Spring 2024 – 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 6, 2024
Week 5

Female Reproductive Tract Development & Function

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

Required Reading

Vue, et al. (2018) Fetal and Postnatal Female Tract Development, in: Encyclopedia of Reproduction (Second Edition), Volume 2, 2018, Pages 261-268

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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

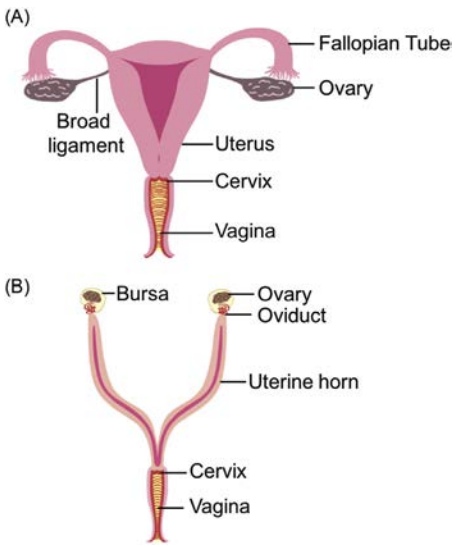

Fig. 1 Schematic illustration of the female reproductive tract in human and mouse. The female reproductive tracts of human (A) and mouse (B) consist of the ovary, Fallopian tube (oviduct in mouse), uterus, cervix and vagina. A bursal membrane surrounds the ovary in the mouse by not in human.

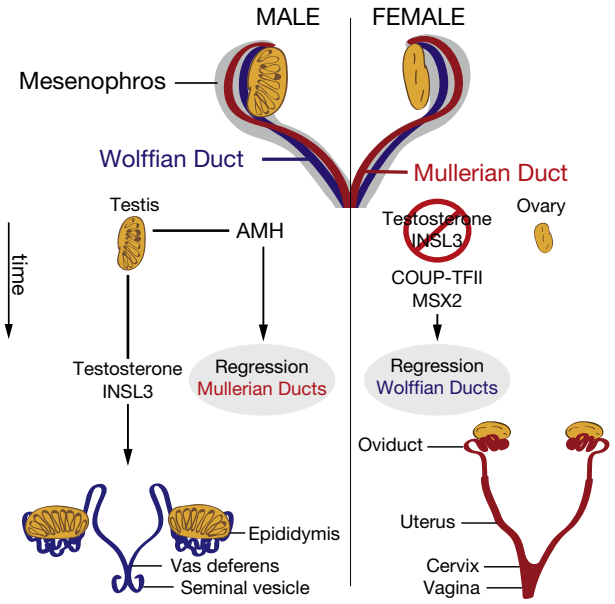

Fig. 2 Reproductive tract sexual differentiation. The reproductive tract progenitor tissues prior to sexual differentiation are equivalent and contain a fully formed Wolffian duct (*blue*) and Müllerian duct (*red*) within the mesenophros (*gray*). Hormones produced by the fetal testis, anti-Müllerian hormone (AMH), testosterone, and insulin-like 3 (INSL3), activate regression of the MD, differentiation of the WD into the male genital tract (vas deferentia, epididymides, and seminal vesicles), and testicular descent, respectively. In females, at this developmental time point the ovary lacks AMH, testosterone, and INSL3. This permits differentiation of the MD into the female reproductive tract (oviducts, uterus and upper vagina), regression of the WD by allowing COUP-TFII signaling and MSX2 expression, and maintenance of the ovaries in an abdominal position, respectively. Modified from Mullen, R.D. and Behringer, R.R. (2014). Molecular Genetics of Mullerian Duct Formation, Regression and Differentiation. *Sex Dev.* **8**, 281–296.

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

MDs form in amniotes, i.e., birds, reptiles and mammals. Current understandings of MD formation are mostly based on studies in the chicken and mammals. The corresponding developmental stages when MDs form are embryonic day (E) 11.5–13.5 in the mouse, E13.5–16.5 in the rat, Hamburger Hamilton stages 20–30 in the chicken, day 25 of gestation to 2–7 days postpartum in the wallaby and gestational week (GW) 6 to 9.5 (Carnegie Collection stages 16–18) in human (Renfree et al., 1996). Although the timing of MD formation varies between species, the process of how the MD forms is likely similar for each organism.

The formation of the MD can be separated into three phases: initiation, invagination, and elongation (Mullen and Behringer, 2014) (Fig. 3). The initiation phase occurs when a thickened placode-like structure forms on the anterior mesonephric epithelium near the WD. These cells are LHX1 positive and specified by an FGF/LHX1 axis, which, in turn, is regulated by a BMP/PAX2 axis (Atsuta and Takahashi, 2016) (Fig. 3).

The invagination phase occurs when the cells in the placode-like structure become elongated and form apical tight junctions, resulting in a depression of the mesonephric epithelium. Some cells appear to detach from the mesonephric epithelium and move into the space between the mesonephric epithelium and the WD. As the depression becomes deeper, it transforms into

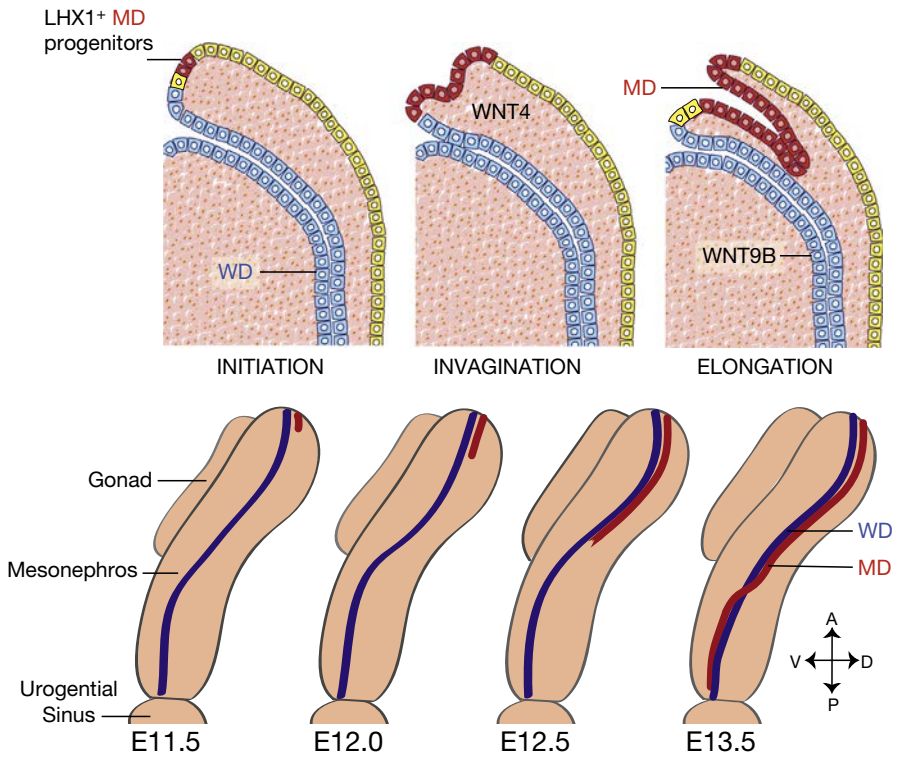

Fig. 3 Müllerian duct formation. (A) MD (*red*) formation occurs in three phases: initiation, invagination, and elongation. Initiation phase: MD progenitor cells in the mesonephric epithelium (*yellow*) are specified and begin to express LHX1. Invagination phase: in response to WNT4 signaling from the 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.

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 MD 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 (Prunskaite-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. Studies in both chicken and mouse have shown that the MD cells are proliferative along the entire anterior to posterior length. In addition, cell migration may play an important role during the elongation process. The tip 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*

is expressed in the WD. Thus, WNT9B secreted from the WD is required for MD elongation, providing a molecular explanation why MD elongation is dependent upon the WD (Mullen and Behringer, 2014).

Interestingly, MD cell differentiation switches between mesenchymal and epithelial states during MD formation. In the initiation phase, the specified MD cells are considered "mesoepithelial" and invade into the mesonephric mesenchyme. The MD cells are histologically epithelial but express mesenchymal molecular markers (Mullen and Behringer, 2014). After MD elongation is completed, the MD cells in female fetuses down-regulate mesenchyme markers and up-regulate epithelial molecular markers.

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 human 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 and 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 by 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 peri-natal 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* and 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 (ex. 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).

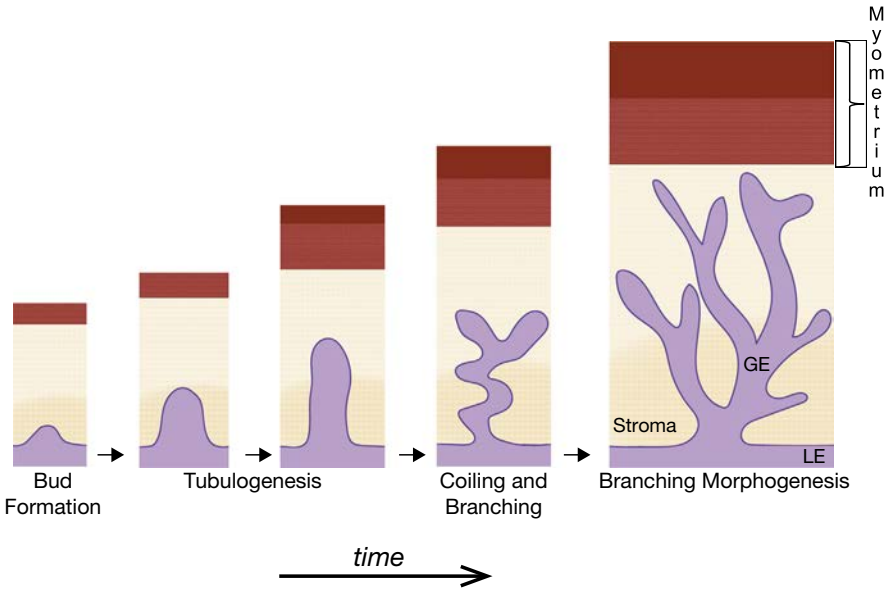

Fig. 4 Schematic illustration of endometrial adenogenesis in the mouse uterus. The uterus consists of two epithelial cell types (*purple*), the luminal epithelium (LE) and glandular epithelium (GE). The myometrium is composed of two smooth muscle layers: the inner circular layer (*pink*) and outer longitudinal layer (*dark red*). Stages of adenogenesis are indicated. *GE*, glandular epithelium; *LE*, luminal epithelium.

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 epitheliomesechymal organs. The communication between the epithelium and stroma appears to be mediated by *Wnt* and *Hox* 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 (*Ctnnb1*, *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: *EMX2*, *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 *EMX2* 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, *EMX2* 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 hyperandrogenism (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.

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Spring 2024 – 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 6, 2024
Week 5

Female Reproductive Tract Development & Function

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

Required Reading

Vue, et al. (2018) Fetal and Postnatal Female Tract Development, in: Encyclopedia of Reproduction (Second Edition), Volume 2, 2018, Pages 261-268

Spring 2024 – 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 8, 2024
Week 5

Female Reproductive Tract Development & Function

Primary Papers:

1. Martin, et al. (2022) J Dairy Sci, 105(10):8189-8198.
2. Du & Taylor (2015) CSH Persp Medicine, 6:a023002.
3. Major, et al. (2021) Biol Reprod, 1-15, ioab166.

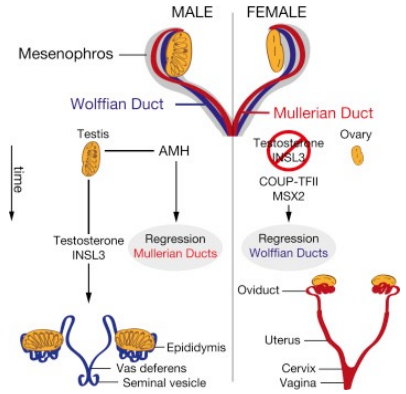
Discussion

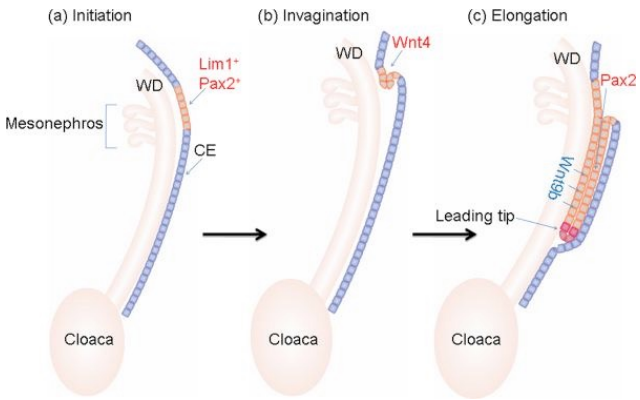
Student 10: Contemporary Paper-Ref #1 above
- What are the organs of the female reproductive tract examined?
- What methods and computational approach was used?
- What aspects of the tract were important and why?

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

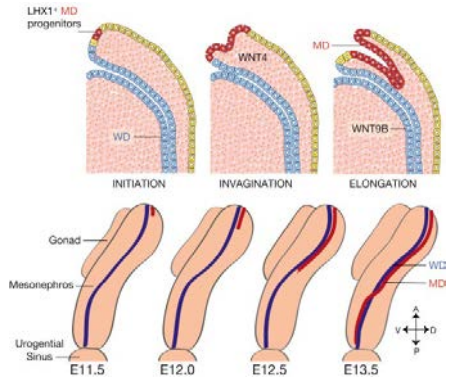
Student 12: Contemporary Paper-Ref #3 above
- What evo-devo approach for female reproductive tract was used?
- What transcription genes involved were discussed?
- What conserved processes are observed in female reproductive tract development?

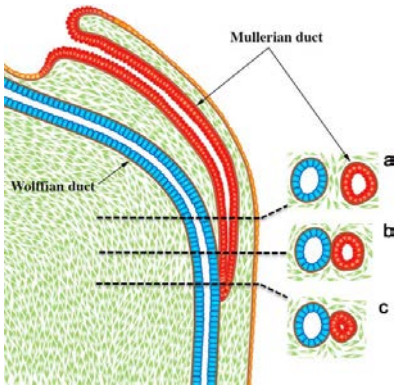
Development





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.





Diagrammatic representation of the caudal growth of the Müllerian duct using the Wolffian duct as a "guide wire". Note in (a) mesenchyme intervening between the Müllerian and Wolffian ducts, (b) contact of the basement membranes of Müllerian and Wolffian ducts, (c) direct contact of the epithelia of the Müllerian and Wolffian ducts. From Robboy et al. (2017) with permission.

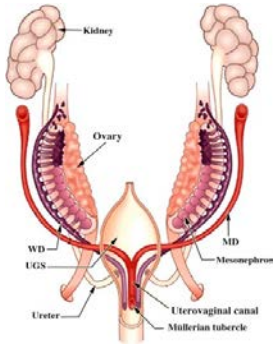
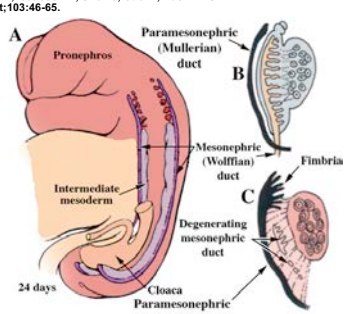
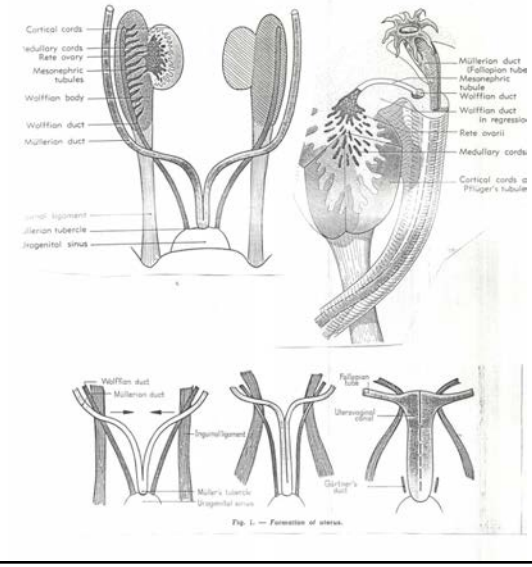


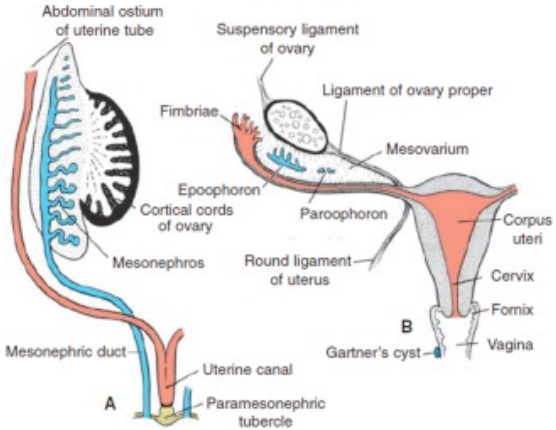
Diagram of developing human female internal genitalia in the indifferent, bisexual stage (~ 54 days of gestation, Carnegie Stage 22). The Müllerian derivatives are red and Wolffian derivatives are purple. Note the changing anatomical relationships between the Müllerian and Wolffian ducts. From Robboy 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.
Cunha GR, Robboy SJ, Kurita T, Isaacson D, Shen J, Cao M, Baskin LS.
Differentiation. 2018 Sep - Oct;103:46-65.

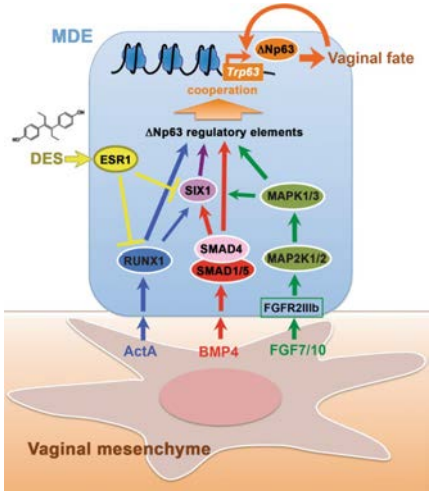


(A) Formation of the Wolffian (mesonephric) duct, which by 24 days has grown caudally to join the cloaca. At 5–6 weeks the paramesonephric (Müllerian) ducts appear as invaginations of the coelomic epithelium. At 7 weeks (B) the MDs have grown caudally towards the urogenital sinus. Subsequently (C, 8 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.



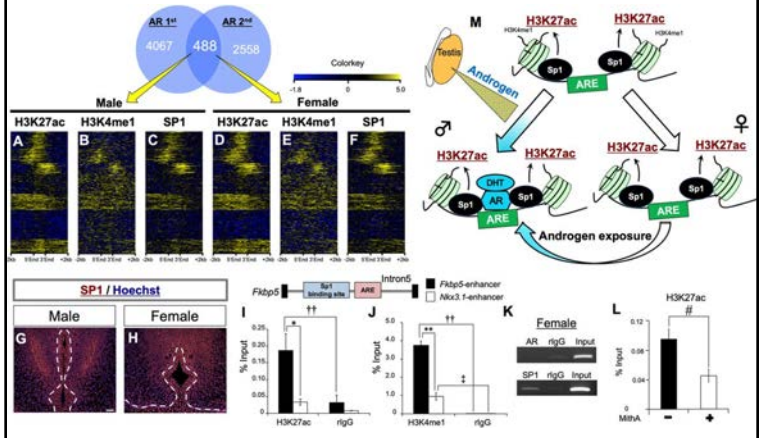


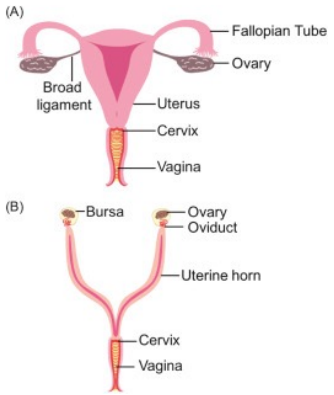
SIX1 cooperates with RUNX1 and SMAD4 in cell fate commitment of Müllerian duct epithelium
Terakawa J, Serna VA, Nair DM, et al.
Cell Death Differ. 2020 Dec;27(12):3307-3320.



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.

Sexual fate of murine external genitalia development: Conserved transcriptional competency for male-biased genes in both sexes.
Kajioka D, Suzuki K, Matsushita S, et al.
Proc Natl Acad Sci U S A. 2021 Jun 8;118(23):e2024067118.





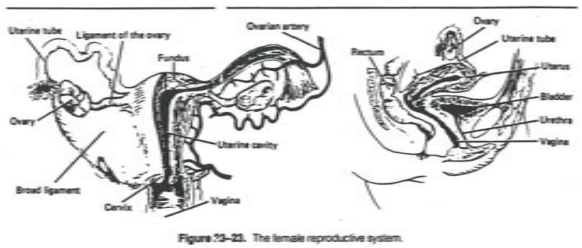
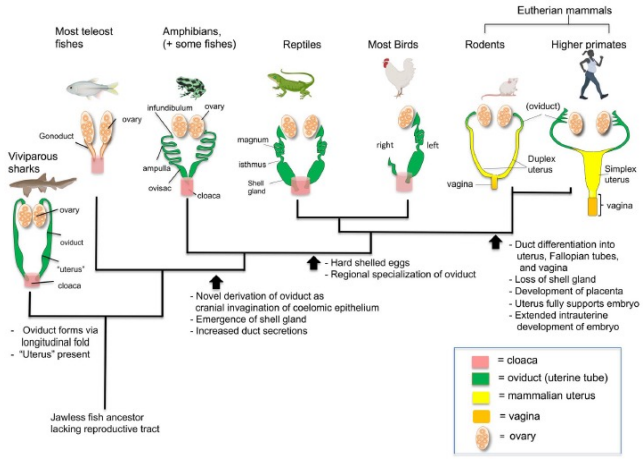
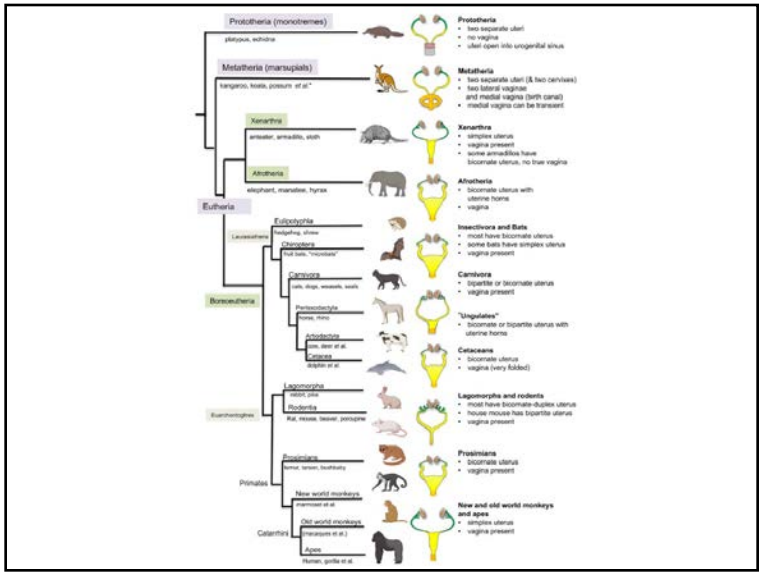


Figure 13–23. The female reproductive system.

An evo-devo perspective of the female reproductive tract.
Major AT, Estermann MA, Roly ZY, Smith CA.
Biol Reprod. 2021 Sep 7:ioab166.





Oviduct

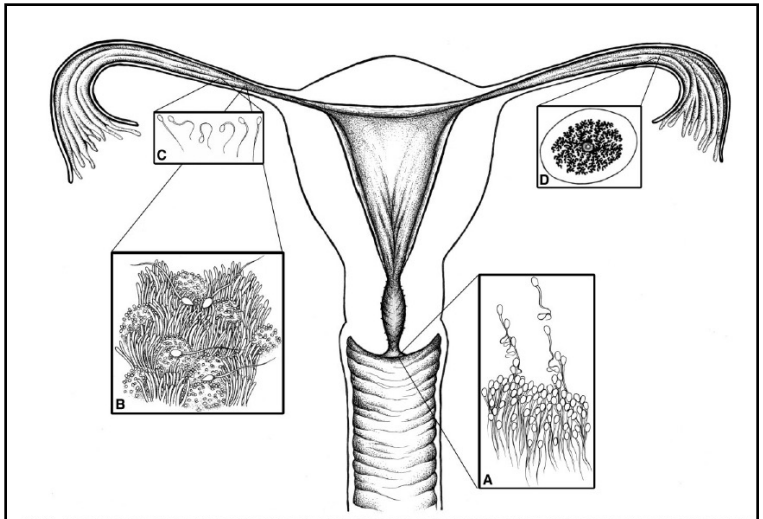
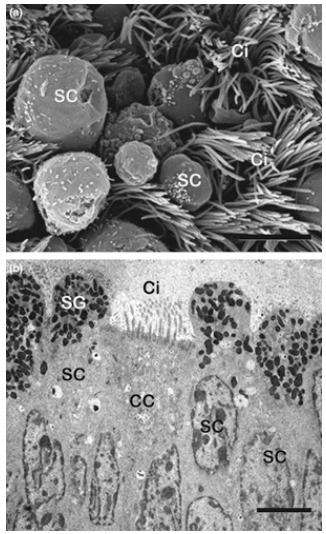


Figure 1. Human female reproductive tract illustrating stages of gamete transport. (A) Sperm entering cervical mucus at external os of cervix. The mucus fills the upper half of the inset. (B) Sperm interacting with endosalpingeal epithelium in Fallopian tube. (C) Hyperactivated motility of sperm in Fallopian tube. (D) Oocyte in cumulus within a transverse section of the tubal ampulla. Artwork by C. Rose Gottlieb.



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

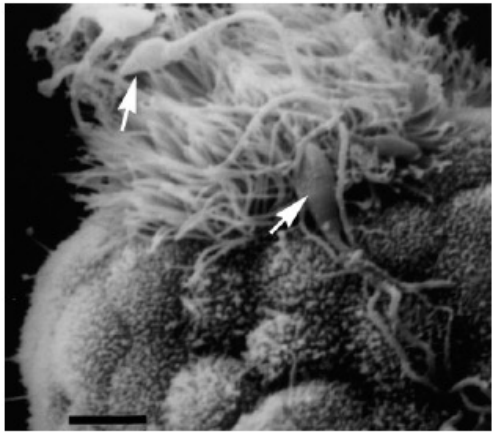
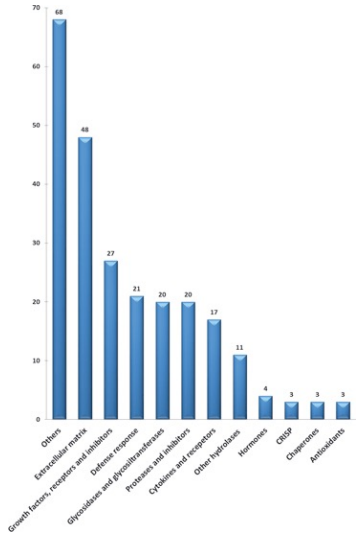
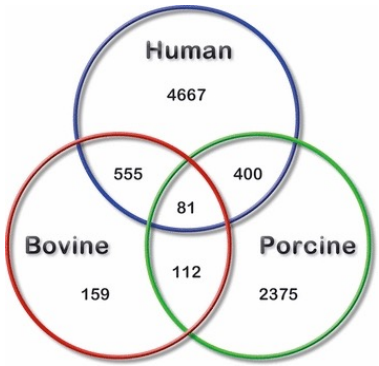


Figure 3. Scanning electron micrograph showing human sperm attached to a ciliated area of Fallopian tube epithelium *in vitro*. Arrows indicate sperm heads associated with cilia. Scale bar, 4 μm. Reproduced from Pacey *et al.* (1995b).

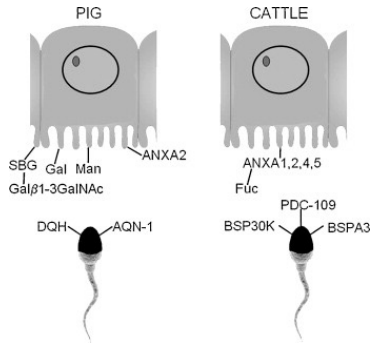


Functional clustering of genes classified as 'secreted' using the DAVID bioinformatic tool using data from normal Fallopian tube reported in Tone et al. (2008)



Venn diagram showing overlapping and non-overlapping gene expression on human, bovine and porcine oviduct

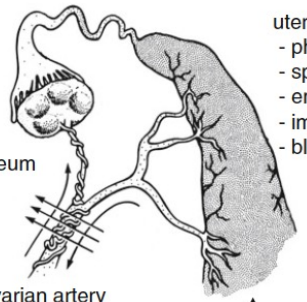
Molecules involved in sperm-oviduct adhesion and release.
Talevi R, Gualtieri R.
Theriogenology. 2010 Apr 1;73(6):796-801.



Schematic drawing of molecules involved in sperm-oviduct binding in pig and cattle. SBG, sperm binding glycoprotein [30]; Galβ1-3GalNAc, galactose-beta 1-3 N-acetylgalactosamine [30]; Gal, galactose [38]; Man, mannose [28]; ANXA2, annexin 2 [33]; DQH [29]; AQN1 [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].

oviduct:
- sperm storage
- embryo development

ovary:
- induction of ovulation
- formation of corpus luteum

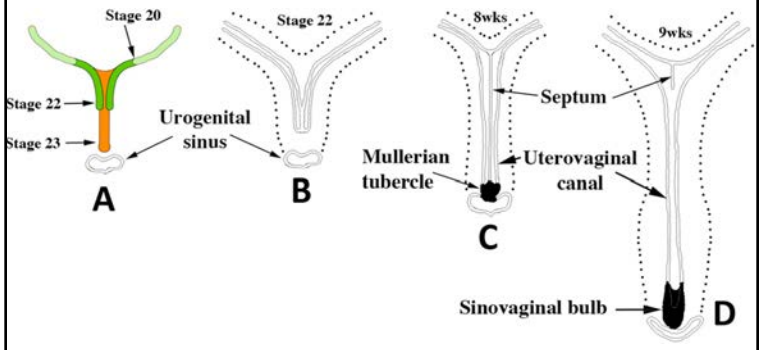


uterus and cervix:
- phagocytic clearance
- sperm selection
- endometrial receptivity
- immune tolerance
- blastocyst development

uterine vein to ovarian artery
counter-current signals

SEMINAL FLUID

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.

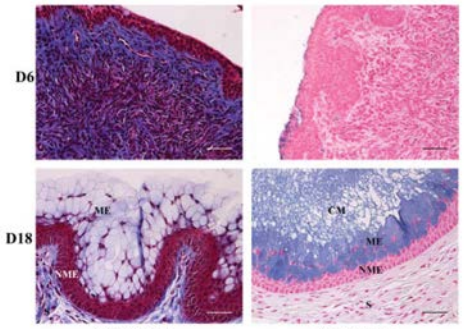


Fig. 2 Structure of mouse cervical epithelium in pregnancy. Mouse cervical sections at gestation day 6 and day18 stained with Trichrome and Alcian blue. In the Trichrome stain, the undifferentiated epithelia appear as red and the differentiated epithelia harbor mucous-laden vacuoles. In the Alcian stain, the differentiated mucosal epithelium and mucinous substances are stained blue, cell cytoplasm is stained pink, and nuclei are stained dark pink to red. Note the marked difference in structure and thickness of epithelium between days 6 and 18 pregnant cervix. Day 6 cervix lacks mucosal epithelium and mucus compared with the day 18 cervix. D6, day 6; D18, day 18; NME, nonmucosal epithelium; ME, mucosal epithelium; S, stroma; CM, cervical mucus. Scale bar: 50μm.

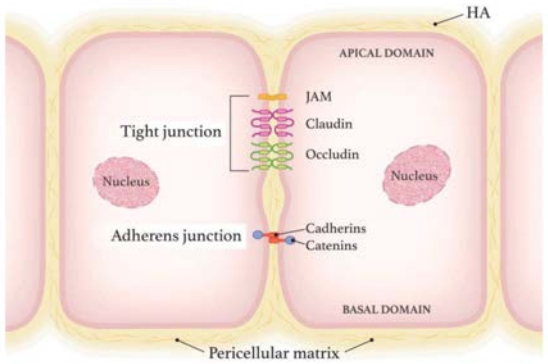


Fig. 3 Diagram depicting the components of cervical epithelial barrier. The cervical epithelial barrier in pregnancy is made up of pericellular matrix rich in hyaluronan (HA) and cell–cell junctional complexes predominantly tight and adherens junctions. The tight junction proteins are claudin family members as well as non-claudin transmembrane proteins (occludin, JAM-A, ZO). These structures maintain integrity and epithelial cell polarity and also regulate pericellular permeability.

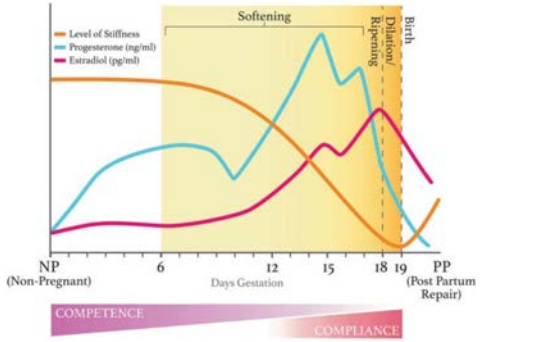
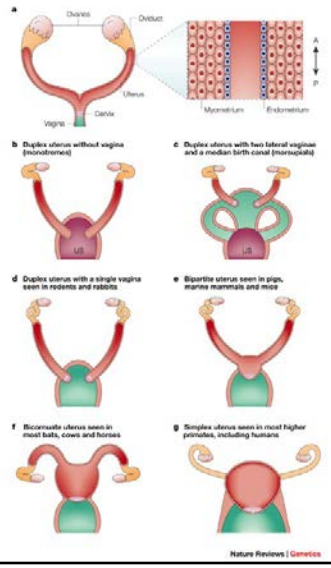
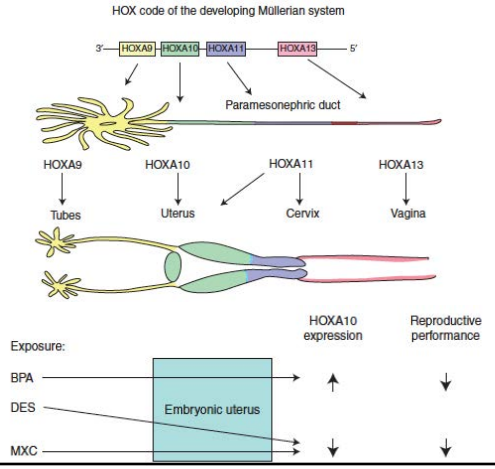


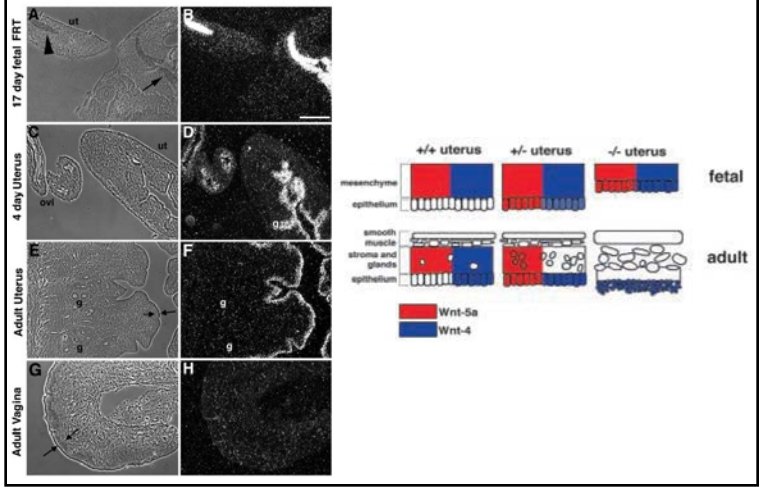
Fig. 5 Schematic representation of cervical remodeling in mice. Biochemical and molecular changes occurring in response to the rise in progesterone after conception trigger transcriptional cascades by gestation day 6 which subsequently result in alterations in the composition and structure of ECM to cause measurable and progressive changes in tissue strength from gestation day 12 until birth. We thus define the softening phase from days 6 to 17. Ripening and dilation occurs with loss of progesterone function on gestation day 18. These sequential events, yet to be completely defined, maintain the delicate balance between cervical competence and compliance.



The Role of Hox Genes in Female Reproductive Tract Development, Adult Function, and Fertility.
Du H, Taylor HS.
Cold Spring Harb Perspect Med. 2015 Nov 9;6(1). pii: a023002.



Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract.
Miller C, Sassoon DA.
Development. 1998 Aug;125(16):3201-11.



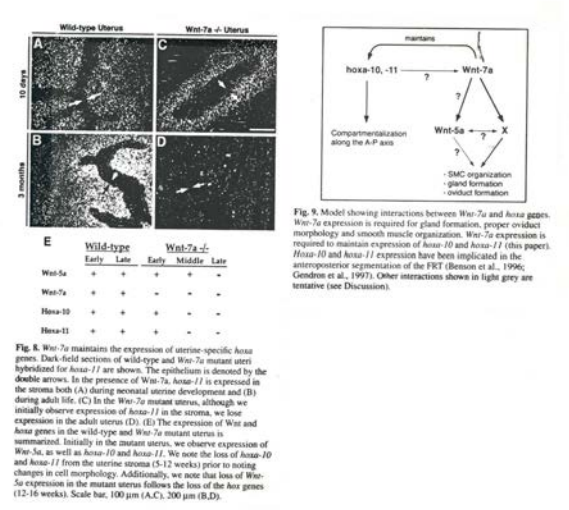


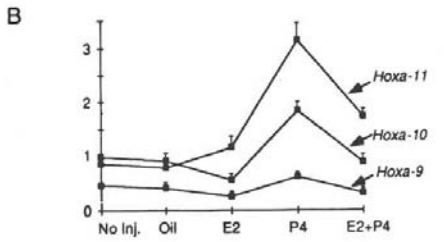
Fig. 8. *Wnt-7a* maintains the expression of uterine-specific *hoxa* genes. Dark-field sections of wild-type and *Wnt-7a* mutant uteri hybridized for *hoxa-11* are shown. The epithelium is denoted by the double arrows. In the presence of Wnt-7a, *hoxa-11* is expressed in the stroma both (A) during neonatal uterine development and (B) during adult life. (C) In the *Wnt-7a* mutant uterus, although we initially observe expression of *hoxa-11* in the stroma, we lose expression in the adult uterus (D). (E) The expression of Wnt and *hoxa* genes in the wild-type and *Wnt-7a* mutant uterus is summarized. Initially in the mutant uterus, we observe expression of *Wnt-5a*, as well as *hoxa-10* and *hoxa-11*. We note the loss of *hoxa-10* and *hoxa-11* from the uterine stroma (5-12 weeks) prior to noting changes in cell morphology. Additionally, we note that loss of *Wnt-5a* expression in the mutant uterus follows the loss of the *hox* genes (12-16 weeks). Scale bar, 100 μm (A,C), 200 μm (B,D).

Fig. 9. Model showing interactions between *Wnt-7a* and *hoxa* genes. *Wnt-7a* expression is required for gland formation, proper oviduct morphology and smooth muscle organization. *Wnt-7a* expression is required to maintain expression of *hoxa-10* and *hoxa-11* (this paper). *Hoxa-10* and *hoxa-11* expression have been implicated in the anteroposterior segmentation of the FRT (Benson et al., 1996; Gendron et al., 1997). Other interactions shown in light grey are tentative (see Discussion).

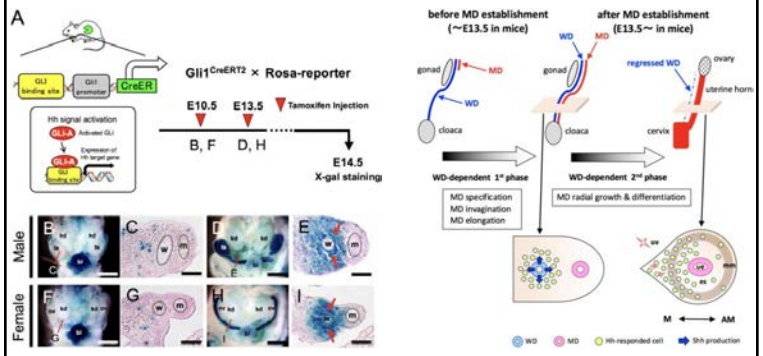
TABLE 1. *HOXA* gene expression in the reproductive tract.

Organ	E15 PMND*	Adult mouse	Adult human
Oviduct	9, 10, 11, 13	9	9
Uterus	9, 10, 11, 13	10, 11	10, 11
Cervix	9, 10, 11, 13	11, 13	11, 13
Vagina	9, 10, 11, 13	13	13

* Embryonic day 15, paramesonephric duct.



New Insights into Development of Female Reproductive Tract-Hedgehog-Signal Response in Wolffian Tissues Directly Contributes to Uterus Development.
Haraguchi R, Yamada G, Murashima A, Matsumaru D, Kitazawa R, Kitazawa S.
Int J Mol Sci. 2021 Jan 26;22(3):1211.



Notch signaling in reproduction.
Moldovan GE, Miele L, Fazleabas AT.
Trends Endocrinol Metab. 2021 Dec;32(12):1044-1057.

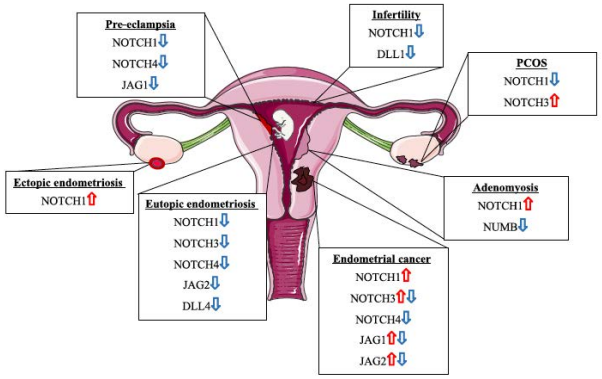
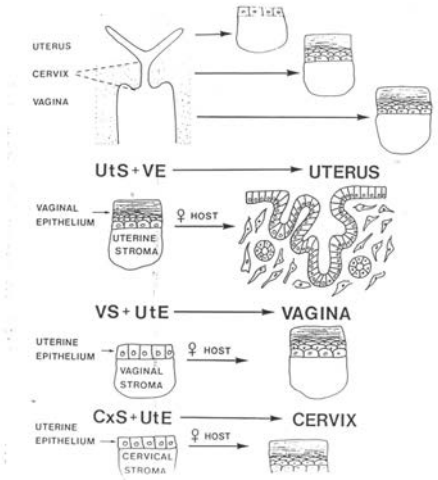


Figure 3. Notch receptor and ligand expression aberrations in gynecological pathologies. Notch receptors and ligand expression are dysregulated in a myriad of gynecological pathologies including pre-eclampsia, infertility, polycystic ovarian syndrome (PCOS), adenomyosis, endometrial cancer, and eutopic and ectopic endometriosis in comparison with disease-free controls. These expression alterations contribute to infertility and disease pathology by compromising decidualization and hormone signaling, inducing recurrent pregnancy loss, increasing epithelial to mesenchymal transition, and contributing to placental defects.

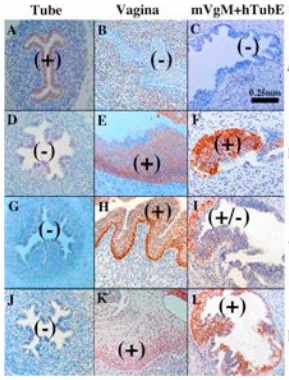
Table 1 | **Mouse genes that are required for female reproductive tract development**

Gene name	Genetic map position	Molecule encoded	Tissue of expression	Female reproductive-tract phenotype abnormality (mode of inheritance)	References
Formation					
Fbx2	Chr19 (43.0 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	8
Lhx1 (Lhx1)	Chr1 (48.0 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	11
Emx2	Chr19 (53.5 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	12
Wnt4	Chr4	Wnt family secreted protein	MM	Absence of FRT (R)	17
Ltap	Chr1 (93.4 cM)	Transmembrane protein with PDZ domain	ND	Imperforate vagina (D)	22,23
Hoxa13	Chr6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Delay or arrested formation (R)	51
Regression					
Mis (Amh)	Chr10 (43.0 cM)	TGFβ superfamily secreted protein	Sertoli cells	Ectopic FRT in males (R)	27,28
Misr2 (Amhr2)	Chr15 (57.4 cM)	TGFβ superfamily type 2 Ser/Thr transmembrane receptor	MM	Ectopic FRT in males (R)	35
Wnt7a	Chr6 (39.5 cM)	Wnt family secreted protein	ME	Ectopic FRT in males (R)	42
Differentiation					
Wnt7a	Chr6 (39.5 cM)	Wnt family secreted protein	ME	Homeotic transformation of oviduct to uterus and uterus to vagina, no uterine glands, abnormal mesenchyme differentiation (SD)	53
Hoxa10	Chr6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Homeotic transformation of anterior uterus to oviduct (R)	49,52
Hoxa11	Chr6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Partial homeotic transformation of uterus to oviduct (SD)	49,99
*Hd (Hoxa13)**	Chr6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Homeotic transformation of cervix to uterus (SD)	100
Ovo1 (Ovol1)	Chr19	C2H2-type zinc-finger protein	ND	Subfertility with dilated uterus and cervix, constricted or imperforate vagina (R)	101

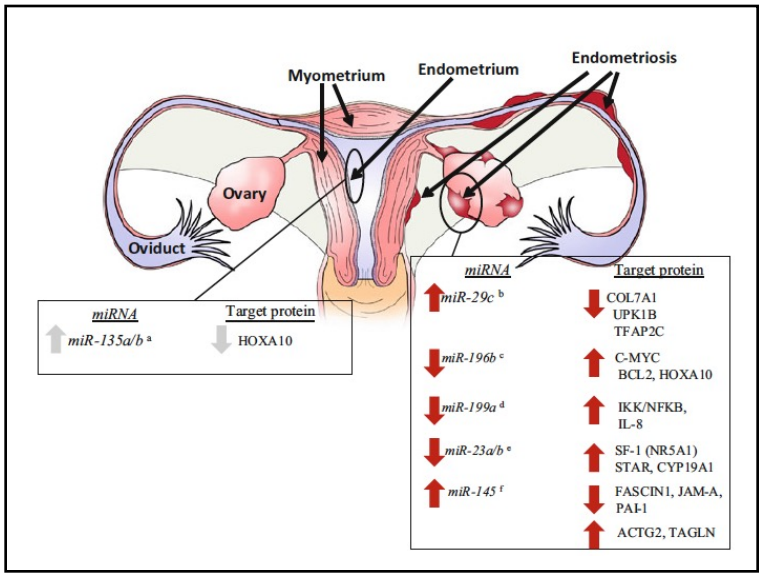
This table lists all of the mouse genes that are known to be involved in female reproductive tract (FRT) development. *The *Hoxa13* mutation in the *Hypodactyly (Hd)* mutant is not a null allele, but is thought to be a dominant-negative allele[51,52]. *Amh*, anti-Müllerian hormone; *Amhr2*, anti-Müllerian hormone type 2 receptor; C2H2, two cysteine two histidine; Ch, chromosome; cM, centimorgan; D, dominant; *Emx*, empty spiracles homologue; *Hoxa*, homeobox A; *Lim1, lin-11, Isl1* and *mec-3* transcription factor homologue; *Lhx1*, LIM homeobox protein; *Ltap*, Lcop-tal-associated protein; ME, Müllerian duct epithelium; *Mis*, Müllerian-inhibiting substance; *Misr2*, Müllerian-inhibiting substance type 2 receptor; MM, Müllerian duct mesenchyme; ND, not determined; *Ovo1*, Ovo homologue-like; *Pax*, paired box gene; R, recessive; SD, semidominant; TGF, transforming growth factor; WE, Wolffian duct epithelium; WM, Wolffian duct mesenchyme; *Wnt*, wingless-related MMTV integration site.

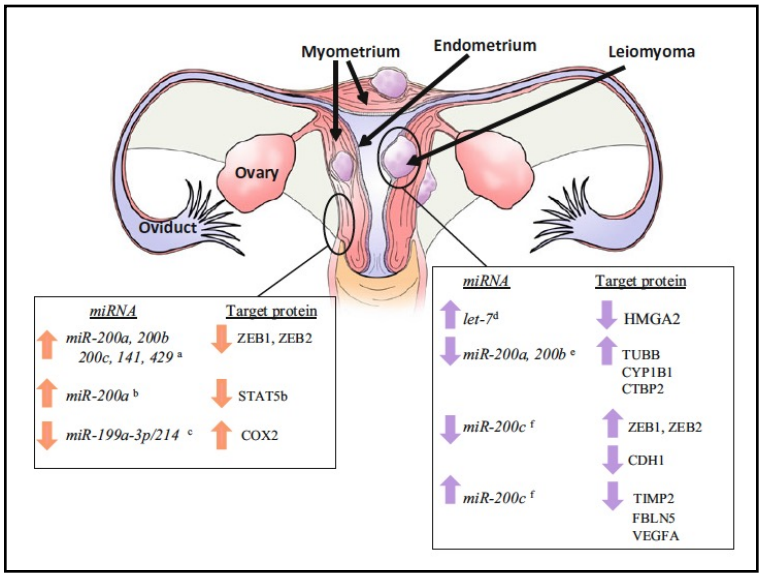


Tissue interactions and estrogenic response during human female fetal reproductive tract development.
Cunha GR, Kurita T, Cao M, Shen J, Cooke PS, Robboy SJ, Baskin LS.
Differentiation. 2018 May - Jun;101:39-45.

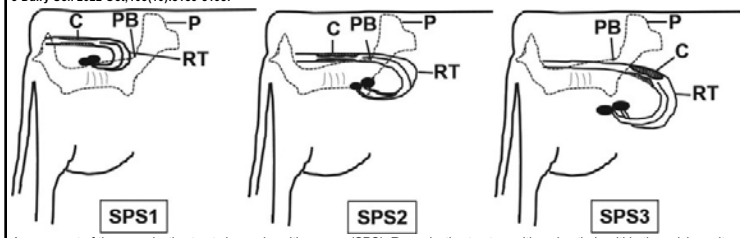


Tissue recombinants composed of neonatal mouse vaginal mesenchyme plus 13 week human fetal uterine tube epithelium (mVgM+hTubE) grown for 4 weeks in DES-treated hosts and immunostained for various vaginal epithelial markers as indicated. Human uterine tube (A, D, G, J) and vagina (B, E, H, K) at 16–18 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.

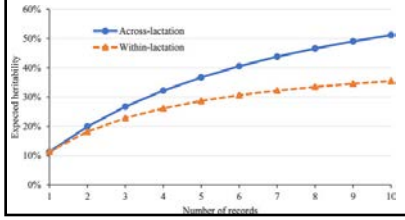




Reproductive tract size and position score: Estimation of genetic parameters for a novel fertility trait in dairy cows.
Martin AAA, Oliveira Jr G, Madureira AML, et al.
J Dairy Sci. 2022 Oct;105(10):8189-8198.



Assessment of the reproductive tract size and position score (SPS). Reproductive tracts positioned entirely within the pelvic cavity were designated SPS 1. Reproductive tracts in which the cervix was within the pelvic cavity, but uterine horns were outside the pelvic cavity, were designated SPS 2. Reproductive tracts in which the cervix and uterine horns lay outside the pelvic cavity were designated SPS 3 (from Young et al., 2017). C = cervix; P = pelvis; RT = reproductive tract; PB = pelvic brim; SPS = size and position score.



Change in expected heritability of reproductive tract size and position score with an increased number of repeated measurements per cow across and within lactation.

Endocrine

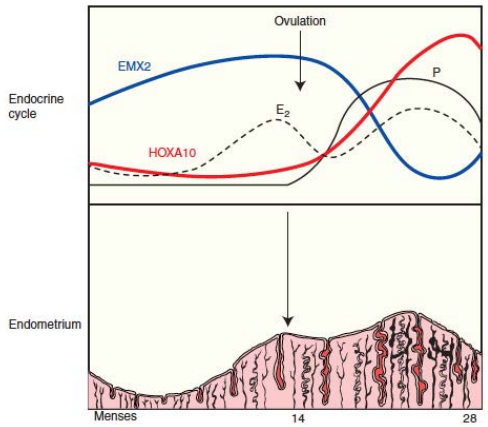


Figure 2. The pattern of *HOXA10* expression in the human endometrium through the menstrual cycle (adapted from Taylor 2000). *HOXA11* expression closely parallels that of *HOXA10*.

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

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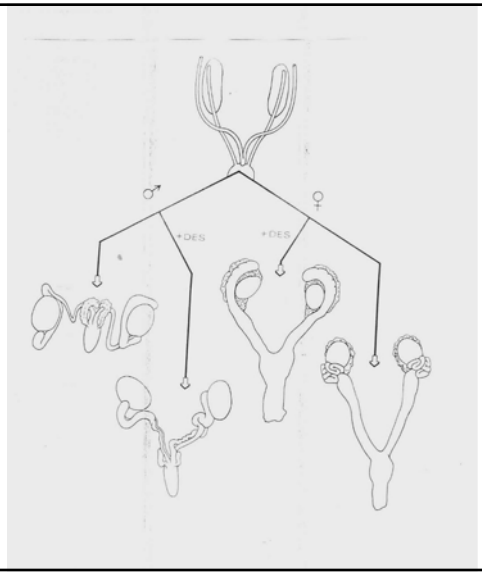
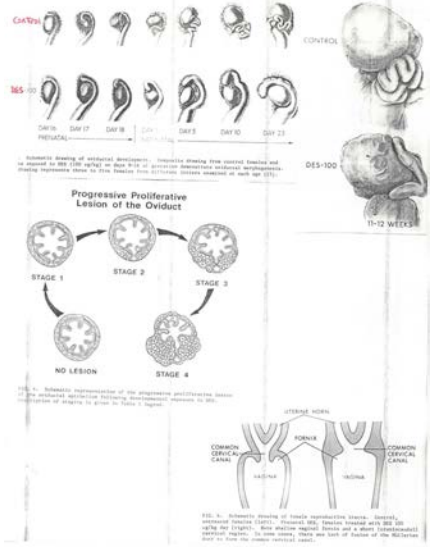
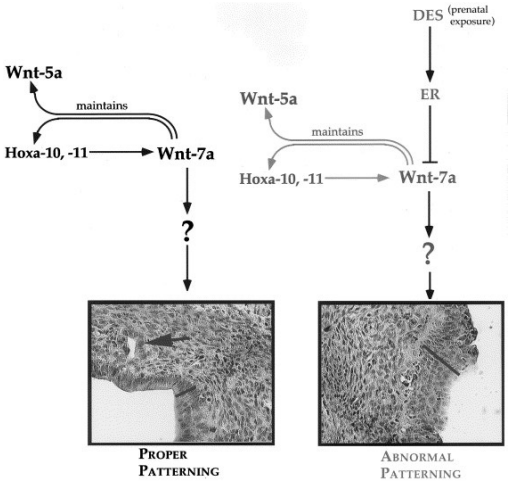
*Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut, 06520, USA; and †Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, 06520 USA

ABSTRACT Diethylstilbestrol (DES) was widely used to treat pregnant women through 1971. The reproductive tracts of their female offspring exposed to DES in *utero* are characterized by anatomic abnormalities. Here we show that DES administered to mice *in utero* produces changes in the expression pattern of several Hox genes that are involved in patterning of the reproductive tract. DES produces posterior shifts in Hox gene expression and homeotic anterior transformations of the reproductive tract. In human uterine or cervical cell cultures, DES induces HOXA9 or HOXA10 gene expression, respectively, to levels approximately twofold that induced by estradiol. The DES-induced expression is not inhibited by cycloheximide. Estrogens are novel morphogens that directly regulate the expression pattern of posterior Hox genes in a manner analogous to retinoic acid regulation of anterior Hox genes. Alterations in HOX gene expression are a molecular mechanism by which DES affects reproductive tract development. Changes in Hox gene expression are a potential marker for the effects of *in utero* drug use that may become apparent only at late stages of development.—Block, K., Kardana, A., Igarashi, P., Taylor, H. S. *In utero* diethylstilbestrol (DES) exposure alters Hox gene expression in the developing müllerian system. *FASEB J.* 14, 1101–1108 (2000)

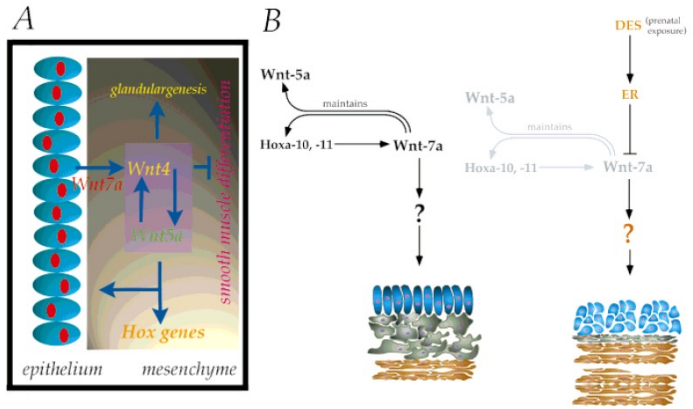




Wnt genes and endocrine disruption of the female reproductive tract: a genetic approach
Sassoon D.
Mol Cell Endocrinol. 1999 Dec 20;158(1-2):1-5.



The emergence of molecular gynecology: homeobox and Wnt genes in the female reproductive tract.
Kitajewski J, Sassoon D.
Bioessays. 2000 Oct;22(10):902-10.



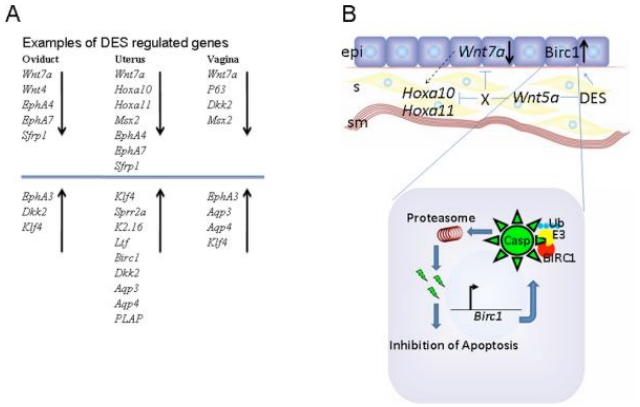


Table 1. Comparison between different DES mouse models.

	Prenatal	Neonatal
DES dosage	100 μg/kg/day	1 mg/kg/day
Treatment	E9.5–E16.5*	PND 1–5
FRT phenotypes		
Oviductal defects (lack of coiling)	Yes	No
Uterine atrophy	Less common	Yes
Squamous metaplasia of uterine epithelium	Yes	Yes
Smooth muscle disorganization	Yes	Yes
Abnormal urethral openings	Yes	No
Enlarged vagina	Yes	Yes
Vaginal adenosis	Less common	Yes
Persistent vaginal epithelial cornification	Yes	Yes
WD remnant	Yes	Yes
Genital tract tumors	Yes	Yes
Relevance to human exposure	A bit early	Mimic

*Other variations of prenatal regimens include treatment from E15-E18 at a dose of 200 μg/day pregnant mother or E10-E18 at a dose of 67 μg/kg/day.

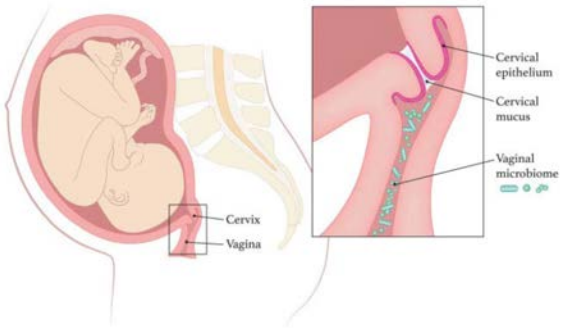
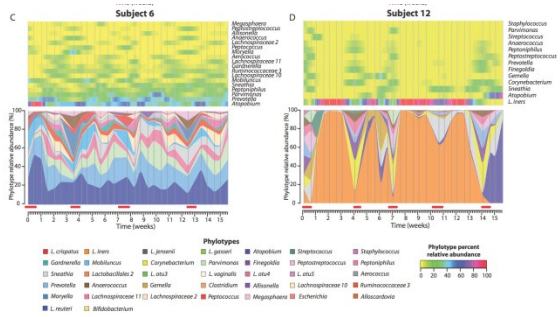


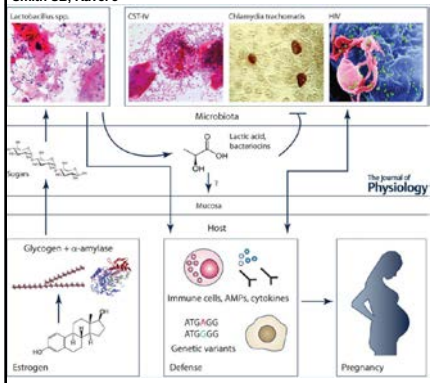
Fig. 1 Diagram depicting barriers of lower female reproductive tract. Three different barriers indicated are vaginal microbiome, cervical mucus, and cervical epithelium. In pregnancy, these barriers play a critical role in protection from ascending infection.

Unraveling the Dynamics of the Human Vaginal Microbiome
Yale J Biol Med. 2016 Sep 30;89(3):331-337.
Nunn KL, Forney LJ.



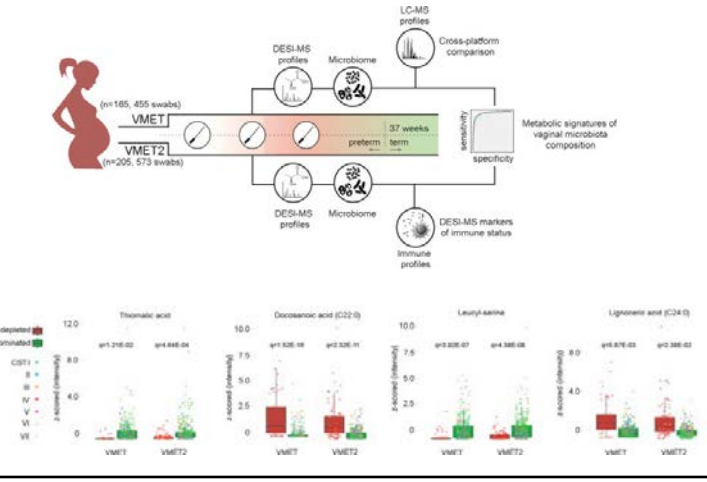
Temporal dynamics of vaginal bacterial communities. Bar plots depict changes in the relative abundances of bacterial taxa in the vaginal communities of four women over 16 weeks. The color code for each taxon is shown below the figure.

The vaginal microbiota, host defence and reproductive physiology
J Physiol. 2017 Jan 15;595(2):451-463.
Smith SB, Ravel J

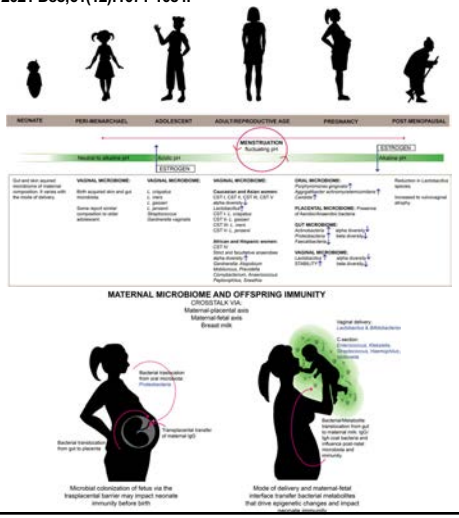


Abstract
The interaction between the human host and the vaginal microbiota is highly dynamic. Major changes in the vaginal physiology and microbiota over a woman's lifetime are largely shaped by transitional periods such as puberty, menopause and pregnancy, while daily fluctuations in microbial composition 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.

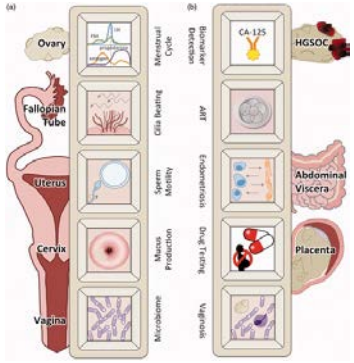
Direct on-swab metabolic profiling of vaginal microbiome host interactions during pregnancy and preterm birth
Pruski P, Correia GDS, Lewis HV, et al.
Nat Commun. 2021 Oct 13;12(1):5967.



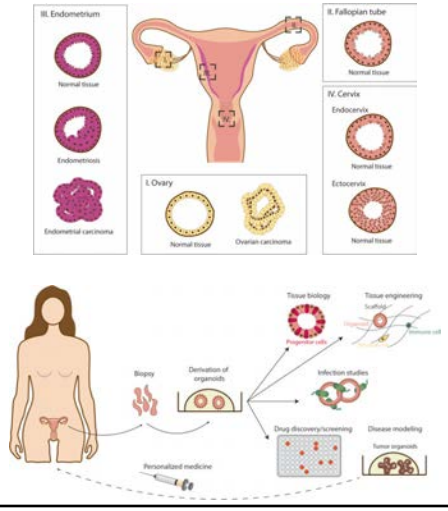
Insights into the role of vaginal microbiome in women's health.
Deka N, Hassan S, Seghal Kiran G, Selvin J.
J Basic Microbiol. 2021 Dec;61(12):1071-1084.



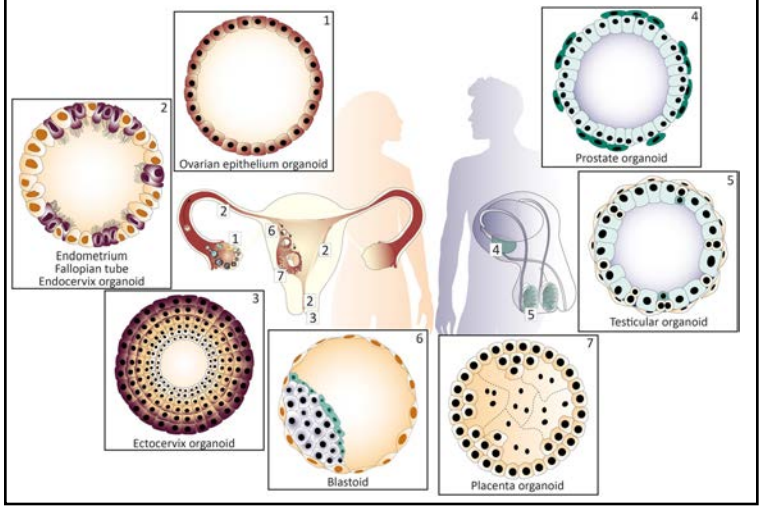
Microphysiologic systems in female reproductive biology.
Exp Biol Med (Maywood). 2017 Nov;242(17):1690-1700.
Young AN, Moyle-Heyrman G, Kim JJ, Burdette JE.



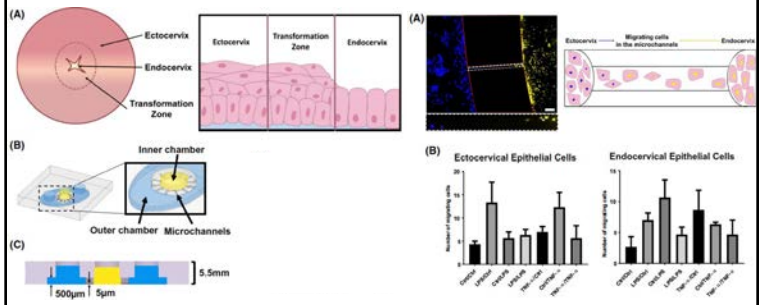
Organoids of the female reproductive tract.
Chumduri C, Turco MY.
J Mol Med (Berl). 2021 Apr;99(4):531-553.



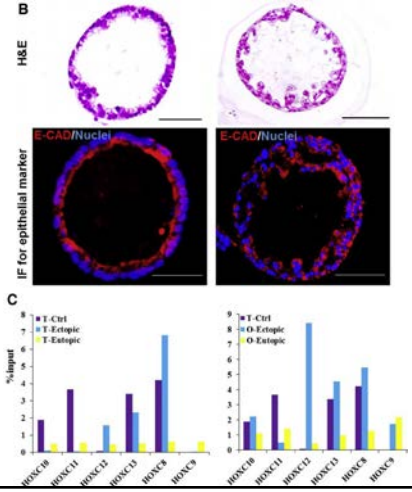
Human organoid systems in modeling reproductive tissue development, function, and disease.
Haider S, Beristain AG.
Hum Reprod. 2023 Aug 1;38(8):1449-1463.



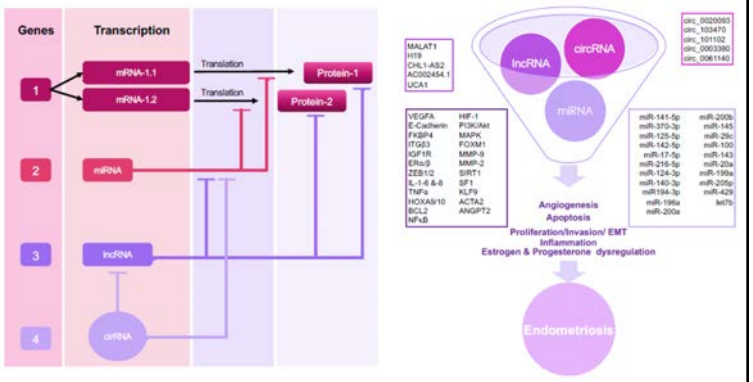
Organ-on-chip of the cervical epithelial layer: A platform to study normal and pathological cellular remodeling of the cervix.
Tantengco OAG, Richardson LS, Medina PMB, Han A, Menon R.
FASEB J. 2021 Apr;35(4):e21463.



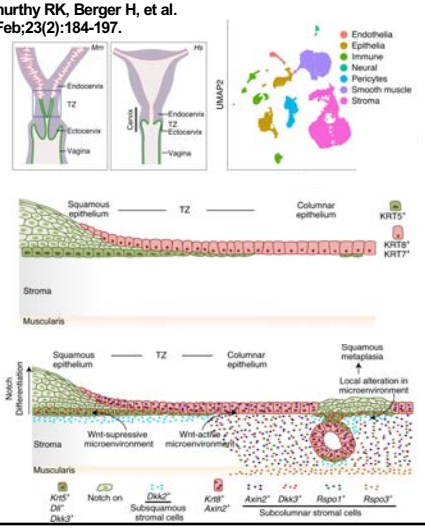
Insight into epigenetics of human endometriosis organoids: DNA methylation analysis of HOX genes and their cofactors.
Esfandiari F, Favaedi R, Heidari-Khoei H, et al.
Fertil Steril. 2021 Jan;115(1):125-137.



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
Int J Mol Sci. 2021 Sep 28;22(19):10498.



Opposing Wnt signals regulate cervical squamocolumnar homeostasis and emergence of metaplasia.
Chumduri C, Gurumurthy RK, Berger H, et al.
Nat Cell Biol. 2021 Feb;23(2):184-197.



Mammary Biology & Disease

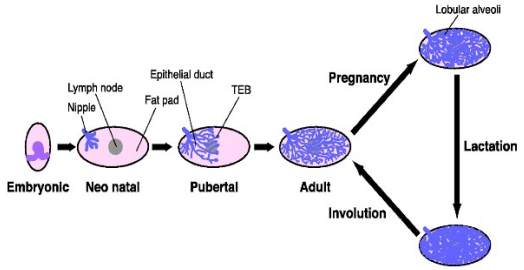
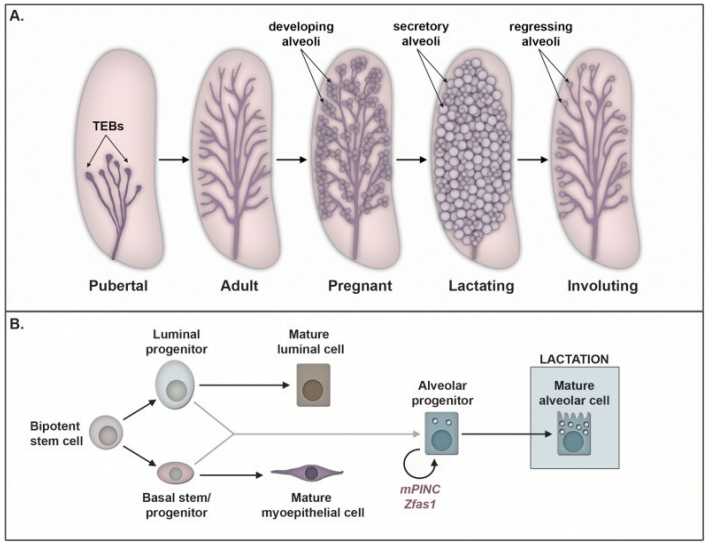


Fig. 1. Stages of mouse mammary gland development. The mouse mammary gland is specified at embryonic day 10. The mammary epithelium invades the fat pad and forms a small, branched ductal network. After birth, the epithelium grows in concert with the mouse but does not begin to fill the fat pad until the release of ovarian hormones at puberty (around 3 weeks of age). With the onset of puberty, TEBs form and the ducts invade, branch, and eventually fill the fat pad by around 10 weeks of age. In the first stage of pregnancy, ducts branch laterally and form side branches with concomitant epithelial proliferation. Alveolar structures then form on the expanded ductal tree and differentiate into lobular alveoli. Finally, the lobular alveoli terminally differentiate and the epithelium becomes secretory, ready to provide milk for suckling pups upon parturition. At this stage, the epithelium has expanded to almost fill the mammary gland and the large fat cells have dedifferentiated into small pre-adipocytes. Upon involution, the secretory epithelium of the mammary gland dies by apoptosis, the fat cells redifferentiate, and the gland is remodeled back to a state resembling that of the adult nulliparous mouse.



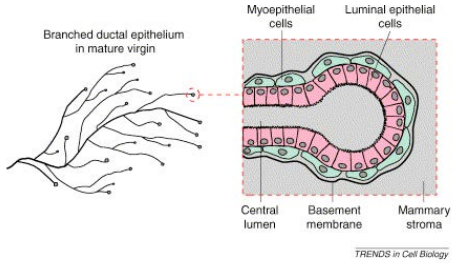
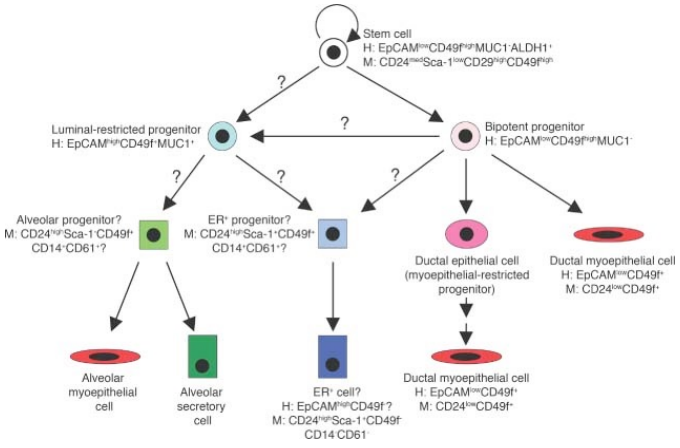
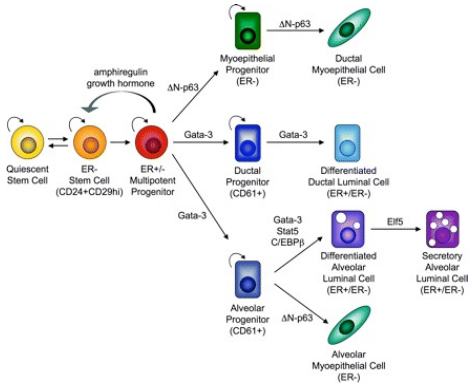


Fig. 2. Minimal structural unit of the mammary alveolus. The mammary ductal network is elaborated from a simpler layered structure. The inner layer consists of a heterogeneous population of luminal epithelial cells surrounding a central lumen, whereas the outer layer contains myoepithelial cells surrounded by the basement membrane. This multilayered structure is embedded in the stroma, consisting of a variety of mesenchymal cells in 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.





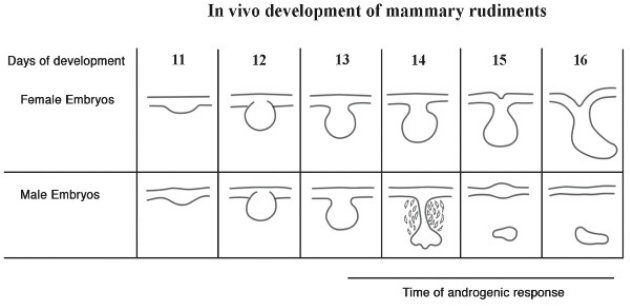


Figure 5 Sexually dimorphic development of female and male mouse mammary glands. The appearance of the mammary placodes and the formation of the buds is identical in males and females. Because of androgens produced by the fetal testes, beginning on embryonic day (E)13, the male mammary gland regresses and is mostly eliminated by E16. The window of sensitivity to androgens is the period E13 to E16, after which androgens cannot elicit mammary gland destruction in female mice. (Adapted from Kratochwil 1977, with permission.)

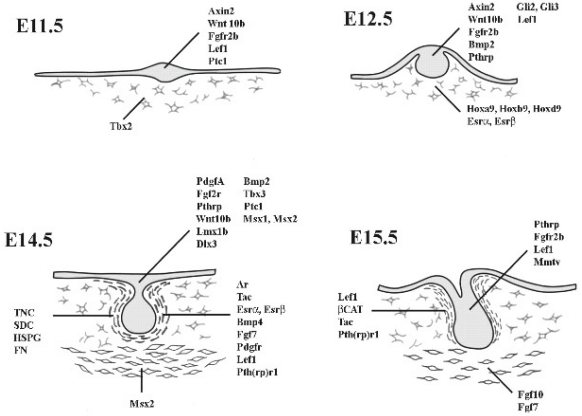


Figure 3 Genes expressed during development of the embryonic mouse mammary gland. Note that genes are expressed in a tissue-specific pattern, with many genes expressed exclusively in epithelium or mesenchyme. E11.5 to E15.5, embryonic days 11.5 to 15.5. (Adapted from Veltmaat et al. 2003, with permission.)

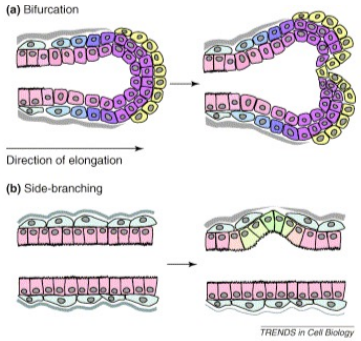
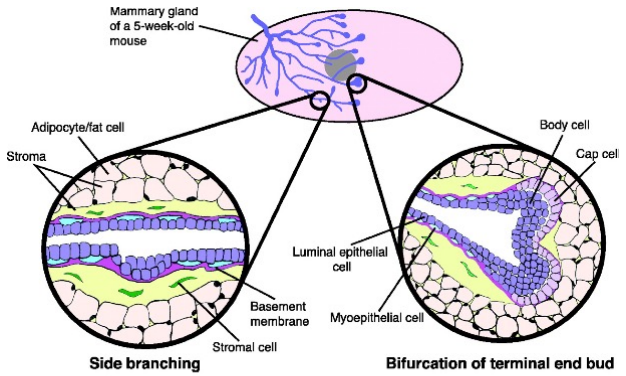


Fig. 3. Alternative methods of branch initiation. (a) Primary branching. Terminal end buds (TEBs) consist of a leading layer of highly proliferative cap cells that differentiate into the luminal epithelial and myoepithelial cells. Branching of TEBs involves bifurcation for separation of the direction of elongation. (b) Side branching. Quiescent ductal structures might initiate side branching, possibly through transient epithelium-to-mesenchyme transition of the luminal epithelial cells, associated with outward migration through the myoepithelial cell layer and degradation of the surrounding basement membrane. These processes can also be distinguished in that elongation of the primary branch and side branches involve different metalloproteinases.



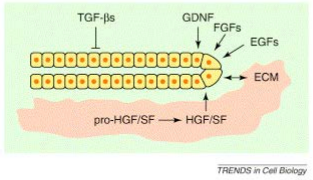


Fig. I. Regulation of tubulogenesis. Mesenchymal cells secrete HGF/SF as an inactive single-chain precursor (pro-HGF/SF) that is cleaved and thus activated by extracellular proteases. Interaction with heparan sulfate proteoglycans restricts HGF/SF action to nearby Met-expressing cells (reviewed in [3]). HGF/SF cooperates with a variety of different ligands such as FGF- and EGF-family members and GDNF to stimulate branching morphogenesis. Epithelial interactions with the surrounding extracellular matrix (ECM) are also required. On the other hand, the ECM is modified by the secretion of new ECM components and ECM-degrading proteases. Branching morphogenesis is probably inhibited in mature tubules by the action of ligands such as TGF-ß. Some naturally occurring truncated forms of HGF/SF, generated by splicing, also directly antagonize HGF/SF signaling [2].

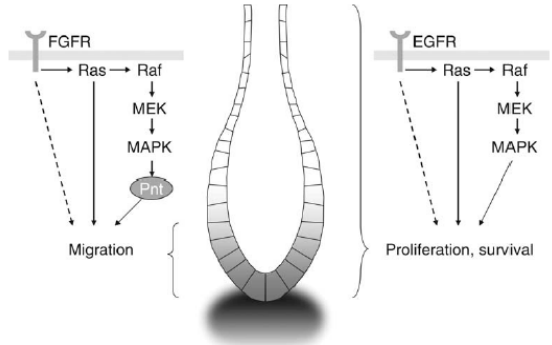


Figure 2 Differential RTK signaling pathways control cell proliferation and migration in *Drosophila* tracheal air sac. Coordination of cell behavior in the outgrowth of a fly larval tracheal air sac. FGF signaling is required for migration of cells at the leading edge. Bnl/Fgf ligands are expressed in the cells adjacent (*dark gray cloud*) to the tracheal epithelium. Activation of Btl/Fgfr function in cells at the leading edge (*gray*) triggers changes in the cytoskeleton and cell shapes, and thus causes cells to migrate toward the source of signal. EGF signaling is required for all epithelial cells to proliferate and/or to survive and thus plays a permissive role in air sac outgrowth. Although sharing major downstream effectors, these two RTKs use different molecules to ensure distinct cellular effects. Adapted from Cabernard and Affolter [35].

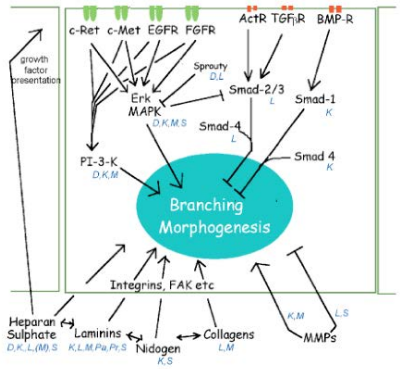


Figure 5. A summary diagram for regulation of branching morphogenesis by extracellular factors. This drawing depicts a generalised cell subject to all of the main extracellular regulators discussed in the text. The blue letters after each named signal refers to organs in which the pathway is known to regulate in the manner indicated; D, Drosophila tracheae; K, kidney; L, lung; M, mammary gland; Pa, pancreas; Pr, prostate; S, salivary gland. Parentheses mean that the data for that organ come only from cell-line models. Encouragement of branching is indicated by an arrow and inhibition of branching by a block.

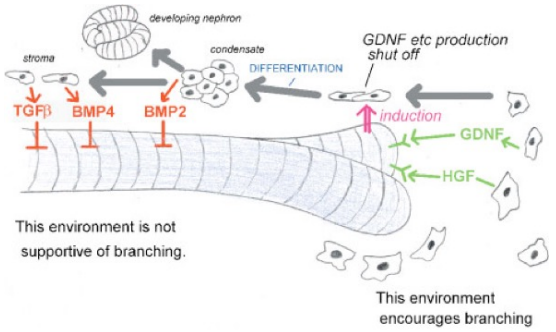
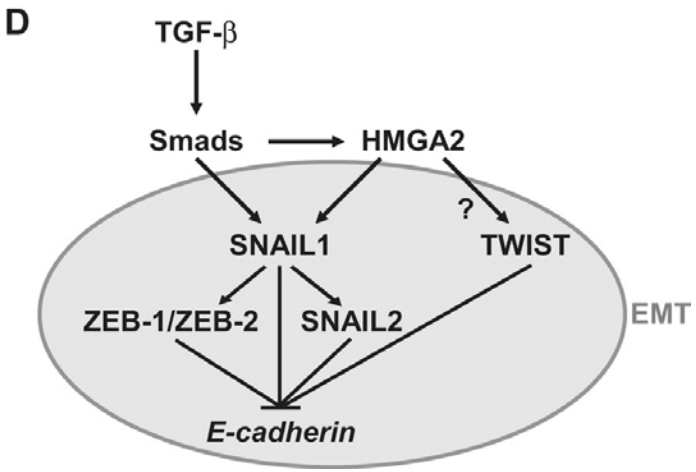


Figure 4.Use of delayed negative feedback to restrict branching activity to collecting duct tips in developing kidneys. Virgin mesechyme produces factors that encourage branching, but contact with collecting ducts induces it to differentiate, a process that results in cessation of production of positive factors and, later, staged production of branch-inhibiting proteins. Downward arrow indicates positive influence and a block indicates inhibition of branching (even if elongation is encouraged; see text).



HMGA2 and Smads Co-regulate SNAIL1 Expression during Induction of Epithelial-to-Mesenchymal Transition
Sylvie Thuault, E-Jean Tan, Hector Peinado, Amparo Cano, Carl-Henrik Heldin, and Aristidis Moustakas
J. Biol. Chem., Vol. 283, Issue 48, 33437-33446, November 28, 2008

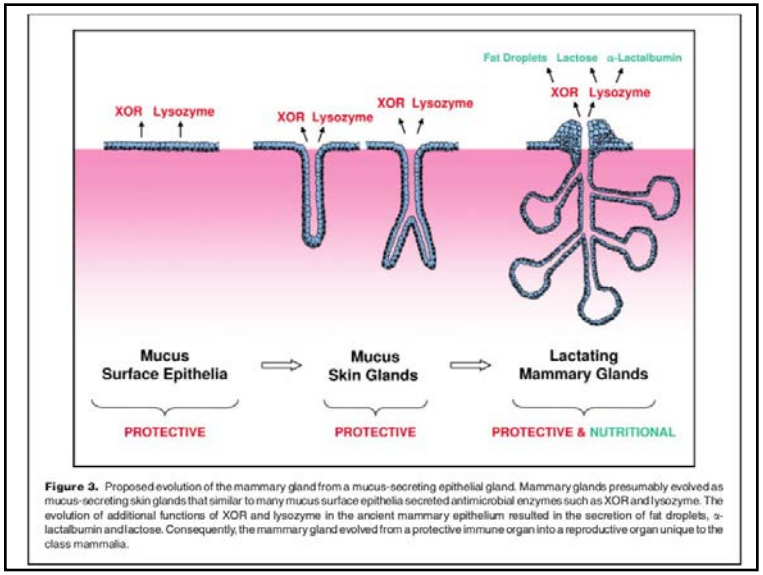


Figure 3. Proposed evolution of the mammary gland from a mucus-secreting epithelial gland. Mammary glands presumably evolved as mucus-secreting skin glands that similar to many mucus surface epithelia secreted antimicrobial enzymes such as XOR and lysozyme. The evolution of additional functions of XOR and lysozyme in the ancient mammary epithelium resulted in the secretion of fat droplets, α-lactalbumin and lactose. Consequently, the mammary gland evolved from a protective immune organ into a reproductive organ unique to the class mammalia.

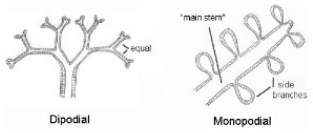
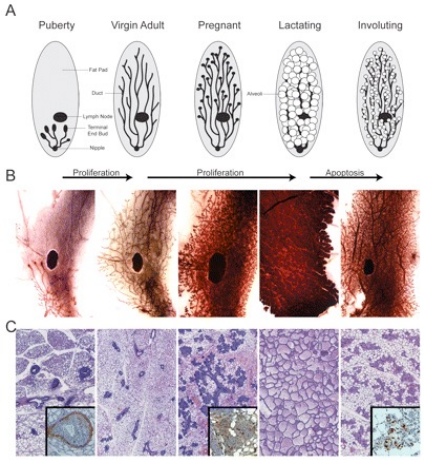


Figure 2. Dipodial and monopodial branching. Many organs use both during their development, typically setting up a large tree by dipodial branching and then using the monopodial pattern of development to attach specialized tissues (e.g. mammary alveoli) to its finest branches.

Figure 3. Branching morphogenesis in the developing kidney. To obtain these images, E11 mouse kidneys were isolated from embryos and were cultured on polycarbonate filters at the gas–medium interface for the times shown. They were then stained for the basement membrane component, laminin.



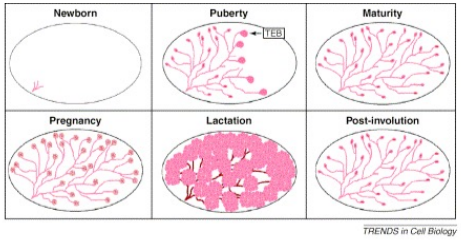
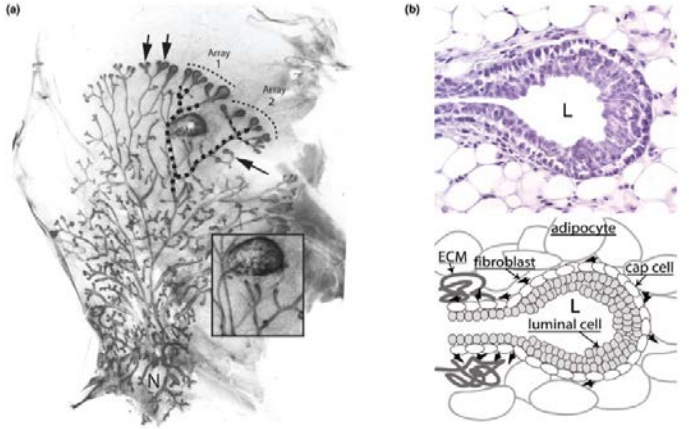
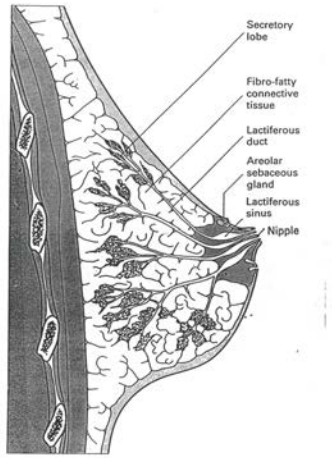
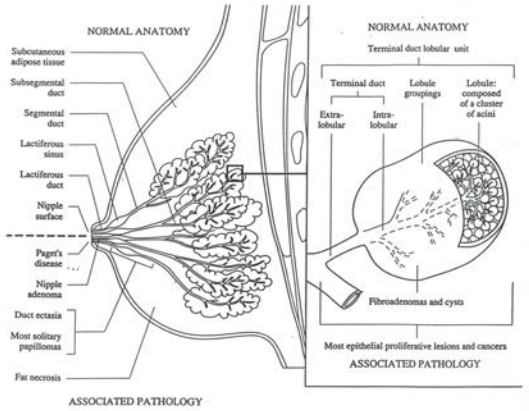


Fig. 1. Postnatal mammary gland development. Prior to birth, mammary placodes and early anlage form in an organ-autonomous fashion, whereas postnatal development occurs under the control of systemic mammogenic hormones. Ductal elongation during puberty occurs through extensive cell division at terminal 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, when 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 prepregnant state.



Photomicrographs illustrating motility and histoarchitecture of end buds. (a) Natural and experimentally induced motility 'behavior' of end buds in the mammary ductal system of a 5-week-old nulliparous mouse. The 'open' ductal architecture of the mammary tree leaves 80% or more of the gland epithelium-free. Large terminal end buds identify the most actively growing region of the gland (top arrows), and progressively smaller lateral end buds extend to each side of the center, indicating slowed forward growth as the end bud encounters a thinning fat pad. End buds may also reverse direction to grow back into accommodating stroma (side arrow). Bifurcating end buds (top arrows) are arrayed along the growth front. Original magnification approx. ×12. (b) Cross-section through end bud with accompanying diagram. End buds are bilayered structures; an outer layer of myoepithelial progenitor cells (cap cells) overlays a multilayered mass of luminal cells fated to form the walls of the ductal lumen (L). Stained with hematoxylin and eosin. Original magnification approx. ×900.





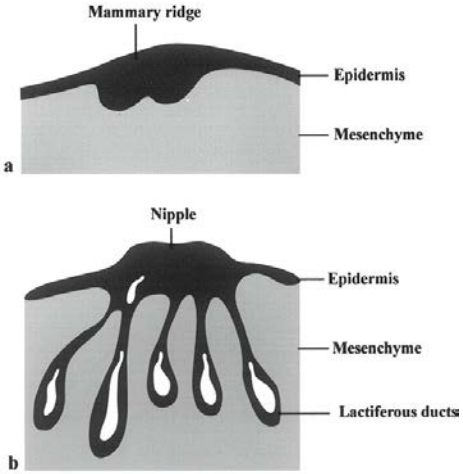


Figure 6 Schematic drawing of embryonic human breast development. (a) Early epidermal mammary ridge. (b) From the mammary ridge, solid epithelial sprouts grow into underlying mesenchyme to form lactiferous ducts. (Adapted from Ronnov-Jessen et al. 1996, with permission.)

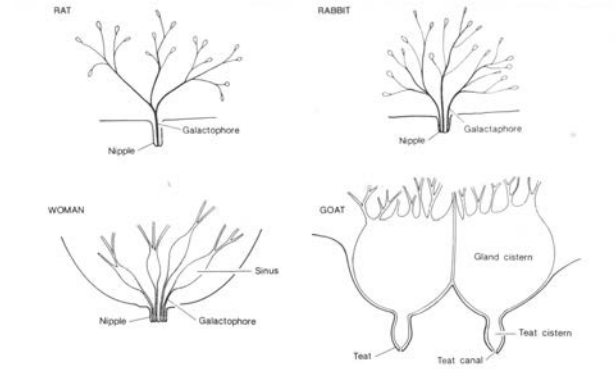


FIG. 1. Examples of the species variation in the arrangement of milk ducts. In the rat the milk ducts join to form a single galactophore within the nipple, whereas in the rabbit the ducts remain separate, such that there are six to eight galactophores opening onto the surface. In the woman, 12 to 20 galactophores are present, and these have a further modification in the form of an enlarged lumen (sinus) close to the nipple. In the goat (as in other ruminants) there is extreme specialization of the duct system to increase storage capacity, with the milk ducts opening into a large gland cistern. The gland cistern connects with a smaller teat cistern, which opens onto the surface via the teat canal. (Based on ref. 241.)

TABLE 17–2. Some Anatomic Characteristics of the Female Reproductive Organs in Laboratory Mammals

Species	Some Anatomic Characteristics	Number of Mammary Glands
Chinchilla	Mesosalpinx tends to enclose ovary; accessory corpora lutea during pregnancy; vaginal closure membrane	6 (2 inguinal and 4 lateral thoracic)
Dog	Ovary is flattened and completely enclosed in a roomy peritoneal pouch. Slender uterine horns are long and straight. Cervix is a short, thick-walled segment. Vagina is wider above (cranially) than below.	10 (arranged in two ventrolateral series)
Guinea pig	Two internal cervical openings, but only one common external os. Intestinal and urinary tracts open into a groove (the "fossa anovaginourethralis"). Lower end of the vagina is closed by an epithelial membrane, but opens periodically at estrus and during parturition.	2 (inguinal)
Hamster	Ovary is compact and encapsulated; oviducts and uterus similar to those of the mouse. Two cervical canals remain separate for about two-thirds of the length of the cervix, but then fuse. Vagina has a mucified type of epithelium; its wall contains urethral glands similar to those in the male prostate.	12 or 14 (thoracic and abdominal)
Mink	Ovary has abundant interstitial tissue; fimbriae only slightly developed. Uterine glands are sparse. External os of the cervix is a transverse uterine slit. Vagina is long and has a transverse fold across its dorsal wall.	6 or 8 (30% nonfunctional)
Mouse	Ovaries lie ventrally just below the kidneys within transparent ovarian capsules. A narrow, tunnel-like passage connects the periovarial space with the peritoneal cavity.	10 (5 thoracic and 4 abdominino-inguinal)
Rabbit	Complete duplication of the uterine segments; two long uterine horns and two entirely separate cervical canals, each of which has an internal and external os. Endometrium arranged in numerous transverse and longitudinal folds, which are particularly prominent along the mesometrial borders. Cervical canals have a narrower lumen and a more extensively folded mucous membrane than the uterine horns. Vaginal portions of the cervical segments are surrounded by a complete ring of fornices.	8 (arranged in ventrolateral series)
Rat	Ovary lies within ovarian bursa. Periovarial space opens into the peritoneal cavity through a slit on the antimesometrial side of the bursa at the tip of each uterine horn.	12 (two ventrolateral series along thoracic and inguinal regions)

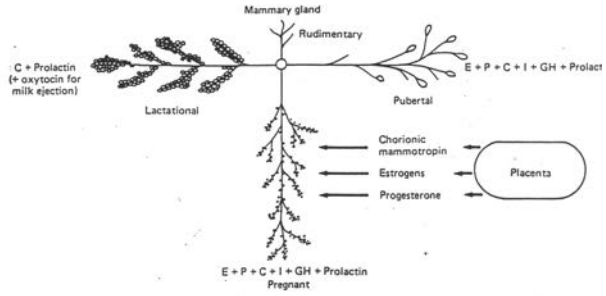


Figure 23–41. Hormonal control of breast development and lactation in rats. Estrogens (E) plus some progesterone (P) and some prolactin in the presence of glucocorticoids (C), insulin (I), and growth hormone (GH) cause duct proliferation and growth at puberty ***(right)***. During pregnancy, all of these hormones bring about full alveolar development and some milk secretion ***(below)***. After delivery, increased secretion of prolactin and a decline in estrogen and progesterone levels bring about copious secretion and, in the presence of oxytocin, ejection of milk ***(left)***. Chorionic mammotropin is the lactogenic hormone presumably secreted by the placenta in rats and is analogous to hCS. It supplements the action of prolactin.

Induction of Mammary Gland Development in Estrogen Receptor- Knockout Mice

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Mammary glands from the estrogen receptor- 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 mammotropic 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, heterozygous ER pituitary isograft under the renal capsule of 25-day-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 luteotropic in the ERKO ovary. By contrast, ovariectomy at the time of pituitary grafting prevented mammary gland development in ERKO mice despite elevated PRL levels. Hormone replacement using pellet implants demonstrated that pharmacological 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 ovarian progesterone levels. Therefore, the manifestation of the ERKO mammary phenotype appears due to the lack of direct estrogen action at the mammary gland and an indirect contributory role of estrogen signaling at the hypothalamic/pituitary axis.

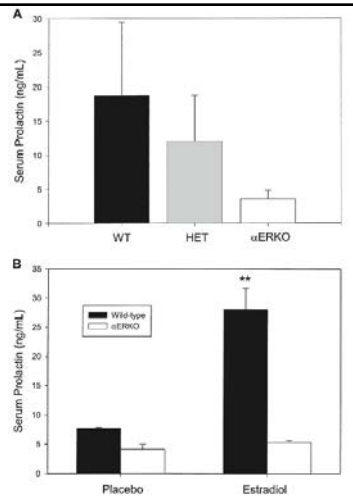


Figure 1. Serum PRL levels in untreated mice and in ovariectomized mice treated with estradiol. A, Serum PRL was measured in untreated WT (n = 8), heterozygous ERa (HET, n = 7) and aERKO (n = 6) mice and expressed as the mean ± SEM. B, Adult WT (n = 5) and aERKO (n = 5) mice were ovariectomized and implanted (sc) with a placebo or estradiol (0.05 mg) pellet. After 21 days, blood was collected and serum PRL was measured and expressed as mean ± SEM (A) One-way ANOVA revealed no significant differences between genotypes. B, Two-way ANOVA revealed significant genotype, treatment and interactive effects ($P < 0.001$). By Fisher's PLSD, **, $P < 0.001$ vs. all other groups.

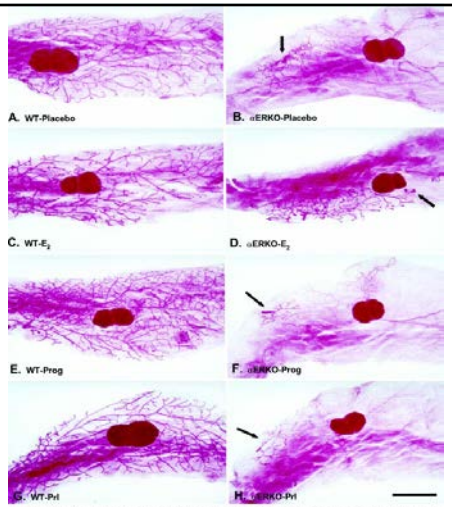
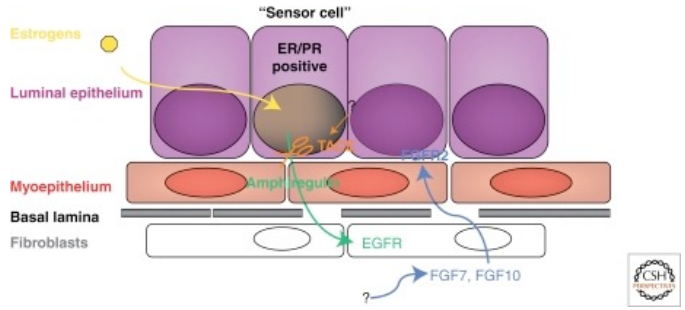


Figure 5. Whole mount analyses of mammary glands from ovariectomized mice treated with various hormones. Twelve-week old WT and ERKO mice were ovariectomized and implanted (sc) with estradiol (E2, 2 x 0.1 mg), progesterone (Prog, 1 x 35 mg) or PRL (Prl, 2 x 5 mg) hormone pellets in various combinations as summarized in Table 1. After 21 days, the mammary glands were excised and stained with carmine red. The arrow in panels B, F, H, and L indicate the unstimulated mammary ductal rudiment. The arrow in panels D, J, N, and P indicate ductal expansion and possible terminal end buds. Scale bar, 2.5 mm for all panels.



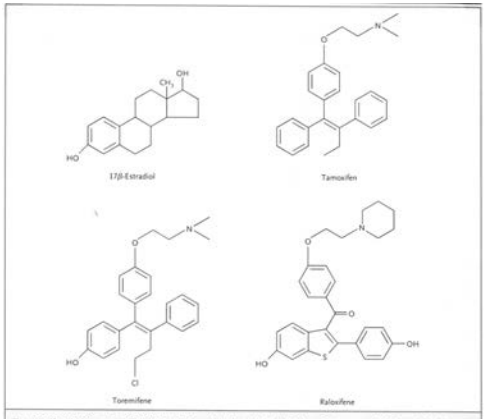


Figure 1. Chemical Structure of 17β-Estradiol, the Main Physiologically Relevant Estrogen, and the Three SERMs Approved by the Food and Drug Administration.

17β-Estradiol has a cyclophenanthrene structure, whereas tamoxifen and toremifene have a triphenylethylene structure and raloxifene has a benzothiophene structure. Although the primary structure of these SERMs differs strikingly from that of estrogens, they have a conformation that allows them to bind to the ligand-binding domain of the estrogen receptor.

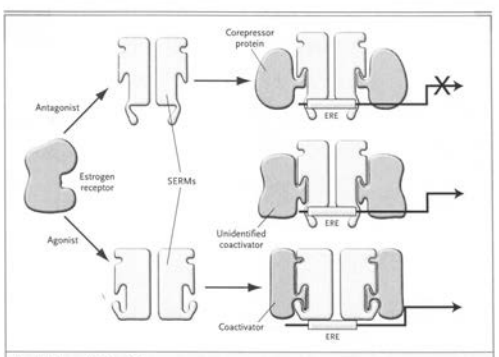


Figure 2. Estrogen-Receptor Action.

On binding an agonist or an antagonist, the estrogen receptor (the α or β isoform) undergoes a conformational change that permits its spontaneous dimerization and facilitates the subsequent interaction of the dimer with estrogen response elements (EREs) located within target genes. It has been determined that estrogen facilitates the interaction of the estrogen receptor with coactivators. An antagonist-activated estrogen receptor, on the other hand, interacts preferentially with a corepressor protein. The binding of different SERMs to the receptor permits it to adopt conformational states distinct from that induced by classic agonists or antagonists. The weight of available evidence suggests that the structure of some SERM–estrogen-receptor complexes favors corepressor recruitment and that of others favors some affinity for known coactivators. Some SERMs may also facilitate the interaction of the estrogen receptor with yet-to-be-identified coactivators with which it would not normally couple. The implication of this model is that SERM activity will be influenced by the relative levels of expression of the cofactors (corepressors and coactivators) in target cells.

Table 1. Comparison of Selected Actions and Side Effects of Estrogen and Clinically Available SERMs.*

Side Effect	Estrogen	Tamoxifen	Toremifene	Raloxifene
Hot flashes	↓↓↓	↑†	↑†	↑†
Uterine bleeding	↑↑↑	↑	↑	↔
Risk of endometrial cancer	↑↑‡	↑	?	↔
Prevention of postmenopausal bone loss	↑↑↑	↑	↔	↑↑
Risk of breast cancer	↑↑	↓↓	↓↓§	↓↓
Favorable pattern of serum lipids	↑↑↑¶	↑	↑↑	↑
Venous thrombosis	↑↑	↑↑	?	↑↑

* Ascending arrows indicate that the drug increases the effect, and descending arrows that it decreases the effect. Horizontal arrows indicate no change. The number of arrows indicates the size of the change.
† In perimenopausal women the action would be ↑↑.
‡ This effect can be prevented by concurrent treatment with a progestin.
§ The only available data are for inhibition of breast-cancer growth.
¶ This effect may be attenuated by concurrent treatment with androgen-derived progestins.[20]

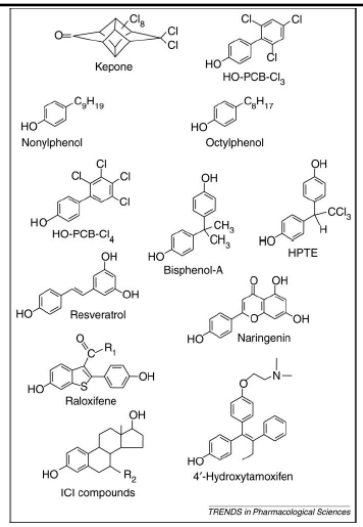
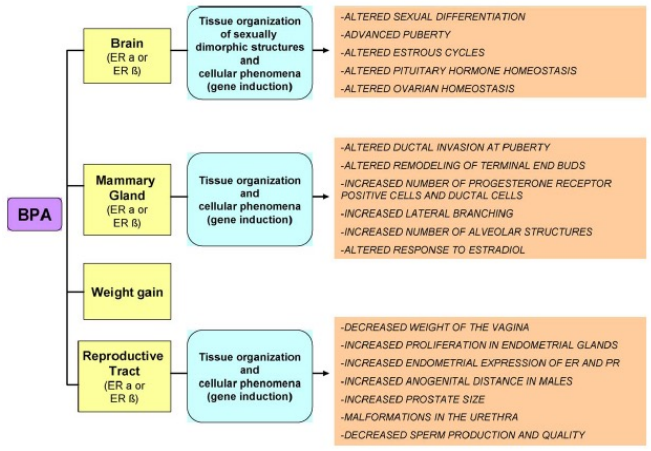


Figure 1. Structures of E2, antiestrogens, xenoestrogens and phytoestrogens. These compounds were selected based on their reported estrogenic activities and structural diversity.



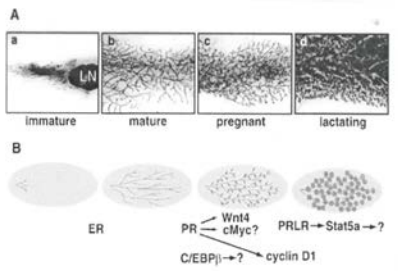
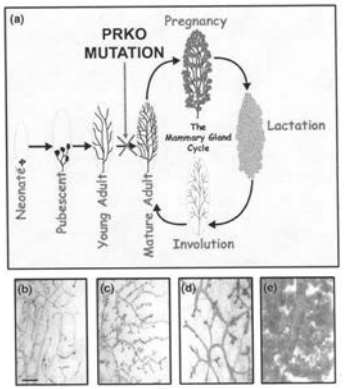


Figure 1. (*A*) Whole-mount analysis of mammary tissue from 4-week-old virgin (*a*), 10-week-old virgin (*b*), day 11 pregnant (*c*), and lactating (*d*) mice. (LN) Lymph node. All pictures are taken at the same magnification. (*B*) Schematic drawings of the mammary fat pad and ductal and alveolar development. A simple ductal tree occupies the fat pad in the immature virgin. The fat pad is filled in mature virgins and substantial side-branching has taken place. Alveolar development occurs during pregnancy and is completed during lactation. Proteins that control defined stages of mammary development (as determined in gene knockout mice) are shown below the corresponding developmental stage. The arrows indicate likely genetic pathways. Although *c-myc* expression is induced by progesterone, it is not clear whether this occurs in MECs. Downstream targets of Stat5a and C/EBPβ in mammary tissue are not known. (ER) Estrogen receptor; (PR) Progesterone receptor; (PRLR) Prolactin receptor. The images are deposited at the NIH HistoBank (http://histology.nih.gov) and can be viewed under the following accession nos.: mammary tissue from an immature virgin, 1345; mammary tissue from a mature virgin, 1346; mammary tissue from day 11 of pregnancy, 1347; mammary tissue from lactation, 1348.

Figure 1



Progesterone receptor function is required for mammary ductal side-branching and alveologenesis. **(a)** The salient postnatal stages of mammary gland development. Whole mounts of **(b)** transplanted progesterone receptor knockout (PRKO) mammary glands and **(c)** wild-type mammary glands taken from a nulliparous host, and **(d)** transplanted PRKO mammary glands and **(e)** wild-type mammary glands taken from a parous host. Scale bar in (b) denotes 500 μm and applies to all whole mounts. Adapted from Lydon *et al.* [5].

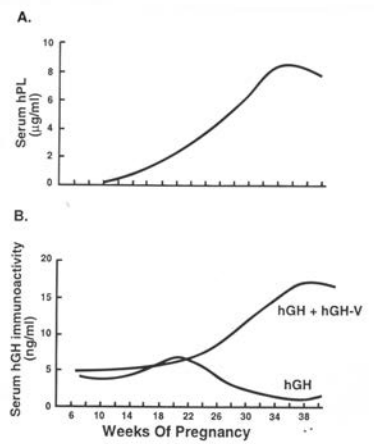
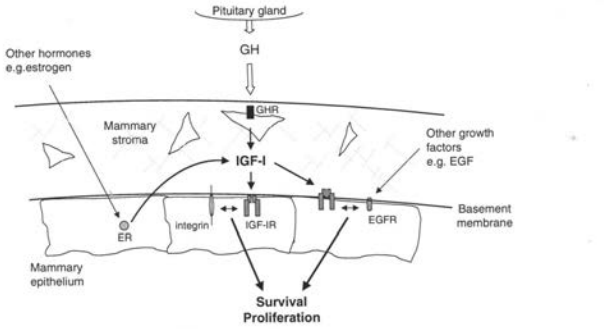


FIG. 4. Changes in human serum placental lactogen (*hPL*, A) and growth hormone (*hGH*) and growth hormone variant (*hGH-V*, B) during pregnancy. Note the decrease in pituitary growth hormone levels and the increase in total growth hormone immunoreactivity, which can be attributed to placental growth hormone variant during the latter half of pregnancy. (Adapted from ref. 153.)



Insulin-like growth factor-I (IGF-I) signalling networks in the mammary gland. Growth hormone (GH) acting on the growth hormone receptor (GHR) on stromal cells induces IGF-I release, which subsequently acts at the type I insulin-like growth factor receptor (IGF-IR) on epithelial cells to mediate survival and proliferation. Oestrogen can also induce IGF-I expression, which may then act on adjacent mammary epithelial cells. The basement membrane provides an interface between stroma and epithelial cells, and it can contribute to the signals required for mammary development *via* integrin receptors. Epidermal growth factor (EGF) can synergize with IGF-I, and IGF-I can transactivate the EGF receptor (EGFR). ER, oestrogen receptor.

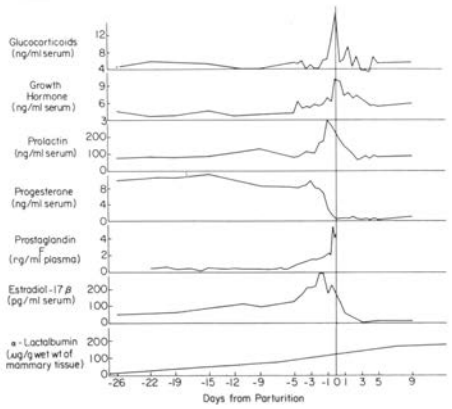


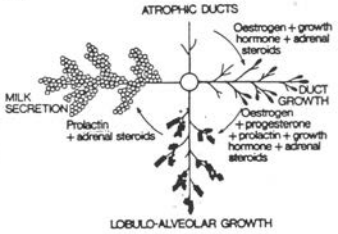
FIG. 1. Changes in concentrations of α-lactalbumin in mammary tissue and hormones in serum or plasma of cows during the periparturient period. (Modified from ref. 18.)

Hormonal Requirements of Breast Development

A. E + F + GH ductal development

B. E + P + F + GH + PROLACTIN lobulo-alveolar development

C. F + PROLACTIN lactation



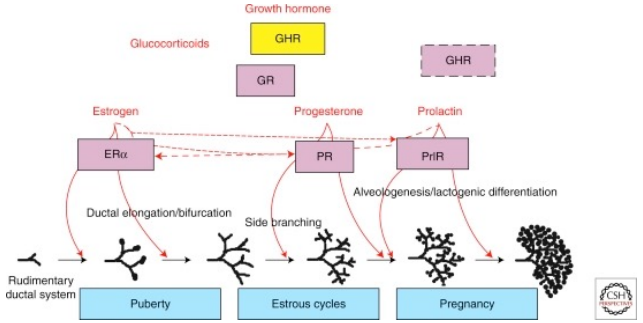
The multihormonal interaction on the growth of the mammary gland and in the initiation of lactogenesis and lactation, delineated in the hypophysectomized-ovariectomized-adrenalectomized rat. (Reproduced from Lyons WR: Hormonal synergism in mammary growth. Proc R Soc (B) 149:303, 1958.)

Hormonal Requirements of Organ Culture

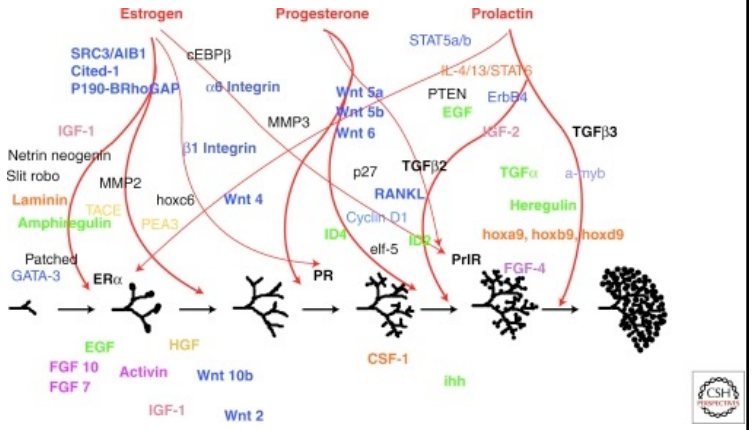
A. Must have insulin

B. Insulin + cortisol (F) rough endoplasmic reticulum

C. Insulin + F + prolactin secretory (protein and lipid droplets)



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

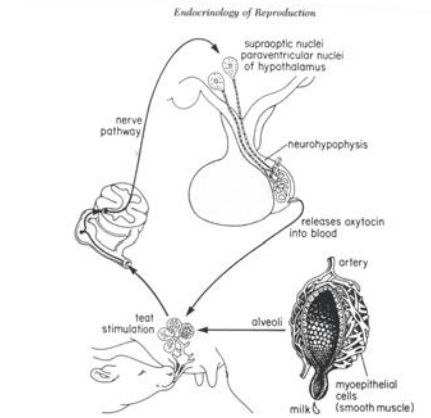


FIG. 5–6. Milk let-down may be considered a neuroendocrine reflex. The stimulation of the teat induces a neural signal to the hypothalamus to release oxytocin from the neurohypophysis, which stimulates the myoepithelial cells to constrict the alveoli resulting in milk secretion.

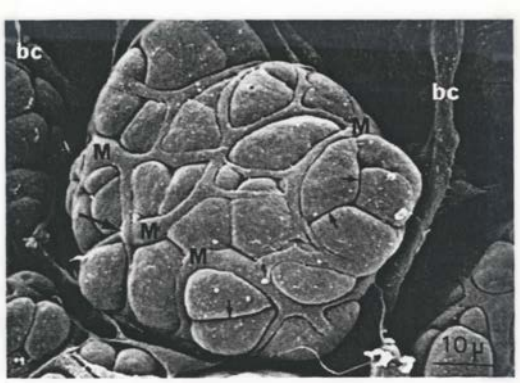


FIG. 2. Scanning electron micrograph of rat mammary gland to show the network of myoepithelial cells (each separate cell labeled **M**) surrounding an alveolus. The large arrow indicates overlap of two adjacent myoepithelial cells; small arrows mark the boundaries of adjacent secretory cells. (bc) Blood capillary. (From ref. 242.)

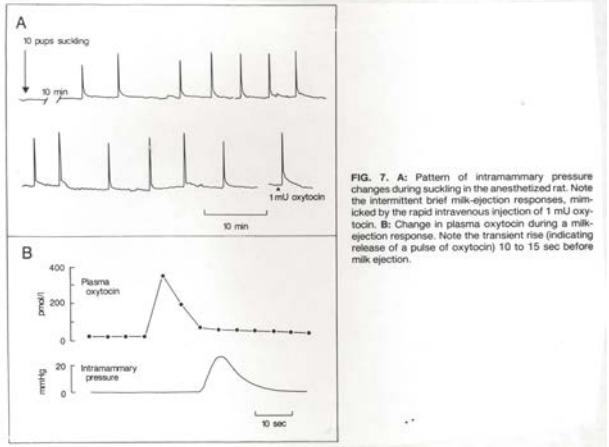


FIG. 7. A: Pattern of intramammary pressure changes during suckling in the anesthetized rat. Note the intermittent brief milk-ejection responses, mimicked by the rapid intravenous injection of 1 mU oxytocin. **B:** Change in plasma oxytocin during a milk-ejection response. Note the transient rise (indicating release of a pulse of oxytocin) 10 to 15 sec before milk ejection.

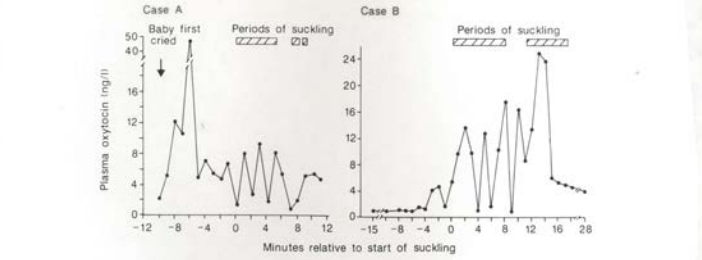


FIG. 8. Plasma oxytocin profiles in the woman during suckling. In Case A (representing the most typical pattern) there was a release of oxytocin before the baby was applied to the breast, presumably reflecting a conditioned release triggered by the sound of the baby crying. Case B shows an example where oxytocin release occurred only after the onset of suckling. The marked fluctuations in oxytocin levels seen in both examples probably reflect the pulsatile pattern of hormone release. (Redrawn from ref. 71.)

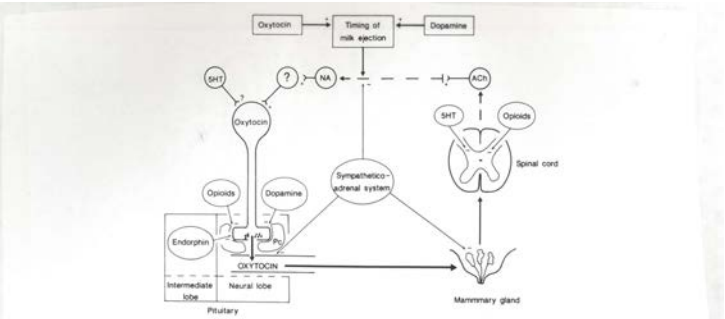


FIG. 23. Schematic summary of some neurotransmitters and neuromodulators involved in the milk-ejection reflex in the rat. Noradrenaline (NA) and acetylcholine (ACh) are both implicated in the neural arc of the reflex. Their sites of action are unknown but are unlikely to be at the level of the oxytocin neurons. 5-Hydroxytryptamine- (5HT) containing neurons project to the vicinity of the oxytocin neurons but their role is unclear. Dopamine and oxytocin both seem to influence the timing of the reflex but relatively little is known about how they influence this complex process. A variety of substances are known to inhibit the reflex. At present the delineated sites of inhibition are at the proximal and distal ends of the neural arc. Opioids and 5HT both suppress the milk-ejection reflex at the level of the spinal cord. At the distal end, a powerful locus of inhibition is provided on oxytocin nerve terminals in the neurohypophysis. Opioids and catecholamines are present and have been shown to inhibit release. Whether they act on the terminals directly, through the adjacent pituicytes (P_C), or modify diffusion or blood flow through the lobe remains to be established. Finally, the sympathetico-adrenal system is known to influence the milk-ejection reflex both at the level of the mammary gland and centrally. (See text for further details.)

Table 23–9. Composition of colostrum and milk.[1]
(Units are weight per deciliter.)

Component	Human Colostrum	Human Milk	Cows' Milk
Water, g	. . .	88	88
Lactose, g	5.3	6.8	5.0
Protein, g	2.7	1.2	3.3
Casein:lactalbumin ratio	. . .	1:2	3:1
Fat, g	2.9	3.8	3.7
Linoleic acid	. . .	8.3% of fat	1.6% of fat
Sodium, mg	92	15	58
Potassium, mg	55	55	138
Chloride, mg	117	43	103
Calcium, mg	31	33	125
Magnesium, mg	4	4	12
Phosphorus, mg	14	15	100
Iron, mg	0.09[2]	0.15[2]	0.10[2]
Vit A, μg	89	53	34
Vit D, μg	. . .	0.03[2]	0.06[2]
Thiamine, μg	15	16	42
Riboflavin, μg	30	43	157
Nicotinic acid, μg	75	172	85
Ascorbic acid, mg	4.4[2]	4.3[2]	1.6[2]

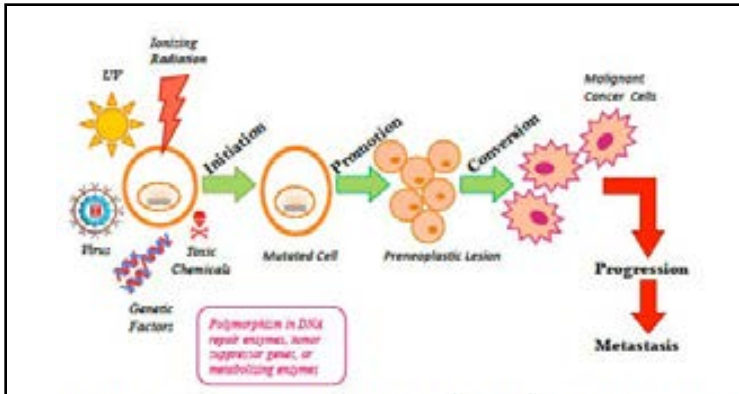
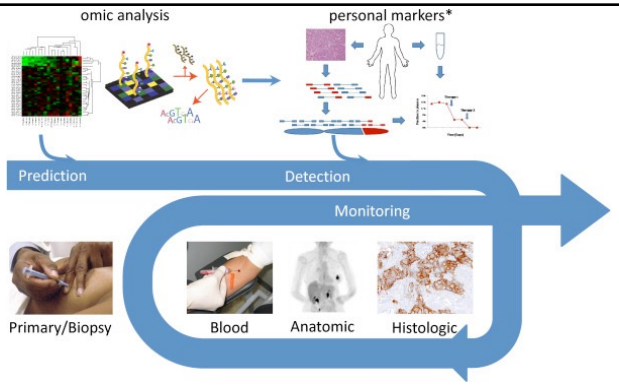
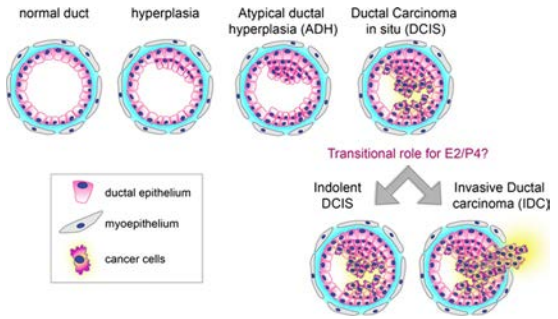


Figure 1. Steps of Carcinogenesis. Initiation requires the exposure of normal cells to carcinogenic factors. This produces genetic damage that, if not repaired, results in irreversible cellular mutations. Mutated cell has an altered response to its environment and a selective growth advantage. Carcinogenic effects result in irreversible cellular mutations leading to deregulations of oncogenes, cell cycle, and DNA transcription. These alterations mediate uncontrolled growth that can progress to tumor invasion into local tissues and the development of metastases.



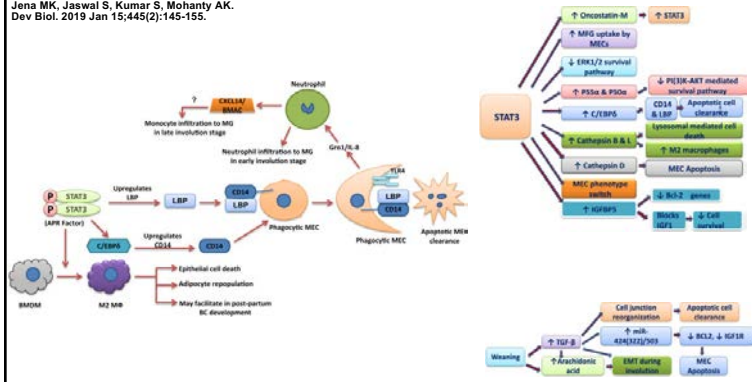
Predicting, detecting and monitoring metastatic breast cancer. The figure portrays an omic-signature-based screening strategy for earlier detection of metastasis-prone lesions and high sensitivity detection of residual disease. This strategy is based on the premise that molecular features can be used 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].

The Emerging Roles of Steroid Hormone Receptors in Ductal Carcinoma in Situ (DCIS) of the Breast.
Villanueva H, Grimm S, Dhamne S, Rajapakshe K, Visbal A, Davis CM, Ehli EA, Hartig SM, Coarfa C, Edwards DP.
J Mammary Gland Biol Neoplasia. 2018 Dec;23(4):237-248.

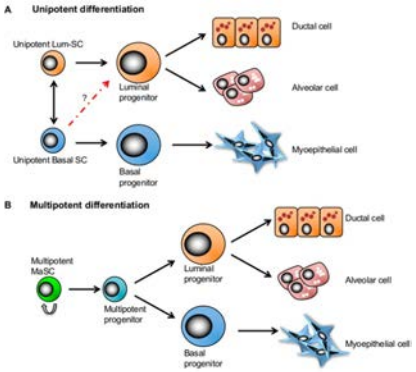


Stages of breast cancer progression. Simplified model of stages of breast cancer progression from normal ductal morphology, advancement to hyperplasia, non-obligate progression through atypical ductal hyperplasia, DCIS, and either arrest at in situ carcinoma or transition to IBC.

Molecular mechanism of mammary gland involution: An update.
Jena MK, Jaswal S, Kumar S, Mohanty AK.
Dev Biol. 2019 Jan 15;445(2):145-155.



Plasticity and Potency of Mammary Stem Cell Subsets During Mammary Gland Development.
Lee E, Piranlioglu R, Wicha MS, Korkaya H.
Int J Mol Sci. 2019 May 13;20(9).



Regulation of mammary epithelial cell homeostasis by lncRNAs.
Shore AN, Rosen JM.
Int J Biochem Cell Biol. 2014 Sep;54:318-30.

LncRNAs associated with mammary development and breast cancer.

LncRNA	Expression	Function	Mechanism
Mammary development			
mPINC	Highly expressed in alveolar cells of pregnant and involuting gland	Inhibits lactogenic differentiation, alternative splice forms regulate cell cycle and survival	Interacts with PRC2
Zfas1	Highly expressed in alveolar and ductal cells of pregnant and involuting gland	Inhibits proliferation and lactogenic differentiation	Unknown
Breast cancer			
BC200	Increased in invasive breast cancer and HG-DCIS	Oncogenic	Translational repression[a]
GAS5	Decreased in breast tumors and breast cancer cell lines	Tumor suppressive-induces growth arrest and apoptosis	Binds and inhibits GR from activating target genes[a]
HOTAIR	Increased in metastatic breast tumors, strong predictor of metastasis and death	Oncogenic-promotes invasion and metastasis	Silences genes in *trans* epigenetically
H19	Increased in stromal cells of breast tumors	Oncogenic-promotes proliferation and tumor growthTumor suppressive-restricts growth[a]	Unknown
LncRNA-JADE	Increased in breast tumors	Oncogenic-promotes proliferation and survival	Binds BRCA1 and enhances transcription of *Jade1* in DDR
LSINCT5	Increased in breast tumors and breast cancer cell lines	Oncogenic-promotes proliferation	Unknown
MALAT1	Increased in breast tumors, mutations in *MALAT1* associated with Luminal B subtype and poor clinical outcome	Oncogenic-promotes metastasis[a]	RNA splicing, regulation of gene expression[a]
MEG3	Expressed in mammary gland, not detected in breast cancer cell lines	Tumor suppressive-inhibits growth, induces apoptosis[a]	Unknown
PTENP1	Focally deleted in breast cancer, undergoes somatic hypermethylation in breast cancer cell lines	Tumor suppressive-represses proliferation[a]	Binds and inhibits miRNAs from targeting and repressing *PTEN*
SRA	Increased in breast tumors, associated with PR+ breast tumors	Oncogenic-promotes proliferation, metastasis Tumor suppressive-induces apoptosis	Co-activator of hormone receptors, scaffold for many transcription factors[a]
treRNA	Increased in paired breast cancer primary and lymph-node metastasis samples	Oncogenic-promotes EMT, invasion and metastasis	Enhances transcription of EMT regulators, represses translation of epithelial markers
UCA1	Increased in breast tumors, negatively correlates with p27 protein levels	Oncogenic-promotes proliferation	Binds hnRNP I, thereby preventing binding and translation of p27
ZFAS1	Decreased in invasive ductal carcinoma	Tumor suppressive-inhibits proliferation	Unknown

[a] Data not shown in breast cancer cells.

"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