Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Gonadal Developmental Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 13, 2024
Week 6

Gonadal Developmental Systems Biology

Early Fetal Gonadal Development
- Morphogenesis
- Transcriptome
- Meiotic Arrest

Testis Gonadal Fetal Development
- Knockout Models and Genes
- Cellular Growth Regulation

Ovary Gonadal Fetal Development
- Developmental Timing and Morphology
- Oocyte Nests and Primordial Follicle Assembly
- Hormone Regulation of Assembly
- Culture Models

Description of Gonadal Development
- Endocrine Disruptors

Required Reading

Pepling and Burton (2018) Fetal/Gonadogenesis, Encyclopedia of Reproduction (Second Edition). Volume 2, Pages 47-51.

Rotgers and Yao, (2018) Formation of the Testis Primordium, Encyclopedia of Reproduction (Second Edition). Volume 1, Pages 84-87.

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OVARY DEVELOPMENT

Fetal/Gonadogenesis

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Glossary

Anti-Müllerian hormone (AMH) A growth factor secreted by Sertoli cells to induce the degeneration of the Müllerian ducts in male embryos.

Coelomic epithelium Mesothelium derived from the splanchnopleura of lateral plate mesoderm that forms the lining of the organs in the coelomic cavity.

Folliculogenesis The growth, development, and maturation of an ovarian follicle.

Genital/gonadal ridge The anlagen of the testis in males and the ovary in females.

Germ cells Cells whose descendants become either sperm or egg.

Granulosa cells Ovary-specific cells that produce estrogen and progesterone, and support female germ cells throughout oogenesis.

Leydig cells The steroidogenic cells of the testis that synthesize testosterone.

Mesonephroi A pair of kidney-like organs derived from the intermediate mesoderm that function temporarily in the embryo, and from which the male and female reproductive tract are derived.

Oogonia Ovarian germ cells derived from PGCs that give rise to primary oocytes.

Primordial germ cell (PGC) Germ cell stage preceding sex differentiation.

Sertoli cells Testis-specific cells that secrete AMH in the developing embryo, and function to support male germ cells throughout spermatogenesis.

Sex-determining region Y (Sry) Gene encoding a transcription factor that initiates male sex determination; also known as testis determining factor (TDF).

Theca cells Endocrine cells associated with ovarian follicles that produce the androgen substrate for estrogen biosynthesis.

Introduction

Mammalian embryonic gonads initially form as a bipotential primordium that is morphologically indistinguishable in male and female embryos. A chromosomally determined sexual fate decision elicits the activation of the testis-specific or the ovary-specific pathway, and initiates the transformation of the common primordial gonads into either the male or female phenotype. The differentiated ovary produces and maintains a pool of oocytes, of which a subset is ovulated for fertilization. Additionally, the ovary functions as an endocrine organ, synthesizing and secreting the steroid hormones estrogen and progesterone that stimulate the development of secondary sex characteristics and prepare the accessory reproductive organs to facilitate pregnancy, birth, and lactation. Consequently, organogenesis of the ovaries is a highly important process for female fertility and reproductive success. Defects during gonad formation and development are pertinent to disorders of sex development (DSDs), and may also contribute to infertility or ovarian cancer. Knowledge of the process of gonadogenesis is therefore crucial to our understanding of reproductive pathologies, and may inform new treatment approaches for associated abnormalities. This article provides an overview of the key events that occur during the formation of the bipotential primordial gonad and the differentiation of the ovary, with particular focus on organogenesis of the ovary in mice, the most extensively studied mammalian model, and in humans.

The Urogenital System

Early in mammalian gestation, the embryo becomes trilaminar as it undergoes gastrulation to form three germ layers: the ectoderm, mesoderm, and endoderm. All embryonic tissues and organs are derived from these germ layers as the cells proliferate, migrate, and differentiate. The mesodermal layer is regionalized into the paraxial, intermediate, and lateral plate mesoderm. The intermediate mesoderm forms the urogenital system which includes the gonads and kidneys, as well as their associated duct systems. The urogenital ridges form bilaterally as longitudinal mesodermal elevations in the dorsal body wall of the embryo. Three sets of kidneys—the pronephroi (transitory and non-functional), mesonephroi (interim kidneys), and the metanephroi (becomes the permanent

kidneys)—develop from the urogenital ridges. Formation of the gonads is concurrent and closely associated with the differentiation of the mesonephroi. Invaginations of the surface epithelium of the mesonephroi give rise to the mesonephric (Wolffian) ducts and the paramesonephric (Müllerian) ducts. In male embryos, the testes produce testosterone which influences the differentiation of the Wolffian ducts to form the internal male genitalia, such as the vas deferens, epididymis, and the seminal vesicle. Additionally, the male gonad produces Müllerian-inhibiting substance (MIS), also known as anti-Müllerian hormone (AMH), which induces the degeneration of the Müllerian ducts. In contrast, the developing female gonad lacks these factors and the Müllerian ducts give rise to the fallopian tubes (oviducts), the uterus, the cervix, and the cranial portion of the vagina while the Wolffian ducts degenerate. Recent evidence suggests that the transcription factor, COUP-TFII, suppresses signaling that is required to maintain the Wolffian ducts and therefore actively regulates differentiation of the female reproductive tract (Zhao et al., 2017).

Formation of the Bipotential Gonad: The Indifferent Stage of Sexual Development

Gonadogenesis begins with the formation of the genital (gonadal) ridges which are the somatic precursor structures of both the testis and ovaries. The genital ridges form as a bulge or outgrowth of the coelomic epithelium on the ventromedial side of the mesonephroi by the fifth week of gestation in humans, and by embryonic day 10 (E10) in mice (Satoh, 1991; Waldeyer, 1870; Kaufman, 1992; Byskov, 1986). This proliferation of the coelomic epithelium and the underlying mesenchyme produces the somatic constituents of the gonads. There are at least two distinct bipotential somatic precursor lineages: (1) supporting cell precursors that differentiate into Sertoli cells in the testis or granulosa cells in the ovary, and (2) steroidogenic progenitors that produce Leydig cells in the testis or theca cells in the ovary (Karl and Capel, 1998; Schmahl et al., 2000). Stromal cells which are not specialized between sexes are also present at the genital ridge, and are responsible for structural patterning and angiogenic vascularization of the gonad. Genital ridge formation is therefore an indispensable prerequisite for development of both testis and ovaries, which remain bipotential at this stage until an intricate network of cellular signals begins to drive sexual differentiation.

Genetic Regulation of Gonad Formation

Several transcription factors and signaling proteins perform crucial regulatory roles in the development of the bipotential gonad. These were recently reviewed (Eggers et al., 2014, Tanaka and Nishinakamura, 2014), and are summarized here (also see **Table 1**). Steroidogenic factor 1 (*Sf1*), also known as *Nr5a1*, encodes an orphan nuclear hormone receptor that is expressed in both the gonad and steroidogenic tissues such as the adrenal glands (Lu and Yamashita, 2017). *Sf1* null mice exhibit agenesis of the gonads and the adrenal glands. *Sf1* is also an important regulator of *Sox9*, an essential gene for mediating testis differentiation. The Wilms tumor gene (*Wt1*) encodes a zinc finger transcription factor that is involved in the regulation of early gonad development, and differentiation of the testis (Kreidberg et al., 1993). *Wt1* null mice fail to undergo gonadogenesis, and their kidneys do not develop. Additionally, the Wt1 protein has two isoforms, one lacking and one containing a KTS (lysine, threonine, and serine) amino acid motif. Mice lacking the Wt1(+KTS) isoform lose the ability to upregulate the testis-determining gene *Sry*, and exhibit male-to-female sex reversal. The Wt1(−KTS) isoform, in conjunction with Lhx9, directly activates the *Sf1* gene promoter and regulates the expression of testis-inducing factors such as *Amh*. This isoform is also important for the maintenance of the gonadal primordium. In humans, *WT1* mutations are also implicated in a form of kidney cancer that primarily affects children. *Lhx9* encodes the Lim/homeobox 9 protein which is another regulator of *Sf1* (Birk et al., 2000). *Lhx9* null mice also fail to undergo gonadogenesis. A deficiency in Lhx9 contributes to the loss of Amh and testosterone, which causes XY mice to develop as females.

Table 1 Genes involved in mouse gonad formation

Gene	*Protein/Function*	*Mutant phenotype*	*References*
Cbx2	Chromobox, chromatin modification and remodeling factor	Impaired ovary development, male to female sex reversal.	Katoh-Fukui et al. (1998)
Emx2	Homeobox transcription factor	Lack kidney, uterer, gonad, reproductive tract.	Miyamoto et al. (1997)
Gata4	Zinc finger transcription factor	Inhibition of genital ridge formation.	Hu et al. (2013)
Igfr1	Insulin like growth factor receptor	Decreased proliferation of somatic progenitor cells.	Nef et al. (2003)
Insr	Insulin receptor	Decreased proliferation of somatic progenitor cells.	Nef et al. (2003)
Lhx9	Lim homeobox	Impaired gonadogenesis	Birk et al. (2000)
Sf1	Steroidogenic factor, orphan nuclear hormone receptor	No gonads or adrenal glands	Lu and Yamashita (2017)
Six1/4	Six family homeobox	Double knockouts have smaller gonads.	Kawakami et al. (2000)
Wt1	Zn finger transcription factor	No gonad development.	Kreidberg et al. (1993)

Empty spiracles homeobox 2 (*Emx2*) encodes a homeobox transcription factor involved in urogenital development (Miyamoto et al., 1997). *Emx2* null mice fail to develop kidneys, ureters, gonads, and genital tracts. Cellular polarity is also lost in the forming gonadal ridges of *Emx2* null mice leading to the abnormal assembly of tight junctions. Two other homeobox containing genes, *Six1* and *Six4*, are mammalian homologs of the *sine oculis homeobox* (*Six*) family in *Drosophila* (Kawakami et al., 2000). Both function redundantly in mouse embryogenesis. *Six1* and *Six4* double-mutant mouse embryos exhibit decreased gonad size in both sexes and abnormal differentiation of the testis in XY gonads.

The chromobox homolog 2 protein encoded by the *Cbx2* gene is a chromatin modification and remodeling factor that is involved in early gonadal development (Katoh-Fukui et al., 1998). Cbx2 has been shown to upregulate *Sf1*, *Wt1*, and *Sry*. Deletion of Cbx2 leads to impaired development of the ovary in XX mouse models, and male-to-female sex reversal in XY mice. *Cbx2* null mice appear to have normal development of the coelomic epithelium, but the gonadal cells later exhibit defective proliferation.

The GATA-binding protein 4 (Gata4) is a zinc finger transcription factor whose deletion is lethal to mouse embryos prior to genital ridge formation (Hu et al., 2013). Conditional knockdown of *Gata4* expression in mouse embryos after E8.75 causes the coelomic epithelium to remain as a morphologically undifferentiated monolayer, thereby preventing genital ridge formation. The insulin/insulin-like growth factor (IGF) signaling pathway is another crucial regulator of gonadogenesis (Nef et al., 2003). Mouse embryos without the insulin receptor (*Insr*) and the IGF receptor (*Igfr1*) have decreased proliferation of somatic progenitor cells in the gonads of both genotypic sexes prior to gonadal sex determination, and the testis fail to develop. Loss of insulin/IGF signaling can induce male-to-female sex reversal, and is also associated with reduced *Sry* expression levels. Proper spatiotemporal expression of each of these genes is imperative for establishing the somatic gonad.

Sex Determination

In mammals, dimorphic sex determination and differentiation progresses in three distinct phases. Firstly, the genetic sex of the embryo is determined at fertilization by the chromosomal complement of the zygote. Females are normally endowed with two X chromosomes, while males have an X and a Y sex chromosome. This genetic information facilitates gonadal sex determination during the second phase, by triggering growth of the bipotential gonad towards a testicular or ovarian fate. The short arm of the Y chromosome contains the gene encoding the mammalian testis-determining factor (TDF) known as *Sry* (sex-determining region Y). The spatiotemporal expression pattern of *Sry* is strictly controlled, and is first detected in the mouse gonad around E11, peaking at E11.5, before disappearing after E12.5 (Tanaka and Nishinakamura, 2014). Sry upregulates *Sox9*, and is both necessary and sufficient to direct the indifferent gonad towards the male phenotype in mice and humans (Koopman et al., 1991; Sinclair et al., 1990). Sry induces testicular morphogenesis by coordinating the differentiation of Sertoli cells and other testis-specific lineages. The presumptive ovarian equivalent of *Sry* has not yet been identified, and there is no evidence of a specific ovary determining factor. However, the ovary differentiation pathway is activated in the absence of functional Sry protein, and is primarily driven by the WNT/β-catenin signaling pathway. Accordingly, although initial morphogenesis of the gonad is macroscopically similar between sexes, the gonadal transcriptome is notably different. The third stage, phenotypic sex determination, begins perinatally and continues throughout sexual maturation as the endocrine products of the gonads stimulate the development of secondary sex characteristics. Mutations or defects that compromise development during any of these three stages could lead to urogenital abnormalities or sex reversal.

The Ovary Differentiation Pathway

Differentiation of the bipotential gonads to the ovaries is not simply a passive process that occurs if there is no Sry. There are several ovary specific genes and signaling pathways that elicit female gonadal determination and development and this gonadal fate requires active repression of the testis pathway (Eggers et al., 2014, Chassot et al., 2014) (see **Table 2**). *Foxl2* encodes the forkhead box L2 transcription factor and is upregulated in the developing ovary (Uhlenhaut et al., 2009). Mutations of this gene in humans lead to blepharophimosis ptosis epicanthus inversus syndrome (BPES), which is characterized by eyelid malformation as well as, in some cases, primary ovarian insufficiency. Conditional knockdown of the *Foxl2* gene causes granulosa cells to become reprogrammed into Sertoli cells.

Table 2 Genes involved in mouse ovary differentiation

Gene	*Protein/function*	*Mutant phenotype*	*References*
Ctnnb1	β-catenin, Wnt signaling pathway	Ectopic expression causes male to female sex reversal.	Liu et al. (2009)
Foxl2	forkhead box transcription factor	Female to male sex reversal of somatic cells	Uhlenhaut et al. (2009)
Fst	Follistatin, activin antagonist	Mutants have female to male sex reversal.	Yao et al. (2004)
Rspo1	R-spondin homolog 1	Mutants have female to male sex reversal	Chassot et al. (2008)
Wnt4	Wnt signaling	Decreased proliferation of somatic progenitor cells.	Tomizuka et al. (2008)

β-Catenin, also known as Catenin beta-1 (Ctnnb1), transcriptionally regulates a variety of genes including *Wnt4* and *Fst* which are important for ovarian development (Liu et al., 2009). Ectopic expression of β-catenin in the developing XY mouse gonad causes male-to-female sex reversal, but the mechanism driving this trans-differentiation is unclear. WNT family members are conserved secreted proteins with roles in various biological processes. WNT3A participates in the stabilization of β-catenin and allows it to transcribe its target genes. WNT4 is known to activate canonical WNT signaling during gonadal development (Tomizuka et al., 2008). Females with heterozygous missense *WNT4* mutations exhibit agenesis of the reproductive tract, while *Wnt4* in the mouse is also important for gonadal morphogenesis via its role in sex determination and female development.

RSPO proteins activate the WNT/β-catenin signaling pathway by binding to the LGR4, LGR5, and LGR6 G protein-coupled receptors, or by binding to ZNRF3 and RNF43 which are negative-feedback regulators of WNT signaling (Chassot et al., 2008). Disruption of human *RSPO1* can lead to female-to-male sex reversal, suggesting that it plays a crucial role in sex determination and female differentiation. At E12.5 in the mouse *Rspo1* and *Wnt4* become expressed in an ovary specific manner, and their gene products are secreted by the somatic cells of the ovary. *Rspo1* null mice exhibit sex reversal, bearing ovotestes. The $Rspo1^{-/-}$ embryo demonstrates abnormalities in the XX gonad such as the presence of testis-like vasculature, and reduced germ cell proliferation. Similarly, a deficiency in Wnt4 is associated with inducing male-like vascularization of the gonad in XX embryos. There is also evidence suggesting that Wnt4 functions as a survival factor in female germ cells, as there are nearly three times as many apoptotic germ cells in the Wnt4 deficient gonad, when compared to wild type gonads.

Follistatin (*Fst*) expression is stimulated by Wnt4 in the XX gonad from E11.5 onwards, but is not expressed in the XY gonad (Yao et al., 2004). *Fst* encodes an activin-binding protein that antagonizes Activin B. This inhibitory action prevents male-like vascularization of the gonad. Subsequent development of the differentiated ovary will involve the production and development of the ovarian follicles.

Primordial Germ Cells: The Embryonic Precursors of Gametes

Primordial germ cells (PGCs) colonize the bipotential gonad in association with the somatic cells, and differentiate to produce haploid eggs or sperm. During gastrulation in mice, at around E7, inductive cell-cell interactions establish the germ line by specifying the allocation of PGCs. The genetic basis for germ cell specification is thought to involve the expression of the *fragilis* and *stella* genes in PGC founder cell lineages, allowing them to retain pluripotency while neighboring cells acquire a mesodermal fate (Saitou et al., 2002). Intercellular signaling involving Bone Morphogenetic Protein 4 (Bmp4) is also critical for initiating the formation of the germ line. *Bmp4* null mouse embryos lack PGCs and an allantois which are both derived from the proximal epiblast (Lawson et al., 1999). The Bmp signaling pathway regulates the expression of two key genes that are essential for germ cell fate: PR domain containing protein 1 (*Prdm1*) which acts to repress the somatic cell fate, and PR domain containing protein 14 (*Prdm14*) which transcriptionally regulates germ cell pluripotency and epigenetic reprogramming (Windley and Wilhelm, 2015).

PGCs migrate via the hindgut and the dorsal mesentery to the developing gonadal ridges from approximately E9.5 to E11.5 in the mouse and from the fifth to seventh week of gestation in humans (Clark and Eddy, 1975; Fujimoto et al., 1977). During this time, the number of PGCs increases from approximately 40 to 250 in mice, and from approximately 100 to 5000 in humans (Cummings and Kavlock, 2004; Saitou and Yamaji, 2012). The PGCs rapidly proliferate by mitosis and increase in number to colonize the developing gonads as oogonia or spermatogonia. This proliferation produces a maximum of almost 7 million germ cells by the 5th month of gestation in humans, and around 25,000 germ cells by E13.5 in mice. At E11.5 in the mouse embryo and in the seventh to ninth week of gestation in humans, PGCs display similar expression patterns of germline and pluripotency genes. These germ cell specific genes include *BLIMP1*, *AP2γ*, *UTF1*, *DAZL*, *Kit*, and *DDX4*, while the pluripotency genes include *OCT4*, *NANOG*, *PRDM14*, and *LIN28* (Dolci et al., 2015). The KIT receptor and its ligand KITL, are well known for promoting cell survival and proliferation during gametogenesis. Loss of KIT function impairs PGC proliferation and migration, results in ectopic PGCs, and affects the growth of early ovarian follicles (Buehr et al., 1993; Huang et al., 1993; McCoshen and McCallion, 1975, Mintz and Russell, 1957). By E13.5 in mice and by the tenth week of gestation in humans, oogonia begin to enter meiosis and are called oocytes. Developing oocytes arrest in the diplotene stage of the first meiotic prophase until ovulation begins during puberty, while spermatogonia enter a state of quiescence and reinitiate development at the onset of puberty.

Development of the Ovaries

The differentiated ovary displays notable complexity in structure and function, and is histologically identifiable around the twelfth week of gestation in humans and E13.5 in mice, by the presence of loose cordlike structures termed ovigerous cords (Loffler and Koopman, 2002; Odor and Blandau, 1969). Ovigerous cords consist of clusters of primordial germ cells that are surrounded by somatic cells. Ovarian PGCs in these clusters are physically interconnected by cytoplasmic bridges, and develop synchronously as germ cell cysts (Pepling and Spradling, 1998). Cysts subsequently undergo programmed breakdown to form primordial follicles, consisting of an individual germ cell or oocyte surrounded by a monolayer of somatic granulosa cells (Pepling and Spradling,

2001). Somatic cells extend thin cytoplasmic prolongations between germ cells in ovigerous cords, thereby facilitating cyst breakdown and follicle assembly (Odor and Blandau, 1969; Pepling and Spradling, 2001). The features of early oocyte development are very similar in mice and humans, with the main difference being that cyst breakdown and primordial follicle formation occur perinatally in the mouse, but before birth in humans (Sarraj and Drummond, 2012).

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DEVELOPMENT

Formation of the Testis Primordium

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Formation of the Testis From the Bipotential Gonad

The testes form from a primitive undifferentiated gonad or genital ridge that has the potential to become either testes or ovary. In this article, we will describe the earliest events in testis development, when gonadogenesis, or formation of the gonads, occurs. Because most of the experimental evidence comes from studies using the mouse as a model organism, the morphological and molecular events during gonad formation are discussed mostly with reference to mouse development.

A functional gonad requires the presence of germ cells and somatic cells in order to produce gametes and hormones. The somatic cells of the gonads originate from the coelomic epithelium, a thin layer of epithelial cells that covers the gonad. While the primitive gonad is establishing, germ cell precursors, or primordial germ cells, migrate into the gonad from elsewhere in the embryo (discussed in the second part of this article). Key steps in forming the gonad are (1) proliferation of the coelomic epithelial cells, (2) ingression of the coelomic epithelium into the gonad proper, and (3) subsequent expansion and sexual differentiation of the somatic cells (**Fig. 1**). This process is controlled by a group of transcription factors and signaling molecules. At the onset of gonadogenesis, the coelomic epithelial cells that are destined to form the gonad become positive for the transcription factor Wilm's tumor 1 (WT1) (Kreidberg et al., 1993). Without *Wt1*, thickening of the coelomic epithelium is reduced dramatically (Kreidberg et al., 1993). The coelomic epithelium proliferates in an anterior to posterior fashion. The anterior-posterior thickening of the coelomic epithelium and the subsequent establishment of the gonad require the action of another transcription factor GATA binding protein 4 (GATA4) (Hu et al., 2013). GATA4 expression in the anterior part of the gonad precedes the initial thickening of the coelomic epithelium, and without *Gata4*, proliferation of the coelomic epithelial cells, and subsequent formation of the gonad fail to occur (Hu et al., 2013). The thickening of the coelomic epithelium is followed by a breakdown of its underlying basement membrane and ingression of the coelomic epithelial cells to form the gonad proper. In addition to WT1 and GATA4, the coelomic epithelial cells also express steroidogenic factor 1 (SF1), an orphan nuclear receptor that is important for gonad formation (Hoivik et al., 2010). Without functional SF1, the gonad does not expand properly and the gonadal cells eventually die (Luo et al., 1994). The early SF1 expression in the coelomic epithelium is promoted by GATA4 and two homeodomain proteins SIX1 and SIX4 (sine oculis homeobox homologs 1 and 4 (Hoivik et al., 2010; Fujimoto et al., 2013).

After its initial thickening, the coelomic epithelium continues to proliferate and provides progenitors to the developing gonad. This proliferation of the coelomic epithelial cells is promoted by Lim homeobox 9 (LHX9) and insulin signaling. Loss of *Lhx9* or insulin receptor and IGF type I receptor (*Igf1r*) result in stunted gonad development due to decreased proliferation of gonadal somatic cells (Birk et al., 2000; Pitetti et al., 2013). What the coelomic epithelial cells become after proliferation is determined by the polarity of the cells. The coelomic epithelium proliferates asymmetrically, where the progenitor cells remain as a part of the coelomic epithelium and maintain an undifferentiated epithelial cell fate. The daughter cells, on the other hand, ingress to the gonad proper, and acquire mesenchymal characteristics. During the ingression process, epithelial cells lose their cell polarity and cell–cell connections, and migrate through the basement membrane. Once they pass through the basement membrane, the ingressing epithelial cells form clusters that become the future somatic cells in the testis (**Fig. 1**). Multiple factors and pathways are involved in the invagination of the coelomic epithelium. Homeodomain transcription factor EMX2 and possibly SIX1/SIX4 are critical for the proliferation and the subsequent migration of the coelomic epithelial cells through the basement membrane. Without *Emx2*, the coelomic epithelial cells accumulate on the gonadal surface and fail to migrate and differentiate (Kusaka et al., 2010). A tight regulation of Notch signaling pathway is also necessary to maintain the asymmetric divisions and proper balance between progenitor and differentiated cell populations. A defect in the Notch signaling pathway results in abnormal accumulation of the progenitors and a failure of them to differentiate into the somatic cells in the testis (Potter et al., 2016; Lin et al., 2017). The coelomic epithelial cells that enter the gonad during sex determination become Sertoli cells. Proliferation and ingression of coelomic epithelial cells continue after sex determination, but cells ingressing at this time contribute to fetal and adult Leydig cell populations in the interstitium (Karl and Capel, 1998).

After the gonad has formed, it is important to ensure that new somatic cell progenitor cells survive and proliferate to form the testes. The survival of gonadal cells is controlled by SF1 and WT1. In the absence *Sf1* and *Wt1*, the coelomic epithelium begins to thicken during the earliest steps of gonad development, but then these structures gradually disappear through apoptosis of the gonadal somatic cells (Luo et al., 1994; Kreidberg et al., 1993). In addition to survival, sufficient proliferation of gonadal somatic cells is necessary for testis development (Schmahl and Capel, 2003). Without proliferation of the somatic cells in this early phase, such as in knockout mouse models for *Pbx1* and *Cbx2*, the resulting gonads are very small and poorly formed (Schnabel et al., 2003; Katoh-Fukui et al., 1998).

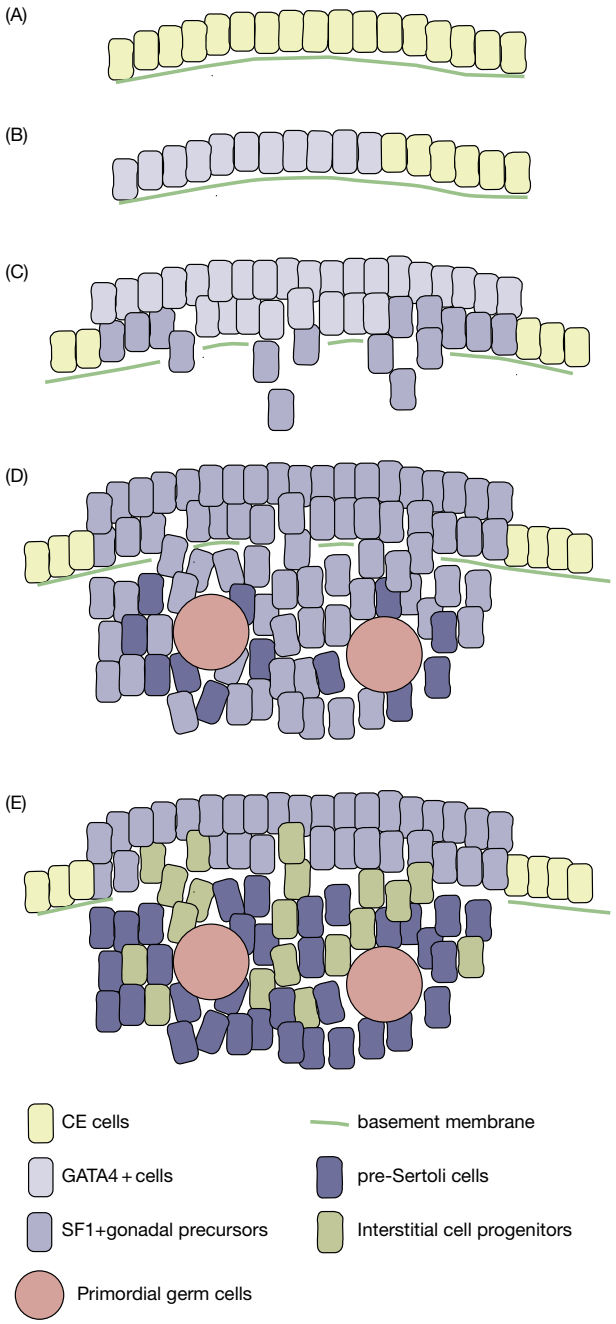

Fig. 1 Formation of the gonad. (A) The coelomic epithelium (CE), which lies on top of the future gonad, has an intact basement membrane prior to gonad formation. (B) Coelomic epithelial cells begin to express GATA4, prior to proliferation and thickening of the coelomic epithelium. (C) The cells that are destined to become gonadal somatic cells begin to express SF1. The basement membrane disintegrates and cells begin to ingress into the gonad. (D) Supporting cell progenitors (pre-Sertoli cells) continue to enter the gonad and proliferate. Primordial germ cells arrive to the gonad and they are surrounded by the pre-Sertoli cells. (E) The proliferating precursor cells from the coelomic epithelium contribute also to interstitial cells. Modified from Piprek, R. P., Kloc, M., and Kubiak, J. Z. 2016. Early development of the gonads: Origin and differentiation of the somatic cells of the genital ridges. *Results and Problems in Cell Differentiation* **58**, 1–22.

Development of the genital ridges lays the foundation for testicular development by ensuring that sufficient numbers of Sertoli cell precursors are established. Once the testis primordium is established, testicular development continues with migration of interstitial cell progenitors, vascular cells and macrophages into the gonad, which is covered in detail in other articles.

Primordial Germ Cell Formation and Migration

While the gonad is forming, primordial germ cells that migrate from the hindgut begin to enter the gonad. Primordial germ cells first gain their identity as a few cells in the epiblast, an embryonic structure next to the embryonic ectoderm (Fig. 2A). Primordial germ

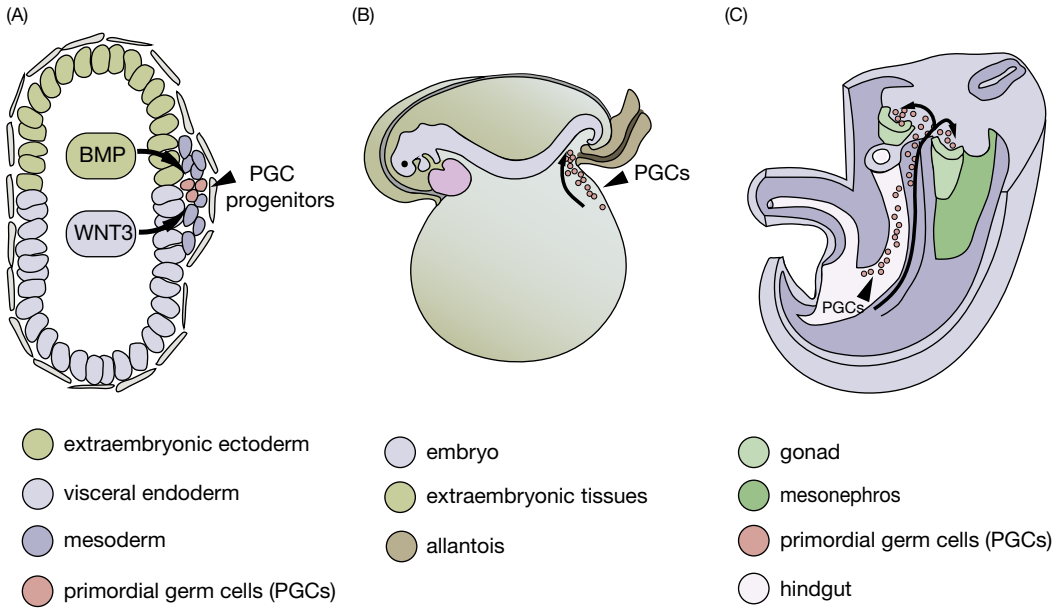

Fig. 2 Specification and migration of primordial germ cells. (A) Primordial germ cells are first specified in the epiblast. This process is controlled by BMP from the extraembryonic ectoderm and WNT3 signaling from the visceral endoderm. (B) As the embryo develops the primordial germ cells migrate to the base of the allantois. (C) When primordial germ cells begin their long migration toward the gonads, they leave the allantois and enter the hindgut. They migrate through the hindgut and mesentery until they reach the gonads. Arrows denote direction of migration. Modified from Tang, W. W., Kobayashi, T., Irie, N., Dietmann, S., and Surani, M. A. 2016. Specification and epigenetic programming of the human germ line. *Nature Reviews. Genetics* ***17***, 585–600; Rosen, M. P., and Cedars, M. I. 2011. Female reproductive endocrinology and infertility, In D. G. Gardner and D. Shoback (eds.), *Greenspan's basic & Clinical endocrinology*, 9th edn. (New York, NY: The McGraw-Hill Companies). Chapter 13.

cell specification is directed by cues from the adjacent structures: bone morphogenetic protein (BMP) signaling from the extraembryonic ectoderm and Wnt family member 3 (WNT3) from the visceral endoderm (Tang et al., 2016). These signals are sensed by the presumptive primordial germ cell progenitors, and activate genes that distinguish them from somatic cells.

As the embryo develops, primordial germ cells relocate to the extraembryonic region at the base of the allantois, a membrane that is important for the development of the umbilical cord and placenta. From the allantois, primordial germ cells migrate through the hindgut and mesentery toward the gonads (**Fig. 2**B). Several factors promote primordial germ cell survival during the long migration process, such as DND microRNA-mediated repression inhibitor 1 (DND1), and Kit ligand (KitL) and stromal cell derived factor 1 (SDF1, also known as CXCL12) (Youngren et al., 2005; Gu et al., 2009; Molyneaux et al., 2003).

There are two theories on the migration process of the primordial germ cells: active migration of the primordial germ cells toward the gonads, which is induced by signals secreted by cells along the migratory path, or passive movement of primordial germ cells due to physical changes of the embryonic structure (Harikae et al., 2013). According to the theory of active migration, primordial germ cells sense chemoattractant signals secreted by somatic cells in the gonad, adjacent mesonephros, and mesentery. In response to these signals with the specific receptors, primordial germ cells actively migrate through the hindgut toward the gonads. Examples of chemoattractant signalling pathways are Kit ligand and c-Kit receptor (also known as Steel and KIT, respectively), and SDF1 and its receptor CXCR4 (chemokine (CXC motif) receptor 4). Kit pathway is involved in primordial germ cell proliferation, survival, and migration throughout the migration period (Gu et al., 2009). Without Kit ligand or its receptor, mice have reduced proliferation of migrating primordial germ cells during fetal life and are sterile as adults. Kit ligands elicit their action within a short range, meaning that the target cell has to be close to the cell secreting the Kit ligand. Interestingly, cells that produce Kit ligand form a path which migrating primordial germ cells follow. Kit ligand-expressing cells surround primordial germ cells that are already in the allantois right after primordial germ cells have become specified (Gu et al., 2009). As migration begins, Kit ligand is expressed both in the gonads and the midline area through which primordial germ cells migrate. Later on, when primordial germ cells reach the gonads, Kit ligand expression is switched off in the migratory path outside the gonads, but maintained in the gonads. Kit ligand not only promotes primordial germ cell survival and proliferation, but also influences their motility. Without Kit ligand, primordial germ cells migrate to the correct direction but at a lower rate than normal, resulting in fewer primordial germ cells reaching the gonads (Gu et al., 2009).

There are several similarities between the roles of Kit/Kit ligand and the SDF1/CXCR signaling pathways in controlling primordial germ cell migration. If SDF1 or its receptor CXCR4 are deleted in mice, very few germ cells reach the gonads and the adult mice are sterile (Ara et al., 2003; Molyneaux et al., 2003). SDF1 also promotes primordial germ cell survival and is expressed in the somatic cells along the migratory path of primordial germ cells in a similar fashion as Kit ligand. However, unlike Kit ligand, SDF1 is involved in establishing the direction of primordial germ cell migration. If ectopic SDF1 is present in embryonic organs other than the gonads, primordial germ cells begin to migrate toward that region erroneously (Molyneaux et al., 2003). Also, in

contrast to Kit ligand which is necessary for primordial germ cell migration from start to finish, SDF1 is dispensable for the early stages of migration, but required for the later stages of migration through the mesentery and hindgut to the gonads (Molyneaux et al., 2003; Ara et al., 2003). In conclusion, KIT and SDF1/CXCR4 signaling pathways play complementary roles in primordial germ cell proliferation, survival, and migration.

The second theory for primordial germ cell migration is the passive theory: when primordial germ cells are first incorporated into the hindgut endoderm, proliferation, and expansion of the hindgut passively propels primordial germ cells forward and toward the gonad (Harikae et al., 2013). If the hindgut does not expand, primordial germ cells are stuck at the hindgut entrance. It has been proposed that both of the active and passive theories are involved: the initial long-range migration of the primordial germ cells is promoted by passive translocation, but the survival and final steps of migration before reaching the gonads are promoted by chemoattractants secreted from the gonads. Interestingly, migration of primordial germ cells to the correct site still occurs even though formation of the gonads is compromised (Hu et al., 2013; Kreidberg et al., 1993). An explanation for this could be that production of chemoattractants in not only the gonads but also the adjacent mesentery and mesonephros is sufficient to guide the migrating primordial germ cells to their final destination.

After primordial germ cells reach the gonads, they cease migration and begin to form cell-to-cell associations with the somatic cells. It is hypothesized that reaching the site of highest concentration of the chemoattractants prompts primordial germ cells to stop migrating (Richardson and Lehmann, 2010). In addition, gonadal somatic cells likely suppress primordial germ cell migration, but the molecular mechanisms are not known. In the fetal testis, primordial germ cells become surrounded by Sertoli cells, which are responsible for the formation of testis cords. While they are enclosed in the testis cords, germ cells undergo rapid proliferation, form aggregates called germ cell cysts, and then cease mitosis (Lei and Spradling, 2013). Germ cell development that follows formation of fetal testes is discussed in detail in the following article on spermatogonial development and spermatogenesis.

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Further Reading

Piprek, R. P., Kloc, M., & Kubiak, J. Z. (2016). Early development of the gonads: Origin and differentiation of the somatic cells of the genital ridges. *Results and Problems in Cell Differentiation, 58*, 1–22.

Rosen, M. P., & Cedars, M. I. (2011). Female reproductive endocrinology and infertility. In D. G. Gardner, & D. Shoback (Eds.), *Greenspan's basic & clinical endocrinology* (9th edn). New York, NY: The McGraw-Hill Companies. Chapter 13.

Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Gonadal Developmental Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 13, 2024
Week 6

Gonadal Developmental Systems Biology

Early Fetal Gonadal Development
- Morphogenesis
- Transcriptome
- Meiotic Arrest

Testis Gonadal Fetal Development
- Knockout Models and Genes
- Cellular Growth Regulation

Ovary Gonadal Fetal Development
- Developmental Timing and Morphology
- Oocyte Nests and Primordial Follicle Assembly
- Hormone Regulation of Assembly
- Culture Models

Description of Gonadal Development
- Endocrine Disruptors

Required Reading

Pepling and Burton (2018) Fetal/Gonadogenesis, Encyclopedia of Reproduction (Second Edition). Volume 2, Pages 47-51.

Rotgers and Yao, (2018) Formation of the Testis Primordium, Encyclopedia of Reproduction (Second Edition). Volume 1, Pages 84-87.

Spring 2024 – Systems Biology of Reproduction
Discussion Outline – Gonadal Developmental Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 15, 2024
Week 6

Gonadal Developmental Systems Biology

Primary Papers:

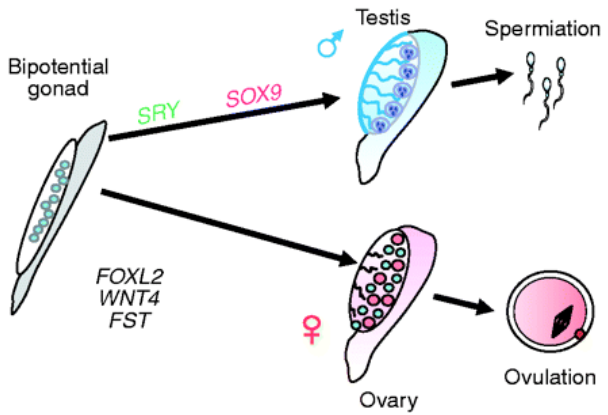
1. Soto and Ross (2021) Reproduction 161:239-253
2. Real, et al. (2023) J Exp Zool B Mol Dev Evol. 340(3):231-244
3. Nilsson, et al. (2013) BMC Genomics 14:496

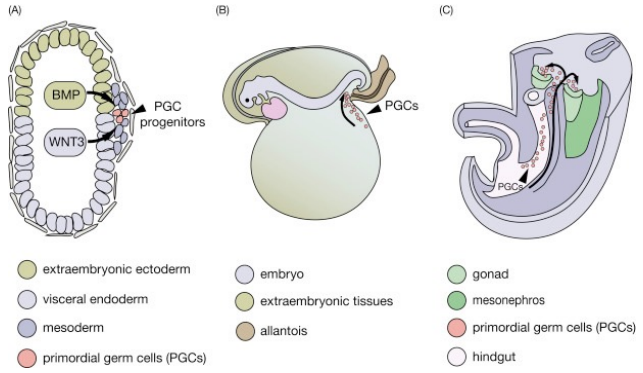
Discussion

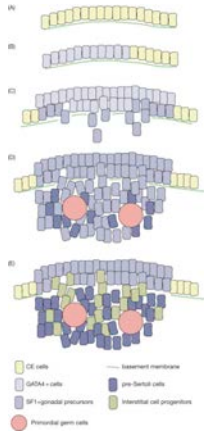
Student 13: Reference #1 above
- What is the technical approach?
- What specific transcriptome observations were made?
- Why is the similarity in cow and human germline development?

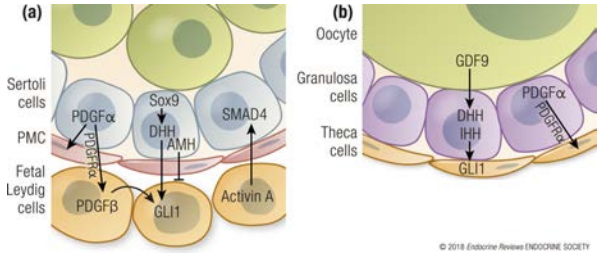
Student 14: Reference# 2 above
- What are the technologies used and objectives?
- What environmental exposures were compared?
- What impacts on testis are observed in seasonal breeders?

Student 15: Reference #3 above
- What is the experimental and systems approach?
- What is a cluster analysis?
  What gene networks were identified for primordial follicle assembly



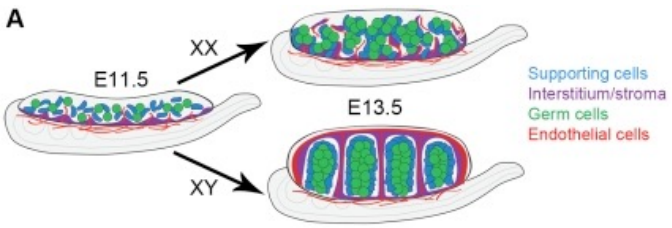




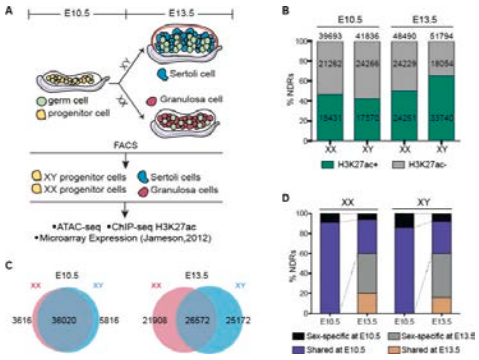


Paracrine regulation of steroidogenic cell development. (a) In the fetal mouse testis, Sertoli cells produce paracrine factors PDGF*α* and DHH that control the appearance and expansion of fetal Leydig cells in the interstitium after E12. Conversely, AMH from Sertoli cells negatively regulates the steroidogenic cell population. Fetal Leydig cells secrete activin A, which promotes proliferation of Sertoli cells via SMAD4 activation. Germ cells do not contribute to paracrine regulation of steroidogenic cells in the testis. (b) Unlike Leydig cells, which differentiate in the testis right after sex determination, the ovarian steroidogenic theca cells differentiate near birth and full steroidogenesis begins after birth. Also, oocytes participate in theca cell recruitment in the ovary through secreting GDF9, which induces expression of the hedgehog ligands DHH and IHH in the granulosa cells. DHH and IHH transform stromal progenitors into the theca cells that surround the follicles around birth in the mouse. Furthermore, granulosa cells promote theca cell recruitment by also producing PDGF*α*.

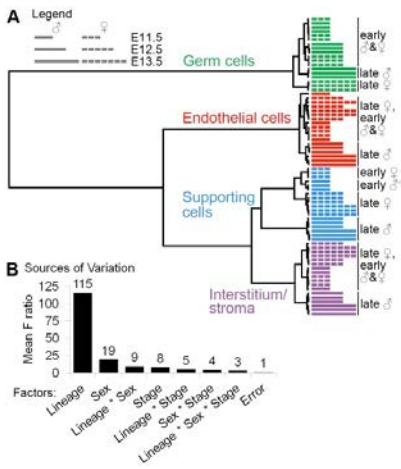
Temporal transcriptional profiling of somatic and germ cells reveals biased lineage priming of sexual fate in the fetal mouse gonad.
Jameson SA, et al. PLoS Genet. (2012) 8(3):e1002575.



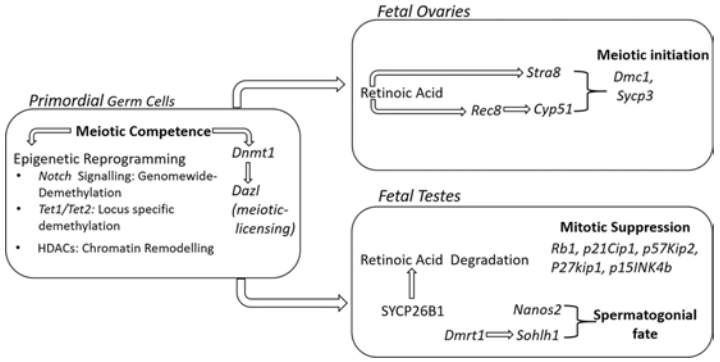
Gonadal supporting cells acquire sex-specific chromatin landscapes during mammalian sex determination.
Garcia-Moreno SA, Futtner CR, Salamone IM, Gonen N, Lovell-Badge R, Maatouk DM.
Dev Biol. 2019 Feb 15;446(2):168-179.



Chromatin architecture is remodeled during mammalian sex determination. A) Overview of sex determination and work flow. Briefly, supporting progenitor cells (yellow) are bipotential and indistinguishable between XX and XY gonads at E10.5. Expression of the Y-linked Sry gene (at E11.5) directs Sertoli cell differentiation in the testis (XY, blue). Absence of Sry directs differentiation of granulosa cells in the ovary (XX, pink). XX and XY progenitor cells (E10.5), Sertoli cells (E13.5), and granulosa cells (E13.5) were FACS-purified and used for ATAC-seq and ChIP-seq for H3K27ac. Further analysis made use of microarray expression data from purified supporting cells (Jameson et al., 2012b). B) Percent (and number) of H3K27ac-negative (grey) and H3K27ac-positive (green) NDRs in XX and XY cells at E10.5 (left) and E13.5 (right). C) Venn diagrams of all NDRs in XX (pink) and XY (blue) supporting cells at E10.5 (left) and E13.5 (right). D) Percent of NDRs that are shared between XX and XY cells at E10.5 (purple) and at E13.5 (orange), or specific to either XX or XY cells at E10.5 (black) or at E13.5 (grey). The purple and black bars at E13.5 represent NDRs that were retained from E10.5, while the orange and grey represent newly acquired NDRs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

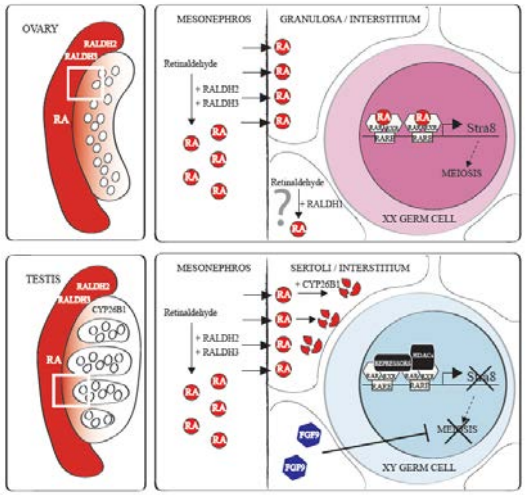


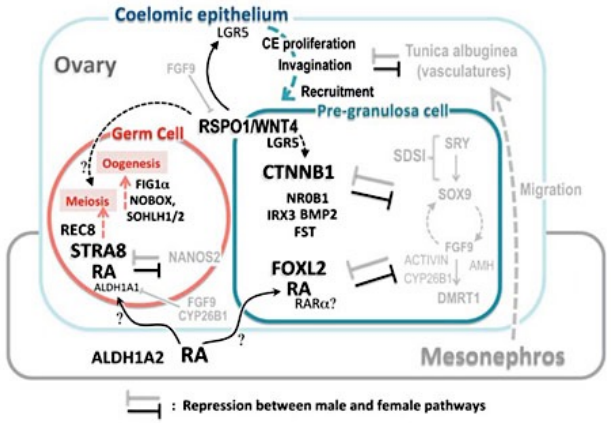
Gene expression was affected by lineage, sex, and stage.
(A) Clustering dendrogram of individual microarray samples. The E11.5, E12.5, and E13.5 samples are represented by short, intermediate, and long bars, respectively. The dashed bars indicate XX samples, and the solid bars indicate XY samples. Ward's method with squared Euclidean distance as the distance metric was used. The arrays cluster primarily by lineage, and secondarily by sex and stage. (B) Analysis of the sources of variation confirmed that the primary source of variation is lineage, and secondarily sex and stage.



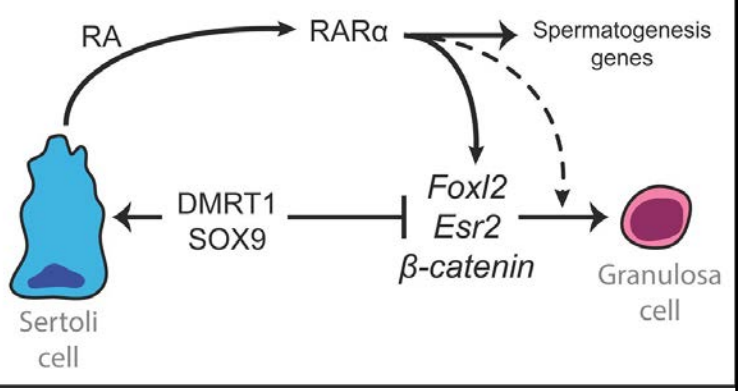
Primordial germ cells acquire competency to respond to RA to initiate meiosis by genetic and epigenetic mechanisms. Dazl contributes to genetic component for meiotic competency, whereas epigenetic reprograming and chromatin modifications contribute to epigenetic components. In fetal ovaries, meiosis is initiated under the influence of RA, which acts via Stra8-dependent and Stra8-independent pathways. In the fetal testis, RA activation is primarily abrogated by CYP26B1-mediated RA degradation. However, genetic factors, such as Nanos2, Dmrt1, and so on, contribute toward maintaining RA refractory state in male germ cells (arrows do not necessarily indicate direct regulation). RA, retinoic acid

Regulation of germ cell meiosis in the fetal ovary.
Spiller CM, Bowles J, Koopman P. Int J Dev Biol. (2012) 56(10-12):779-87.

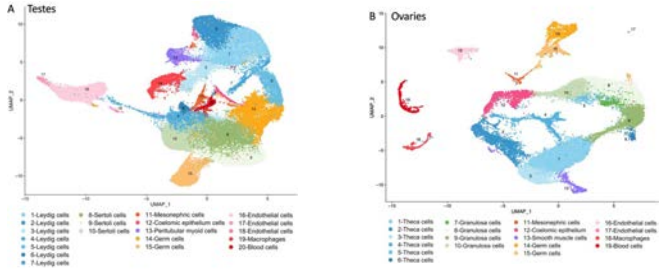




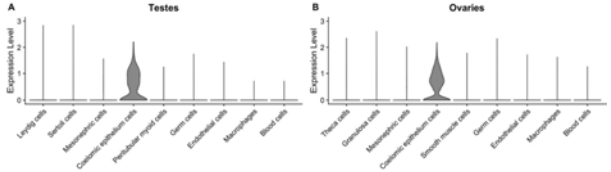
DMRT1 protects male gonadal cells from retinoid-dependent sexual transdifferentiation.
Minkina A, Matson CK, Lindeman RE, Ghyselinck NB, Bardwell VJ, Zarkower D.
Dev Cell. 2014 Jun 9;29(5):511-20.



MYRF: A New Regulator of Cardiac and Early Gonadal Development-Insights from Single Cell RNA Sequencing Analysis
Calonga-Solís V, Fabbri-Scallet H, Ott F, et al.
J Clin Med. 2022 Aug 18;11(16):4858.



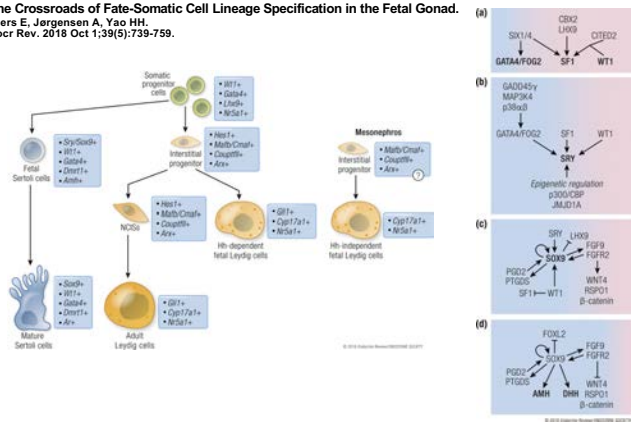
UMAP plots of cell clusters in human (**A**) testes from embryonic stage to adults; and (**B**) ovaries from embryonic and foetal stages identified by single cell RNA sequencing. UMAP: uniform manifold approximation and projection.



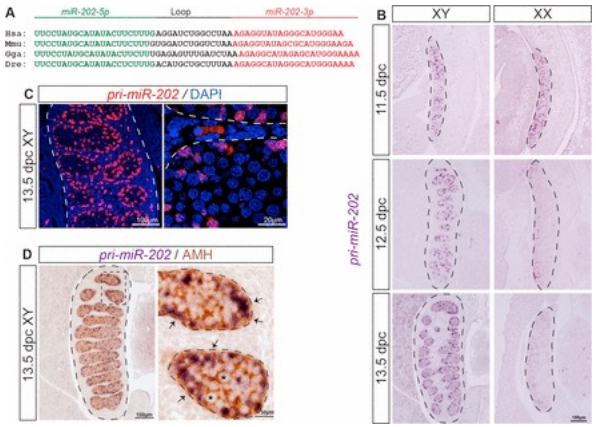
Expression levels of MYRF in testes (**A**) and ovaries (**B**) at embryonic and fetal stages, showing that it is highly expressed in coelomic epithelium cells.

Testis Development

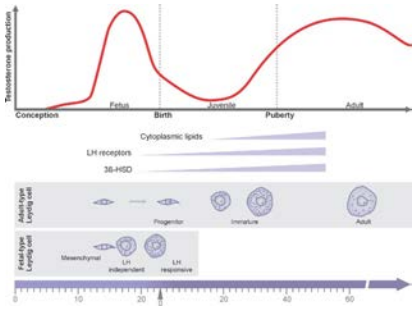
At the Crossroads of Fate-Somatic Cell Lineage Specification in the Fetal Gonad.
Rotgers E, Jørgensen A, Yao HH.
Endocr Rev. 2018 Oct 1;39(5):739-759.



SOX9 regulates microRNA miR-202-5p/3p expression during mouse testis differentiation.
Wainwright EN, et al. Biol Reprod. (2013) 15;89(2):34.

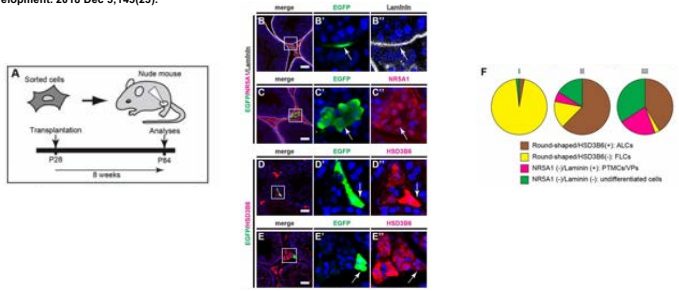


Leydig cells: formation, function, and regulation.
Zirkin BR, Papadopoulos V.
Biol Reprod. 2018 Jul 1;99(1):101-111.



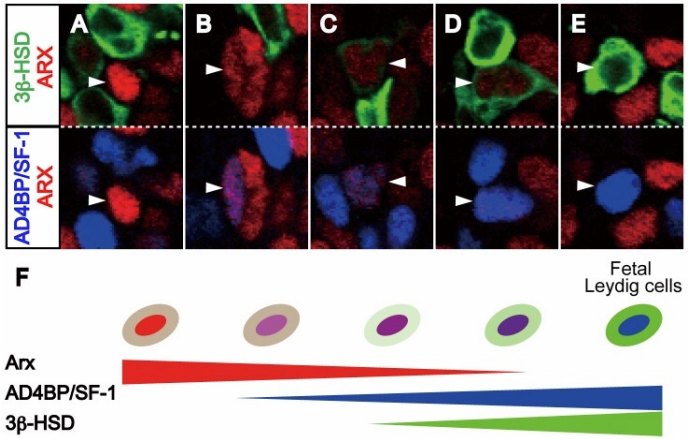
Fetal and adult periods of testosterone production. Fetal Leydig cells produce the high levels of testosterone that are required for the differentiation of the male genitalia and for brain masculinization. Testosterone production declines with the postnatal decline in numbers of the fetal Leydig cells, reaching a nadir early in the postpartum period. Thereafter, testosterone gradually increases to high levels with the development of the adult Leydig cells from stem cells of the neonatal testis. LH is not required either for the development of fetal Leydig cells or for their initial testosterone production. Later, however, the fetal Leydig cells express LH receptor and respond to LH stimulation.

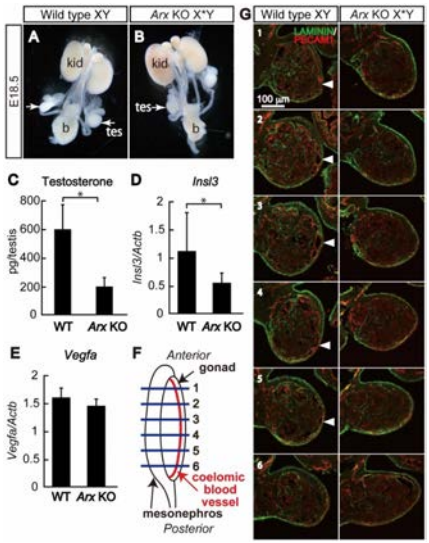
Fetal Leydig cells dedifferentiate and serve as adult Leydig stem cells.
Shima Y, Miyabayashi K, Sato T, Suyama M, Ohkawa Y, Doi M, Okamura H, Suzuki K.
Development. 2018 Dec 5;145(23).



Transplantation of cell populations isolated from P10 testis. (A) Schematic of the strategy for cell transplantation and fate tracing. Cells isolated from P10 testes were transplanted into the interstitial space of the P28 nude mouse testis and analyzed at 8 weeks after transplantation. (B-C") Testis sections at P84 were immunostained for EGFP (green), NR5A1 (red) and laminin (white). The boxed area in B was enlarged, and EGFP and laminin signals are shown in B' and B", respectively. The boxed area in C was enlarged, and EGFP and NR5A1 signals are shown in C' and C", respectively. Arrows in B' and B" indicate laminin$^+$ PTMCs. Arrows in C' and C" indicate NR5A1$^+$ Leydig cells. (D-E") Testis sections at P84 were immunostained for EGFP (green) and HSD3B6 (red). The boxed area in D was enlarged, and EGFP and HSD3B6 signals are shown in D' and D", respectively. The boxed area in E was enlarged, and EGFP and HSD3B6 signals are shown in E' and E", respectively. Arrows in D' and D" indicate HSD3B6$^-$ ALCs, and arrows in E' and E" indicate HSD3B6$^-$ FLCs. (F) Percentages of the indicated cell populations in the recipient testis. We counted 6-70 cells in each section (three distinct sections from each sample; *n*=3 for population I and II, *n*=2 for population III). Scale bars: 50 μm.

Aristaless related homeobox gene, Arx, is implicated in mouse fetal Leydig cell differentiation possibly through expressing in the progenitor cells.
Miyabayashi K, et al. PLoS One. (2013) 28;8(6):e68050.



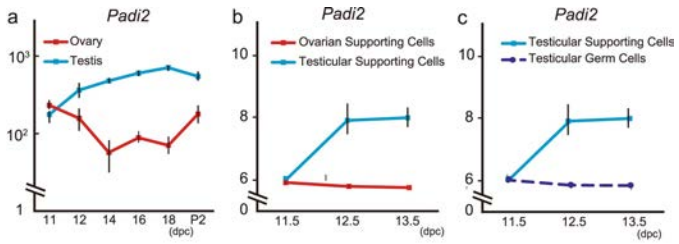


Reprogramming of Sertoli cells to fetal-like Leydig cells by Wt1 ablation.
Zhang L, Chen M, Wen Q, Li Y, Wang Y, Wang Y, Qin Y, Cui X, Yang L, Huff V, Gao F.
Proc Natl Acad Sci U S A. 2015 Mar 31;112(13):4003-8.

Significance

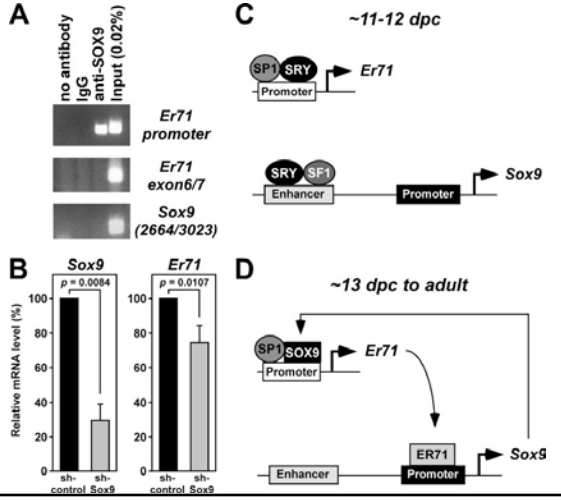
Genetic control of the differentiation between Sertoli cells and granulosa cells has been reported previously. However, the relationship between Sertoli cells and Leydig cells in the testis has not yet been definitively determined. In the present study, we demonstrate for the first time, to our knowledge, that these two cell types can be mutually reprogrammed and that Wilms' Tumor Gene 1 (Wt1) plays a critical role in this process. This study provides a novel concept for cell fate determination in testis development that will improve our understanding of the regulatory mechanisms of gonad development.

Peptidyl arginine deiminase 2 (Padi2) is expressed in Sertoli cells in a specific manner and regulated by SOX9 during testicular development.
Tsuji-Hosokawa, Kashimada, Kato, Ogawa, Nomura, Takasawa, Lavery, Coschiera, Schlessinger, Harley, Takada, Morio.
Sci Rep. 2018 Sep 5;8(1):13263.



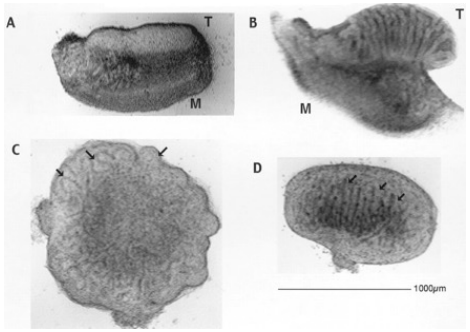
Padi2 was exclusively expressed in Sertoli Cells in fetal developing testes. (a–c) Expression profile of *Padi2* in fetal mice gonads obtained from the GEO Profiles database. During fetal period, testicular supporting cells expressed *Padi2*. P2, postnatal day 2. Data accessible at NCBI GEO database[12], accession GSE27715[13], GSE4818, and GSE5334; Gaido K, Lehmann K et al., 2006.

Transcription factors ER71/ETV2 and SOX9 participate in a positive feedback loop in fetal and adult mouse testis.
DiTacchio L, et al. J Biol Chem. (2012) 6;287(28):23657-66.

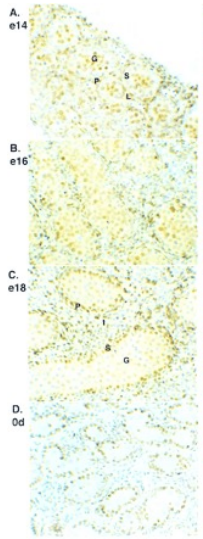


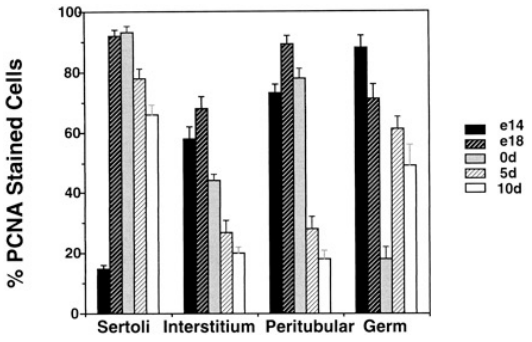
Role of transforming growth factor-alpha and the epidermal growth factor receptor in embryonic rat testis development.

Levine E, Cupp AS, Miyashiro L, Skinner MK.
Biol Reprod. 2000 Mar;62(3):477-90.

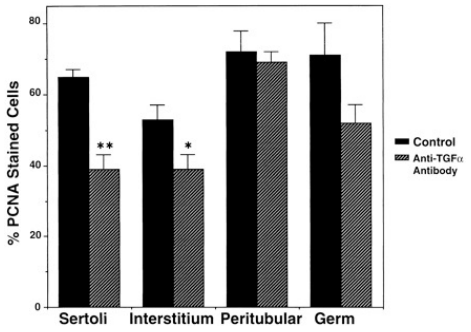


E13 testis + mesonephros organ cultures after 1 day (A) and after 3 days (B) in culture. The upper structure (T) is the testis attached to the lower, dark-colored mesonephros (M). Representative of more than 10 different experiments. The diameter of the organ was approximately 1000 µm. E14 testis organ cultures treated with control nonimmune IgG (C) or with anti-TGF-α IgG antibody S574 (D) after 4 days in culture. Representative cords are marked by arrows placed on the lighter-colored structures and are shown in C and D. This is a microdissected testis devoid of mesonephros with some manipulation in comparison to that shown in A and B. Representative of 10 experiments

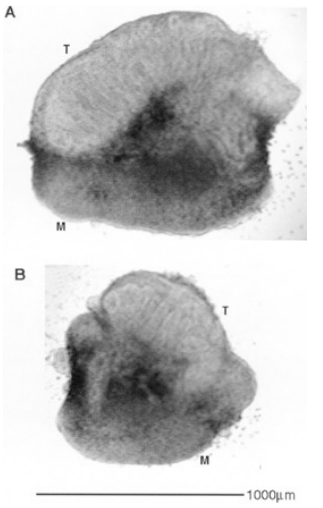


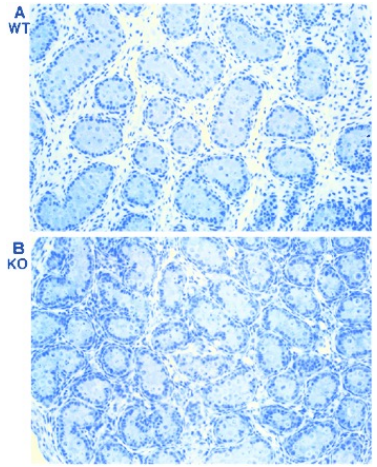


Labeling indices of the percentage of PCNA-positive cells for each cell type at various stages of development. Labeling indices were calculated by counting stained and unstained cells in random regions of replicate slides from at least 3 different sections in 3 different experiments. Data are presented as the mean ± SEM.



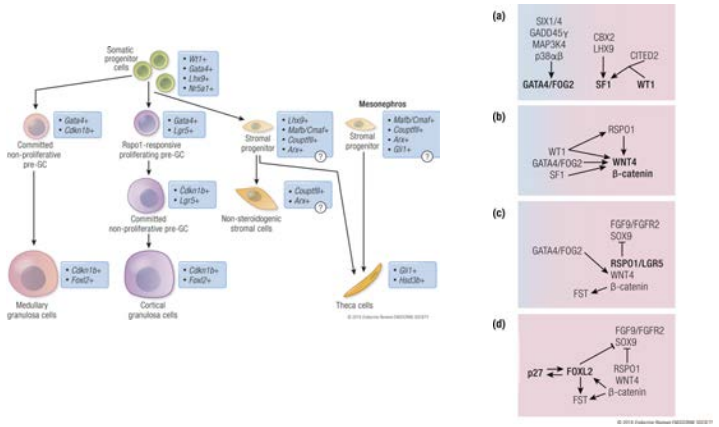
Labeling index for the percentage of each cell type positive for PCNA on e14 testis organ cultures treated with control nonimmune or anti-TGF-α antibody for 4 days. Labeling indices were calculated by counting stained and unstained cells in random regions of at least 3 different sections from 3 different experiments. ** Statistically significant difference from control ($P < 0.001$); * statistically significant difference from control ($P < 0.02$) as determined by a t-test

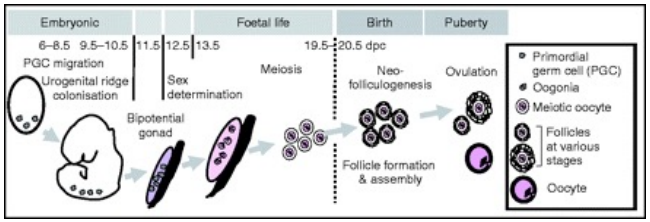


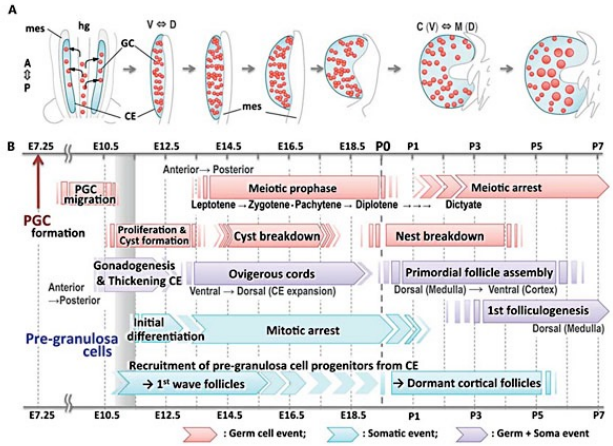


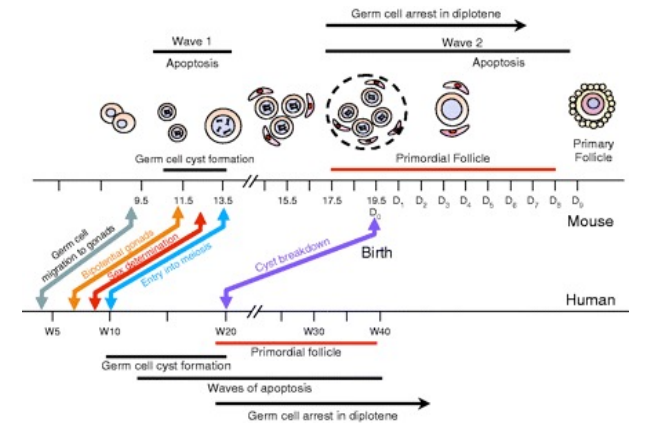
A) E18 wild-type (WT) and B) e18 knockout (KO) mice testes. JB4 sections were stained with toluidine blue. ×200 (published at 80%). Data are representative of more than 10 sections from different testes

Ovary Development

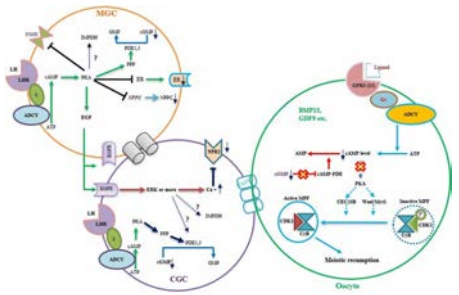






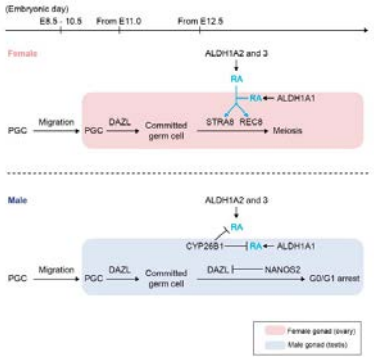


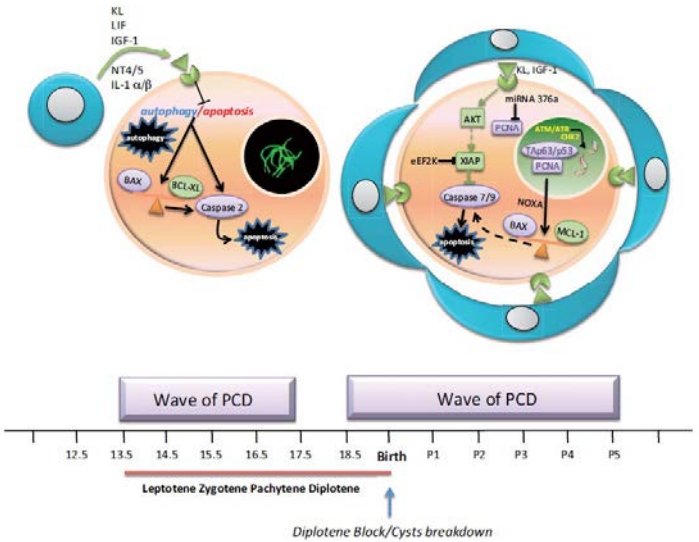
The art of oocyte meiotic arrest regulation.
Pan B, Li J.
Reprod Biol Endocrinol. 2019 Jan 5;17(1):8.



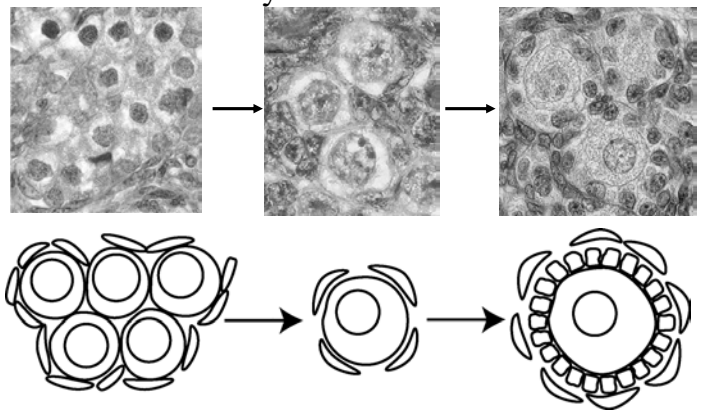
Schematic model depicting the proposed signaling pathway in LH/hCG-induced the resumption of meiosis. LH surge results in the dephosphorylation and deactivation of NPR2 via the phosphoprotein of phosphatase (PPP)-family in the cumulus granulosa cell [96], NPPC production and secretion in the mural granulosa cell. The LH/LHR-induced activation of cAMP/PKA increases the following compounds in the mural granulosa cell: phosphorylation of the cGMP-PDE5, EGF and EGF-like factors production and secretion, and ER expression [103]. The rapid phosphorylation of the cGMP-PDE5 is responsible for converting cGMP into 5′GMP, causes the outward diffusion of intraoocyte cGMP, leads to the release of cAMP-PDE activity within the oocyte, in turn, the activated cAMP-PDE catalyses the cAMP into AMP, ultimately results in meiotic resumption [99]; The production of EGF and EGF-like factors activates the EGFR signaling elevates calcium of cumulus granulosa cells further to inactivate NPR2 [106]; Furthermore, the decrease of ER serves as an augment step [103]. cGMP-PDE5: cGMP phosphodiesterase 5; EGF-like factors: amphiregulin (AREG), epiregulin (EREG), and β-cellulin (BTC)

Retinoic Acid and Germ Cell Development in the Ovary and Testis.
Endo T, Mikedis MM, Nicholls PK, Page DC, de Rooij DG.
Biomolecules. 2019 Nov 24;9(12).





Follicular Assembly and Primordial to Primary Follicle Transition



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

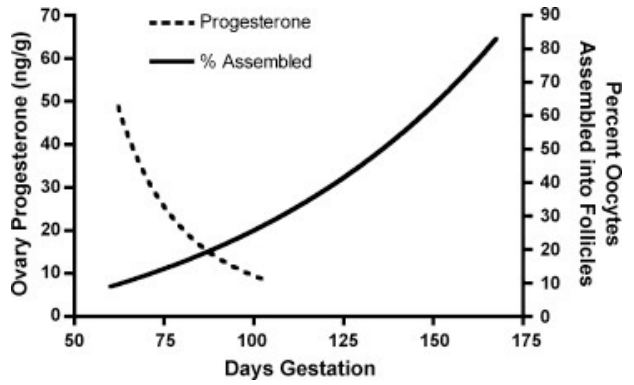
Only 500 human follicles ovulate.

Perinatal Steroids and Follicular Assembly

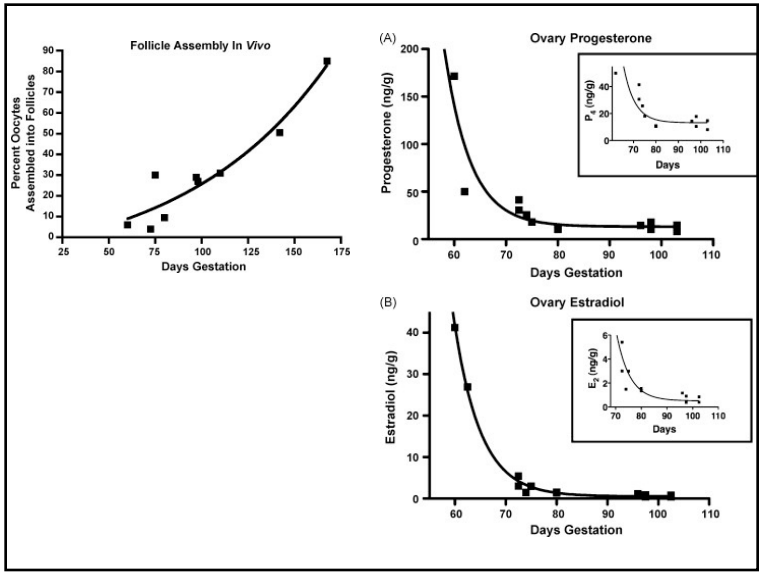
	Time of Initiation	[Steroid]	Time of Completion	[Steroid]
Rodent	Birth	5x10-7	Day 4 Post-Natal	2x10-8
Primate	Mid Gestation	4x10-7	Birth	NA

Steroids might be endocrine factor that coordinates follicular assembly.

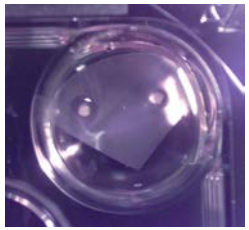
Progesterone regulation of primordial follicle assembly in bovine fetal ovaries.
Nilsson EE, Skinner MK. Mol Cell Endocrinol. (2009) 10;313(1-2):9-16.

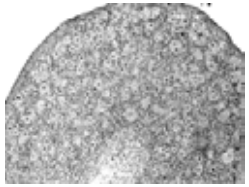


Correlation of progesterone concentration in fetal bovine ovaries and the percentage (%) of oocytes assembled into primordial follicles as a function of estimated gestational age (days). Follicle assembly is initiated as ovarian progesterone concentration declines.



Ovarian Floating Filter Culture System

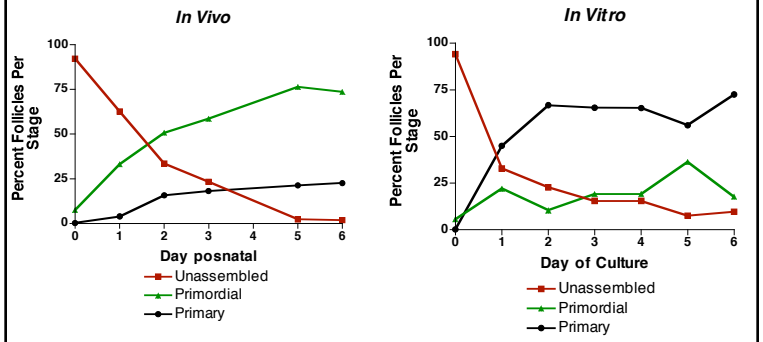




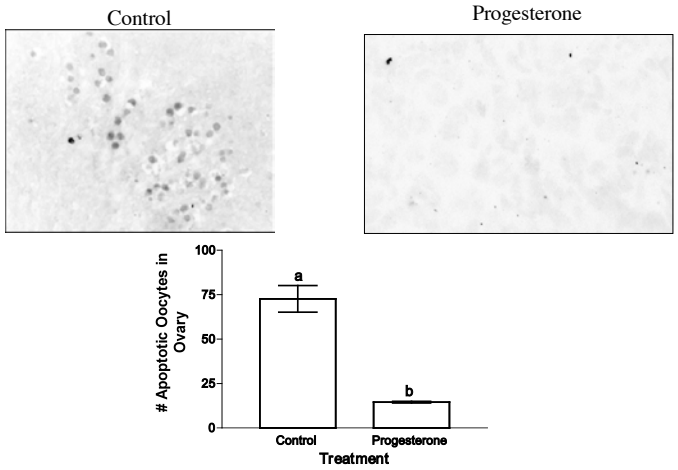
Culture & Treatment
(1 or 2 weeks)

Analysis

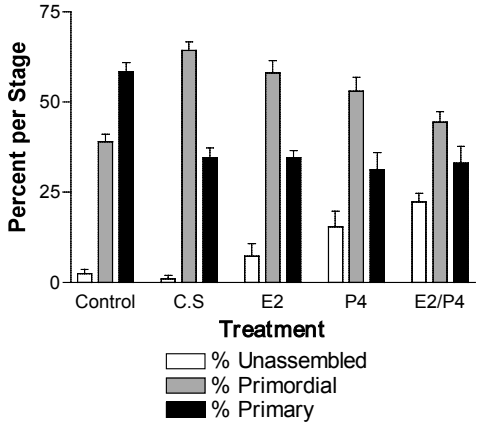
Follicular Kinetics *in vivo/in vitro*.



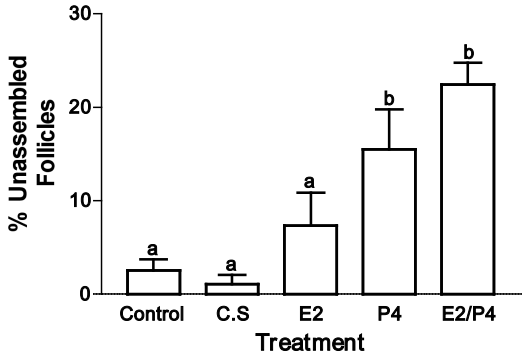
Oocyte Apoptosis in Progesterone Treated and Control Neonatal Ovary.



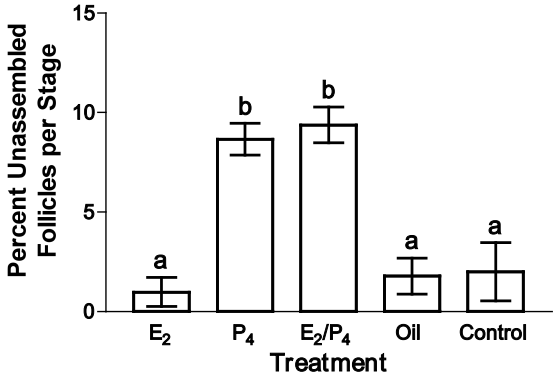
Effect of Steroids on Neonatal Ovary Culture



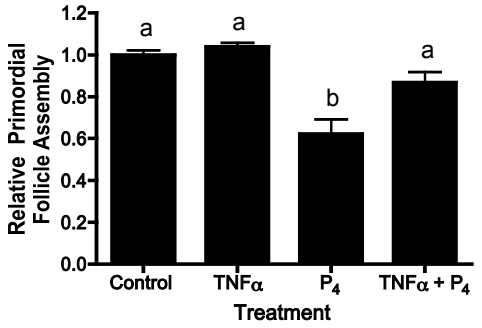
Effect of Steroids on Follicular Assembly *in vitro*



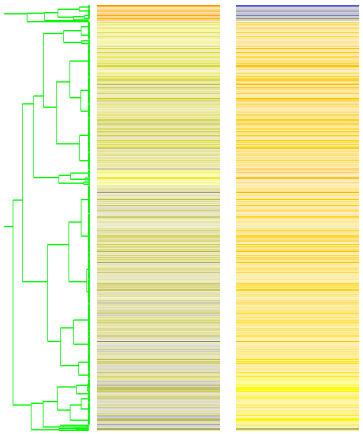
Effect on Folliculogenesis of Neonatal Steroid Treatment *in vivo*.



TNFα and Progesterone Interactions

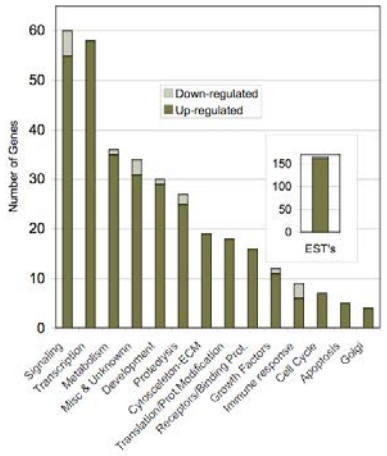


PROGESTERONE ACTIONS ON PRIMORDIAL FOLLICLE ASSEMBLY

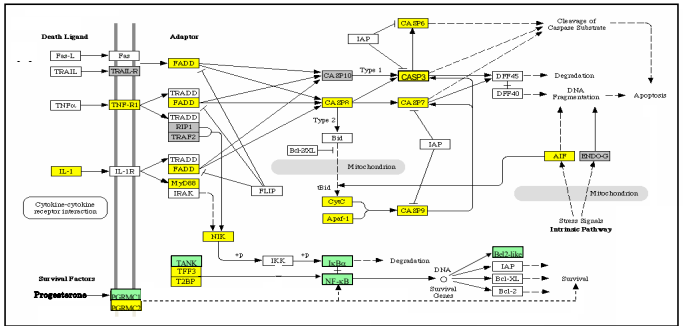


491 Genes Increased
17 Genes Decreased

Progesterone Regulated Follicle Assembly



Progesterone Inhibited Oocyte Apoptosis



Model for Primordial Follicle Assembly

Fetal Environment (Progesterone)

Tumor Necrosis Factor (TNFα)

Oocyte "Nest" → **Programmed Oocyte Death** → **Primordial Follicles**

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DEV3721

4939

FIGα, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes

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SUMMARY

The mouse zona pellucida is composed of three glycoproteins, ZP1, ZP2 and ZP3, encoded by single-copy genes whose expression is temporally and spatially restricted to oocytes. All three proteins are required for the formation of the extracellular zona matrix and female mice with a single disrupted zona gene lack a zona and are infertile. An E-box (CANNTG), located approximately 200 bp upstream of the transcription start sites of *Zp1*, *Zp2* and *Zp3*, forms a protein-DNA complex present in oocytes and, to a much lesser extent, in testes. It has been previously shown that the integrity of this E-box in *Zp2* and *Zp3* promoters is required for expression of luciferase reporter genes microinjected into growing oocytes. The presence of the ubiquitous transcription factor E12 in the complex was used to identify a novel basic helix-loop-helix protein, FIGα (Factor In the Germline alpha) whose expression was limited to oocytes within the ovary. The ability of FIGα to transactivate reporter genes coupled to each of the three mouse zona promoters in heterologous 10T½ embryonic fibroblasts suggests a role in coordinating the expression of the three zona pellucida genes during oogenesis.

Key words: FIGα, basic helix-loop-helix transcription factor, zona pellucida, oocyte-specific gene expression, mouse

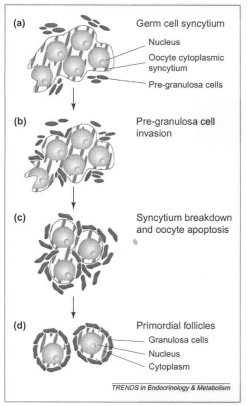


Fig. 2. Formation of primordial follicles. **(a) Germline cell clusters** arise at E10.5–E13.5 as a result of synchronous mitotic divisions and incomplete cytokinesis. (b) Perinatally, somatic (pre-granulosa) cells appear to invade the syncytia and, (c) the subsequent breakdown of the syncytia correlates with massive germline apoptosis. (d) The surviving germ cells, arrested in the prophase of the first meiotic division, become surrounded by a single layer of granulosa cells and contained within an outer basal lamina. These primordial follicles represent the **lifetime complement of germ cells available to females.**

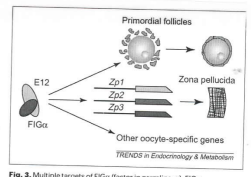
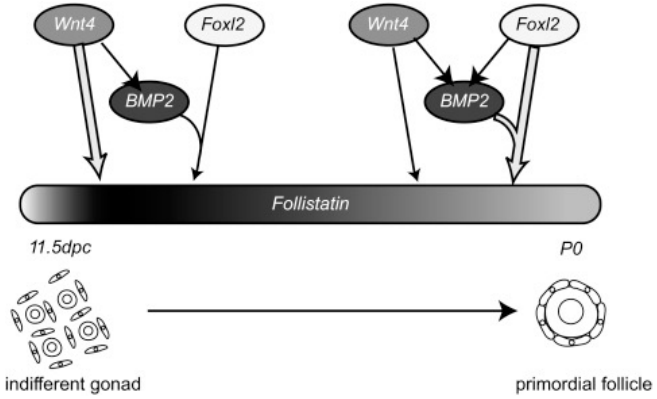
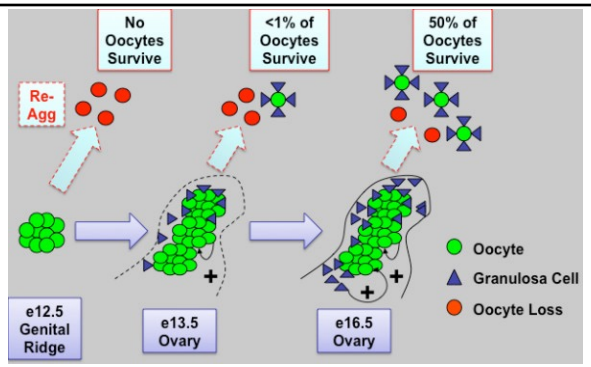


Fig. 3. Multiple targets of FIGα (factor in germline, α). FIGα heterodimerizes with E12, a ubiquitous basic helix- loop- helix protein, and binds upstream of one or more genes, the expression of which is required for primordial follicle formation. In addition, FIGα is required for the expression of the three zona pellucida genes (*Zp1-3*), without which the zona matrix is not formed. The persistence of FIGα in oocytes from embryonic day 13 until they are fully grown suggests that it could modulate additional genes important for normal folliculogenesis [39].





Model of intact ovarian cord formation and maturation promoting oocyte survival and development in follicles. Prior to ovary development on e13.5, germ cells located in cysts in the e12.5 female genital ridge are not yet competent to survive and form follicles when re-aggregated (re-agg). However, ovary maturation on e13.5 and development of intact ovarian cord structures containing oocyte and somatic granulosa cell clusters are sufficient to permit some oocyte survival and follicle formation upon re-aggregation. By e16.5, intact ovarian cord-enclosed meiotic oocyte clusters and granulosa somatic cells are now primed to undergo robust follicle formation and oocyte development in follicles when re-aggregated. The percentage of surviving re-aggregated oocytes was calculated in comparison to intact transplant controls. Oocyte (green) and granulosa cell (blue) contacts and paracrine signaling factors may promote oocyte survival (+) and possibly facilitate programmed ovarian germ cell cyst break down into follicles. The cord and/or oocyte-mediated recruitment of granulosa cell clusters during ovary differentiation may also provide somatic cell signaling factors such as WNT4, R-spondin1, and Follistatin that promote oocyte survival and maturation. In addition, intact ovarian cords may facilitate the protection of oocytes from re-aggregation induced inhibitory signaling from somatic cells in the genital ridge. Thus, we define a critical window from e13.5 to e16.5 of oocyte enclosure in intact fetal ovarian cord structures for the intrinsic programming of oocytes with competence to survive and undergo further development.

Table 1 Summary of analysis and results following e12.5 female genital ridge transplantation.

Days of Transplantation	Analysis[a]	Re-Agg Gonad	Intact Gonad	In Vivo[b] Control
3	Meiosis	Zygotene-pachytene of meiosis I	Zygotene-pachytene of meiosis I	Zygotene-pachytene of meiosis I [18]
5	Ovarian Cord Formation	Ovarian cords do not form and ovary disorganized	Ovarian cords form with distinct organization of oocyte and granulosa cell clusters	Ovarian cords form with distinct organization of oocyte and granulosa cell clusters [1]
7	Oocyte Survival	Oocyte numbers significantly reduced compared to intact gonad	Oocyte numbers reduced compared to day 5	Oocyte numbers decline due to programmed breakdown [10]
12-21	Oocyte Development in Follicles	Oocytes do not survive to day 12 and follicles not observed	Oocytes survive, form follicles, and mature	Oocytes survive, form follicles, and mature [2]

[a] For all experiments, n = 3 transplants with 4 embryos or 8 gonads per transplant
[b] In vivo control refers to e15.5, e17.5, e19.5, and P4 - P14 stages

Model for Primordial Follicle Assembly

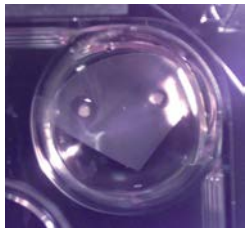
Oocyte "Nest" ⟶ **Programmed Oocyte Death** ⟶ **Primordial Follicles**

Objective: Identify the genes involved in follicle assembly.

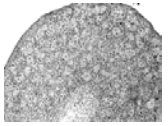
Experimental approach:

- Take ovaries from 0-day old rats.
- Culture for 24h with one of several growth factors.
- Extract RNA.
- Affymetrix microarrays.

OVARIAN FLOATING FILTER CULTURE SYSTEM



2 days



Morphological Analysis

1 day

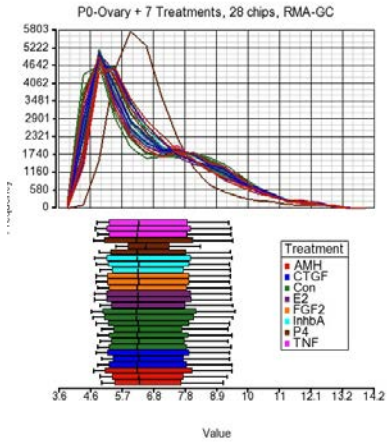
Culture & Treatment

Collect RNA
Microarrays

Treatments

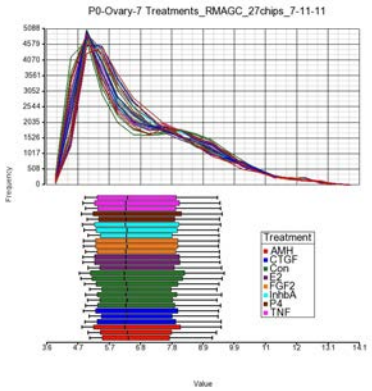
AMH	Anti-Mullerian hormone
CTGF	Connective tissue growth factor
E2	Estradiol
FGF2	Fibroblast growth factor 2
INHBa	Activin A (inhibin alpha subunit)
P4	Progesterone
TNFa	Tumor necrosis factor alpha
Cont	Control untreated

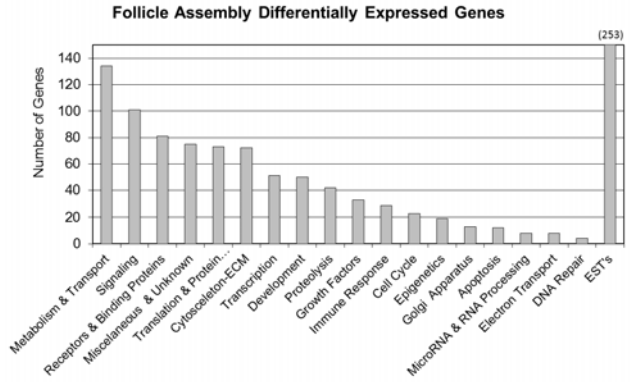
RMA-GC pre-processing of all 28 chips



Conclusion:
we have one outlier (P4 sample)
To do : omit this sample and pre-process again

RMA-GC pre-processing of 27 chips (minus an outlier)





Analysis Approach

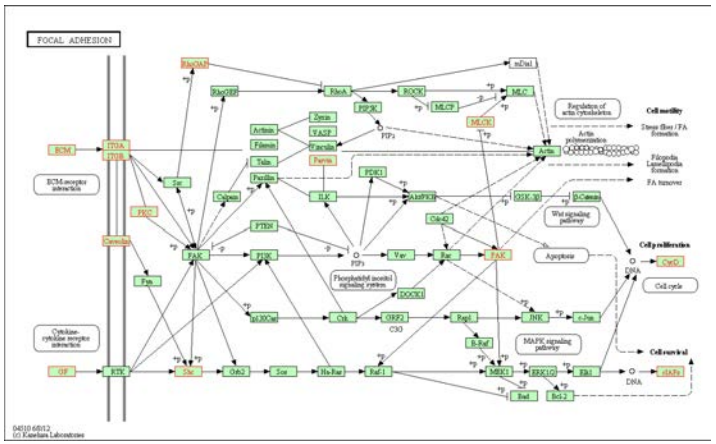
1. Microarray analysis by treatment.
2. Coexpression analysis (modules).
3. Automated literature network analysis.

Number of genes and KEGG pathways overlaped between signature lists

	#PW	AMH	CTGF	E2	FGF2	INHBa	P4	TNFa		3+ Total
# genes		73	9	79	115	83	85	63		138
AMH	158		4	45	63	43	41	29		73
CTGF	50	2		7	8	7	7	5		9
E2	120	10	0		69	49	49	38		79
FGF2	303	13	4	9		71	70	50		115
INHBa	287	11	1	4	14		52	40		83
P4	167	5	3	8	11	14		43		85
TNFa	116	6	4	4	7	9	3			63
Total	1081									

P0ovaries, 7treatments, 1081 gene list, KEGG from Entrez ID, gene overlaps frc

Number of overlaps with only KEGG 3+ start list

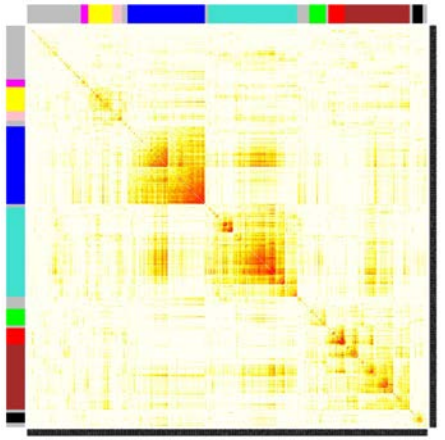


			AMH	CTGF	E2	FGF2	INHBa	P4	TNFa
			158	50	120	303	287	167	116
KEGG ID	Pathway name	total # genes							
rno01100	Metabolic pathways	59	9		6	11	23	9	10
rno05200	Pathways in cancer	19	3		3	7	3	3	2
rno04740	Olfactory transduction	19	2	3	1		2	11	1
rno04010	MAPK signaling pathway	15			2	7	2	3	1
rno04510	Focal adhesion	15	1		4	5	4	3	
rno04062	Chemokine signaling pathway	15	3		2	6	4	1	1
rno04144	Endocytosis	15			5	3	3	2	2
rno00980	Metabolism of xenobiotics by cytoch	14		1	1	3	3	6	
rno04145	Phagosome	13	1		4	3	4	2	3
rno04020	Calcium signaling pathway	12	5		3	4	1	1	1
rno04514	Cell adhesion molecules	12	1		1	4	2	1	3
rno04360	Axon guidance	11	1			6	3	3	1
rno04810	Regulation of actin cytoskeleton	11	2		3	4	2	2	
rno00480	Glutathione metabolism	11		1	1	3	3	3	1
rno00230	Purine metabolism	11	3		3	3	2		1
rno03010	Ribosome	11					11		
rno04060	Cytokine-cytokine receptor interacti	11				6	3	2	
rno04080	Neuroactive ligand-receptor interac	10	2		1	6	3	1	
rno04976	Bile secretion	10	2		2	4	1	1	1
rno04380	Osteoclast differentiation	9				5	3	1	1
rno04640	Hematopoietic cell lineage	9	3	1	2	2	1	1	1
rno04110	Cell cycle	8	1			5	1		1
rno04512	ECM-receptor interaction	7	1		2	2	2	1	
rno04612	Antigen processing and presentatio	7				1		3	3
rno04972	Pancreatic secretion	7			1	3	2	1	
rno04350	TGF-beta signaling pathway	7	1		1	3	2		

Analysis Approach

1. Microarray analysis by treatment.
2. Coexpression analysis (modules).
3. Automated literature network analysis.

Co-expression heat map



Module	Database	Function	# module genes	p-value
turquoise	KEGG pathway	Ribosome	10	7.52E-08
turquoise	GO: Cell Component	cytosolic ribosome	7	0.0000024
turquoise	GO: Biological Process	response to virus	6	0.00013
turquoise	GO: Biological Process	neural tube closure	4	0.00041
turquoise	GO: Biological Process	negative regulation of binding	5	0.00045
turquoise	Panther Biological Process	Protein biosynthesis	15	0.00071
turquoise	KEGG pathway	Glutathione metabolism	5	0.0011
turquoise	KEGG pathway	TGF-beta signaling pathway	3	0.09
blue	GO: Biological Process	response to oxidative stress	12	0.0000013
blue	GO: Biological Process	regulation of anatomical structure morphogene	13	0.0000022
blue	Panther Biological Process	Mesoderm development	18	0.000019
blue	Panther Biological Process	Angiogenesis	6	0.000022
blue	GO: Biological Process	response to carbohydrate stimulus	6	0.000036
blue	GO: Biological Process	regulation of axonogenesis	6	0.00005
blue	GO: Cell Component	membrane raft	9	0.000078
blue	GO: Biological Process	negative chemotaxis	3	0.0001
blue	KEGG pathway	Axon guidance	6	0.0037
blue	KEGG pathway	Glutathione metabolism	3	0.031
blue	KEGG pathway	Focal adhesion	6	0.032
blue	KEGG pathway	Fc gamma R-mediated phagocytosis	4	0.036
blue	KEGG pathway	Calcium signaling pathway	5	0.067
blue	KEGG pathway	Cytokine-cytokine receptor interaction	5	0.074
brown	GO: Cell Component	germ cell nucleus	3	0.0007
brown	GO: Biological Process	male meiosis	3	0.0015
brown	GO: Biological Process	neurite regeneration	3	0.0031
brown	GO: Cell Component	condensed nuclear chromosome	3	0.0041
brown	KEGG pathway	Cell cycle	4	0.026
brown	KEGG pathway	Olfactory transduction	12	0.065

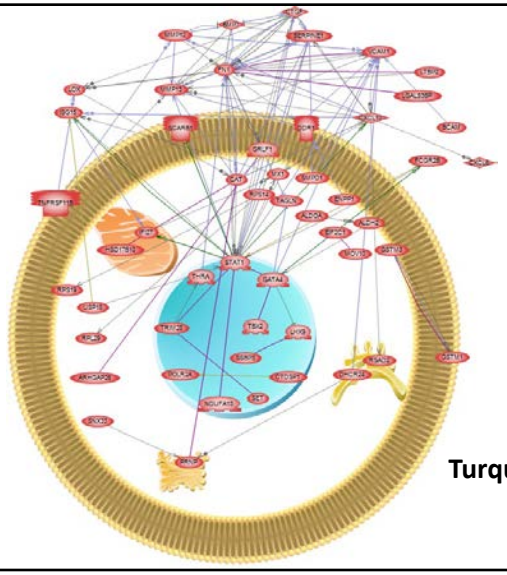
Number of genes overlaped between signatures (treatments) and modules

		turquoise	blue	brown	yellow	green	red	black	pink	magenta
	# genes	240	209	176	65	45	43	27	26	20
AMH	158	14	7	28	19	**25**	0	0	7	3
CTGF	50	0	4	8	1	1	5	2	3	4
E2	120	1	6	3	20	8	**14**	**15**	9	4
FGF2	303	4	**169**	44	28	9	1	0	2	4
INHBa	287	**184**	24	26	5	2	**15**	0	**14**	1
P4	167	16	5	58	11	0	8	6	7	4
TNFa	116	36	10	19	6	4	2	4	0	6

Number of overlaps with all probeset IDs
Bold numbers heavily represented by module.
Underlined numbers heavily represented by signature (treatment).

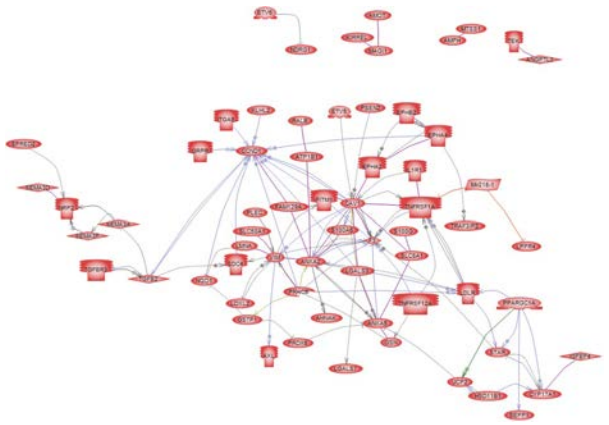
Analysis Approach

1. Microarray analysis by treatment.
2. Coexpression analysis (modules).
3. Automated literature network analysis.

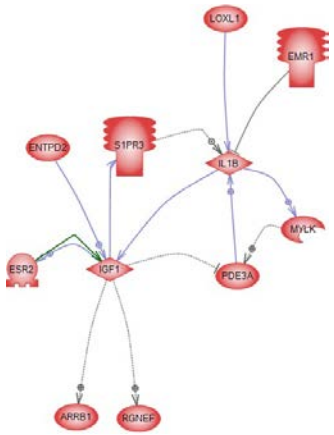


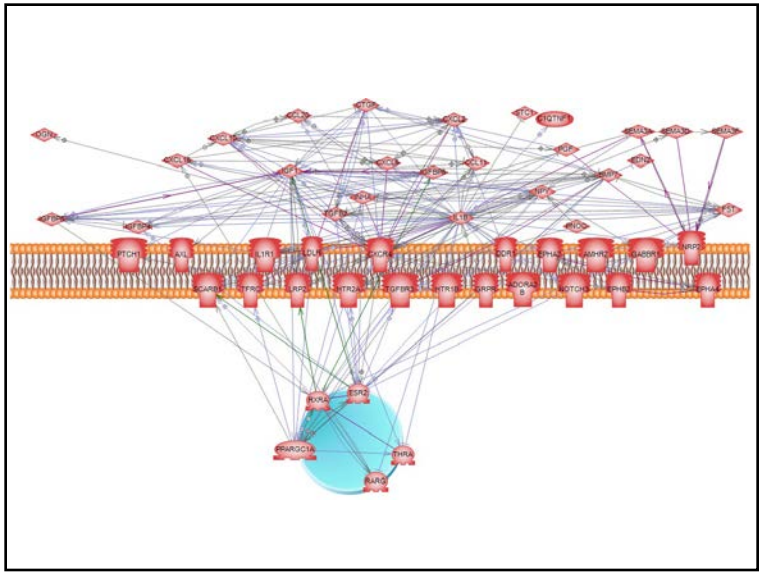
Turquoise

Blue

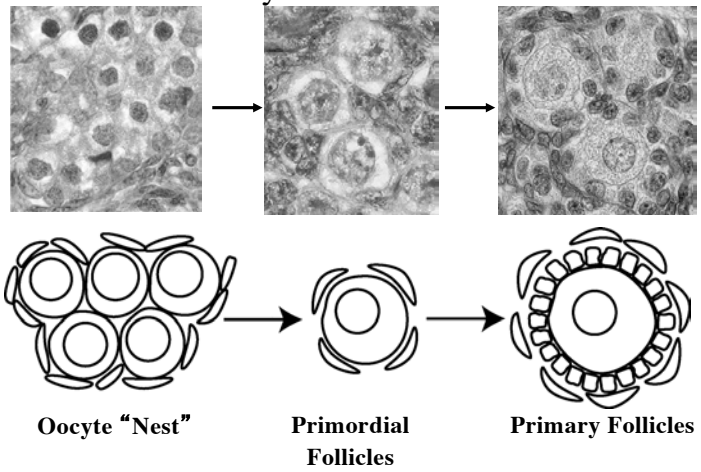


Black

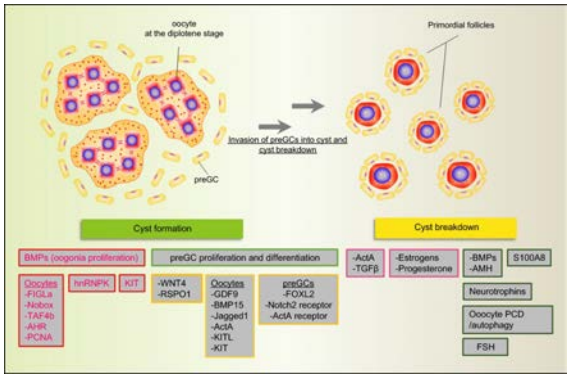


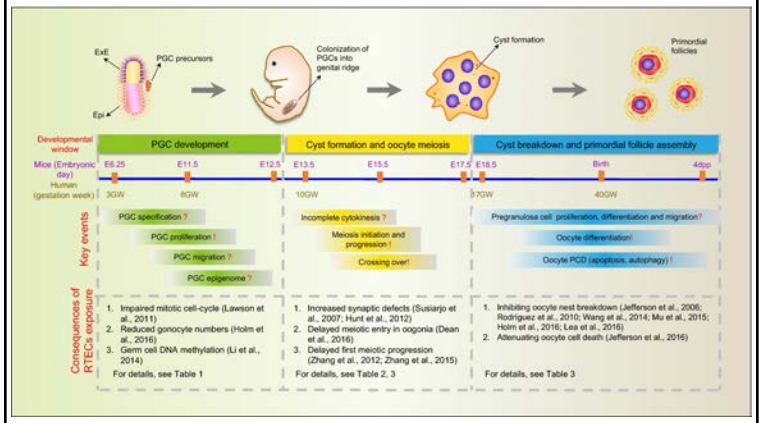


Follicular Assembly and Primordial to Primary Follicle Transition

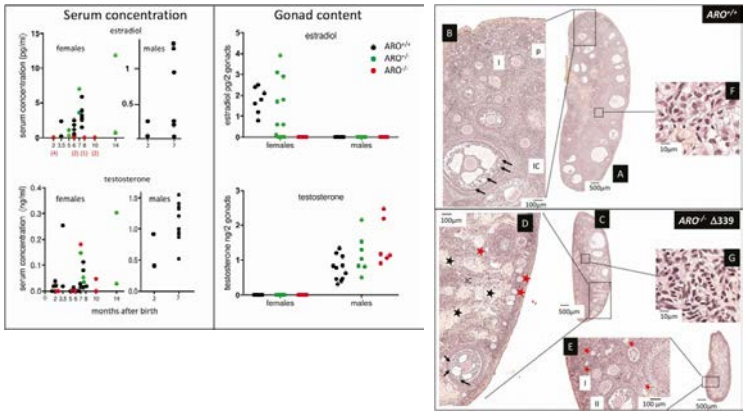


Establishment and depletion of the ovarian reserve: physiology and impact of environmental chemicals.
Ge W, Li L, Dyce PW, De Felici M, Shen W.
Cell Mol Life Sci. 2019 May;76(9):1729-1746.

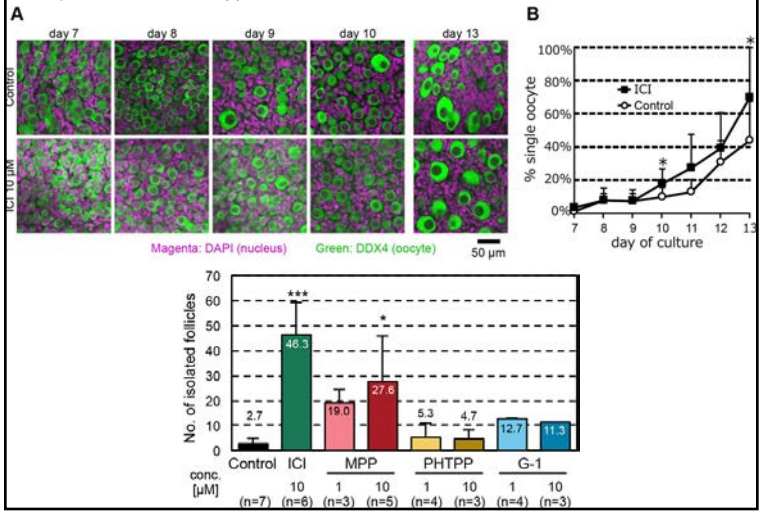




Fetal Estrogens are not Involved in Sex Determination But Critical for Early Ovarian Differentiation in Rabbits.
Jolivet G, Daniel-Carlier N, Harscoët E, et al.
Endocrinology. 2022 Jan 1;163(1):bqab210.

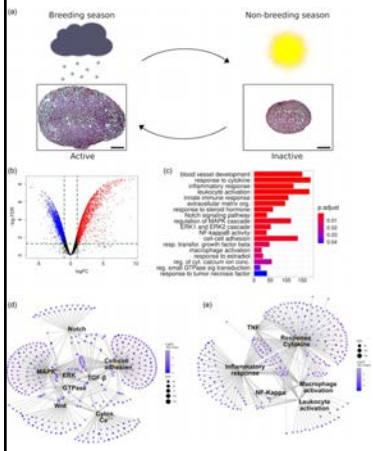


Blocking estrogen-induced AMH expression is crucial for normal follicle formation.
Tanimoto R, Sekii K, Morohaku K, et al.
Development. 2021 Mar 19;148(6):dev197459.

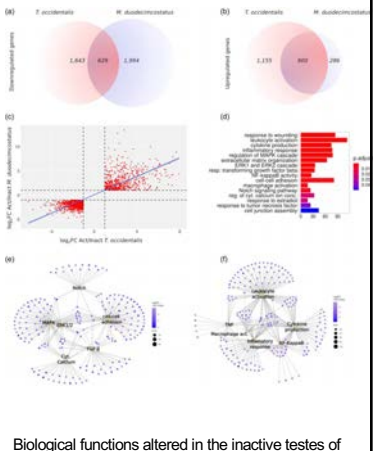


Gonadal Disruption

Cell adhesion and immune response, two main functions altered in the transcriptome of seasonally regressed testes of two mammalian species.
Real FM, Lao-Pérez M, Burgos M, et al.
J Exp Zool B Mol Dev Evol. 2023 May;340(3):231-244.

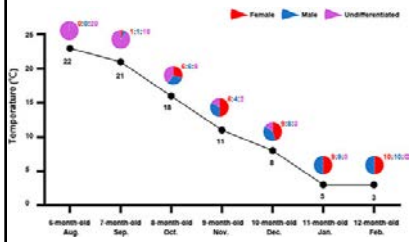


Transcriptomic analysis of seasonally active and inactive testes of *Talpa occidentalis*

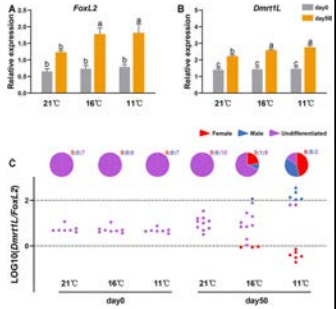


Biological functions altered in the inactive testes of both *Talpa occidentalis* and *Microtus duodecimcostatus*.

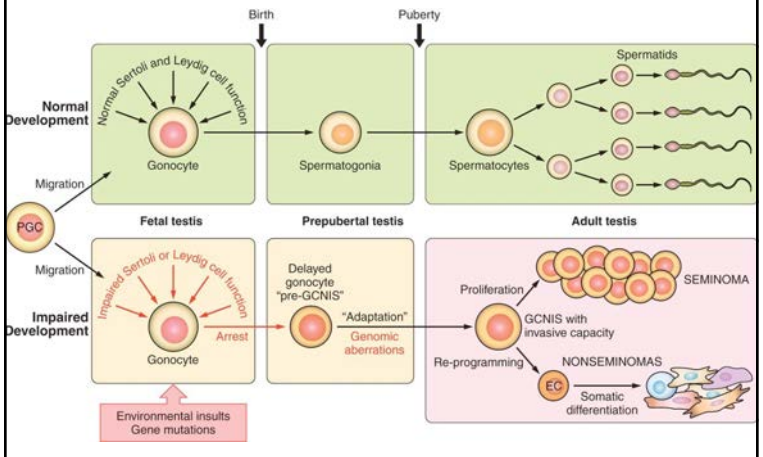
The Effect of Temperature on Gonadal Sex Differentiation of Yesso Scallop Patinopecten yessoensis.
Liu T, Li R, Liu L, Wu S, Zhang L, et al.
Front Cell Dev Biol. 2022 Jan 31:9:803046.



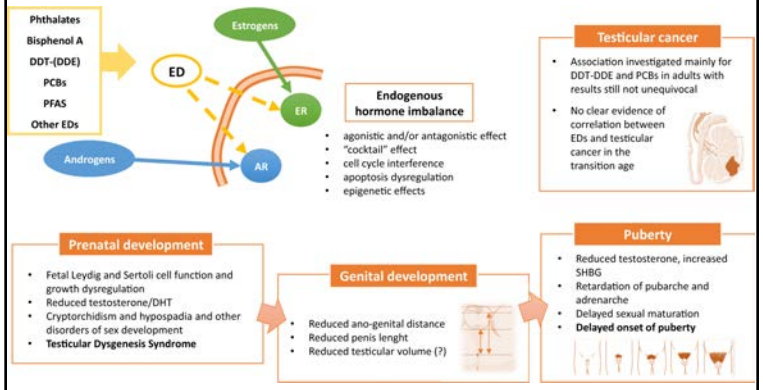
Sex differentiation ratio of 6–12 month-old scallops and the corresponding seawater temperature in each month. For each month, 12–20 individuals were examined. The pie charts show the percentages of female (red), male (blue) and undifferentiated samples (purple) in each month. The values above the pie charts are the corresponding numbers of female (red), male (blue) and undifferentiated samples (purple). The line indicates the seawater temperatures for each month.

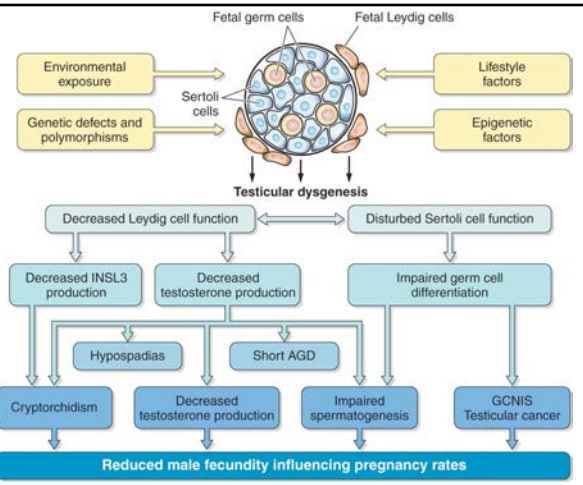


Relative expression of *FoxL2* and *Dmrt1L* in the three temperature groups at day 0 and 50. Relative expression levels of *FoxL2* **(A)** and *Dmrt1L* **(B)** in the three temperature groups (21, 16 and 11° C) at day 0 and 50. The vertical bars represent the means ± SEM (N = 7–13). Different letters indicate significant differences ($p < 0.05$). **(C)** The LOG10 (*Dmrt1L*/*FoxL2*) values and sex differentiation ratio for each group on day 0 and 50. Each dot represents a sample. The dashed lines indicate the threshold for sex differentiation, with values below 0 for ovary and values higher than two for testis. The pie charts indicate the percentages of female (red), male (blue) and undifferentiated samples (purple) in each month. The values above the pie charts are the corresponding numbers of female (red), male (blue) and undifferentiated samples (purple).



Effects of endocrine disruptors on fetal testis development, male puberty, and transition age
Cargnelutti F, Di Nisio A, Pallotti F, et al.
Endocrine. 2021 May;72(2):358-374.

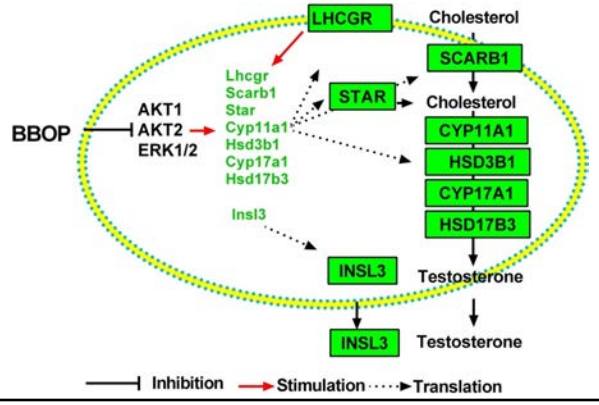




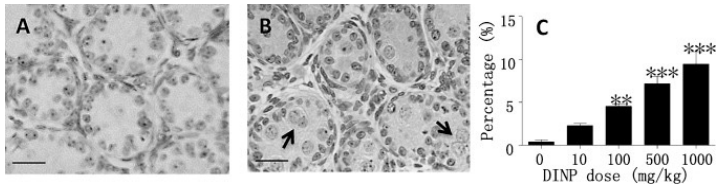
The hypothesis of testicular dysgenesis syndrome (TDS) and signs that might be linked to it: poor spermatogenesis, testicular cancer, hypospadias, cryptorchidism, and short ano-genital distance (AGD). The single symptoms and combinations thereof are risk factors for reduced fecundity. [Updated from Skakkebaek et al. (387).]

Endocrine Disrupting Chemicals and Reproductive Health in Boys and Men.
Rodprasert W, Toppari J, Virtanen HE.
Front Endocrinol (Lausanne). 2021 Oct 7;12:706532.

Effects of bis(2-butoxyethyl) phthalate exposure in utero on the development of fetal Leydig cells in rats.
Liu M, Chen H, Dai H, et al.
Toxicol Lett. 2021 Oct 15;351:65-77.

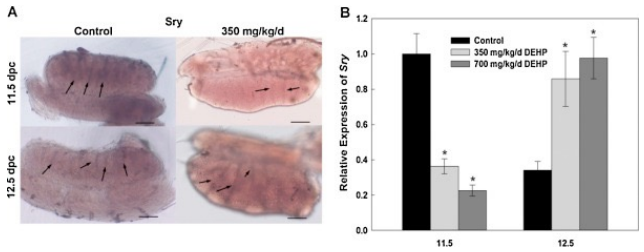


Inutero exposure to diisononyl phthalate caused testicular dysgenesis of rat fetal testis.
Li L, Bu T, Su H, Chen Z, Liang Y, et al.
Toxicol Lett. 2015 Jan 22;232(2):466-74.



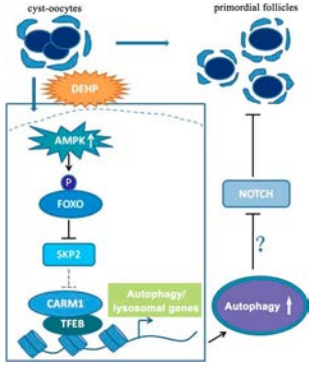
Occurrence of multinucleated gonocytes (MNGs) in representative photomicrographs of testis section and percentage of seminiferous cords exhibiting MNGs. (A) Control group and (B) DINP 1000 mg/kg group. Sections were stained with hematoxylin-eosin stains. Arrow indicates MNG. Scale bar = 50 μm. (C) Percentage of seminiferous cords exhibiting MNGs on GD21.5 (n = 6); Mean ± SEM. Significant differences at *P < 0.05, **P < 0.01 and ***P < 0.001, respectively, were shown, when compared to control.

Di (2-ethylhexyl) phthalate exposure during pregnancy disturbs temporal sex determination regulation in mice offspring.
Wang Y, Liu W, Yang Q, Yu M, Zhang Z.
Toxicology. 2015 Oct 2;336:10-6.

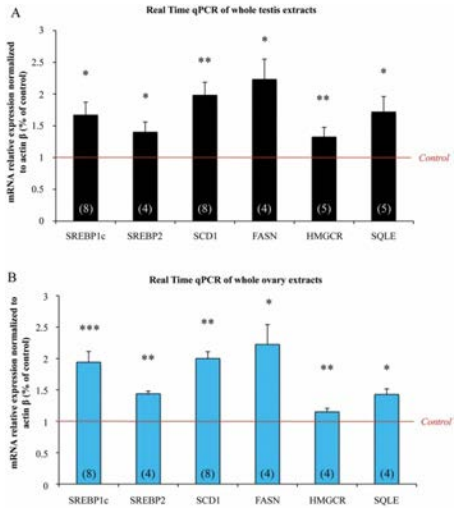


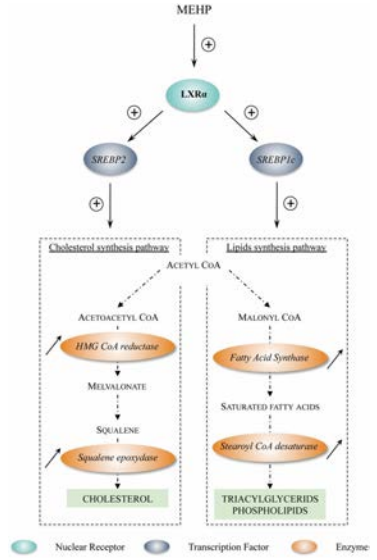
Expression levels and patterns of Sry in male genital ridges in offspring from control and embryonal DEHP treatment group during 11.5–12.5 dpc. A: Expression pattern of Sry examined by whole-mount in situ hybridization (WISH) in male genital ridges. Antisense probe is active versus sense probe (data no shown). Arrows indicate the sex cord in gonad. Bars: 100 μm. B: Relative expression levels of Sry normalized to the β-actinin male genital ridges analyzed by RT-qPCR. Values significantly different from control at $p < 0.05$ are indicated by asterisks, Values are litter mean ± SE (n = 4–6 for control, 350 and 700 mg/kg/d groups).

Foetal-neonatal exposure of Di (2-ethylhexyl) phthalate disrupts ovarian development in mice by inducing autophagy.
Zhang Y, Mu X, Gao R, Geng Y, Liu X, Chen X, Wang Y, Ding Y, Wang Y, He J.
J Hazard Mater. 2018 Sep 15;358:101-112.

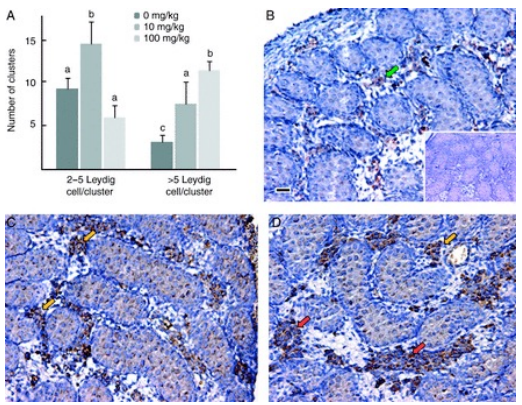


Cellular and molecular effect of MEHP Involving LXRα in human fetal testis and ovary.
Muczynski V, et al. PLoS One. (2012) 7(10):e48266.

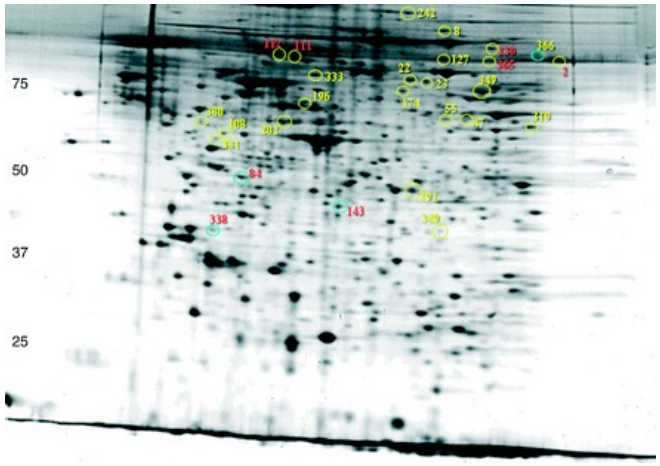




Working model of LXRα transcriptional up-regulation induced by MEHP exposure and effects on downstream genes involved in cholesterol/lipid synthesis.
First, exposure to MEHP up-regulates *LXRα* expression. Increased *LXRα* activity then stimulates the mRNA expression of *SREBP1c* and *SREBP2* that positively regulate, respectively, the transcriptional level of lipid and cholesterol synthesis enzymes, therefore potentially leading to an increase in cholesterol and lipid synthesis in cells.



(A) Data showing frequency of Leydig cell clusters expressed as the mean number observed in a testis cross section (mean±s.e.m., n=8 males/treatment group; ^{a,b,c} denote significance at P<0.05). Clusters were classified as those containing 2–5 contiguous Leydig cells (small clusters) and those containing more than five Leydig cells (large clusters). Note the significant increase in small cluster following exposure to 10 mg and the significant increase in large clusters following exposure to 100 mg. (B) A GD19 control testis immunostained for 3β-HSD; Leydig cells are clearly indicated (green arrow). (C) Testes in the 10 mg group showed a dramatic increase in small Leydig cell clusters (orange arrows). (D) The Leydig cell clusters in testes in the 100 mg group were noticeably large (red arrows). Magnification bar shown in (A)=20 μm.



Two-dimensional gel profile of the GD19 testis proteome. Proteins which were significantly altered by both the 10 and 100 mg exposures are indicated. Proteins whose expression was upregulated are circled in cyan, all other proteins were downregulated. Proteins that were either correlated with and/or predictive of treatment, testosterone production, or Leydig cell clustering are indicated by the red numbers.

Table 1 Proteins found to be significantly down- or up-regulated following both 10 and 100 mg/kg DEHP exposures.

Spot	Symbol	Accession numbers	Protein name	Protein MW (kDa)	Percent coverage	Dose 0	Dose 10	Dose 100	Molecular function
2	**STIP1**	Q3ZCU9	Stress-induced-phosphoprotein 1	63	66	100	58*	52	Protein–protein binding
8	SYG	Q580G4	Glycyl t-RNA synthetase	72	15	100	56	66	Protein biosynthesis
22	LMAN1	Q62902	Lectin mannose binding 1	55	20	100	61	71	Protein transport
23	PDIA3	P11598	Protein disulfide-isomerase A3	57	60	100	67	71	Cellular redox homeostasis
47	ENO1	P04764	Alpha enolase	47	29	100	60	60	Glycolysis/plasminogen activation
55	ENO1	P04764	Alpha enolase	47	37	100	78	73	Glycolysis/plasminogen activation
84	**HSPA8**	P63018	Heat shock protein 71 kDa	71	38	100	143	162	Chaperone/transcriptional repressor
108	ACTG	P63259	Actin, cytoplasmic 2	42	33	100	40	37	Responder to calcium/ATP binding
111	**HSP90B1**	Q66HD0	Heat shock protein 90 kDa, subunit 1	90	7	100	53	59	Chaperone in the endoplasmic reticulum
112	**HSP90B1**	Q66HD0	Heat shock protein 90 kDa, subunit 1	90	7	100	51	50	Chaperone in the endoplasmic reticulum
127	TCP1	P28480	T complex protein 1 subunit alpha	60	40	100	62	60	Chaperone involved in protein folding
130	**TRAP1**	Q5XHZ0	Heat shock protein 75 kDa, Mito.	74	26	100	68	58	Chaperone involved in protein folding
143	**SRM**	Q99M85	Spermadine synthase	34	18	100	132	140	Spermadine biosynthesis
174	ATP6V1-B2	P62815	V type proton ATPase subunit B	57	12	100	49	47	Hydrogen ion transport/ ATPase activity
191	RPLP0	P19945	60S acidic ribosomal protein P0	34	34	100	67	63	Translational elongation
196	PDIA6	Q63081	Protein disulfide isomerase A6	46	33	100	78	70	Chaperone/platelet aggregation
202	CCT5	Q68FQ0	T complex protein 1 subunit epsilon	59	23	100	71	76	Chaperone involved in protein folding
242	GSTM4	P08009	Glutathione S-transferase	26	6	100	72	73	Conjugation of reduced glutathione
319	TUFM	P49411	Elongation factor Tu, Mito.	45	67	100	78	74	Translation of proteins
333	TRAP1	Q5XHZ0	Heat protein 75 kDa, Mito	74	27	100	62	67	Chaperone/ATPase activity
338	**YWHAE**	P62260	14-3-3 epsilon	32	32	100	142	177	Signal transduction via protein binding
349	CCT2	Q5XIM9	T complex protein 1 subunit beta	57	57	100	80	75	Chaperone involved in protein folding
365	**DPYL2**	P47942	Dihydropyrimidinase-related protein 2	62	28	100	70	76	Differentiation/cell migration
366	RPL13A	Q5RKI0	WD repeat containing protein 1	66	26	100	134	152	Disassembly of actin
380	VIM	P31000	Vimentin	54	24	100	59	74	Intermediate filament of cytoskeleton
381	RPSA	P38983	40 S ribosomal protein SA	33	33	100	73	67	Cell surface receptor for laminin/morphogenesis

Statistical results represent the analysis of 16 I per treatment group (n=16); one testis from four GD19 males pooled and extracted/litter.
*The background-corrected, integrated optical density of each spot was averaged within group and expressed as percent of control. The symbols of proteins that were found to be significantly (P<0.05) correlated with and/or predictive of treatment or measured endpoints (bold and underlined).

Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems.
Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA.
Environ Toxicol Pharmacol. 2017 Apr;51:56-70.

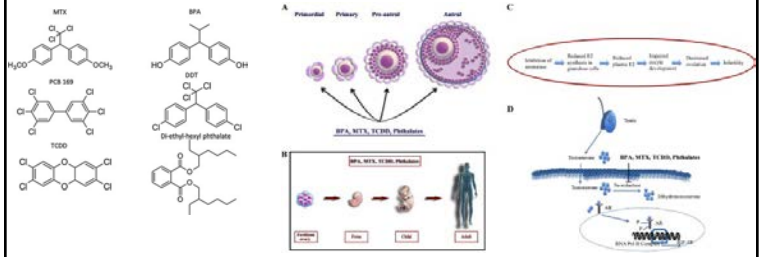
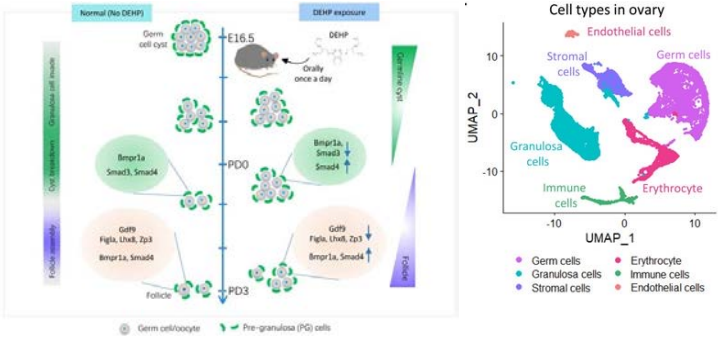


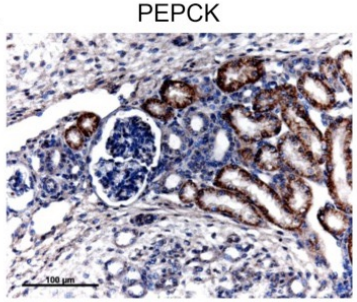
Table 1
A summary of the studies that have assessed the associations between the exposure of human populations to pesticides and EDC agents and the incidence of disorders affecting the female reproductive system. (CS: cross sectional study; CC: case-control study; CHS: Cohort study).

Authors, Year	Chemical	N	Results	Variables	Study Type
Kandaraki et al., 2011	BPA	171 (100 PCOS, 71 Normal)	Higher BPA levels in PCOS, association of BPA with androgens	Hormonal parameters, BMI	CS
Caserta et al., 2013	BPA, phthalates	61 women	Higher BPA and receptor levels in infertile group	Urinary BPA, EDC levels in serum, oestrogen and nuclear receptors	CS
Souter et al., 2013	BPA	209 women on fertility treatment	Higher BPA levels associated with lower AFC	Urinary BPA, Antral follicle count (AFC), FSH	CHS
Ehrlich et al., 2012a,b	BPA	147 women with IVF cycles	Negative association between urinary BPA and serum peak E2 and oocyte yield	Urinary BPA, oocyte maturation, fertilization, embryo quality	CHS
Ehrlich et al., 2012a,b	BPA	137 women	Positive association between urinary BPA and implantation failure	Urinary BPA, β-hCG, implantation failure	CHS
Mok-Lin et al., 2010	BPA	84 women	Urinary BPA is inversely correlatedwith serum peak E2	Urinary BPA, E2 levels, number of oocytes	CHS
Bloom et al., 2011	BPA	44 women with IVF	Serum BPA is associated with reduced E2 levels	Serum BPA, E2 levels, number of oocytes	CHS
Fujimoto et al., 2011	BPA	58 infertile females, 37 male partners	Inverse association between BPA and fertilization	Serum BPA, fertilization	CHS
Yang et al., 2009	BPA	167 women with breast cancer, 152 controls	No association with BPA and breast cancer	Blood BPA, incidence of breast cancer	CC
Aschengrau et al., 1998	BPA, BHA, benzyl phthalate, nonylphenol	261 women with breast cancer, 753 controls	Association with PCBs, 4-octylphenol and breast cancer	Levels of EDCs and pesticides and incidence of breast cancer	CC
Philippat et al., 2012	BPA, phenols, phthalates	288 mother-newborn pairs (72 cases, 216 controls)	Positive/Negative association of benzophenone-3/2,5 DCP with male birth weight, respectively	Levels of EDC and pesticides, birth weight, head circumference, birth length	CC
Sugiura-Ogasawara et al., 2005	BPA	77 women (45 cases with miscarriages, 32 controls)	Serum BPA is associated with recurrent miscarriage	Serum BPA, ANA, NK cells, prolactin, progesterone, TSH, fT4	CC
Buck Louis et al., 2013	BPA, phthalates	626 women	miCPP, miEHP, miEOHP, miEHP, miBP and meMHP were associated with endometriosis	BPA, 14 phthalate metabolites, endometriosis, age, BMI, creatinine	CHS
Kannan et al., 2015	DAP, DE, DM	359 mothers and children	Early life exposure (1–7 years) to DAP, DE, DM is associated with respiratory symptoms	Urinary DAP, DE, DM and metabolites, respiratory symptoms in childhood	CHS
Mahalingaiah et al., 2012	HCB, DDT, DDE	720 women with IVF	Serum HCB is associated with failed implantation	Serum HCB, DDT, DDE and metabolites, fertilization	CHS

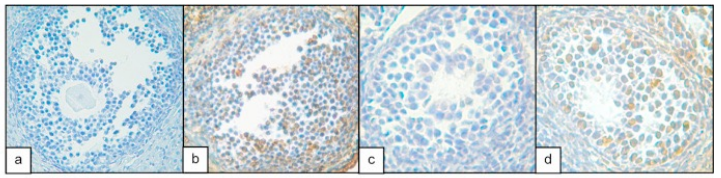
Single-cell transcriptome dissection of the toxic impact of Di (2-ethylhexyl) phthalate on primordial follicle assembly.
Wang J-J, Tian Y, Li M-H, et al.
Theranostics. 2021 Mar 5;11(10):4992-5009.

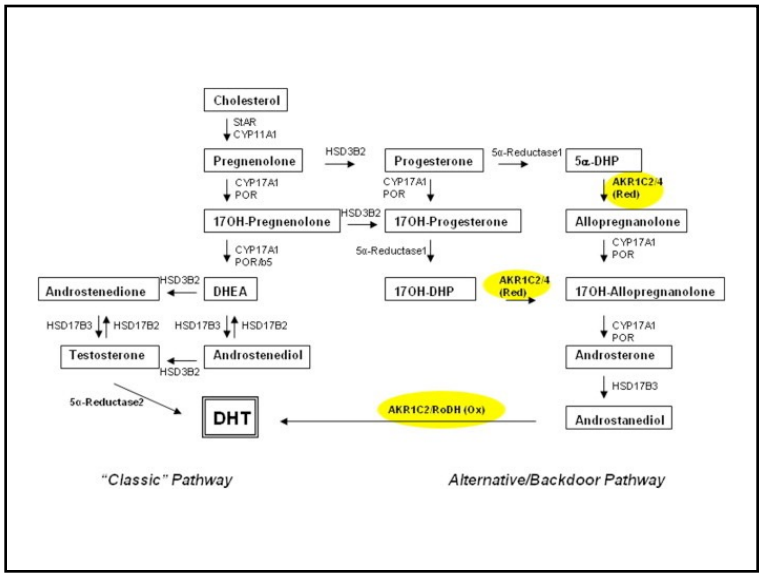


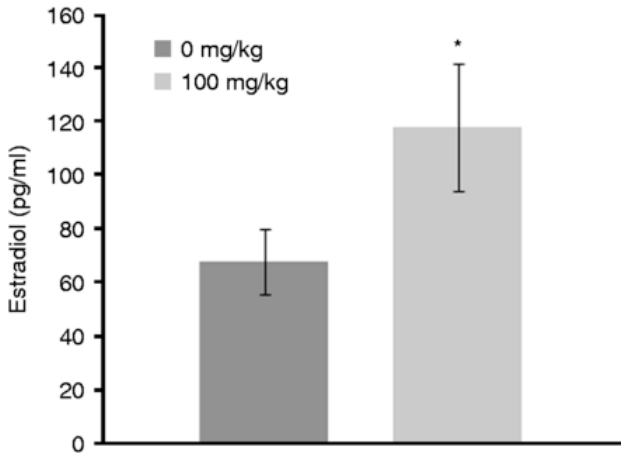
In an Ovine Model of Polycystic Ovary Syndrome (PCOS) Prenatal Androgens Suppress Female Fetal Renal Gluconeogenesis.
Connolly F, Rae MT, Späth K, Boswell L, McNeilly AS, Duncan WC.
PLoS One. 2015 Jul 6;10(7):e0132113.



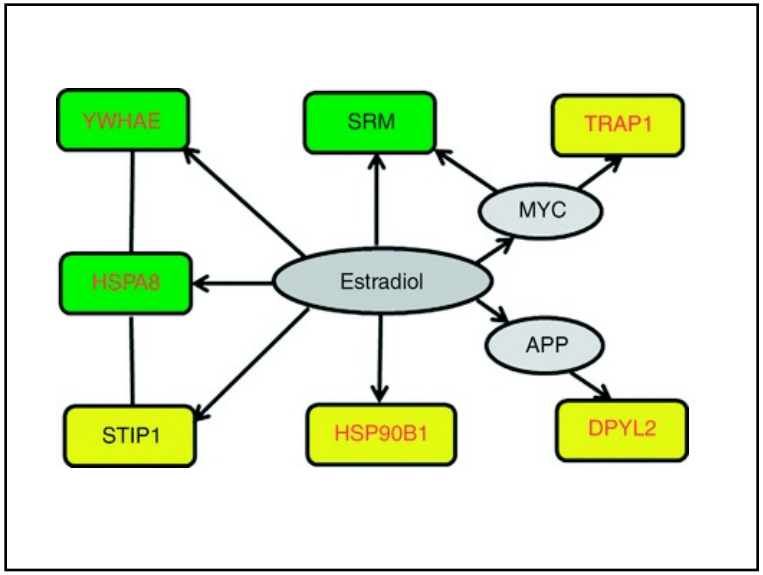
Impaired development of female mouse offspring maternally exposed to simazine.
Park S, Kim S, Jin H, Lee K, Bae J.
Environ Toxicol Pharmacol. 2014 Nov;38(3):845-51.







Plasma estradiol concentrations in GD19 males. The observed increase in the estradiol level in males exposed to 100mg DEHP from GD13 to GD19 was significant (*P<0.05) compared with control males; mean±s.e.m., n=7 males/group.



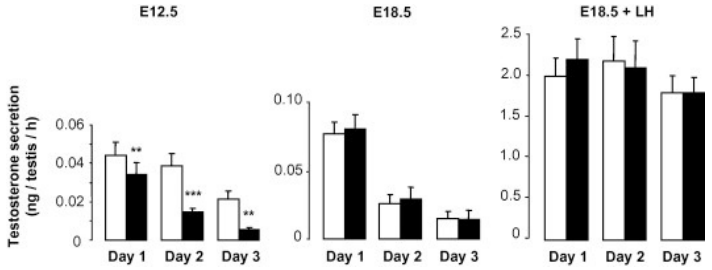
Developmental programming: gestational testosterone treatment alters fetal ovarian gene expression. Luense LJ, et al. (2011) 152(12):4974-83.

TABLE 1. microRNA differentially expressed in control *vs.* T- or T plus flutamide-treated fetal ovine ovaries

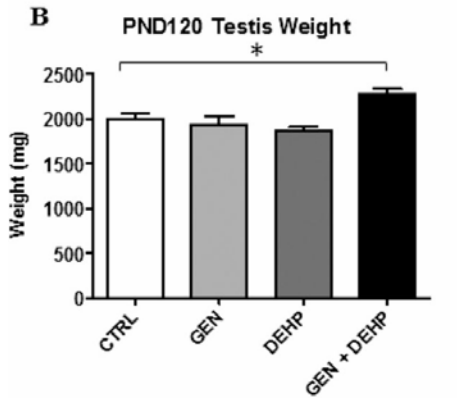
miRNA	C vs. T FC	C vs. TF FC	Functional analysis literature based[a]	Predicted targets (Targetscan)
miR-497	3.83	3.71	Type 2 diabetes rat [miR-15b/497 family (1)	PAPPA, IR, GHR, IGF2R, IRS2, IGF1R, furin
miR-29a	2.98		Diabetes/insulin signaling (1–5), FSH regulated (6)	IGF1, INSIG1, leptin
miR-192	2.19		Diabetic neuropathy (4, 7, 8)	IGF1
miR-24-2*	2.12		Diabetes (9), Insulin signaling (10), bovine ovary (11)	INSIG1, PPARa, Furin, IGFBP5, IGF2BP2
miR-15b	2.06	2.93	Type 2 diabetes rat FSH (1), regulated in ovary (6)	PAPPA, IR, GHR, IGF2R, IRS2, IGF1R, furin
miR-101	1.90		AR regulated (12)	PGRMC2, PPARa
miR-212	1.87		LH regulated in ovary (13)	
miR-451	1.85		Sex dependent in liver (14)	
miR-186	1.82		Type 1 diabetes (15)	INSM1, LEPR, IGF1, IGF1R
miR-672	1.82		Mouse ovary (16)	IGF1R
miR-7	1.67		Insulin signaling (17, 18)	IRS2, IRS1, IGF1R, PAPPA
miR-30b-5p	1.58		Estrogen regulated (19)	LEPR, IGF2R, INSIG2, IRS1, IRS2, LDLR, IGF1R, IGF1
miR-22*	1.52		Fetal ovine gonad (20), androgen regulated (21), represses ESR1 (22)	ESR1, GHRHR, PTGS1, IGF2BP1, furin
miR-378	−4.13		Lipid/fatty acid metabolism (23), regulates estrogen production (24)	
miR-760	−2.80		Estrogen regulated (25)	IR
miR-10a	−2.76	−3.36	Androgen regulated (26), ovary (27)	PPARa
miR-182	−2.71		Insulin signaling (10), ovary (27)	INSIG1, IGF2BP1
miR-129*	−2.22		Diabetes (28)	IGF1, GHR, ESR1, INSIG2
miR-132	−1.82		LH regulated in ovary (13)	
miR-223	−1.69		Diabetes (9)	IGF1R
miR-363		4.50	Sex differentiation (29)	PTGER4, IRS2, INSIG1
miR-20b		3.24	Diabetes (9), ESR1 regulated (30)	PPARa, LDLR, IGF2BP1, ADIPOR2, PPARd
miR-330		1.71	Fetal programming (31)	IGF2BP1
miR-29c		1.64	Diabetic neuropathy (32), diabetes (2)	IGF1, INSIG1, leptin
miR-29b		1.62	Diabetes (2, 9), sex dependent in liver (14)	IGF1, INSIG1, leptin
miR-191*		1.56	Diabetes (9)	
miR-101a*		1.53	AR regulated (12)	PGRMC2, PPARa
miR-105		1.52	Human ovary (27)	
miR-133a		−1.85	Insulin signaling (33, 34)	IR, IGF1R

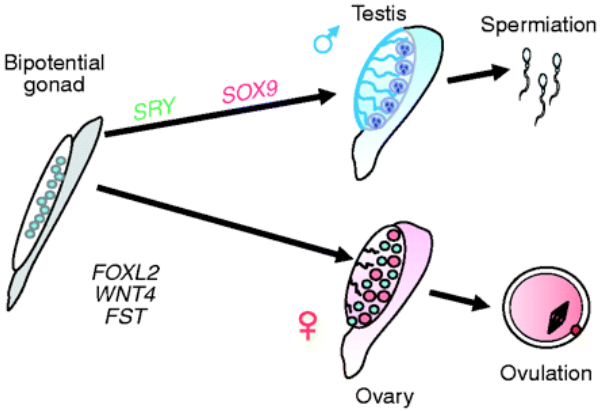
C, Control; ER, estrogen receptor; FC, Fold change; TF, testosterone + flutamide.
References for table are included in the Supplemental Online Materials.

Genistein impairs early testosterone production in fetal mouse testis via estrogen receptor alpha.
Lehraiki A, et al. Toxicol In Vitro. (2011) 25(8):1542-7.



Disruption of rat testis development following combined in utero exposure to the phytoestrogen genistein and antiandrogenic plasticizer di-(2-ethylhexyl) phthalate.
Jones S, Boisvert A, Duong TB, Francois S, Thrane P, Culty M.
Biol Reprod. 2014 Sep;91(3):64.





"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