



Histomorphological and ultrastructural characterization of Granulosa Cells

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Abstract: The granulosa cells are central for female reproduction. These cells establish a very close relationship with the female gamete even before oogonia differentiation during embryogenesis. This relationship continues for months or years and during the maturation of primary and secondary oocytes. After ovulation, cumulus granulosa cells escort the oocyte to the oviduct, whereas the mural granulosa cells that remain in the ovary are transformed into luteal cells. Luteal cells are necessary for implantation and early development of the zygote. The understanding of the intricate mechanisms, pathway activation, and gene expression in the granulosa cells at each of these steps is still evolving.

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Introduction:

Granulosa cells display different phenotypes within the follicle, depending on their location. 108-111 The mural granulosa cells, antral granulosa cells, and cumulus granulosa cells each have distinguishing features that are likely determined by their proximity to the oocyte and theca cells and by the paracrine substances that they produce. The mural granulosa cells in the antral follicle express the greatest steroidogenic activity (Fig. 9.7). In addition, mural granulosa cells in the preovulatory follicle have the highest level of LH receptors. The granulosa cells closest to the antral cavity have a lower expression of steroidogenic enzymes, whereas those in the middle region have greater mitotic activity than the antral and mural granulosa cells.

The cumulus cells, which are released with the oocyte at ovulation, do not express aromatase, and their LH receptor content and level of LH responsiveness are substantially lower than those of their mural counterparts. In mice, they have distinguishing gene expression patterns that include expression of *Slc38a3*, which encodes a sodium coupled neutral amino acid transporter, and higher expression of the anti-müllerian hormone gene, *Amh*. These cells proliferate after the LH surge and are active in producing an extracellular matrix consisting of hyaluronan, proteoglycans, and proteoglycan-binding proteins when stimulated by the prostaglandins generated in response to the ovulatory stimulus. The elaboration of this matrix leads to the preovulatory expansion of the cumulus-oocyte complex, which appears to be essential for ovulation

(Fig. 9.8). Differential patterns of expression of prostaglandin E (EP) receptors allow granulosa cell subpopulations to respond uniquely to PGE2 during ovulation. Cumulus cells appear to respond via EP2 and EP3, whereas rupture of a specific region of the follicle is through EP1 in granulosa cells near the apex. A growing body of literature suggests that cumulus cell viability (e.g., apoptosis) and expression of certain proteins and mRNAs correlates with oocyte competence and success in assisted reproduction. 112

Luteinized granulosa cells of the ovulated follicle undergo terminal differentiation to give rise to the large luteal cell population of the corpus luteum. These cells have an increased capacity to synthesize progesterone, resulting from up-regulation of the machinery required to acquire cholesterol from the circulating lipoproteins, which now have access to the granulosa-lutein cells as a result of neovascularization of the developing corpus luteum. The granulosa-lutein cells also retain the capacity to synthesize estrogens from androgen precursors produced by theca lutein cells.

Granulosa cells, their precursors, or their final differentiation stages are critical components of the ovary that nurture germ cells, sustain oocyte maturation, provide the hormonal milieu required to synchronize uterus receptivity with the release of the mature eggs, and support early pregnancy. Granulosa cells are the only somatic cells that closely interact with the oocyte from the moment the follicle forms until the release of the oocyte at ovulation. Throughout this long relationship, both the oocyte and the granulosa cells go

through significant functional and morphological changes.

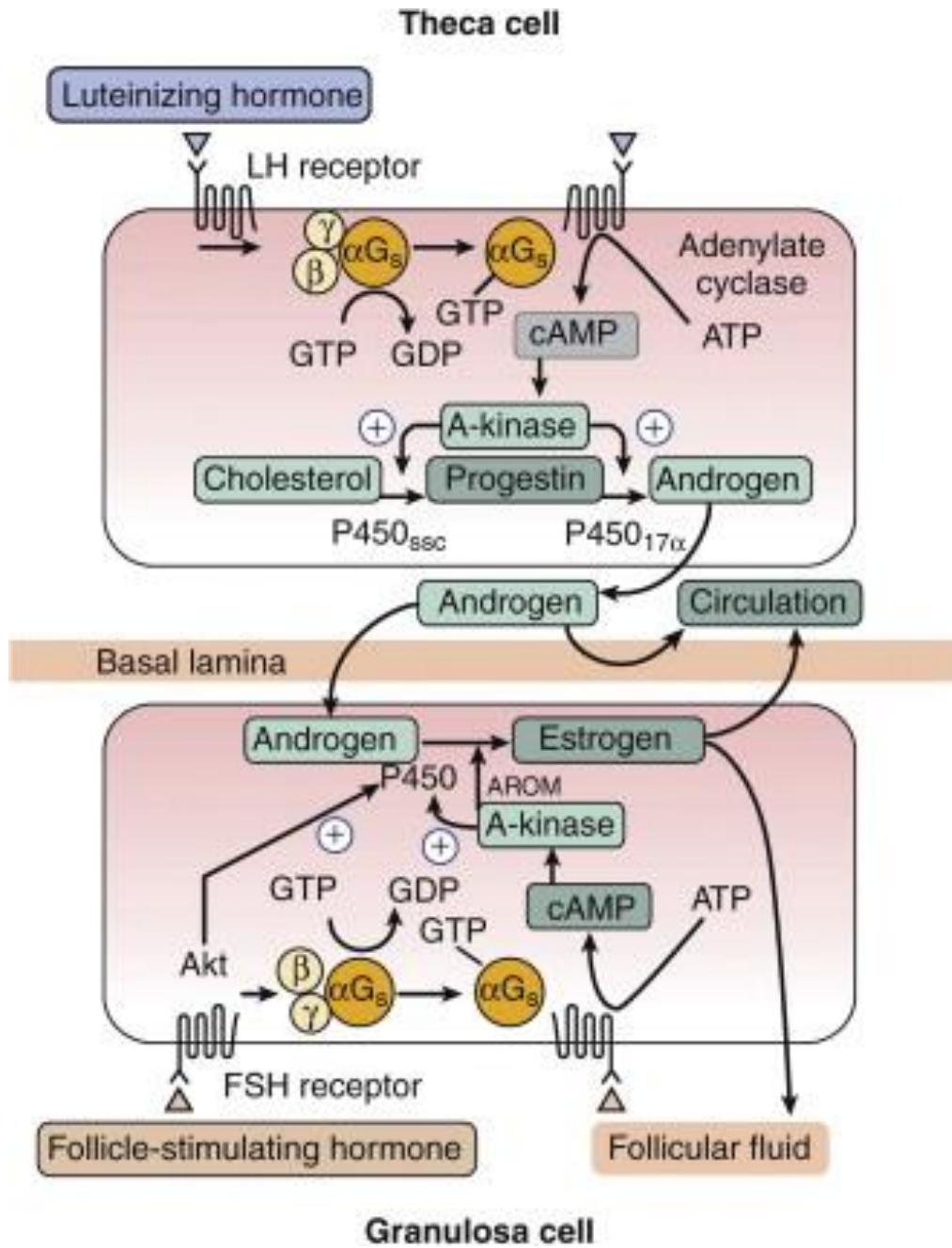


Figure 1. The two-cell–two-gonadotropin system for estradiol synthesis in the follicle.

What do granulosa cells do?

Granulosa cells play a key role in the female reproductive system. They help you get and stay pregnant. Granulosa cells produce reproductive hormones and support ovarian follicles. These follicles are small sacs of fluid in your ovaries that contain eggs. Here’s the process:

From the first day of your period (menstruation) to the release of an egg (ovulation), your anterior pituitary releases follicle-stimulating hormone (FSH). FSH stimulates the granulosa cells to change androgen sex hormones, released by theca cells, to estrogen sex hormones.

As your follicles grow, granulosa cells continue to produce more estrogen. This results in a surge in

lutinizing hormone (LH) from your anterior pituitary. The LH surge leads to the release of an egg from your dominant follicle.

After ovulation, the follicle collapses and becomes a structure called the corpus luteum. The granulosa cells inside the corpus luteum (granulosa lutein cells) produce the hormone progesterone. This helps to maintain a possible pregnancy.

Granulosa cells also produce other hormones and chemicals including anti-Müllerian hormone (AMH). Since levels of AMH decrease as follicle numbers decrease, AMH testing may help measure egg count. Low levels of AMH in your blood may mean that you have fewer remaining eggs (diminished ovarian reserve).

Where are granulosa cells located?

You can find granulosa cells inside a person's ovarian follicles. Besides granulosa cells, these follicles contain: Immature egg (oocyte).

Theca cells. These cells respond to LH by producing androgens and progesterone.

What are the types of granulosa cells?

There are two types of granulosa cells:

Cumulus cells (CC): These cells surround the oocyte and give it nutrients. During ovulation, cumulus cells stay with the egg when it travels through the fallopian tube.

Mural granulosa cells (MGC): These cells line the walls of the follicles and surround the part of the follicle filled with fluid (antrum). Mural granulosa cells support the follicles' growth.

What conditions affect granulosa cells?

Conditions that affect granulosa cells include:

Granulosa cell tumors: This rare type of ovarian cancer makes up 2% of all ovarian tumors. It often occurs around ages 50 to 55. Granulosa cell tumors (or granulosa theca cell tumors) tend to grow slowly and have high recovery rates. Healthcare providers usually use surgery to treat these tumors.

Juvenile granulosa cell tumors: This type of tumor occurs in 5% of children. Providers can often successfully treat these tumors with surgery in their early stages.

What conditions are related to granulosa cells?

Conditions that may relate to granulosa cells include:

Infertility: Providers use AMH levels when deciding how much medication they should use to stimulate egg production during treatments for infertility, such as IVF. But low AMH levels don't give information about the quality of the remaining eggs.

Polycystic ovarian syndrome (PCOS): Providers don't know the exact cause of PCOS. But studies suggest that problems with granulosa cells may affect follicle growth and ovulation.

Primary ovarian insufficiency (POI): Increasing levels of FSH and decreasing quality of granulosa cells may lead to a decrease in the number of a woman's eggs. POI can impact your ability to get pregnant.

What are common tests to check the health of granulosa cells?

Common tests to check the health of granulosa cells include:

Blood tests: Granulosa cell tumors often produce chemicals or hormones that providers can measure in your blood. These include inhibin, AMH, estradiol, testosterone and high levels of CA-125.

CT scan: A CT scan uses X-rays and a computer to produce a 3D image of any tumors. A CT scan usually shows larger tumors better than smaller ones.

Laparoscopy: During laparoscopy, your provider passes a thin tube through a small incision in your abdomen. They can look at your ovaries and take a sample of cells (biopsy) if needed.

Pelvic ultrasound: A pelvic ultrasound uses sound waves to look for growths on your ovaries.

PET scan: A PET scan uses an injectable chemical and a PET scanner to produce images of your ovaries.

What are simple lifestyle changes to keep granulosa cells healthy?

People who've had children or use birth control pills have a lower risk of developing ovarian cancer. If ovarian cancer runs in your family, talk to your healthcare provider about your options.

You can also lower your risk of granulosa cell tumors and ovarian cancer by maintaining a healthy lifestyle, including:

Eating a balanced diet.

Exercising regularly.

Limiting your alcohol intake.

Maintaining a healthy weight.

Visiting your doctor for annual checkups.

Gonadotropin-Independent Activation and Growth Activation The pregranulosa cells and the oocytes of cortical primordial follicles remain dormant for months in rodents or for decades in humans. Primordial follicles then transition to primary follicles. During this transition, the single layer of flattened granulosa cells changes morphology to become cuboidal granulosa cells. Primordial follicle activation occurs independently of pituitary gonadotropins. Instead, it is regulated by factors produced within the ovary and requires the interaction between pregranulosa cells and oocyte. In FOXL2-null mice, granulosa cells remain flattened resulting in the absence of follicle growth suggesting that the activation of these cells in adult ovaries requires FOXL2. Similarly, inhibition of mTORC1 in granulosa cells blocks dormant follicle activation, while its overactivation increases this process. These findings indicate that granulosa cells play a major role in the activation of primordial follicles. An

interaction between the flattened granulosa cells and the oocyte is also critical for follicle activation and is carried out by the KIT and KIT ligand (KITL) system. The oocyte expresses KIT, a tyrosine kinase receptor, on its surface, whereas flattened granulosa cells and later the granulosa cells express KITL, which is able to bind to KIT to promote follicle activation. Preventing KIT/KITL interaction in mice pauses follicle growth at the primordial stage, and few, if any, follicles transition to the primary stage. On the other hand, *in vitro* experiments demonstrated that if ovaries are treated with KITL, a significant number of follicles transition from the primordial to the primary stage (Saatcioglu et al., 2016; Zhang and Liu, 2015). However, the molecular changes involved in the transition from flattened to cuboidal granulosa cells remain to be determined.

Proliferation to Preantral Stage As the ovarian follicle progresses from the primary stage to the preantral stage, the layer of cuboidal granulosa cells proliferates to form multiple layers. Granulosa cell proliferation occurs independently of gonadotropins. However, it is possible that the oocyte influences proliferation. Thus, mouse studies demonstrated that oocytes from follicles with two layers of granulosa cells could stimulate granulosa cell proliferation and differentiation in primordial follicles, suggesting that oocyte-secreted factors are crucial for follicle growth. One such factor is growth differentiation factor 9 (GDF9). For instance, folliculogenesis is arrested at the primary stage in animals lacking GDF9. Direct communication between the granulosa cells and the oocyte is also essential for follicle maturation beyond the primary stage. For instance, knockout of connexins 43 and 37 (CX43 and CX37), which both are components of gap junctions between the oocyte and surrounding granulosa cells, halts folliculogenesis at the primary (unilaminar) stage, and granulosa cell differentiation is impaired. The full spectrum of molecules and signals that travel through gap junctions to promote follicle growth remains to be determined.

Final Differentiation:

Luteinization Mural and cumulus cells have different destinies after the preovulatory surge of LH. The mural cells remain in the ovary, luteinize, and participate in the formation of the corpus luteum, whereas the expanded cumulus cells accompany the metaphase II (secondary) oocyte to the oviduct. During luteinization, the granulosa cells express high levels of StAR, CYP11a1, and 3 β -HSD, which are needed for the production of progesterone. After luteinization, luteinized granulosa cells are called luteal cells and produce high amounts of progesterone. Progesterone stimulates blood vessel growth and nutrient secretion in the endometrium of the uterus to support the implantation of the embryo. FSH stimulates proliferation of granulosa cells; however, after the LH surge, granulosa cells stop dividing and exit

the cell cycle. Luteal cells are found arrested predominantly at the G0/G1 phase of the cell cycle. Cessation of cell proliferation during luteinization is associated with a progressive loss of positive cell-cycle regulators, including cyclins and Cdk2, and with increased expression of the Cdk inhibitors p21cip1 and p27kip. In addition, luteinizing granulosa cells express factors that stimulate the development of an extensive web of blood vessels, which provide not only the substrates for progesterone synthesis but also a mechanism to transfer of the high amounts of progesterone produced by luteal cells to the systemic circulation. One such factor is VEGF, which is expressed in the granulosa compartment only at the preovulatory stage. After ovulation, luteal cells continue expressing VEGF. The block of VEGF before ovulation inhibits luteal angiogenesis (Stocco et al., 2007).

Conclusion

Granulosa cells are inside your ovaries. These cells produce estrogen, progesterone and other hormones. The hormones play a large part in the female reproductive system, from menstruation to ovulation to egg implantation. Conditions related to granulosa cells include granulosa cell tumors and juvenile granulosa cell tumors. PCOS, POI and female infertility may also relate to granulosa cells. You can reduce your risk of granulosa cell disorders by living a healthy lifestyle and making regular visits to your healthcare provider. In the ovary, the corpus luteum (CL) forms a temporal structure. Luteinized mural granulosa cells (GCs), which stem from the ruptured follicle, are the main cells of the CL. They can be isolated from follicular fluid of woman undergoing *in vitro* fertilization. In culture, human GCs are viable for several days and produce progesterone, yet eventually steroid production stops and GCs with increasing time in culture undergo changes reminiscent of the ones observed during the demise of the CL *in vivo*. This short review summarizes the general use of human GCs as a model for the primate CL and some of the data from our lab, which indicate that viability, functionality, survival and death of GCs can be regulated by local signal molecules (e.g., oxytocin and PEDF) and the extracellular matrix (e.g., via the proteoglycan decorin). We further summarize studies, which identified autophagocytotic events in human GCs linked to the activation of an ion channel. More recent studies identified a form of regulated cell death, namely necroptosis. This form of cell death may, in addition to apoptosis, contribute to the demise of the human CL. We believe that human GCs are a unique window into the human CL. Studies employing these cells may lead to the identification of molecular events and novel targets, which may allow to interfere with CL functions.

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