Ovarian Systems Biology

Cell Biology of the Ovary
- Cell types/organization
- Developmental stages (Folliculogenesis)
- Atresia/apoptosis
- Oogenesis

Regulation of Folliculogenesis
- Growth properties of ovarian follicles
- Local production and action of growth factors
- Growth regulations during development
- Primordial follicle transition

Endocrine Regulation of Tissue Function
- Gonadotropin actions (Pituitary/Gonadal Axis)
- Steroid production and action
- Two cell theory modifications
- Hormone actions during development

Cell-Cell Interactions
- Categorization of different cell-cell interactions in the ovary
- Growth factor regulation follicle development
- Oogenesis and systems biology

Required Reading


REFERENCES


Findlay JK. et.al. (2002) Recruitment and development of the follicle; the roles of the transforming growth factor-β superfamily. Molecular and cellular endocrinology 191:35-43.
**Introduction**

Ovaries are female gonads responsible for the generation of female gametes (oocytes) and synthesis of hormones necessary for the regulation of reproductive functions. Since the first description of the ovary reported by Aristotle more than 2000 years ago, information about the ovary has expanded significantly. Knowledge of the formation of the ovary and its endocrine function is essential to understand the mystery of the regeneration of life.

**Anatomy of the Ovary**

Most vertebrates develop a pair of ovaries with the exception of some birds, reptiles and a few mammals that only have one ovary. Ovaries lie on either side of the upper pelvic cavity and against the pelvic wall. They are held in place by a mesentery (mesovarium) connected to a broad ligament. Ovaries are one of the most vascular organs in the body. The ovarian artery (or utero-ovarian artery) which arises from the abdominal aorta reaches the ovary along with the mesovarium. Branches of the ovarian artery enter the ovary through the hilus, the same site at which the venous blood exits. Adrenergic and cholinergic nerves also enter the ovary through the hilus.

Even though the size of the ovary varies, the structure of the ovary is similar among mammalian species (Fig. 1). The ovary consists of an inner medulla, containing a rich vascular bed within loose connective tissue and an outer cortex, where the ovarian follicles are located. The outermost lay of the cortex is a single squamous or cuboidal surface epithelium derived from the peritoneum. Under the surface epithelium lies the tunica albuginea, a poorly delineated layer of dense connective tissue that gives the ovary a whitish color. The cortex of the ovary is made up of numerous follicles of varies sizes and stages of development embedded in the stroma. The stroma is composed of at least three different cell types: connective tissue cells (fibroblasts) performing support functions, smooth muscle cells regulating the contraction of blood vessels and interstitial cells including undifferentiated theca cells and degenerated cells from atretic follicles and regressed corpora lutea. The follicles (follicle is Latin for “little bag”) are structurally very conspicuous because of their variation in size. The microscopic appearance of follicles is different depending on the stage of follicular development whereas the basic cellular organization of follicles is the same. A follicle consists of an oocyte and surrounding follicular wall. Between the oocyte and surrounding follicular wall is a thin transparent membrane, the zona pellucida. The follicular wall contains an inner granulosa layer and an outer theca layer. The granulosa layer surrounds the oocyte and is separated from the theca layer by the basement membrane. The number and function of the granulosa cells changes during follicular growth. In mature follicles, the theca layer can be divided in the theca externa and interna. The theca externa consists of concentrically arranged smooth muscles cells innervated with autonomic nerves. The theca interna has epithelioid cells called interstitial cells, which are steroid producing cells. These cells contain LH and insulin receptors.
and synthesize primarily androgens, of which the predominant steroid is androstenedione. The theca interna has both blood vessels and nerves. The granulosa layer is devoid of blood vessels and nerves at all times.

Once ovulation of the Graafian follicle (tertiary) has occurred, blood derived from torn blood vessels of the theca layer infiltrates the collapsed follicle and results in the formation of the corpus hemorrhagicum, a developing corpus luteum with a bloody core. Luteinizing granulosa and thecal cells begin to divide and invade the antral cavity, which remains after ovulation of the oocyte, forming the corpus luteum (Latin for "yellow body"). Blood vessels from the theca layer grow and penetrate the developing luteal cell mass. If pregnancy does not occur, the corpus luteum degenerates after a certain length of time depending upon the species. The connective tissues replaces the luteal cells and forms the corpus albicans (Latin for "white body"). The ovarian medulla devoid of follicles, contains large, spirally arranged blood vessels, lymphatic vessels and nerves.

Functions of the Ovary

Generation of the Female Gametes

Oogenesis

Female gametes, or oocytes, provide the maternal genetic material for the formation of an embryo. The ovary nurtures thousands of oocytes and functions as an incubator for their development. The development of oocytes (oogenesis) starts with primordial germ cells, residing in sex cords which divide mitotically producing oogonia. Oogonia then become primary oocytes and undergo the first meiosis. The primary oocytes are arrested at the diplotene stage of the first meiosis until they experience the preovulatory LH surge. Then the first meiosis is reinitiated and the membrane of the oocyte nucleus (germinal vesicle) disintegrates, which is called germinal vesicle breakdown. Meiosis of the oocyte is unequal producing a large haploid secondary oocyte and a tiny haploid first polar body. This polar body can divide again or remain single; in either case, it degenerates. Then the secondary oocyte begins the second meiotic division but this division is arrested at metaphase until after sperm penetration of the oocyte, which occurs in the oviduct. Completion of the second meiosis results in a haploid ovum and the second polar body.

Folliculogenesis

Folliculogenesis is a developmental sequence regulated by a number of genes, transcription factors and hormones. During fetal development of humans and postnatal development of mice, oocytes are present in clusters or nests. Majority of these oocytes enter meiosis during embryonic life. As the oocytes separate into individual oocytes, they form primordial follicles and undergo further development called oogenesis. Maturation of oocytes (oogenesis) is closely associated with the development of follicles because factors produced by the oocytes have a major impact on the development of the granulosa and theca layers. Folliculogenesis always begins in the innermost part of the ovarian cortex in mammals. Primordial follicles consist of primary oocytes surrounded by flat squamous pre-granulosa cells. Primordial follicles are the only available source of oocytes during the entire reproductive period of the female. As primordial follicles develop into primary follicles, there are changes in the oocyte. It
increases in diameter and develops an extracellular matrix, the zona pellucida. Reactivation of the oocyte genome causes the oocyte to secrete growth factors which play a crucial role in the growth of the follicle. As primary follicles grow, the granulosa cells divide mitotically so that secondary follicles have two to six layers of cuboidal-shaped granulosa cells. Secondary follicles also acquire an additional somatic layer, the theca. There are at least two sources of the theca progenitor cells: somatic precursors of the fetal ovary and mesenchymal cells in the neighboring mesonephros. The formation of this theca layer is dependent upon the presence of growth differentiation factor-9 (GDF-9) produced by the oocyte. The theca layer forms around the basement membrane in secondary follicles and ultimately forms the theca interna and theca externa. Follicular growth from primordial to secondary follicles is gonadotrophin-independent. During the formation of tertiary follicles or preantral follicles, follicles continue to grow in size. As follicles progress from secondary follicles to antral follicles, granulosa cells secrete a fluid that accumulates between cells. Large amounts of additional fluid diffuse out of the thecal blood vessels and are added to the fluid which is called follicular fluid. Follicular fluid contains steroid and protein hormones, anticoagulants, enzymes, and electrolytes and is similar to blood serum in appearance and contents. The follicle filled with follicular fluid is the tertiary or preovulatory follicle. These follicles have a mural granulosa layer of four to six layers and the theca layer is differentiated into an inner theca interna and an outer theca externa. Oocytes in preovulatory follicles are suspended in follicular fluid by a stalk of granulosa cells, the cumulus oophorus. Immediately surrounding the oocyte is a thin ring of granulosa cells, the corona radiata. At this state the follicle is called the Graafian follicle and appears as a transparent vesicle that bulges from the surface of the ovary.

Even though one of the function of the ovary is to produce oocytes, the majority of oocytes never ovulate. The number of oocytes reaches its maximum soon after the ovaries are formed. After that time oocyte number decreases dramatically. At birth, a female has all the oocytes she will have in her life; no new oocytes are formed after birth. The vast majority of oocytes, enclosed in follicles, around 99.9%, are eliminated before ovulation through a process called atresia which is due to the activation of apoptosis in the oocyte and granulosa cells. Follicles can become atretic at any stage of development.

**Production of Hormones**

Another function of the ovary is to secrete hormones which act on the hypothalamus and pituitary to regulate the secretion of hormones by these two tissues, thus establishing the hypothalamic-pituitary-ovarian axis. The ovarian hormones also regulate the function of the reproductive tract and ultimately reproduction.

**Protein and peptide hormones**

(i) **Inhibin and activin**: Inhibin and activin were first isolated from gonadal fluids because of their effects on production of follicle stimulating hormone (FSH) by the pituitary in mammals. Inhibins consist of two disulfide-bridged subunits, the α and β subunits, whereas activins consist of two β subunits. The primary source of inhibin and activin in the ovary is the maturing follicles and the corpus luteum. The function of inhibins is to modulate FSH secretion at the level of the pituitary, whereas the function of activins is to increase FSH secretion at the level of the pituitary. Inhibins and activins have antagonistic actions. Inhibins and activins also function as intraovarian hormones.

(ii) **Follistatin**: Follistatin is a FSH-modulating polypeptide not related to TGF-β. Follistatin acts as a binding protein and a functional antagonist of activin. Granulosa cells in antral follicles and luteal cells secrete follistatin.

(iii) **Relaxin**: Relaxin is produced by the corpus luteum. The structure of relaxin is very similar to that of insulin but has < 20% amino acid homology. In the human, relaxin is the highest during the first trimester of pregnancy after which the concentrations are relative stable. In the rat and the pig, relaxin reaches the highest concentration prior to parturition. Relaxin in these species functions to soften the cervix and vagina for the passage of the fetus during parturition and to promote the growth of nipples. Relaxin also acts on nonreproductive tissues, such as skin and the gastrointestinal tract.

(iv) **Growth factors**: The ovary not only secretes endocrine hormones to regulate functions of other reproductive organs but also produces growth factors to coordinate the activities of different ovarian compartments. Many growth factors, such as insulin-like growth factors, transforming growth factors and epidermal growth factor are produced by the oocyte and somatic cells in the ovary. This complex intraovarian regulation system is less important than the extraovarian regulation by the pituitary hormones. These growth factors form a delicate interactive communication web inside the ovary. Without them, the ovarian cells cannot interact with each other and the growth of the ovary is halted.

**Steroid hormones**

The ovary uses cholesterol as the precursor for steroid synthesis. Cholesterol is metabolized into progestins, androgens, and estrogens by different compartments of the follicles (Fig. 2).

(i) **Progestins pregnenolone**: Is the most important progestins (C21 pregnane family) produced by follicles because of its key position as the precursor of all steroid hormones. The most abundant progestin is progesterone, produced as a biosynthetic intermediate by follicles at all growing stages of development and as a secretary end product of the corpus luteum. In the developing follicles, the theca layer is the primary site of progesterin productions. Immediately prior to ovulation, the granulosa cells stimulated by LH also synthesize progesterone. After ovulation the corpus luteum
synthesizes copious amounts of progesterone needed to prepare the uterus for implantation and later for the maintenance of pregnancy.

(ii) **Androgens**. The follicle is a significant source of ovarian androgens (C19 androstane family). Pregnenolone and progesterone are converted into androgen metabolites, dehydroepiandrosterone and androstenedione, respectively. These are two metabolites are then transformed into testosterone. The theca layer of the follicle is the primary source of ovarian androgens.

(iii) **Estrogens**. Physiologically, the estrogens (C18 estrane family) especially estrone and estradiol-17-β, are the most important of the ovarian steroids. Androstenedione and testosterone are the immediate biosynthetic precursors of estrone and estradiol-17-β, respectively. Their names reflect their roles in the induction of sexual receptivity (estrus) in female mammals. Estrone was the first sex steroid isolated and identified. The granulosa layer is the major site of estrogen synthesis in the mammalian ovary.

**Fig. 2** Biosynthesis of steroid hormones from cholesterol. This scheme provides a simplistic view of a highly organized and complicated process that requires multiple enzymes. Adapted from Hafez, E.S.E (1993) Folliculogenesis, egg maturation, and ovulation. In Reproduction in farm animals, 6th ed., pp. 114–143. Lea and Febiger, Philadelphia, p. 79.

Regulation of Ovarian Functions

**Regulation of Folliculogenesis**

Growth of primordial follicles to the preantral stage is independent of gonadotropins and is controlled by intraovarian growth factors. Growth of follicles after the preantral stage depends on appropriate patterns of secretion, sufficient concentrations and adequate ratios of FSH and LH in the blood. FSH plays a major role in early follicular development. FSH stimulates granulosa cell mitosis and accumulation of follicular fluid. Granulosa cells synthesize estrogens in response to FSH which further enhance the mitotic effect of FSH. Moreover, FSH induces granulosa cell sensitivity to LH by increasing LH receptor expression. Abundant LH receptors in granulosa cells prepare the luteinization of granulosa cells in response to the ovulatory LH surge in mammals. In contrast, theca cells are stimulated only by LH and LH receptors are present from the beginning of the formation of the theca layer.
Regulation of Steroidogenesis

The steroidogenic output of the ovary is a function of coordinated actions of theca and granulosa cells. Differences in gonadotropin receptors on the membrane, in the activity of steroidogenic enzymes and in compartmentalization in the follicle result in a unique partnership in steroid synthesis between theca and granulosa cells. The principal site of estrogen synthesis in the mammalian ovary is granulosa cells under the control of FSH. Androgen production appears to be the primary steroidogenic function of theca cells in response to LH. Androgens from theca cells provide substrates for granulosa cells to synthesize estrogens. The action of LH on theca androgen production, together with the action of FSH on granulosa estrogen synthesis, forms the basis of the "two-cell, two-hormone" theory for the control of steroidogenesis in the ovary (Fig. 3).

Further Reading

“Systems Biology of Reproduction”
Spring 2022 (Even Years) – Course Syllabus
Biol 475/575 Level Undergraduate/Graduate (3 Credit)
SLN: (475) – 05504, (575) – 05505
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and on Canvas/Panopto and Discussion Sessions in person and on WSU Zoom for all campuses
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Schedule/Lecture Outline –
January 11 & 13 Week 1
Systems Biology Introduction
18 & 20 Week 2
Molecular/ Cellular/ Reproduction Systems
25 & 27 Week 3
Sex Determination Systems
February 1 & 3 Week 4
Male Reproductive Tract Development & Function
8 & 10 Week 5
Female Reproductive Tract Development & Function
15 & 17 Week 6
Gonadal Developmental Systems Biology
22 & 24 Week 7
Testis Systems Biology
March 1 & 3 Week 8
Ovary Systems Biology
8 & 10 Week 9
Epigenetics and Transgenomterial Gamend Disease
14 – 18 Week 10
Spring Break
22 & 24 Week 11
Gonadogenesis/Stem Cells/Clining
29 & 31 Week 12
Hypothalamus/Pituitary Development & Function
April 5 & 7 Week 13
Reproductive Endocrinology Systems
12 & 14 Week 14
Fetalt Development & Birth Systems
19 & 21 Week 15
Ovarian Systems Biology
26 & 28 Week 16
Reproductive/Hypothalamus
May 3 & 5 Week 17
Assisted Reproduction/Contraception

Spring 2022 – Systems Biology of Reproduction
Lectures Outline – Ovarian Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
March 1, 2022
Week 8

Ovarian Systems Biology

Primary Papers:

Discussion
Student 20: Reference 1 above
• What approach and technology was used?
• What gene categories and networks were identified?
• What oocyte maturation and folliculogenesis insights were identified?

Student 21: Reference 2 above
• What are the technology used and objectives?
• What epigenetic regulation and gene network were identified?
• What insights are provided into the development of polycystic ovarian disease?

Student 22: Reference 3 above
• What is the experimental and systems approach?
• What new insights provided on polycystic ovary disease?
• What gene signaling networks were identified for primordial follicle development?
The Mammalian Ovary

- Primordial Follicles
- Graffian Follicle
- Ovulatory Follicle
- Corpus Luteum
- Antral Follicle
- Primordial Follicles

Follicle maturation and ovulation

- Primordial Follicle
- Primary Follicle
- Secondary Follicle
- Tertiary Follicle
- Graffian Follicle
- Ovulatory Follicle
- Corpus Luteum
- Germ cell
- Cyst formation
- Oocyte
- Granulosa cells
- Pre-granulosa cells
- Fetal ovary
- Adult ovary
- Primary follicle
- Secondary follicle
- Antral follicle
- Ovulatory follicle
Cell Death

1858 Virchow - discuss process active & passive

Process = necrosis & degenerative

1860’s-1870’s: Weigert & Cohnheim - Students Virchow

Describe further - physiological need

1885 - Walter Flemming - ovary model used

1st micrograph apoptosis

previously he described - chromatin & mitosis

termed cell death process - chromatolysis

• Apoptosis termed -1914 for programmed cell death

• Rediscovered in 1980’s

• Term not best
Influences of pro- and anti-apoptotic effectors on death receptor-mediated apoptosis. Apoptosis is controlled by several pro-(green) and anti-(red) apoptotic proteins. The balance of these proteins are important to ensure tissue homeostasis.


Bubble chart of potential signaling pathways generated by DEGs. Pathway analysis was performed to associate the unique DEGs with pathways using the KEGG database. The size and color of each bubble represent number of DEGs in each pathway and P-value respectively. DEG, differentially expressed gene.


The Mammalian Ovary

- Graffian Follicle
- Ovulatory Follicle
- Corpus Luteum
- Antral Follicle
- Primordial Follicles

Text: 1. Morphological and biochemical aspects of ovary

A. Graffian Follicle
   - Primordial follicle
   - Development (growth, maturation, ovulation)
   - Increased aromatase production
   - Increased progesterone production (luteinization)
   - Increased LH and FSH synthesis and secretion
   - Increased estradiol production
   - Increased FSH receptor expression
   - Increased oocyte nuclear maturation
   - Increased sperm penetration

B. Ovulatory Follicle
   - Graffian follicle matures
   - Ovulation occurs
   - Corpus luteum forms

C. Corpus Luteum
   - Stores and secretes additional hormones
   - Post-ovulatory follicle becomes corpus luteum
   - Secretion of progesterone and estrogen
   - Regression and formation of secondary sex cells in some species

D. Ovary
   - Glandular vesicles/ductules
Number of Oocytes During Stages of Early Folliculogenesis

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<th>Proliferation</th>
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<td>Primate</td>
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<td>1,000,000</td>
<td>700,000</td>
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</table>

Only 500 human follicles ovulate.

Oogonial Stem Cell

The controversial existence and functional potential of oogonial stem cells.
Cyclic epithelial remodelling in the ovary and fimbria

Follicular Assembly and Primordial to Primary Follicle Transition

SP1 governs primordial folliculogenesis by regulating pregranulosa cell development in mice.

Nuclear exclusion of SMAD2/3 in granulosa cells is associated with primordial follicle activation in the mouse ovary.

Hardy K, Mora JM, Dunlop C, Carzaniga R, Franks S, Fenwick MA.


Interaction of Follicle-Stimulating Hormone and Stem Cell Factor to Promote Primordial Follicle Assembly in the Chicken.

Guo C, Liu G, Zhao D, Mi Y, Zhang C, Li J.


Effects of FSH treatment on chicken folliculogenesis in vivo. (A,B) Morphology of the primordial and growing follicles in 6-day-old chicken ovaries. Scale bar: 20 μm. Arrowheads and arrows represent the somatic cells and oocytes, respectively. (C) Changes in the primordial and growing follicle numbers after FSH treatment. (D) The c-KIT and SCF mRNA expressions were measured by qRT-PCR in ovaries from the 6-day-old chickens after FSH treatment at day 4. GAPDH was used as the normalization control. T-tests were used to determine statistically significant differences. The values are the mean ± SEM of six experiments. Asterisks indicate significant differences (*P<0.05, **P<0.01, ***P<0.001).
FOXO1/3 depletion in granulosa cells alters follicle growth, death and regulation of pituitary FSH.

Forkhead box O member FOXO1 regulates the majority of follicle-stimulating hormone responsive genes in ovarian granulosa cells
Herndon MK, Lee NC, Donaubauer EM, Kyriazis B, Hunzicker-Dunn M.

Somatic cells initiate primordial follicle activation and govern the development of dormant oocytes in mice.
JAK signaling regulates germline cyst breakdown and primordial follicle formation in mice
Biol Open. 2018 Jan 17;7(1).
In Vivo Follicular Kinetics

In Vitro Follicular Kinetics

Candidate Factors for Primordial to Primary Follicle Transition

- KL (Stem Cell Factor)(Kit Ligand)
- bFGF (basic Fibroblast Growth Factor)
- LIF (Leukemia Inhibitory Factor)
- GDF-9 (Growth and Differentiation Factor 9)
- BMP-4 (Bone Morphogenic Protein 4)
- EGF (Epidermal Growth Factor)
- HGF (Hepatocyte Growth Factor)
- KGF (FGF-7)(Karotinocyte Growth Factor)
- IGF-1 (Insulin Like Growth Factor 1)
- VEGF (Vascular Endothelial Growth Factor)
- TNFα (Tumor Necrosis Factor)
Current Model of Early Folliculogenesis

Neurotrophin NT3 promotes ovarian primordial to primary follicle transition.
Nilsson E, Dole G, Skinner MK.

Lhx8 regulates primordial follicle activation and postnatal folliculogenesis.

Roles of Gremlin 1 and Gremlin 2 in regulating ovarian primordial to primary follicle transition.
Nilsson EE, Larsen G, Skinner MK.
Dynamic expression patterns of Irx3 and Irx5 during germline nest breakdown and primordial follicle formation promote follicle survival in mouse ovaries.


Dazl determines primordial follicle formation through the translational regulation of Tex14.


ELAVL2-directed RNA regulatory network drives the formation of quiescent primordial follicles.

Sirtuins in female meiosis and in reproductive longevity
Berta N Vazquez, Alejandro Vaquero, Karen Schindler
Mol Reprod Dev. 2020 Dec;87(12):1175-1187.

Identification of a unique epigenetic profile in women with diminished ovarian reserve.
Systems Biology Approach:

1. Comparative mRNA expression with several treatments.
2. Network analysis.
3. Unbiased literature analysis.

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<tr>
<th>Used Compound</th>
<th>Official Gene Symbol</th>
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<td>Fgf2</td>
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All compounds had effect on Primordial to Primary Follicle Transition

Oocyte “Nest”

Number of Genes and Pathways Overlapped

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Gene Co-expression Network

(Hierarchical Clustering over the module-module similarity matrix heatmap derived from a network.)
“Short” list of 55 most connected in network genes
CTGF, connective tissue growth factor

Organ culture experiments to test if CTGF regulates follicle transition:

Table 1. Intraovarian Paracrine Hormones Act
Through RTKs, RSKs, GPCRas, Guanylyl Cyclase Receptor
NPRs, and Integrins to Regulate Presenal Follicle Growth.

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Proposed role of WNT signaling in ovulation. WNT-responding pre-GCs produce Wnt4, Wnt6, and Wnt11 in primordial follicles and induce the Wnt agonist RSPO2. Activation of canonical WNT signaling in pre-GCs promotes their transition into GCs during primordial follicle activation (PFA). In primary follicles, GCs induce the withdrawal of oocytes from a dormant state, as reflected by the translocation of FOXO3 from the nucleus to the cytoplasm.

Intraovarian control of early folliculogenesis.

WNT signaling in pre-granulosa cells is required for ovarian folliculogenesis and female fertility
Habara O, Logan CT, Kanai-Azuma M, Nusse R, Takase HM.
Development. 2021 May 1;148(9):dev198846.

Habara O, Logan CT, Kanai-Azuma M, Nusse R, Takase HM.
Development. 2021 May 1;148(9):dev198846.


Fig. 4. Schematic of the proposed steroid-mediated interactions between ovarian theca cells and granulosa cells. The steroids involved include androgens (A), estrogens (E), and progestins (P) with both positive (+) and negative (-) effects on steroidogenesis. The gonadotropins involved include luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

"FSH Actions on Granulosa Cells"

<table>
<thead>
<tr>
<th>Table 1: FSH-stimulated functional parameters in cultured granulosa cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Enhancement of aromatization</td>
</tr>
<tr>
<td>a. Estrone synthesis</td>
</tr>
<tr>
<td>b. Progesterone and the G-O-P interconversion</td>
</tr>
<tr>
<td>Stimulation of thecal cell activity</td>
</tr>
<tr>
<td>Reduction of cholesterol and calcium metabolism</td>
</tr>
<tr>
<td>Inhibition of LH hydrolysis activity</td>
</tr>
<tr>
<td>2. Modulation of ovarian follicle responses</td>
</tr>
<tr>
<td>a. Inhibition of membrane receptor formation</td>
</tr>
<tr>
<td>b. Increase in LH receptor binding</td>
</tr>
<tr>
<td>c. Downregulation of LH receptor</td>
</tr>
<tr>
<td>3. Modulation of granulosa cell functions</td>
</tr>
<tr>
<td>a. Stimulation of granulosa cell activity</td>
</tr>
<tr>
<td>b. Modulation of granulosa cell activity</td>
</tr>
<tr>
<td>4. Modulation of general cell function</td>
</tr>
<tr>
<td>a. Increase in protein synthesis</td>
</tr>
<tr>
<td>b. Increase in glycosynthesis</td>
</tr>
<tr>
<td>c. Alterations of cell shape</td>
</tr>
<tr>
<td>d. Reduction in size of granulosa cells</td>
</tr>
<tr>
<td>5. Effect on cumulus-oocyte complexes</td>
</tr>
<tr>
<td>a. Stimulation of cumulus-oocyte complexes</td>
</tr>
<tr>
<td>b. Modulation of cumulus-oocyte activity</td>
</tr>
</tbody>
</table>

"Actions of LH on Granulosa Cells"

<table>
<thead>
<tr>
<th>Table 2: LH-modulated functional parameters in cultured granulosa cells</th>
</tr>
</thead>
<tbody>
<tr>
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<td>a. Estrone synthesis</td>
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<td>b. Modulation of cumulus-oocyte activity</td>
</tr>
<tr>
<td>6. Increase in plasma corticosteroid concentration</td>
</tr>
</tbody>
</table>

"FSH Actions on Granulosa Cells"
Transforming Growth-Beta Factor Family

- PGC
- PGC
- oocyte/ follicle
- Granulosa
- Theca/Granulosa
Growth differentiation factor-9 is required during early ovarian folliculogenesis

Jennifer Fang, David F. Alberts, Katerina Nikolaou, E. Regadera Koror, Kating Lo* & Martin M. Matzuk*

Department of Pathology, Cell Biology and Biochemistry and Human Genetics, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Growth factors involved in ovarian follicle assembly consist of growth and survival signaling networks that act in concert to maintain the pool of quiescent follicles. Recent advances have revealed the importance of growth factor signaling in the regulation of follicular development. Here, we have developed a comprehensive understanding of the signaling networks that govern follicle assembly.


"Insulin-Like Growth Factors"

<table>
<thead>
<tr>
<th>Insulin-like Growth Factor</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAME</td>
<td>Fibroblast Growth Factor (FGF)</td>
<td>Vascular Endothelial Growth Factor (VEGF)</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>~14kDa</td>
<td>Active in fmol (pg) levels</td>
</tr>
<tr>
<td>Interacts with heparin components of ECM</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Promotes vascularization</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Growth factor</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Ovary -
postulated GC FGF
- act as growth factor GC
- act as angiogenic factor for follicle
**KGF** - keratinocyte growth factor  
28kDA mesenchymal cell derived growth factor (FGF-7); receptor FGFR-2 splice variant  
Receptor only on epithelial type cells  

**HGF** - hepatocyte growth factor  
28kDA mesenchymal cell derived growth factor (scatter factor); promote kidney tubulogenesis  
Receptor is c-met protooncogene  
only on epithelium  
Both mediate mesenchymal-epithelial interactions  

Theca cells - express/secrete KGF & HGF  
Granulosa cells - respond + growth  

---

**Table 1: List of immune response-related genes and their functions in ovary**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Proposed Site</th>
<th>Proposed Site of Action</th>
<th>Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>Granulosa</td>
<td>Targeting</td>
<td>+growth</td>
<td>+growth differentiation</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Granulosa</td>
<td>Targeting</td>
<td>+growth</td>
<td>+growth differentiation</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Theca</td>
<td>Targeting</td>
<td>+growth</td>
<td>+growth differentiation</td>
</tr>
<tr>
<td>BMP15</td>
<td>Theca</td>
<td>Targeting</td>
<td>+growth</td>
<td>+growth differentiation</td>
</tr>
<tr>
<td>GDF9</td>
<td>Oocyte</td>
<td>Targeting</td>
<td>+growth</td>
<td>+growth differentiation</td>
</tr>
<tr>
<td>OXT</td>
<td>Granulosa</td>
<td>Targeting</td>
<td>+growth</td>
<td>+growth differentiation</td>
</tr>
</tbody>
</table>

---

**The role of Notch signaling in the mammalian ovary**  
*Parrott et al (1994)*  
*Endocrinology*  
*135:569*
Procr-expressing granulosa cells are highly proliferative and are important for follicle development

Granulosa cells (GCs) play a critical role in folliculogenesis. It remains unclear how GCs expand during follicle development and whether there is a subpopulation of cells that is responsible for GCs growth. Here, we observed that a small population of GCs expressed stem cell surface marker Procr (Protein C receptor). Procr GCs displayed higher proliferation ability and lower levels of hormone receptors compared with Procr- GCs. Knockdown of Procr inhibited proliferation. Lineage tracing experiments demonstrated that they contribute to increasing numbers of GCs during folliculogenesis. Targeted ablation of Procr+ cells disrupted ovarian follicle development, leading to phenotypes of polycystic ovary syndrome. Our findings suggest that Procr-expressing GCs are endowed with high proliferative capacity that is critical for follicle development.
Genetics of ovarian insufficiency and defects of folliculogenesis.
França MM, Mendonca BB.
Best Pract Res Clin Endocrinol Metab. 2021 Oct 14;101594.

Proteomics-based systems biology modeling of bovine germinal vesicle stage oocyte and cumulus cell interaction.
Peddinti D, et al.

Proteome of fluid from human ovarian small antral follicles reveals insights in folliculogenesis and oocyte maturation.
Pla I, Sanchez A, Pors SE, et al.

The dormant and the fully competent oocyte: comparing the transcriptome of human oocytes from primordial follicles and in metaphase II.
Grøndahl ML, et al.
Transcriptome profile of goat folliculogenesis reveals the interaction of oocyte and granulosa cell in correlation with different fertility population.


Follicle Online: an integrated database of follicle assembly, development and ovulation.


Cell-specific network analysis of human folliculogenesis reveals network rewiring in antral stage oocytes.


A single-cell atlas reveals unanticipated cell type complexity in Drosophila ovaries.

Polycystic ovary syndrome as a paradigm for prehypertension, prediabetes, and preobesity.
Lyuque-Ramírez M, Escobar-Morreale HF.

Table 1  Effect of obesity on cardiometabolic risk factors in women with PCOS compared with lean patients

- ↑ Insulin resistance
- ↑ Hyperinsulinism
- ↑ Fasting glucose
- ↑ 2 h glucose after OGTT
- ↑ Prevalence of abnormal glucose tolerance
- ↓ HDL cholesterol levels
- ↑ Triglycerides levels
- ↑ Office and ABPM systolic and diastolic blood pressure values
- ↑ Prevalence of office and ABPM hypertension
- ↑ Prevalence of NAFLD
- ↑ Prevalence of metabolic syndrome

ABPM ambulatory blood pressure monitoring, NAFLD nonalcoholic fatty liver disease, OGTT standard 75 g oral glucose tolerance test

Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome.
Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS.

Pathophysiology of PCOS—a vicious circle. Several theories have been proposed to explain the pathogenesis of PCOS. One of these is that neuroendocrine defects lead to increased pulse frequency and amplitude of LH and relatively low FSH. This causes intrinsic defects in ovarian androgen production. Also, there may be an alteration in cortisol metabolism and excessive adrenal androgen production. Insulin resistance with compensatory hyperinsulinemia further increases ovarian androgen production both directly and indirectly via the inhibition of hepatic SHBG production. Obesity, insulin resistance, and high circulating androgens are associated with increased sympathetic nerve activity. F, estradiol.


The concentration of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) levels in FF and serum of control and women with polycystic ovary syndrome (PCOS). FF (n = 20 in each group) and serum (n = 10 in each group) were used to measure levels of VEGF (A and B) and FGF2 (C and D) in women with PCOS compared to controls. Statistical comparison was performed using the Mann-Whitney U test. Data are represented as mean ± SEM and *P < 0.05; **P < 0.01 considered significant. FF, follicular fluid.


Networks of putative target genes regulated by differentially expressed miRNAs. Validated target genes (grey nodes) mapped to pathway annotations derived from literature and gene ontology using Ingenuity Pathway Analysis (IPA, http://www.ingenuity.com). A. Mir143 regulatory network. B. Mir145 regulatory network. The solid lines connecting molecules represent a direct relation and dotted lines an indirect relation. IPA constructs networks that optimize for both interconnectedness and number of Focus Genes (the grey nodes) under the constraint of a maximal network size. White nodes are added by the algorithm to build a highly connected molecular network between Focus Genes.


Methylome and transcriptome profiling revealed epigenetic silencing of LPCAT1 and PCYT1A associated with lipidome alterations in polycystic ovary syndrome.

J Cell Physiol. 2021 Sep;236(9):6362-6375.

<table>
<thead>
<tr>
<th>Spring 2022 (Even Years) – Course Syllabus</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIOL 475/575 Level Undergraduate/Graduate (3 Credit)</td>
</tr>
<tr>
<td>SLN: (475) – 05504, (575) – 05505</td>
</tr>
<tr>
<td>Time - Tuesday and Thursday 10:35 am-11:50 am</td>
</tr>
<tr>
<td>Course Lectures in person and on Canvas/Panopto and Discussion Sessions in person and on WSU Zoom for all campuses</td>
</tr>
<tr>
<td>Room – CUE 418</td>
</tr>
<tr>
<td>Course Director – Michael Skinner, Abelson Hall 507, 335-1524, <a href="mailto:skinner@wsu.edu">skinner@wsu.edu</a></td>
</tr>
<tr>
<td>Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, <a href="mailto:nilsson@wsu.edu">nilsson@wsu.edu</a></td>
</tr>
</tbody>
</table>

| Learning Objective - |

| Schedule/Lecture Outline – |
| January |
| 11 & 13 Week 1 |
| Systems Biology Introduction |
| 16 & 20 Week 2 |
| Molecular/Cellular Reproduction Systems |
| 25 & 27 Week 3 |
| Sex Determination Systems |

| February |
| 8 & 10 Week 4 |
| Male Reproductive Tract Development & Function |
| 15 & 17 Week 5 |
| Female Reproductive Tract Development & Function |
| 22 & 24 Week 7 |
| Testis Systems Biology |

| March |
| 8 & 10 Week 8 |
| Ovary Systems Biology |
| 14 – 18 Week 10 |
| Spring Break |
| 22 & 24 Week 11 |
| Genitotesticular Stem Cells/Cloning |
| 29 & 31 Week 12 |
| Hypothalamo-Pituitary Development & Function |

| April |
| 7 & 9 Week 13 |
| Reproductive Endocrinology Systems |
| 12 & 14 Week 14 |
| Fertilization & Implantation Systems |
| 19 & 21 Week 15 |
| Fetal Development & Birth Systems |
| 26 & 28 Week 16 |
| Assisted Reproduction/Contraception |

| May |
| 7 & 9 Week 17 |
| Exam or Grant Review |