 h earlier (Martin & Talbot, 1981). Another indicator of smooth muscle contraction is phosphorylation of the myosin regulatory light chain protein (MRLC, also known as myosin light chain 2 or MYL9) on its regulatory serine (Ito, Okamoto, Ito, Zhe, & Dohi, 2022). Measurements at 6 and 12 h after hCG application to isolated follicles or injection into mice have shown that MRLC is phosphorylated, but its phosphorylation state before LH exposure has not been examined (Thomas, Marx, Penir, & Schuh, 2024). Spatiotemporal imaging of Ca^{2+} in the theca smooth muscle cells might provide information as to whether or not the theca smooth muscle cells show an increase in contraction just preceding ovulation.

Measurements of contractile force generated by application of endothelin to rat or mouse ovary tissue strips indicate that ovarian smooth muscle cells have receptors for endothelin (Cacioppo et al., 2017; Ko et al., 2006), and endothelin-2 mRNA is synthesized in mural granulosa cells at ~11 h after hCG injection (Palanisamy et al., 2006). Thus, endothelin from the granulosa cells could cause theca smooth muscle cells to contract in the final minutes before the follicle ruptures. Mice lacking endothelin in their granulosa cells ovulate fewer oocytes, supporting the hypothesis that endothelin-induced smooth muscle contraction could help to expel the oocyte (Cacioppo et al., 2017). However, endothelin synthesis is too slow to explain the earlier surface invaginations. Contraction of theca smooth muscle could also result from activation of other $G_{q/11}$-linked receptors by agonists from the mural cells that are synthesized earlier, such as prostaglandins (Land, Lane, Fugate, & Hannon, 2021) and neurotensin (Shrestha et al., 2023).

Alternatively, the surface invaginations may result because as the antral volume expands, the wall of the follicle buckles inwards due to its confinement by the stiff ovarian tissue around it (see (Trushko et al., 2020)). The LH-induced invaginations are dependent on the ADAMTS1 protease (Brown et al., 2010), and protease action on the extracellular matrix of the follicle could weaken the theca and mural layers, allowing them to buckle.

Forces generated by the inward migration of mural granulosa cells that adhere to the basal lamina have also been proposed to contribute to pulling in of the follicular surface (Owen & Jaffe, 2024). In summary, the factors causing the LH-induced invaginations in the follicle surface are uncertain, and whether the invaginations and/or theca smooth muscle contraction enable the release of the oocyte remains to be determined.

8. Increases in vascular permeability, capillary diameter, and angiogenesis

In response to LH, the blood vessels in the theca rapidly increase their permeability to proteins. Before LH receptor stimulation, the permeability of blood capillaries is limited to proteins smaller than ~200 kDa and is also determined by protein charge (Hess et al., 1998; Powers et al., 1995). However, by 10 min after intraperitoneal injection of mice with hCG, a 220 kDa protein (inter-α-trypsin inhibitor protein) that was previously unable to pass from the serum into the follicular fluid, appears in the follicular fluid (Hess et al., 1998; Powers et al., 1995). Proteins larger than ~700 kDa are retained within the capillaries, even after the ovulatory stimulus (Hess et al., 1998). The permeability increase has been confirmed by injection of mice with fluorescently labeled Ficoll (Mitsube, Brännström, & Haraldsson, 2013).

As mentioned above (see Section 5), inter-α-trypsin inhibitor protein is critical for linking hyaluronic acid, allowing formation of the cumulus matrix, and in the absence of this protein, the number of oocytes found in the oviduct is reduced (Zhuo et al., 2001). In addition, after mating, very few eggs from mice lacking inter-α-trypsin inhibitor protein undergo cleavage, suggesting that they failed to fertilize (Zhuo et al., 2001). These observations indicate the LH-induced increase in vascular permeability serves the essential function of allowing transfer from the serum to the follicle of a protein needed for successful reproduction.

Whether LH acts to increase capillary permeability by way of LH receptors in the endothelial cell membranes, as suggested by studies of monkey ovarian endothelial cell lines (Lund et al., 2023), or by way of paracrine signaling from LH receptors in nearby theca or mural granulosa cells, is unknown. Nitric oxide may be a mediator of the LH-induced permeability increase, based on mimicking of the permeability increase by the nitric oxide donor sodium nitroprusside and inhibition of the increase by inhibitors of nitric oxide synthase (Powers et al., 1995).

LH receptor stimulation also regulates capillary diameter (Fig. 1B). Blood volume in the rat ovary starts to increase by 4 h after hCG injection (Tanaka, Espey, & Okamura, 1989), associated with an increase in diameter of the capillaries in the theca (Kranzfelder, Reich, Abisogun, & Tsafriri, 1992; Macchiarelli et al., 2006), which is detected by 3 h after hCG (Kranzfelder et al., 1992). The LH-induced increases in capillary permeability and diameter within the follicle could reflect an increased need for gas exchange, nutrient supply and waste removal during the dynamic process of ovulation. A recent study showed that just before ovulation, blood capillaries in the follicular apex constrict, such that blood flow is inhibited, and it was suggested that the decrease in blood flow could cause apical thinning and follicular rupture (Migone et al., 2016). However, isolated follicles that are disconnected from the blood circulation can also ovulate in response to LH (Egbert et al., 2019; Tsafriri, Lindner, Zor, & Lamprecht, 1972; Vigone et al., 2018; Thomas, Marx, Penir, & Schuh, 2024).

In addition to its regulation of capillary permeability and diameter, LH stimulates angiogenesis in the follicle. Angiogenic genes are upregulated in granulosa cells within 4 h of injecting mice with hCG, and the number of endothelial cells increases by 8 h (Guzmán, Hughes, & Murphy, 2022). The angiogenesis that begins before ovulation increases dramatically after ovulation, as blood vessels grow into the interior of the forming corpus luteum (Stocco, Telleria, & Gibori, 2007; Stouffer & Hennebold, 2015).

9. Conclusions

Despite intensive investigation over the past century, much remains mysterious about the structural changes in the ovarian follicle that result in ovulation in response to luteinizing hormone. Both the theca and mural granulosa layers of the follicle thin, and clearly this is essential for the follicle to rupture. However, the events causing apical thinning, such as the role of proteolysis of the extracellular matrix, are not well understood. Whether the signals initiating the thinning arise solely from LH receptors in the mural cells, or also from those in the theca, is uncertain. There are indications that contraction of smooth muscle cells in the basal region of the theca might provide pressure that breaks the thinned follicle wall. However, direct evidence as to whether this smooth muscle contraction is essential, when it occurs, and what controls it, is lacking. Thinning of the apical mural granulosa region and invaginations in the basolateral region

begin many hours before the rupture of the follicle, accompanied by inward migration of mural granulosa cells. However, the functional significance of these early structural changes for ovulation, and for the subsequent formation of the corpus luteum, remains to be determined.

Acknowledgments

We thank John Eppig his insightful comments on the manuscript, and Jeremy Egbert, Lisa Mehlmann, Rachael Norris, Siu-Pok Yee, and Mark Terasaki for helpful discussions. Research in our laboratory is supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (F31 HD107918 to C.M.O., and R37 HD014939 to L.A.J.).

References

Abbara, A., Eng, P. C., Phylactou, M., Clarke, S. A., Richardson, R., Sykes, C. M., ... Dhillo, W. S. (2020). Kisspeptin receptor agonist has therapeutic potential for female reproductive disorders. *The Journal of Clinical Investigation, 130*, 6739–6753. https://doi.org/10.1172/JCI139681.

Bächler, M., Menshykau, D., De Geyter, C., & Iber, D. (2014). Species-specific differences in follicular antral sizes result from diffusion-based limitations on the thickness of the granulosa cell layer. *Molecular Human Reproduction, 20*, 208–221. https://doi.org/10.1093/molehr/gat078.

Baena, V., Owen, C. M., Uliasz, T. F., Lowther, K. M., Yee, S.-P., Terasaki, M., ... Jaffe, L. A. (2020). Cellular heterogeneity of the luteinizing hormone receptor and its significance for cyclic GMP signaling in mouse preovulatory follicles. *Endocrinology, 161*, bqaa074. https://doi.org/10.1210/endocr/bqaa074.

Baena, V., & Terasaki, M. (2019). Three-dimensional organization of transzonal projections and other cytoplasmic extensions in the mouse ovarian follicle. *Scientific Reports, 9*, 1–13. https://doi.org/10.1038/s41598-018-37766-2.

Blandau, R. J. (1955). Ovulation in the living albino rat. *Fertility and Sterility, 6*, 391–404. https://doi.org/10.1016/S0015-0282(16)32100-8.

Bortolussi, M., Marini, G., & Dal Lago, A. (1977). Autoradiographic study of the distribution of LH(HCG) receptors in the ovary of untreated and gonadotrophin-primed immature rats. *Cell and Tissue Research, 183*, 329–342. https://doi.org/10.1007/BF00220640.

Breen, S. M., Andric, N., Ping, T., Xie, F., Offermans, S., Gossen, J. A., & Ascoli, M. (2013). Ovulation involves the luteinizing hormone-dependent activation of G(q/11) in granulosa cells. *Molecular Endocrinology (Baltimore, Md.), 27*, 1483–1491. https://doi.org/10.1210/me.2013-1130.

Brown, H. M., Dunning, K. R., Robker, R. L., Boerboom, D., Pritchard, M., Lane, M., & Russell, D. L. (2010). ADAMTS1 cleavage of versican mediates essential structural remodeling of the ovarian follicle and cumulus-oocyte matrix during ovulation in mice. *Biology of Reproduction, 83*, 549–557. https://doi.org/10.1095/biolreprod.110.084434.

Cacioppo, J. A., Lin, P.-C. P., Hannon, P. R., McDougle, D. R., Gal, A., & Ko, C. (2017). Granulosa cell endothelin-2 expression is fundamental for ovulatory follicle rupture. *Scientific Reports, 7*, 817. https://doi.org/10.1038/s41598-017-00943-w.

Camp, T. A., Rahal, J. O., & Mayo, K. E. (1991). Cellular localization and hormonal regulation of follicle-stimulating hormone and luteinizing hormone receptor messenger RNAs in the rat ovary. *Molecular Endocrinology (Baltimore, Md.), 5*, 1405–1417. https://doi.org/10.1210/mend-5-10-1405.

Casarini, L., Lispi, M., Longobardi, S., Milosa, F., La Marca, A., Tagliasacchi, D., ... Simoni, M. (2012). LH and hCG action on the same receptor results in quantitatively and qualitatively different intracellular signalling. *PLoS One, 7*, e46682. https://doi.org/10.1371/journal.pone.0046682.

Clarke, H. J. (2022). Transzonal projections: Essential structures mediating intercellular communication in the mammalian ovarian follicle. *Molecular Reproduction and Development, 89*, 509–525. https://doi.org/10.1002/mrd.23645.

Conti, M., Hsieh, M., Zamah, A. M., & Oh, J. S. (2012). Novel signaling mechanisms in the ovary during oocyte maturation and ovulation. *Molecular and Cellular Endocrinology, 356*, 65–73. https://doi.org/10.1016/j.mce.2011.11.002.

Curry, T., & Smith, M. (2006). Impact of extracellular matrix remodeling on ovulation and the folliculo-luteal transition. *Seminars in Reproductive Medicine, 24*, 228–241. https://doi.org/10.1055/s-2006-948552.

Curry, T. E., Song, L., & Wheeler, S. E. (2001). Cellular localization of gelatinases and tissue inhibitors of metalloproteinases during follicular growth, ovulation, and early luteal formation in the rat. *Biology of Reproduction, 65*, 855–865. https://doi.org/10.1095/biolreprod65.3.855.

Czieselsky, K., Prescott, M., Porteous, R., Campos, P., Clarkson, J., Steyn, F. J., ... Herbison, A. E. (2016). Pulse and surge profiles of luteinizing hormone secretion in the mouse. *Endocrinology, 157*, 4794–4802. https://doi.org/10.1210/en.2016-1351.

Daane, T. A., & Parlow, A. F. (1971). Periovulatory patterns of rat serum follicle stimulating hormone and luteinizing hormone during the normal estrous cycle as revealed by radioimmunoassays: Effects of pentobarbital. *Endocrinology, 88*, 653–663. https://doi.org/10.1210/endo-88-3-653.

Dekel, N., Sherizly, I., Tsafriri, A., & Naor, Z. (1983). A comparative study of the mechanism of action of luteinizing hormone and a gonadotropin releasing hormone analog on the ovary. *Biology of Reproduction, 28*, 161–166.

Diaz, F. J., Wigglesworth, K., & Eppig, J. J. (2007). Oocytes determine cumulus cell lineage in mouse ovarian follicles. *Journal of Cell Science, 120*, 1330–1340. https://doi.org/10.1242/jcs.000968.

Duffy, D. M., Ko, C., Jo, M., Brannstrom, M., & Curry, T. E. (2019). Ovulation: Parallels with inflammatory processes. *Endocrine Reviews, 40*, 369–416. https://doi.org/10.1210/er.2018-00075.

Dumont, J. N., & Brummett, A. R. (1978). Oogenesis in *Xenopus laevis* (Daudin). V. Relationships between developing oocytes and their investing follicular tissues. *Journal of Morphology, 155*, 73–97. https://doi.org/10.1002/jmor.1051550106.

Egbert, J. R., Fahey, P. G., Reimer, J., Owen, C. M., Evsikov, A. V., Nikolaev, V. O., ... Jaffe, L. A. (2019). Follicle-stimulating hormone and luteinizing hormone increase Ca^{2+} in the granulosa cells of mouse ovarian follicles. *Biology of Reproduction, 101*, 433–444. https://doi.org/10.1093/biolre/ioz085.

Eppig, J. J. (1982). The relationship between cumulus cell-oocyte coupling, oocyte meiotic maturation, and cumulus expansion. *Developmental Biology, 89*, 268–272. https://doi.org/10.1016/0012-1606(82)90314-1.

Eppig, J. J., Wigglesworth, K., Pendola, F., & Hirao, Y. (1997). Murine oocytes suppress expression of luteinizing hormone receptor messenger ribonucleic acid by granulosa cells. *Biology of Reproduction, 56*, 976–984. https://doi.org/10.1095/biolreprod56.4.976.

Espey, L. L., Yoshioka, S., Russell, D. L., Robker, R. L., Fujii, S., & Richards, J. S. (2000). Ovarian expression of a disintegrin and metalloproteinase with thrombospondin motifs during ovulation in the gonadotropin-primed immature rat. *Biology of Reproduction, 62*, 1090–1095. https://doi.org/10.1095/biolreprod62.4.1090.

Fan, X., Bialecka, M., Moustakas, I., Lam, E., Torrens-Juaneda, V., Borggreven, N. V., ... de, S. (2019). Single-cell reconstruction of follicular remodeling in the human adult ovary. *Nature Communications, 10*, 1–13. https://doi.org/10.1038/s41467-019-11036-9.

Feng, Y., Piñon Gonzalez, V. M., Lin, M., Egertová, M., Mita, M., & Elphick, M. R. (2023). Localization of relaxin-like gonad-stimulating peptide expression in starfish reveals the gonoducts as a source for its role as a regulator of spawning. *The Journal of Comparative Neurology, 531*, 1299–1316. https://doi.org/10.1002/cne.25496.

Furman, A., Rotmensch, S., Kohen, F., Mashiach, S., & Amsterdam, A. (1986). Regulation of rat granulosa cell differentiation by extracellular matrix produced by bovine corneal endothelial cells. *Endocrinology, 118*, 1878–1885. https://doi.org/10.1210/endo-118-5-1878.

Gosden, R. G., & Telfer, E. E. (1987). Scaling of follicular sizes in mammalian ovaries. *Journal of Zoology, 211*, 157–168. https://doi.org/10.1111/j.1469-7998.1987.tb07459.x.

Gothié, S. (1967). Contribution à l'etude cinétique de l'ovulatoin chez la Souris prépubère. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales, 161*, 554–556.

Guzmán, A., Hughes, C. H. K., & Murphy, B. D. (2022). Liver receptor homolog 1 (LRH-1) regulates follicle vasculature during ovulation in mice. *Reproduction (Cambridge, England), 164*, 283–289. https://doi.org/10.1530/REP-22-0135.

Hess, K. A., Chen, L., & Larsen, W. J. (1998). The ovarian blood follicle barrier Is both charge- and size-selective in mice. *Biology of Reproduction, 58*, 705–711. https://doi.org/10.1095/biolreprod58.3.705.

Huang, R., Kratka, C. E., Pea, J., McCann, C., Nelson, J., Bryan, J. P., ... Goods, B. A. (2024). Single-cell and spatiotemporal profile of ovulation in the mouse ovary. *BioRxiv Prepr. Serv. Biol.*, 2024.05.20.594719. https://doi.org/10.1101/2024.05.20.594719.

Hughes, C. H. K., & Murphy, B. D. (2021). Nuclear receptors: Key regulators of somatic cell functions in the ovulatory process. *Molecular Aspects of Medicine, 78*, 100937. https://doi.org/10.1016/j.mam.2020.100937.

Hunzicker-Dunn, M., & Mayo, K. (2015). *Gonadotropin signaling in the ovary. Knobil and Neill's physiology of reproduction*. Elsevier, 895–945. https://doi.org/10.1016/B978-0-12-397175-3.00020-X.

Ichikawa, S., Ohta, M., Morioka, H., & Murao, S. (1983). Blockage of ovulation in the explanted hamster ovary by a collagenase inhibitor. *Reproduction (Cambridge, England), 68*, 17–19. https://doi.org/10.1530/jrf.0.0680017.

Ito, M., Okamoto, R., Ito, H., Zhe, Y., & Dohi, K. (2022). Regulation of myosin light-chain phosphorylation and its roles in cardiovascular physiology and pathophysiology. *Hypertension Research, Official Publication of the Japanese Society of Hypertension, 45*, 40–52. https://doi.org/10.1038/s41440-021-00733-y.

Jaffe, L. A., & Egbert, J. R. (2017). Regulation of mammalian oocyte meiosis by intercellular communication within the ovarian follicle. *Annual Review of Physiology, 79*, 237–260. https://doi.org/10.1146/annurev-physiol-022516-034102.

Kim, J., Bagchi, I. C., & Bagchi, M. K. (2009). Control of ovulation in mice by progesterone receptor-regulated gene networks. *Molecular Human Reproduction, 15*, 821–828. https://doi.org/10.1093/molehr/gap082.

Ko, C., Gieske, M. C., Al-Alem, L., Hahn, Y., Su, W., Gong, M. C., Iglarz, M., & Koo, Y. (2006). Endothelin-2 in ovarian follicle rupture. *Endocrinology, 147*, 1770–1779. https://doi.org/10.1210/en.2005-1228.

Kovacic, N., & Parlow, A. F. (1972). Alterations in serum FSH/LH ratios in relation to the estrous cycle, pseudopregnancy, and gonadectomy in the mouse. *Endocrinology, 91*, 910–915. https://doi.org/10.1210/endo-91-4-910.

Kranzfelder, D., Reich, R., Abisogun, A. O., & Tsafriri, A. (1992). Preovulatory changes in the perifollicular capillary network in the rat: role of eicosanoids. *Biology of Reproduction, 46*, 379–385. https://doi.org/10.1095/biolreprod46.3.379.

Land, K. L., Lane, M. E., Fugate, A. C., & Hannon, P. R. (2021). Ovulation is inhibited by an environmentally relevant phthalate mixture in mouse antral follicles in vitro. *Toxicological Sciences: Official Journal of the Society of Toxicology, 179*, 195–205. https://doi.org/10.1093/toxsci/kfaa170.

Larsen, W. J., Wert, S. E., & Brunner, G. D. (1987). Differential modulation of rat follicle cell gap junction populations at ovulation. *Developmental Biology, 122*, 61–71. https://doi.org/10.1016/0012-1606(87)90332-0.

Lipner, H., & Cross, N. L. (1968). Morphology of the membrana granulosa of the ovarian follicle. *Endocrinology, 82*, 638–641. https://doi.org/10.1210/endo-82-3-638.

Lund, M., Pearson, A. C., Sage, M. A. G., & Duffy, D. M. (2023). Luteinizing hormone receptor promotes angiogenesis in ovarian endothelial cells of Macaca fascicularis and Homo sapiens. *Biology of Reproduction, 108*, 258–268. https://doi.org/10.1093/biolre/ioac189.

Lydon, J. P., DeMayo, F. J., Funk, C. R., Mani, S. K., Hughes, A. R., Montgomery, C. A., ... O'Malley, B. W. (1995). Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes & Development, 9*, 2266–2278. https://doi.org/10.1101/gad.9.18.2266.

Lyga, S., Volpe, S., Werthmann, R. C., Götz, K., Sungkaworn, T., Lohse, M. J., & Calebiro, D. (2016). Persistent cAMP signaling by internalized LH receptors in ovarian follicles. *Endocrinology, 157*, 1613–1621. https://doi.org/10.1210/en.2015-1945.

Macchiarelli, G., Jiang, J., Nottola, S. A., & Sato, E. (2006). Morphological patterns of angiogenesis in ovarian follicle capillary networks. A scanning electron microscopy study of corrosion cast. *Microscopy Research and Technique, 69*, 459–468. https://doi.org/10.1002/jemt.20305.

Mantri, M., Zhang, H. H., Spanos, E., Ren, Y. A., & De Vlaminck, I. (2024). A spatio-temporal molecular atlas of the ovulating mouse ovary. *Proceedings of the National Academy of Sciences, 121*, e2317418121. https://doi.org/10.1073/pnas.2317418121.

Martin, G. G., & Talbot, P. (1981). The role of follicular smooth muscle cells in hamster ovulation. *The Journal of Experimental Zoology, 216*, 469–482. https://doi.org/10.1002/jez.1402160316.

Martin, G. G., & Talbot, P. (1987). Formation of the rupture site in preovulatory hamster follicles: Morphological and morphometric analysis of thinning of the granulosa and thecal layers. *Gamete Research, 17*, 303–320. https://doi.org/10.1002/mrd.1120170404.

McKey, J., Cameron, L. A., Lewis, D., Batchvarov, I. S., & Capel, B. (2020). Combined iDISCO and CUBIC tissue clearing and lightsheet microscopy for in toto analysis of the adult mouse ovary. *Biology of Reproduction. 102*, 1080–1089. https://doi.org/10.1093/biolre/ioaa012.

Migone, F. F., Cowan, R. G., Williams, R. M., Gorse, K. J., Zipfel, W. R., & Quirk, S. M. (2016). In vivo imaging reveals an essential role of vasoconstriction in rupture of the ovarian follicle at ovulation. *Proceedings of the National Academy of Sciences of the United States of America, 113*, 2294–2299. https://doi.org/10.1073/pnas.1512304113.

Mita, M. (2023). Relaxin-like gonad-stimulating peptides in asteroidea. *Biomolecules, 13*, 781. https://doi.org/10.3390/biom13050781.

Mitsube, K., Brännström, M., & Haraldsson, B. (2013). Modulation of microvascular permeability in the preovulatory rat ovary by an ovulatory gonadotropin stimulus. *Fertility and Sterility, 99*, 903–909. https://doi.org/10.1016/j.fertnstert.2012.11.017.

Mittaz, L., Russell, D. L., Wilson, T., Brasted, M., Tkalcevic, J., Salamonsen, L. A., ... Pritchard, M. A. (2004). Adamts-1 is essential for the development and function of the urogenital system. *Biology of Reproduction, 70*, 1096–1105. https://doi.org/10.1095/biolreprod.103.023911.

Mogessie, B., Scheffler, K., & Schuh, M. (2018). Assembly and positioning of the oocyte meiotic spindle. *Annual Review of Cell and Developmental Biology, 34*, 381–403. https://doi.org/10.1146/annurev-cellbio-100616-060553.

Morris, M. E., Meinsohn, M.-C., Chauvin, M., Saatcioglu, H. D., Kashiwagi, A., Sicher, N. A., ... Pépin, D. (2022). A single-cell atlas of the cycling murine ovary. *eLife, 11*, e77239. https://doi.org/10.7554/eLife.77239.

Nicol, B., Rodriguez, K., & Yao, H. H.-C. (2020). Aberrant and constitutive expression of FOXL2 impairs ovarian development and functions in mice. *Biology of Reproduction, 103*, 966–977. https://doi.org/10.1093/biolre/ioaa146.

Norman, R. L., & Greenwald, G. S. (1972). Follicular histology and physiological correlates in the preovulatory hamster. *The Anatomical Record, 173*, 95–107. https://doi.org/10.1002/ar.1091730108.

Norris, R. P., Freudzon, M., Mehlmann, L. M., Cowan, A. E., Simon, A. M., Paul, D. L., ... Jaffe, L. A. (2008). Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: One of two paths to meiotic resumption. *Development (Cambridge, England), 135*, 3229–3238. https://doi.org/10.1242/dev.025494.

Ohnishi, J., Ohnishi, E., Shibuya, H., & Takahashi, T. (2005). Functions for proteinases in the ovulatory process. *Biochimica et Biophysica Acta, 1751*, 95–109. https://doi.org/10.1016/j.bbapap.2005.05.002.

Omony, J. B., Biran, J., Kahwa, D., Aizen, J., Golan, M., Nyatia, E., ... Rutaisire, J. (2022). Cloning of gonadotropin Gph-alpha, FSH-beta and LH-beta subunits and seasonal profiles of steroid hormones in wild-caught Nile perch, Lates niloticus. *General and Comparative Endocrinology,* 323–324. https://doi.org/10.1016/j.ygcen.2022.114035.

Owen, C. M., & Jaffe, L. A. (2024). Luteinizing hormone stimulates ingression of mural granulosa cells within the mouse preovulatory follicle. *Biology of Reproduction, 142*. https://doi.org/10.1093/biolre/ioad142.

Owen, C. M., Zhou, X., Bernard, D. J., & Jaffe, L. A. (2021). Kisspeptin-54 injection induces a physiological luteinizing hormone surge and ovulation in mice. *Biology of Reproduction, 104*, 1181–1183. https://doi.org/10.1093/biolre/ioab067.

Oxberry, B. A., & Greenwald, G. S. (1982). An autoradiographic study of the binding of 125 I-labeled follicle-stimulating hormone, human chorionic gonadotropin and prolactin to the hamster ovary throughout the estrous cycle. *Biology of Reproduction, 27*, 505–516. https://doi.org/10.1095/biolreprod27.2.505.

Pakarainen, T., Zhang, F.-P., Nurmi, L., Poutanen, M., & Huhtaniemi, I. (2005). Knockout of luteinizing hormone receptor abolishes the effects of follicle-stimulating hormone on preovulatory maturation and ovulation of mouse graafian follicles. *Molecular Endocrinology (Baltimore, Md.), 19*, 2591–2602. https://doi.org/10.1210/me.2005-0075.

Palanisamy, G. S., Cheon, Y.-P., Kim, J., Kannan, A., Li, Q., Sato, M., ... Bagchi, I. C. (2006). A novel pathway involving progesterone receptor, endothelin-2, and endothelin receptor B controls ovulation in mice. *Molecular Endocrinology (Baltimore, Md.), 20*, 2784–2795. https://doi.org/10.1210/me.2006-0093.

Parr, E. L. (1974). Histological examination of the rat ovarian follicle wall prior to ovulation. *Biology of Reproduction, 11*, 483–503. https://doi.org/10.1095/biolreprod11.5.483.

Pibuel, M. A., Poodts, D., Sias, S. A., Byrne, A., Hajos, S. E., Franco, P. G., & Lompardía, S. L. (2023). 4-Methylumbelliferone enhances the effects of chemotherapy on both temozolomide-sensitive and resistant glioblastoma cells. *Scientific Reports, 13*, 9356. https://doi.org/10.1038/s41598-023-35045-3.

Powers, R. W., Chen, L., Russell, P. T., & Larsen, W. J. (1995). Gonadotropin-stimulated regulation of blood-follicle barrier is mediated by nitric oxide. *American Journal of Physiology – Endocrinology and Metabolism, 269*, E290–E298. https://doi.org/10.1152/ajpendo.1995.269.2.E290.

Quiroga Artigas, G., Lapébie, P., Leclère, L., Bauknecht, P., Uveira, J., Chevalier, S., ... Houliston, E. (2020). A G protein–coupled receptor mediates neuropeptide-induced oocyte maturation in the jellyfish Clytia. *PLoS Biology, 18*, e3000614. https://doi.org/10.1371/journal.pbio.3000614.

Richards, J. S., & Ascoli, M. (2018). Endocrine, paracrine, and autocrine signaling pathways that regulate ovulation. *Trends in Endocrinology & Metabolism TEM, 29*, 313–325. https://doi.org/10.1016/j.tem.2018.02.012.

Richards, J. S., Ireland, J. J., Rao, M. C., Bernath, G. A., Midgley, A. R., & Reichert, L. E. (1976). Ovarian follicular development in the rat: Hormone receptor regulation by estradiol, follicle stimulating hormone and luteinizing hormone. *Endocrinology, 99*, 1562–1570. https://doi.org/10.1210/endo-99-6-1562.

Robker, R. L., Hennebold, J. D., & Russell, D. L. (2018). Coordination of ovulation and oocyte maturation: A good egg at the right time. *Endocrinology, 159*, 3209–3218. https://doi.org/10.1210/en.2018-00485.

Salustri, A., Campagnolo, L., Klinger, F. G., & Camaioni, A. (2019). Molecular organization and mechanical properties of the hyaluronan matrix surrounding the mammalian oocyte. *Matrix Biology: Journal of the International Society for Matrix Biology, 78–79*, 11–23. https://doi.org/10.1016/j.matbio.2018.02.002.

Salustri, A., Yanagishita, M., Underhill, C. B., Laurent, T. C., & Hascall, V. C. (1992). Localization and synthesis of hyaluronic acid in the cumulus cells and mural granulosa cells of the preovulatory follicle. *Developmental Biology, 151*, 541–551. https://doi.org/10.1016/0012-1606(92)90192-J.

Shrestha, K., Al-Alem, L., Garcia, P., Wynn, M. A. A., Hannon, P. R., Jo, M., Drnevich, J., ... Curry Jr, T. E. (2023). Neurotensin expression, regulation, and function during the ovulatory period in the mouse ovary. *Biology of Reproduction, 108*, 107–120. https://doi.org/10.1093/biolre/ioac191.

Sirois, J., Sayasith, K., Brown, K. A., Stock, A. E., Bouchard, N., & Doré, M. (2004). Cyclooxygenase-2 and its role in ovulation: A 2004 account. *Human Reproduction Update, 10*, 373–385. https://doi.org/10.1093/humupd/dmh032.

Skorupskaite, K., George, J. T., & Anderson, R. A. (2014). The kisspeptin-GnRH pathway in human reproductive health and disease. *Human Reproduction Update, 20*, 485–500. https://doi.org/10.1093/humupd/dmu009.

Skory, R. M., Xu, Y., Shea, L. D., & Woodruff, T. K. (2015). Engineering the ovarian cycle using in vitro follicle culture. *Human Reproduction (Oxford, England), 30*, 1386–1395. https://doi.org/10.1093/humrep/dev052.

Stocco, C., Telleria, C., & Gibori, G. (2007). The molecular control of corpus luteum formation, function, and regression. *Endocrine Reviews, 28*, 117–149. https://doi.org/10.1210/er.2006-0022.

Stouffer, R. L., & Hennebold, J. D. (2015). *Structure, function, and regulation of the corpus luteum. Knobil and Neill's physiology of reproduction*. Elsevier Inc, 1023–1076. https://doi.org/10.1016/B978-0-12-397175-3.00023-5.

Su, Y.-Q., Sugiura, K., Wigglesworth, K., O'Brien, M. J., Affourtit, J. P., Pangas, S. A., ... Eppig, J. J. (2008). Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP15 and GDF9 control cholesterol biosynthesis in cumulus cells. *Development (Cambridge, England), 135*, 111–121. https://doi.org/10.1242/dev.009068.

Szołtys, M., Galas, J., Jabłonka, A., & Tabarowski, Z. (1994). Some morphological and hormonal aspects of ovulation and superovulation in the rat. *The Journal of Endocrinology, 141*, 91–100. https://doi.org/10.1677/joe.0.1410091.

Takahashi, T., Hagiwara, A., & Ogiwara, K. (2018). Follicle rupture during ovulation with an emphasis on recent progress in fish models. *Reproduction (Cambridge, England)*. https://doi.org/10.1530/REP-18-0251.

Tanaka, N., Espey, L. L., & Okamura, H. (1989). Increase in ovarian blood volume during ovulation in the gonadotropin-primed immature rat. *Biology of Reproduction, 40*, 762–768. https://doi.org/10.1095/biolreprod40.4.762.

Thomas, C., Marx, T. L., Penir, S. M., & Schuh, M. (2024). Ex vivo imaging reveals the spatiotemporal control of ovulation. *Nature Cell Biology* (in press).

Trushko, A., Di Meglio, I., Merzouki, A., Blanch-Mercader, C., Abuhattum, S., Guck, J., ... Roux, A. (2020). Buckling of an epithelium growing under spherical confinement. *Developmental Cell, 54*, 655–668.e6. https://doi.org/10.1016/j.devcel.2020.07.019.

Tsafriri, A., Lindner, H. R., Zor, U., & Lamprecht, S. A. (1972). In vitro induction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E2. *Reproduction (Cambridge, England), 31*, 39–50. https://doi.org/10.1530/jrf.0.0310039.

Vigone, G., Shuhaibar, L. C., Egbert, J. R., Uliasz, T. F., Movsesian, M. A., & Jaffe, L. A. (2018). Multiple cAMP phosphodiesterases act together to prevent premature oocyte meiosis and ovulation. *Endocrinology, 159*, 2142–2152. https://doi.org/10.1210/en.2018-00017.

Zhuo, L., Yoneda, M., Zhao, M., Yingsung, W., Yoshida, N., Kitagawa, Y., ... Kimata, K. (2001). Defect in SHAP-hyaluronan complex causes severe female infertility: A study by inactivation of the bikunin gene in mice. *The Journal of Biological Chemistry, 276*, 7693–7696. https://doi.org/10.1074/jbc.C000899200.