Female Reproductive Tract Development & Function

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Required Reading


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FEMALE REPRODUCTIVE TRACT

Fetal and Postnatal Female Tract Development
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Nomenclature
AKT  Protein kinase B
AMH  Anti-Müllerian hormone
AR   Androgen receptor
BMP  Bone morphogenetic protein
DES  Diethylstilbestrol
FGF  Fibroblast growth factor
GE   Glandular epithelium
GW   Gestational week
LE   Luminal epithelium
MD   Müllerian duct
MODY5 Maturity-onset diabetes of the young type 5
MRKH Mayer-Rokitansky-Küster-Hauser
PI3K Phosphatidylinositol-4,5-bisphosphate 3-kinase
WD   Wolffian duct

Introduction
The female reproductive tract organs form and differentiate during fetal and postnatal stages of development (Kobayashi and Behringer, 2003) (Fig. 1). The oviducts, uterus, cervix and upper portion of the vagina are derived from the paramesonephric ducts or

Fig. 1  Schematic illustration of the female reproductive tract in human and mouse. The female reproductive tracts of human (A) and mouse (B) consist of the ovary, Fallopian tube (oviduct in mouse), uterus, cervix and vagina. A bursal membrane surrounds the ovary in the mouse by not in human.
Müllerian ducts (MD) and adjacent mesenchyme that form within the fetal kidneys, the mesonephroi (Fig. 2). The MD is an epithelial tube with adjacent mesenchyme cells. The MDs are located adjacent and lateral to the mesonephric ducts or Wolffian ducts (WD) that also reside within the mesonephroi (Fig. 2). The WDs can give rise to male reproductive tract organs, including the seminal vesicles, vasa deferentia and epididymides. In male fetuses, the MDs are eliminated by the action of anti-Müllerian hormone (AMH), whereas the WDs differentiate in response to androgens. However, during female fetal development, the ovaries do not secrete AMH or androgens. Thus, in females the MDs differentiate, whereas the WDs regress (Fig. 2).

Once the MDs have formed, they will become regionalized into the oviduct, uterus, cervix and vagina. Depending on the species, the posterior region of the MDs will fuse to various extents, leading to different uterine morphologies (Kobayashi and Behringer, 2003). At birth, the uterus is composed of a lumen lined by a single layer of epithelial cells with a surrounding undifferentiated mesenchyme. Subsequently, the mesenchyme differentiates into an inner stromal compartment surrounded by inner circular and outer longitudinal smooth muscle layers, the myometrium. The adult uterus contains endometrial glands that produce factors required for uterine receptivity, embryo implantation, embryo survival and development (Gray et al., 2001). Endometrial glands from the luminal epithelium will invade into the uterine stroma in a process called adenogenesis (Spencer et al., 2005). Thus, the development of the fetal and postnatal female reproductive tract organs is complex and essential for the fertility of an individual female.

Formation of the Müllerian Duct

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a funnel-like structure. The invagination process is possibly driven by Wnt4 expressing cells in the mesonephric mesenchyme because Wnt4 mutant mice have Lhx1-specified cells but do not form the MD (Fig. 3).

As the specified cells move posteriorly, MD formation enters the elongation phase. The posterior tip cells of the MD, which have shown to be Wnt4 positive, will invade through the common basal lamina between the mesonephric epithelium and the lateral side of the WD (Prunskaitė-Hyyrylainen et al., 2016). Following the tip cells, the rest of the MD cells will move along the WD in an anterior to posterior manner. When the MD elongates past the middle of the WD (posterior to the gonad), the MD will elongate dorsomedially across the WD, but will remain in close contact with it. After reaching the medial side of the WD, the MD resumes its anterior-posterior elongation along the medial side of the WD. At the end of the elongation phase, the MD tip reaches the urogenital sinus and fuses (Fig. 3).

Although the cellular mechanisms of MD formation are not fully understood, recent studies have shown that both cell proliferation and migration are involved in MD elongation. In both chicken and mouse, the MD cells are proliferative and migrate along the entire anterior to posterior length. In addition, cell migration may play an important role during the elongation process. The tips cells extend prominent processes, suggesting that the tips cells are actively investigating their environment for MD elongation (Huang et al., 2014). PI3K/AKT activity has been shown to be required for MD cell migration and elongation in rat embryos (Mullen and Behringer, 2014). It is also possible that cell shape changes may also contribute to MD elongation.

The relatively rapid elongation of the MD during mouse development has led to speculation that cells may be contributed from neighboring tissues, such as the adjacent WD, the mesonephric mesenchyme or the mesonephric epithelium. However, recent studies show that cell contributions from neighboring tissues are not found in both chicken and mouse (Mullen and Behringer, 2014). Therefore, cell recruitment is not a major cellular mechanism that contributes to the elongation of the MD.

The MD elongates in a unique manner, i.e., tube-dependent tubulogenesis. In 1937, Grünwald found that the MD elongation is dependent on the presence of the WD (Grünwald, 1937). It was found that the Wnt9b mutant mouse lacked MD formation. Wnt9b

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**Fig. 3** Müllerian duct formation. (A) MD (red) formation occurs in three phases: initiation, invagination, and elongation. Initiation phase: MD progenitor cells in the mesonephric epithelium (yellow) are specified and begin to express LHX1. Invagination phase: in response to WNT4 signaling from the mesonephric mesenchyme, LHX1 positive (LHX1⁺) MD progenitor cells invaginate posteriorly into the mesonephros towards the WD (blue). Elongation phase: the tip of the MD contacts the WD and elongates caudally in close proximity to the WD requiring WNT9B signaling from the WD. The formation of the MD begins at around E11.5 in the mouse (B) The MD invaginates from the anterior mesonephric epithelium and extends posteriorly guided by the WD. During elongation, mesenchymal cells separate the WD and MD anterior to growing tip. However, at the MD tip, the MD and WD are in physical contact. At around E13.0 the MD crosses over the WD to become located medially. Elongation is complete by E13.5 with the MD reaching the urogenital sinus. E, embryonic day in mouse; D, dorsal; MD: Müllerian duct; P, posterior (caudal); V, ventral; WD, Wolffian duct. Adapted from Kobayashi, A., and Behringer, R.R. (2003). Developmental Genetics of the Female Reproductive Tract in Mammals. *Nature Reviews Genetics* 4, 969–980.
Wolffian Duct Regression

In amniotes, the initial formation of the reproductive tracts of genetic male and female embryos is identical with two pairs of simple epithelial tubes, the WD and MD, surrounded by mesenchymal cells. However for proper sexual differentiation, only one of these pairs of tubes will differentiate while the other is eliminated. As discussed above, this is regulated by the presence or absence of fetal gonadal hormones. The fetal male gonad secretes androgens, causing the WD to differentiate into the mature male reproductive tract organs. In females, it is necessary to eliminate or regress the WD. The absence of androgens in female fetuses leads to the elimination or regression of the WD. In female rodents, without androgens, degeneration of the WD is observed beginning midway between the gonads and point of contact with the urogenital sinus and proceeds cranial (head) to caudal (tail). Lower, caudal segments of the WD remain and fuse with the MD and urogenital sinus to form the lower portion of the vagina (Fig. 2). The ability of androgens (from the testis) to block WD regression in females has been shown in tammar wallaby. Grafting of a testis in female tammar pouch young resulted in a block of WD regression and differentiation of the WD. Similarly, mutations in the androgen receptor (AR) gene in humans and rodents result in intersex phenotypes and genetically male (XY) individuals lack WD-derived tissues. Further, observations in rodent models indicate androgen signaling in the WD mesenchyme may allow cell survival and differentiation of the adjacent WD epithelial tube and lack of androgen signaling in the mesenchyme results in cell death, thus facilitating WD regression (Shaw and Renfree, 2014).

Early studies of female reproductive tract differentiation during WD regression were limited to two-dimensional analyses in animal models. Recently, light-sheet microscopy has made it possible to quickly generate high-resolution three-dimensional images of fluorescently-labeled fetal organs. Light-sheet microscopy was used to visualize the developing female reproductive tract at GW 10.5, 11.5 and 13 weeks. The human embryos were immuno-fluorescently stained with PAX2 antibody (which binds WD and MD epithelial cells) and imaged. At GW 10.5 fusion of the MD to form the uterovaginal canal was observed in female embryos. The WD was still intact however there was initial regression of the mesonephric tubules. At GW 11.5 WD regression was apparent and the MDs had grown in length. By GW 13, the WD was fragmented and completely regressed distally (Belle et al., 2017). WD regression has long been considered a passive process, where lack of androgens in female fetuses fails to support the differentiation of the WD. However, several recent studies suggest that WD regression requires active signaling to promote cell death of the epithelium. MSX2, a transcription factor expressed in the WD epithelium, and orphan nuclear receptor chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) found in the WD mesenchyme have both been identified as potential mediators of WD regression in female reproductive tract differentiation. Down-regulation of Msx2 expression in the WD epithelium either in response to diethylstilbestrol (DES) exposure or in a Msx2 mutant mouse model in females results in persistent WD remnants dorsal to the vagina and reduced apoptosis (programmed cell death) in the WD epithelium (Yin et al., 2006). COUP-TFII, a mesenchyme specific transcriptional regulator, is required for WD regression during differentiation of the female reproductive tract in the mouse. Loss of the Coup-tfii gene in the WD mesenchyme results in retention of the WD independent of androgen signaling. In fetal males, androgens secreted from the testis presumable antagonize COUP-TFII function and prevent WD regression (Zhao et al., 2017).

Oviduct Development

The oviduct, or Fallopian tube in women, is a paired organ that is essential for fertility. In mature animals, the oviduct is the conduit for oocyte or embryo transfer to the uterus and is the site of fertilization. The ovulated oocyte enters the oviduct through the infundibulum, which is the most anterior region of the oviduct, and travels through the ampulla, which contains numerous longitudinal epithelial folds and abundant cilia to aid in oocyte transport. Upon fertilization, the zygote will travel through the isthmus region of the oviduct. The isthmus has fewer epithelial folds and cilia than the ampulla, but thicker smooth muscle layers. To leave the oviduct, the zygote must travel through the uterotubal junction to enter into the uterine horn/body. This junction is an ovarian hormone-controlled valve that controls the movement of spermatozoa/zygotes between the oviduct and uterus.

Defects in oviduct formation or the formation of occlusions can cause infertility. This may be overcome via superovulation, in vitro fertilization and embryo transfer into the uterus, but these are costly methods with demanding hormonal regimens and relatively low success rates. Tubal occlusions are caused most frequently by infections, but structural abnormalities arising during perinatal development can have the same result. Very little is known of how and what regulates oviduct development.

The study of Fallopian tube development in women is limited, requiring the use of other animal models including both mammals and birds. However, there are some striking differences in the gross morphology and histology of various species. Oviduct coiling is observed in some species (e.g., mice), but not others (e.g., women, sheep, chickens). A bursa surrounds the oviduct and
ovary (e.g., mice) in certain species, which is absent in others (e.g., women). Oviduct epithelial folding, particularly in the ampulla region can be minimal (e.g., mice) or very extensive (e.g., women, sheep). Despite these differences, the oviducts function in a very similar manner.

Mammalian female reproductive organs, including the oviduct, uterus, cervix, and anterior vagina, are all derived along the anterior-posterior axis of the MD during embryonic development. The most anterior aspect of the MD gives rise to the oviduct. The developing MD forms a shepherd’s crook shape around the ovary. The end of the curved portion of the “crook,” posterior to the ovary, is referred to as the *flexura medialis* is proposed to define the border between the region of the MD that will become the oviduct and that of the uterus (Agduhr, 1927).

The TGFβ, WNT and mTOR signaling pathways have been identified as potential regulators of oviduct development. TGFβ may play a key role in controlling cell proliferation, differentiation and apoptosis during oviduct development (Conery et al., 2004; Elliott and Blobe, 2005; Li et al., 2011; Rodriguez et al., 2016). Regulation of TGFβ signaling during oviduct development likely involves extracellular matrix proteins, including matrix metalloproteinases and tissue inhibitors of metalloproteinases (e.g., MMP-2, -9, TIMP-2) which act via enzymatic cleavage and activation or repression of signal transducers (Hu et al., 2004; Imai et al., 1997; Lesniak-Walentyn and Hrabia, 2016).

In addition to TGFβ signaling, the WNT pathway appears to play a direct role in oviduct development. Oviduct development and formation is regulated tightly by correct expression of canonical WNT signaling pathway members in both the epithelia (Wnt7a) and mesenchyme (Wnt4, Wnt5a, Ctnnb1). WNT signaling during oviduct development is associated with the appearance of coiling and initial formation of the anterior region of the MD, suggesting that this pathway plays a key role in anterior-posterior oviduct extension and differentiation.

mTOR signaling appears to play a key role in smooth muscle differentiation and function in the oviduct. mTOR signaling is downstream of PI3K/AKT signaling and regulates cell growth and proliferation in response to growth factors and nutrients and is negatively regulated by a heterodimeric complex of TSC1 and TSC2. In the mouse, conditional deletion of Tsc1 in both the MD mesenchyme and in all MD cell types results in infertility related to oviduct hyperplasia and formation of occlusions and hydrosalpinx in the ampulla (Daikoku et al., 2013; Tanaka et al., 2012). Conditional deletion of Tsc2 in the MD mesenchyme resulted in infertility that may be related to the formation of oviductal blockages, but oviductal histology was not reported. The uterine phenotype was characterized by the presence of myometrial hyperplasia (Kaneko-Tarui et al., 2014). It is possible that this also occurred in the oviductal smooth muscle layers, which would adversely affect oocyte/zygote transport, resulting in a phenotype similar to oviduct blockage.

**Uterine Development**

In eutherian mammals, the majority of the development and differentiation of the female reproductive tract is completed by birth. However, the uterus is not fully developed or differentiated by birth and the histoarchitecture of this organ is established postnatally. Postnatal radial patterning morphogenesis establishes two functional compartments, the endometrium and the myometrium, surrounded by the perimetrium. The endometrium consists of two epithelial cell types, luminal epithelium (LE) and glandular epithelium (GE), and two stratified stromal compartments including a densely organized stromal zone, blood vessels and immune cells. The myometrium includes the smooth muscle layers of the uterine wall, an inner circular layer and an outer longitudinal layer (Gray et al., 2001). Morphogenic events common to morphogenesis of the uterus include: (1) organization and stratification of the endometrial stroma, (2) differentiation and growth of the myometrium and (3) coordinated development of the endometrial glands. The LE will invaginate into the stroma to generate the GE (endometrial or uterine glands), resulting in an extensive network of glands that extends towards the myometrium (Gray et al., 2001; Spencer et al., 2005).

Humans have a simplex uterus that consists of a single uterine body. The endometrium is lined by a LE that contains glands that radiate from the surface to the endometrial-myometrial interface. The endometrium is divided into two functional layers, the upper *stratum functionalis* (containing glands and is surrounded by loose stroma) and the lower *stratum basalis* (containing branched glands and dense stroma). During menses, the endometrial *stratum functionalis* is shed. The *stratum basalis* includes a zone that contains loose stroma and endometrial glands and another zone where endometrial glands terminate and endometrial progenitor and stem cells are thought to reside (Spencer et al., 2005).

During pregnancy, uterine glands secrete histotroph that is essential for endometrial receptivity of the embryo, conceptus survival, implantation, development and growth in sheep, cattle, pigs, horses and rodents (Gray et al., 2001). Histotroph is present in the uterine luminal fluid and is a complex, undefined mixture of ions, amino acids, carbohydrates, proteins, lipids, and other substances that are selectively transported into the uterine lumen by the epithelium, as well as specific secretory products encoded by genes and expressed in the LE and GE. Evidence shown in mouse and sheep suggests that uterine glands are required for female fertility, with defects resulting in abnormal implantation and early pregnancy loss (Filant and Spencer, 2014; Spencer et al., 2005).

Knowledge of prenatal uterine development is most complete in rodents. However, the basic biology of this process is assumed to be similar across mammalian species and the morphogenesis of the postnatal uterus is dependent on the maturity of the uterus at birth (e.g., gestational length) and perhaps the interval between birth and puberty (Gray et al., 2001). For example, in rodents, at birth, the uterus has not yet differentiated into endometrial stroma and myometrium, whereas in certain domestic animals and humans, the endometrial stroma and myometrium are present at birth (Spencer et al., 2005).
Uterine adenogenesis is the process of endometrial gland formation from the LE. It includes epithelial budding, extension and penetration into the stroma with coiling and branching. In humans, rodents and livestock, this process is completed postnatally (Gray et al., 2001). In mice, at birth, the uterus is comprised of a simple epithelium surrounded by undifferentiated mesenchyme with no endometrial glands. At Postnatal Day (P) 5, three mesenchymal layers are radially oriented and segregated into the endometrial stroma and inner circular and prospective outer longitudinal myometrial layers and the formation of epithelial buds by epithelial invaginations. Between P9 to P15, simple tubular glands develop that are not tightly coiled or branched (Fig. 4). By P10, the outer longitudinal layer of the myometrium becomes organized into bundles. At P15, the adult configuration of the mouse uterus is already established and as females mature, the glands lengthen as the uterus grows (Gray et al., 2001). P21 marks the end of the postnatal stage of gland formation. Many of these studies were performed using two-dimensional histological analyses. Recently, the three-dimensional morphology and organization of adult uterine glands has been examined (Arora et al., 2016).

Knowledge of prenatal and postnatal female reproductive tract development in humans is limited. By GW 12, the uterine corpus and cervix is has formed and the LE invaginates to give rise to epithelial buds. By GW 20—22, the myometrium is well defined but endometrial gland development has not progressed beyond epithelial buds. At birth, the uterine histoarchitecture is similar to that of an adult, but less developed. From birth to the onset of puberty, the glands develop slowly. A female at 6 years of age will have endometrial glands that will extend from one-third to one-half of the distance of the stroma to the myometrium. The mature uterine histoarchitecture is observed at puberty with glands extending to the inner circular layer of the myometrium. Endometrial gland formation in humans (fetus and neonate) involves differentiation of the GE from the LE, followed by radial development of the tubular glands through the endometrial stroma extending to the myometrium.

Multiple studies have established that prenatal urogenital tract development in female mammals is an ovary (hormonal) independent process (Gray et al., 2001). These studies have shown that uterine development and endometrial adenogenesis can proceed in the absence of the ovary for varying periods of time during early postnatal development. In rats, circulating estrogens increase between P9 and P11 in association with gland remodeling, but early postnatal uterine development and adenogenesis are both ovary- and adrenal-independent (Gray et al., 2001; Spencer et al., 2005). In mice, the introduction of hormones during a critical postnatal window causes a delay in gland formation or the loss of glands (Filant and Spencer, 2014).

Gland morphogenesis is highly complex and mediated by diverse mechanisms (hormonal, cellular and molecular). Despite being studied for decades, very little details are available, compared with other epitheliummesenchymal organs. The communication between the epithelium and stroma appears to be mediated by Wnt and Hex genes, intrinsic growth factors systems and the extracellular matrix (Spencer et al., 2005). In recent years, knockout (Hoxa10, Hoxa11, Lef1, Wnt4, Wnt5a) and conditional knockout (Ctmb1, Foxa2, Wnt7a) mutants mouse models have been used to identify genes involved in uterine gland development (Filant and Spencer, 2014). Although some cellular events and molecular pathways have been identified through gene expression and mouse models, there is still a significant gap in knowledge of how glands develop and their morphogenesis.
Malformations of the Uterus

Uterine malformations can be classified into three main groups, (1) formation defects, (2) fusion defects, and (3) septal absorption defects (Jacquinet et al., 2016). The actual prevalence of uterine malformations has been difficult to evaluate because some defects may be considered normal variants of uterine anatomy, for example, arcuate uterus. Chan et al. (2011) reported a 5.5% prevalence of uterine malformations in an unselected population. 8.0% in infertile women, 13.3% in women with a history of miscarriage, and 24.5% in patients with a history of miscarriage and infertility. This lead to the conclusion that women who are infertile and/or have had spontaneous abortions are more likely to have a uterine malformation (Chan et al., 2011).

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome or Müllerian agenesis is characterized by the absence of the uterus, cervix and upper portion of the vagina in a 46,XX female; it is the second most common cause of primary amenorrhea (Fontana et al., 2017). It is divided into two main subtypes: (1) MRKH type 1 in which only the female reproductive tract is affected and (2) MRKH type 2 which can manifest with malformations of other organs systems such as: renal, skeletal (spine and limb) and less frequently auditory and heart defects. Even though MRKH is most severe in the spectrum of uterine defects, its incidence is relatively low, with only 1 in every 4500–5000 newborn females being affected. However, the association of MRKH type 2 with other organ system defects suggests that abnormal MD development involves the disruption of developmental pathways important for structures derived from the intermediate mesoderm of the embryo (Fontana et al., 2017).

The cause of uterine malformations is thought to be multifactorial and in the case of MRKH, the mode of transmission is thought to be autosomal dominant with incomplete penetrance and variable expressivity. First-degree relatives of patients presenting with a uterine anomaly are said to have a 1%–5% recurrence risk. There are reports of familial cases suggesting a predisposing genetic background. Conversely, there have been studies of monozygotic twins that show discordant phenotypes: MRKH vs. normal uterine anatomy, suggesting nongenetic mechanisms that point towards epigenetic and/or environmental factors (Jacquinet et al., 2016).

Relatively little is known about the genetic pathways that regulate the development of the female reproductive tract and lead to uterine malformations in humans. However mutation or deletion of certain genes have been found to be associated with reproductive tract defects in humans including: EMT2, HNF1β, LHX1, PBX1, WNT4, WNT7A, and WNT9B. In patients with MRKH syndrome, a rare pathogenic deletion in region 17q12 containing LHX1, as well as HNF1β, has been found to be statistically significant compared to a control population (Jacquinet et al., 2016). Mutations in HNF1β are the cause of a form of maturity-onset diabetes of the young type 5 (MODY5). MODY5 clinically manifests with diabetes, renal disease and genital malformations (MRKH syndrome). Mutations in HNF1β have only been found in patients with both renal and uterine malformations, and are rare in cases of isolated uterine defects (Fontana et al., 2017). Recently, in a case control study of 517 Chinese women with incomplete Müllerian fusion, a novel nonsense mutation in the EMT2 gene (p.E142X) was detected in one patient (0.19%). The authors report functional studies in cultured cells, suggesting a dominant negative effect of the mutation (Jacquinet et al., 2016). Even though this mutation is uncommon in the studied population, EMT2 is the first gene to be identified suggestive of a cause for an isolated uterine malformation (Jacquinet et al., 2016). An association study performed in a Chinese Han female population with MRKH found two susceptibility SNPs (single nucleotide polymorphism) in WNT9B and PBX1 associated with MRKH syndrome risk (Ma et al., 2015). In humans, WNT4 was the first gene to be associated with uterine defects accompanied by hyperandrogeinism (Fontana et al., 2017). WNT4 mutations are more commonly associated to an MRKH-like syndrome because of the concomitant virilization. WNT7A mutations have been linked to Al-Awadi/Raas-Rothschild and Fuhrmann syndromes which are characterized by skeletal dysplasia, hypoplastic pelvis and females may present with an absent uterus (Jacquinet et al., 2016).

Prenatal exposure of fetuses to endocrine disruptors can affect the development of the uterus in mice and humans. Diethylstilbestrol (DES) is a synthetic estrogen that was used from 1938 to 1971 to prevent miscarriages in millions of pregnant women. However, it was later discovered that prenatal and perinatal exposure to DES disturbs the development of the reproductive tract in both humans (males and females) and mice (Spencer et al., 2005). Prenatal exposure of human fetuses to DES alters the organizational program of the female reproductive tract tissues and disrupts the normal expression or function of genes in an epigenetic manner. These induced abnormalities have set the stage for infertility, cervicovaginal cancer and other complications in exposed females and their offspring in a transgenerational manner.

References


Spring 2022 – Systems Biology of Reproduction
Lecture Outline – Female Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesdays & Thursdays
February 8, 2022
Week 5

Female Reproductive Tract Development & Function

- Female Urogenital Tract Organogenesis
- Development of Vagina/Cervix
- Mesenchymal-Epithelial Interactions
- Role of Hormones
  a. Organ Culture
  b. Fetal Castration
  c. Estrogen Receptor Knockout
- Molecular Control of Wnt and HOX Genes
- DES Story
- Mammary Biology and Disease
  a. Cell Types
  b. Structure
  c. Gland Development
d. Disease

Required Reading

Spring 2022 – Systems Biology of Reproduction
Discussion Outline – Female Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesdays & Thursdays
February 10, 2022
Week 5

Female Reproductive Tract Development & Function

Primary Papers:

Discussion

Student 10: Contemporary Paper-Ref #1 above
- What are the functions of the oviduct?
- What methods were used?
- Are secretions important?

Student 11: Contemporary Paper-Ref #2 above
- What are HOX genes and role in development?
- What are endocrine disrupters and mechanisms?
- How do they alter female reproductive tract?

Student 12: Contemporary Paper-Ref #3 above
- What are mesenchymal-epithelial interactions?
- What transcription factors were involved?
- What conserved processes are observed in female reproductive tract development?
A schematic diagram depicting Müllerian duct formation. (a) In the first phase, initiation, *Lim1* expressing cells (orange) in the coelomic epithelium (CE) are specified to Müllerian duct fate. (b) In the second phase, invagination, Wnt4 functions downstream of *Lim1* possibly to induce Müllerian duct invagination to reach the Wolffian duct (WD). (c) In the third phase, elongation, the leading tip cells (pink) proliferate and deposit cells to form the Müllerian duct (orange) until it reaches the cloaca (more specifically the urogenital sinus). WD serves as a guide and secretes WNT9b to promote Müllerian duct elongation. *Pax2* is required for elongation and Müllerian duct maintenance. Genes specifically expressed in the MD (red), WD (blue) or both (purple) are shown.
Diagram of developing human female internal genitalia in the indifferent, bisexual stage (~54 days of gestation, Carnegie Stage 22). The Mullerian derivatives are red and Wolffian derivatives are purple. Note the changing anatomical relationships between the Mullerian and Wolffian ducts. From Robboy et al. (2017) with permission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Development of the human female reproductive tract.

(A) Formation of the Wolffian (mesonephric) duct, which by 24 days has grown caudally to join the cloaca. At 5–6 weeks the paramesonephric (Mullerian) ducts appear as invaginations of the coelomic epithelium. At 7 weeks (B) the MDs have grown caudally towards the urogenital sinus. Subsequently (C, 8 weeks), the opening of the MDs into the coelomic cavity is fimbriated, and with further growth the MDs reach the UGS, while the Wolffian ducts degenerate. Modified from (Park, 2016) with permission.

Signals of vaginal mesenchymal factors are transduced to downstream transcription factors, and the transcription factors dose-dependently activate enhancers of ΔNp63 in MDE. Upon differentiation of VgE, ΔNp63 itself maintains the transcriptional activity of ΔNp63 locus in VgE fate independently of vaginal mesenchymal factors. DES-ESR1 activity within MDE causes vaginal adenosis by blocking the vaginal cell fate commitment of MDE interfering the signal transduction.


Figure 13-23. The female reproductive system.
An evo-devo perspective of the female reproductive tract.
Major AT, Estermann IA, Roly ZY, Smith CA.
Biol Reprod. 2021 Sep 7:ioab166.

Oviduct
Epithelial cells of the ampullary-isthmic junction (AIJ) of bovine oviduct. (a) Scanning electron micrographs of the epithelial surface of the ampulla of bovine oviduct in late follicular phase. (b) Electron micrograph of the epon-embedded AIJ. Ciliated cells (CC), secretory cells (SC), secretory granules (SG) and cilia (Ci). Bar: 5 μm

Functional clustering of genes classified as ‘secreted’ using the DAVID bioinformatic tool using data from normal Fallopian tube reported in Tone et al. (2008)

Venn diagram showing overlapping and non-overlapping gene expression on human, bovine and porcine oviduct
Molecules involved in sperm-oviduct adhesion and release.
Talevi R, Gualtieri R.
Theriogenology. 2010 Apr 1;73(6):796-801.

Schematic drawing of molecules involved in sperm-oviduct binding in pig and cattle. SBG, sperm binding glycoprotein [30]; Gaaccessible to galNAc, galactose-beta 1-3 N-acetylgalactosamine [30]; Gal, galactose [38]; Man, mannose [28]; ANXA2, annexin 2 [33]; DOH [29]; AQNI [28]; ANXA 1,2,4,5, annexins 1, 2, 4, 5 [34]; Fuc, fucose [34]; BSP30K and BSPA3, bovine seminal plasma protein 30K and A3 [47]; PDC-109, protein with N-terminus aspartic acid and carboxy terminus cystine, having 109 amino acids [45].

Uterus, Vagina and Cervix

Early Müllerian duct growth and fusion to form the midline uterovaginal canal. Length of the uterovaginal canal increases with developmental age. In (A) the extent of MD caudal extension is depicted at Carnegie Stages 20–23 (50–56 days). (B-D) depict fusion of the right and left MDs to form the midline uterovaginal canal, formation of the septum and its subsequent disappearance. From Robboy et al. (2017) with permission.
The Role of Hox Genes in Female Reproductive Tract Development, Adult Function, and Fertility.
Du H, Taylor HS.

Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract.
Miller C, Sassoon DA.

Tissue recombinants composed of neonatal mouse vaginal mesenchyme plus 13 week human fetal uterine tube epithelium (mVgM+hTubE) grown for 4 weeks in DES-treated hosts and immunostained for various vaginal epithelial markers as indicated. Human uterine tube (A, D, G, J) and vagina (B, E, H, K) at 16–18 weeks of gestation serve as controls. Note induction of KRT6, TP63 and RUNX1 and down regulation of AR in epithelium of the mVgM+hTubE recombinants, indicative of an effect of mouse vaginal mesenchyme on expression of differentiation markers in human tubal epithelium (+) and (-) indicate epithelial marker expression.

Endocrine
In utero diethylstilbestrol (DES) exposure alters Hox gene expression in the developing Müllerian system

KAREN BLOCK, ANDREW KARANJA, PETER SCARELLI, AND RICH S. TAYLOR

OBJECTIVE: Diethylstilbestrol (DES) was widely used to treat pregnant women through DES. The reproductive systems of female offspring exposed to DES in utero are characterized by anatomical abnormalities. Here we show that DES exposure in utero results in changes in expression patterns of Hox genes that are involved in the control of Müllerian development.

RESULTS: The expression of Hox genes is essential for the normal development of the Müllerian system. In DES-exposed offspring, specific Hox genes were overexpressed, indicating a potential role in the etiology of Mullerian defects.

CONCLUSION: The altered expression of Hox genes in DES-exposed offspring may contribute to the development of Mullerian abnormalities, providing a potential molecular basis for understanding the pathogenesis of DES-related disorders.

REFERENCES: Further research is required to elucidate the role of Hox genes in Mullerian development and the mechanisms underlying the effects of DES exposure on Hox gene expression.
Wnt genes and endocrine disruption of the female reproductive tract: a genetic approach

The emergence of molecular gynecology: homeobox and Wnt genes in the female reproductive tract.

Table 1. Comparison between different DES mouse models.

<table>
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<tr>
<th>Treatment</th>
<th>Prenatal</th>
<th>Neonatal</th>
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<tr>
<td>Treatment</td>
<td>E3S-18.5</td>
<td>PMO-1.5</td>
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<tr>
<td>DES dosage</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ovarian defects (testis)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Uterine atrophy</td>
<td>Less common</td>
<td>Yes</td>
</tr>
<tr>
<td>Squamous metaplasia of uterine epithelium</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Smooth muscle disorganization</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Abnormal uterine openings</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Gravid vagina</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Vaginal atresia</td>
<td>Less common</td>
<td>Yes</td>
</tr>
<tr>
<td>Persistent vaginal epithelial stratification</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>WD resistance</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ovarian tumours</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reference to human exposure</td>
<td>40 weeks</td>
<td>16 weeks</td>
</tr>
</tbody>
</table>

Other variants of prenatal exposure include treatment from $\text{ES}5-18.5$ at a dose of 250 μg/kg pregnant mother or $\text{ES}5-18.5$ at a dose of 45 μg/kg/day.
Unraveling the Dynamics of the Human Vaginal Microbiome
Nunn KL, Forney LJ.

The vaginal microbiota, host defence and reproductive physiology
Smith SB, Ravel J

Abstract
The interaction between the human host and the vaginal microbiota is highly dynamic. Major changes in the vaginal physiology and microbiota over a woman's lifetime are largely shaped by transitional periods such as puberty, menopause and pregnancy, while daily fluctuations in microbial composition observed through culture-independent studies are more likely to be the results of daily life activities and behaviours. The vaginal microbiota of reproductive-aged women is largely made up of at least five different community state types. Four of these community state types are dominated by lactic-acid producing Lactobacillus spp., while the fifth is commonly composed of anaerobes and strict anaerobes and is sometimes associated with vaginal symptoms. The production of lactic acid has been associated with contributing to the overall health of the vagina due to its direct and indirect effects on pathogens and host defence. Some species associated with non-Lactobacillus vaginal microbiota may trigger immune responses as well as degrade the host mucosa, processes that ultimately increase susceptibility to infections and contribute to negative reproductive outcomes such as infertility and preterm birth. Further studies are needed to better understand the functional underpinnings of how the vaginal microbiota affect host physiology but also how host physiology affects the vaginal microbiota. Understanding this fine-tuned interaction is key to maintaining women's reproductive health.

Direct on-swab metabolic profiling of vaginal microbiome host interactions during pregnancy and preterm birth
Insights into the role of vaginal microbiome in women’s health.

Microphysiologic systems in female reproductive biology.
Young AN, Moyle-Heyrman G, Kim JJ, Bulteje J.

Organoids of the female reproductive tract.

Organ-on-chip of the cervical epithelial layer: A platform to study normal and pathological cellular remodeling of the cervix.
Tantengco OAG, Richardson LS, Medina PMB, Han A, Menon R.

New Therapeutics in Endometriosis: A Review of Hormonal, Non-Hormonal, and Non-Coding RNA Treatments
Geraldine Brichant, Ines Laraki, Laurie Henry, Carine Munaut, Michelle Nisolle


Mammary Biology & Disease
Fig. 1. Stages of human mammary gland development. The human mammary gland is specialized at embryonic day 15. The mammary epithelium invades the fat capsule and forms a small branching duct network. After birth, the epithelium grows in concert with the mammary ducts, which do not begin to be formed until the 12th week. After birth, the mammary gland is specialized at puberty around 15 weeks of age. The onset of puberty, if no sex could be detected, is based on the onset of menarche, which is generally around 15 years of age. At this stage, the size of the mammary ducts increases rapidly, and the cells in the milk glands undergo differentiation to form lactating cells. The ductal structures are not fully developed until after prepuberty, and the ductal structures are not fully developed until after puberty. The mammary gland is further specialized at pregnancy around 17 weeks of age. At this stage, the mammary gland begins to differentiate into a structure similar to the adult mammary gland. The ductal structures are not fully developed until after pregnancy, and the mammary gland is fully differentiated into a structure similar to the adult mammary gland.

Fig. 2. Minimal structural unit of the mammary alveolus. The mammary ductal network is elaborated from a simpler layered structure. The inner layer consists of a heterogeneous population of luminal epithelial cells surrounding a central lumen, whereas the outer layer contains myoepithelial cells. This multilayered structure is embedded in the stroma, consisting of a variety of mesenchymal cells and a fibrous extracellular matrix. The precise spatial relationship of epithelial and myoepithelial cells is not constant and changes as a function of hormonal and reproductive status, and some epithelial cells contact the basement membrane.
In vivo development of mammary rudiments

<table>
<thead>
<tr>
<th>Days of development</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Embryos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male Embryos</td>
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</tbody>
</table>

Time of androgenic response

Figure 3. Sexually dimorphic development of female and male mouse mammary glands. The appearance of the mammary placodes and the formation of the buds is identical in males and females. Because of androgens produced by the fetal testes, beginning on embryonic day (E)13, the male mammary gland regresses and is mostly eliminated by E16. The window of sensitivity to androgens is the period E13 to E16, after which androgens cannot illicit mammary gland destruction in female mice. (Adapted from Kuroda et al. 1997, with permission.)

Figure 3. Genes expressed during development of the embryonic mouse mammary gland. Note that genes are expressed in a tissue-specific, pattern, with many genes expressed exclusively in epithelium or mesenchyme E11.5 to E13.5. Embryonic days 11.5 to 15.5. (Adapted from Hynes et al. 2001, with permission.)

(a) Branching
- Direction of elongation
- Side branching

Fig. 3. Alternative methods of branch initiation. (a) Primary branching. Terminal end buds (TEBs) consist of a leading layer of highly proliferative cap cells that differentiate into the luminal epithelial and myoepithelial cells branching of TEBs involves bifurcation for separation of the direction of elongation. (b) Side branching. Quiescent ductal structures might initiate side branching, possibly through transient epithelium to mesenchyme transition. These processes can also be distinguished in that elongation of the primary branch and side branch involve different metalloproteins.
Figure 1. Regulation of tubulogenesis. Mammalian cell-secreted HGF/SF is an active single-chain precursor (pro-HGF/SF) that is cleaved and thus activated by extracellular proteases. Interaction with hepatic substrate proteases or extracellular matrix proteins results in HGF/SF, which binds the epidermal growth factor receptor (EGFR) on the luminal surface of the ductal epithelial cells. HGF/SF acts on a variety of different ligands such as FGFR and EGF family members and GBF to promote branching morphogenesis. Epithelial-structure ECM interaction with the surrounding vasculature results in ECM, which also require.
Fig. 1. Postnatal mammary gland development. Prior to birth, mammary glands and milk ejection form in an organ-autonomous fashion, whereas postnatal development occurs under the control of systemic mammogenic hormones. Ductal elongation during puberty occurs through extensive cell division at terminal end buds (TEBs). Specialized structures that can produce both luminal and myoepithelial cell populations, TEBs are lost at maturity and replaced by end buds. The gland becomes functionally differentiated during pregnancy, where extensive proliferation leads to development of lobuloalveoli, followed by conversion of the lobuloalveolar epithelium to a secretory phenotype during lactation. Cessation of nursing leads to milk stasis and consequent glandular involution, in which extensive epithelial apoptosis returns the gland to a state that is similar but not identical to the preganant state.
Figure 1: Schematic showing of mammary, lactiferous sinus, and mammary ridge.

(a) Mammary ridge

(b) Lactiferous sinus

Table 17-2: Some Anatomic Characteristics of the Female Reproductive Organs in Laboratory Mammals

<table>
<thead>
<tr>
<th>Species</th>
<th>Some Anatomic Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>A pouch-lined pocket with a nipple is present, and the mammary glands are well developed.</td>
</tr>
<tr>
<td>Dog</td>
<td>Mammography reveals a well-developed mammary gland with multiple lobules and ducts.</td>
</tr>
<tr>
<td>Cat</td>
<td>The mammary glands are well-developed and have a prominent nipple.</td>
</tr>
<tr>
<td>Rabbit</td>
<td>The mammary glands are well-developed and have a prominent nipple.</td>
</tr>
<tr>
<td>Mouse</td>
<td>The mammary glands are poorly developed and have a small, papilliform nipple.</td>
</tr>
<tr>
<td>Rat</td>
<td>The mammary glands are poorly developed and have a small, papilliform nipple.</td>
</tr>
</tbody>
</table>
Induction of Mammary Gland Development in Estrogen Receptor-Knockout Mice

Wayne P. Bolzschlau, Jonathan K. Lindsey, Sylvia Curtis Hewitt, James A. Clark, Page H. Myers, Ralph Cooper and Kenneth S. Kerch

Mammary glands from the estrogen receptor (ER) knockout (ERKO) mouse do not undergo ductal morphogenesis or alveolar development. Disrupted ER signaling may result in reduced estrogen-responsive gene products in the mammary gland or reduced mammaryotropic hormones that contribute to the ERKO mammary phenotype. We report that circulating PRL is reduced in the female ERKO mouse. Implantation of an age-matched, 12-gene-selected, heterologous ER reporter under the nuclear capsule of 3-week-old or 12-week-old ERKO mice increased circulating PRL and progesterone levels, and induced mammary gland development. Grafted ERKO mice also possessed hypertrophied corpora lutea demonstrating that PRL is luteotrophic in the ERKO ovary. By contrast, luteectomy at the time of parathyroid grafting prevented mammary gland development in ERKO mice despite elevated PRL levels. Hormone replacement using pellet implants demonstrated that pharmacologically doses of estradiol induced limited mammary ductal elongation, and estradiol in combination with progesterone stimulated lobuloalveolar development. PRL alone or in combination with progesterone or estradiol did not induce ERKO mammary growth. Estradiol and progesterone are required for the structural development of the ERKO mammary gland, and PRL contributes to this development by inducing ovine progesterone levels. Therefore, the manifestation of the ERKO mammary phenotype appears to be due to the lack of direct estrogen action at the mammary gland and an indirect contributory role of estrogen signalling at the hypothalamic-pituitary axis.

![Diagram of mammary gland development](image_url)

![Graphs showing hormone levels](image_url)
Figure 1. Structure of ICI compounds, estrogenic and phytoestrogenic. These compounds were selected based on their estrogenic activities and structural aromatics.

Figure 2. (A) Whole-mount analysis of mammary tissue from 4-week-old virgin (a), 10-week-old virgin (b), 4-week-pregnan (c), and lactating (d, e, f). (d, e, f) Lactating mammary glands. All pictures are taken at the same magnification. (a) Immunohistochemical staining of the mammary fat pad and ductal and alveolar development. A simple ductal tree occurs on the fat pad in the immature virgin. The fat pad is filled in mature virgin and subcutaneous sub-branching has taken place. Alveolar development occurs during pregnancy and is completed during lactation. (B) Stages of mammary development as determined in gene knockout mice are shown below the corresponding developmental stage. The arrows in disease genes indicate pathways. Although estrogen activity is reduced in postnatal mammary glands, ERα and 25BPA are still present. It is not clear whether this occurs in mice. Dose-response targets of 25BPA and C/EBP are not known. ERα: Estrogen receptor α, PR: Progesterone receptor, PRLR: Prolactin receptor. The images are deposited in the NIH/Unichem (http://unichem.nih.gov) and can be viewed under the following accession nos: mammary tissue from an immature virgin, 1386; mammary tissue from a mature virgin, 1386; mammary tissue from an immature virgin, 1386; mammary tissue from a mature virgin, 1386; mastitis tissue from a mature virgin, 1386.

Figure 3. BPA exposure affects mammary gland development and function. (A) Pregnant and lactating mice were exposed to 25BPA (1 or 100 mg/kg) from gestation day 7 (GD7) to lactation day 21 (L21). The effects of 25BPA on mammary gland development were assessed by measuring mammary gland weights, DNA content, and protein content. (B) Effect of 25BPA on mammary gland development and function. The effects of 25BPA on mammary gland development were assessed by measuring mammary gland weights, DNA content, and protein content. (C) Effect of 25BPA on mammary gland development and function. The effects of 25BPA on mammary gland development were assessed by measuring mammary gland weights, DNA content, and protein content. (D) Effect of 25BPA on mammary gland development and function. The effects of 25BPA on mammary gland development were assessed by measuring mammary gland weights, DNA content, and protein content.
Control of mammary gland development by hormones. Different stages of mammary gland development are depicted. All hormone receptors are required in the mammary epithelium (pink boxes) with the exception of the GHR that is required in the stroma (yellow box) but also signals in the epithelium (dotted pink box). Red arrows indicate when different hormones are limiting with growth hormone and glucocorticoids being required throughout mammary gland development. Dotted arrows illustrate hormonal regulation of HR expression.

VI Milk Ejection Reflex
A. Stimulation sensory endings in nipple
B. Afferent impulses via sensory nerves
C. Stimulation neurosecretory neurons in the paraventricular nucleus
D. Release of oxytocin from posterior pituitary
E. Oxytocin stimulates contraction of myoepithelial cells surrounding mammary alveoli and ducts
F. Milk forced into larger ducts under pressure
FIG. 2. Scanning electron micrograph of rat mammary gland to show the network of myoepithelial cells (each separate cell labeled M) surrounding an alveolus. The large arrow indicates overlap of two adjacent myoepithelial cells; small arrows mark the boundaries of adjacent secretory cells. (bc) Blood capillary. (From ref. 242.)

FIG. 3. Schematic summary of some neuromodulators and neurotransmitters involved in the milk-ejection reflex. Hypothalamic oxytocin-secreting neurons (OTN) are thought to be involved in the neural arc of the reflex. These axons are thought to contain oxytocin and organum vasculosum laminae terminalis (OVLT) neurons that send their axons to the hypothalamus and brainstem. A variety of neuropeptides are known to inhibit the reflex, to prevent the release of oxytocin from the hypothalamus. (See text for further details.)
Predicting, detecting and monitoring metastatic breast cancer. The figure portrays an omic-signature-based screening strategy for earlier detection of metastasis-prone lesions and high sensitivity detection of residual disease. This strategy is based on the premise that molecular features can be used to define breast cancer subtypes that are at high risk of progressing to metastatic disease. Molecular features associated with metastasis discovered through analysis of metastatic breast cancers are used to develop sensitive assays for disease. This involves a multi-step process in which low-cost blood-based assays of molecular signatures associated with metastasis-prone disease are applied routinely to identify high-risk individuals who are then screened using more expensive but sensitive and specific anatomic assays followed by histopathological and omic assays to identify and characterize even the smallest lesions. The molecular information in individual tumors detected in this way can then be used to develop sensitive blood or imaging-based ‘individualized’ assays for recurrent disease that might be used to guide early detection and treatment. *Image from [80] reprinted with permission from AAAS. All other images were obtained from Wikimedia Commons and are available under public domain, Creative Commons Attribution 3.0 Unported license [92], or Creative Commons Attribution-Share Alike 3.0 Unported license [93].


Stages of breast cancer progression. Simplified model of stages of breast cancer progression from normal ductal morphology, advancement to hyperplasia, non-obligate progression through atypical ductal hyperplasia (ADH), Ductal Carcinoma in situ (DCIS), and either arrest at in situ carcinoma or transition to IBC.
Molecular mechanism of mammary gland involution: An update.
Jena MK, Jaswal S, Kumar S, Mohanty AK.

Plasticity and Potency of Mammary Stem Cell Subsets During Mammary Gland Development.
Lee E, Piranlioglu R, Wicha MS, Korkaya H.

Regulation of mammary epithelial cell homeostasis by IncRNAs.
Shore AN, Rosen JM.

Schedule/Lecture Outline –

<table>
<thead>
<tr>
<th>Month</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
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<th>Week 11</th>
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Note: The schedule outlines the topics covered each week. The lecture topics are as follows:
- **Week 1**: Systems Biology Introduction
- **Week 2**: Molecular/Cellular Reproduction Systems
- **Week 3**: Sex Determination Systems
- **Week 4**: Male Reproductive Tract Development & Function
- **Week 5**: Female Reproductive Tract Development & Function
- **Week 6**: Gonadal Developmental Systems Biology
- **Week 7**: Testis Systems Biology
- **Week 8**: Ovary Systems Biology
- **Week 9**: Epigenetics and Transgenerational Gonadal Disease
- **Week 10**: Spring Break
- **Week 11**: Gametogenesis/ Stem Cells/ Cloning
- **Week 12**: Hypothalamus-Pituitary Development & Function
- **Week 13**: Reproductive Endocrinology Systems
- **Week 14**: Fertilization & Implantation Systems
- **Week 15**: Fetal Development & Birth Systems
- **Week 16**: Assisted Reproduction/Contraception
- **Week 17**: Exam or Grant Review