Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Male Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
January 30, 2024
Week 4

Male Reproductive Tract Development & Function

Embryonic Development and Reproductive Tract Organogenesis
- Overview
- Development of Mullerian Duct vs. Duct Wolffian Duct Derivatives
- Mullerian Inhibiting Substance (MIS)

Male Urogenital Tract Organogenesis
- Prevention of Programmed Cell Death in the Wolffian Duct
- UGS/Prostate/Seminal Vesicle
    1. Prostate Morphogenesis (ductal branching)
    2. Cell-Cell Interactions and Paracrine Factors
    3. Prostate Cancer
- Epididymis/Ductus Deferens
- Role of Androgens (T versus DHT)
    1. Androgen Metabolism
    2. 5 α Reductase Inhibitors
    3. Organ Culture
- Endocrine Disruption

Required Reading

Moses MM and Behringer RR. (2019) Environ Epigenet. 25;5(3):dvz017.

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REVIEW ARTICLE

A gene regulatory network for Müllerian duct regression

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Abstract

Mammalian embryos initially develop progenitor tissues for both male and female reproductive tract organs, known as the Wolffian ducts and the Müllerian ducts, respectively. Ultimately, each individual develops a single set of male or female reproductive tract organs. Therefore, an essential step for sex differentiation is the regression of one duct and growth and differentiation of the other duct. In males, this requires Müllerian duct regression and Wolffian duct growth and differentiation. Müllerian duct regression is induced by the expression of *Amh*, encoding anti-Müllerian hormone, from the fetal testes. Subsequently, receptor-mediated signal transduction in mesenchymal cells surrounding the Müllerian duct epithelium leads to duct elimination. The genes that induce *Amh* transcription and the downstream signaling that results from *Amh* activity form a pathway. However, the molecular details of this pathway are currently unknown. A set of essential genes for AMH pathway function has been identified. More recently, transcriptome analysis of male and female Müllerian duct mesenchyme at an initial stage of regression has identified new genes that may mediate elimination of the Müllerian system. The evidence taken together can be used to generate an initial gene regulatory network describing the *Amh* pathway for Müllerian duct regression. An *Amh* gene regulatory network will be a useful tool to study Müllerian duct regression, sex differentiation, and its relationship to environmental influences.

Key words: sex differentiation; anti-Müllerian hormone; transcription

Introduction

Classic experiments by Alfred Jost in fetal rabbits identified a Müllerian inhibitor associated with the testis that was required for the regression of the Müllerian ducts [1, 2]. The Müllerian inhibitor was subsequently identified as anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance (MIS) or factor (MIF) [3]. Molecular studies led to the cloning of the genes encoding AMH and its type II receptor (AMHR2) [4–7]. Human studies have identified mutations in the *AMH* and *AMHR2* genes, leading to a condition known as persistent Müllerian duct syndrome (PMDS), a rare recessive intersex condition [8]. Males with PMDS have a uterus and fallopian tubes and can have testicular descent abnormalities. Mutations in these two genes have been shown to result in PMDS in human, mouse, and dog [9]. In addition, gene knockout studies in mice have led to the identification of *Amh*, *Amhr2*, and other genes required for Müllerian duct regression (Table 1). Together, there is now

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Table 1: genes that participate in AMH GRN for Müllerian duct regression

Gene	Mutation	Phenotype	Reference
Acvr1	Conditional KO	Normal MD regression	Orvis *et al.* [36]
Amh	Null	PMDS	Behringer [21]; Arango *et al.* [22]
Amhr2	Null	PMDS	Mishina *et al.* [30]; Jamin *et al.* [31]; Arango *et al.* [17]
Bmpr1a	Conditional KO	PMDS	Jamin *et al.* [31]; Orvis *et al.* [36]
Ctnnb1 (*beta-catenin*)	Conditional KO	PMDS	Kobayashi *et al.* [41]
Gata4	Binding site mutant or deletion	Normal MD regression	Bouchard *et al.* [26]
Mmp2	Null	Normal MD regression	Roberts *et al.* [44]
Smad1/5/8	Conditional KO	PMDS	Orvis *et al.* [36]
Sp7 (*Osterix*)	Null	Delayed MD regression	Mullen *et al.* [42]
Wif1	Null	Normal MD regression	Park *et al.* [46]
Wnt7a	Null	PMDS	Parr and McMahon [34]

Conditional KO, Müllerian duct mesenchyme-specific knockout; MD, Müllerian duct; null, full body knockout; PMDS, persistent Müllerian duct syndrome.

sufficient information to describe the first gene regulatory network (GRN) for Müllerian duct regression. This GRN should provide a useful framework to understand the genetic interactions that lead to Müllerian duct regression, sex differentiation, and its relationship to environmental influences.

Müllerian Ducts Form in Both Male and Female Embryos

During fetal development, the Wolffian and Müllerian ducts form in both males and females. The Müllerian ducts can differentiate into the oviduct, uterus, and a portion of the vaginal canal. The Wolffian ducts can differentiate into vasa deferentia, epididymides, seminal vesicles, and the ejaculatory ducts. The Müllerian duct is a mesoepithelial tissue that requires the Wolffian duct for its development [10, 11]. Müllerian duct formation begins shortly after the Wolffian duct forms, taking place in three phases: initiation, invagination, and elongation [11]. Initiation consists of the specification of mesonephric epithelial cells to become Müllerian duct cells, characterized by *Lhx1* expression, at about E11.75 in mice. During the invagination phase, the *Lhx1*+ cells form an invagination that makes contact with the Wolffian duct. The Müllerian duct then elongates along the Wolffian duct [12]. During Müllerian duct elongation, cell proliferation is observed throughout the duct with more proliferating cells at the growing tip. The Müllerian duct crosses the Wolffian duct at E12.5 to gain a medial position and subsequently fuses with the urogenital sinus at E13.5 [13]. *Wnt9b* is expressed in the Wolffian duct [14]. *Wnt9b* knockout mice form Wolffian ducts but do not elongate the Müllerian ducts, suggesting a molecular mechanism for the requirement of the Wolffian duct for Müllerian duct development [14]. Shortly after the Müllerian ducts form, sex differentiation proceeds. In females, the Müllerian system continues to develop into the uterus, oviducts and a portion of the vagina. However, in males the Müllerian ducts are actively eliminated.

Müllerian Duct Regression Occurs in Male Embryos

A major event in male sex differentiation is Müllerian duct regression. Mesenchyme–epithelia interactions mediate this process to ensure that oviducts and a uterus do not develop within the male body [15]. The cells of the Müllerian duct have a mesoepithelial character during regression [11]. Regression begins shortly after the Müllerian duct connects to the urogenital sinus at E13.5 in the mouse and is completed by birth. Early stages of regression can be observed by tightly condensed mesenchymal cells surrounding the Müllerian duct with intercellular spaces located more radially [16, 17]. Müllerian duct regression is proposed to occur through at least three mechanisms: epithelial cell migration, epithelial to mesenchymal transformation, and apoptosis [18–20]. Apoptosis is initially detected in the rostral region of the Müllerian duct and subsequently in intermediate and caudal regions [18]. Disruptions in the Müllerian duct basement membrane are followed by epithelial cell entrance into the mesenchymal compartment [18]. The regression of the Müllerian duct is proposed to occur in a rostral to caudal manner [15].

Genes That Regulate Müllerian Duct Regression

Anti-Müllerian Hormone (*Amh*)

The first gene knockout in the AMH signaling pathway was in the *Amh* locus [21]. Males homozygous for the targeted *Amh* mutation did not regress the Müllerian ducts, resulting in the development of a uterus, oviducts, and vaginal tissue (Fig. 1). These mutant males had correctly descended testes and Wolffian duct derivatives, including seminal vesicles, vasa deferentia, and epididymides. Only ~10% of the mutant males were fertile. However, sperm from the mutant epididymides were competent to fertilize oocytes *in vitro*. These genetic findings demonstrate that *Amh* produced by the fetal testes is essential for Müllerian duct regression.

The *cis*-regulation of *Amh* transcription was studied *in vitro* and *in vivo* [22, 23]. *In vitro* studies identified interactions of the nuclear hormone receptor steroidogenic factor 1 (SF1) also known as Ad4-binding protein (Ad4BP) or NR5A1 with a 20-bp sequence just upstream of the TATAA motif that is required for *Amh* transcription [23]. About 50-bp upstream of the SF1-binding site is a conserved binding site for the high-mobility group transcription factor SOX9 for activation of *Amh* transcription [24]. SF1- and SOX9-binding site mutations were introduced into the endogenous *Amh* locus by gene targeting in embryonic stem (ES) cells [22]. Surprisingly, males homozygous for the SF1-binding site mutation had normal Müllerian duct regression. Molecular studies showed that *Amh* transcript levels in fetal and postnatal testes were 3-fold lower in comparison to wild type. Thus, the SF1-binding site regulates *Amh* transcript levels. In contrast, males homozygous for the SOX9-binding site mutation were a phenocopy of the *Amh*-null male phenotypes, i.e. PMDS. These findings suggest that SF1 regulates *Amh* transcript

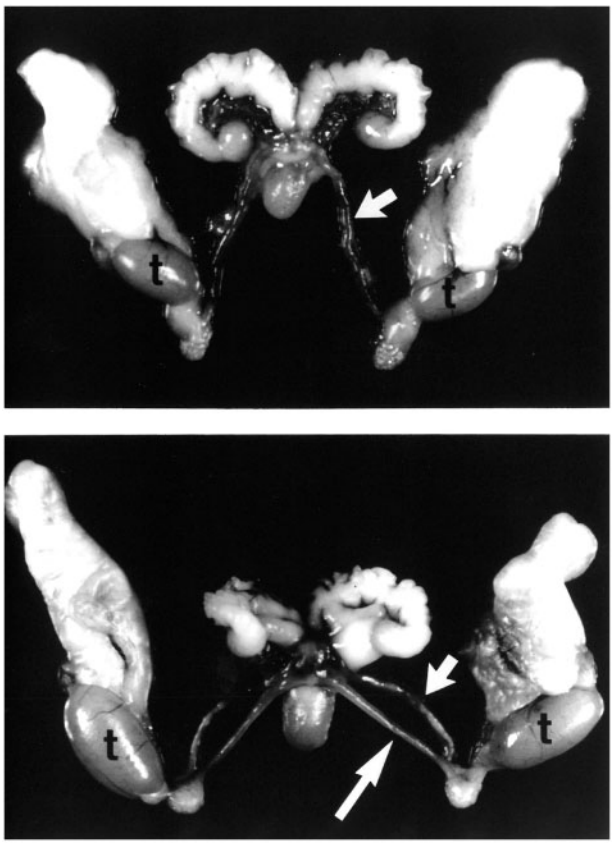

Figure 1: PDMS in the mouse. Dissected reproductive tract organs from control (top) and *Amh* homozygous mutant (bottom) males. In the mutant, the uterine horns (long arrow) and vas deferens (short arrow) parallel each other down to the testes (t) because of a common connective tissue. In this dissection, the connective tissue has been cut to reveal the dual nature of the reproductive tract. Note that because of the physical constraints imposed by the vas deferens, the uterine horns project caudally instead of rostrally. Images from Behringer [21]

levels and SOX9 is essential for the activation of *Amh* transcription.

There are also multiple GATA-binding sites 5′ of the *Amh* transcriptional start site [25]. Recently, male mice homozygous for a 2-bp mutation in one of the GATA-binding sites that abolishes GATA binding (termed *GATAmut*) were generated, using CRISPR genome editing [26]. *GATAmut* homozygotes were found to have a 50% reduction in *Amh* transcripts in their testes [26]. In addition, a 40-bp deletion that included the GATA-binding site and an adjacent SF1-binding site resulted in a 90% reduction in testicular *Amh* transcripts. Although there was a dramatic reduction in *Amh* transcripts, both types of adult mutant males did not retain Müllerian duct derivatives. Apparently, there are still sufficient levels of AMH for Müllerian duct regression in these mouse mutants.

Anti-Müllerian Hormone Receptor 2 (*Amhr2*)

A 21-day-old rat Sertoli cell cDNA, encoding the type II receptor for AMH, *Amhr2*, was first reported by Baarends *et al.* [5]. Their conclusion was based on protein domain structure, indicating a transmembrane serine/threonine kinase receptor and expression localized in the Müllerian duct of male and female fetuses. *Amhr2* transcripts were detected in the mesenchyme adjacent to the Müllerian duct epithelium during embryogenesis, suggesting that the target cell for AMH action is the Müllerian duct mesenchyme. *Amhr2* is also expressed in the fetal and postnatal gonads, specifically in Sertoli and granulosa cells [5, 22, 27–29].

A targeted mutation in the mouse *Amhr2* gene was generated by gene targeting in ES cells [30]. Approximately 4.4 kb of *Amhr2*, including exons 1–6, was deleted, replacing these sequences with a neomycin resistance gene expression cassette. Homozygous mutant males were normal in size, had correctly descended testes, and differentiated derivatives of the Wolffian ducts. All of the homozygous mutant males also developed a uterus, oviducts, and partial vagina in addition to their male reproductive organ system. This was a phenocopy of *Amh*-null male mice. Some of the homozygous mutant males were fertile. In addition, two other *Amhr2* loss-of-function alleles have been generated, including *Cre* and *lacZ* knock-ins [17, 31]. Both alleles result in a persistence of Müllerian duct derivatives in homozygous mutant males. These findings demonstrate that *Amhr2* is required for Müllerian duct regression.

The transcriptional regulation of *Amhr2* has been explored [32, 33]. *Wt1* encodes a zinc finger transcription factor. A microarray analysis of ∼E11.0 *Wt1* wild-type and null urogenital ridges identified *Amhr2* as a candidate gene regulated by *Wt1*. *Wt1* and *Amhr2* were found to be co-expressed in the urogenital ridge. Three WT1-binding sites are within 100-bp of the *Amhr2* transcriptional start site. Biochemical and *in vitro* studies showed that these sequences bind WT1 and act together to regulate *Amhr2* transcription. More studies are required to determine if these sequences are required for *Amhr2* transcription *in vivo* for Müllerian duct regression.

Wnt7a in the Müllerian Duct Epithelium Induces *Amhr2* Expression in Adjacent Mesenchyme

Wnt7a was identified as a gene required for Müllerian duct regression [34]. *Wnt7a* is expressed in the Müllerian duct epithelium in both male and female mice from E12.5 to E14.5 [34]. Expression continues in the Müllerian ducts of females as they differentiate into the oviducts and uterus. *Wnt7a*-null males are born with Müllerian duct derivatives [34]. *In-situ* hybridization analysis of the mutant males showed that *Amhr2* expression was detected in testes but absent in the Müllerian duct mesenchyme [34]. This suggests that the essential function of *Wnt7a* in the *Amh* pathway is to activate *Amhr2* transcription in the Müllerian duct mesenchyme [34]. Thus, *Wnt7a* expressed in the Müllerian duct epithelium signals to the adjacent mesenchyme that induces *Amhr2* transcription making the Müllerian ducts competent to respond to AMH for regression [34].

Bmpr1a and *Acvr1* Encode Type I Receptors That Mediate AMH Signaling

The transforming growth factor (TGF)-beta superfamily consists of >30 cytokines [35]. However, only seven type I receptors have been identified. Thus, TGF-beta family members must share type I receptors to mediate their signal transduction. Two TGF-beta type 1 superfamily receptor genes, *Acvr1* and *Bmpr1a*, have been identified as AMH receptors for Müllerian duct regression [36]. *Acvr1*- and *Bmpr1a*-null mice are embryonic lethal before the Müllerian ducts form [37, 38]. Thus, tissue-specific knockouts were generated. When *Bmpr1a* was knocked out in the Müllerian duct mesenchyme of male mice, Müllerian duct retention was observed in ∼50% of the mutants [31, 36]. This Müllerian duct retention phenotype was identical to the phenotype observed for *Amh* and *Amhr2* knockout males. All males with a conditional knockout of *Acvr1* in the Müllerian duct

mesenchyme had Müllerian duct regression [36]. However, when both *Acvr1* and *Bmpr1a* were both knocked out in the Müllerian duct mesenchyme, 100% of the male mutants retained the Müllerian ducts, forming the uterus and oviducts [36]. These results suggest that *Acvr1* and *Bmpr1a* act redundantly in the *Amh*-induced Müllerian duct regression pathway.

Smad1, *Smad* 5, and *Smad8* Act Redundantly to Mediate AMH Signaling

Smad activity has been shown to contribute to Müllerian duct regression within the *Amh* pathway. *Smad1*, *Smad5*, and *Smad8* (also known as *Smad9*) are all expressed in the Müllerian duct mesenchyme [39, 40]. A conditional knockout of *Smad1* in the Müllerian duct mesenchyme resulted in proper Müllerian duct regression, as did a conditional *Smad1/Smad8* knockout [36]. Conditional knockouts of *Smad1* or *Smad8* combined with a *Smad5* conditional knockout resulted in partial Müllerian duct retention [36]. This consisted of only part of the Müllerian duct being retained: caudally, rostrally, and/or on one side. The triple conditional knockout of *Smad1/Smad5/Smad8*, however, resulted in fully retained Müllerian duct derivatives [36]. Therefore, the three *Smad* genes function redundantly within the pathway, likely downstream of *Acvr1* and *Bmpr1a*.

Beta-Catenin Is Required for MD Regression

Multiple *Wnt* genes are expressed in the mesonephros, including *Wnt4*, *Wnt5a*, *Wnt7a*, and *Wnt9b* (gudmap.org). To determine if the canonical WNT pathway was required for Müllerian duct regression, a Müllerian duct mesenchyme-specific knockout of β-catenin was performed [41]. Loss of beta-catenin in the Müllerian duct mesenchyme resulted in the persistent Müllerian duct phenotype in all male mutants. Additionally, AMH was found to be expressed in the Sertoli cells of the mutant testes, implying that loss of β-catenin disrupts the pathway downstream of AMH expression [41]. These results suggest that β-catenin functions specifically in the Müllerian duct mesenchyme to mediate Müllerian duct regression downstream of AMH signaling [41].

Osterix Is an AMH-Induced Regulator of Müllerian Duct Regression

A transcriptome analysis of RNA-seq data generated from purified E14.5 male and female Müllerian duct mesenchyme identified *Osterix* (*Osx*), also known as *Sp7*, as a male-enriched gene [42]. *Osx* was originally identified as a gene required for osteoblast differentiation [43]. *Osx* is expressed in a sexually dimorphic manner in the Müllerian duct mesenchyme. At E14.5, *Osx* expression is detected in the Müllerian ducts of male fetuses, whereas no expression was detected in the Müllerian ducts of female fetuses [42]. This male-specific expression continues throughout the remaining regression process [42]. In addition, *Osx* expression is lost in male mice lacking *Amhr2* [42]. In contrast, overexpression of human AMH in females, using an *MT-hAMH* transgene stimulates *Osx* transcription [42]. This *Amh*- and *Amhr2*-dependent, sex-specific expression implies a role for *Osx* in the *Amh* signaling pathway. Additionally, loss of *ß-catenin* expression leads to a reduction in *Osx* transcripts, implying that *Osx* is downstream of *ß-catenin* in the regression pathway [42]. *Osx* knockout males have a 24-h delay in Müllerian duct regression [42]. When compared to wild-type males, *Osx* knockout males showed longer and thicker segments of the Müllerian duct at E15.5, E16.5, and E17.5 but complete regression by E18.5 [42]. Taken together, the data suggest that *Osx* is an AMH-induced gene that contributes to Müllerian duct regression.

Other Genes That May Be Involved in Müllerian Duct Regression

Mmp2 (matrix metallopeptidase 2) transcripts are detected in the male Müllerian duct mesenchyme [44]. *Mmp2* expression in the Müllerian duct mesenchyme is lost in $Amh^{-/-}$ male mouse fetuses at E13 and E14 [44]. Pharmacological inhibition of *Mmp2* *in vitro* blocks Müllerian duct regression in male urogenital organ culture [44]. Knockdown of *Mmp2* using morpholino oligonucleotides also resulted in Müllerian duct regression defects in male urogenital organ culture. In contrast, activation of MMPs resulted in Müllerian duct regression in female urogenital organ culture. However, male *Mmp2* mutant mice have normal Müllerian duct regression [44], suggesting that *Mmp2* is not essential for Müllerian duct regression *in vivo* or may act redundantly perhaps with other *Mmp* genes.

Wif1 (WNT inhibitory factor 1) is a secreted frizzled-related protein that inhibits WNT signaling by binding WNT, blocking binding to receptors [45]. *Wif1* expression is detected in the male Müllerian duct but not the female at E13.5 and E14.5 [46]. *Wif1* transcripts are not detected in $Amhr2^{-/-}$ male fetuses at E13.5 [46]. Exogenous AMH can induce *Wif1* expression in the Müllerian duct mesenchyme in female urogenital organ culture. Knockdown of *Wif1* expression by siRNA *in vitro* inhibits Müllerian duct regression [46]. However, newborn and 4-week-old *Wif1* knockout male mice did not have residual Müllerian tissues, suggesting that *Wif1* is not essential for Müllerian duct regression *in vivo* or there is gene redundancy.

GRNs Describe Pathways That Regulate Biological Processes

GRNs can be described as control systems, at the genetic level, for living creatures. They can include, but are not limited to, DNA sequence-specific transcription factors and downstream genes. The network consists of the processes by which gene products and sequences function collectively to fulfill a biological task. The gene sequences in this case are targets of transcription factors, including enhancers, insulators, and silencers [47]. GRNs are particularly paramount in development. GRNs, for example, specify morphological structures in organisms by dictating the timing and development of cells and tissues that make up a structure [48]. GRNs, therefore, can be uncovered and visualized to illuminate the proper development and function of an organism. When mapped, a GRN can display nodes, feedback loops, enhancement, inhibition, and much more to represent genetic regulation. Regulatory information placed into GRNs is first uncovered and supported through experimentation. Nodes are often defined from knockout or knockdown experiments, which show that a gene is required for function of the network. Further experimentation then specifies genes that encode regulators of those required genes or regulatory sequences within the genome. Once a GRN has begun to be mapped, it can be used to generate hypotheses for the GRN or biological process affected by the GRN. A GRN from one species can be compared to other GRNs across species for evolutionary study, as GRNs hold the modifications that differentiate one species from another [48]. Medically, GRNs dictate normal bodily function and are, therefore, effective aids in finding causes and solutions for disease. Considering sexual development particularly, GRNs provide information needed to understand how

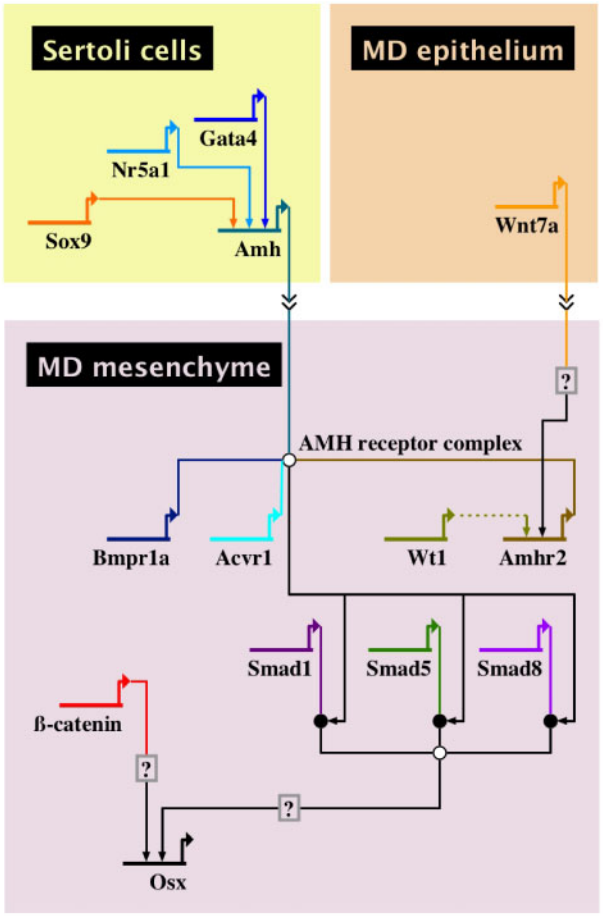

Figure 2: a GRN for AMH-induced Müllerian duct regression. The GRN is divided into domains, representing specific fetal cell types. The interactions contained within a domain occur within that cell type. Each gene in the GRN is depicted as a short horizontal line from which extends a bent arrow indicating transcription. The name of each gene is below the horizontal line. Arrows extending from the transcription arrow of one gene to the horizontal line of another gene indicate transcription factor binding to *cis*-regulatory elements to enhance transcription. Double arrows between cell-type domains indicate intercellular signaling of a protein. Circles in the GRN indicate intracellular protein activity. White circles indicate multiprotein complexes. Black circles indicate phosphorylation of a protein. Arrows formed by dotted lines indicate interactions that have been observed *in vitro* but not yet confirmed *in vivo*. Boxes containing question marks indicate a predicted site of regulation that has not yet been determined

organisms become sexually differentiated. In the case of this review, we will define our GRN using the studies by Eric Davidson as a guide [49].

A GRN for Müllerian Duct Regression

Based on the genetic and molecular evidence presented above, we present the first GRN for AMH-induced Müllerian duct regression (Fig. 2). Sertoli cells of the fetal testes express *Sf1/Nr5a1*, *Gata4*, and *Sox9*, encoding transcription factors that directly bind the 5′ region of the *Amh* locus. These are among the very few direct interactions between transcription factors and *cis*-regulatory elements for genes in the GRN. Müllerian duct epithelial cells express and secrete WNT7A that interacts with the adjacent mesenchyme cells to induce the expression of *Amhr2*, making the mesenchyme competent to respond to AMH. AMH secreted by fetal Sertoli cells interacts with AMHR2/BMPR1A/ACVR1, resulting in phosphorylation of SMAD1/5/8. The transcriptional targets of these SMADs are currently unknown. The requirement of beta-catenin in mesenchyme for Müllerian duct regression suggests that canonical WNT signaling may be required. Beta-catenin regulates *Osx* transcript levels but there are other inputs for *Osx* transcription. Transcriptome comparisons between male and female Müllerian duct mesenchyme have identified numerous genes that are upregulated in males relative to females [42, 46]. These provide candidate genes to be investigated for their roles in Müllerian duct regression. The GRN indicates that environmental influences that alter Amh transcriptional regulators (SOX9, NR5A1, and GATA4) could alter Müllerian duct regression. However, more studies are required to investigate how environmental factors might alter transcriptional outputs within the Müllerian duct mesenchyme. In conclusion, this *Amh*-regulated GRN provides a tool to investigate Müllerian duct regression, male sex differentiation, and how it may relate to environmental influences.

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MALE REPRODUCTIVE TRACT

Male Reproductive Tract: Development Overview

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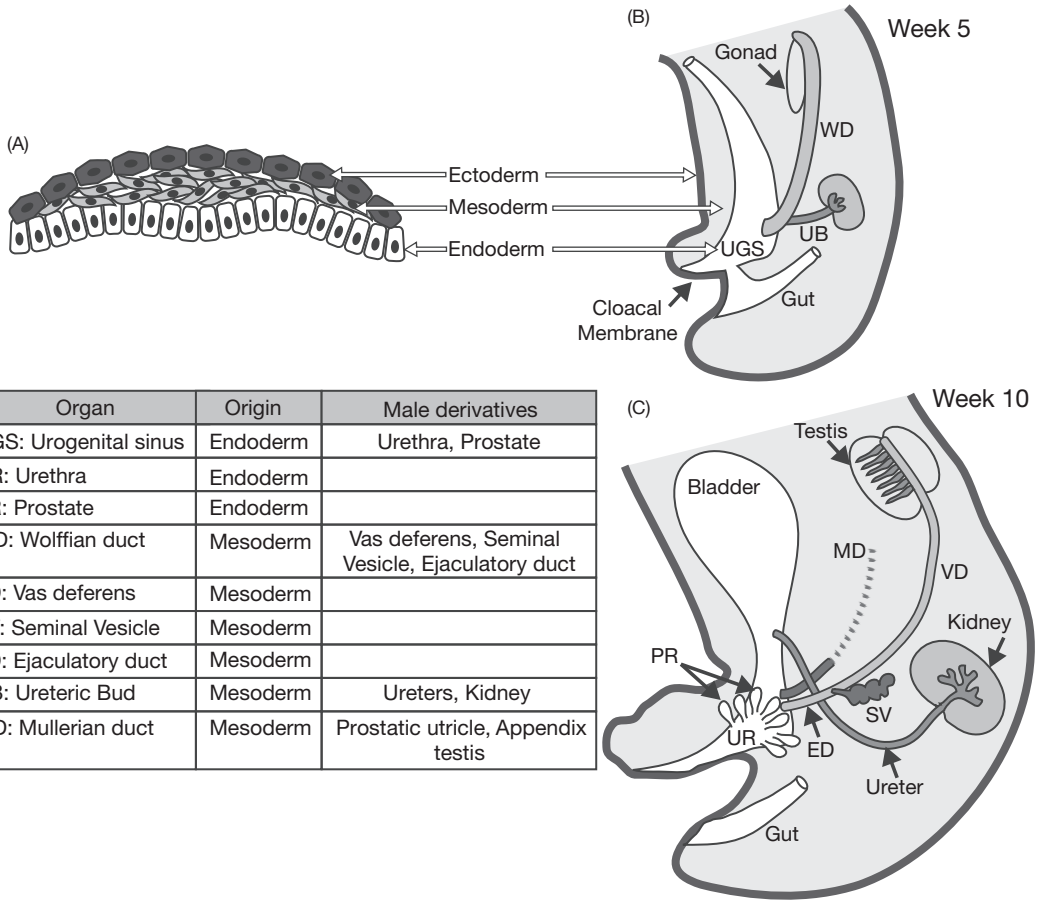
Introduction

Human male reproductive tract development is described in this chapter. The male reproductive tract consists of organs involved in the storage, maintenance and transportation of male reproductive cells. The major structures of the male reproductive tract originate from the endoderm-derived cloaca and the mesoderm-derived Wolffian duct. Male reproductive tract and urinary system development are closely linked and together their structures comprise the urogenital system. All human developmental ages mentioned in this chapter are counted from the day of fertilization.

Developmental Origins of the Male Reproductive Tract

Gastrulation gives rise to three germ layers: the endoderm, ectoderm and mesoderm. Male reproductive tract development begins early in gestation and involves cells from all three germ layers (Fig. 1). The cloaca is a transient pouch-like structure at the terminal



Organ	Origin	Male derivatives
UGS: Urogenital sinus	Endoderm	Urethra, Prostate
UR: Urethra	Endoderm	
PR: Prostate	Endoderm	
WD: Wolffian duct	Mesoderm	Vas deferens, Seminal Vesicle, Ejaculatory duct
VD: Vas deferens	Mesoderm	
SV: Seminal Vesicle	Mesoderm	
ED: Ejaculatory duct	Mesoderm	
UB: Ureteric Bud	Mesoderm	Ureters, Kidney
MD: Mullerian duct	Mesoderm	Prostatic utricle, Appendix testis

Fig. 1 Germ cell layer of origin of male reproductive tract structures. (A) Cross sectional view of a gastrulated embryo showing the three germ layers: endoderm (*white*), mesoderm (*light gray*) and ectoderm (*dark gray*). (B) Sagittal view of the male reproductive tract in a week 5 embryo during the bi-potential stage. (C) Sagittal view of the male reproductive tract in a week 10 embryo after the onset of sexual differentiation. Structures of the reproductive tract are colored according to germ layer of origin.

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portion of the endoderm-derived hindgut in the early embryo. The cloaca (Latin for "sewer") differentiates into the urogenital sinus, bladder, urethra and prostate in males. Paired epithelial Wolffian ducts derive from the mesoderm and insert into the cloaca. Testicular factors including testosterone masculinize male reproductive structures and drive cellular differentiation within them. The Wolffian duct gives rise to the epididymis, vas deferens, seminal vesicle and ejaculatory duct. In the mature adult, sperm from the testes are transported through the epididymis, vas deferens and ejaculatory duct to the urethra. At the urethra, the seminal vesicle and prostate contribute secretions to the ejaculate that promote sperm health. The urethra is the conduit for deposition of sperm during reproduction (Moore and Persaud, 2003).

Urogenital Sinus, Urethra, and Prostate

Growth and re-positioning of mesenchymal tissue divides the cloaca into the urogenital sinus ventrally and the anorectal sinus dorsally by week 7 of gestation.

Urogenital sinus

The ventral portion of the cloaca gives rise to the urogenital sinus (UGS). The UGS is a transient structure comprised of a simple epithelial tube featuring a balloon-shaped central cavity (**Fig. 1**). Paired Wolffian ducts insert into the UGS around week 4 of gestation. The UGS is divided into three parts:

- The cranial or upper part of the UGS differentiates into the urinary bladder.
- The middle or pelvic portion develops into the pelvic urethra and prostate.
- The caudal or lower part develops into the phallic urethra.

Urethra

The urethra develops from the middle and caudal regions of the UGS. The pelvic urethra extends from the bladder to the body wall. The phallic urethra extends from the body wall to the tip of the external genitalia. Urethral glands (Littre's glands) and bulbourethral glands (Cowper's glands) emerge as urethral outgrowths around week 12 of gestation.

Lineage tracing studies following the fate of endodermal cells within the developing urogenital tract show that the entire urethral epithelium derives from endoderm (Seifert et al., 2008). The developing urethra forms a stratified epithelium comprised of basal, intermediate, and superficial layers. Epithelial–mesenchymal interactions are crucial for urethral morphogenesis. Sonic hedgehog (SHH) peptide is secreted from epithelium and activates GLI transcription factors in nearby mesenchyme. GLI transcription factors drive bone morphogenetic protein 4 (BMP4) transcription and mesenchymal differentiation. Molecular mapping studies in the mouse embryo reveal a multilayered pelvic urethral mesenchyme consisting of lamina propria, muscularis mucosa, submucosa, and muscularis propria (Abler et al., 2011). Male urethra morphogenesis is guided by androgens, principally by androgen-induced signals from the male urethral mesenchyme that drive urethral epithelium differentiation and remodeling.

Prostate

The prostate is a male accessory sex gland positioned at the base of the bladder and surrounding the pelvic urethra. Prostate secretions contribute to the ejaculate and promote sperm health. Prostate ductal development initiates in utero as solid epithelial buds deriving from UGS epithelium. The epithelial buds elongate, branch, and canalize to form a complex ductal system draining into the urethral lumen. Most studies on early prostate development have been carried out in rodents. The early development program of prostate budding is remarkably conserved between rodents and humans, even though their prostates differ anatomically at sexual maturity.

In the human fetus, prostate development occurs in response to testosterone production by testicular Leydig cells around week 7 of gestation. Testosterone acts on androgen receptor (AR)-expressing UGS mesenchymal cells. AR activation increases the abundance of UGS mesenchymal steroid 5 alpha reductase type 2 (SRD5A2). SRD5A2 converts testosterone to the more potent dihydrotestosterone (DHT). DHT binding to AR amplifies androgen signaling in the UGS mesenchyme, which evokes paracrine signaling mechanisms that instruct UGS epithelium to form prostatic buds. Prostate bud outgrowth begins around week 10 of gestation and continues until week 24.

Studies in mice have shown that prostatic bud number and location are precisely controlled. The Nk-3 transcription factor locus-1 (NKX3–1) is the earliest marker of prostate specified UGS epithelial cells. Although prostatic bud formation cannot occur in the absence testosterone, several other factors including SHH, SOX9, HOXB13, and WNT5A are also required for prostatic bud formation and subsequent prostate ductal development.

Prostate formation is dependent on complex epithelial–mesenchymal interactions. Tissue recombination experiments have shown that androgen signaling deriving from UGS mesenchyme not UGS epithelium directs prostate bud formation. However, epithelial AR is required for prostate epithelial cell differentiation and secretory protein production. UGS mesenchyme is organized into distinct zones, and some zones serve as signaling centers to guide prostate morphogenesis. UGS mesenchymal condensations (mesenchymal pads) lie on the UGS periphery, are characterized by *FGF10* mRNA expression, and guide directional outgrowth of prostatic buds (Thomson and Cunha, 1999). Epigenetic mechanisms including DNA methylation and histone acetylation regulate expression of key genes involved in prostate development. DNA methylation controls E-cadherin (CDH1) and AR abundance,

which in turn control prostate bud elongation and timing of bud formation respectively (Keil et al., 2014a,b). Histone acetylation controls BMP2 expression to regulate prostatic ductal branching (Keil et al., 2015).

In later stages, the prostate undergoes branching, canalization and differentiation to form a pseudo-stratified epithelium consisting of basal cells, secretory luminal cells and rare neuroendocrine cells. The prostate increases in size following an upsurge in testosterone production during puberty. The study of early prostate development is receiving renewed interest as the reawakening of embryonic processes has been implicated in the pathogenesis of prostate cancer and benign prostatic hyperplasia.

Rete Testis, Epididymis, Vas Deferens, Seminal Vesicle

The intermediate mesoderm lies between paraxial and lateral plate mesoderm of the fetus and gives rise to ductal structures of the urogenital system. Crests of intermediate mesoderm called urogenital ridges form near the midline and along the cranio-caudal body axis. Early in week 4 of gestation, a non-functional, transient excretory organ called the pronephros develops within the urogenital ridge at the position of the thorax. The rudimentary tubular structures of the pronephros feed into the pronephric duct, which joins the cloaca at approximately week 4 of gestation. The pronephric duct is formed by mesenchymal to epithelial transition of intermediate mesoderm. The pronephros undergoes degeneration through an apoptotic program. The mesonephros forms caudal to the degenerating pronephros late in week 4 of gestation. The mesonephros contains glomeruli and mesonephric tubules that function as temporary kidneys until week 10 of gestation, when the metanephros permanently assumes kidney function. The mesonephric tubules open into mesonephric ducts (also known as Wolffian ducts) which drain into the cloaca. The mesonephros degenerates in the cranial to caudal direction around week 8, leaving a few residual tubules that will give rise to efferent ductules of the testes (Rao and Burnett, 2013).

The Wolffian ducts are precursors for ductal structures of the male reproductive and urinary tracts. The ureteric bud emerges as a Wolffian duct outgrowth near its insertion into the cloaca (**Fig. 1**). The ureteric bud undergoes branching and differentiation within a specialized mesenchyme called the metanephric blastema to form the metanephros or permanent kidneys. The ureteric bud is also the precursor for the ureters, which connect the kidneys to the bladder. The caudal portion of the Wolffian duct between the ureteric bud and the insertion site into the UGS is called the common nephric duct. Common nephric duct apoptosis positions the ureters at their final insertion site within the bladder, spatially separating the ureteral and Wolffian duct openings to the lower urinary tract. Ureter separation from the Wolffian duct is completed by week 7 of gestation. Paired box 2 (PAX2) expression marks the intermediate mesoderm early after gastrulation. Lineage tracing studies show that the PAX2 expressing intermediate mesoderm gives rise to Wolffian ducts, the ureteric bud and the metanephric blastema (Bouchard et al., 2002).

During the ambisexual stage, two sets of paired genital ducts are present in male and female embryos. The paramesonephric or Müllerian ducts extend in the craniocaudal direction and lie lateral to the Wolffian ducts. Müllerian duct formation occurs between week 6 and 7 of gestation. Lineage tracing studies in chick and mouse models demonstrate that Müllerian ducts develop from the coelomic epithelial layer, which derives from lateral plate mesoderm. Although Wolffian ducts lie in close apposition to and stimulate Müllerian duct formation, the Wolffian ducts do not contribute epithelial cells to the Müllerian ducts (Guioli et al., 2007; Orvis and Behringer, 2007).

Sexual differentiation of the male reproductive tract begins around week 7 of gestation. Müllerian inhibiting substance (MIS) produced by Sertoli cells and testosterone produced by the interstitial Leydig cells of the fetal testis act on the reproductive tract to induce male differentiation. MIS is a glycoprotein of the TGF-β family of growth factors which causes irreversible regression of the Müllerian ducts in males. Testicular MIS production commences by week 8 of gestation and Müllerian duct regression occurs between weeks 8 and 10. The prostatic utricle near the UGS and the appendix testis near the male gonads are Müllerian duct remnants in males. Female reproductive structures including the uterus, vagina and oviducts form in the absence of MIS. Testosterone-mediated AR activation supports Wolffian duct survival in males. Reproductive tract structures derived from the Wolffian ducts are formed between week 9 and 13 of gestation. Regional expression of homeobox (HOX) genes drives segmental differentiation of the Wolffian duct into the epididymis, vas deferens and seminal vesicle. The Wolffian ducts regress in females due to insufficient testosterone to support cell survival (Rao and Burnett, 2013).

Rete testis

After the mesonephros undergoes regression, the remaining mesonephric tubules form the efferent ductules. The seminiferous tubules, which contain sperm cells, are connected to the efferent ductules by a maze-like network of interconnecting tubes called the rete testis. The ciliated cells lining the rete testis guide sperm into the efferent ductules.

Epididymis

The efferent ductules, which receive sperm from the testis, drain into the epididymis. The epididymis is a convoluted series of tubules that derives from the portion of the Wolffian duct adjacent to the testis. The epididymis stores and transports sperm.

Vas deferens

The medial Wolffian duct segment forms the vas deferens. Androgens drive Wolffian duct differentiation into the vas deferens around week 12 of gestation. Smooth muscle surrounding the vas deferens contracts during ejaculation to propel sperm from the epididymis to the urethra.

Seminal vesicle

The seminal vesicles develop as lateral outgrowths from the caudal Wolffian duct segment. The seminal vesicles form around week 10 of gestation, after the onset of testosterone synthesis by the fetal testis. Seminal vesicles contribute secretions to the ejaculate. The most caudal Wolffian duct portion, positioned between the seminal vesicle and the urethra, is called the ejaculatory duct. Ejaculatory ducts drain the contents of the seminal vesicle and vas deferens into the urethra.

External Genitalia

Internal fertilization requires specialized male and female external reproductive organs. Lateral plate mesoderm, endoderm, and surface ectoderm cells contribute to the external genitalia. The initial phase of external genitalia development is essentially the same in males and females. In the later phase, androgens drive masculinization of the external genitalia in males (Blaschko et al., 2012; Yamada et al., 2003).

Early phase: Formation of ambisexual external genitalia

The early phase of external genitalia development, which occurs between 4 and 7 weeks of gestation, is essentially the same in males and females. This phase occurs independently of androgen action as it happens before the onset of testicular testosterone production. The cloacal membrane, formed by direct contact of the endoderm and surface ectoderm, is intact at 4 weeks of gestation. Proliferating lateral plate mesenchymal cells form paired lateral swellings above the cloacal membrane. These swellings fuse at the midline to form the genital tubercle. The genital tubercle is a bi-potential structure which is the precursor of the penis in males and clitoris in females. Paired mesenchymal swellings called urogenital folds and labio-scrotal swellings form on either side of the cloacal membrane. After cloacal septation around week 7, the cloacal membrane becomes the urogenital membrane ventrally and the anal membrane dorsally. The cloacal membrane ruptures at two sites to form the urethral orifice and the anal opening. The urogenital membrane is bounded by urogenital folds and lies within a temporary indentation on the ventral genital tubercle surface called the urethral groove. The urethral groove is lined by a solid cord of endodermal cells comprising the urethral plate epithelium. The urethral plate epithelium is the region of the phallic urethra distal to the UGS.

Studies in mice have shown that early patterning of the genital tubercle does not depend on androgens but does require Sonic hedgehog (SHH) signaling. Mice harboring inactivating mutations in the SHH gene fail to form external genitalia. SHH signaling from the urethral plate epithelium coordinates cell movements during external genitalia development (Perriton et al., 2002).

Later phase: Sexual differentiation of the external genitalia

Male testicular androgens induce genital tubercle differentiation into the penis. In females, the genital tubercle fails to elongate and forms the clitoris in the absence of androgens. Testosterone synthesis begins by week 7 and maximal concentrations in the fetus are achieved between weeks 10 and 15 of gestation. Early signs of sexual differentiation in the external genitalia can be detected by week 9 and complete differentiation is achieved by weeks 12–13.

The steroid hormone dihydrotestosterone (DHT) masculinizes the external genitalia. Testosterone is converted to DHT by the action of steroid metabolizing enzymes like SRD5A2 which are expressed in genital tubercle mesenchymal cells. DHT initiates androgen signaling in androgen receptor expressing mesenchymal cells. Interactions between androgen activated mesenchyme and urethral plate epithelium initiate male external genitalia differentiation.

Androgen exposure elongates the genital tubercle into the penis. Early in sexual differentiation, the proximal phallic urethra is a closed hollow tube but the distal portion comprising the urethral plate epithelium remains a solid mass of cells. The urogenital folds lining the penile ventral surface guide midline fusion of the urethral plate, forming a hollow urethral tube that extends the whole length of the penis. The fusion of urogenital folds occurs in a proximal to distal direction, positioning the urethral orifice at the tip of the penis. Hypospadias, a common birth defect, occur from defective fusion of urogenital folds. Specialized mesenchyme induces the differentiation of the distal urethral into a stratified squamous epithelium. The labio-scrotal swellings fuse in the midline to form the scrotum. The skin covering the developing penis is derived from surface ectoderm. Specialized structures of the penis, the corpus cavernosa and the corpus spongiosum, derive from the proliferation and differentiation of mesoderm derived cells.

The Genitourinary Development Molecular Anatomy Project (GUDMAP) website provides curated information on reproductive tract anatomy, histology, mRNA and protein expression over developmental time (www.gudmap.org).

Signaling Pathways in Male Reproductive Tract Development

Studies in rodents have greatly advanced our knowledge of signaling pathways involved in male reproductive tract development. **Table 1** provides a parallel chronology of the major events in male reproductive tract development in human and mouse.

Sonic Hedgehog Signaling

Sonic hedgehog (SHH) peptide is a developmental morphogen secreted by epithelial cells. The secreted SHH peptide relieves the repression of smoothened (SMO) by binding to its inhibitor patched (PTC1). SMO activation initiates transcription of GLI transcription factors which are involved in several developmental and morphogenetic processes. SHH is expressed in

Table 1 Chronology of male reproductive tract development in human and mouse

Human	*Internal reproductive tract*	*External genitalia*	*Mouse*
Week 3	Gastrulation; cloacal membrane forms		E6-E6.5
Week 4	Nephrogenic cord forms pronephros	Genital tubercle forms	E8.5
	forms cloaca develops from hindgut		E9
	mesonephros forms Wolffian duct fuses		E9.5
	with cloaca (day 26)		E9.5–E11.5
			E9.5
			E11–E11.5
Week 5	Ureteric bud forms (day 28)		E10.5–E11.5
	Cloacal septation begins		E10.5
	Common nephric duct apoptosis		E11–E12
Week 6	Müllerian duct forms		E12–E13
		Urogenital and labio-scrotal folds form	E14
Week 7	Cloacal septation complete	Cloacal membrane rupture	E13–E13.5
	Ureters join bladder		E13–E14
	Onset of testosterone synthesis		E13–E13.5
Week 8	Müllerian ducts reach UGS		E13.5
Onset of sexual dimorphism			
Week 9	Müllerian duct degeneration		E16.5
	Wolffian duct differentiation		E16.5-P1
Week 10	Müllerian duct degeneration complete		E16.5
	Seminal vesicle forms		E16.5
	Prostate forms		E16.5–E18.5
	Wolffian duct differentiation		E16.5–P1
Week 11	Wolffian duct differentiation		E16.5–P1
Week 12	Wolffian duct differentiation		E16.5–P1
Week 13		Urethral tube closure	E16.5
2nd Trimester		Growth of external genitalia	E15.5–P1
		Inguinal descent (week 23)	E15.5–E17.5
3rd Trimester		Growth of external genitalia	E15.5–P1
		Scrotal descent (weeks 24–34)	E17.5–P20

Provides a parallel chronology of male internal reproductive tract and external genitalia development in the human and mouse. Developmental age in humans is depicted here in weeks from the time of fertilization. Developmental age in the mouse embryo is depicted as days (E) from the time of fertilization.

hindgut-derived structures including the cloacal epithelium, where it patterns the surrounding mesenchyme. SHH induces mesenchymal GLI2, which regulates epithelial and mesenchymal proliferation and apoptosis during cloacal development. SHH regulates cloacal septation by promoting proliferation of mesenchymal cells in the urorectal septum (Seifert et al., 2009). SHH signaling is required for prostate formation and external genitalia development. Treatment with SHH inhibitors impairs prostate ductal growth and morphogenesis (Podlasek et al., 1999). SHH knockout mice show a complete absence of external genitalia (Haraguchi et al., 2001).

WNT-Beta Catenin Signaling

WNT ligand binding to cell surface Frizzled receptors stabilizes and activates the transcription factor beta-catenin. WNT signaling is involved in several aspects of urogenital development including the septation of the cloaca into the urogenital and anorectal sinuses. Disruption of WNT signaling results in rectourethral fistulas (abnormal connection between the urethra and rectum). Epithelial beta-catenin is also required for prostate development and growth (Mehta et al., 2013). The WNT-beta catenin pathway acts downstream of SHH signaling to regulate external genitalia development (Miyagawa et al., 2009).

Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) are growth factors belonging to the TGF-beta superfamily. BMPs bind to serine threonine kinase receptors and initiate intracellular signaling through SMAD proteins. BMP7 expression in the urorectal septum is required for cloacal septation. In addition, BMP7 expression maintains proliferation and cell survival in the cloacal epithelium (Xu et al., 2012). Expression of BMP7 in the UGS mesenchyme restricts prostate ductal budding and prevents excessive branching of elongating ducts (Grishina et al., 2005).

Fibroblast Growth Factors

Fibroblast growth factors are a family of secreted growth factors that signal through tyrosine kinase fibroblast growth factor receptors to regulate proliferation, differentiation and morphogenesis during embryonic development. FGF signaling is required for cell survival during the early stages of genital tubercle outgrowth. FGF signaling in the ectoderm is required for urethral tube formation. Deletion of FGF10 or its receptor FGFR2 results in severe hypospadias (Harada et al., 2015). FGFR2, a critical regulator of prostate development, is required for branching and growth of prostate buds. FGF10 is a paracrine mediator of epithelial to mesenchymal signaling during prostate bud formation. FGF10 knockout mice fail to form prostate, seminal vesicle, bulbourethral glands and caudal vas deferens (Thomson and Cunha, 1999).

PAX Genes

Paired box (PAX) genes are tissue specific transcriptions factors that determine lineage specification in the early embryo. PAX2 and PAX8 are required for Wolffian duct formation from the intermediate mesoderm. In PAX2 and PAX8 double mutant mice, the intermediate mesoderm fails to undergo the mesenchymal to epithelial transition required for Wolffian duct formation (Bouchard et al., 2002). GATA3, a downstream effector of PAX2, regulates Wolffian duct growth and caudal extension which is required for fusion with the cloaca and formation of definitive kidneys (Grote et al., 2006).

EPH Receptors/Ephrins

EPH receptors are a family of receptor tyrosine kinases with plasma membrane bound ligands called ephrins. EPH receptor/ephrins are involved in the maintenance of cell–cell adhesion and communication between similar or different cell types during developmental processes. The ephrin receptors EPHA4 and EPHA7 are expressed in the mesenchyme surrounding the cloaca and Wolffian duct where they mediate Wolffian duct fusion with the cloaca (Weiss et al., 2014). Signaling from EPHA4 and EPHB2 is required for apoptosis of the common nephric duct for proper separation of ureters from Wolffian ducts (Peuckert et al., 2016). EphrinB1 expressed by prostatic mesenchyme regulates prostate growth and branching (Ashley et al., 2010).

Vitamin A/Retinoic Acid Signaling

Retinoic acid, a derivative of vitamin A (retinol), binds to nuclear retinoic acid receptors to activate transcriptional programs for differentiation and organogenesis. The spatial expression of retinaldehyde dehydrogenases, which convert retinaldehyde to retinol, is tightly regulated in a tissue specific manner. Apoptosis induced by retinoic acid signaling is required for ureter separation from the Wolffian duct and proper positioning in the bladder (Batourina et al., 2005). Retinoic acid is a powerful inducer of prostate budding (Vezina et al., 2008a). Retinoic acid signaling regulates external genitalia formation by maintaining SHH and BMP4 expression in the genital tubercle (Liu et al., 2012).

Müllerian Inhibiting Substance

Müllerian inhibiting substance (MIS) or anti-Müllerian hormone (AMH) is a gonadal hormone secreted by Sertoli cells of the developing testis. The secreted glycoprotein MIS belongs to the TGF-beta family of transcription factors. MIS acts through AMH Type II receptors expressed by Müllerian duct mesenchyme to initiate apoptosis and degeneration of the Müllerian duct (Behringer, 1995; Abler et al., 2011).

Androgens, INSL3

Testosterone synthesis initiates from fetal Leydig cells during week 7 of gestation. Testosterone is converted to the more potent dihydrotestosterone (DHT) by the enzyme SRD5A2. DHT acts on AR expressing cells to initiate androgen-dependent transcriptional programs. DHT regulates prostate and seminal vesicle formation, external genitalia masculinization and formation of the vas deferens and epididymis. The Leydig cell specific insulin-like peptide INSL3 binds to relaxin/insulin like family peptide receptor 2 (RXFP2) to promote testicular descent into the scrotum (Barsoum and Yao, 2006).

Endocrine Disruptors

Environmental toxins with the capability of interfering with endocrine signaling are known as endocrine disruptors (Prusinski et al., 2016). In utero exposure to endocrine disruptors adversely affects hormone-dependent development and increases risk of adulthood disease. Maternal exposure to low doses of Bisphenol A, an estrogenic compound found in plastics, has been shown to increase prostate size in rodent models (Gupta, 2000; Dolinoy et al., 2007). Early exposure to Bisphenol A can also increase the risk of prostate cancer in rodent models of estrogen-induced carcinogenesis by inducing long-term changes to the DNA methylome (Cheong et al., 2016). The anti-androgenic endocrine disruptor vinclozolin found in fungicides, can induce

hypospadias in mice (Buckley et al., 2006). In utero exposure to persistent environmental pollutants called 2,3,7,8 tetrachlorodibenzo-*p*-dioxin impairs reproductive function of male and female rodents (Gray and Ostby, 1995; Bjerke and Peterson, 1994). In utero dioxin exposure disrupts mouse prostate formation (Vezina et al., 2008b) and sensitizes mice to hormone-mediated urinary dysfunction (Ricke et al., 2016).

Congenital Anomalies of the Male Reproductive Tract

Congenital anomalies of the male reproductive tract can reduce fertility. Hypospadias are the most common congenital anomaly of the male reproductive tract, with an occurrence of 1 in 250 live male births. Hypospadias occur when the urethral opening is not at the tip of the penis, but instead on the ventral surface or scrotal region. Defects in urethral tube closure result in hypospadias (Baskin and Ebbers, 2006). Genetic, endocrine and environmental factors have been implicated in the occurrence of hypospadias. Epispadias, which occur when the urethral opening is on the dorsal surface of the penis, is a much rarer condition (affecting 1 in 117,000 males) (Gearhart and Jeffs, 1992). Unlike hypospadias, epispadias result from defects in the cloacal membrane (Suzuki et al., 2017). Another congenital anomaly called chordee is associated with increased curvature of the penis. A congenital condition called posterior urethral valves is associated with the occurrence of flaps of urethral tissue that obstruct urine flow and impair reproductive function (Agarwal, 1999).

Persistent Müllerian duct syndrome is a rare anomaly of the reproductive tract in which Müllerian duct derivatives (uterus and oviduct) persist in males. The persistence of Müllerian duct derivatives can be due to insufficient production of Müllerian inhibiting substance (MIS) or insensitivity of the Müllerian duct to MIS (Elias-Assad et al., 2016).

Congenital anomalies of Wolffian duct derivatives include ectopic insertion of the ureter into the urethra, seminal vesicle, ejaculatory duct or vas deferens. Ectopic ureters are a result of abnormal ureteric bud formation and abnormal separation from the Wolffian duct during kidney development. Ectopic ureter insertion into the seminal vesicle results in the development of congenital seminal vesicle cysts. Seminal vesicle anomalies on their own do not contribute to male infertility. However, these defects are often observed with other Wolffian duct defects that affect fertility (Kroovand and Perlmutter, 1981).

Defects in vas deferens development are a major cause of male infertility. Congenital bilateral absence of the vas deferens results in male infertility from obstructive azoospermia (lack of sperm in semen). This condition is highly prevalent in males who have abnormal mucus production from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Abnormal mucus production in affected individuals results in obstruction and destruction of the vas deferens, leading to infertility in later life (Stuhrmann and Dork, 2000). Other congenital anomalies of Wolffian duct derivatives include agenesis of the epididymis, epididymal cysts with loss of continuity, agenesis of the seminal vesicle and agenesis of the ejaculatory duct.

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"Systems Biology of Reproduction"

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

Spring 2022 – Systems Biology of Reproduction
Lecture Outline – Male Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 1, 2022
Week 4

Male Reproductive Tract Development & Function

Embryonic Development and Reproductive Tract Organogenesis
- Overview
- Development of Mullerian Duct vs. Duct Wolffian Duct Derivatives
- Mullerian Inhibiting Substance (MIS)

Male Urogenital Tract Organogenesis
- Prevention of Programmed Cell Death in the Wolffian Duct
- UGS/Prostate/Seminal Vesicle
  1. Prostate Morphogenesis (ductal branching)
  2. Cell-Cell Interactions and Paracrine Factors
  3. Prostate Cancer
- Epididymis/Ductus Deferens
- Role of Androgens (T versus DHT)
  1. Androgen Metabolism
  2. 5 α Reductase Inhibitors
  3. Organ Culture
- Endocrine Disruption

Required Reading

Moses MM and Behringer RR. (2019) Environ Epigenet. 25;5(3):dvz017.

Joseph and Vezina (2018) Male Reproductive Tract: Development Overview. in: Encyclopedia of Reproduction 2nd Ed. Vol. 1, Pages 248-255.

Spring 2022 – Systems Biology of Reproduction
Discussion Outline – Male Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 3, 2022
Week 4

Reproduction Tract Development & Function

Primary Papers:

1. Murashima, et al. (2015) Asian J Andrology 17:749-755
2. Zhao, et al. (2017) Science 357:717-720
3. Sakib, et al. (2020) Andrology 8(4):835-841
4. Richer, et al. (2020) Andrology 8(4):879-891

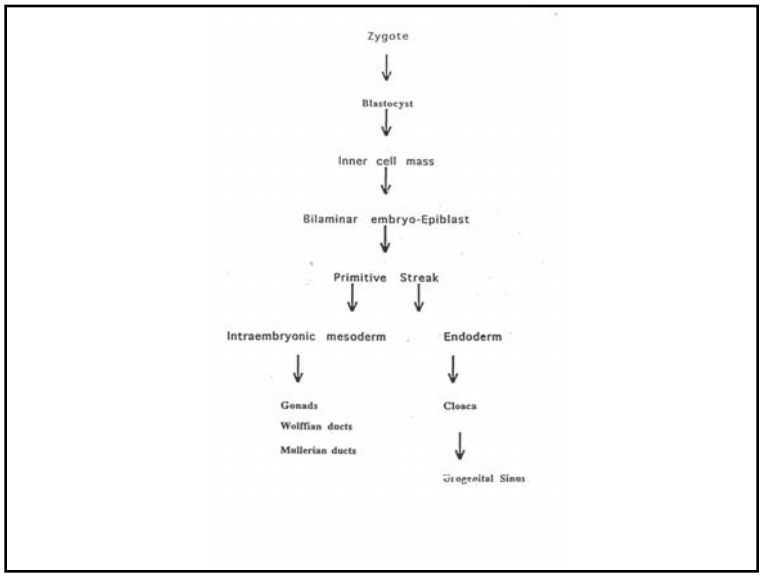
Discussion

Student 7: Classic Reference #1 above
- What are the developmental steps of the Wolffian/epididymal duct?
- What are the Phenotypes of knockouts that explain the development?
- What technology was used

Student 8: Reference #2 above
- What is the technology used?
- Where is the expression pattern of the COUP-TF11?
- What does the knockout phenotypes show on regional actions of COUP-TF11?

Student 9: Reference #3 and #4 above
- What is the technology used and how different?
- What organoid cell structures were observed?
- What basic information on male reproductive tract development was obtained?

Development


Zygote
Blastocyst
Inner cell mass
Bilaminar embryo-Epiblast
Primitive Streak
Intraembryonic mesoderm
Endoderm
Gonads
Wolffian ducts
Mullerian ducts
Cloaca
Urogenital Sinus

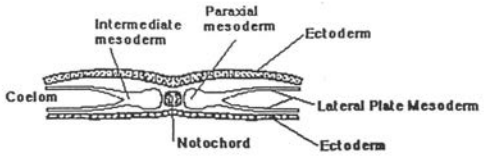
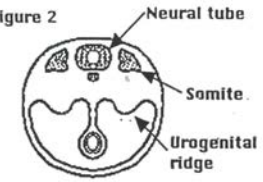
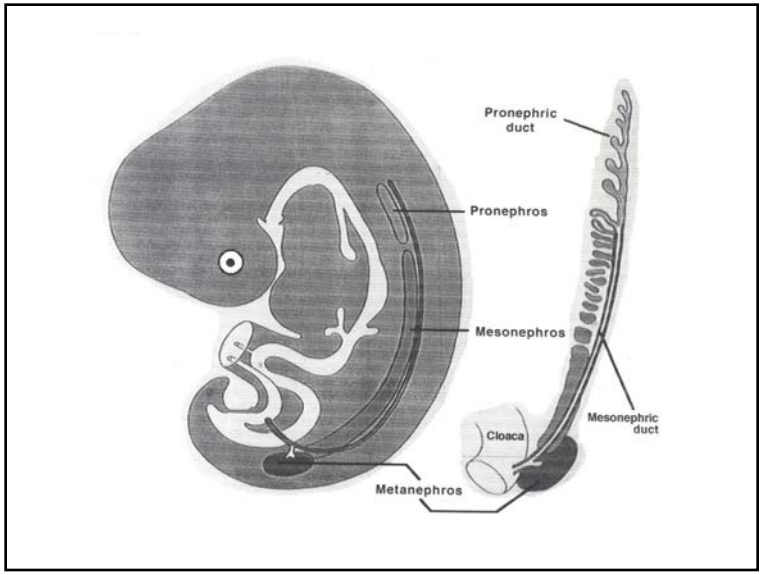
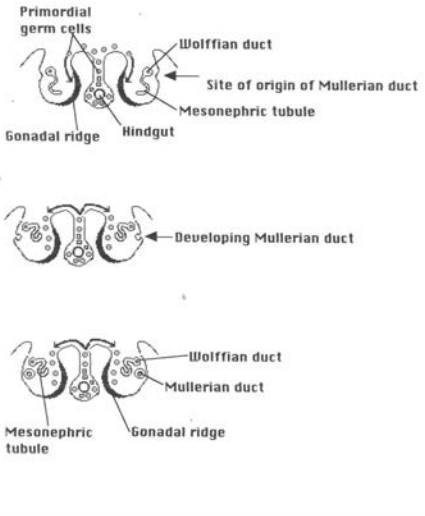

Intermediate mesoderm
Paraxial mesoderm
Ectoderm
Coelom
Lateral Plate Mesoderm
Notochord
Ectoderm

Figure 2


Neural tube
Somite
Urogenital ridge


Pronephric duct
Pronephros
Mesonephros
Mesonephric duct
Cloaca
Metanephros


Primordial germ cells
Wolffian duct
Site of origin of Mullerian duct
Mesonephric tubule
Gonadal ridge
Hindgut
Developing Mullerian duct
Wolffian duct
Mullerian duct
Mesonephric tubule
Gonadal ridge

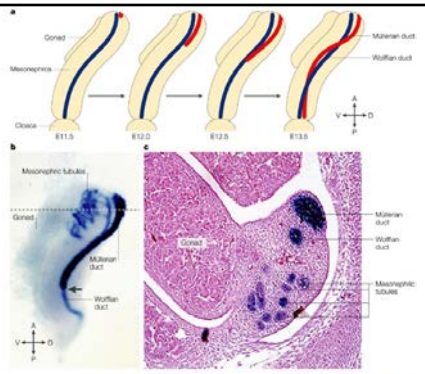
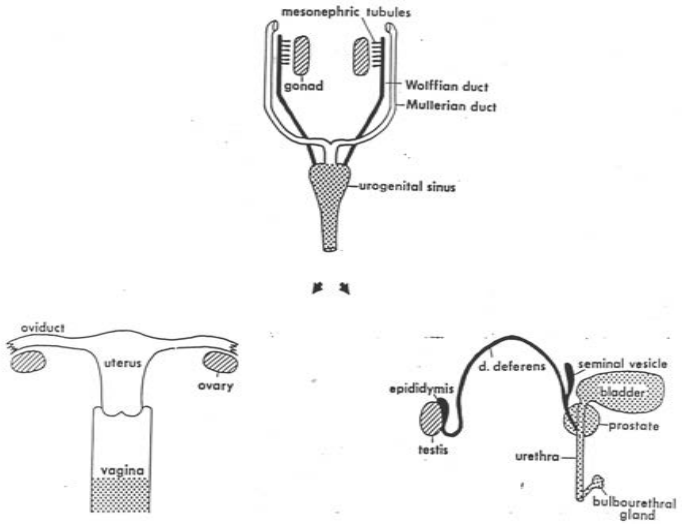
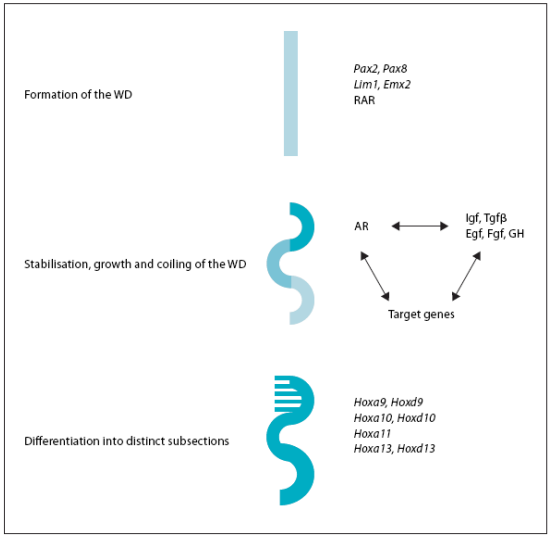
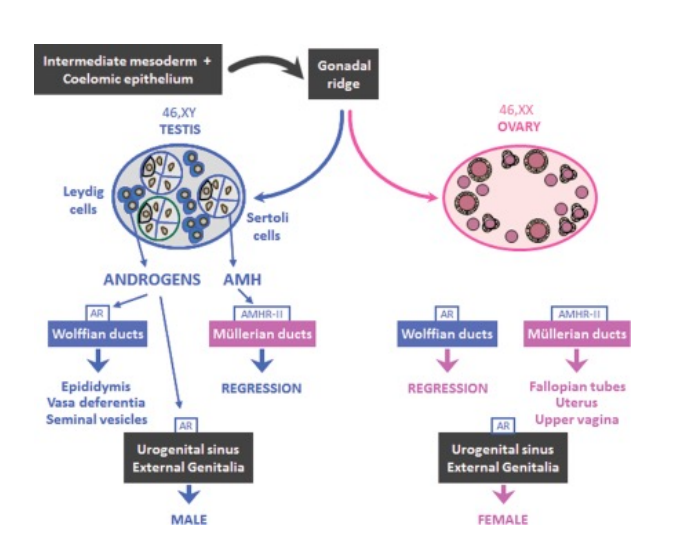


Figure 2 | **Formation of the Müllerian ducts.** **a** | Schematic diagram of Müllerian duct formation in mammals. The Müllerian duct forms as an invagination of the surface epithelium of the MESONEPHROS at around embryonic day (E) 11.5 in mice and this epithelial invagination extends posteriorly until it reaches the CLOACA at E13.5. **b** | The extending epithelium of the Müllerian duct is visualized at E12.5 by *Lim1 (Lhx1)-lacZ* expression11. Note that the Wolffian duct (blue) has reached the cloaca posteriorly, but the Müllerian duct is still in the process of extending posteriorly. The grey arrow points to the posterior tip of the extending Müllerian duct. **c** | Cross section of the gonadal/mesonephric region (dashed line in **b**). Blue staining by *Lim1–lacZ* expression is observed in the epithelium of the Wolffian and Müllerian ducts and the mesonephric tubules. A, anterior (cranial); D, dorsal; *Lim*, *lin-11*, *Isl1* and *mec-3* transcription-factor homologue; P, posterior (caudal); V, ventral. Panel **c** adapted from Ref. 11 © (2003) The Company of Biologists Ltd.



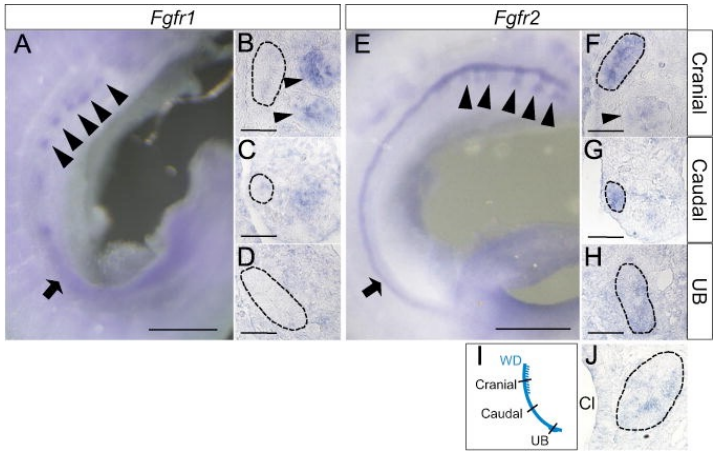


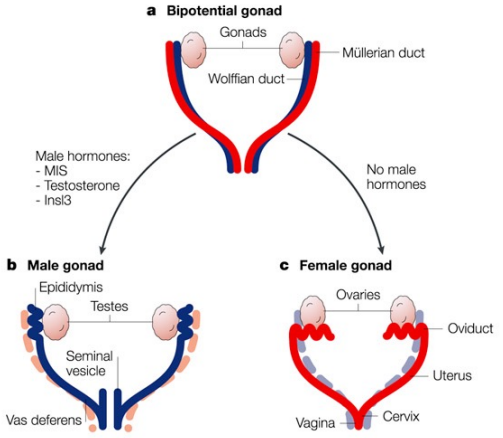


Region-specific regulation of cell proliferation by FGF receptor signaling during the Wolffian duct development.

Okazawa M, et al.

Dev Biol. 2015 Apr 1;400(1):139-47.





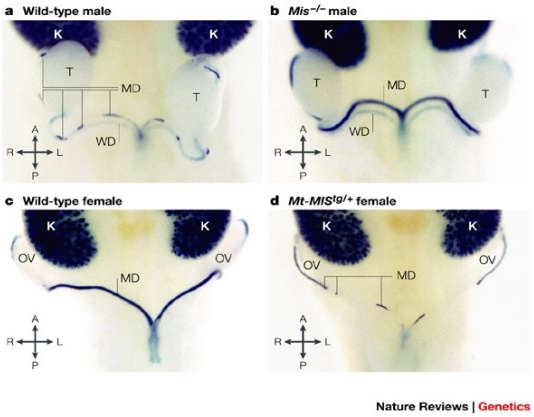
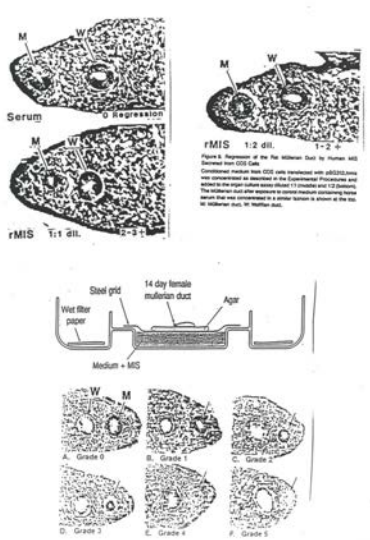


Figure 3 | **Müllerian duct regression.** The developing Müllerian ducts are visualized by *Lim1–lacZ* expression11 in the mouse embryo at embryonic day (E) 15. **a** | In XY male mice, Müllerian-inhibiting substance (MIS) is produced by the testes and eliminates the Müllerian ducts. The regressing Müllerian ducts (MD) have a fragmented pattern at this stage. **b** | When *Mis* is mutated by gene targeting in XY mice, there is no Müllerian duct regression. **c** | There is no Müllerian duct regression in the absence of MIS in XX female mice. **d** | When human *MIS* (*AMH*) is overexpressed using a metallothionein (*Mt*) promoter in XX mice, ectopic regression of the Müllerian duct is observed. A, anterior (cranial); K, kidney; L, left; *Lim*, *lin-11*, *Isl1* and *mec-3* transcription-factor homologue; OV, ovary; P, posterior (caudal); R, right; T, testis; WD, Wolffian duct. Panels **c** and **d** adapted from



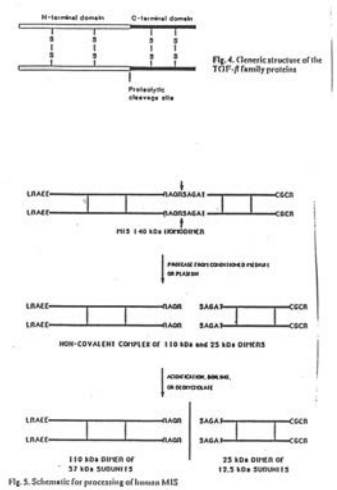


Fig. 4. Generic structure of the TGF-β family proteins

Fig. 5. Schematic for processing of human MIS

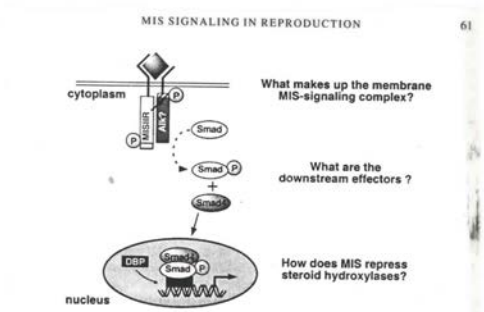


FIG. 5. MIS signaling cascade (hypothetical). Binding of MIS to the MISIIR triggers subsequent binding to a type I receptor. Cross-phosphorylation of the type I receptor upon ligand binding results in phosphorylation of SMAD cytoplasmic proteins that, in turn, are shuttled into the nuclei, where they heterodimerize with other DNA-binding proteins. This heterodimeric complex binds cis-elements to affect gene expression. Identification of MIS target genes in the embryo and adult awaits further work.

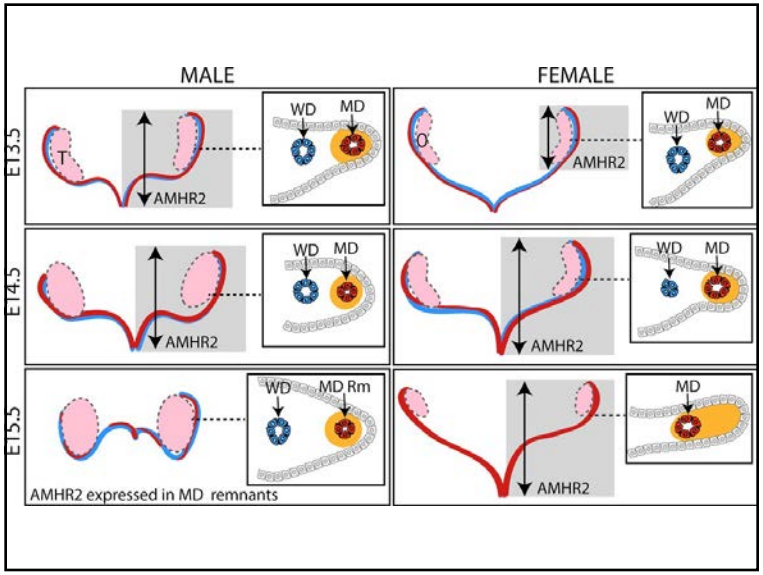


Table 1. Effect of protease inhibitors on Müllerian duct regression

Protease inhibitor	Target protease	% Regressed*	n†
Control analog	None	95	19
GM6001	Metalloproteinases	5	22
Phosphoramidon	Metalloproteinases	50	8
Ecotin	Serine proteases	45	11
Aprotinin	Serine proteases	86	7
Leupeptin	Serine proteases	100	7
Boc-D-FMK	Caspases	0	9

*Genital ridges with bo Müllerian duct.
†Total number of ridges tested. Experiments were repeated on 3-11 separate litters. Control analog and GM6001 were tested on male and female ‡MIS cultures; all other inhibitors were tested on males.

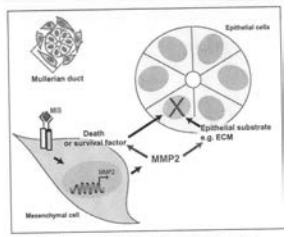
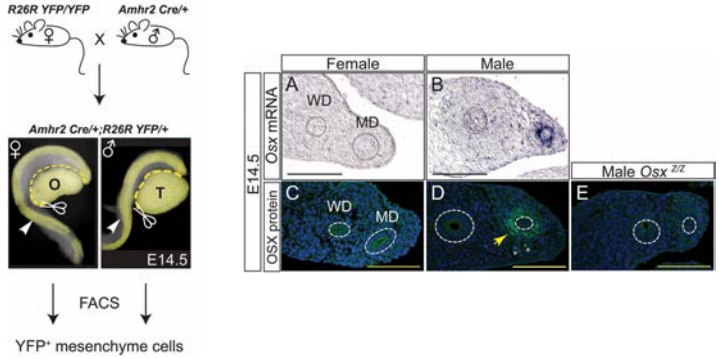
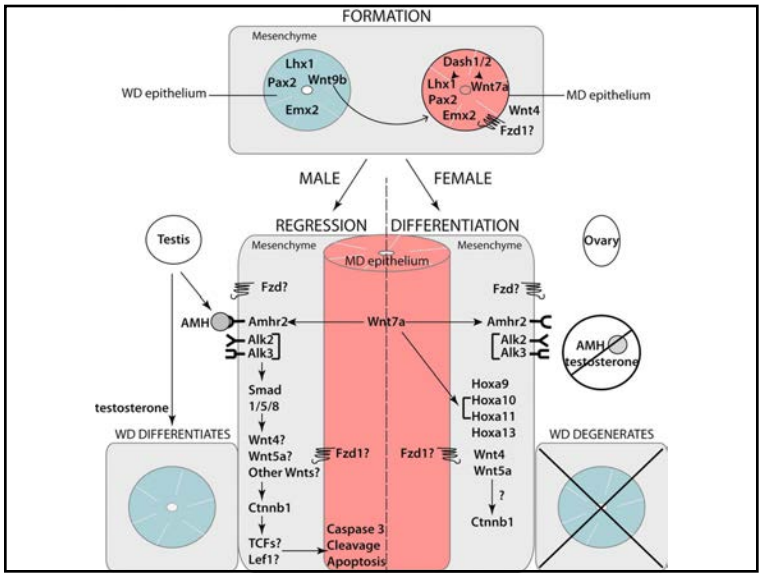
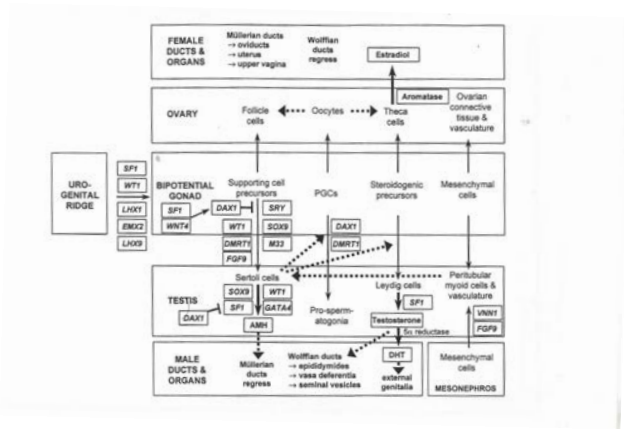


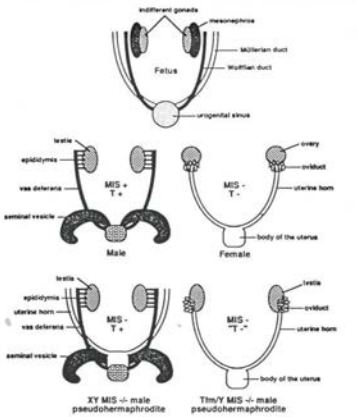
Fig. 6. Potential roles of MMP2 in mediating Müllerian duct regression. Activation of the MIS signaling cascade induces expression of MMP2 in the Müllerian duct mesenchyme. Latent or pro-MMP2 is secreted into the extracellular space, where it is activated to its mature form by membrane bound metalloproteinases such as MMMP14. Cell death of the Müllerian duct epithelium may occur by MMP2 cleavage of a factor secreted by the mesenchyme. MMP2 activity may result in the degradation of a survival factor or activation of a death factor. Alternatively, MMP2 could cause apoptosis by cleaving substrates on the epithelial cell, such as the epithelial cell basement membrane.

Osterix functions downstream of anti-Müllerian hormone signaling to regulate Müllerian duct regression.
Mullen RD, Wang Y, Liu B, Moore EL, Behringer RR.
Proc Natl Acad Sci U S A. 2018 Aug 14;115(33):8382-8387.

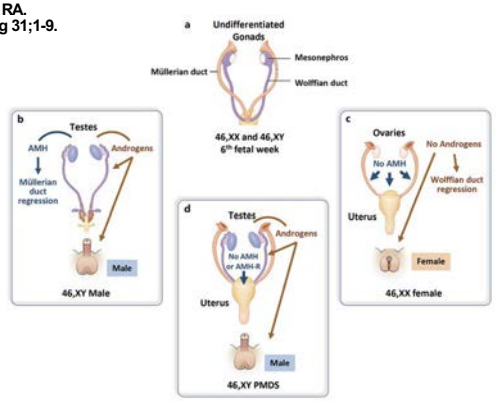








AMH and AMHR2 Involvement in Congenital Disorders of Sex Development
Brunello FG, Rey RA.
Sex Dev. 2021 Aug 31;1-9.



Hormonal control of fetal sex differentiation. **a** In the human embryo, before the 7th week, the primordia of the gonads and of the external genitalia are undifferentiated and sexually bipotential, while 2 duct systems coexist, the müllerian and the wolffian ducts, which are unipotential. **b** In the male, the testes secrete anti-müllerian hormone (AMH), responsible for müllerian duct regression, and androgens, responsible for wolffian duct differentiation into the epididymis, vas deferens, and seminal vesicle, as well as for the virilization of the external genitalia. **c** In the female, the ovaries do not secrete AMH or testosterone during the sex differentiation window, which leads the müllerian ducts to form the fallopian tubes, the uterus, and the upper portion of the vagina, the wolffian ducts to regress, and the external genitalia to feminize. **d** In 46,XY individuals with mutations resulting in impaired expression of AMH or the AMH receptor type II, the müllerian duct derivatives develop, leading to the persistent müllerian duct syndrome (PMDS). Modified, with permission, from Josso and Rey [2020].

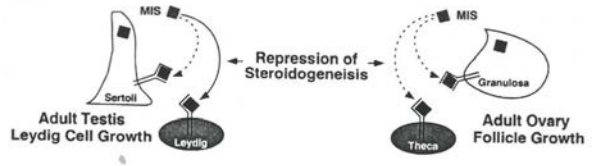


FIG. 2. MIS: a cellular signal in the adult reproductive system. In the adult testis and ovary, MIS is expressed in Sertoli and granulosa cells, respectively. The MISIIR is co-expressed with the MIS ligand in granulosa cells as well as the internal theca layer and in Leydig cells. Proposed functions for the MIS in the ovary have been implied from loss-of-function MIS or MISIIR mouse mutants (Durlinger *et al.*, 1999). In the testis, MIS represses Leydig cell growth. For both gonads, MIS is implicated in repression of steroid production.

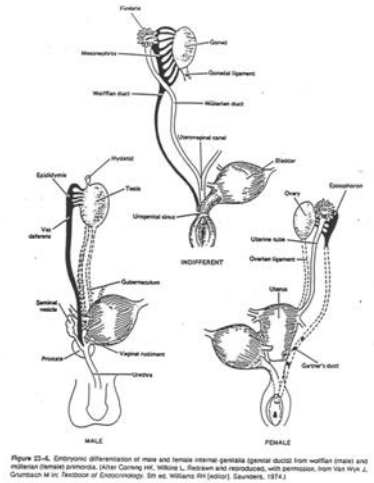
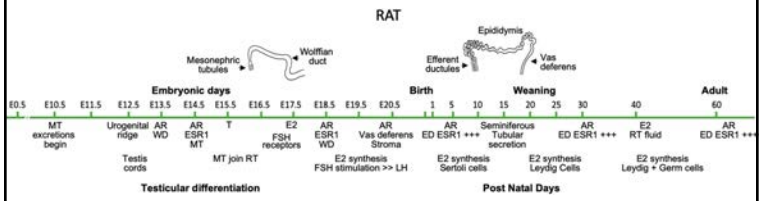
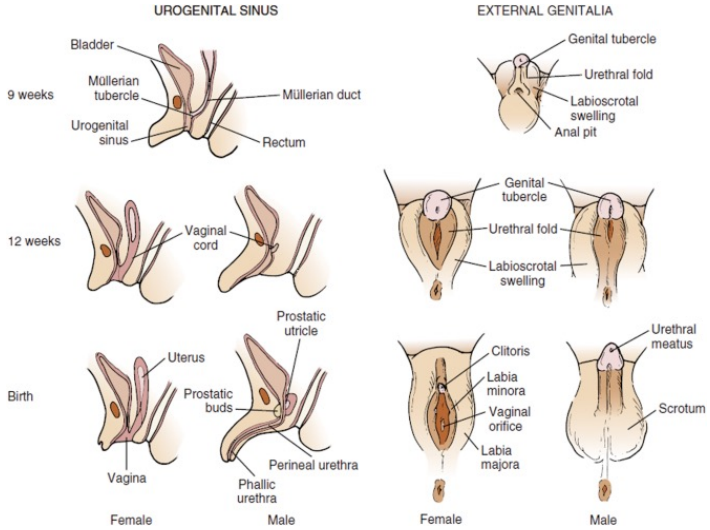


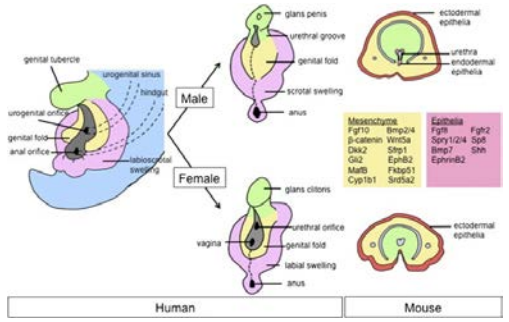
Figure 23-4. Embryonic differentiation of male and female internal genitalia (genital ducts) from wolffian (male) and müllerian (female) primordia. (After Corliss HK, Wilkins L, Redrawn and reproduced, with permission, from Van Wyk J, Grumbach M in: *Textbook of Endocrinology*. 5th ed. Williams RH (editor). Saunders, 1974.)

Estrogens and development of the rete testis, efferent ductules, epididymis and vas deferens
Hess RA, Sharpe RM, Hinton BT.
Differentiation. Mar-Apr 2021;118:41-71.



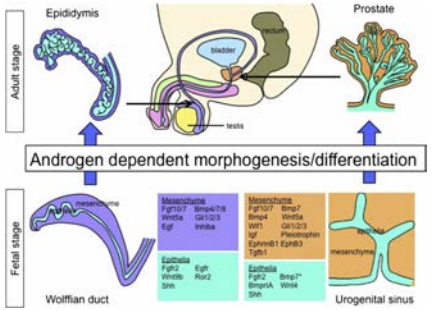
\Time course major events in development and differentiation of the rat male reproductive system. Although efferent ductules were emphysized for ESR1 expression, epididymal epithelium has positive expression in select cell types (see section 3). MT, mesonephric tubules; AR, androgen receptor; ESR, estrogen receptor; WD, Wolffian duct; T, testosterone; E2, estrogen; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; ED, efferent ductules; RT, rete testis. Adapted from Hinton and Avellar (2018).





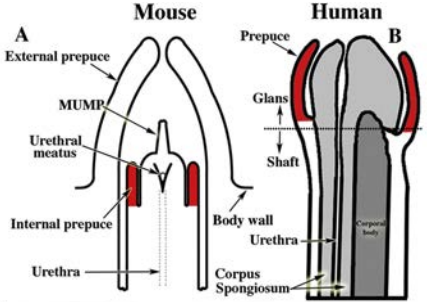
A schematic diagram of sexual differentiation of external genitalia. In the mouse, the first morphological differences between the male and female GT are evident from E16.5. The fusion of the urethral folds in the ventral midline leads to the formation of the urethral groove, which subsequently becomes the mature urethra (human). This fold is prominent in the male GT after E16.5 (mouse). The canalization of the urethral plate proceeds proximo-distally in the male GT. Genes expressed in the mesenchyme and epithelia are shown. The bilateral mesenchyme is the ventral (lower) side of embryonic GT mesenchyme adjacent to the endodermal epithelia which develops to the urethra (mouse).

Androgens and mammalian male reproductive tract development.
Murashima A, Kishigami S, Thomson A, Yamada G.
Biochim Biophys Acta. 2015 Feb;1849(2):163-70.



A schematic diagram of male genital tract masculinization. WD epithelium is stabilized by androgen exposure, and the epididymis elongates and coils three-dimensional manner (left low and upper illustration). Prostate develops from the UGS. During sexual differentiation, solid buds from the urogenital sinus epithelium invade into the urogenital sinus mesenchyme where subsequent branching occurs (right low and upper illustration). Circulating or testis-derived androgens initiate these developmental processes through the mesenchymally expressed AR which regulates paracrine signaling to epithelia. Growth factor related genes which show tissue specific expression patterns are summarized in the scheme. *lobe-specific expression.

Reproductive tract biology: Of mice and men.
Cunha GR, Sinclair A, Ricke WA, Robboy SJ, Cao M, Baskin LS.
Differentiation. 2019 Nov - Dec;110:49-63.



The mouse penile glans (A) lies within an extensive preputial space defined by the hair-bearing external prepuce, which forms the prominent elevation in the perineum. Comparison of (A & B) suggests homology between the human prepuce and the mouse “internal prepuce” (both red) in so far as both are integral to the distal aspect of the penis and encircle the glans. The MUMP (male urogenital mating protuberance) is a fibrocartilaginous process that extends ~1 mm beyond the urethral meatus in mice (A). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

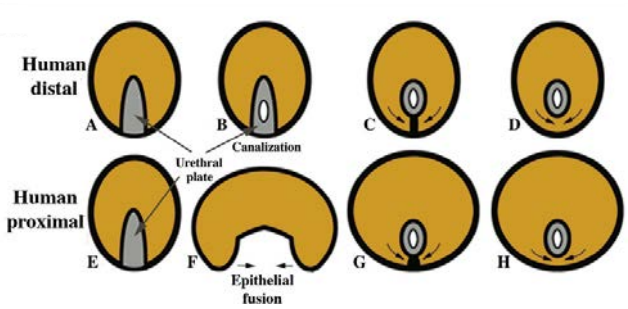
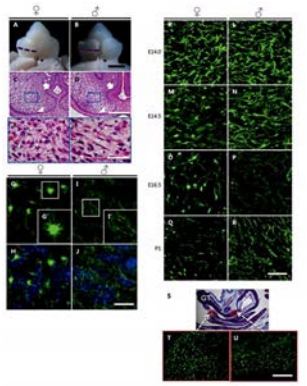
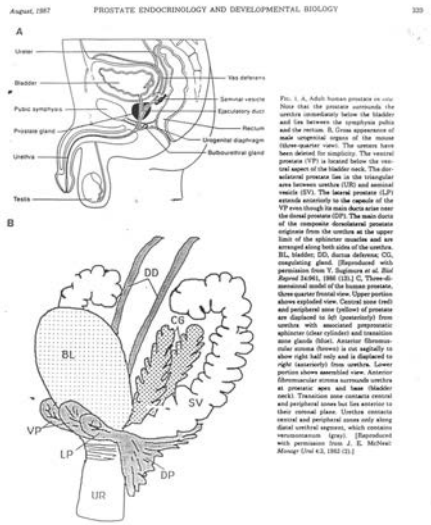


Diagram of divergent development of human penile urethral development. The distal portion of the human penile urethra (within the glans) develops via direct canalization of the urethral plate, while the proximal portion of the human penile urethra within the shaft forms via fusion of the urethral folds.

Androgen Regulates Dimorphic F-Actin Assemblies in the Genital Organogenesis.
Liu L, Suzuki K, Chun E, Murashima A, Sato Y, Nakagata N, Fujimori T, Yonemura S, He W, Yamada G.
Sex Dev. 2017;11(4):190-202.



Prostate and Seminal Vesicle



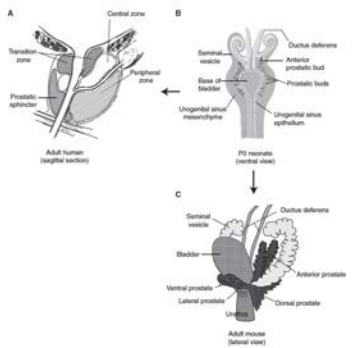
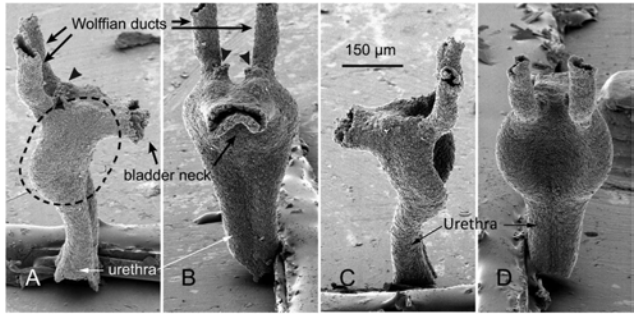
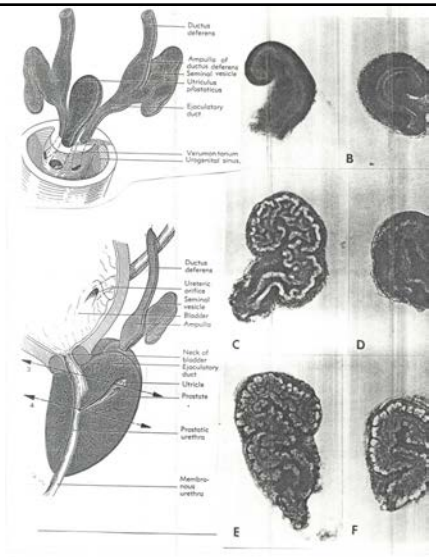


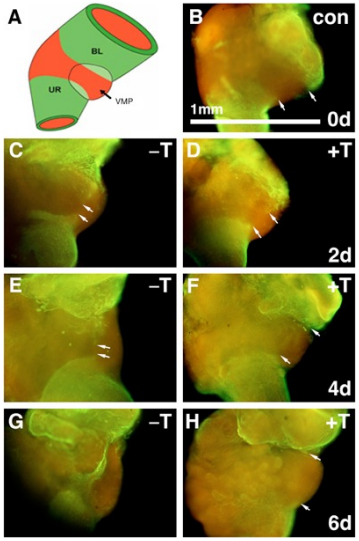
Figure 1. Schematic diagram showing the structures of the male neonatal urogenital sinus (UGS) that develops into the adult human prostate and adult mouse prostate. (*A*) The adult human prostate is acorn-shaped and composed of three zones: transition zone, central zone, and peripheral zone. (*B*) In both the human and mouse prostate, the developing UGS epithelium buds out into the surrounding mesenchyme. (*C*) The mouse prostate has four prostatic lobes named after their position relative to the bladder: anterior prostate, lateral prostate, dorsal prostate, and ventral prostate. (Panel *A* is based on data in McNeal 1968. Panel *B* is reprinted from Bhatia-Gaur et al. 1999, courtesy of Creative Commons License, Attribution—NonCommercial 4.0 International License. Panel *C* is reprinted from Cunha et al. 1987; adapted, with permission, from Oxford University Press © 2008.)

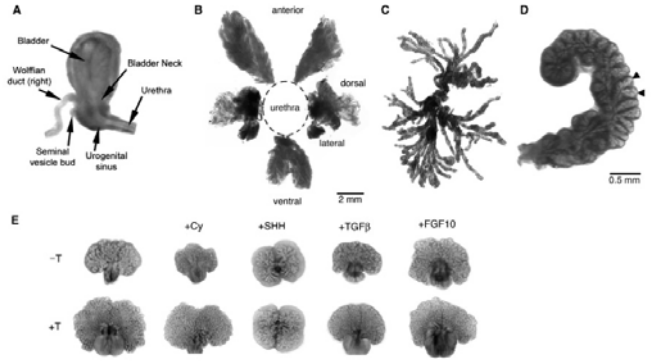
Development of the human prostate.
Cunha GR, Vezina CM, Isaacson D, Ricke WA, Timms BG, Cao M, Franco O, Baskin LS. Differentiation. 2018 Sep - Oct;103:24-45.



UGS epithelium from a fetal mouse on gestation day 15 after removal of the mesenchyme. Images were taken by scanning electron microscopy (SEM) at successive 90˚ rotations of the sample, starting with a lateral view (A). Dotted line in (A) indicates that portion of the UGS from which prostatic buds will emerge. Arrowheads show where the Müllerian ducts entered the UGS epithelium. From Lin et al. (2003) with permission.







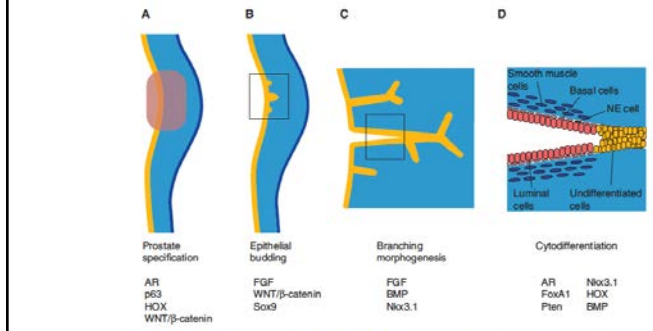
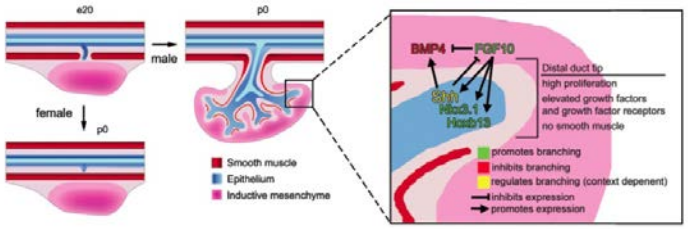


Figure 3. Morphological processes that occur during prostate organogenesis and associated genes and signaling pathways. (*A*) Prostate cell-fate specification and organ initiation. Red box denotes an area where urogenital sinus (UGS) cells have been specified to become prostate. (*B*) The UGS epithelium buds out into the surrounding mesenchyme. (*C*) Branching morphogenesis of the prostate epithelia and canalization to produce lumen. (*D*) Differentiation of the epithelial cells into luminal cells, basal cells, and rare neuroendocrine (NE) cells and mesenchyme into smooth muscle cells. Yellow is the epithelium and light blue is the mesenchyme.



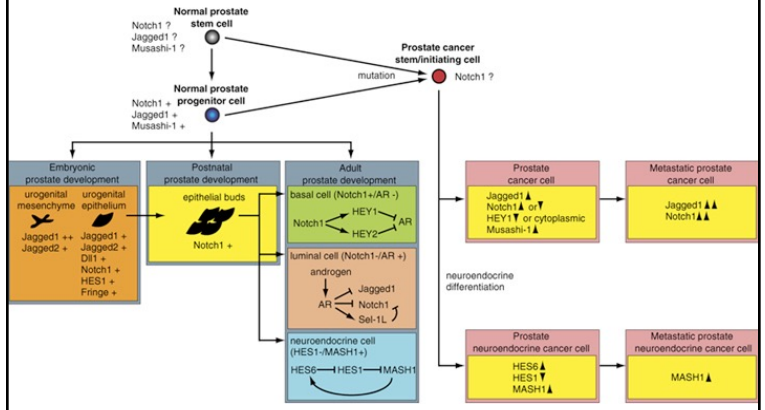
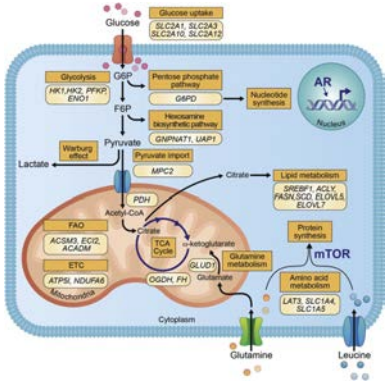


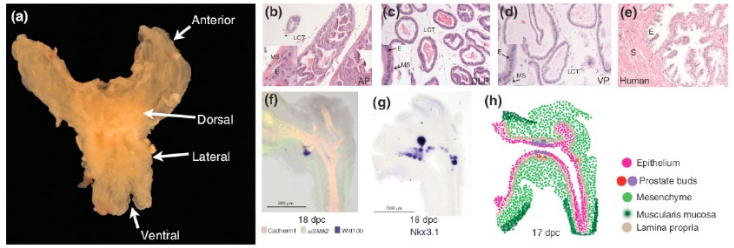
Table 1 Proteins affecting branching morphogenesis in the prostate or seminal vesicles

Protein	Role in branching?	Supporting data for		Supporting evidence		References
		Prostate	Seminal vesicles	*In vitro* studies	Genetic studies	
AR	Promote	X	X	X	X	Takeda et al. (1986), Brown et al. (1988), Lubahn et al. (1989), Charest et al. (1991), Gaspar et al. (1991), He et al. (1991)
BMP4	Inhibit	X		X	X	Lamm et al. (2001)
BMP7	Inhibit	X		X	X	Grishina et al. (2005)
FGF7	Promote	X	X	X		Alarid et al. (1994), Sugimura et al. (1996)
FGF10	Promote	X	X	X	X	Thomson and Cunha (1999), Donjacour et al. (2003)
FST	Promote	X		X		Cancilla et al. (2001)
GDF7	Promote		X		X	Settle et al. (2001)
GHR	Promote	X			X	Ruan et al. (1999)
GLI2	Promote	X		X	X	Doles et al. (2006)
HOXA10	Promote	X	X		X	Podlasek et al. (1999c)
HOXA13	Promote	X	X		X	Podlasek et al. (1999b)
HOXB13	Promote	X			X	Economides and Capecchi (2003)
HOXD13	Promote	X	X		X	Podlasek et al. (1997), Economides and Capecchi (2003)
IGF1	Promote	X			X	Ruan et al. (1999)
INHBA	Inhibit	X		X		Cancilla et al. (2001)
NKX3.1	Promote	X			X	Bhatia-Gaur et al. (1999), Schneider et al. (2000), Tanaka et al. (2000)
p63	Promote	X			X	Signoretti et al. (2000)
SFRP1	Promote	X		X		Joesting et al. (2005)
TGFβ	Inhibit	X		X		Itoh et al. (1998), Tomlinson et al. (2004)
SHH	Context dependent regulator	X		X	X	Podlasek et al. (1999a), Freestone et al. (2003), Wang et al., (2003), Lamm et al., (2002), Berman et al. (2004), Doles et al. (2006)
SMO	Context dependent regulator	X		X	X	(Podlasek et al. (1999a), Freestone et al. (2003), Wang et al. (2003), Lamm et al. (2002), Berman et al. (2004), Doles et al. (2006)
SRD5A2	Promote	X	X		X	Andersson et al. (1991), Mahendroo et al. (2001)

BMP, bone morphogenetic protein; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β; SHH, sonic hedgehog.

Functional genomic studies reveal the androgen receptor as a master regulator of cellular energy metabolism in prostate cancer.
Gonthier K, Poluri RTK, Audet-Walsh É.
J Steroid Biochem Mol Biol. 2019 Jul;191:105367.





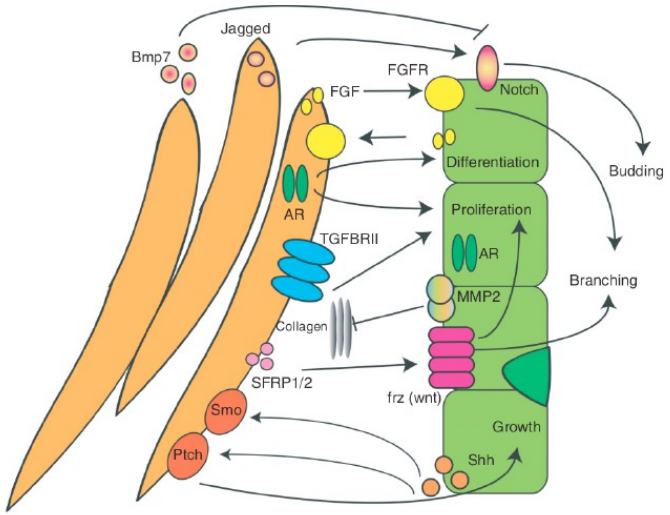


TABLE 1 | Recent Advances in Paracrine Regulation of Prostate Development

Name	Process	Reference
Axin2	Expressed in budding and branching epithelium	18
Bmp2	Marker of the ventral prostate	16
Bmp7	Mesenchymal expression inhibits Notch and restricts budding	19
Lef1	Expressed in budding and branching epithelium	18
FGF10	Stromal expression promotes branching	20, 21
FGFR2	Epithelial expression is required for proper branching and optimal androgen responsiveness	20, 22
MMP2	Epithelial expression required for branching and reducing collagen deposition of stroma	23
Notch	Required for terminal differentiation of epithelium	24
SFRP1	Prostate initiation gene signature and branching	12, 13
Shh	Required for epithelial growth	25
SOX9	Promotes prostate budding (particularly VP and AP) and deletion reduces FGFR2 expression	26
Sulf1	Inhibits ductal branching and FGFR signaling	27
Wnt4	Prostate epithelium marker	18
Wnt7a	Prostate epithelium marker	18
Wnt9b	Prostate epithelium marker	18
Wnt10b	Marker for prostate buds and epithelium	16, 18

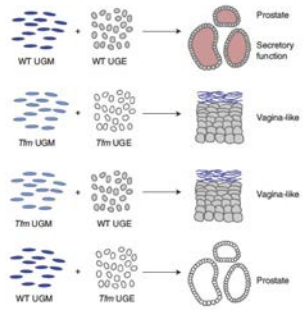
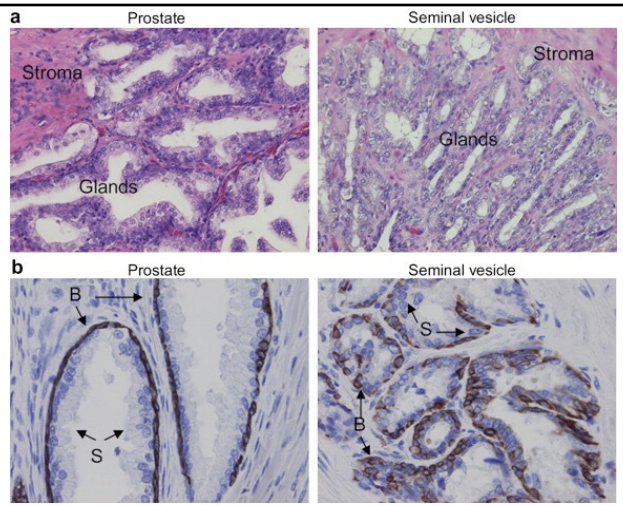


Figure 4. Tissue recombination experiments demonstrating the essential role of androgen receptor (AR) during prostate development. Prostate glands form that produce secretory products when wild-type (WT) urogenital sinus (UGS) mesenchyme (UGM) and WT UGS epithelia (UGE) are combined and grafted in a male host animal. When *Testicular feminization* (*Tfm*), AR-mutant, embryonic UGM is combined with either WT UGE or *Tfm* UGE, no prostate structure forms and vagina-like differentiation occurs, demonstrating the essential role of mesenchymal AR in the early stages of prostate organogenesis acting in a paracrine fashion to promote epithelial bud growth. UGE from *Tfm* mutant embryos combined with WT UGM forms prostate structures with no secretory function. (From Cunha 2008; adapted, with permission, from Elsevier © 2008.)



Histology of SVs and the prostate. The glandular structure of SVs and the prostate is visualized in standard paraffin-embedded tissue sections. The sections were stained with hematoxylin-eosin (HE) (A) and for basal cytokeratins 5/14 (B) as described in Jäämaa et al. (2010). The epithelium of both tissues consists of two major cell types, basal (B) and secretory (S) cells. Note the discontinuous layer of basal cells in SVs.

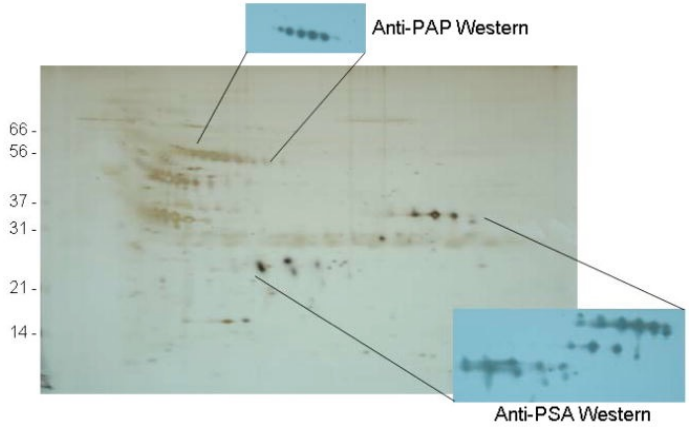


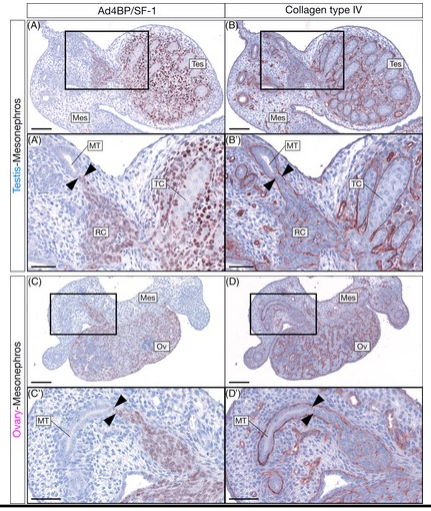
Table 1 – Abundant EPS urine proteins identified following 2D-gel separation.

Protein name*	Score	Peptide matches
AMBP/Alpha-1-microglobulin	1160	254
Gelsolin	1097	97
Serotransfertin	1001	32
Basement membrane-specific HS proteoglycan	981	60
Serum albumin	890	66
Ig kappa chain C regions	673	102
Inter-alpha-trypsin inhibitor heavy chain H4	657	45
Alpha-1B-glycoprotein	518	28
Prostate-specific antigen	478	31
Vesicular integral-membrane protein, VIP36	477	12
Ig lambda chain C regions	455	41
Complement C3	432	9
Actin, cytoplasmic 1	391	18
Epithelial cadherin	352	7
Prostatic acid phosphatase	349	17
Protein S100-A9	312	11
Cell adhesion molecule 4	294	5
Apolipoprotein D	230	9
Prostaglandin-H2 D-isomerase	227	5
Carboxypeptidase E	217	6
Zinc-alpha-2-glycoprotein	208	7
Triosephosphate isomerase	202	4
Cathepsin H	185	7
Alpha-1-antitrypsin	151	2
Histidine-rich glycoprotein	134	2
Annexin A5	133	5
Uromodulin precursor	124	5
14-3-3 protein epsilon	119	3
Proactivator polypeptide/Saposin-A	118	10
Monocyte differentiation antigen CD14	85	2
Endothelial protein C receptor	77	3
Metalloproteinase inhibitor 1	76	2
14-3-3 protein beta/delta	70	2
Neural cell adhesion molecule 1	68	2

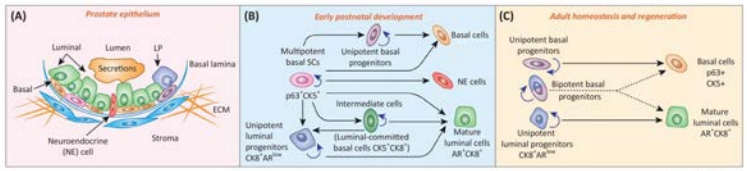
*Gel spots were excised from 2D gels, then reduced, alkylated and digested with trypsin to release peptides. Mass spectrometric analysis was performed on an LTQ™ Linear Ion Trap (ThermoFinnigan, San Jose, CA) mass spectrometer in the data-dependent acquisition mode. Survey full scan MS spectra (from m/z 300 to 1800) were acquired and the four most intense ions in a scan were sequentially isolated and fragmented in the linear ion trap (MS/MS). The peptide sequences were identified from their tandem mass spectra using Mascot a probability based search engine (www.matrixscience.com) using the SwissProt database. The following search criteria were used: variable modifications; carbamido-methylation of cysteine and oxidation of methionine residues, 1 missed enzyme cleavage site and an error tolerance of 1.5 Da for MS and 0.8 Da for MS/MS.

Prostate Disease and Cancer

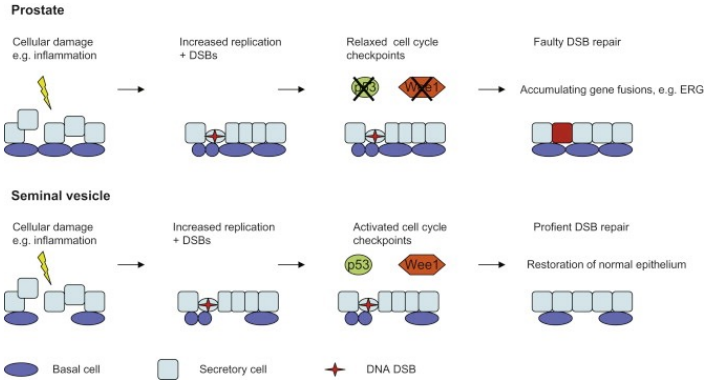
Progenitors in prostate development and disease
Joseph DB, Turco AE, Vezina CM, Strand DW.
Dev Biol. 2021 May;473:50-58.



Prostate Luminal Progenitor Cells in Development and Cancer.
Zhang D, Zhao S, Li X, Kirk JS, Tang DG.
Trends Cancer. 2018 Nov;4(11):769-783.



Hierarchy of Prostate Epithelial Cell Lineages. (A) A simplified cartoon illustrating the epithelial cell composition and glandular structure of the adult prostate. (B,C) Hierarchy of epithelial cell lineages during early prostate development (B) and homeostasis and regeneration (C), revealed by genetic lineage-tracing studies. Blue-curved arrows denote self-renewal and broken arrows indicate inconsistent results reported by different groups. Abbreviations: ECM, extracellular matrix; LP, luminal progenitor; SC, stem cell.



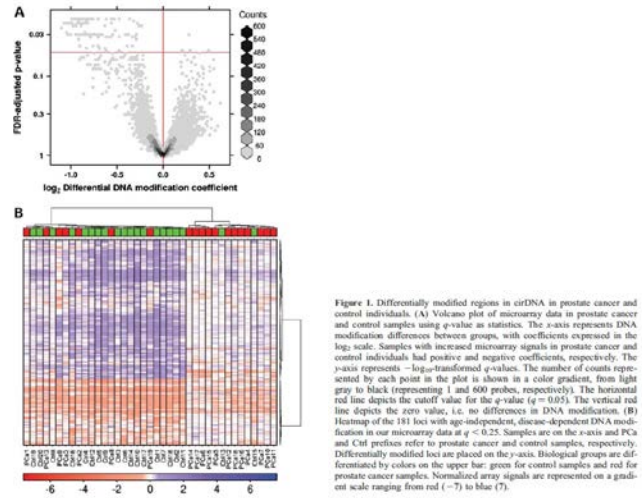
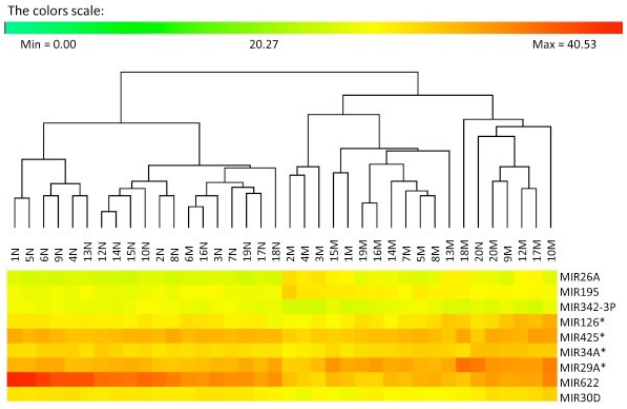
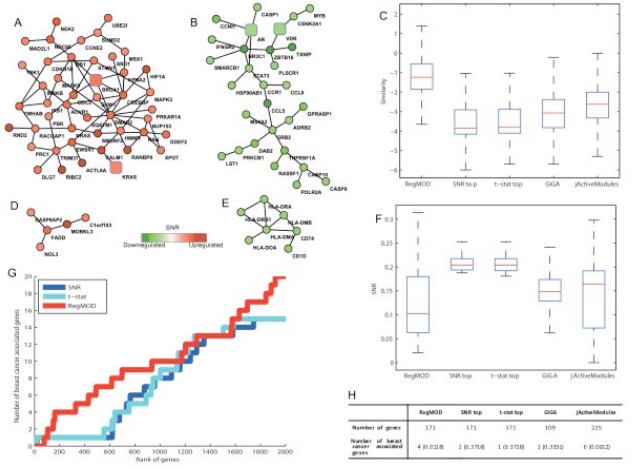


Figure 1. Differentially modified regions in cirDNA in prostate cancer and control individuals. (**A**) Volcano plot of microarray data in prostate cancer and control samples using q-value as statistics. The x-axis represents DNA modification differences between groups, with coefficients expressed in the $\log_2$ scale. Samples with increased microarray signals in prostate cancer and control individuals had positive and negative coefficients, respectively. The y-axis represents $-\log_{10}$-transformed q-values. The number of counts represented by each point in the plot is shown in a color gradient, from light gray to black (representing 1 and 600 probes, respectively). The horizontal red line depicts the cutoff value for the q-value ($q = 0.05$). The vertical red line depicts the zero value, i.e. no differences in DNA modification. (**B**) Heatmap of the 181 loci with age-independent, disease-dependent DNA modification in our microarray data at $q < 0.25$. Samples are on the x-axis and PCa and Ctrl prefixes refer to prostate cancer and control samples, respectively. Differentially modified loci are placed on the y-axis. Biological groups are differentiated by colors on the upper bar: green for control samples and red for prostate cancer samples. Normalized array signals are represented on a gradient scale ranging from red (−7) to blue (7).

A miRNA expression signature that separates between normal and malignant prostate tissues.

Carlsson J, Davidsson S, et al. Cancer Cell Int. 2011 May 27;11(1):14.





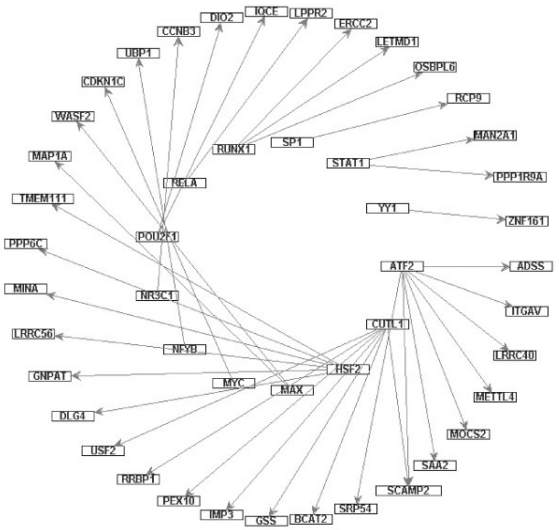


TABLE II. Genes Identified in the Genetic Prognostic Signature and the Hybrid Genetic and Clinical (Marked by ¶) Predictive Model

Gene symbol	Gene title	Mean expression in recurrent tumors	*P*-value	Occurrence frequencies
PAK3¶	P21 (CDKN1A)-activated kinase 3	Under-expressed	<9.0e−6	78 (79)
RPL23¶	Ribosomal protein L23	Over-expressed	<5.0e−5	79 (79)
E124¶	Etoposide-induced 2.4 mRNA	Over-expressed	<3.0e−7	79 (79)
TGFB3¶	Transforming growth factor, beta 3	Under-expressed	<1.0e−5	79 (3)
RBM34¶	RNA-binding motif protein 34	Over-expressed	<3.0e−4	62 (8)
PCOLN3	Procollagen (type III) N-endopeptidase	Under-expressed	<3.0e−5	78
FUT7	Fucosyl transferase 7 (alpha (1,3) fucosyl transferase)	Under-expressed	<3.0e−3	30
RICS Rho	GTPase-activating protein	Over-expressed	<3.0e−6	8
MAP4K4	Mitogen-activated protein kinase 4	Over-expressed	<3.0e−5	5
CUTL1	Cut-like 1, CCAAT displacement protein (*Drosophila*)	Over-expressed	<3.0e−5	2
ZNF324B	Zinc finger protein 324B	Under-expressed	<5.0e−4	1

The *P*-values, computed using a *t*-test, quantify the up- or down-regulation of a gene between patients with, and without recurrence. The value inside and outside of the brackets in the last column is the number of iterative models in which a gene was selected in the hybrid and genetic models, respectively.

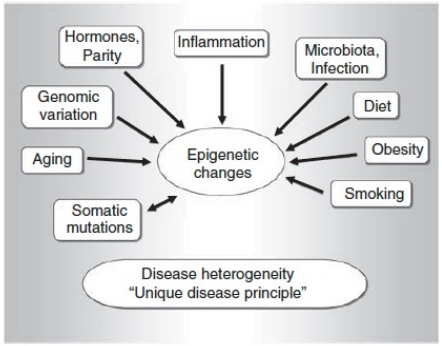


Figure 1 A variety of endogenous and exogenous etiological factors contribute to epigenetic changes leading to heterogeneity of disease processes, which is implicated by the 'unique disease principle'. To simplify, only selected examples of those etiological factors are demonstrated. There are numerous interactions between the factors, which are not depicted for simplicity.

Cortese R, Kwan A, et al. (2012) Epigenetic markers of prostate cancer in plasma circulating DNA. Hum Mol Genet. 15;21(16):3619-31.

Table 1. Summary of pyrosequencing results

Loci	Sample set 1 % Mod. Pca[a]	% Mod. Ctrl[a]	Disease p$_{LME}$[b]	Age p$_{LME}$[b]	Sample set 2 % Mod. Pca[a]	% Mod. Ctrl[a]	Disease p$_{LME}$[b]	Age p$_{LME}$[b]
DLG2	48.7 ± 22.0	45.6 ± 23.6	**0.02**	**0.02**	42.9 ± 14.7	41.9 ± 18.3	0.85	0.07
GNG7	38.4 ± 3.0	38.2 ± 2.4	0.74	0.40	37.9 ± 11.1	36.8 ± 7.8	0.93	0.21
HPSE2	22.7 ± 13.7	16.1 ± 8.3	0.13	**0.02**	40.6 ± 30.0	29.6 ± 27.1	0.32	0.82
KIAA1539	38.8 ± 5.8	37.1 ± 10.9	**1×10^{-3}**	**8×10^{-4}**	30.8 ± 10.0	36.2 ± 6.4	**1×10^{-4}**	0.05
NUDCD3	59.4 ± 6.1	63.6 ± 9.0	0.09	0.19	67.6 ± 11.6	69.7 ± 11.1	0.47	0.76
PCDHB1	66.6 ± 23.3	50.9 ± 36.2	0.51	0.69	58.4 ± 27.6	58.0 ± 25.0	0.97	0.77
RNF219	35.0 ± 18.1	55.0 ± 17.6	**3×10^{-41}**	**3×10^{-9}**	35.3 ± 31.8	46.2 ± 23.7	**1×10^{-4}**	0.14

Statistically significant differences ($p < 0.05$) are in bold.
[a]Mean cirDNA modification per amplicon.
[b]LME model *p*-value.

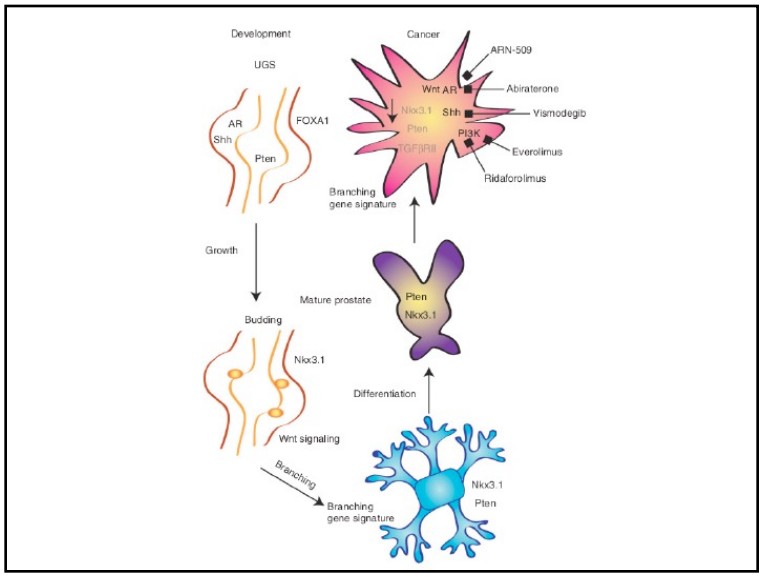
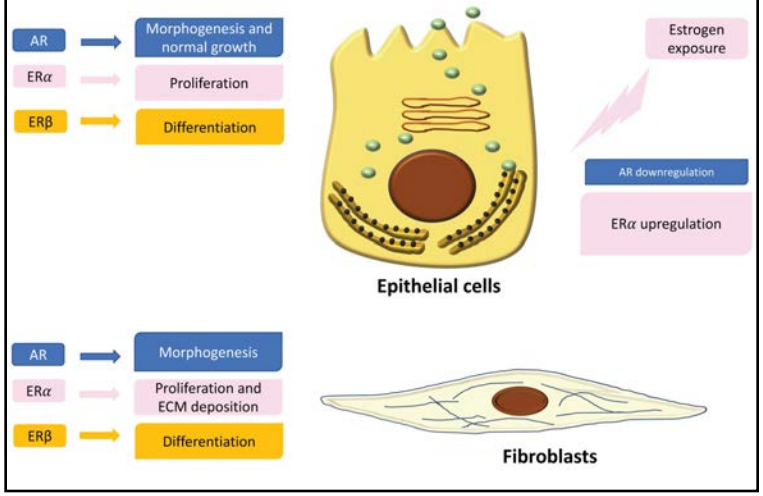


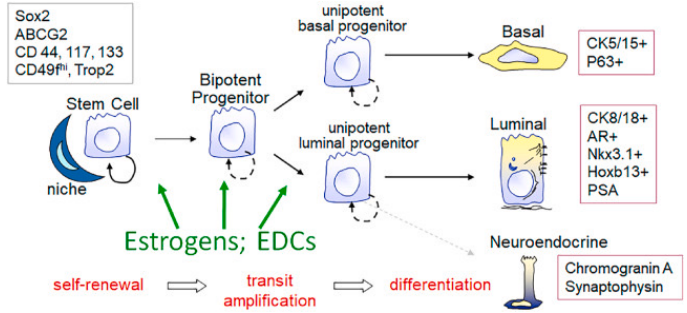
TABLE 2 | Drugs Targeting Prostate Development Pathways

Drug Name	Target	Phase of Development	Reference
Abiraterone	CYP17A1 inhibitor	Approved for castration-resistant prostate cancer, current trials with various drug combinations	58
ARN-509	AR antagonist	Phase I (NCT01171898) and Phase II (NCT01709734) trials in castration-resistant prostate cancer	57
Bicalutamide	AR antagonist	Approved for prostate cancer, current trials in combination with other drugs	62
Dutasteride	5α reductase type II inhibitor	Approved BPH, current trials for combination therapy for BPH and prostate cancer	63
Enzalutamide	AR antagonist	Approved for castration-resistant prostate cancer, current trials in combination with abiraterone, leuprolide, and bicalutamide	64
Everolimus	PI3 kinase/mTOR inhibitor	Phase I (NCT01642732) and II (NCT01313559) clinical trials for advanced prostate cancer	59
Finasteride	5α reductase type II inhibitor	Approved for BPH	63
Flutamide	AR antagonist	Approved for prostate cancer, current trials in combination therapies	62
Leuprolide	GnRH antagonist	Approved for prostate cancer, current trials in various combination therapies	62
Ridaforolimus	mTor inhibitor	Phase II prostate cancer trial (NCT00777959 and NCT00110188)	60
Vismodegib	Shh inhibitor	Phase I/II prostate cancer trial (NCT01163084) FDA approved for metastatic basal cell carcinoma	61

Combined Effects of Different Endocrine-Disrupting Chemicals (EDCs) on Prostate Gland
De Falco M, Laforgia V.
Int J Environ Res Public Health. 2021 Sep 16;18(18):9772.

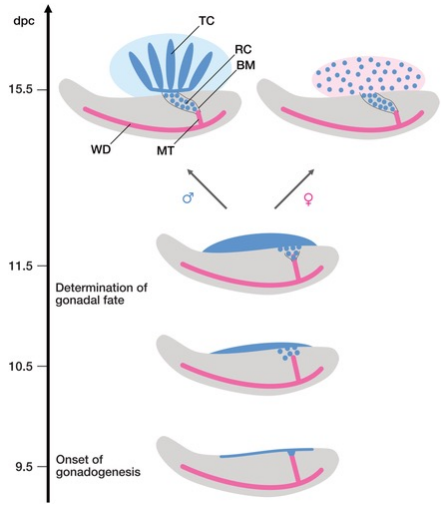


Developmental estrogenization: Prostate gland reprogramming leads to increased disease risk with aging
Prins GS.
Differentiation. Mar-Apr 2021;118:72-81.



Epididymis

Connection between seminiferous tubules and epididymal duct is originally induced before sex differentiation in a sex-independent manner
Omotehara T, Wu X, Kuramasu M, Itoh M.
Dev Dyn. 2020 Jun;249(6):754-764.



A diagram summarizing the present study in regard to the interaction between the Adrenal-4 binding protein/Steroidogenic factor-1 (Ad4BP/SF-1)-positive gonadal cells and mesonephric tubules (MT) during early gonadogenesis. Ad4BP/SF-1-positive cells (blue dots) are adjacent to the MT in all developmental stages, but serial localization of the basal membrane (BM) from MT to the Ad4BP/SF-1-expressing cells is found in both sexes after 11.5 dpc, indicating that the connection is induced before sex differentiation in a sex-independent manner. RC, rete cord; TC, testis cord; WD, Wolffian duct.

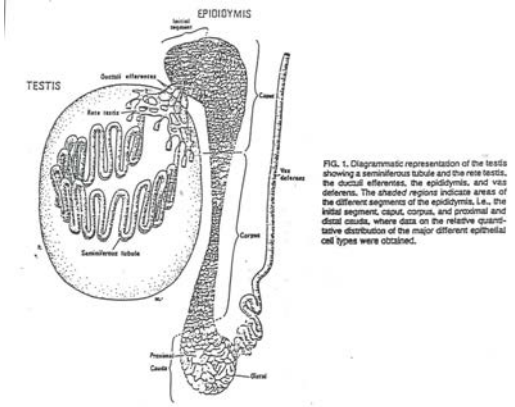
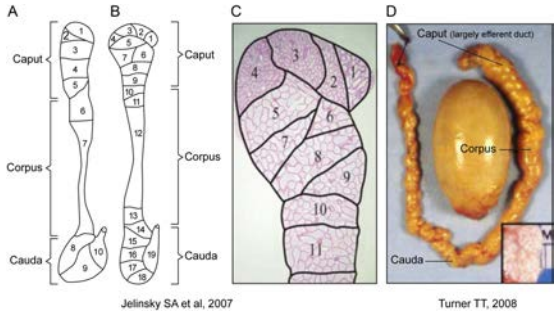


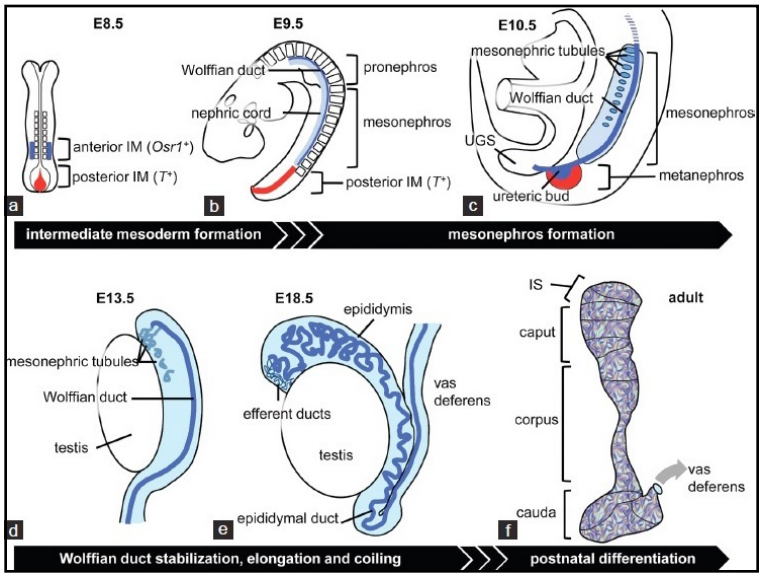
FIG. 1. Diagrammatic representation of the testis showing a seminiferous tubule and the rete testis, the ductuli efferentes, the epididymis, and vas deferens. The shaded regions indicate areas of the different segments of the epididymis, i.e., the initial segment, caput, corpus, and proximal and distal cauda, where data on the relative quantitative distribution of the major different epithelial cell types were obtained.

The human epididymis: its function in sperm maturation.
Sullivan R, Mieusset R.
Hum Reprod Update. 2016 Sep;22(5):574-87.



Jelinsky SA et al, 2007 Turner TT, 2008

Anatomy of the epididymis. Schematic representation of the mouse (A) and rat (B) epididymis illustrating the partitioning of epididymal interstitium by connective tissue septae. (C) Micrograph of longitudinal histological section of the proximal region of the rat epididymis. The connective tissue septae are drawn according to (B). (D) Photograph of the human epididymis dissected from the testis. Inset: higher magnification illustrating the organization of the epididymal tubule. (A), (B) and (C) Reprinted with permission from Jelinsky et al. (2007) and (D) Reprinted with permission from Turner (2008).

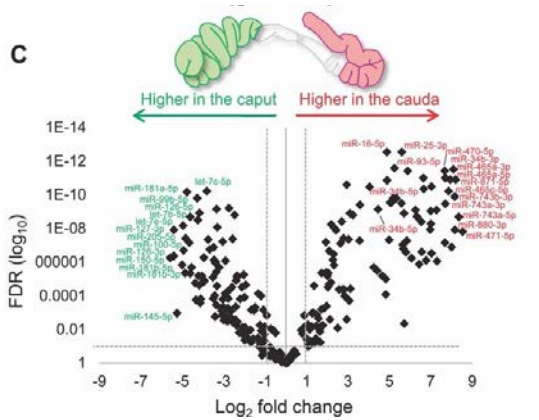


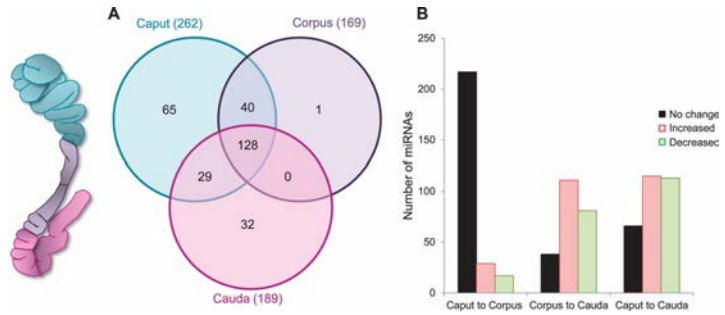
Mouse and human epididymides.

Mouse: 1, initial segment, proximal caput; 2, midcaput; 3, distal caput; 4, corpus; 5, cauda. Adapted (regional divisions by the author) from Trevor Cooper, University of Muenster (Yeung and Cooper, 2002), with permission of author and publisher, Springer. Human: ED, efferent ducts; 1, anterior caput; 2, posterior caput; 3, anterior corpus; 4, midcorpus; 5, posterior corpus; 6, anterior cauda; 7, posterior cauda; DD, ductus deferens. Reprinted by permission of the author (Dacheux et al., 2006), UMR INRA-CNRS and publisher, Elsevier Limited. Scale bar, 1 cm.

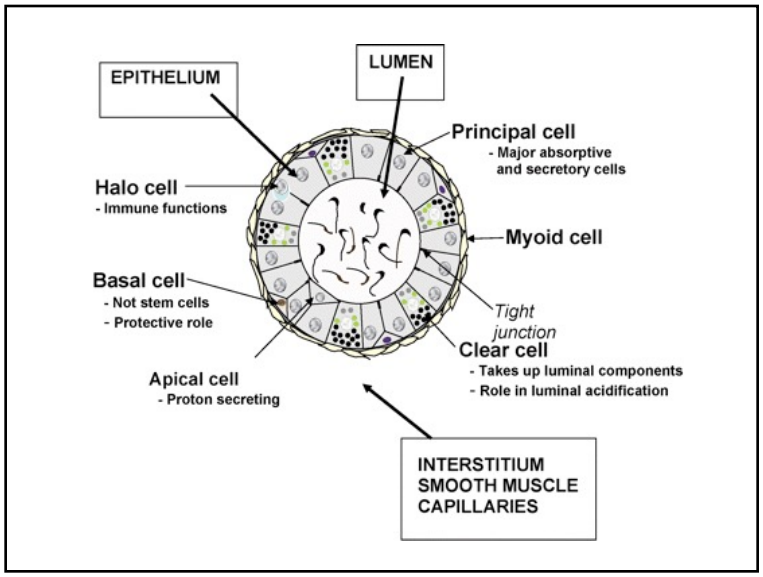
The microRNA signature of mouse spermatozoa is substantially modified during epididymal maturation.

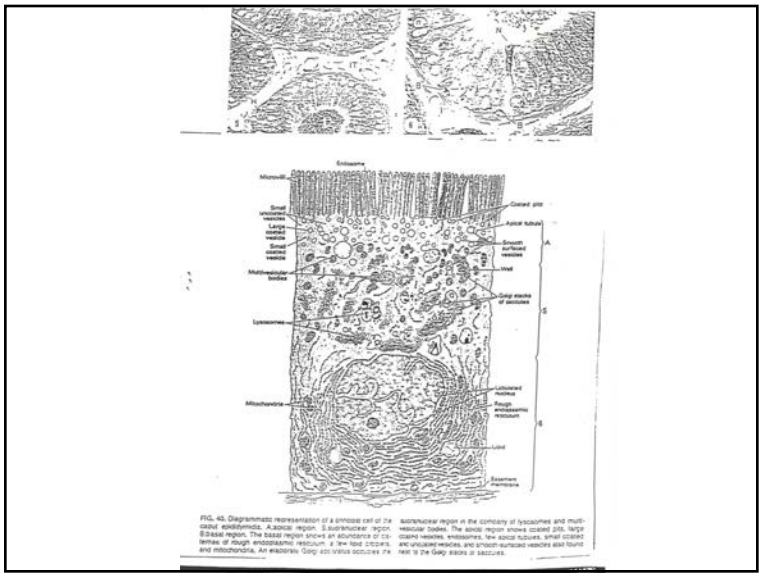
Nixon B, Stanger SJ, Mihalas BP, Reilly JN, Anderson AL, Tyagi S, Holt JE, McLaughlin EA. Biol Reprod. 2015 Oct;93(4):91.

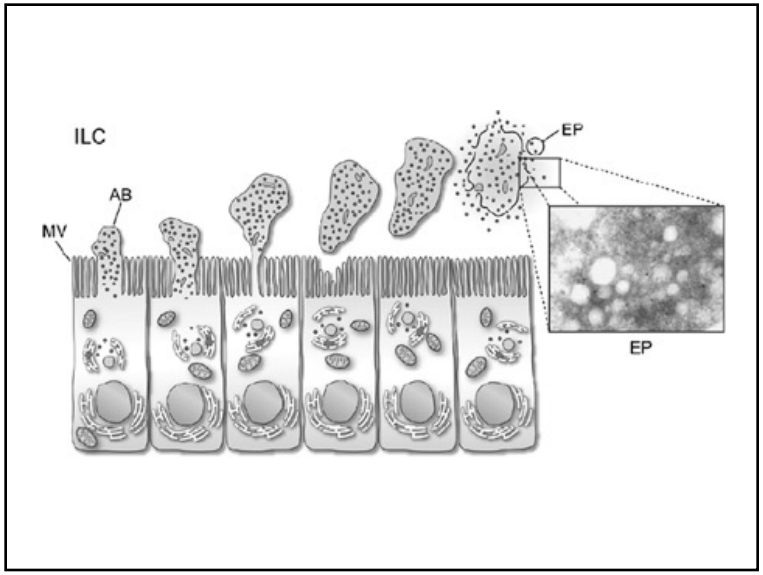




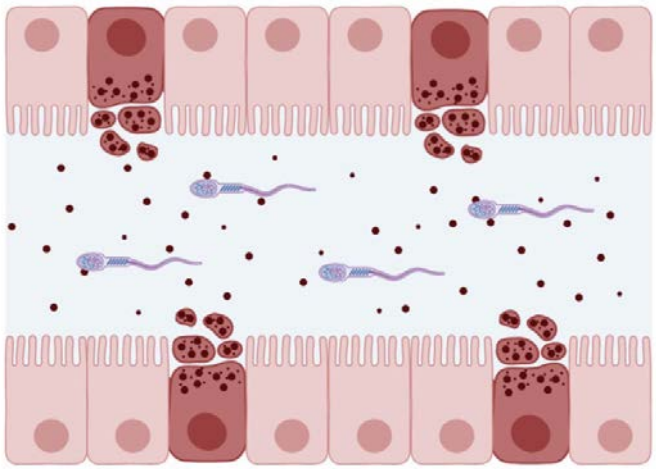
Determination of the miRNA signature present in mouse epididymal spermatozoa. A) Venn diagram illustrating the number of miRNAs that were identified by next-generation sequencing and their disruption within spermatozoa sampled from the caput, corpus, and cauda regions of the adult mouse epididymis. B) Graphical representation of miRNA distribution highlighting the number of significantly up- and down-regulated (threshold = ± ≥2-fold change and FDR of <0.05) miRNAs positively identified in spermatozoa between each epididymal region. For the purpose of these analyses, an average count value of >10 across two biological replicates (with each replicate comprising pooled miRNA from a minimum of nine animals) was used as the threshold for positive identification of all miRNAs.







The Role of Extracellular Vesicles in Sperm Function and Male Fertility
Foot NJ, Kumar S.
Subcell Biochem. 2021;97:483-500.

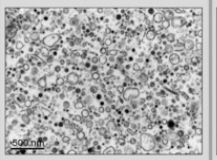
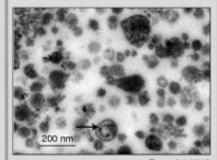


Sullivan R, Saez F. (2013) Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology. Reproduction. 2013 Jun 14;146(1):R21-35.

Table 1 Proteins associated with epididymosomes[a]. Proteins from epididymosomes with known or proposed functions once transferred to spermatozoa during maturation.

Name	Abbreviation	Functions	Particularities	References
Macrophage migration inhibitory factor	MIF	Associated with sperm dense fibers; involved in motility	Chelation of Zn; disulfide-bound formation	Eickhoff *et al.* (2004, 2006) and Frenette *et al.* (2002, 2003, 2004, 2005, 2006, 2010)
Liprin α3	Ppfia3	Acrosome reaction	Estrogen-responsive element in the 5′UTR	Joshi *et al.* (2012)
Kinases cSrc	cSrc	Signaling cascade of capacitation	Essential in cauda epididymal development	Krapf *et al.* (2012)
Glutathione peroxidase 5	GPX5	Protection against oxidative stress (DNA integrity)	Seleno-independent GPX	Chabory *et al.* (2009)
Ubiquitin	UBC	Elimination of defective spermatozoa	Involved in proteasome activity	Fraile *et al.* (1996) and Sutovsky *et al.* (2001)
Epididymal sperm binding protein 5	CD52 (HE5)	Protection against immune response	Highly glycosylated GPI anchored to sperm surface	Kirchhoff & Hale (1996), for review
Epididymal sperm binding protein 1	ELSPBP1	Elimination of defective spermatozoa	Zn-dependent transfer from epididymosomes to spermatozoa	D'Amours *et al.* (2012*a*, 2012*b*)
P26h (hamsters), P25b (bovine)	P26h/P25b	Sperm–zona pellucida interaction	GPI-anchored to sperm surface	Legare *et al.* (1999) and Frenette & Sullivan (2001)
Sperm adhesion molecule 1	SPAM1 (PH-20)	Different roles in fertilization	GPI-anchored to sperm surface	Martin-DeLeon (2006) and Griffiths *et al.* (2008)
Glioma pathogenesis-related protein 1	GLIPR1L1	Roles in fertilization	Belongs to the CAP family, GPI-anchored to sperm surface	Caballero *et al.* (2012) and Gibbs *et al.* (2010)
A desintegrin metalloproteases	ADAM2, ADAM3, ADAM7	Involved in fertilization	Behave as integral membrane proteins once transferred to sperm	Oh *et al.* (2005)
Methylmalonate-semialdehyde dehydrogenase	MMSDH	Unknown	Behave as peripheral and integral membrane protein once transferred to sperm	Suryawanshi *et al.* (2012)

[a]Proteomes of ram epididymosomes (exosomes-like), bovine caput, and cauda epididymosomes and of human 'epididymosomes' collected from the proximal vas deferens during vasectomy procedures have been published by Gatti *et al.* (2005), Thimon *et al.* (2008*a*, 2008*b*) and Girouard *et al.* (2011) respectively.

Epididymosomes		Prostasomes	
		 Ronquist 2012	
	Reference		Reference
Size: 25–300 nm	(1)	Size: 30–500 nm	(2)
Cellular origin: epididymal principal cells	(3)	Cellular origin: prostate acinar cells	(2)
Extracellular release: Apocrine secretion	(3, 4)	Extracellular release: Exocytosis of storage vesicles	(5)
Function: Sperm maturation, transfer of proteins to the sperm surface	(6)	Function: Sperm motility, immunosuppressive, and antioxydant factor, and sperm capacitation	(7)
Lipids: cholesterol: phospholipid ratio = 0.5	(8, 9)	Lipids: cholesterol: phospholipid ratio = 2. High amount of sphingomyelin.	(10)

Proteomic Profiling of Mouse Epididymosomes Reveals their Contributions to Post-testicular Sperm Maturation.
Nixon B, De Iuliis GN, Hart HM, et al.
Mol Cell Proteomics. 2019 Mar 15;18(Suppl 1):S91-S108.

TABLE I
Summary of mouse epididymosome proteome data set

	Total proteins identified	Av. peptide hits/protein	Av. unique peptide hits/protein	Av. protein coverage (%)	Number of differentially accumulated proteins (fold change > 1.5)		
					Corpus vs Caput	Cauda vs Corpus	Cauda vs Caput
Mouse epididymosomes	1640	13.1	11.8	29.9	146	344	474

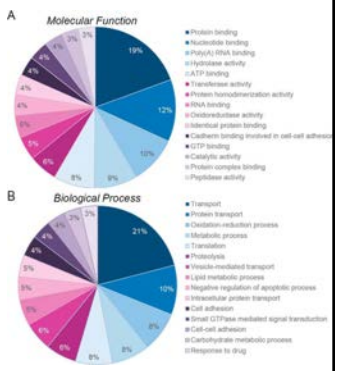


Table 1 Epididymal sperm proteins

Sperm proteins modified or relocalized during epididymal transit	Epididymal proteins that interact with spermatozoa
Spam1[1]	CRISP1[11]
ADAM2[2], ADAM3[3], ADAM15[4], ADAM24[5]	P26h[12]
α-mannosidase[6]	Clusterin[13]
CE9[7]	HE1[14], HE2[15], HE4[16], HE5[17], HE12[18]
β-galactosidase[8]	HEL75[19]
Basigin[9]	SPAG11[20]
α-enolase[10]	Eppin[21]
Grp78/Hsp70[10]	Cystatin 11[22]
Endoplasmin[10]	SED1[23]
Phosphatidylethanolamine binding protein[10]	
Lactate dehydrogenase 3[10]	
Testis lipid-binding protein[10]	
Cytokeratin[10]	
β-subunit F1-ATPase[10]	

[1](Phelps *et al.*, 1990); [2](Lum and Blobel, 1997); [3](Frayne *et al.*, 1998); [4](Pasten-Hidalgo *et al.*, 2008); [5](Zhu *et al.*, 2001); [6](Tulsiani *et al.*, 1995); [7](Nehme *et al.*, 1993); [8](Scully *et al.*, 1987); [9](Saxena *et al.*, 2002); [10](Baker *et al.*, 2005); [11](Cohen *et al.*, 2000); [12](Legare *et al.*, 1999); [13](Sylvester *et al.*, 1991); [14](Kirchhoff *et al.*, 1996); [15](Osterhoff *et al.*, 1994); [16](Kirchhoff *et al.*, 1991); [17](Kirchhoff and Hale, 1996); [18](Saalmann *et al.*, 2001); [19](Lin *et al.*, 2008); [20](Yenugu *et al.*, 2006); [21](Richardson *et al.*, 2001); [22](Hamil *et al.*, 2002); [23](Ensslin and Shur, 2003).

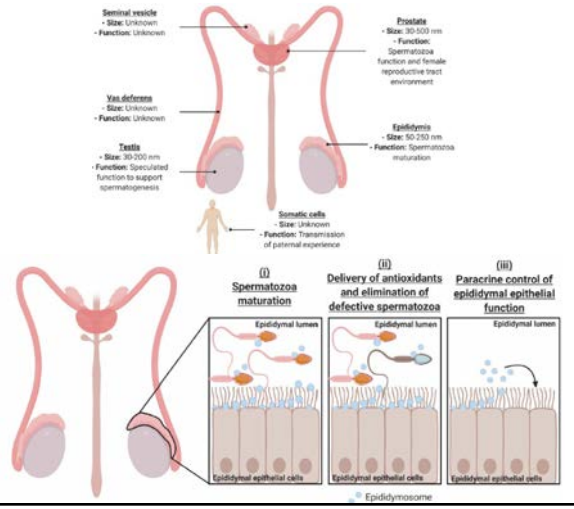
Region-specific microRNA signatures in the human epididymis.
Browne JA, Leir SH, Eggener SE, Harris A.
Asian J Androl. 2018 Nov-Dec;20(6):539-544.

Table 1: Differentially expressed miRNAs comparing the caput, corpus and cauda epididymis cells

miRNA	*FPKM comparison*		*Log2 fold change*	*Actual fold change*	*Differential*
A.	Caput	Corpus			
miR-573	10.28	0.40	-4.68	25.7	Cap>Corp
miR-155	3.11	0.61	-2.36	5.1	Cap>Corp
miR-30c2	3.92	1.10	-1.83	3.6	Cap>Corp
B.	Caput	Cauda			
miR-196a1	15.03	0.44	-5.10	34.4	Cap>Cau
miR-573	10.28	0.58	-4.15	17.8	Cap>Cau
miR-155	3.11	0.45	-2.77	6.8	Cap>Cau
miR-let7i	0.72	8.67	3.59	12.0	Cau>Cap
miR-770	2.12	24.47	3.53	11.5	Cau>Cap
miR-1204	8.54	89.13	3.38	10.4	Cau>Cap
C.	Corpus	Cauda			
miR-4730	21.14	0.00	infinity	infinity	Corp>Cau
miR-196a1	72.23	0.44	-7.37	165.2	Corp>Cau
miR-let7d	12.80	1.64	-2.97	7.8	Corp>Cau
miR-3916	34.98	9.98	-1.81	3.5	Corp>Cau
miR-1204	7.84	89.13	3.51	11.4	Cau>Corp
miR-675	0.42	2.90	2.79	6.9	Cau>Corp

A: caput *vs* corpus; B: caput *vs* cauda; C: corpus *vs* cauda. Cap: caput; Corp: corpus; Cau: cauda; FPKM: fragments per kilobase of transcript per million mapped reads

Roles of male reproductive tract extracellular vesicles in reproduction
Tamessar CT, Trigg NA, Nixon B, Skerrett-Byrne DA, et al.
Am J Reprod Immunol. 2021 Feb;85(2):e13338.



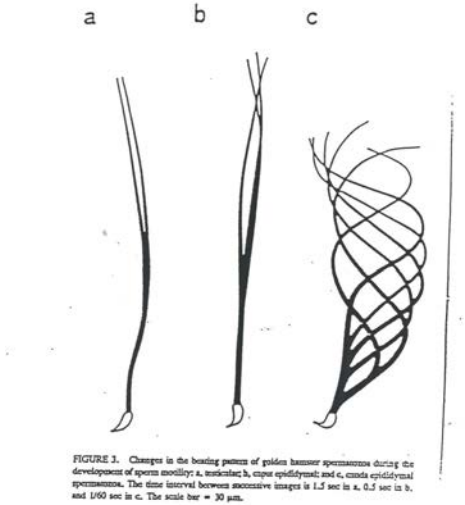


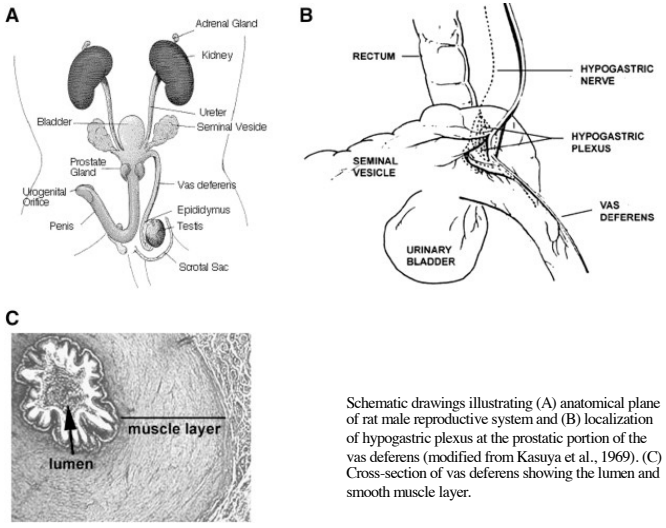
FIGURE 3. Changes in the beating pattern of golden hamster spermatozoa during the development of sperm motility: a, testicular; b, caput epididymal; and c, cauda epididymal spermatozoa. The time interval between successive images is 1.5 sec in a, 0.5 sec in b, and 1/60 sec in c. The scale bar = 30 μm.

Vas Deferens

Mechanisms of adaptive supersensitivity in vas deferens.

Quintas LE, Noël F.
Auton Neurosci. 2009 Mar 12;146(1-2):38-46.

Adaptive supersensitivity is a phenomenon characteristic of excitable tissues and discloses as a compensatory adjustment of tissue's response to unrelated stimulatory endogenous and exogenous substances after chronic interruption of excitatory neurotransmission. The mechanisms underlying such higher postjunctional sensitivity have been postulated for a variety of cell types. In smooth muscles, especially the vas deferens with its rich sympathetic innervation, the mechanisms responsible for supersensitivity are partly understood and appear to be different from one species to another. The present review provides a general understanding of adaptive supersensitivity and emphasizes early and recent information about the putative mechanisms involved in this phenomenon in rodent vas deferens.



Schematic drawings illustrating (A) anatomical plane of rat male reproductive system and (B) localization of hypogastric plexus at the prostatic portion of the vas deferens (modified from Kasuya et al., 1969). (C) Cross-section of vas deferens showing the lumen and smooth muscle layer.

Table 1
Alteration of signaling proteins in supersensitive vas deferens

Protein	Species	Model	Technique	Change	Reference
α1-adrenoceptor	Rat	Res	[^{3}H]WB4101 binding	=	Watanabe et al., 1982
		Den	[^{125}I]BE-2254 binding	=	Abel et al., 1985
		Res	[^{125}I]BE-2254 binding	=	Nasseri et al., 1985
		Den	[^{3}H]WB4101 binding	↓	Hata et al., 1981
	Guinea pig	Den	[^{3}H]WB4101 binding	=	Hata et al., 1980
		Den, Res	[^{3}H]WB4101 binding	=	Cowan et al., 1985
α2-adrenoceptor	Rat	Den, Res, OHDA	[^{3}H]Clonidine binding	↑	Watanabe et al., 1982
		Den	[^{3}H]Rauwolscine binding	ND	Abel et al., 1985
		Res	[^{3}H]Rauwolscine binding	ND	Nasseri et al., 1985
Muscarinic receptor	Rat	Den	[^{3}H]QNB binding	=	Hata et al., 1981
		Guan	[^{3}H]QNB binding	↑	Higuchi et al., 1985a
	Guinea pig	Den	[^{3}H]QNB binding	↑	Hata et al., 1980
Na$^+$/K$^+$-ATPase α1 (or αIT) isoform	Rat	Den	Immunoblot	=	Quintas et al., 2000
	Guinea pig	Res	Immunoblot	=	Hershman et al., 1993
Na$^+$/K$^+$-ATPase α2 isoform	Rat	Den	Immunoblot, [^{3}H] ouabain binding	↓	Quintas et al., 2000
	Guinea pig	Den, Dec, Res, OHDA	[^{3}H]Ouabain binding	↓	Wong et al., 1981
		Res	Immunoblot	↓	Hershman et al., 1993
		Res	Immunoblot	↓	Hershman et al., 1995
SERCA 2	Rat	Den	Immunoblot	↓	Quintas et al., 2005
PMCA	Rat	Den	Immunoblot	=	Quintas et al., 2005
Ryanodine receptor	Rat	Den	[^{3}H]Ryanodine binding	↓	Quintas et al., 2005
L-Type Ca^{2+}-channel	Rat	Den	[3H]Isradipine binding	↓	Jurkiewicz et al., 1994

Den – denervation; Dec – decentralization; Res – reserpine; Guan – guanetidine; OHDA – 6-hydroxydopamine; ND – not detected; WB4101 – 2-[(2′,6′-dimethoxy)-phenoxyethylamino]methylbenzodioxane; QNB – quinuclidinyl benzilate; BE – (2-[(l-(4-hydroxyphenyl)ethylaminomethyl]-tetralone); SERCA – sarco(endo)plasmic reticulum Ca^{2+}-ATPase; PMCA – plasma membrane Ca^{2+}-ATPase.

Endocrine

Role Testosterone -

1) Wolffian Duct Development
2) Male ReproductionGenetalia
3) External Genetalia

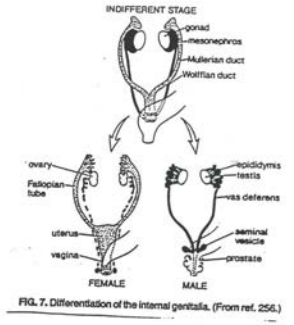


FIG. 7. Differentiation of the internal genitalia. (From ref. 256.)

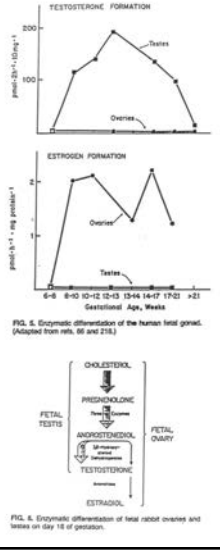
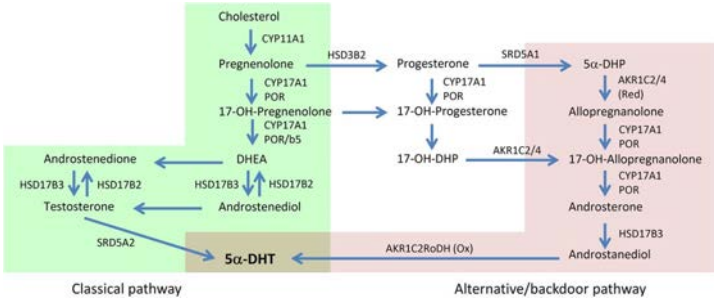


FIG. 5. Enzymatic differentiation of the human fetal gonad. (Adapted from refs. 66 and 218.)

FIG. 6. Enzymatic differentiation of fetal rabbit ovaries and testes on day 18 of gestation.



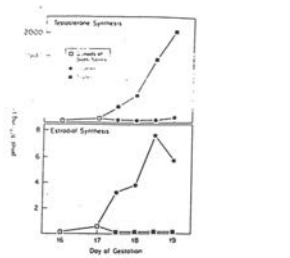


Fig. 4. Onset of endocrine function in the fetal testis and ovary of the rabbit embryo. Each gonad begins to synthesize its characteristic hormone at approximately the same time, beginning on day 17.5 (Milewich et al. 1977)

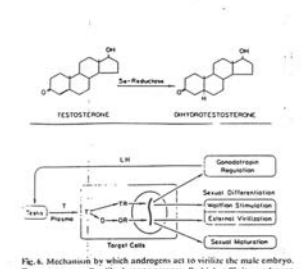
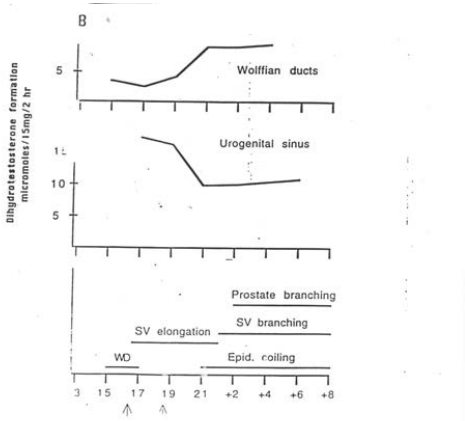
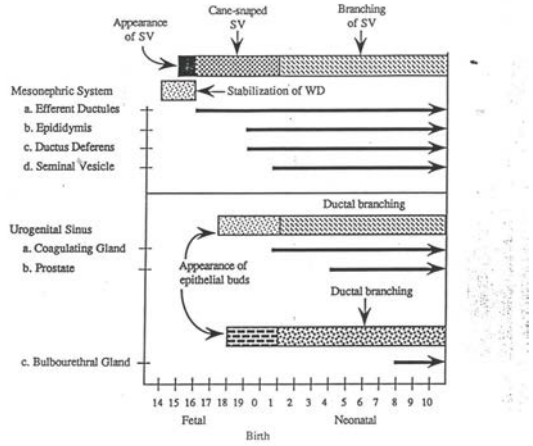
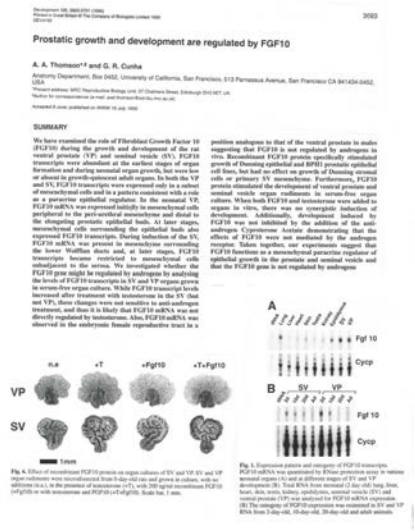


Fig. 6. Mechanism by which androgens act to virilize the male embryo. *T*, testosterone; *D*, dihydrotestosterone; *R*, high affinity androgen receptor protein. Three types of mutations in this pathway have been particularly useful in proving the applicability of this model to the events in the male embryo, namely those that result in abnormalities in the 5α-reductase enzyme, disorders of the androgen receptor, and so-called receptor positive resistance







Prostatic growth and development are regulated by FGF10

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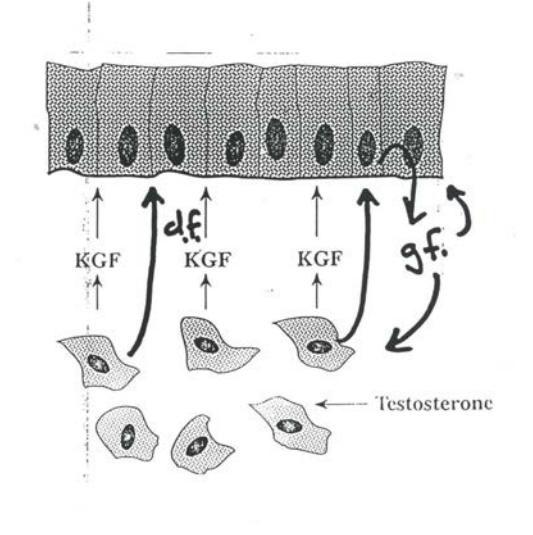
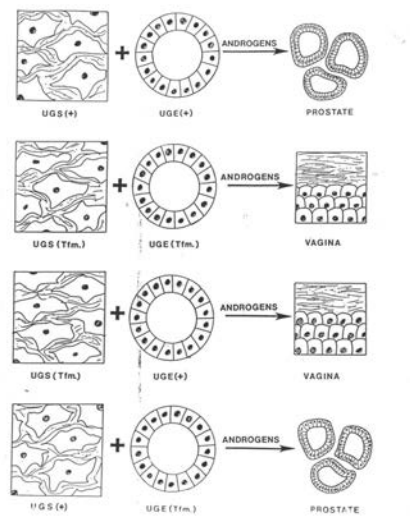
Accepted 8 June, published on WWW 15 July 1999

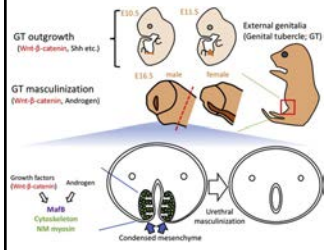
SUMMARY

We have examined the role of Fibroblast Growth Factor 10 (FGF10) during the growth and development of the rat ventral prostate (VP) and seminal vesicle (SV). FGF10 transcripts were abundant at the earliest stages of organ formation and during neonatal organ growth, but were low or absent in growth-quiescent adult organs. In both the VP and SV, FGF10 transcripts were expressed only in a subset of mesenchymal cells and in a pattern consistent with a role as a paracrine epithelial regulator. In the neonatal VP, FGF10 mRNA was expressed initially in mesenchymal cells peripheral to the peri-urethral mesenchyme and distal to the elongating prostatic epithelial buds. At later stages, mesenchymal cells surrounding the epithelial buds also expressed FGF10 transcripts. During induction of the SV, FGF10 mRNA was present in mesenchyme surrounding the lower Wolffian ducts and, at later stages, FGF10 transcripts became restricted to mesenchymal cells subadjacent to the serosa. We investigated whether the FGF10 gene might be regulated by androgens by analysing the levels of FGF10 transcripts in SV and VP organs grown in serum-free organ culture. While FGF10 transcript levels increased after treatment with testosterone in the SV (but not VP), these changes were not sensitive to anti-androgen treatment, and thus it is likely that FGF10 mRNA was not directly regulated by testosterone. Also, FGF10 mRNA was observed in the embryonic female reproductive tract in a position analogous to that of the ventral prostate in males suggesting that FGF10 is not regulated by androgens in vivo. Recombinant FGF10 protein specifically stimulated growth of Dunning epithelial and BPH1 prostatic epithelial cell lines, but had no effect on growth of Dunning stromal cells or primary SV mesenchyme. Furthermore, FGF10 protein stimulated the development of ventral prostate and seminal vesicle organ rudiments in serum-free organ culture. When both FGF10 and testosterone were added to organs in vitro, there was no synergistic induction of development. Additionally, development induced by FGF10 was not inhibited by the addition of the anti-androgen Cyproterone Acetate demonstrating that the effects of FGF10 were not mediated by the androgen receptor. Taken together, our experiments suggest that FGF10 functions as a mesenchymal paracrine regulator of epithelial growth in the prostate and seminal vesicle and that the FGF10 gene is not regulated by androgens





Regulatory roles of epithelial-mesenchymal interaction (EMI) during early and androgen dependent external genitalia development. Hyuga T, Suzuki K, Acebedo AR, Hashimoto D, Kajimoto M, Miyagawa S, Enmi JI, Yoshioka Y, Yamada G. Differentiation. 2019 Nov - Dec;110:29-35.



Early and late (androgen dependent) processes for GT develpment

	outgrowth	sexual differentiation
Early-stage	**Hedgehog** Haraguchi, Development, 2001 Perriton, Dev Biol, 2002 **Wnt, Fgf** Lin, ProS Genet, 2013 Miyagawa, Development, 2009 Cell Death Differ, 2014 **Fgf** Haraguchi, Development, 2000 Petiot, Development, 2005 Ching, Dev Biol, 2014 **Bmp** Suzuki, Development, 2003 Kajioka, Congenit Anom, 2019	Late-stage (androgen dependent) **Hedgehog** Miyagawa, Endocrinology, 2011 Chen Y, Bio Reprod, 2018 Zheng, PNAS, 2015 **Wnt, AP-1** Miyagawa, Mol Endocrinol, 2009 Matsushita, Nat Rev Urol, 2018 Suzuki, PNAS, 2014 **AP-1; Atf3** Tannour-Louet, Nat Med, 2014 van der Zanden, J Clin Endorinol Metab, 2010 Liu, Horm Res, 2006 Pediatr Dev Pathol, 2007 **Non-muscle myosin II** Acebedo, Commun Biol, 2019

Endocrine Disruption and Disruptors

Compounds that alter with hormone receptor and/or signal transduction to alter hormone actions.

Anti-androgenic chemicals that impact the androgen signaling pathway can affect male reproductive development via several different mechanisms of action resulting in slightly differing profiles of effects

		↓ Testosterone production			
Compound	Binds AR	↓ Insl3	↓mRNA	↓activity w/no ↓mRNA	'Low dose' prominent malformations
Vinclozolin	X	0	0	0	Retained nipples; Hypospadias; Agenesis of ventral prostate
Procymidone	X	0	0	0	Similar to Vinclozolin
Linuron	X	0	0	X	Epididymal and testis abnormalities; No gubernacular agenesis
Prochloraz	X	0	0	X	Similar to Vinclozolin
Dibutyl phthalate	0	X	X	-	All three phthalates produce epididymal and testis abnormalities' Gubernacular agenesis
Benzybutyl phthalate	0	X	X	-	
Diethylhexyl phthalate	0	X	X	-	

↓: decrease, X: a known mechanism, 0: does not act through this mechanism, ↓activity w/no ↓mRNA: an x here indicates enzyme activity is decreased but expression levels of mRNA for the enzyme are not affected.

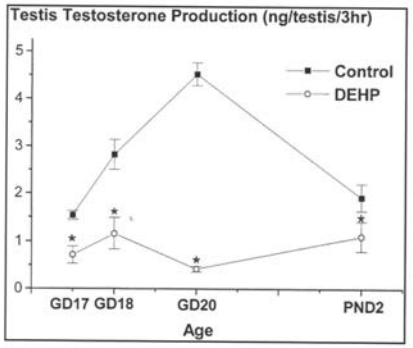


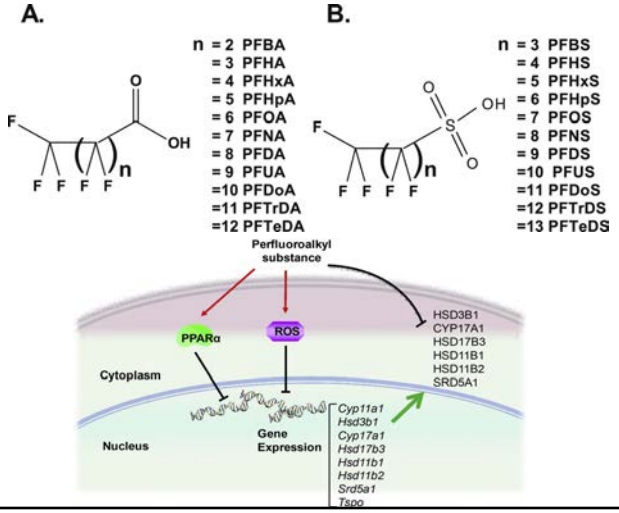
FIG. 2. Basal levels of testosterone production (ng/testis/3 h) during incubation of one testis (GD 17–20) or paired testes (PND 3) for control and DEHP (750 mg/kg/day from GD 14–PND 3) treatment groups from gestational day 17 to postnatal day 2. Graph values represent litter means with standard error bars, and asterisks indicate statistical significance at the $p \leq 0.05$ level, based on litter means analyses ($n = 4$ litters for GDs 17, 18, and 20, and $n = 5$ litters for PND 2).

Bisphenol A induces a shift in sex differentiation gene expression with testis-ova or sex reversal in Japanese medaka (Oryzias latipes).
Horie Y, Kanazawa N, Takahashi C, Tatarazako N, Iguchi T.
J Appl Toxicol. 2020 Jun;40(6):804-814.

Gestational vinclozolin exposure suppresses fetal testis development in rats.
Wu K, Li Y, Pan P, Li Z, et al.
Ecotoxicol Environ Saf. 2020 Oct 15;203:111053.

Bisphenol B stimulates Leydig cell proliferation but inhibits maturation in late pubertal rats.
Li Y, Yan H, Yu Y, Zou C, et al.
Food Chem Toxicol. 2021 Jul;153:112248.

Perfluoroalkyl substances cause Leydig cell dysfunction as endocrine disruptors
Zhu Q, Li H, Wen Z, Wang Y, et al.
Chemosphere. 2020 Aug;253:126764.

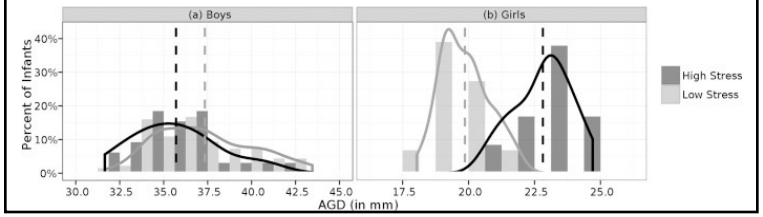


Stress and Androgen Activity During Fetal Development

Endocrinology. 2015 Oct;156(10):3435-41
Barrett ES, Swan SH

Abstract

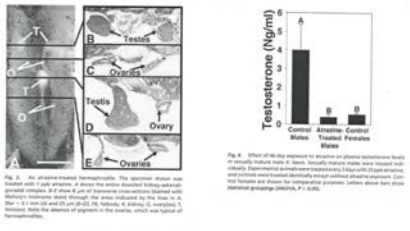
Prenatal stress is known to alter hypothalamic-pituitary-adrenal axis activity, and more recent evidence suggests that it may also affect androgen activity. In animal models, prenatal stress disrupts the normal surge of testosterone in the developing male, whereas in females, associations differ by species. In humans, studies show that (1) associations between prenatal stress and child outcomes are often sex-dependent, (2) prenatal stress predicts several disorders with notable sex differences in prevalence, and (3) prenatal exposure to stressful life events may be associated with masculinized reproductive tract development and play behavior in girls. In this minireview, we examine the existing literature on prenatal stress and androgenic activity and present new, preliminary data indicating that prenatal stress may also modify associations between prenatal exposure to diethylhexyl phthalate, (a synthetic, antiandrogenic chemical) and reproductive development in infant boys. Taken together, these data support the hypothesis that prenatal exposure to both chemical and nonchemical stressors may alter sex steroid pathways in the maternal-placental-fetal unit and ultimately alter hormone-dependent developmental endpoints.



Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses

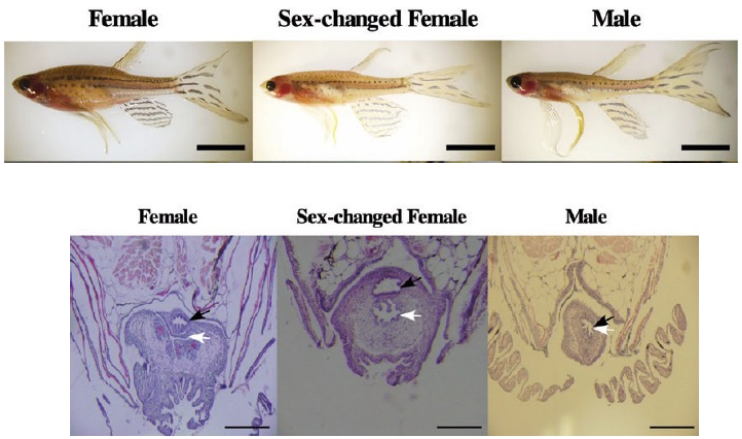
Tyrone B. Hayes*, Arif Collins, Melissa Lee, Magdalena Mendoza, Nigel Noriega, A. Ali Stuart, and Aaron Vonk

Atrazine is the most commonly used herbicide in the U.S. and probably the world. It can be present at several parts per million in agricultural runoff and can reach 40 parts per billion (ppb) in precipitation. We examined the effects of atrazine on sexual development in African clawed frogs (*Xenopus laevis*). Larvae were exposed to atrazine (0.01–200 ppb) by immersion throughout larval development, and we examined gonadal histology and laryngeal size at metamorphosis. Atrazine (≥0.1 ppb) induced hermaphroditism and demasculinized the larynges of exposed males (≥1.0 ppb). In addition, we examined plasma testosterone levels in sexually mature males. Male *X. laevis* suffered a 10-fold decrease in testosterone levels when exposed to 25 ppb atrazine. We hypothesize that atrazine induces aromatase and promotes the conversion of testosterone to estrogen. This disruption in steroidogenesis likely explains the demasculinization of the male larynx and the production of hermaphrodites. The effective levels reported in the current study are realistic exposures that suggest that other amphibian species exposed to atrazine in the wild could be at risk of impaired sexual development. This widespread compound and other environmental endocrine disruptors may be a factor in global amphibian declines.



Rapid Induction of Female-to-Male Sex Change in Adult Zebrafish by Injection of an Aromatase Inhibitor

Rahaman MM, Kumagai R-I, Tokumoto T.
Zebrafish. 2020 Aug;17(4):261-267.



Reproductive Biology and Endocrinology

Bio Med Central

Review

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Estrogen in the adult male reproductive tract: A review

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Abstract

Testosterone and estrogen are no longer considered male only and female only hormones. Both hormones are important in both sexes. It was known as early as the 1930's that developmental exposure to a high dose of estrogen causes malformation of the male reproductive tract, but the early formative years of reproductive biology as a discipline did not recognize the importance of estrogen in regulating the normal function of the adult male reproductive tract. In the adult testis, estrogen is synthesized by Leydig cells and the germ cells, producing a relatively high concentration in rete testis fluid. Estrogen receptors are present in the testis, efferent ductules and epididymis of most species. However, estrogen receptor-? is reported absent in the testis of a few species, including man. Estrogen receptors are abundant in the efferent ductule epithelium, where their primary function is to regulate the expression of proteins involved in fluid reabsorption. Disruption of the ? -receptor, either in the knockout (? ERKO) or by treatment with a pure antiestrogen, results in dilution of cauda epididymal sperm, disruption of sperm morphology, inhibition of sodium transport and subsequent water reabsorption, increased secretion of Cl-, and eventual decreased fertility. In addition to this primary regulation of luminal fluid and ion transport, estrogen is also responsible for maintaining a differentiated epithelial morphology. Thus, we conclude that estrogen or its ? -receptor is an absolute necessity for fertility in the male.

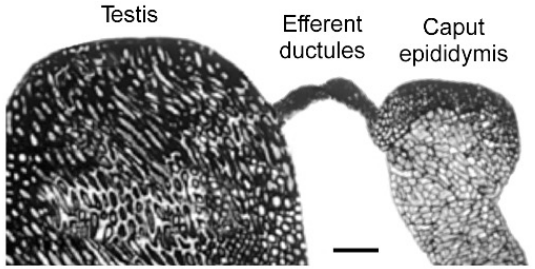


Figure 4
Testis, efferent ductules and epididymis. The surrounding fat pad was dissected away so show the efferent ductules that lie between the testis and caput epididymis. Bar = 2 mm.

Reproductive Biology and Endocrinology 2003, 1 http://www.RBEJ.com/content/1/1/52

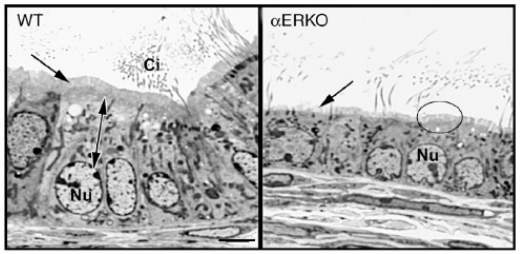


Figure 6
Histology of the efferent ductule epithelium in ? ERKO mouse. The wild-type (WT) ductule epithelium is columnar in shape with nonciliated cells that contain large spherical to oblong shaped nuclei (Nu) and extensive apical cytoplasm (double arrow). The nonciliated cell has a tall microvillus brush border (arrow) and extensive endocytotic apparatus. The ciliated cells have motile cilia (Ci) that extend into the lumen. The ? ERKO efferent ductule epithelium has a low cuboidal shape, with the apical cytoplasm reduced in size and the nucleus (Nu) also smaller. Microvilli are sparse on some cells (arrow) and reduced in height in other cells (circle). Bar = 10 μm.

Reproductive Biology and Endocrinology 2003, 1 http://www.RBEJ.com/content/1/1/52

Estrogen and Its Inhibition in the Male Reproductive Tract

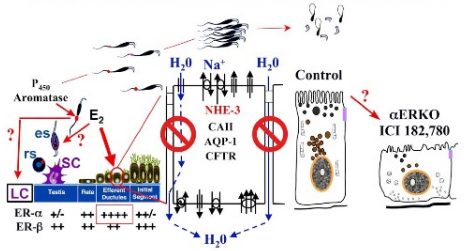
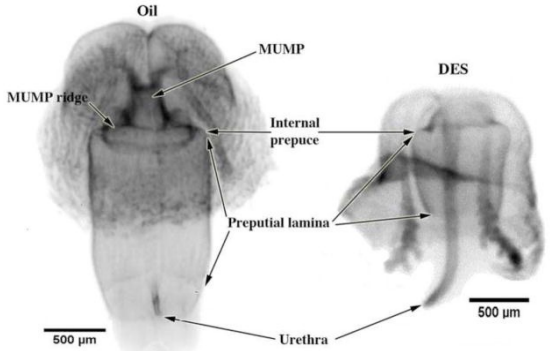


Figure 7
Estrogen and its inhibition in the male reproductive tract: a summary. In adult males, germ cells, as well as Leydig cells (LC) contain P450 aromatase and actively synthesize estrogen (E2), which produces a relatively high concentration in rete testis fluid. This luminal estrogen targets estrogen receptors that are abundant throughout the male reproductive tract, but particularly ER? that is localized in the efferent ductule epithelium, where its expression is more abundant than even the female reproductive tract. In the testis, E2 may also feedback to influence the function of LC and spermatids, either round spermatids (rs) or elongated spermatids (es). Estrogen's primary function in the male tract is the regulation of fluid reabsorption in the efferent ductules via ER? , which increases the concentration of sperm prior to entering the epididymis. Disruption of ER? , either in the knockout (? ERKO) or by treatment with a pure antiestrogen ICI 182,780, results in a decrease in Na^+ transport from lumen to interstitium and thus a decrease in water (H_2O) and fluid reabsorption. This inhibition is mediated by a decrease in the expression of NHE3 mRNA and protein and also decreases in carbonic anhydrase II (CAII) and aquaporin I (AQP-1) proteins. There is also an increase in cystic fibrosis transmembrane conductance regulator protein and mRNA, which adds to the NHE3 effect by secreting Cl^- into the lumen by the cystic fibrosis transmembrane conductance regulator (CFTR) [64]. This inhibition of fluid reabsorption results in the dilution of cauda epididymal sperm, disruption of sperm morphology, and eventual decreased fertility. In addition to this primary regulation of luminal fluids and ions, estrogen is also responsible for maintaining a differentiated epithelial morphology through an unknown mechanism.

Prenatal diethylstilbestrol induces malformation of the external genitalia of male and female mice and persistent second-generation developmental abnormalities of the external genitalia in two mouse strains.
Mahawong P, et al.
Differentiation. 2014 Sep-Oct;88(2-3):51-69.



Optical projection tomography images stained with anti-E-cadherin of day 5 postnatal penises derived from mice treated prenatally with oil or DES as indicated. Note overall reduction in size of all structures, specifically reduction in overall length of the preputial lamina and truncation of distal structures destined to form the penile urethral meatus.

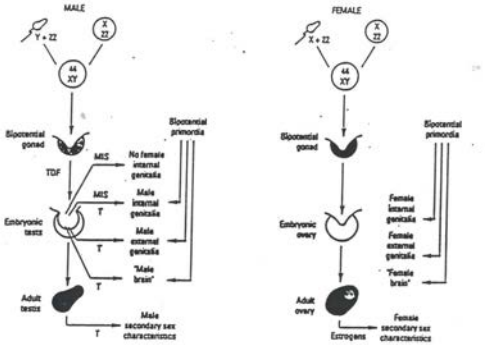
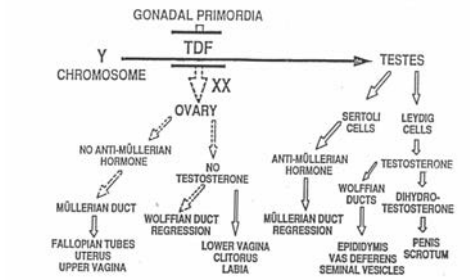
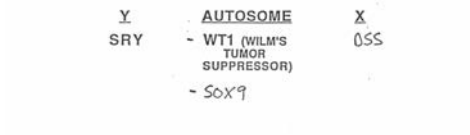
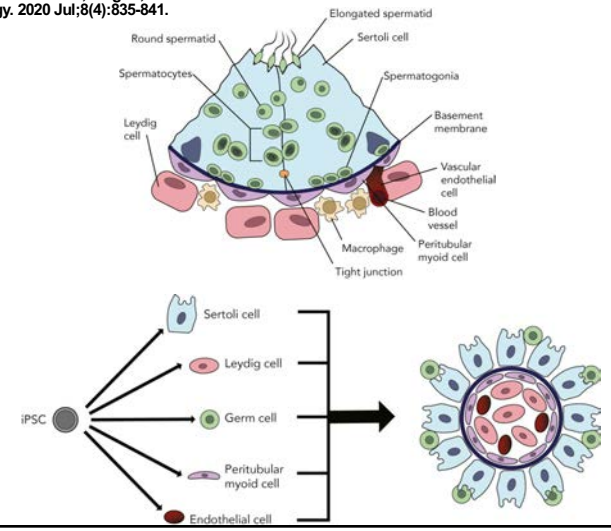


Figure 23–5. Diagrammatic summary of normal sex determination, differentiation, and development in humans. MIS, müllerian inhibiting substance; T, testosterone or other androgen.

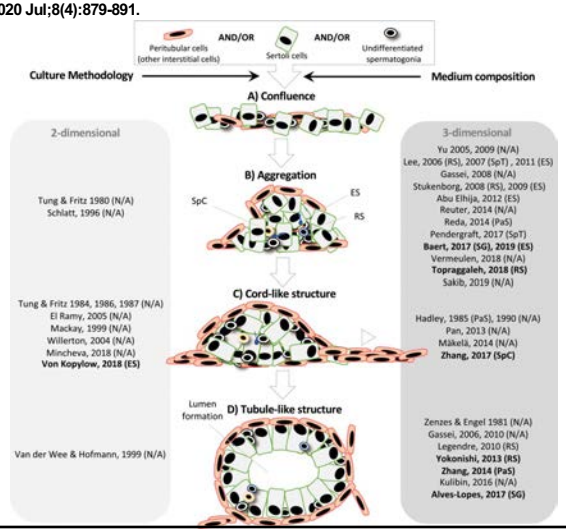




Testicular organoids to study cell-cell interactions in the mammalian testis
Sakib S, Goldsmith T, Voigt A, Dobrinski I.
Andrology. 2020 Jul;8(4):835-841.



In-vitro spermatogenesis through testis modelling: Toward the generation of testicular organoids
Richer G, Baert Y, Goossens E.
Andrology. 2020 Jul;8(4):879-891.



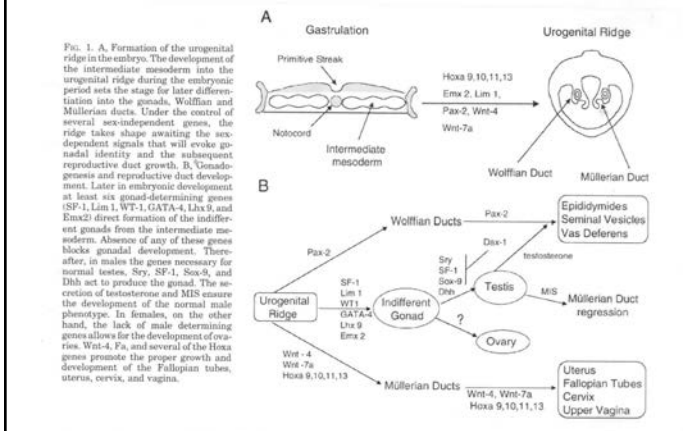


FIG. 1. A, Formation of the urogenital ridge in the embryo. The development of the intermediate mesoderm into the urogenital ridge during the embryonic period sets the stage for later differentiation into the gonads, Wolffian and Müllerian ducts. Under the control of several sex-independent genes, the ridge takes shape awaiting the sex-dependent signals that will evoke gonadal identity and the subsequent reproductive duct growth. B, Gonadogenesis and reproductive duct development. Later in embryonic development at least six gonad-determining genes (SF-1, Lim 1, WT-1, GATA-4, Lhx 9, and Emx2) direct formation of the indifferent gonads from the intermediate mesoderm. Absence of any of these genes blocks gonadal development. Thereafter, in males the genes necessary for normal testes, Sry, SF-1, Sox-9, and Dhh act to produce the gonad. The secretion of testosterone and MIS ensure the development of the normal male phenotype. In females, on the other hand, the lack of male determining genes allows for the development of ovaries. Wnt-4, Fa, and several of the Hoxa genes promote the proper growth and development of the Fallopian tubes, uterus, cervix, and vagina.

Schedule/Lecture Outline –

January	14 & 16	Week 1	Systems Biology Introduction
	21 & 23	Week 2	Molecular/ Cellular/ Reproduction Systems
	28 & 30	Week 3	Sex Determination Systems
February	4 & 6	Week 4	Male Reproductive Tract Development & Function
	11 & 13	Week 5	Female Reproductive Tract Development & Functio
	18 & 20	Week 6	Gonadal Developmental Systems Biology
	25 & 27	Week 7	Testis Systems Biology
March	3 & 5	Week 8	Ovary Systems Biology
	10 & 12	Week 9	Epigenetics and Transgenerational Gonadal Disease
	16 – 20	**Week 10**	**Spring Break**
	24 & 26	Week 11	Gametogenesis/ Stem Cells/ Cloning
	31 & 2	Week 12	Hypothalamus-Pituitary Development & Function
April	7 & 9	Week 13	Reproductive Endocrinology Systems
	14 & 16	Week 14	Fertilization & Implantation Systems
	21 & 23	Week 15	Fetal Development & Birth Systems
	28 & 30	Week 16	Assisted Reproduction/Contraception
May	5 & 7	Week 17	Exam or Grant Review

"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
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	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
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	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