Spring 2026 – Systems Biology of Reproduction
Lecture Outline – Ovarian Systems Biology
Eric Nilsson – Biol 475/575
10:35-11:50 am, Tuesday & Thursday
March 3, 2026
Week 8

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

Bahr JM. (2018) Ovary, Overview. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 2: 3-7.

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OVARY

Ovary, Overview

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Glossary

Corpus luteum An endocrine gland formed from the granulosa and theca layers of an ovulated follicle.

Follicle A structure in the ovary consisting of the oocyte and surrounding granulosa and theca cell layers.

Granulosa cells Somatic cells directly surrounding the oocyte.

Meiosis A type of cell division which the oocyte undergoes reducing the number of chromosomes so that the oocyte has one copy of each chromosome.

Oocyte The female gamete.

Ovary The female gonad.

Steroids Molecules with a basic structure similar to that of cholesterol.

Theca cells Layer of steroidogenic cells and connective tissue surrounding the granulosa cells and forming the outer layer of the follicle.

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

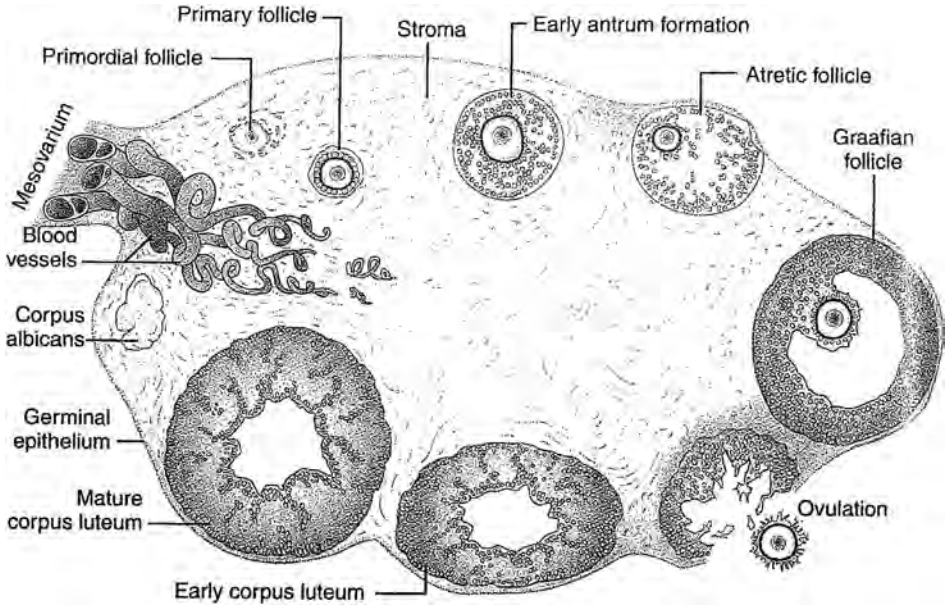


Fig. 1 A cross-section of the ovary illustrating follicles at different stages of development (from primordial to Graafian follicles), corpus hemorrhagicum, corpus luteum, and corpus albicans. The microscopic structures of follicles are also shown. Adapted from Jones, R.E. (1991) The ovaries in human reproductive biology, pp. 39–53. Academic Press, San Diego. p. 42.

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 Graffian 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 cell 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 no 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 secretory end product of the corpus luteum. In the developing follicles, the theca layer is the primary site of progestin productions. Immediately prior to ovulation, the granulosa cells stimulated by LH also synthesize progesterone. After ovulation the corpus luteum

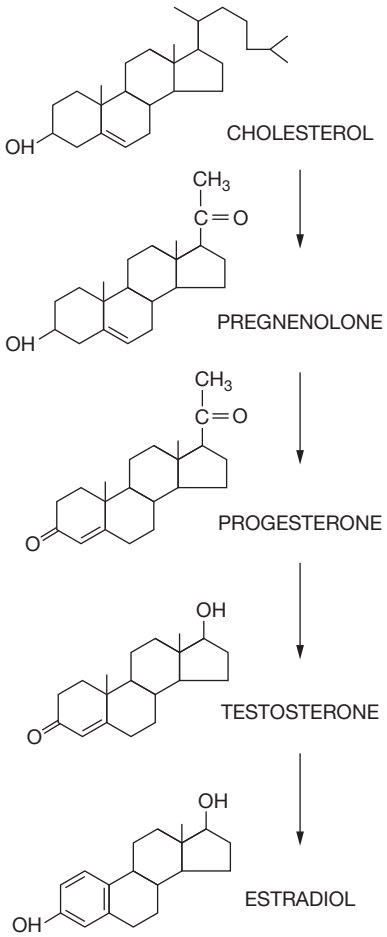


Fig. 2 Biosynthesis of steroid hormones from cholesterol. This scheme provides a simplistic view of a highly organized and complicated process that requires multiple enzymes. Adapted from Hafez, E.S.E (1993) Folliculogenesis, egg maturation, and ovulation. In Reproduction in farm animals, 6th ed., pp.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

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.

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.

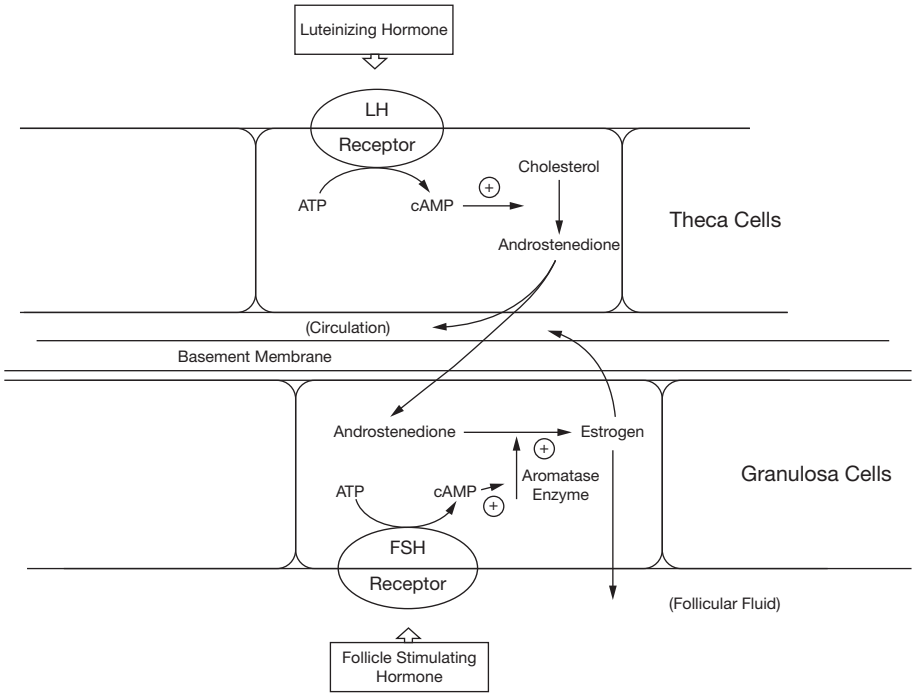


Fig. 3 "Two-cell, two-hormone" theory of follicular steroidogenesis. LH binds to specific membrane receptors on theca calls and stimulates cyclic AMP production and the conversion of cholesterol to androgens, primarily androstenedione and testosterone. These androgens diffuse into the circulation and across the basement membrane into granulosa cells. FSH binds to specific membrane receptors on granulosa cells and stimulates cyclic AMP production, which leads to increased aromatase enzyme activity and the conversion of theca androgens to estrogens. Adapted from Yen, S. S. C. and Jaffe, R. B. (1986). Reproductive Endocrinology (2nd ed.), Philadelphia: Saunders.

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

Dong, J., Albertini, D. F., Nishimori, K., Kumar, T. R., Lu, N., & Matzuk, M. M. (1996). Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature, 383*, 531–535.

Hafez, E. S. E. (1993). Folliculogenesis, egg maturation, and ovulation. In *Reproduction in farm animals* (6th ed., pp. 114–143). Philadelphia: Lea and Febiger.

Jones, R. E. (1991). *The ovaries in human reproductive biology*. San Diego: Academic Press. pp. 39–53.

Liu, C., Peng, J. Matzuk, M.M. and Yao, H.H. (2015). Nature Communications. https://doi.org/10.1038/ncomms7934.

Rajkovic, A., Panagas, S. A., & Matzuk, M. M. (2006). Follicular development: Mouse, sheep and human models. In J. D. Neill (Ed.), *Knobil and Neill's physiology of reproduction* (3rd edn). Amsterdam: Elsevier.

Strauss, J.F. III and Williams, C.J. (2009). The ovarian life cycle, Strauss, J.F. III, Barberi, R.L. eds., Yen and Jaffe's reproductive endocrinology, physiology, pathophysiology, and management, 6th edn, Saunders, Philadephia, PA.

Williams, C. J., & Erickson, G. F. (2012). In L. J. De Groot, G. Chrousos, & K. Dungan (Eds.), *Morphology and physiology of the ovary*. South Dartmouth, MA: Endotext.

"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
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 -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review

Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Ovarian Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 27, 2024
Week 8

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

Bahr JM. (2018) Ovary, Overview. in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 2: 3-7.

Spring 2024 – Systems Biology of Reproduction
Discussion Outline – Ovary Systems Biology
Michael K. Skinner – Biol 475/575
Zoom/CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 29, 2024
Week 8

Ovary Systems Biology

Primary Papers:

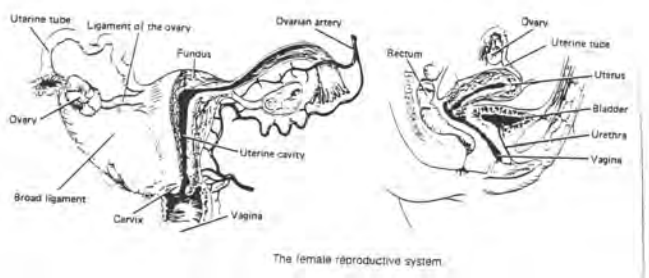
1. Pla, et al. (2021) Hum Reprod. 18;36(3):756-770
2. Sagvekar, et al. (2019) Clinical Epigenetics 11:61
3. Nilsson, et al. (2010) PLoS ONE 7:e11637

Discussion

Student 8: Reference 1 above
- What approach and technology was used?
- What gene categories and networks were identified?
- What oocyte maturation and folliculogenesis insights were identified?

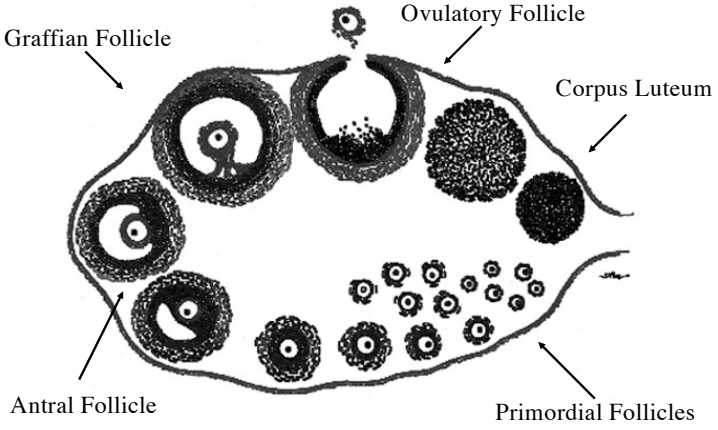
Student 9: Reference 2 above
- What are the technology used and objectives?
- What epigenetic regulation and gene network were identified?
- What insights are provided into the development of polycystic ovarian disease?

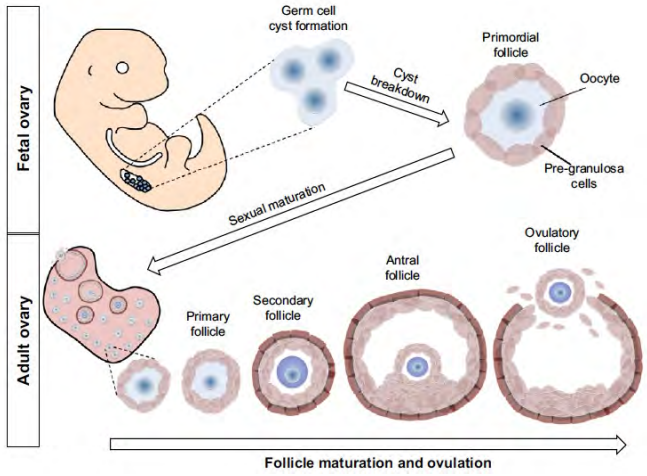
Student 10: Reference 3 above
- What is the experimental and systems approach?
- What new insights provided on primordial follicle development?
- What gene signaling networks were identified for primordial follicle development?



The female reproductive system.

The Mammalian Ovary





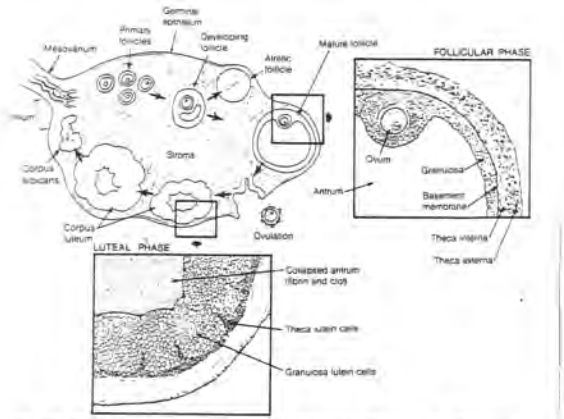
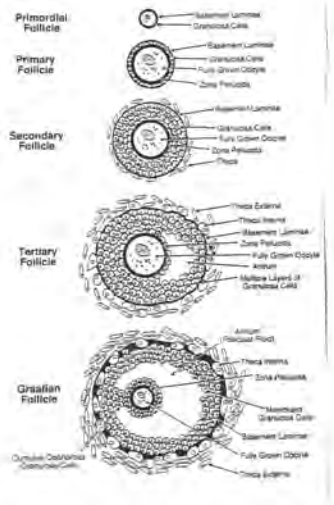
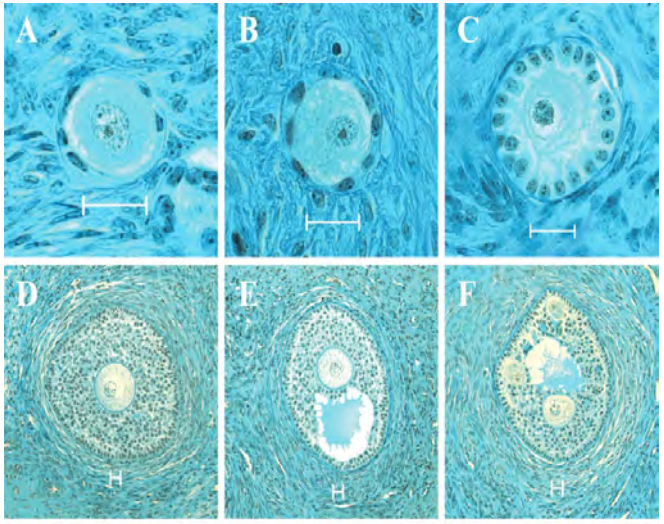


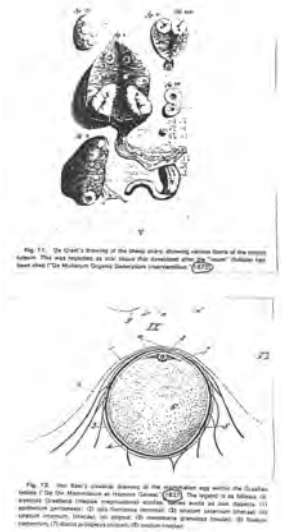
Figure 11–5. Developmental changes in the adult ovary during a complete menstrual cycle. (Reproduced, with permission, from Carr BR, Wilson JD. Disorders of the ovary and female reproductive tract. In: Wilson JD, Braunwald E, Isselbacher K, et al (eds). Harrison's Principles of Internal Medicine, 12th Ed. New York, N.Y., McGraw-Hill, 1991, pp 1778–1793.)





De Graaf 1672

First Anatomy
Ovary



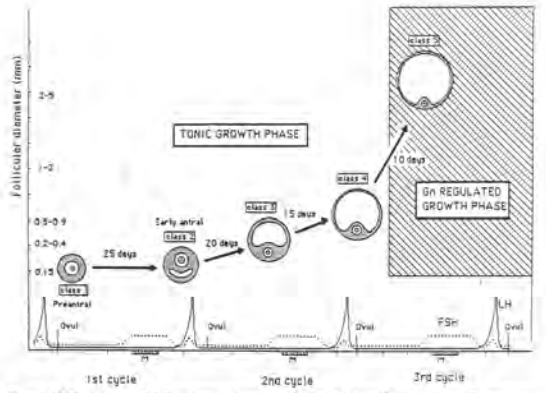


Figure 6–14 ■ Tonic (periantral) follicular growth: a conversion of a class 1 to a class 4 follicle. M, menses; Gn, gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone. (Courtesy of A. Gougeon.)

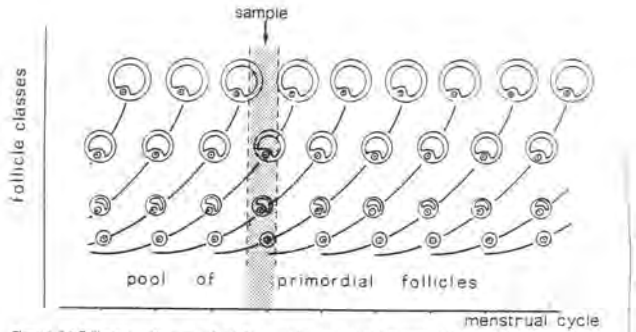


Figure 6–8 ■ Follicular growth waves underlie the nonsynchronous state of development of ovarian follicles at any given point in time. (From Gougeon A. Dynamics of follicular growth in the human: A model from preliminary results. Human Reprod 1:81, 1986.)

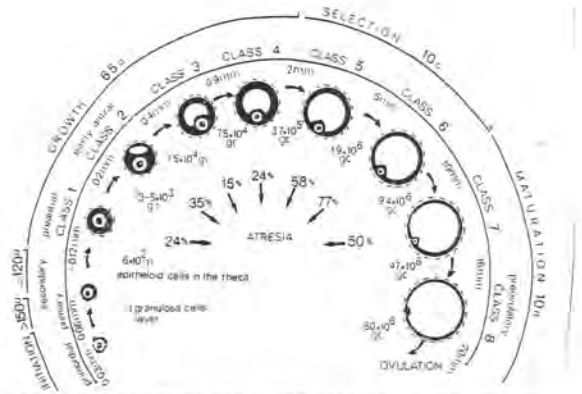


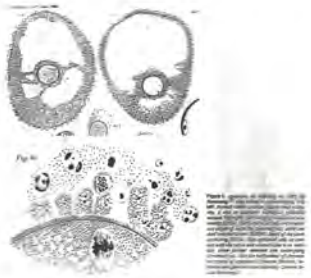
Figure 6–6 ● Stages of folliculogenesis in the adult human ovary and level of atresia in the eight classes of growing follicles. The classes under study are defined by the granulosa cell (gc) numbers and the corresponding estimated follicular diameter (mm).
(From Gougeon A. Dynamics of follicular growth in the human: A model from preliminary results. Human Reprod 1:81, 1986.)

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

TABLE 2. Cellular and molecular parameters that distinguish apoptosis from necrosis

Apoptosis	Necrosis
Affects scattered individual cells	Affects tracts of contiguous cells
Chromatin and cytoplasmic condensation, cell shrinkage	Cell swelling and rupture of plasma membrane
May require mRNA and protein synthesis	Not dependent upon new mRNA or protein synthesis
Normal ATP level No inflammation	Decreased ATP level Elicits inflammatory responses
Endonuclease activation and internucleosomal DNA cleavage (ladder pattern)	Activation of nonspecific DNases with generalized DNA breakdown (smearing)

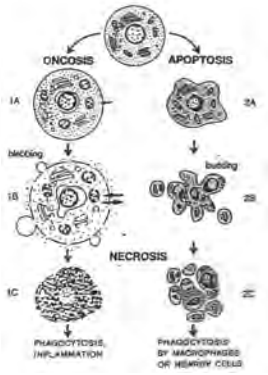


TABLE 3. The *bcl-2* gene family

	Method of identification	Function
bcl-2	Overexpressed in B-cell lymphoma found at junction of t(14;18) chromosomal translocation	Suppresses apoptosis
BAX	Coprecipitate with *Bcl-2* protein	Prevents *bcl-2* action and increases apoptosis
bcl-X	Homologous screening of cDNA library	
Long form		Prevents apoptosis
Short form		Increases apoptosis
A1	Differential screening (hemopoietic cells)	???
MCL1	Subtraction screening (early response gene in myeloid cells)	???
ICE	Protease homolog of ced-3 (interleukin 1*d*-converting enzyme)	Promotes apoptosis
ced-9	Genetic analysis (*C. elegans*)	Prevents apoptosis
BHRF1	Epstein-Barr viral sequence	Prevents apoptosis
LMW5-HL	African swine fever virus gene	???

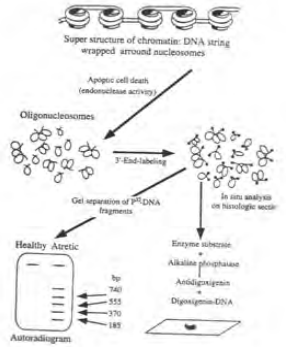
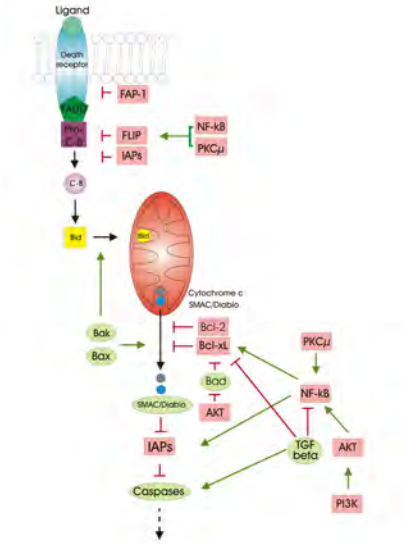
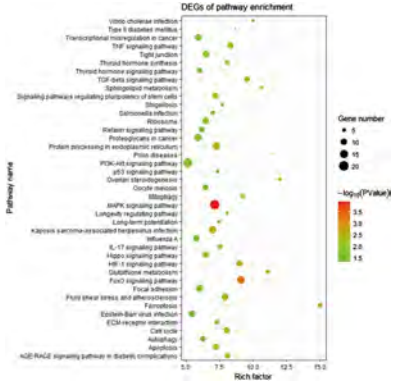


Figure 2

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.

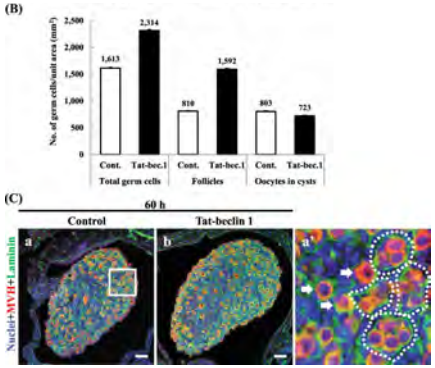


Initiation of follicular atresia: gene networks during early atresia in pig ovaries.
Zhang J, Liu Y, Yao W, Li Q, Liu H, Pan Z.
Reproduction. 2018 Jul;156(1):23-33.



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 genes.

Activation of autophagy in early neonatal mice increases primordial follicle number and improves lifelong fertility.
Watanabe R, Sasaki S, Kimura N.
Biol Reprod. 2019 Sep 30. [Epub ahead of print]



Autophagy participates in cyst breakdown and primordial folliculogenesis by reducing reactive oxygen species levels in perinatal mouse ovaries.
Zhihan T, Xinyi M, Qingying L, Rufei G, Yan Z, Xuemei C, Yanqing G, Yingxiong W, Junlin H.
J Cell Physiol. 2019 May;234(5):6125-6135.

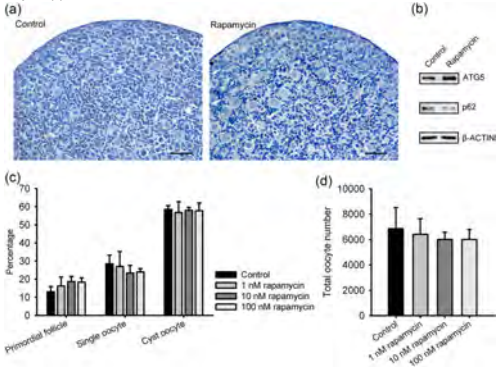
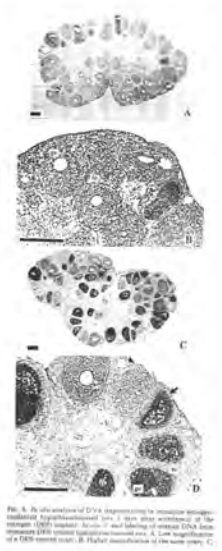


TABLE 1. Morphological and biochemical correlates of atresia

A. Granulosa cells
- Pyknotic nuclei
- Decreased DNA turnover (thymidine uptake)
- Decreased estrogen production
- Increased progesterone production (loss of 17α-hydroxylase)
- Decreased gonadotropin receptors and responsiveness
- Increased IGF binding protein expression
- Increased SGP-2 expression
- Increased angiotensin II receptor expression
- Increased cathepsin-D activity
- Decreased expression of gap junction protein connexin-43
- Detachment from basement membrane

B. Thecal cells
- Hypertrophy and formation of secondary interstitial cells in some species, formation of pyknotic nuclei in others

C. Oocyte
- Germinal vesicle breakdown



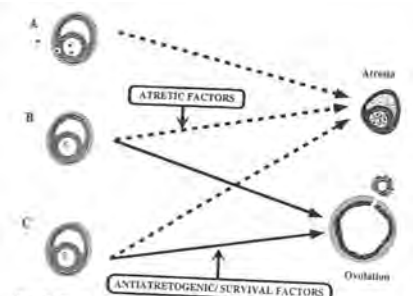
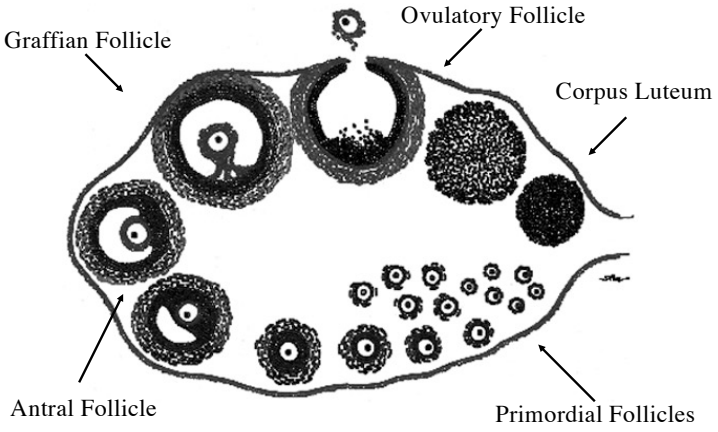
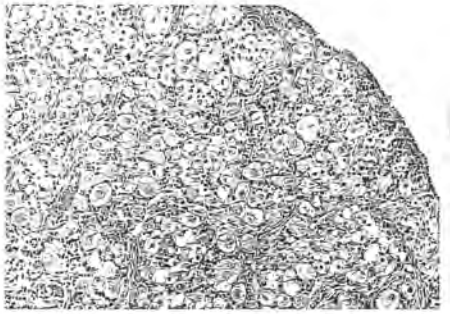
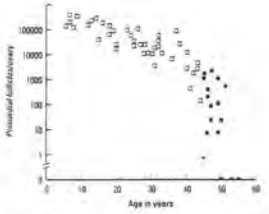


FIG. 5. Three theoretical models for the determination of follicular atresia. A, Follicular fate is predetermined by inherent deficiency in the genetic or cytoplasmic component of the oocyte, the somatic cells, or the follicular environment (*). B, Normally, all follicles may reach ovulation unless exposed to an atretogenic factor(s) that channels them into the degenerative pathway. C, All follicles are doomed to atresia unless protected at a critical stage by survival factors.

The Mammalian Ovary







Number of Oocytes During Stages of Early Folliculogenesis

	Proliferation	Assembly	Puberty
Rodent	75,000	27,000	10,000
Primate	6,800,000	1,000,000	700,000

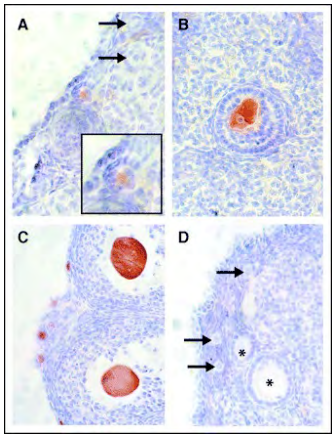
Only 500 human follicles ovulate.

Oogonial Stem Cell

Journal of Clinical Oncology, Vol 25, No 22 (August 1), 2007: pp. 3198-3204
© 2007 American Society of Clinical Oncology.
DOI: 10.1200/JCO.2006.10.3028

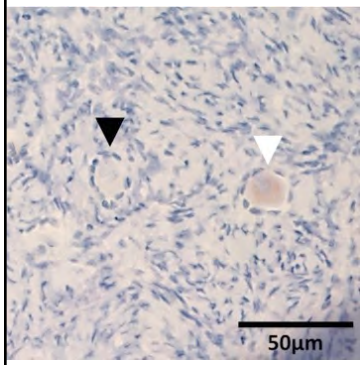
Bone Marrow Transplantation Generates Immature Oocytes and Rescues Long-Term Fertility in a Preclinical Mouse Model of Chemotherapy-Induced Premature Ovarian Failure

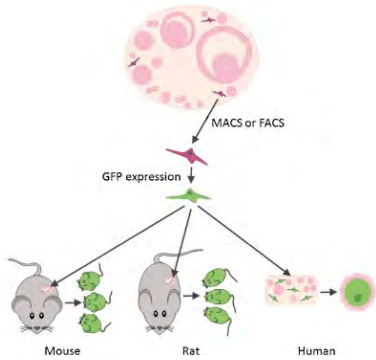
Ho-Joon Lee, Kaisa Selesniemi, Yuichi Niikura, Teruko Niikura, Rachael Klein, David M. Dombkowski, Jonathan L. Tilly



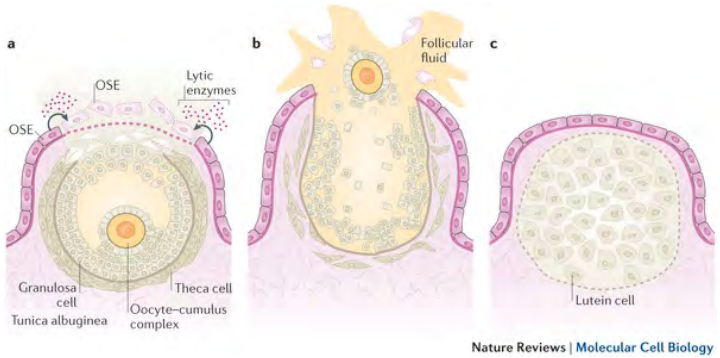
The controversial existence and functional potential of oogonial stem cells.

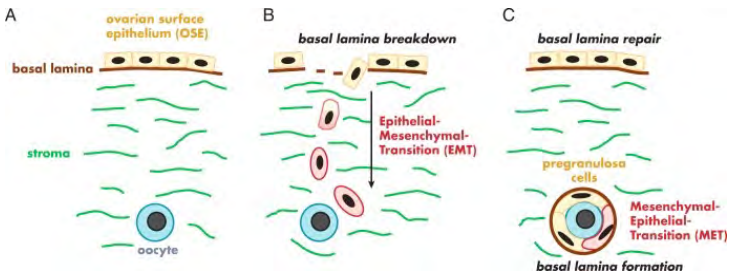
Grieve KM, McLaughlin M, Dunlop CE, Telfer EE, Anderson RA.
Maturitas. 2015 Nov;82(3):278-81.



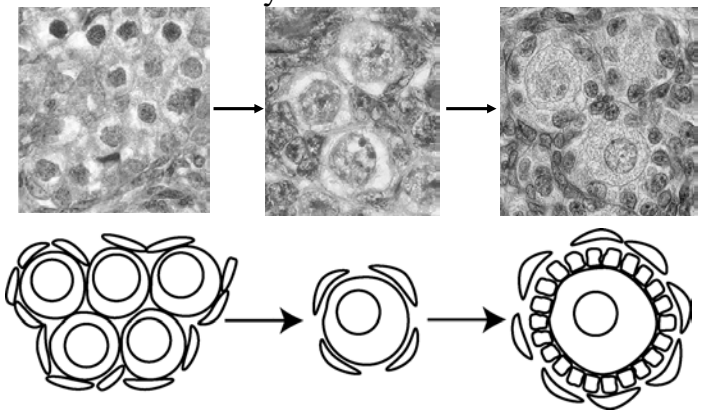


Cyclic epithelial remodelling in the ovary and fimbria

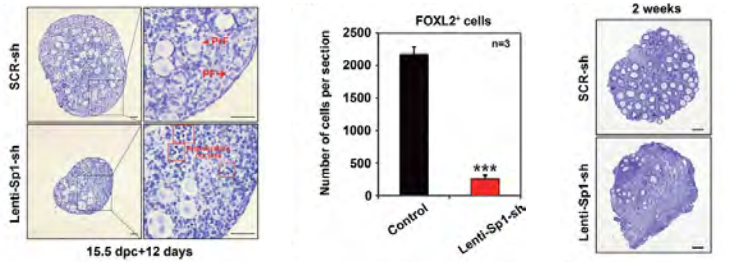




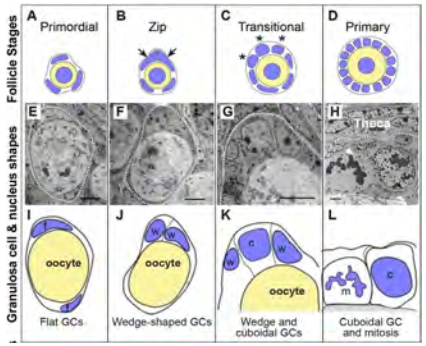
Follicular Assembly and Primordial to Primary Follicle Transition



SP1 governs primordial folliculogenesis by regulating pregranulosa cell development in mice.
Cai H, Liu B, Wang H, Sun G, Feng L, Chen Z, Zhou J, Zhang J, Zhang T, He M, Yang T, Guo Q, Teng Z, Xin Q, Zhou B, Zhang H, Xia G, Wang C. J Mol Cell Biol. 2019 Jul 8. pii: mjz059. doi: 10.1093/jmcb/mjz059. [Epub ahead of print]



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.
J Cell Sci. 2018 Sep 7;131(17). pii: jcs218123.



Kit-Ligand/Stem Cell Factor Induces Primordial Follicle Development and Initiates Folliculogenesis*

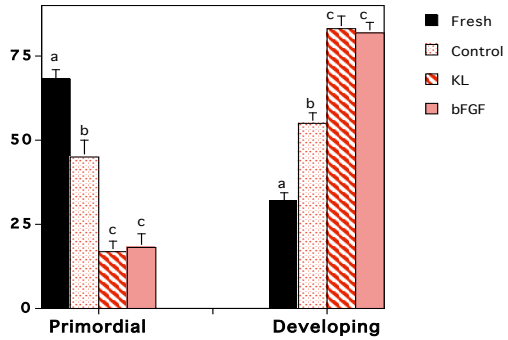
JEFF A. PARROTT AND MICHAEL K. SKINNER
Center for Reproductive Biology, Department of Genetics and Cell Biology, Washington State University, Pullman, Washington 99163-4231

ABSTRACT

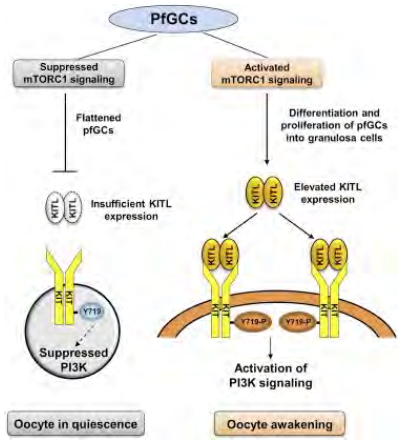
Initiation of folliculogenesis through the induction of primordial follicle development in the ovary has an important role in determining the fertility and reproductive fitness of most mammalian species. The factors that control this critical process are largely unknown. The hypothesis tested in the current study was that kit-ligand/steel cell factor (KL) promotes the initiation and progression of primordial follicle development in the ovary. Ovaries from 4-day-old rats were maintained in organ culture for 5 and 14 days and treated with no factor (control), recombinant kit-ligand (KL), or gonadotropins (FSH and hCG). Follicles in ovarian sections were counted and histologically classified as primordial (stage 0), early primary (stage 1), primary (stage 2), transitional (stage 3), or preantral (stage 4). Fresh ovaries from 4-day-old rats contained 68% primordial follicles (stage 0) and 32% developing follicles (stages 1–4) per section. After 5 and 14 days in culture, sections from control ovaries contained approximately 41% and 55%, respectively, developing follicles (stage 1–4) per section due to spontaneous development of primordial follicles. Spontaneous primordial follicle development was consistently blocked by ACK-2, a c-kit antibody that blocks KL actions. This observation suggests that endogenous KL is necessary for primordial follicle development in vitro. After 14 days of KL treatment, ovaries from ovaries contained 17% primordial follicles (stage 0) and 83% developing follicles (stage 1–4) per section demonstrating a dramatic induction of primordial follicle development by KL. Gonadotropins (FSH and hCG) did not induce primordial follicle development but did increase the percentage of preantral follicles (stage 4) per section. This small increase in preantral follicles in response to gonadotropins was blocked by ACK-2 suggesting that KL may in part mediate gonadotropin actions after the initiation of primordial follicle development. Ovaries contained an average of 369 ± 10 follicles per section. The total number of follicles per section did not significantly vary between treatments suggesting that the effects of KL were not due to an alteration in follicle number (i.e. survival). KL appears to be one of the first factors identified to be involved in the promotion of primordial follicle development. Results suggest that KL is necessary and sufficient to induce primordial follicle development and initiate folliculogenesis. (*Endocrinology* 140: 4262–4271, 1999)

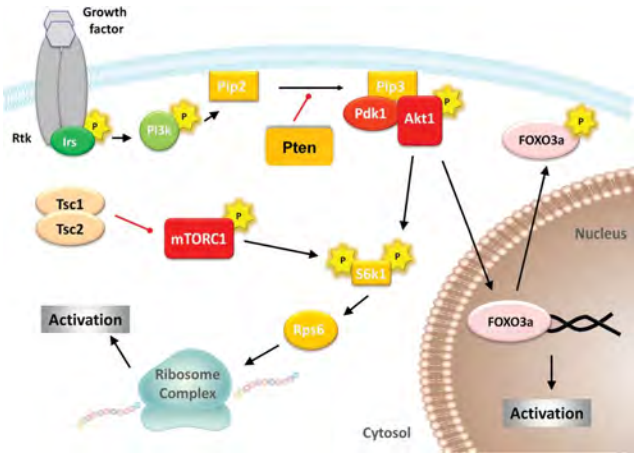
LONG-TERM CULTURE

RAT OVARY ORGAN CULTURE

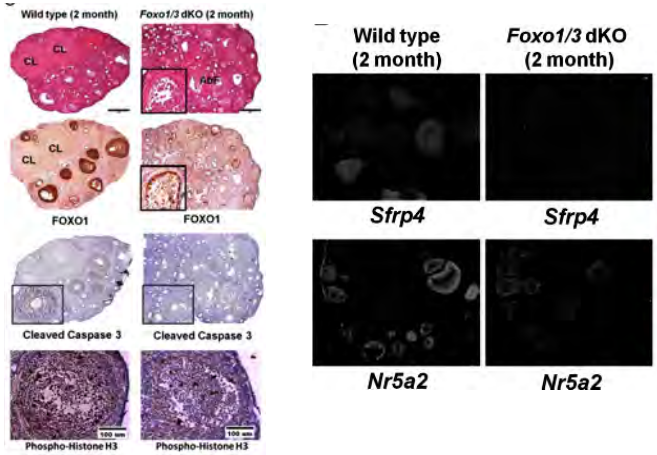


Somatic cells initiate primordial follicle activation and govern the development of dormant oocytes in mice.
Zhang H, Risal S, Gorre N, Busayavalasa K, Li X, Shen Y, Bosbach B, Brännström M, Liu K.
Curr Biol. 2014 Nov 3;24(21):2501-8.





FOXO1/3 depletion in granulosa cells alters follicle growth, death and regulation of pituitary FSH.
Liu Z, et al.
Mol Endocrinol. (2013) Feb;27(2):238-52.



Forkhead box O member FOXO1 regulates the majority of follicle-stimulating hormone responsive genes in ovarian granulosa cells
Mol Cell Endocrinol. 2016 Oct 15;434:116-26.
Herndon MK, Law NC, Donaubauer EM, Kyriss B, Hunzicker-Dunn M.

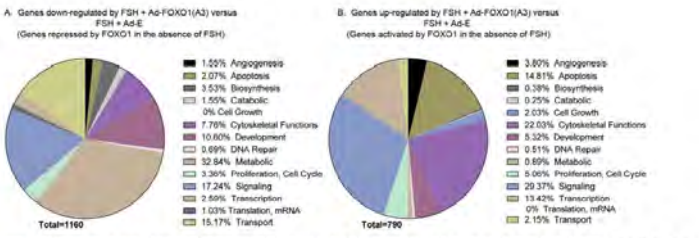
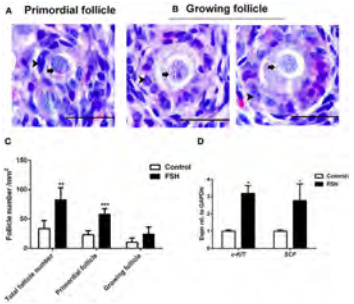
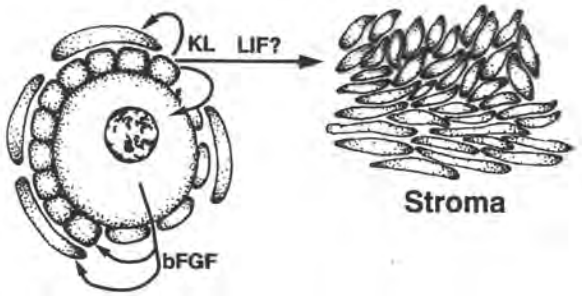


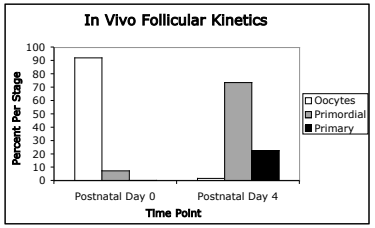
Fig. 2. Summary of the predominant biological functions of genes repressed or activated by FOXO1 in the absence of FSH identified by Pathway Studio Analysis. Panels are pie charts showing the percentage of genes down-regulated by FSH + Ad-FOXO1(A3) versus FSH + Ad-E (i.e., genes repressed by FOXO1 in the absence of FSH; Panel A) and of genes up-regulated by FSH + Ad-FOXO1(A3) versus FSH + Ad-E (i.e., genes activated by FOXO1 in the absence of FSH; Panel B). Genes identified for each biological function in Supplemental Tables 4 and 5 were counted and are reflected in Panels A and B, respectively. Biological functions that contained genes highly duplicated in other groups within the same biological function category were deleted as were enriched groups with p values > 0.01 (based on Fisher's Exact Test), as explained in Materials and Methods. A number of genes appear in different functional categories. As a result, total gene numbers do not align with results presented in Fig. 1.

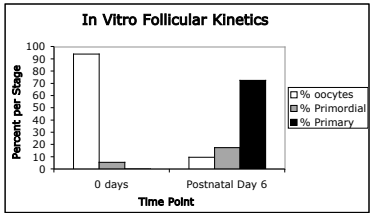
Interaction of Follicle-Stimulating Hormone and Stem Cell Factor to Promote Primordial Follicle Assembly in the Chicken.
Guo C, Liu G, Zhao D, Mi Y, Zhang C, Li J.
Front Endocrinol (Lausanne). 2019 Feb 19;10:91.

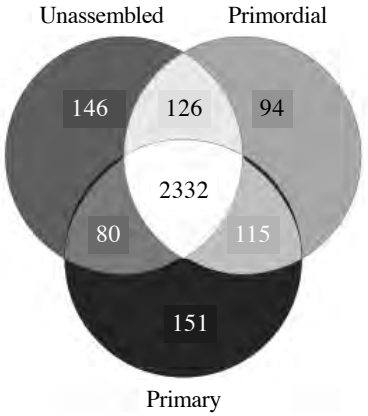


Effects of FSH treatment on chicken folliculogenesis *in vivo*. **(A,B)** Morphology of the primordial and growing follicles in 6-day-old chicken ovaries. Scale bar: 20 μm. Arrowheads and arrows represent the somatic cells and oocytes, respectively. **(C)** Changes in the primordial and growing follicle 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 (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).







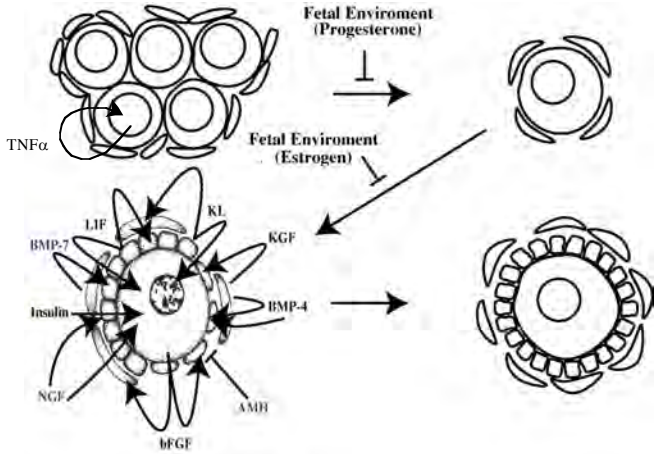


	Unassembled to Primordial	Primordial to Primary
Total Genes (>2X)	64 Genes Up Regulated	62 Genes Up Regulated
Total Genes (>2X)	28 Genes Down Regulated	121 Genes Down Regulated

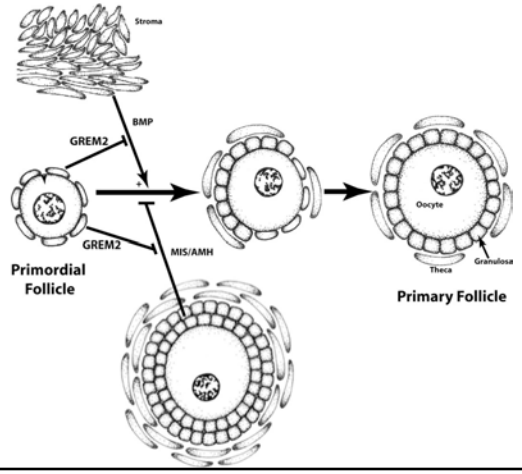
Candidate Factors for Primordial to Primary Follicle Transition

- 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)

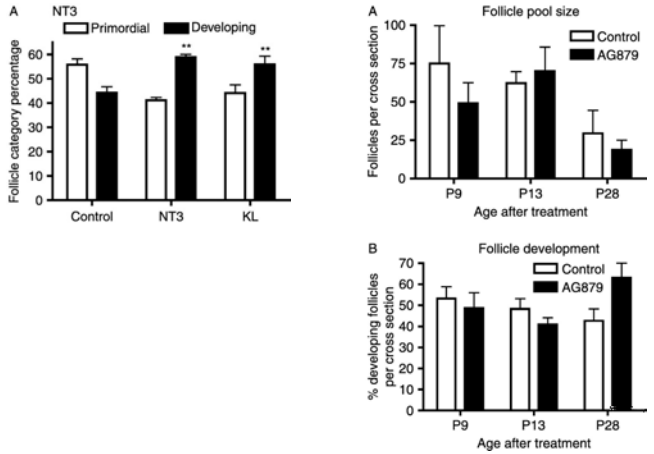
Current Model of Early Folliculogenesis

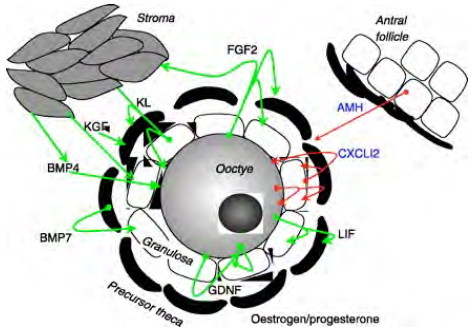


Roles of Gremlin 1 and Gremlin 2 in regulating ovarian primordial to primary follicle transition.
Nilsson EE, Larsen G, Skinner MK.
Reproduction. 2014 Jun;147(6):865-74.



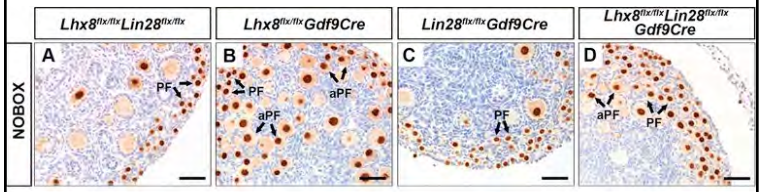
Neurotrophin NT3 promotes ovarian primordial to primary follicle transition.
Nilsson E, Dole G, Skinner MK.
Reproduction. 2009 Oct;138(4):697-707.





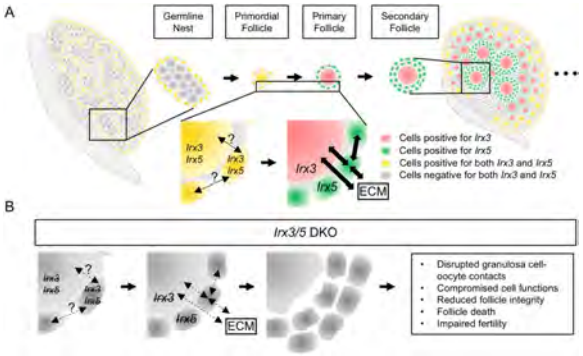
Lhx8 regulates primordial follicle activation and postnatal folliculogenesis.

Ren Y, Suzuki H, Jagarlamudi K, Golnoski K, McGuire M, Lopes R, Pachnis V, Rajkovic A
BMC Biol. 2015 Jun 16;13:39.



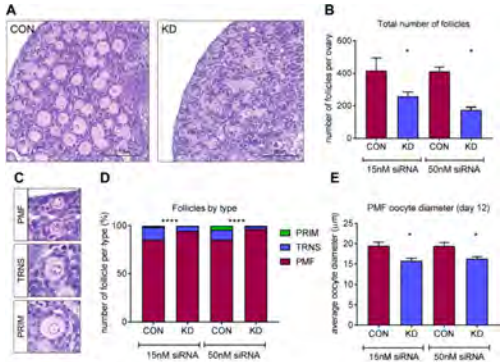
Dynamic expression patterns of Irx3 and Irx5 during germline nest breakdown and primordial follicle formation promote follicle survival in mouse ovaries.

Fu A, Oberholtzer SM, Bagherl-Fam S, et al.
PLoS Genet. 2018 Aug 2;14(8):e1007488.

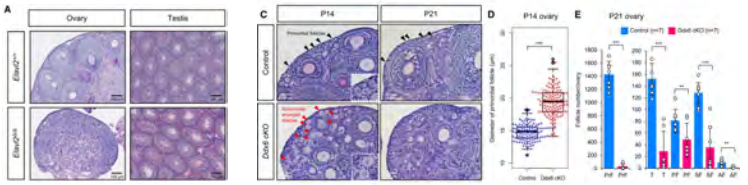


Dazl determines primordial follicle formation through the translational regulation of Tex14.

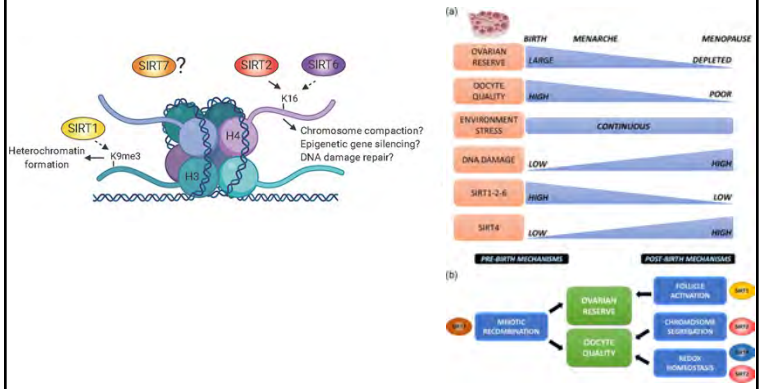
Rosario R, Crichton JH, Stewart HL, Childs AJ, Adams IR, Anderson RA.
FASEB J. 2019 Dec;33(12):14221-14233.



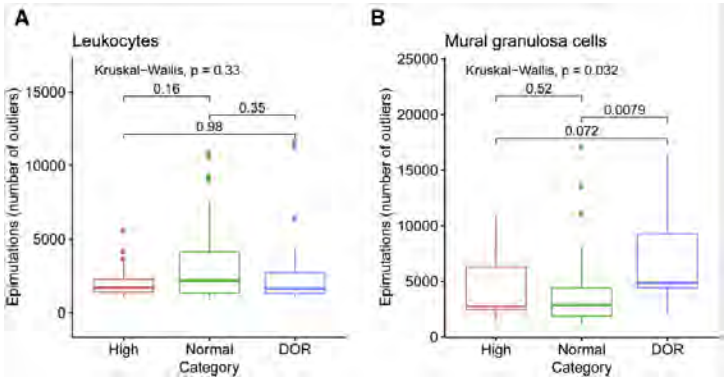
ELAVL2-directed RNA regulatory network drives the formation of quiescent primordial follicles.
Kato Y, Iwamori T, Ninomiya Y, Kohda T, Miyashita J, Sato M, Saga Y.
EMBO Rep. 2019 Dec 5;20(12):e48251.



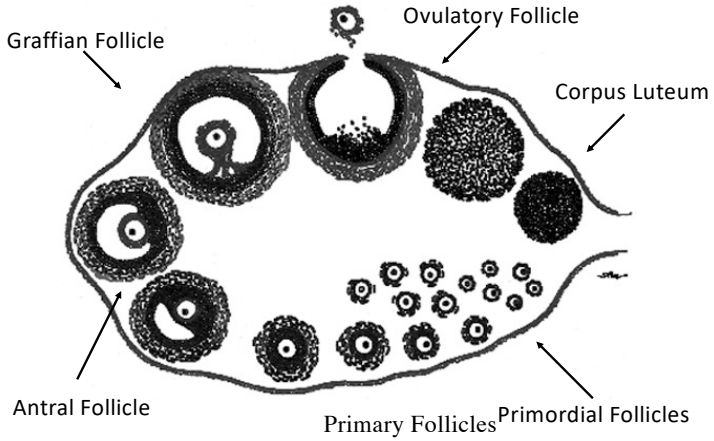
Sirtuins in female meiosis and in reproductive longevity
Berta N Vazquez, Alejandro Vaquero, Karen Schindler
Mol Reprod Dev. 2020 Dec;87(12):1175-1187.

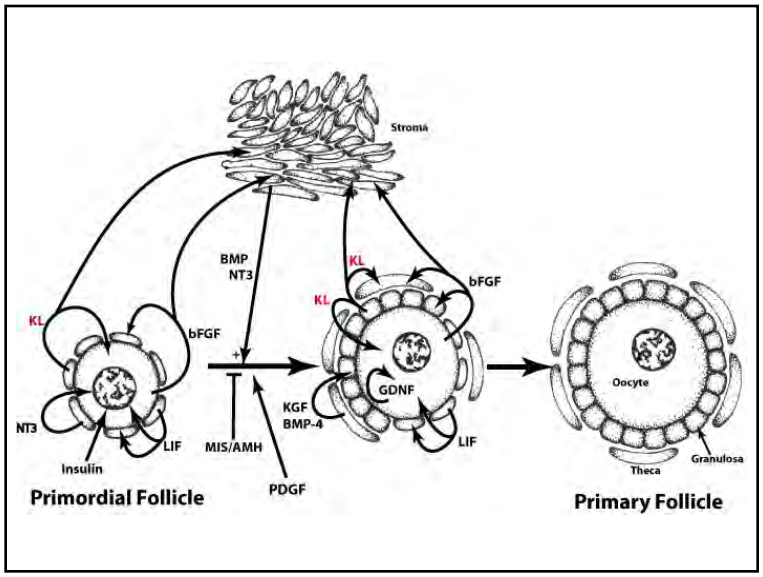


Identification of a unique epigenetic profile in women with diminished ovarian reserve.
Olsen KW, Castillo-Fernandez J, Chan AC, et al.
Fertil Steril. 2021 Mar;115(3):732-741.



The Mammalian Ovary





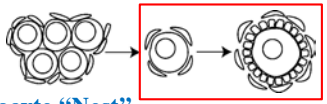
Systems Biology Approach:

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

Regulatory Growth Factors

Used Compound	Official Gene Symbol	Gene Title
bFGF	Fgf2	fibroblast growth factor 2
PDGF	Pdgfb	platelet-derived growth factor beta polypeptide
LIF	Lif	Leukemia inhibitory factor
KGF	Fgf7	fibroblast growth factor 7
BMP4	Bmp4	bone morphogenetic protein 4
AMH	Amh	anti-Mullerian hormone
KL	Kitlg	KIT ligand
GDNF	Gdnf	glial cell derived neurotrophic factor
NT3	Ntf3	neurotrophin 3

All compounds had effect on **Primordial** to **Primary Follicle** Transition

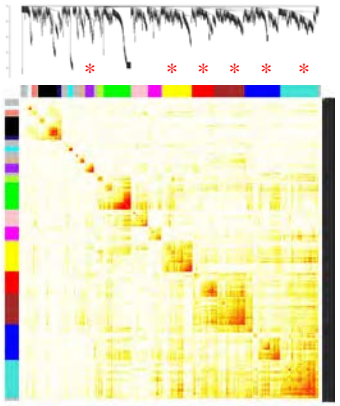


Oocyte "Nest"

Number of Genes and Pathways Overlapped

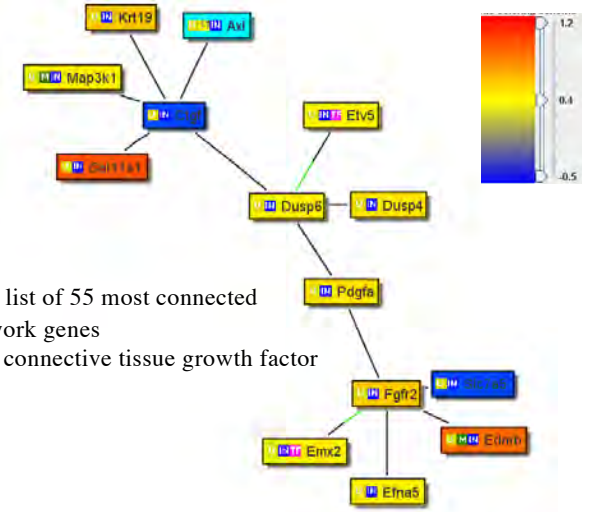
	#PW	AMH	FGF2	BMP4	GDNF	FGF7	KITLG	LIF	PDGFab	CTGF
# genes		56	41	22	20	36	54	56	41	12
AMH	268		28	18	14	29	37	40	32	7
FGF2	248	10		13	11	19	27	29	17	7
BMP4	79	4	5		9	11	14	16	13	6
GDNF	148	14	7	3		9	9	11	11	3
FGF7	123	36	5	1	5		28	25	17	5
KITLG	271	8	5	3	1	2		39	24	8
LIF	349	7	18	13	4	3	18		30	9
PDGFab	275	18	22	3	14	5	7	10		6
CTGF	155	5	7	2	1	2	6	2	6	

Gene Co-expression Network



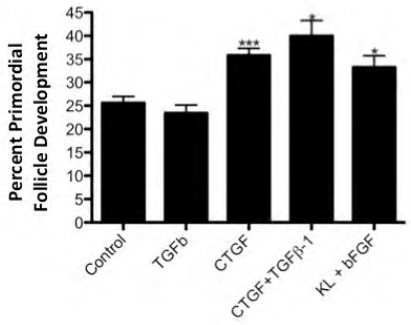
Module	Size, # genes
turquoise *	194
blue *	182
brown *	158
yellow *	150
green	139
red *	112
black	99
pink	85
magenta	68
purple *	45
greenyellow	32
tan	29
salmon	28
cyan	22
midnightblue	20
lightcyan	20
* - chosen	

(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:



Intraovarian control of early folliculogenesis.
Hsueh AJ, Kawamura K, Cheng Y, Fauser BC.
Endocr Rev. 2015 Feb;36(1):1-24.

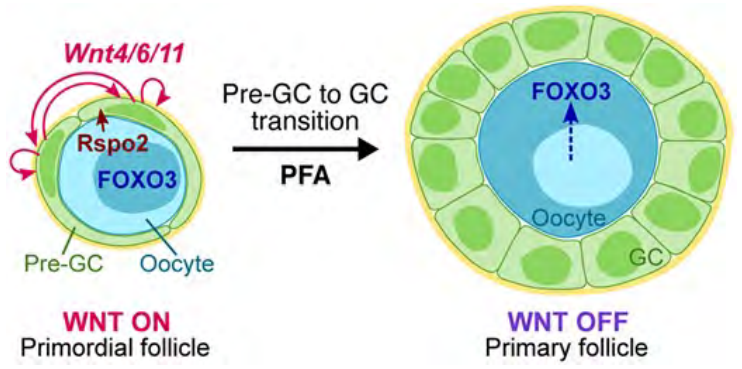
Table 1. Intraovarian Paracrine Hormones Act Through RTKs, RSKs, GPCRs, Guanylyl Cyclase Receptor NPRB, and Integrins to Regulate Preantral Follicle Growth[a]

Ligands	Receptors
IGF-1, KGF, VEGF, FGF2, FGF10	RTKs
Activins, BMP6, AMH	RSKs (types I and II)
PACAP, VIP	GPCRs
CNP	Guanylyl cyclase (NPRB)
CCN2/CTGF	Integrins

Abbreviations: CTGF, connective tissue growth factor.

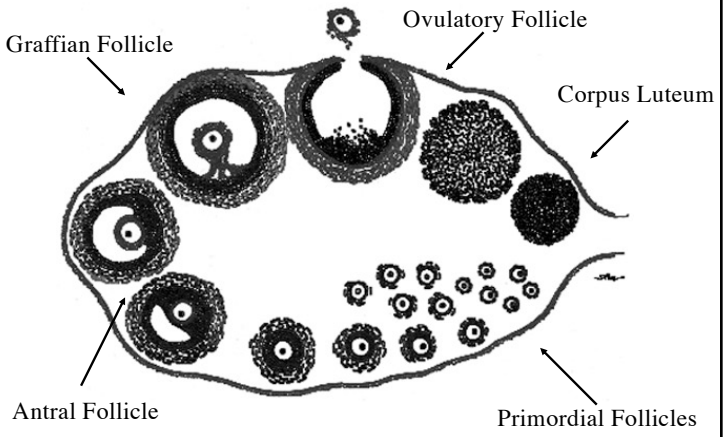
[a] Diverse paracrine growth factors are secreted by granulosa cells; they act through several distinct intracellular signaling pathways to promote follicle development. IGF-1, KGF, VEGF, FGF2, and FGF10 act through their respective RTKs in granulosa cells to regulate follicle development. In contrast, activins, AMH, and BMP6 synthesized by granulosa cells act though type I and type II RSKs in granulosa cells to regulate follicle development. Also, both PACAP and VIP produced by granulosa cells increase cAMP production by granulosa cells to regulate follicular functions. CNP secreted by granulosa cells binds to the guanylyl cyclase NPR8 to increase cGMP production and promote follicle development. In contrast, CCN2/CTGF, produced by granulosa cells in response to Hippo signaling disruption, interacts with membrane-bound integrins in granulosa cells to promote follicle growth.

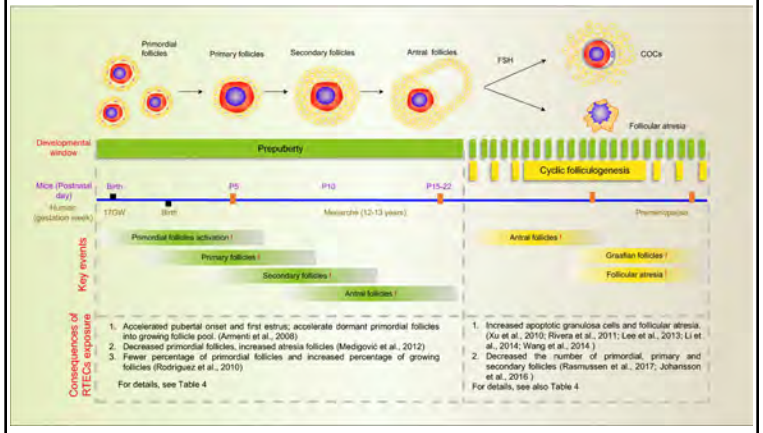
WNT signaling in pre-granulosa cells is required for ovarian folliculogenesis and female fertility
Habara O, Logan CY, Kanai-Azuma M, Nusse R, Takase HM.
Development. 2021 May 1;148(9):dev198846.

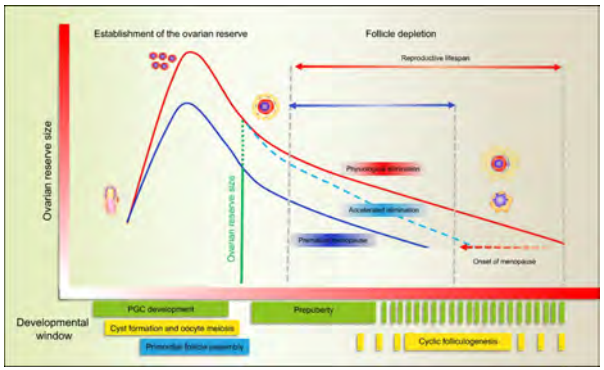


Proposed role of WNT signaling in folliculogenesis. WNT-responding pre-GCs produce *Wnt4*, *Wnt6* and *Wnt11* in primordial follicles and oocytes secrete the WNT agonist RSPO2. Activation of canonical WNT signaling in pre-GCs promotes their transition into GCs during primordial follicle activation (PFA). 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.

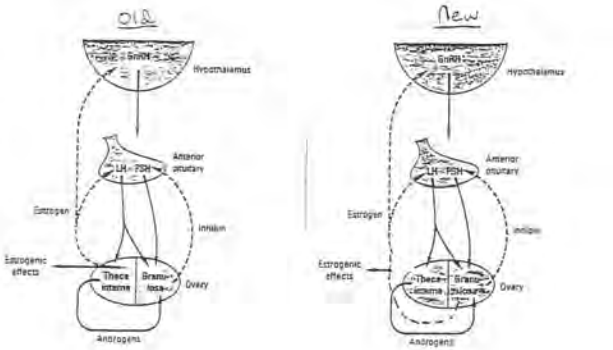
The Mammalian Ovary







Hypothetical diagram of RTEC induced earlier menopause. The red line represents normal ovarian dynamics during aging while the blue line represents ovarian dynamics in a premature menopause scenario.



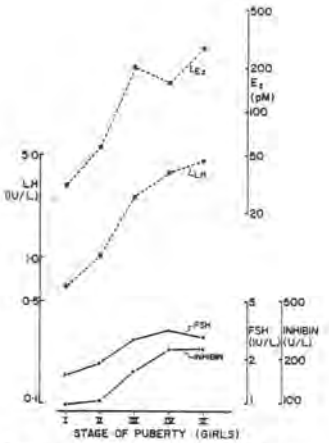


Figure 11–L. The semilogarithmic plot of mean serum FSH, inhibin, LH, and estradiol (E_2) levels during the stages of puberty in girls. (*Reproduced, with permission, from Burger HG, et al. 1988, p. 689.*[36])

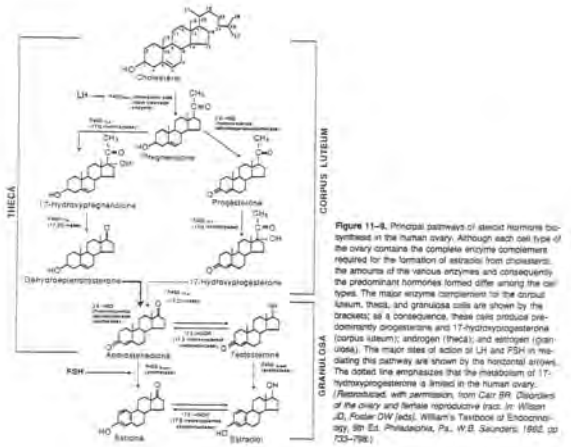


Figure 11–9. Principal pathways of steroid hormone biosynthesis in the human ovary. Although each cell type of the ovary contains the complete enzyme complement required for the formation of estradiol from cholesterol, the amounts of the various enzymes and consequently the predominant hormones formed differ among the cell types. The major enzyme complement for the corpus luteum, theca, and granulosa cells are shown by the brackets; as a consequence, these cells produce predominantly progesterone and 17-hydroxyprogesterone (corpus luteum); androgen (theca); and estrogen (granulosa). The major sites of action of LH and FSH in mediating this pathway are shown by the horizontal arrows. The dotted line emphasizes that the metabolism of 17-hydroxyprogesterone is limited in the human ovary. (*Reproduced, with permission, from Carr BR. Disorders of the ovary and female reproductive tract. In: Wilson JD, Foster DW [eds]. Williams Textbook of Endocrinology, 8th Ed. Philadelphia, Pa., W.B. Saunders, 1992, pp 703–796.*)

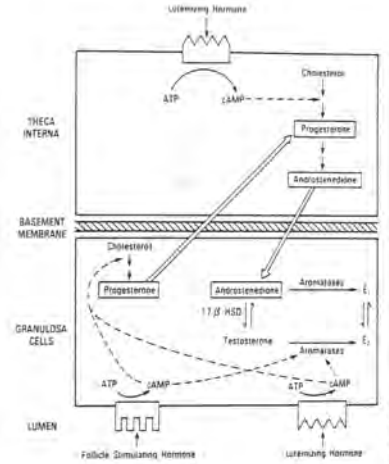


Figure 11–11. Two-cell, two-gonadotropin hypothesis of gonadotropin control of ovarian estrogen biosynthesis. (*Reproduced, with permission, from Hsueh AJW, Adashi EY, Jones PBC, et al. 1984, p 76.*[101])

Rapid Paper

Mesenchymal-epithelial cell interactions in the ovary: estrogen-induced theca cell steroidogenesis

Andrew J. Roberts and Michael K. Skinner

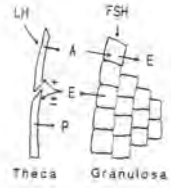


Fig. 4. Schematic of the prpoosed steroid-mediated interactions between ovarian theca cells and granulosa cells. The steroids involved include androgens (A), estogens (E) and progestins (P) with both positive (+) and negative (-) effects on steroidogenesis. The gonadotropins involved include luteinizing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

"FSH Actions on Granulosa Cells "

TABLE 1. FSH-stimulated functional parameters in cultured granulosa cells

1. Enhancement of steroidogenesis
   a. Estrogen biosynthesis
      Induction of aromatases
   b. Progesterone and 20α-OH-P biosynthesis
      Induction of cholesterol side-chain cleavage enzymes and mitochondrial cytochrome P450 activity
      Induction of 3β-hydroxysteroid dehydrogenase
      Induction of 20α-hydroxysteroid dehydrogenase
2. Induction of specific plasma membrane receptors
   a. LH receptor formation
   b. Prolactin (lactogenic) receptor formation
   c. β_2-adrenergic receptor formation (coupling)
   d. Lipoprotein receptor formation
   e. FSH receptor formation
   f. EGF receptor formation
3. Secretion of nonsteroidal cell products
   a. Inhibin
   b. Plasminogen activator
   c. Prostaglandins
   d. Proteoglycans (mucopolysaccharides)
4. Stimulation of general cell functions
   a. DNA synthesis
   b. Protein synthesis
   c. Glucose uptake and lactate formation
   d. Cell roundup and aggregation
   e. Gap junction formation
   f. Microvilli formation
5. Plasma membrane-related processes
   a. Adenyl cyclase activation and cAMP formation
   b. Formation of cAMP binding protein
   c. Phosphodiesterase activation
   d. Increases in plasma membrane microviscosity

"Actions of LH on Granulosa Cells"

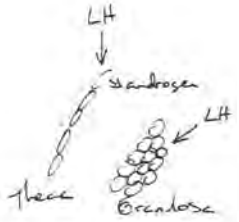
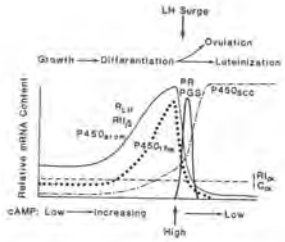
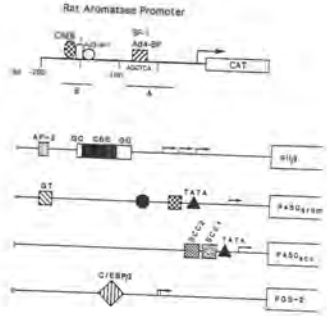


TABLE 3. LH-modulated functional parameters in cultured granulosa cells

1. Enhancement of steroidogenesis
   a. Estrogen biosynthesis
   b. Progesterone and 20α-OH-P biosynthesis
      Stimulation of cholesterol esterase activity
      Induction of cholesterol side-chain cleavage enzyme
      Induction of 3β-hydroxysteroid dehydrogenase
2. Modulation of plasma membrane receptors
   a. Increases in prolactin (lactogenic) receptor formation
   b. Increases in β_2-adrenergic receptor formation (coupling)
   c. Down-regulation or stimulation of LH receptors
   d. Increases in lipoprotein receptor formation
3. Secretion of non-steroidal cell products
   a. Plasminogen activator
   b. Mucopolysaccharides
   c. Prostaglandins
   d. Relaxin
4. Modulation of general cell functions
   a. Increases in protein synthesis
   b. Increases in glycolysis
   c. Alterations of cell shape
   d. Reduction in size of gap junctions
   e. Inhibition of DNA synthesis
   f. Stimulation of ornithine decarboxylase activity
5. Plasma membrane-related processes
   a. Adenyl cyclase activation and cAMP formation
   b. Stimulation of cAMP-dependent protein kinase activity
   c. Stimulation of phospholipid turnover





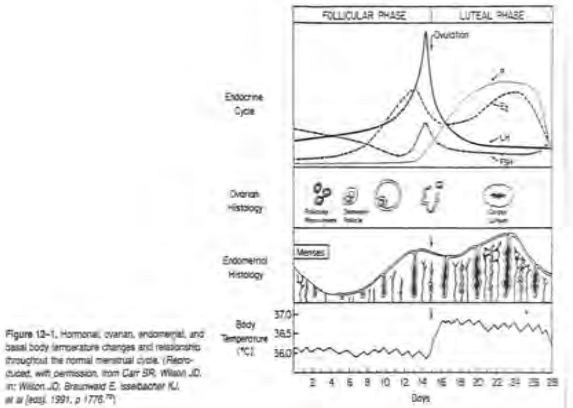


Figure 12–1. Hormonal, ovarian, endometrial, and basal body temperature changes and relationship throughout the normal menstrual cycle. (Reproduced, with permission, from Carr BR, Wilson JD. In: Wilson JD, Braunwald E, Isselbacher KJ, et al [eds]. 1991, p 1778.[T])

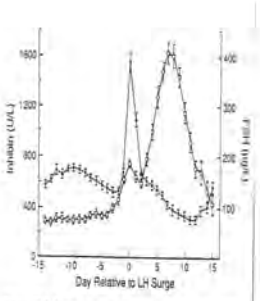


Figure 12–6. Serum levels of inhibin and FSH during the normal menstrual cycle. Day of LH surge = 0. Key: (○), inhibin; (●), FSH. (Adapted, with permission, from McLachlan RI, Cohen NL, Dahl KD, et al. Serum inhibin in normal women: Relationship with ratio steroid and gonadotropin levels. Clin Endocrinol. 32:39, 1990.)

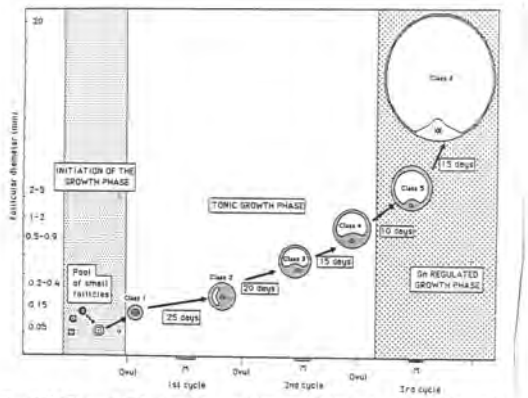


Figure 6–15 ■ The complete follicular growth trajectory: the conversion of a class 1 to a class 6 follicle. M, menses; Gn, gonadotropin. (Courtesy of A. Gougeon.)

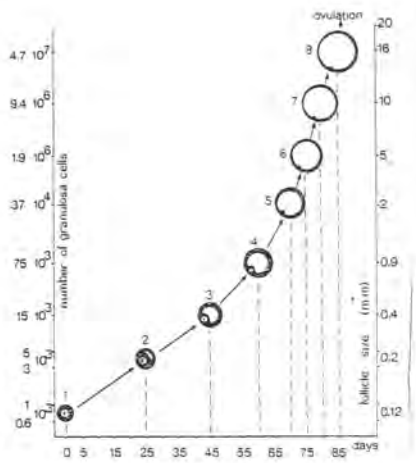
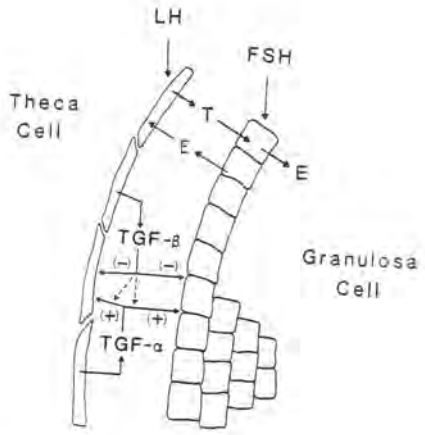


Figure 6–17 ■ Rate of follicular growth (classes 1 to 8) in the human ovary. (From Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. Human Reprod 1:81, 1986.)

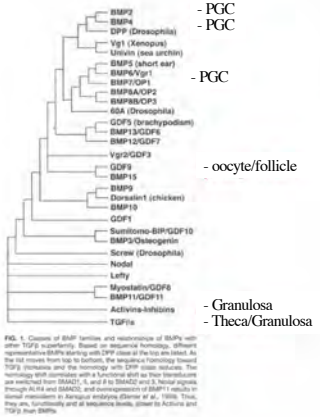
TABLE I

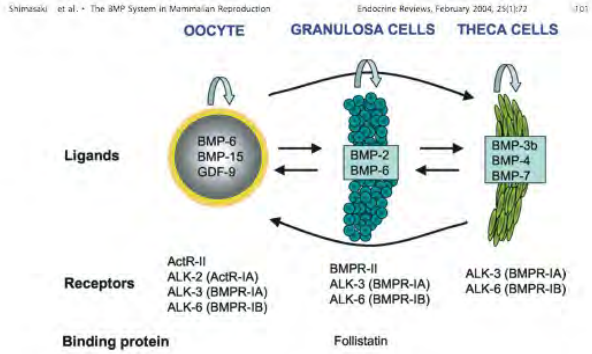
PROPERTIES AND NOMENCLATURE OF SEVERAL COMMON GROWTH FACTORS

Growth Factor		Approx. Size (kDa)	Examples of Physiological Action
Insulin-like Growth Factor-I	IGF-I	7.5	Skeletal Growth
Insulin-like Growth Factor-II	IGF-II	7.5	Fetal Development
Epidermal Growth Factor	EGF	6	Tissue Growth
Transforming Growth Factor Alpha	TGF-α	5	Tissue Growth
Transforming Growth Factor Beta	TGF-β	25/dimer	Growth Inhibition/ Tissue Repair
Fibroblast Growth Factor	FGF	17	Angiogenesis/Tissue Growth
Vascular Endothelial Growth Factor	VEGF	25-50/dimer	Angiogenesis/Tissue Growth
Nerve Growth Factor	NGF	13	Neuronal Development
Interleukin-1	IL-1	17	Immune Response/ Inflammation
Platelet Derived Growth Factor	PDGF	30/dimer	Tissue Growth
Stem Cell Factor (c-kit ligand)	SCF	30	Tissue Growth/Fetal Development

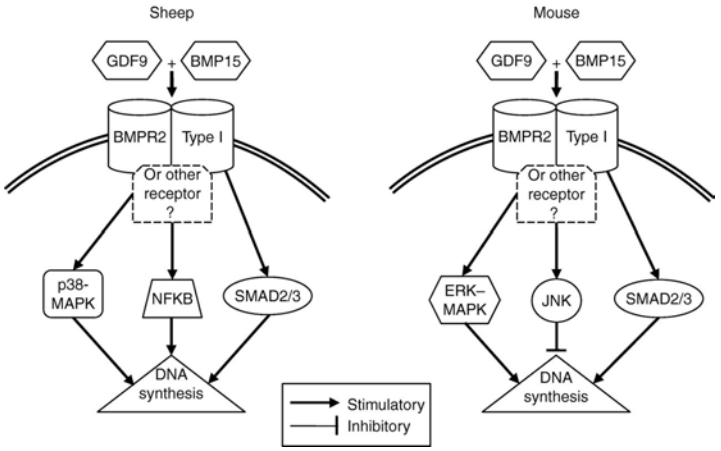


Transforming Growth-Beta Factor Family





Signalling pathways involved in the cooperative effects of ovine and murine GDF9+BMP15-stimulated thymidine uptake by rat granulosa cells.
Reader KL, et al.
Reproduction. (2011) Jul;142(1):123-31.



The epidermal growth factor network: role in oocyte growth, maturation and developmental competence
Hum Reprod Update. 2018 Jan 1;24(1):1-14.
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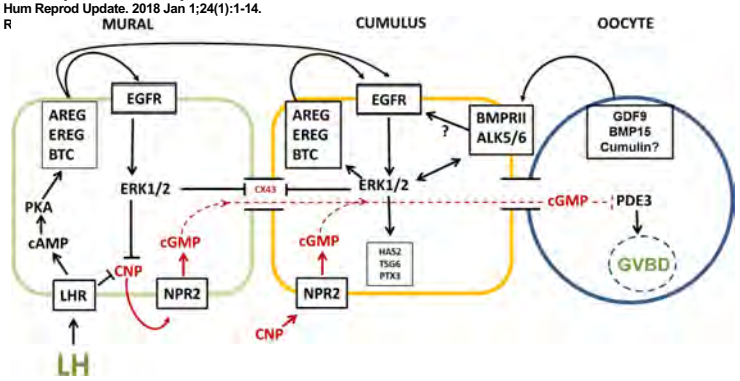


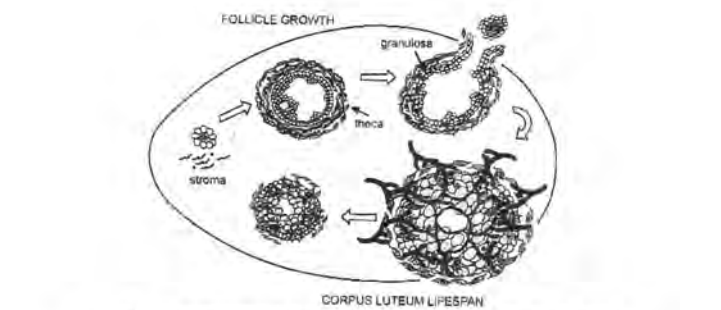


Figure 1. Schematic representation of the changes in vasculature (black) during the life of a single follicle selected to mature and ovulate (clockwise from the left). Initially, vascular support for the primordial and primary follicle is indirect from stromal capillaries. As the follicle forms an antrum, a vascular network develops and is restricted to the theca layer. Around ovulation, capillaries grow into the avascular granulosa layer. Rampant capillary growth continues during development of the corpus luteum. At luteal regression, the vascular network decreases until the corpus luteum becomes part of the ovarian stroma.

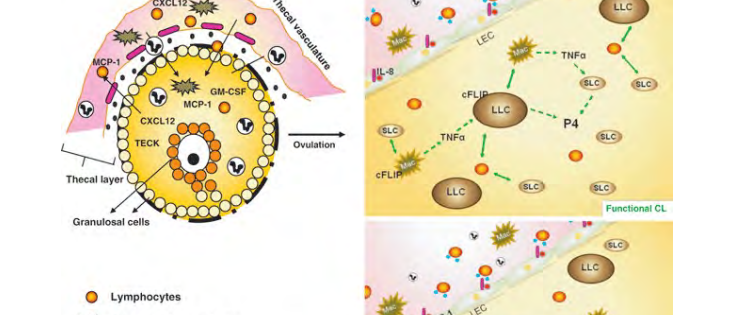


Table 1. List of immune response-related genes and their functions in ovulation

Gene	Product	Function
Amphiregulin (Areg)	EGF-like factor	Binds EGFR and activates ERK1/2
A disintegrin and metalloproteinase with thrombospondin-like repeats (Adamts1)	Protein	Secreted protease cleaves versican
Cathepsin L (Ctsl)	Protein	Protease
Chondroitin sulfate proteoglycan (Cspg2)	Versican	Binds HA
Cell differentiation14 (Cd14)	Adaptor molecule	Binds LPS
CD34 (Cd34)	Membrane protein	Immune cell stem cell marker
CD36 (Cd36)	Membrane protein	Scavenger receptor
CD52 (Cd52)	GPI anchored protein	Unknown
Complement factor Q1 (C1q)	C1q	Adaptor for TLR2/4 binds HA and PTX3
HA synthase 2 (Has2)	HA	Polymer of matrix of COCs
Interleukin 6 (Il6)	IL-6, cytokine	Inflammation, innate immune
Pentraxin 3 (Ptx3)	Matrix protein	Binds TNFAIP6
Progesterone receptor (Pgr)	Nuclear protein	Transcription factor
Programmed cell death1 (Pdcd1)	Membrane protein	Anti-autoimmune regulator
Prostaglandin synthase 2 (Ptgs2)	PGE2, prostaglandins	Binds (prostaglandin E2 receptor subtype) EP2 receptorand induces AREG
Runx1	Nuclear protein	Transcription factor
Toll-like receptors (Tlr2, Tlr4)	Membrane proteins	Bind LPS and Pam3Cys and HA fragments
TNF-a-induced protein 6 (Tnfaip6)	Matrix protein	Binds and stabilizes HA matrix

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

Parrott et al (1994)
Endocrinology
135:569

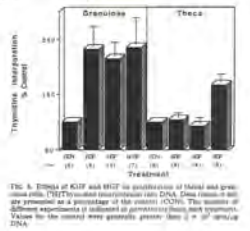


TABLE 2

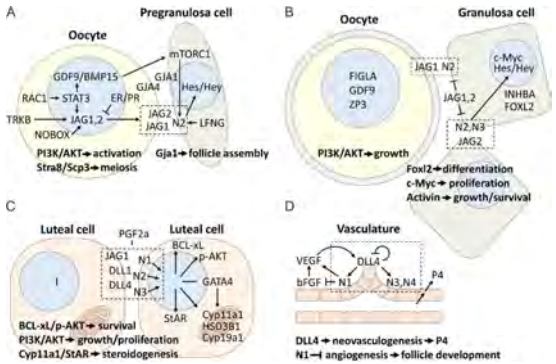
GROWTH FACTORS IN THE OVARY

Growth Factor	Proposed Site Synthesis	Proposed Site of Action	Proposed Function*
IGF-1	Granulosa	Granulosa Theca	+Growth/+Differentiation +Growth/+Differentiation
FGF	Granulosa	Granulosa Endothelium	+Growth Angiogenesis
TGF-ß	Theca Granulosa	Granulosa Theca	-Growth/+Differentiation -Growth/+Differentiation
TGF-α	Theca	Granulosa Theca	+Growth/-Differentiation +Growth
VEGF	Granulosa	Endothelium	Angiogenesis/+Growth
NGF	Ovary	Neurons	Innervation
SCF (kit ligand)	Granulosa	Oocyte	Oocyte Maturation

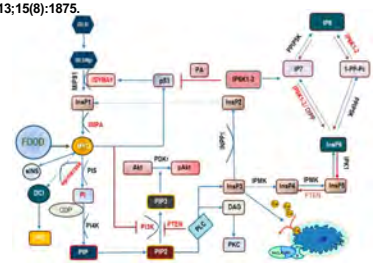
*A (+) denotes an increase and (-) indicates a decrease.

KGF	Theca	Granulosa	+growth
HGF	Theca	Granulosa	+growth
GDF9	Oocyte	Granulosa/ Theca	+growth/ +differentiation
BMP15	Oocyte	Granulosa	+growth

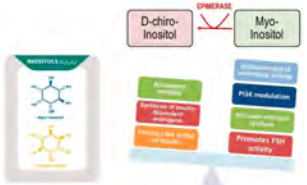
The role of Notch signaling in the mammalian ovary
Reproduction. 2017 Jun;153(6):R187-R204.
Vanorny DA, Mayo KE.



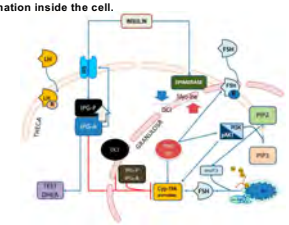
Myo-Inositol and D-Chiro-Inositol as Modulators of Ovary Steroidogenesis: A Narrative Review.
Bizzarri M, Monti N, Piombarolo A, Angeloni A, Verna R.
Nutrients. 2023 Apr 13;15(8):1875.



Main metabolic pathways related to myo-inositol transformation inside the cell.



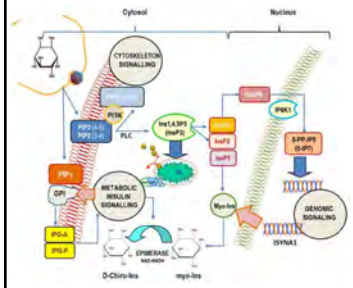
Opposite functions exerted by myo-inositol and D-chiro-inositol.



Main pathways showing the involvement of myo-Ins and D-chiro-Ins (DCI) upon endocrine signaling pathways in theca and granulosa cells within the ovary.

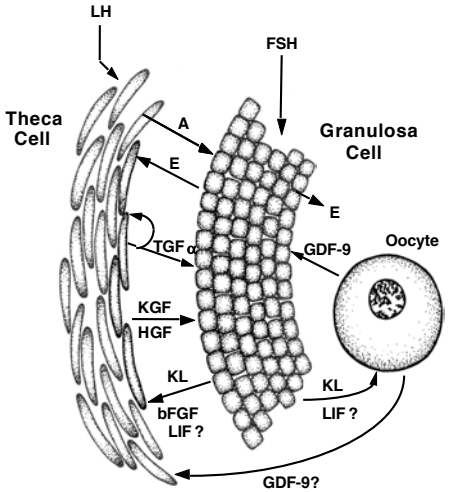
The Role of Inositols in the Hyperandrogenic Phenotypes of PCOS: A Re-Reading of Larner's Results.
Fedeli V, Catizone A, Querqui A, Unfer V, Bizzarri M.
Int J Mol Sci. 2023 Mar 27;24(7):6296.

Main intracellular signaling pathways in which myo-inositol participates.

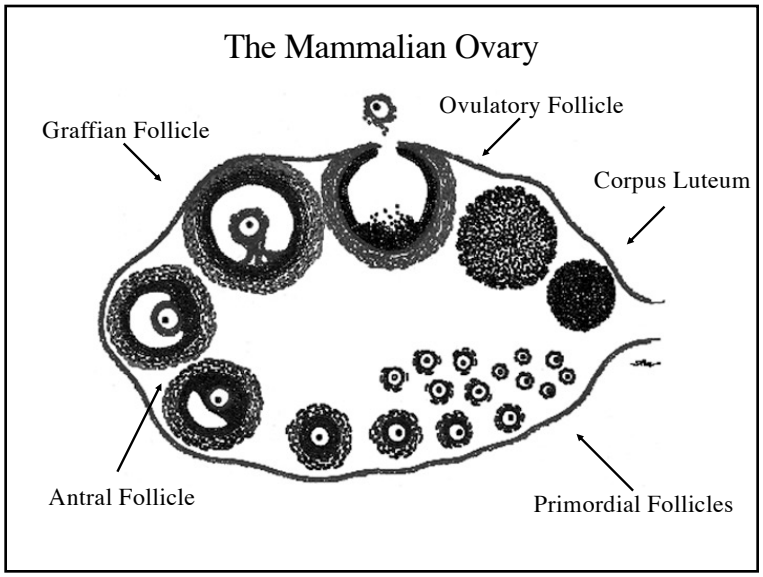


Opposite effects upon ovary steroidogenesis of myo-Ins and D-Chiro-Ins.

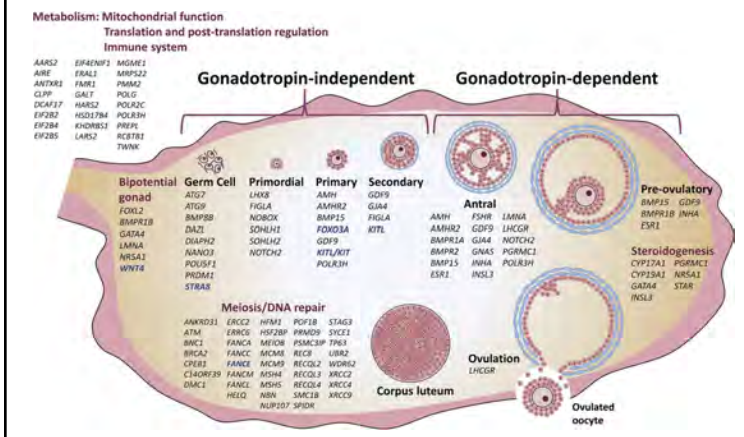
CELL CELL INTERACTIONS IN THE ANTRAL FOLLICLE



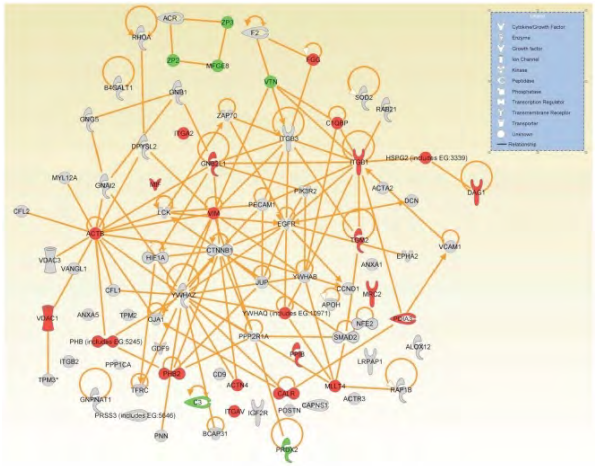
	Environmental	Nutritional	Regulatory
Theca-Granulosa	ECM	Androgen	Estrogen Progestin TGFα TGFβ HGF KGF
Granulosa-Oocyte	Minimal ECM Cytoarchitectural Support	Gap Junctions (<800mw)	cAMP Xanthine Dirivatives OMI Kit-ligand (SCF) GDF-9
Granulosa-Granulosa	Minimal ECM Gap Junctions	Gap Junctions (cAMP)	Estrogen IGF FRP



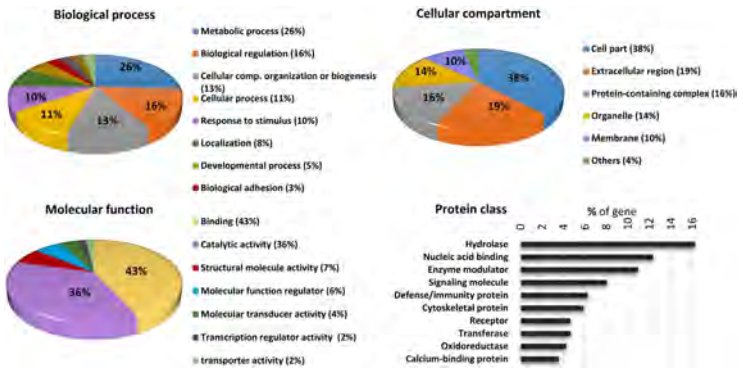
Genetics of ovarian insufficiency and defects of folliculogenesis.
França MM, Mendonca BB.
Best Pract Res Clin Endocrinol Metab. 2021 Oct 14;101594.



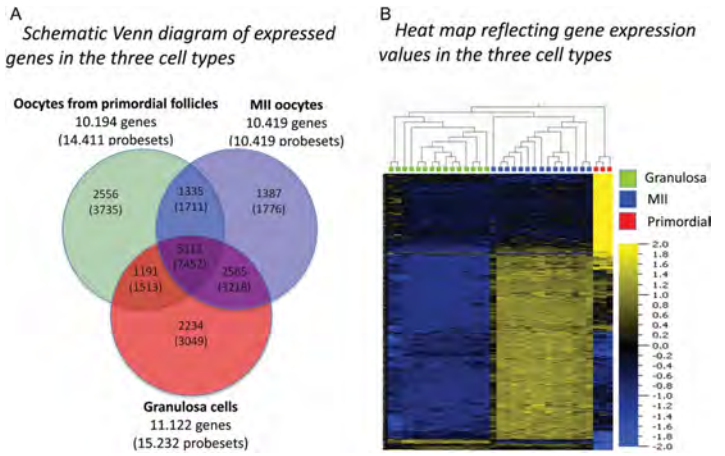
Proteomics-based systems biology modeling of bovine germinal vesicle stage oocyte and cumulus cell interaction.
Peddinti D, et al.
PLoS One. (2010) Jun 21;5(6):e11240.



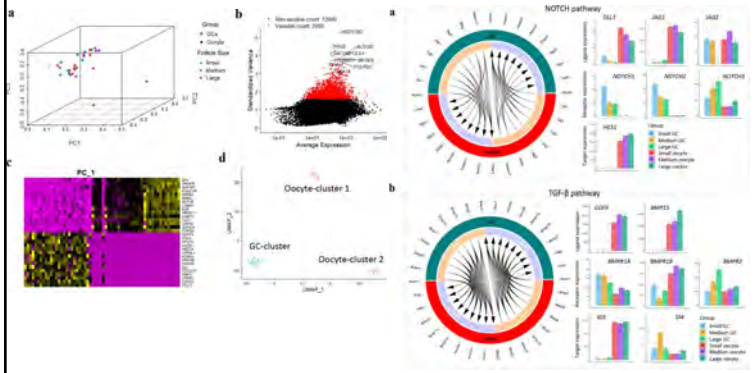
Proteome of fluid from human ovarian small antral follicles reveals insights in folliculogenesis and oocyte maturation.
Pla I, Sanchez A, Pors SE, et al.
Hum Reprod. 2021 Feb 18;36(3):756-770.



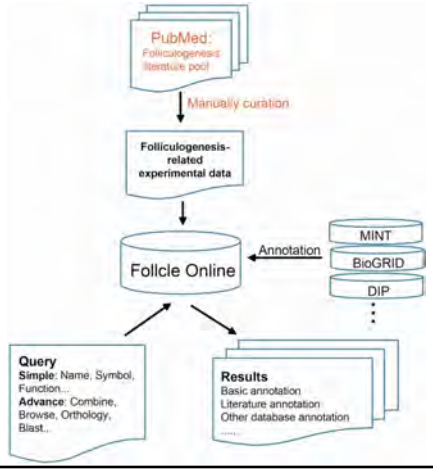
The dormant and the fully competent oocyte: comparing the transcriptome of human oocytes from primordial follicles and in metaphase II.
Grøndahl ML, et al.
Mol Hum Reprod. (2013) 19(9):600-17.



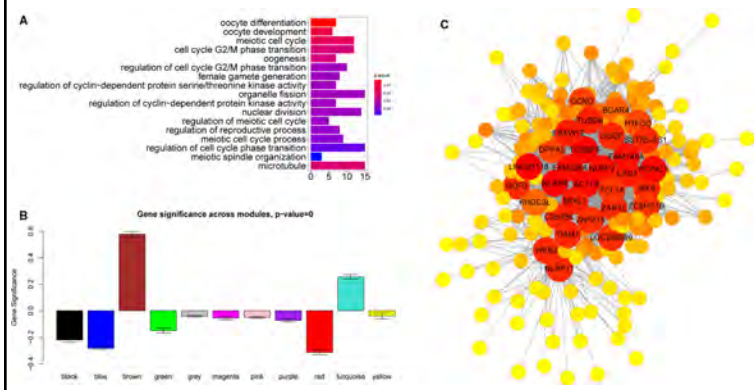
Transcriptome profile of goat folliculogenesis reveals the interaction of oocyte and granulosa cell in correlation with different fertility population.
Li S, Wang J, Zhang H, et al.
Sci Rep. 2021 Aug 3;11(1):15698.



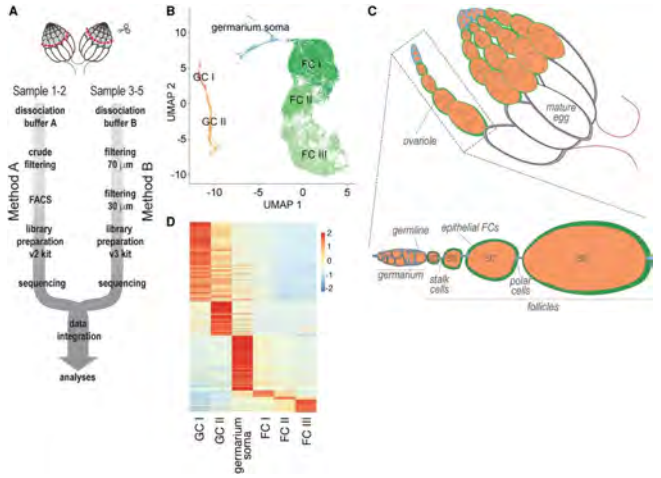
Follicle Online: an integrated database of follicle assembly, development and ovulation.
Hua J, Xu B, Yang Y, Ban R, Iqbal F, Cooke HJ, Zhang Y, Shi Q.
Database (Oxford). 2015 Apr 29;2015:bav036.



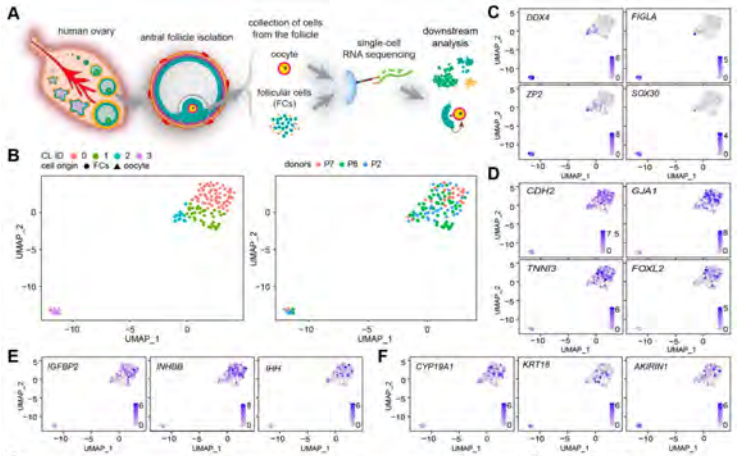
Cell-specific network analysis of human folliculogenesis reveals network rewiring in antral stage oocytes.
Wang S, Gong Y, Wang Z, et al.
J Cell Mol Med. 2021 Mar;25(6):2851-2860.



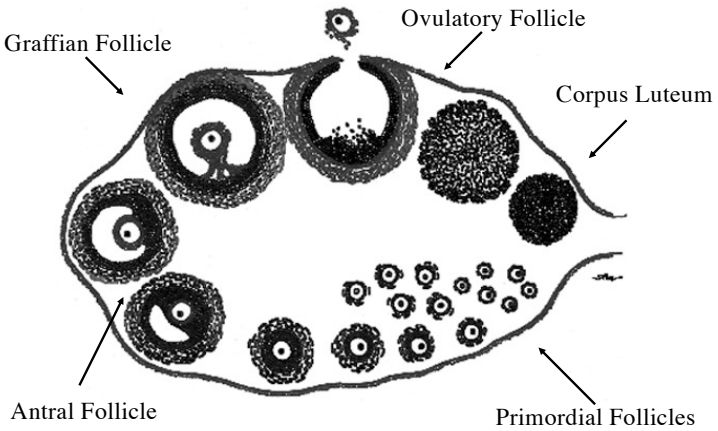
A single-cell atlas reveals unanticipated cell type complexity in Drosophila ovaries.
Slaidina M, Gupta S, Banisch TU, Lehmann R.
Genome Res. 2021 Oct;31(10):1938-1951.



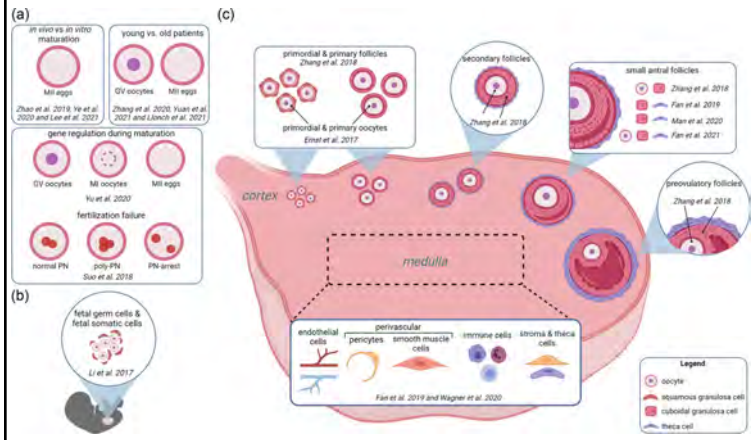
Single-Cell Transcriptomics Analysis of Human Small Antral Follicles.
Fan X, Moustakas I, Bialecka M, et al.
Int J Mol Sci. 2021 Nov 4;22(21):11955.



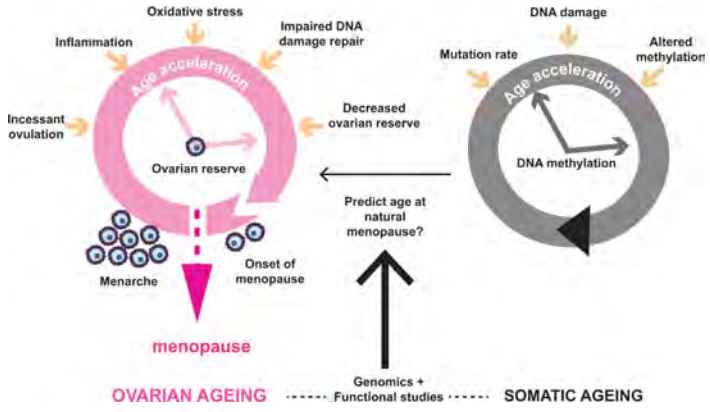
The Mammalian Ovary



Single-cell RNA-sequencing of retrieved human oocytes and eggs in clinical practice and for human ovarian cell atlasing.
Machlin JH, Shikanov A.
Mol Reprod Dev. 2022 Dec;89(12):597-607.

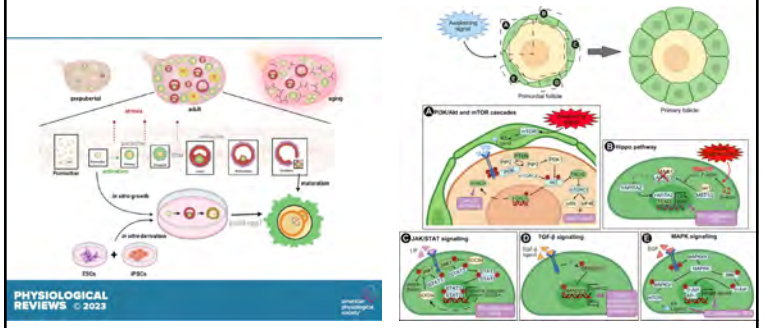


Novel insights into reproductive ageing and menopause from genomics.
Das A, Destouni A.
Hum Reprod. 2023 Feb 1;38(2):195-203.



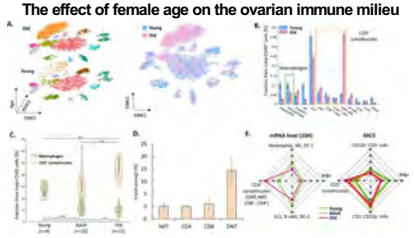
Ovarian and somatic biological ageing clocks and their putative relationship.

Making a good egg: human oocyte health, aging, and in vitro development.
Telfer EE, Grosbois J, Odey YL, Rosario R, Anderson RA.
Physiol Rev. 2023 Oct 1;103(4):2623-2677.

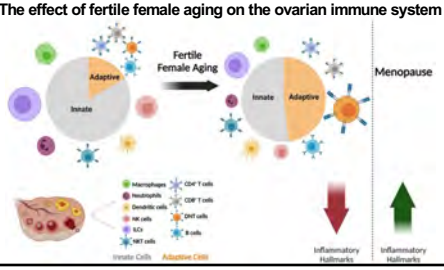


Single-cell analysis of the aged ovarian immune system reveals a shift towards adaptive immunity and attenuated cell function.
Ben Yaakov T, Wasserman T, Aknin E, Savir Y.
Elife. 2023 Apr 25;12:e74915.

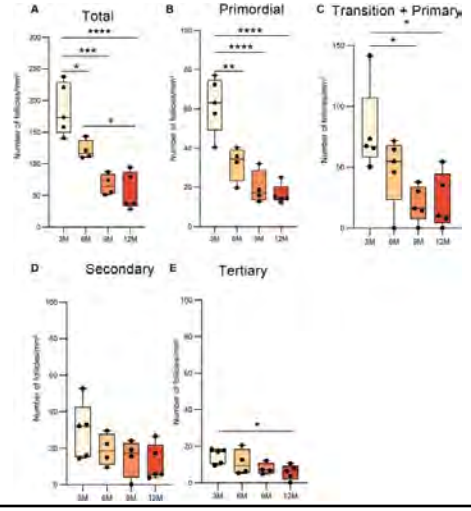
The effect of female age on the ovarian immune milieu



The effect of fertile female aging on the ovarian immune system



Cellular hallmarks of aging emerge in the ovary prior to primordial follicle depletion.
Ansere VA, Ali-Mondal S, Sathiaseelan R, et al.
Mech Ageing Dev. 2021 Mar;194:111425.



Polycystic ovary syndrome as a paradigm for prehypertension, prediabetes, and preobesity.

Luque-Ramírez M, Escobar-Morreale HF.

Curr Hypertens Rep. 2014 Dec;16(12):500.

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

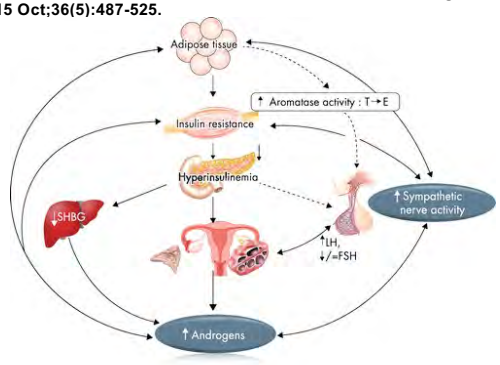
- ↑ Insulin resistance
- ↑ Hyperinsulinism
- ↑ Fasting glucose
- ↑ 2 h glucose after OGTT
- ↑ Prevalence of abnormal glucose tolerance
- ↓ HDL-cholesterol levels
- ↑ Triglycerides levels
- ↑ Office and ABPM systolic and diastolic blood pressure values
- ↑ Prevalence of office and ABPM hypertension
-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

Endocr Rev. 2015 Oct;36(5):487-525.

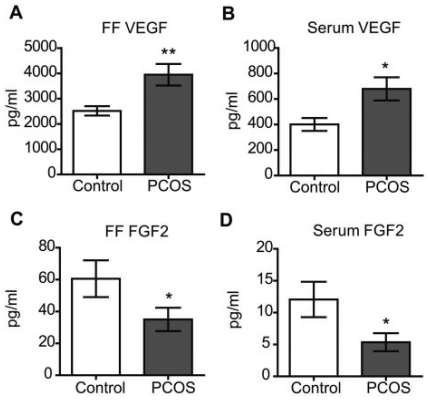


Pathophysiology of PCOS—a vicious circle. Several theories have been proposed to explain the pathogenesis of PCOS. One of these is that neuroendocrine defects lead to increased pulse frequency and amplitude of LH and relatively low FSH. This causes intrinsic defects in ovarian androgen production. Also, 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. E, estradiol.

Alteration in angiogenic potential of granulosa-lutein cells and follicular fluid contributes to luteal defects in polycystic ovary syndrome.

Patil K, Hinduja I, Mukherjee S.

Hum Reprod. 2021 Mar 18;36(4):1052-1064.

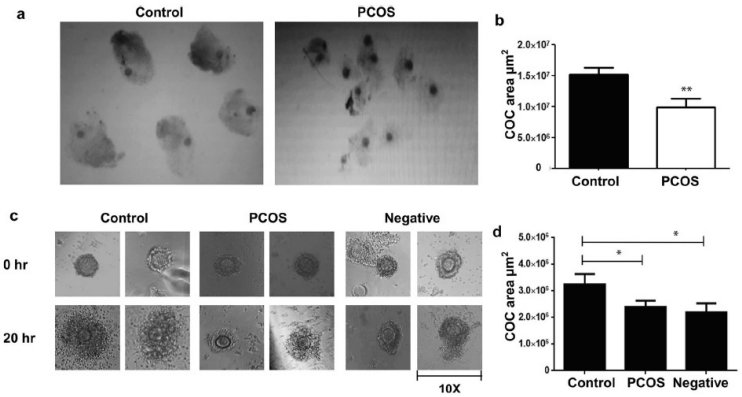


The concentration of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) levels in FF and serum of control and women with polycystic ovary syndrome (PCOS). FF (n ¼ 20 in each group) and serum (n ¼ 10 in each group) were used to measure levels of VEGF (A and B) and FGF2 (C 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.

Compromised Cumulus-Oocyte Complex Matrix Organization and Expansion in Women with PCOS

Patil K, Shinde G, Hinduja I, Mukherjee S.

Reprod Sci. 2021 Nov 8. Online ahead of print.

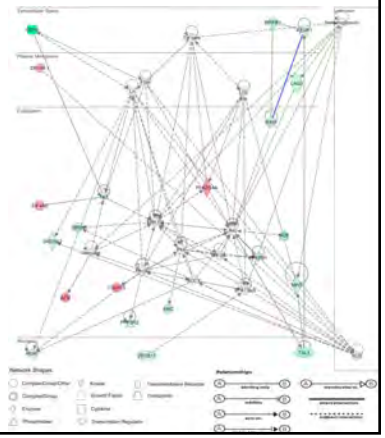


Functional microarray analysis of differentially expressed genes in granulosa cells from women with polycystic ovary syndrome related to MAPK/ERK signaling.

Lan CW, Chen MJ, Tai KY, Yu DC, Yang YC, Jan PS, Yang YS, Chen HF, Ho HN.

Sci Rep. 2015 Oct 13;5:14994.

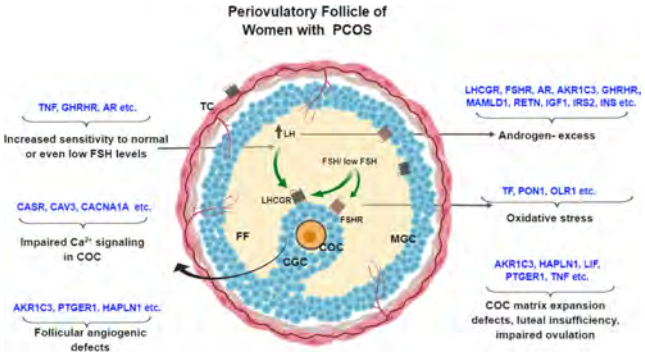
Genes Symbol	Genes Name	log fold change	adj *P* value
Upregulated			
AOX1	aldehyde oxidase 1	1.475	0.001813**
C1orf54	chromosome 1 open reading frame 54	1.311	0.002377**
SNORD59A	small nucleolar RNA, C/D box 59A	1.257	0.002768**
TRIM22	tripartite motif-containing 22	1.212	0.002481**
RN7SK	RNA, 7SK small nuclear	1.180	0.001813**
ANKRD36B	ankyrin repeat domain 36B	1.157	0.001583**
ENPP5	ectonucleotide pyrophosphatase/phosphodiesterase 5	1.113	0.002160**
SLC25A30	solute carrier family 25, member 30	1.085	0.002598**
SNORD21	small nucleolar RNA, C/D box21	1.028	-0.001583**
ALPK1	alpha-kinase 1	1.008	0.001583**
Downregulated			
DHX16	DEAH box polypeptide 16	−0.762	0.004345**
FBXO5	F-box protein 5	−0.709	0.013218*
OR14C36	olfactory receptor, family 14, subfamily C,member 36	−0.627	0.007862**
PCOTH	prostate collagen triple helix	−0.613	0.001583**
RPL4	ribosomal protein L4	−0.563	0.003669**
BCL2L15	BCL2-like15	−0.552	0.002969**
OR52E8	olfactory receptor, family 52, subfamily E, member 8	−0.453	0.014957*
GTF2H1	general transcription factor IIH, polypeptide 1	−0.430	0.025261*
OR5A2	olfactory receptor, family 5, subfamily A, member 2	−0.429	0.011600*
PTH2R	parathyroid hormone 2 receptor	−0.403	0.011824*



DNA methylome profiling of granulosa cells reveals altered methylation in genes regulating vital ovarian functions in polycystic ovary syndrome.

Sagvekar P, Kumar P, Mangoli V, Desai S, Mukherjee S.

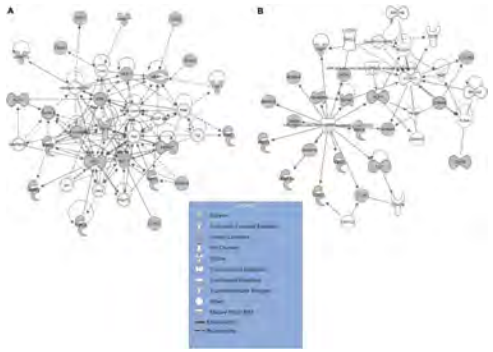
Clin Epigenetics. 2019 Apr 11;11(1):61.



Transcriptional landscape of mouse-aged ovaries reveals a unique set of non-coding RNAs associated with physiological and environmental ovarian dysfunctions.

Cuomo D, Porreca I, Ceccarelli M, Threadgill DW, et al.

Cell Death Discov. 2018 Dec 5;4:112.

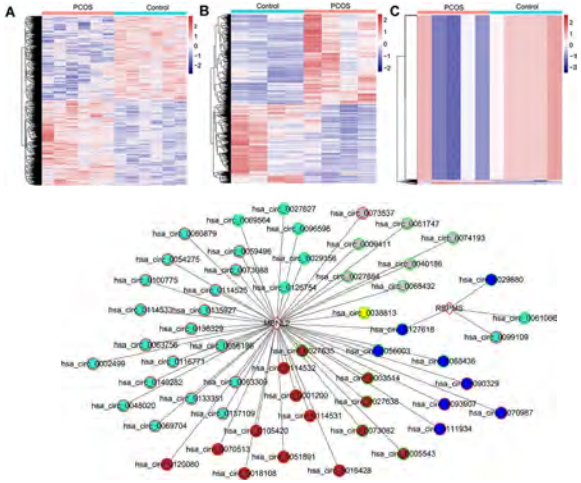


Networks of putative target genes regulated by differentially expressed miRNAs. Validated target genes (grey nodes) mapped to pathway annotations derived from literature and gene ontology using Ingenuity Pathway Analysis (IPA, http://www.ingenuity.com) **A** *Mir143* regulatory network **B** and *Mir145* regulatory network. The solid lines connecting molecules represent a direct relation and dotted lines an indirect relation. IPA constructs networks that optimize for both interconnectivity 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.

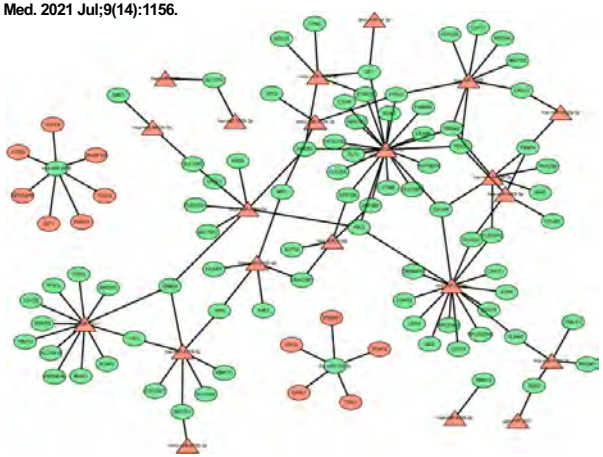
WGCNA Analysis Identifies Polycystic Ovary Syndrome-Associated Circular RNAs That Interact with RNA-Binding Proteins and Sponge miRNAs.

Li M, Zeng Z, Zhang A, et al.

Int J Gen Med. 2021 Nov 23;14:8737-8751.

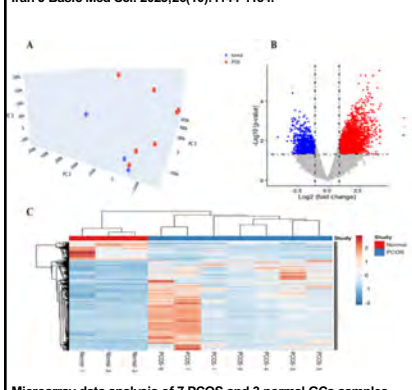


The lncRNA-miRNA-mRNA ceRNA network in mural granulosa cells of patients with polycystic ovary syndrome: an analysis of Gene Expression Omnibus data.
Chen H, Cheng S, Xiong W, Tan X.
Ann Transl Med. 2021 Jul;9(14):1156.

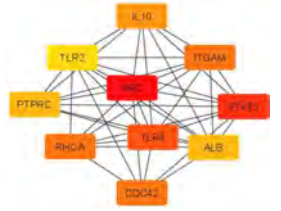


Flowchart of data processing and analysis. LncRNA, long non-coding RNA; miRNA, microRNA; mRNA, messenger RNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

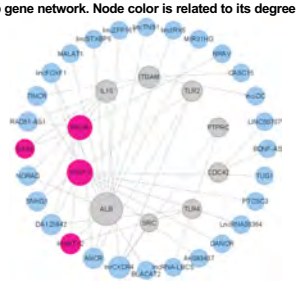
Evaluation of the expression profile of mRNAs and lncRNAs in cumulus cells associated with polycystic ovary syndrome and pregnancy.
Hammami B, Mostafavi FS, Akbari A, Mousavi SR, Kazemi M.
Iran J Basic Med Sci. 2023;26(10):1144-1154.



Microarray data analysis of 7 PCOS and 3 normal GCs samples

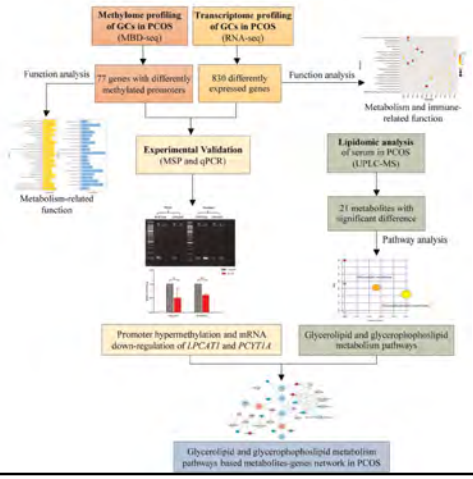


Hub gene network. Node color is related to its degree



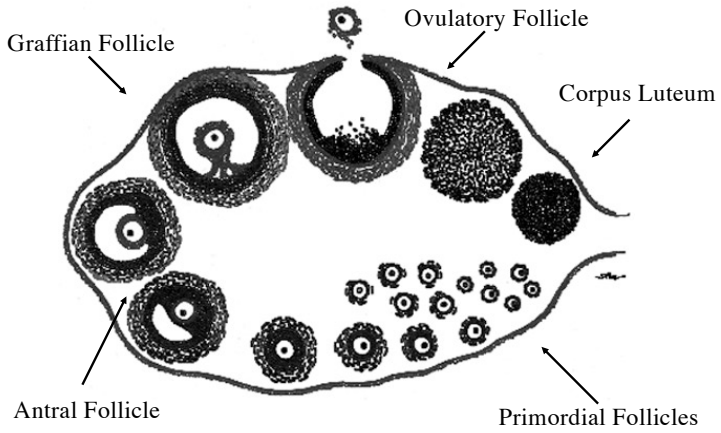
lncRNA-mRNA interaction network obtained from cytoscape

Methylome and transcriptome profiling revealed epigenetic silencing of LPCAT1 and PCYT1A associated with lipidome alterations in polycystic ovary syndrome.
Mao Z, Li T, Zhao H, Wang X, Kang Y, Kang Y.
J Cell Physiol. 2021 Sep;236(9):6362-6375.



Systems biology and in silico-based analysis of PCOS revealed the risk of metabolic disorders.
Hossain MA, Al Ashik SA, Mahin MR, Al Amin M, Rahman MH, Khan MA, Emran AA.
Heliyon. 2022 Dec 22;8(12):e12480.

The Mammalian Ovary



"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
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 -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review