ANSC 630
Biology of Sex
PART 2

Male and Female Reproductive Tract Development

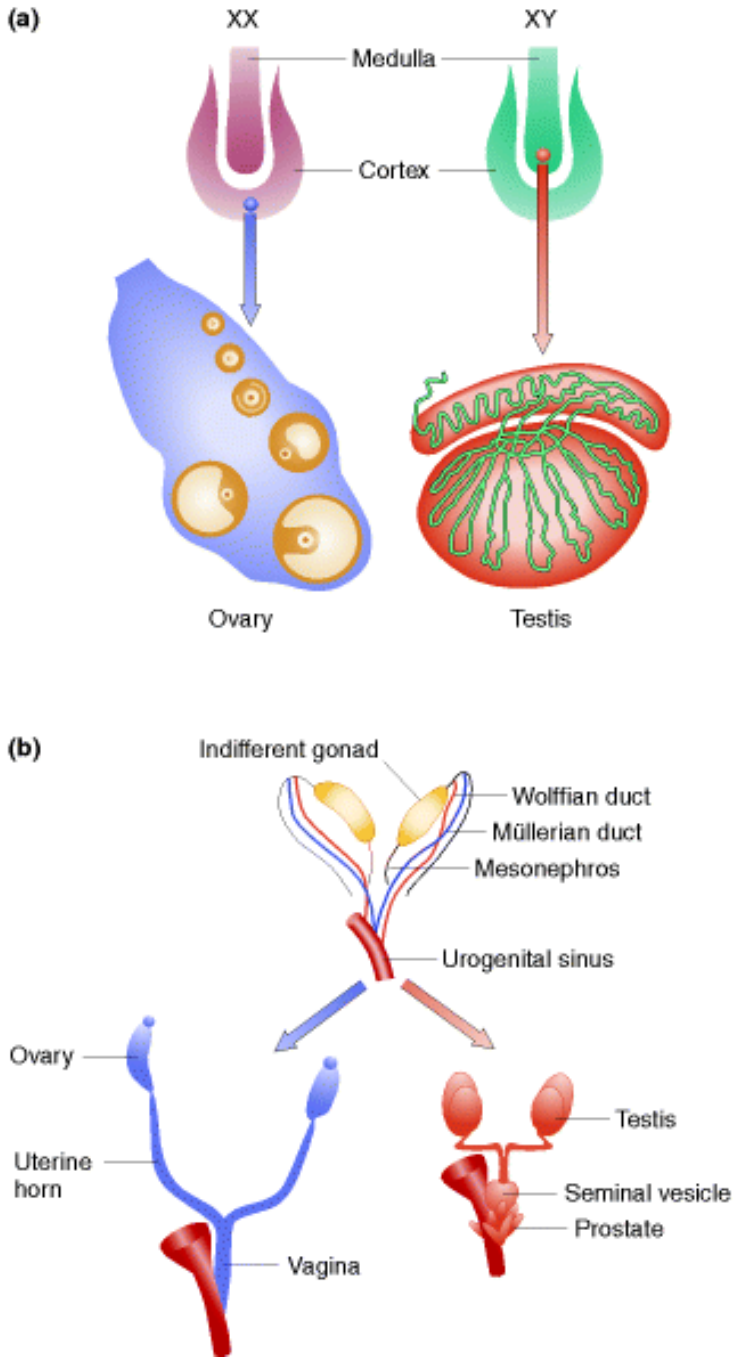
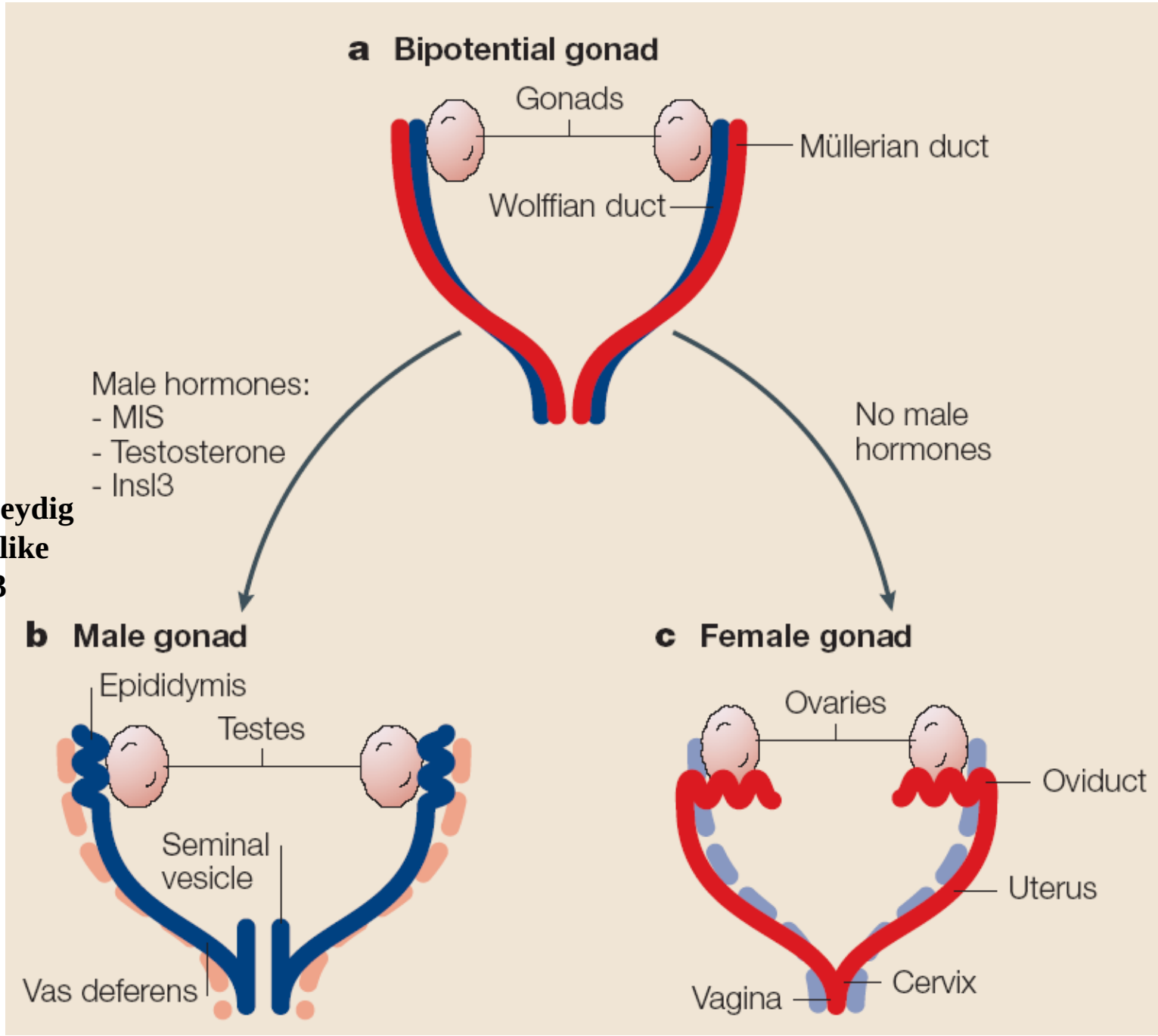


Figure 23-9. Development of the mammalian urogenital system. (a) The embryonic genital ridge consists of a medulla surrounded by a cortex. Female germ cells migrate into the cortex and become organized into an ovary. Male germ cells migrate into the medulla and become organized into a testis. (b) In the initial urogenital organization at the indifferent gonad stage, precursors of both male (Wolffian) and female (Müllerian) ducts are present. If a testis is present, it secretes two hormones, testosterone and a polypeptide hormone called Müllerian-inhibiting substance (MIS, or anti-Müllerian hormone or AMH). These hormones cause the Müllerian ducts to regress and the Wolffian ducts to develop into the male reproductive ducts. If an ovary is present, testosterone and MIS are absent and the opposite happens: the Wolffian ducts regress and the Müllerian ducts develop into the female reproductive ducts. (From U. Mittwoch, *Genetics of Sex Differentiation.*)



Insl3- Leydig Insulin-like Factor 3

Müllerian Ducts

- Two paired Müllerian ducts ultimately develop into the structures of the female reproductive tract. The structures involved include the **fallopian tubes, uterus, cervix, and the upper two-thirds of the vagina**.
- The ovaries and lower one third of the vagina are **not** derived from the Müllerian system.

Female Reproductive Tract

- Complete formation and differentiation of the Müllerian ducts into the segments of the female reproductive tract depend on completion of 3 phases of development as follows:

1. **Organogenesis**
2. **Fusion, laterally and vertically**
3. **Resorption**

Müllerian Development

- Wolffian ducts largely degenerate
- **Cephalic ends** of the Müllerian ducts form **fallopian tubes**
- **Caudal portion** then fuses to form the **uterus**
- ~9 weeks- uterine cervix is recognizable
- ~17 weeks- myometrium formation is complete

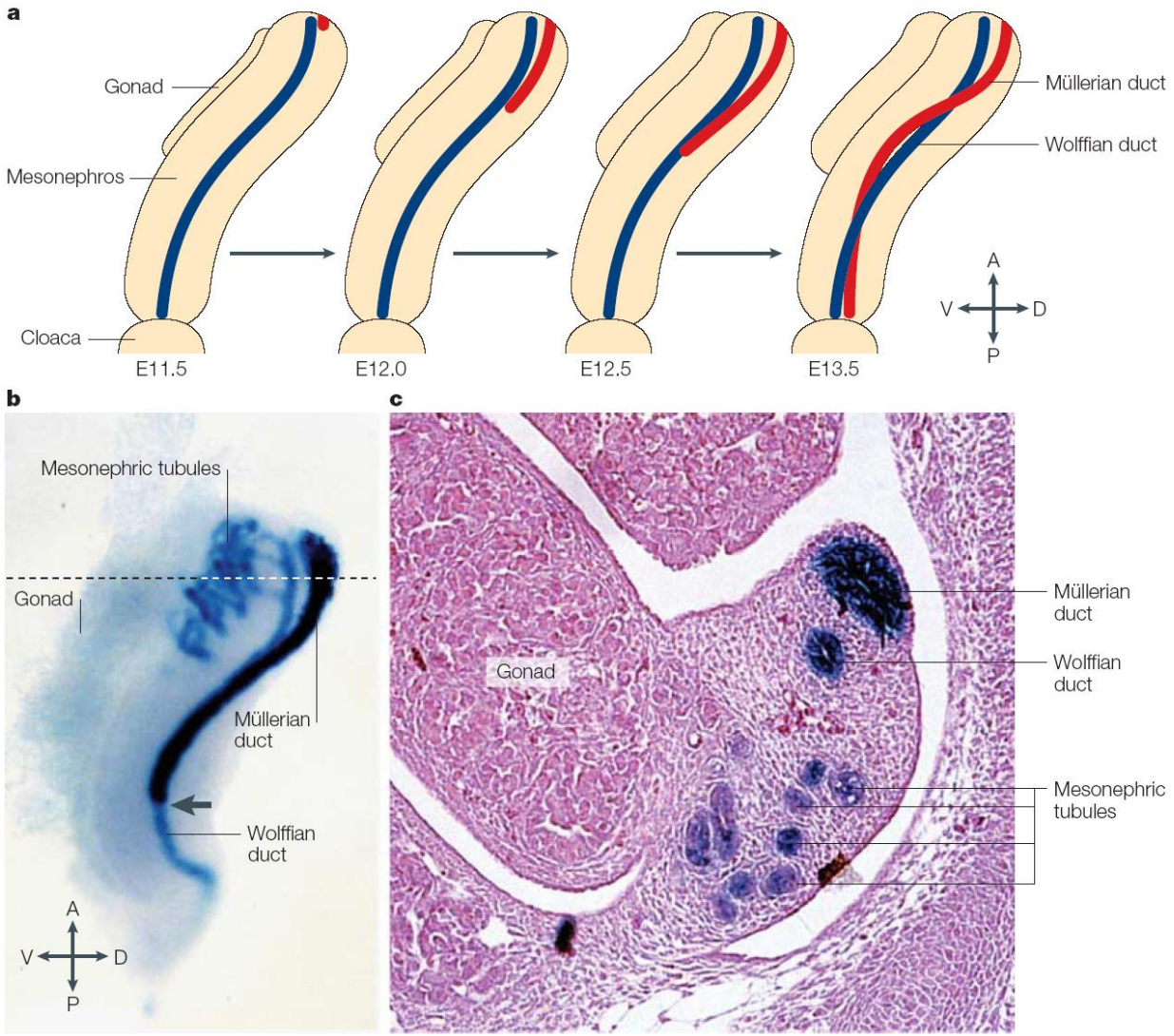


Figure 2 | **Formation of the Müllerian ducts. a** | Schematic diagram of Müllerian duct formation in mammals. The Müllerian duct forms as an invagination of the surface epithelium of the MESONEPHROS at around embryonic day (E) 11.5 in mice and this epithelial invagination extends posteriorly until it reaches the CLOACA at ~E13.5. **b** | The extending epithelium of the Müllerian duct is visualized at E12.5 by *Lim1* (*Lhx1*)–*lacZ* expression[11]. 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.

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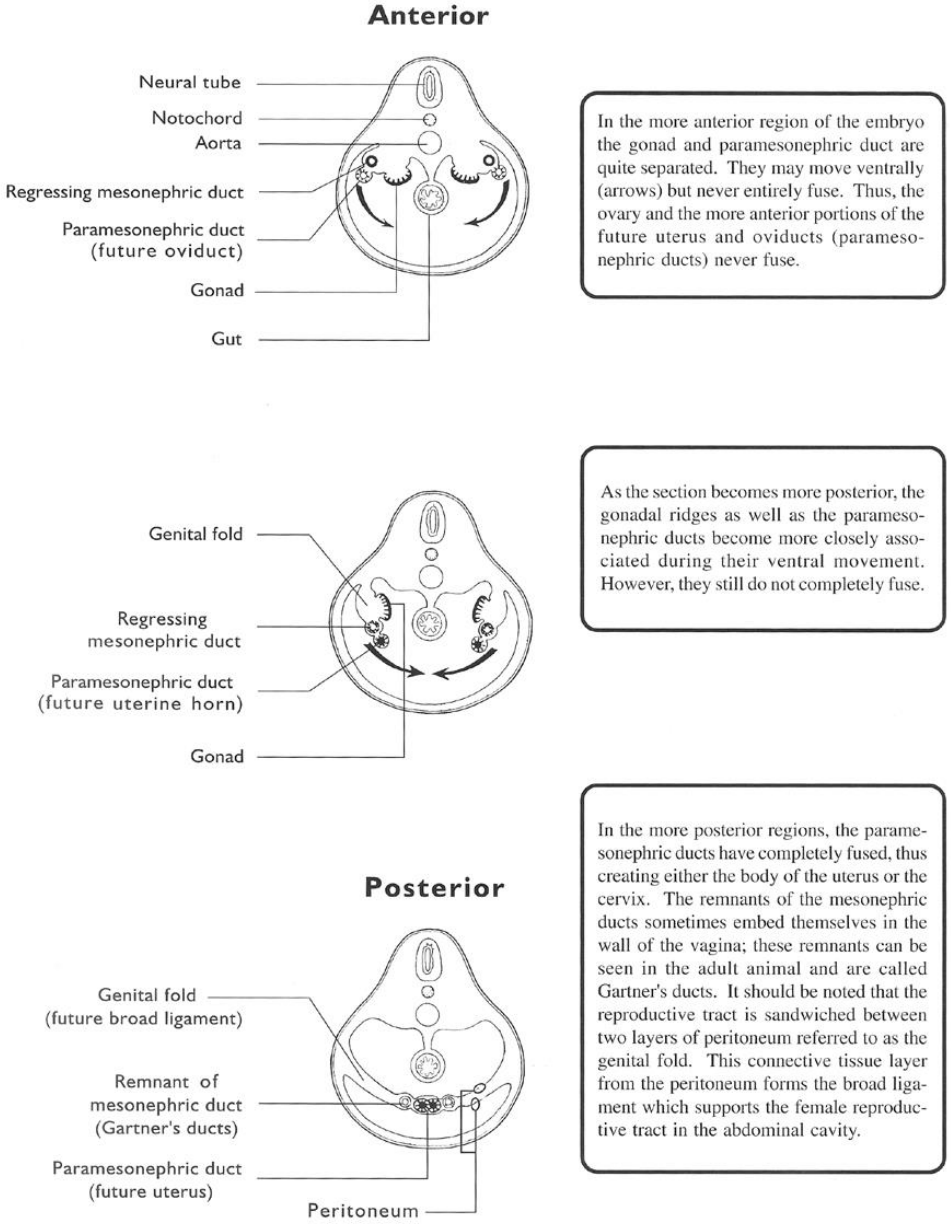
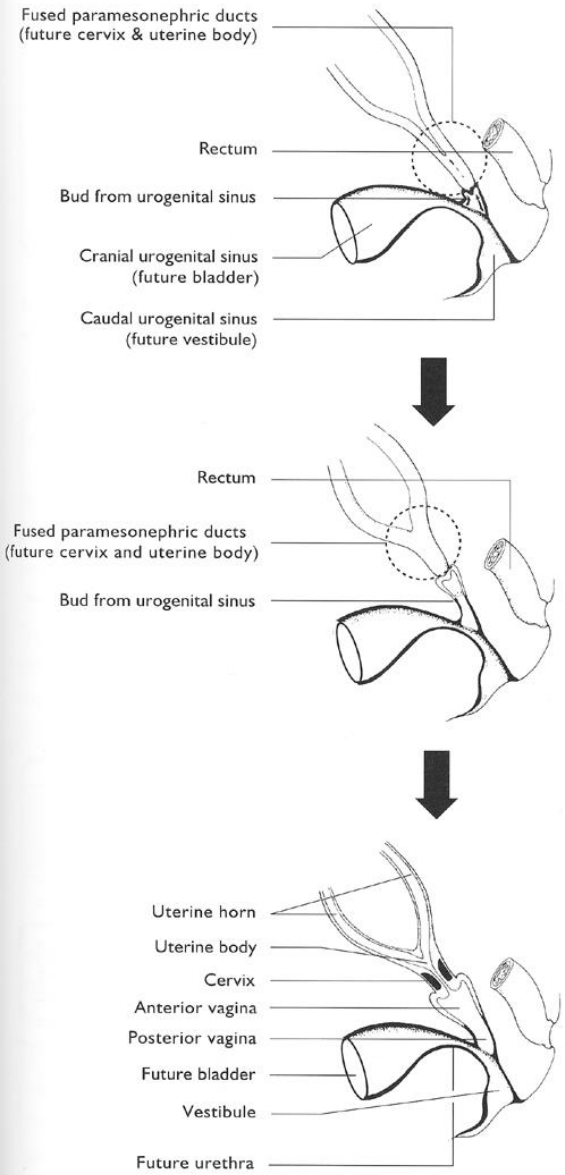


Figure 4-11. Transverse sections from anterior (top) to posterior (bottom) illustrating the formation of the supportive structures of the female tract from the genital fold. Modified from Dyce, Sack and Wensing, *Textbook of Veterinary Anatomy*, 2nd Edition, with permission from W.B. Saunders Co.



The paramesonephric ducts are beginning to fuse (circled area) and will form the cervix and uterine body. A small bud evaginates from the urogenital sinus and attaches to the fused paramesonephric ducts.

The paramesonephric ducts continue to fuse, forming a more defined cervix and a more developed vagina. The bud of the urogenital sinus elongates.

Points of fusion have now formed a definitive cervix and an anterior and a posterior vagina. Note that the anterior vagina originates from the paramesonephric duct after paramesonephric tubule fusion and thus has a different tissue type than the posterior vagina and vestibule. The posterior vagina and vestibule have stratified squamous epithelium because they originate from the urogenital sinus which invaginates from the exterior.

Figure 4-10. Fusion of paramesonephric ducts with the urogenital sinus to form the vagina. The purpose of this figure is to illustrate how the paramesonephric ducts fuse with a bud of the urogenital sinus. This results in the anterior vagina, cervix and uterus being of one tissue origin (mesoderm) and the posterior vagina and vestibule from another (ectoderm). Modified from Dyce, Sack and Wensing, *Textbook of Veterinary Anatomy*, 2nd Edition, with permission from W.B Saunders Co.

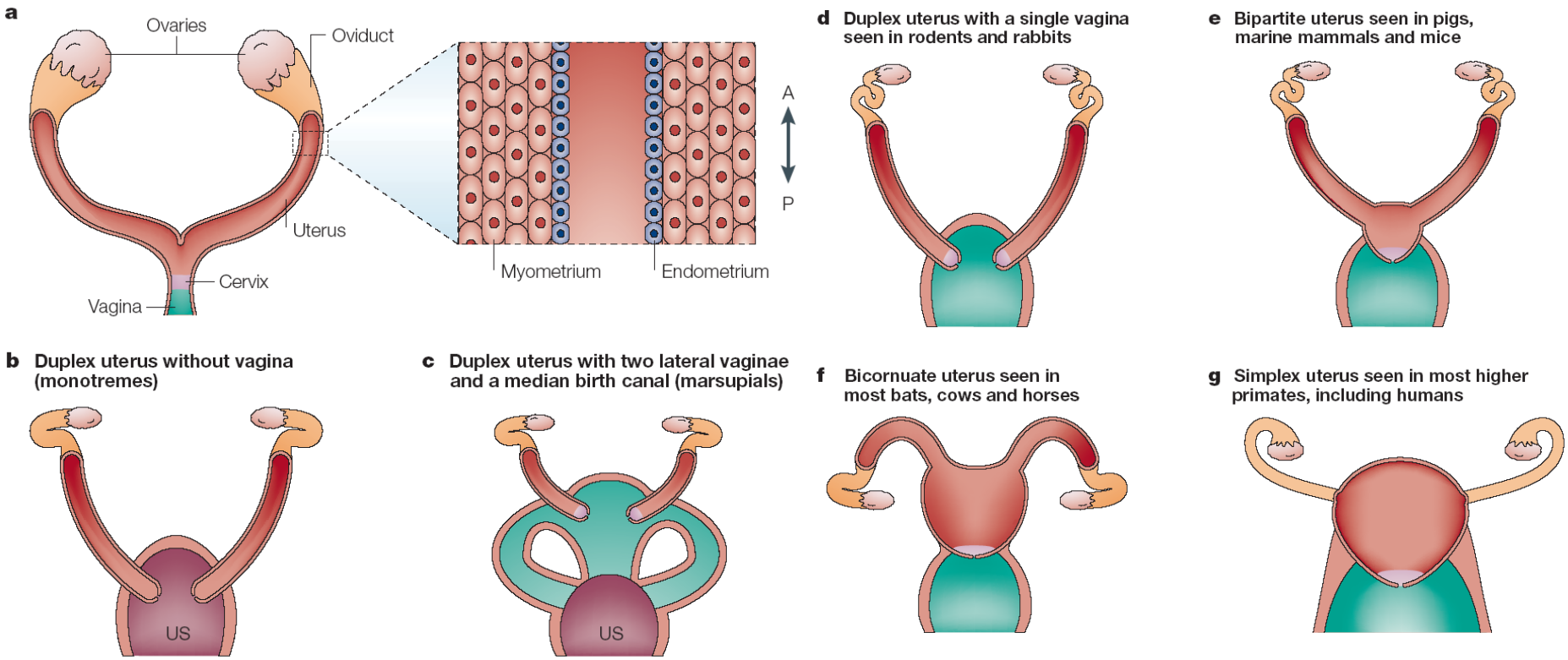
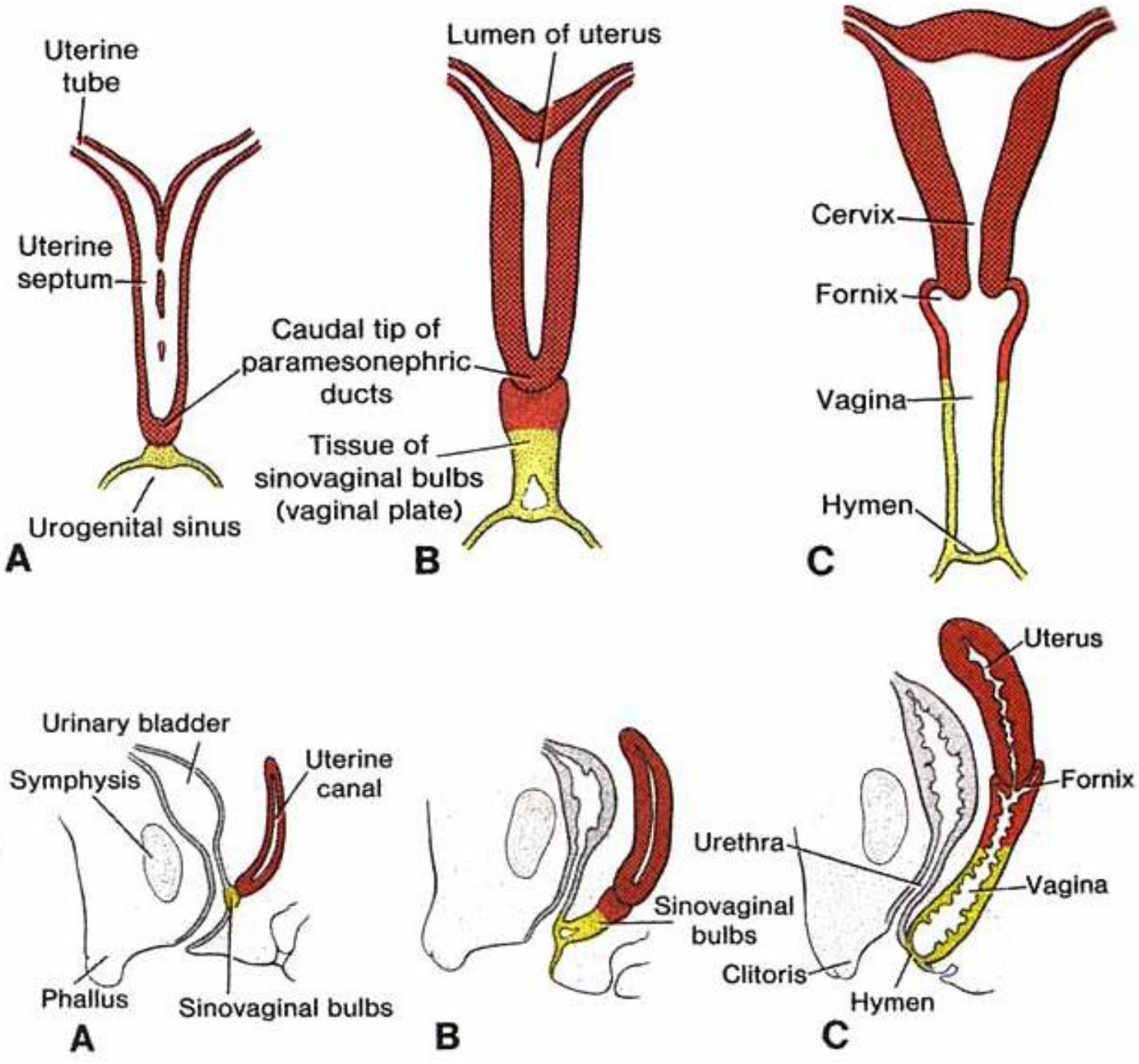


Figure 1 | **Female reproductive-tract variation in mammals. a** | Basic anatomical features of the female reproductive tract. Oocytes leave the ovaries and move into the oviduct, where fertilization occurs. The cervix is the boundary between the uterus and the vagina or urogential sinus. With the exception of in the egg-laying mammals (monotremes), embryos implant in the uterus and are delivered through the vagina. The developing female reproductive tract has two layers, the epithelium and the surrounding mesenchyme, which differentiate into the endometrium and the myometrium, respectively, in the uterus. **b**,**c** | Absent (or limited) fusion of the Müllerian ducts leads to the presence of two uteri. The urogenital sinus (US) is connected to the female reproductive tract (**b**). Müllerian duct fusion is physically blocked by the presence of the ureters, which leads to the formation of three vaginae (**c**). **d** | The duplex uterus shown here has a pair of cervices. **e** | In the duplex bipartite uterus seen in many mammalian species, Müllerian fusion in the uterine region does not occur, or is limited, which leads to the formation of a pair of uterine horns that can support the development of many fetuses. **f** | A larger portion of the uterus forms the uterine body. **g** | Müllerian ducts fuse anteriorly to generate a single uterine body that supports a single fetus or a small number of fetuses per pregnancy. A, anterior (cranial); P, posterior (caudal). Panels **b**–**g** adapted with permission from REF. 5 © (2003) McGraw-Hill.


Uterine tube
Uterine septum
Urogenital sinus
A
Lumen of uterus
Caudal tip of paramesonephric ducts
Tissue of sinovaginal bulbs (vaginal plate)
B
Cervix
Fornix
Vagina
Hymen
C
Urinary bladder
Symphysis
Uterine canal
Phallus
Sinovaginal bulbs
A
Sinovaginal bulbs
B
Uterus
Fornix
Urethra
Vagina
Clitoris
Hymen
C

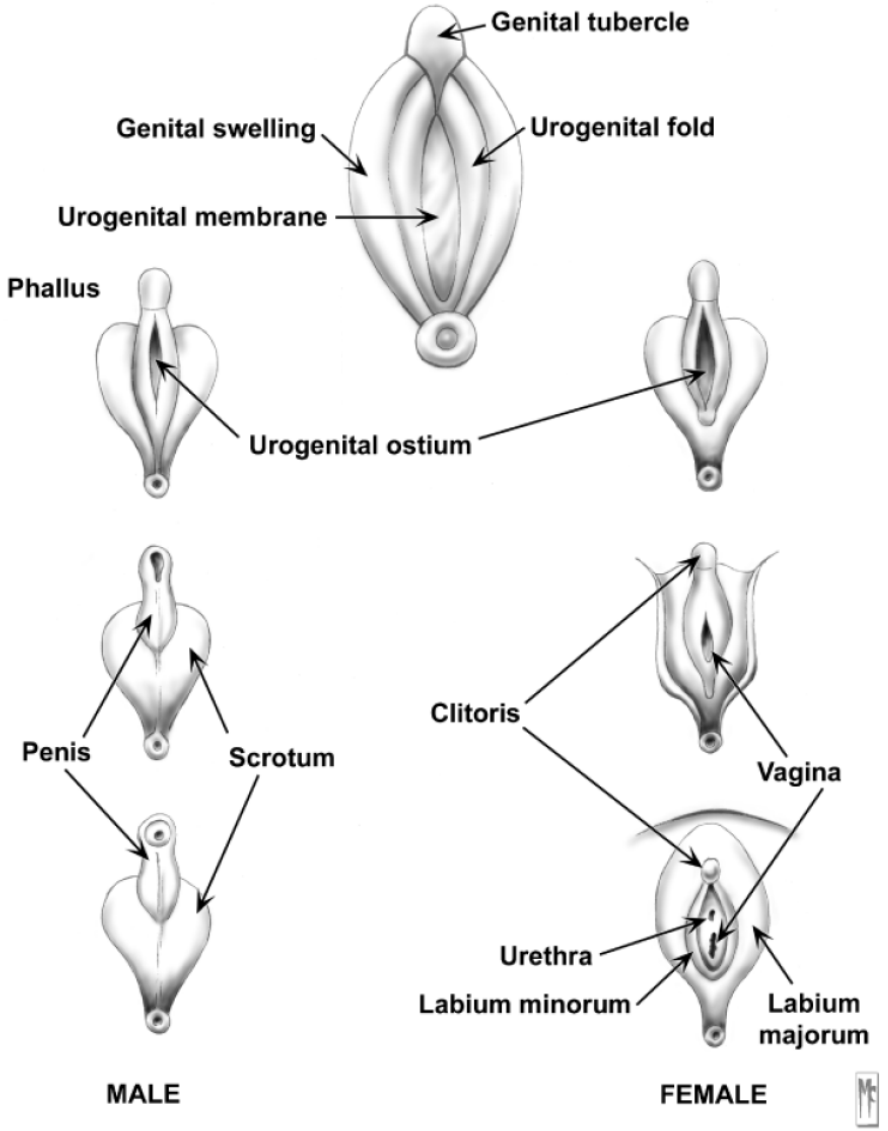


Fig. 3 Sex differentiation of the external genitalia. The ambisexual stage (top center) consists of the genital tubercle, the urogenital folds bounding the urogenital ostium, and the more laterally placed genital swellings. During male development (left), the genital tubercle elongates to form the penis, while the genital swellings migrate caudally and fuse in the midline to form the scrotum. As the phallus elongates, the urethral groove forms on the ventral aspect of the genital tubercle. The urethral groove extends distally as a result of canalization of the solid urethral plate. Finally, the urethral folds bounding the urethral groove grow to the midline and fuse with each other, thus forming a tubular urethra. During female development (right), only minor changes occur from the original ambisexual stage. The genital tubercle undergoes minimal growth to form the clitoris. The urogenital folds and genital swellings remain unfused to form the labia minora and majora, respectively. The urogenital folds (labial minora) define the vestibule of the vagina into which open the urethra and vagina.

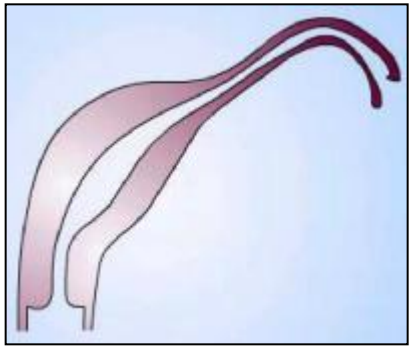
Female External Development

- After 10 weeks gestation:
- The genital tubercle begins to bend caudally forming the clitoris
- The side portions of the genital swellings enlarge to become labia majora
- The posterior portions of labia majora fuse becoming posterior fourchette [fold of skin]
- The urethral folds persist to form labia minora

Organogenesis

- One or both Müllerian ducts may not develop fully, resulting in abnormalities such as uterine agenesis or hypoplasia (bilateral) or unicornuate uterus (unilateral).

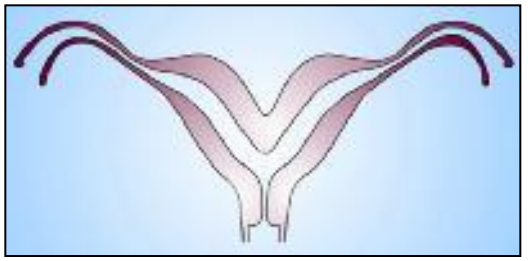
Normal



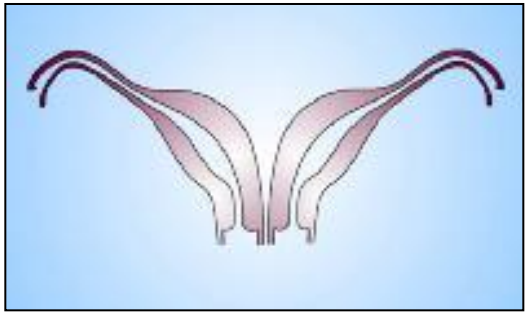
Unicornuate

Fusion

- Lateral Fusion: The process during which the lower segments of the paired müllerian ducts fuse to form the uterus, cervix, and upper vagina. **Failure of fusion results in anomalies such as bicornuate or didelphys uterus.**
- Vertical fusion refers to fusion of the ascending sinovaginal bulb with the descending müllerian system (ie, fusion of the lower one third and upper two thirds of the vagina). Complete vertical fusion forms a normal patent vagina, while **incomplete vertical fusion results in an imperforate hymen.**



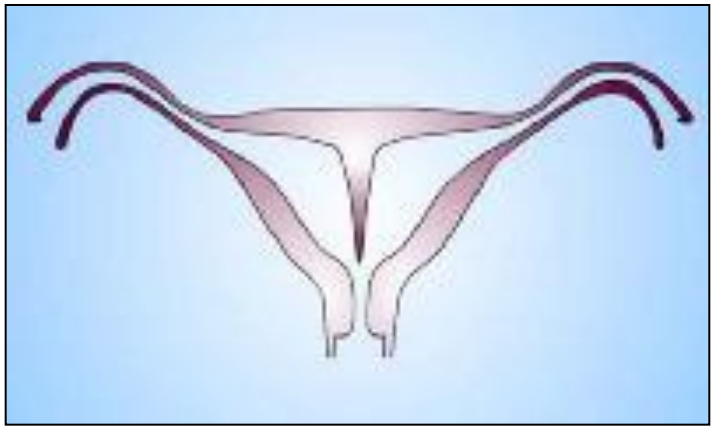
Bicornuate



Didelphys

Septal resorption

- After the lower Müllerian ducts fuse, a central septum is present, which subsequently must be resorbed to form a single uterine cavity and cervix.
- Failure of resorption is the cause of septate uterus



Frequency

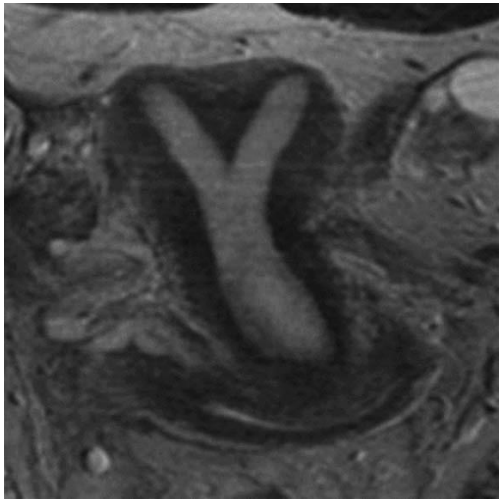
- **In the US:** Müllerian duct anomalies are estimated to occur in 0.1-0.5% of women. The true prevalence is unknown because the anomalies usually are discovered in patients presenting with infertility. Full-term pregnancies have occurred in patients with forms of bicornuate, septate, or didelphys uteri; therefore, true prevalence may be slightly higher than currently estimated.
- In the healthy fertile population, Müllerian duct anomalies have a prevalence of 3.2%

Check this excellent web site out for an **in-depth** explanation of every variant, with recommendations of imaging studies, great graphics…

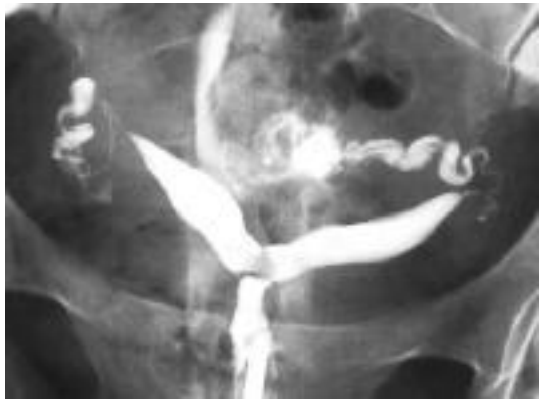
http://www.emedicine.com/radio/topic738.htm

Mullerian Duct Abnormalities

http://www.emedicine.com/radio/topic738.htm



Uterus, müllerian duct abnormalities. T2 fast spin-echo MRI image of septate uterus acquired in the oblique plane along the long axis of the uterus. Note that the outer fundal contour (superior border) is flat or slightly concave, which is sufficient to make the diagnosis of septate uterus.



Uterus, müllerian duct abnormalities. Surgically proven case of bicornuate uterus. Correct diagnosis may be suggested based on hysterosalpingography findings, which are, most notably, the widened intercornual distance (>4 cm) and the widened intercornual angle (>60°).

Table 2 | **A selection of human syndromes that affect female reproductive tract development**

Syndrome name	OMIM*	FRT abnormalities in patients	Mode of inheritance	Genomic location	Gene mutated	Molecule encoded	References
Formation							
Maturity-onset diabetes of the young type V (MODY5)	604284	Vaginal aplasia and rudimentary uterus	AD	17cen–q21.3	*TCF2* (*HNF1β*)	Homeodomain transcription factor	58
McKusick–Kaufman syndrome (MKKS)	236700	Hydrometrocolpos by vaginal atresia	AR	20p12	*MKKS* (*BBS6*)	Chaperonin	68,102, 103
Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome	277000	Absence of the vagina and uterus	AR	ND	ND	ND	104
MURCS association	601076	Müllerian duct aplasia	SP	ND	ND	ND	105
Regression							
Persistent Müllerian duct syndrome (PMDS) type I	261550	Persistence of Müllerian derivatives	AR	19p13.3 –p13.2	*MIS* (*AMH*)	TGFβ superfamily secreted molecule	69
Persistent Müllerian duct syndrome (PMDS) type II	261550	Persistence of Müllerian derivatives	AR	12q13	*MISR2* (*AMHR2*)	TGFβ superfamily type 2 Ser/Thr transmembrane receptor	69
Urioste syndrome	235255	Persistence of Müllerian derivatives	AR	ND	ND	ND	70,106, 107
Differentiation							
Hand–foot–genital (HFG) syndrome	140000	Longitudinal vaginal septum	AD	7p15–p14.2	*HOXA13*	Homeodomain transcription factor	75
Cat eye syndrome (CES)	115470	Hypoplastic uterus, vaginal atresia	SP	21q11	ND	ND	108
Fryns syndrome (FRNS)	229850	Uterus bicornis or hypoplasia	AR	ND	ND	ND	109

*Reference number for the entry in the online Mendelian inheritance in man (OMIM) database of genetic disorders (see online links box). AD, autosomal dominant; *AMH*, anti-Müllerian hormone; *AMHR2*, anti-Müllerian hormone type 2 receptor; AR, autosomal recessive; *BBS*, Bardet–Biedl syndrome; cen, centromere; FRT, female reproductive tract; *HNF*, hepatocyte nuclear factor; *HOXA*, homeobox A; *MIS*, Müllerian-inhibiting substance; *MISR2*, Müllerian-inhibiting substance type 2 receptor; MURCS, Müllerian duct aplasia, unilateral renal aplasia and cervicothoracic somite dysplasia; ND, not determined; SP, sporadic; *TCF*, transcription factor; *TGF*, transforming growth factor.

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

Gene name	Genetic map position	Molecule encoded	Tissue of expression	Female reproductive-tract phenotype abnormality (mode of inheritance)	References
Formation					
Pax2	Ch19 (43.0 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	8
Lim1 (*Lhx1*)	Ch11 (48.0 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	11
Emx2	Ch19 (53.5 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	12
Wnt4	Ch4	Wnt family secreted protein	MM	Absence of FRT (R)	17
Ltap	Ch1 (93.4 cM)	Transmembrane protein with PDZ domain	ND	Imperforate vagina (D)	22,23
Hoxa13	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Delay or arrested formation (R)	51
Regression					
Mis (*Amh*)	Ch10 (43.0 cM)	TGFβ superfamily secreted protein	Sertoli cells	Ectopic FRT in males (R)	27,28
Misr2 (*Amhr2*)	Ch15 (57.4 cM)	TGFβ superfamily type 2 Ser/Thr transmembrane receptor	MM	Ectopic FRT in males (R)	35
Wnt7a	Ch6 (39.5 cM)	Wnt family secreted protein	ME	Ectopic FRT in males (R)	42
Differentiation					
Wnt7a	Ch6 (39.5 cM)	Wnt family secreted protein	ME	Homeotic transformation of oviduct to uterus and uterus to vagina, no uterine glands, abnormal mesenchyme differentiation (SD)	53
Hoxa10	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Homeotic transformation of anterior uterus to oviduct (R)	49,52
Hoxa11	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Partial homeotic transformation of uterus to oviduct (SD)	49,99
Hd (*Hoxa13*)*	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Homeotic transformation of cervix to uterus (SD)	100
Ovo1 (*Ovol1*)	Ch19	C2H2-type zinc-finger protein	ND	Subfertility with dilated uterus and cervix, constricted or imperforate vagina (R)	101

This table lists all of the mouse genes that are known to be involved in female reproductive tract (FRT) development. *The *Hoxa13* mutation in the *Hypodactyly* (*Hd*) mutant is not a null allele, but is thought to be a dominant-negative allele[73,74]. *Amh*, anti-Müllerian hormone; *Amhr2*, anti-Müllerian hormone type 2 receptor; C2H2, two cysteine two histidine; Ch, chromosome; cM, centimorgan; D, dominant; *Emx*, empty spiracles homologue; *Hoxa*, homeobox A; *Lim1*, *lin-11*, *Isl1* and *mec-3* transcription factor homologue; *Lhx1*, LIM homeobox protein; *Ltap*, Loop-tail-associated protein; ME, Müllerian duct epithelium; *Mis*, Müllerian-inhibiting substance; *Misr2*, Müllerian-inhibiting substance type 2 receptor; MM, Müllerian duct mesenchyme; ND, not determined; *Ovol*, Ovo homologue-like; *Pax*, paired box gene; R, recessive; SD, semidominant; TGF, transforming growth factor; WE, Wolffian duct epithelium; WM, Wolffian duct mesenchyme; *Wnt*, wingless-related MMTV integration site.

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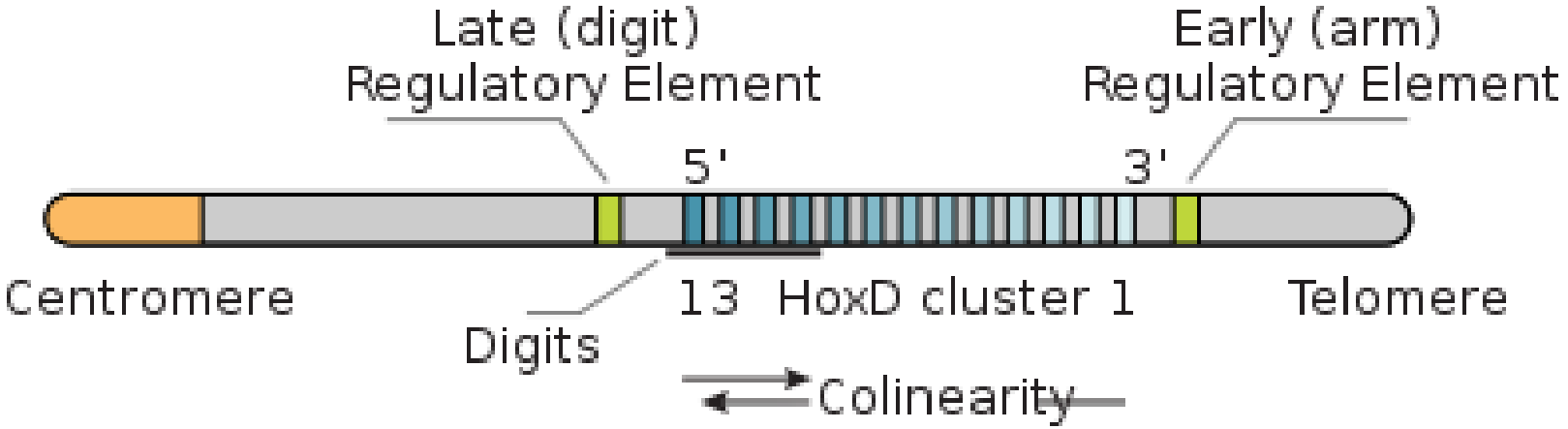
Hox Genes

Hox genes are a group of related genes that specify the anterior-posterior axis and segment identity of metazoan organisms during early embryonic development. These genes are critical for the proper number and placement of embryonic segment structures (such as legs, antennae, and eyes).



http://en.wikipedia.org/wiki/Hox_gene

Hox Genes Regulate Female Reproductive Tract (FRT) Development

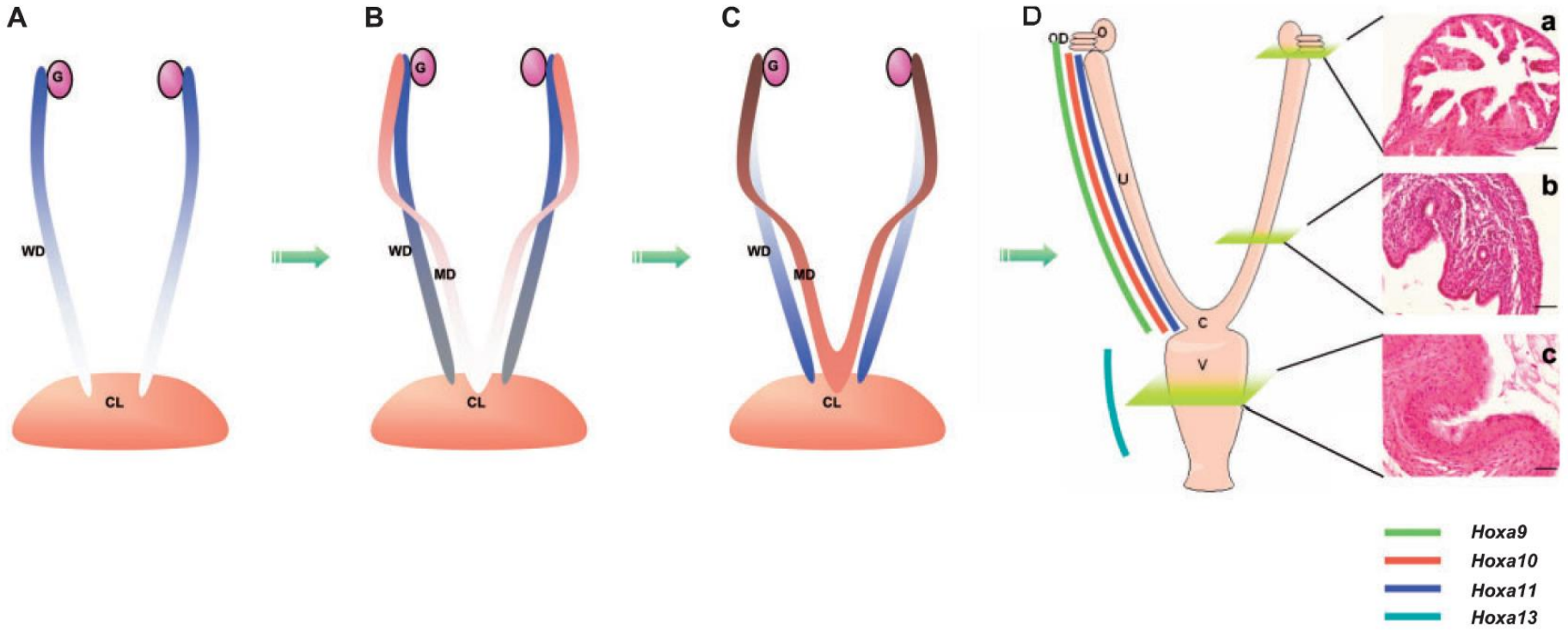


Fig. 1. **A schematic representation of FRT development in the mouse.** A, on E9.5, Wolffian ducts are readily distinguishable from the surrounding mesonephroi and are growing caudally. B, Müllerian ducts grow in parallel to Wolffian ducts at around E11.5 and reach the cloaca by E13.5. At this time, the embryo possesses both the male and female reproductive tract primordials regardless of its genetic sex. C, in females, Wolffian ducts start to degenerate in the absence of androgen, while Müllerian ducts develop in the absence of anti-Müllerian hormone. D, eventually Müllerian ducts differentiate into oviduct, uterus, cervix and upper part of vagina. *AbdB* like *Hox* genes have a nested expression pattern along the A-P axis of the FRT, as illustrated in D. Panels a–c in D, H&E staining of oviductal, uterine and vaginal sections showing morphological differences among the epithelia. G, gonad; WD, Wolffian duct; CL, cloaca; MD, Müllerian duct; OD, oviduct; U, uterus; C, cervix; V, vagina. Scale bars = 50 μm.

Yin and Ma, J Biochem 2005; 137:677

Hox Genes

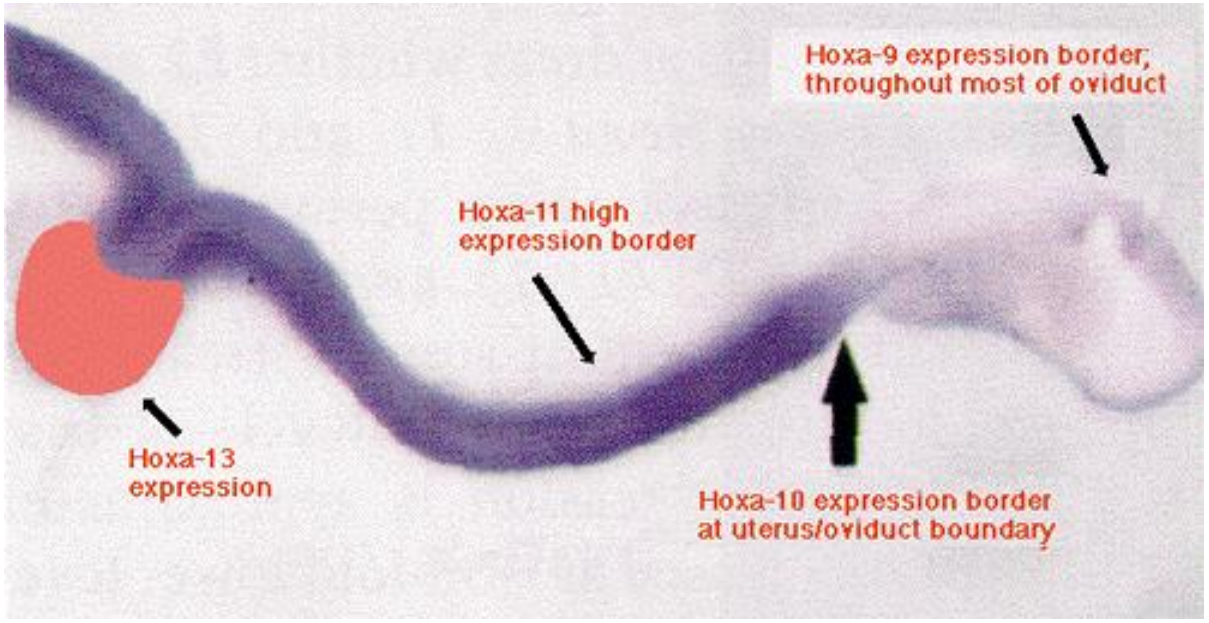


Figure 1. 5' HoxA gene expression in the normal 16.5 day embryonic female mouse reproductive system. The whole mount in situ hybridization of the *Hoxa-13* probe is seen here. *Hoxa-9* goes from the cervix through the uterine anlage to about halfway up the Müllerian duct. *Hoxa-10* expression has a sharp anterior border of expression at the transition between presumptive uterus and oviduct (thick arrow). *Hoxa-11* has the same anterior border as *Hoxa-10,* but its expression diminishes closer to the cervix. In the Müllerian ducts, the expression of *Hoxa-13* is found only in the cervix and upper vagina. (After Ma et al., 1998).

Table 1. **Genes involved in FRT development and phenotype of the reproductive tract in their mutants.**

Gene	Knockout phenotype in reproductive tract	Reference
Lim1	Absence of derivatives of Müllerian duct	(*3*)
Pax2	Absence of entire FRT	(*8*)
Emx2	Absence of derivatives of Müllerian duct	(*9*)
Wnt4	Absence of derivatives of Müllerian duct	(*11*)
MIS	Persistence of Müllerian duct derivatives in male mutants	(*16*)
Misr2	Same as MIS-null mice	(*22*)
Alk3	Oviduct and uterus in male mutants	(*26*)
Hoxa10	Homeotic transformation of the anterior part of the uterus into oviduct-like structure	(*36*, *37*)
Hoxa11	Hypoplastic uterus, reduced gland number	(*74*, *75*)
Hoxa13	Agenesis of the posterior portion of the Müllerian ducts	(*37*)
P63	Persistence of columnar epithelium at lower genital tract sites that normally undergo squamous and urothelial differentiation.	(*76*)
Wnt7a	Absence of oviducts and uteri exhibiting a cytoachitecture typical of vagina; failure to form uterine glands	(*28*, *40*)
Wnt5a	Coiled and shortened uterus and poorly-defined cervix and vagina; failure to form uterine glands	(*41*)
Lif1	Failure of blastocyst invasion and stromal decidulization	(*45*)
IGF	Dramatic hypoplasia of the uterus	(*53*)
TIMP1	Accelerated endometrial gland formation	(*46*)
ERα	Hypoplastic uteri, nonovulatory ovaries and reduced gland number	(*61*, *62*)

Yin and Ma, J Biochem 2005; 137:677

Diethylstilbestrol (DES)

- Diethylstilbesterol was a drug given to women to ease their pregnancies.
- Unfortunately, it was later found to alter the reproductive tract of female fetuses.
- The mechanism of DES action is thought to involve the repression of the Hox genes that instruct the regional specificity of the Müllerian duct.

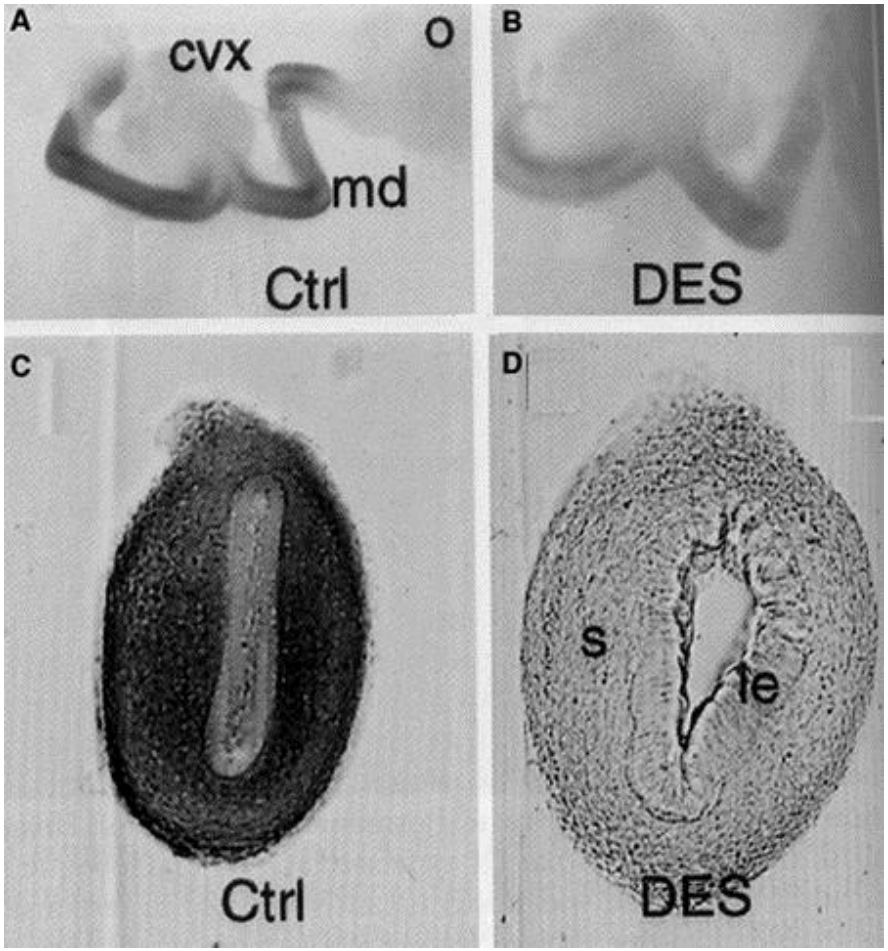


Figure 2. In situ hybridization of a *Hoxa-10* probe shows that DES exposure represses *Hoxa-10*. (A) In 16.5 day embryonic female mice, those mice not exposed to DES had *Hoxa-10* expression from the boundary of the cervix through the uterus primordium and through most of the oviduct. (B) In those mice exposed prenatally to DES, this expression was severely repressed. (C) In control female mice at day 5, sections through the uterus show abundant expression of the *Hoxa-10* gene in the uterine stroma. (D) In female mice who were given higher doses of DES at 5 days after birth (while their reproductive tissues are still forming), DES almost completely suppresses *Hoxa-10* gene expression in the mesenchyme. cvx, cervix; md, Müllerian duct; o, ovary; le, luminal epithelium; s, stroma. (After Ma et al., 1998.)

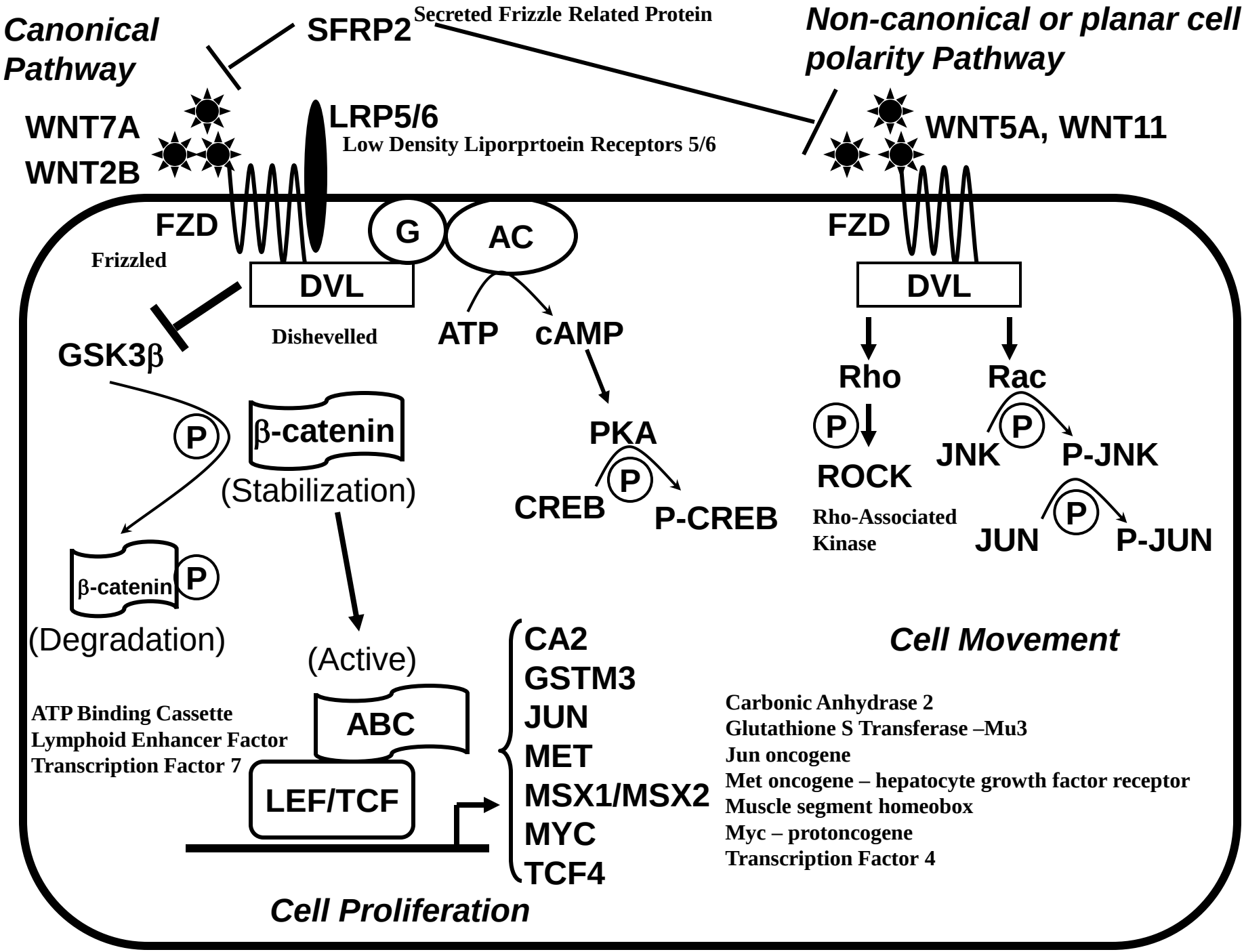
TABLE 1. Expression patterns of Wnt-4, -5a, *and* -7a

	Wnt-4	Wnt-5a	Wnt-7a
During embryogenesis	Ovary Future uterine horn mesenchyme Sinus vagina epithelium	In the genital tubercle Genital tract mesenchyme	Müllerian duct epithelium
After birth	Uterine horn mesenchyme Oviduct mesenchyme	Genital tract mesenchyme	Cervix, vagina, and uterine horn epithelium
Few days after birth	Vagina epithelium	Uterine stroma Oviduct mesenchyme and epithelium	Uterine horn epithelium and forming glands Oviduct epithelium Vagina epithelium
During estrous cycle			
Diestrus	Undetectable	Uterine epithelium and stroma (low levels)	Luminal epithelium of uterine horn (all stages)
Proestrus	Uterine epithelium and stroma (low levels)	Uterine and stroma	
Estrus	Uterine stroma and epithelium	Uterine stroma and epithelium	
Metestrus	Uterine stroma	Uterine stroma	

TABLE 2. Defects in female reproductive system in Wnt *mutant phenotypes*

	Wnt-4	Wnt-5a	Wnt-7a
Defects	Lack of Müllerian duct Partial masculinization of gonads and ectopic expression of testosterone biosynthesis genes Loss of oocytes	Stunted genital tubercle→lack of external genitalia	Abnormal oviduct Abnormal uterus
Viability	<24 hr	<24 hr	Viable, sterile

Heikkila et al. J Exp Zool 2001; 290:616


Canonical Pathway
SFRP2
Secreted Frizzle Related Protein
Non-canonical or planar cell polarity Pathway
WNT7A
WNT2B
LRP5/6
Low Density Liporprtoein Receptors 5/6
WNT5A, WNT11
FZD
Frizzled
G
AC
DVL
Dishevelled
ATP
cAMP
GSK3β
P
β-catenin
(Stabilization)
β-catenin
P
(Degradation)
PKA
P
CREB
P-CREB
FZD
DVL
Rho
Rac
P
ROCK
Rho-Associated Kinase
P
JNK
P-JNK
P
JUN
P-JUN
(Active)
ABC
LEF/TCF
ATP Binding Cassette
Lymphoid Enhancer Factor
Transcription Factor 7
CA2
GSTM3
JUN
MET
MSX1/MSX2
MYC
TCF4
Cell Proliferation
Cell Movement
Carbonic Anhydrase 2
Glutathione S Transferase –Mu3
Jun oncogene
Met oncogene – hepatocyte growth factor receptor
Muscle segment homeobox
Myc – protoncogene
Transcription Factor 4

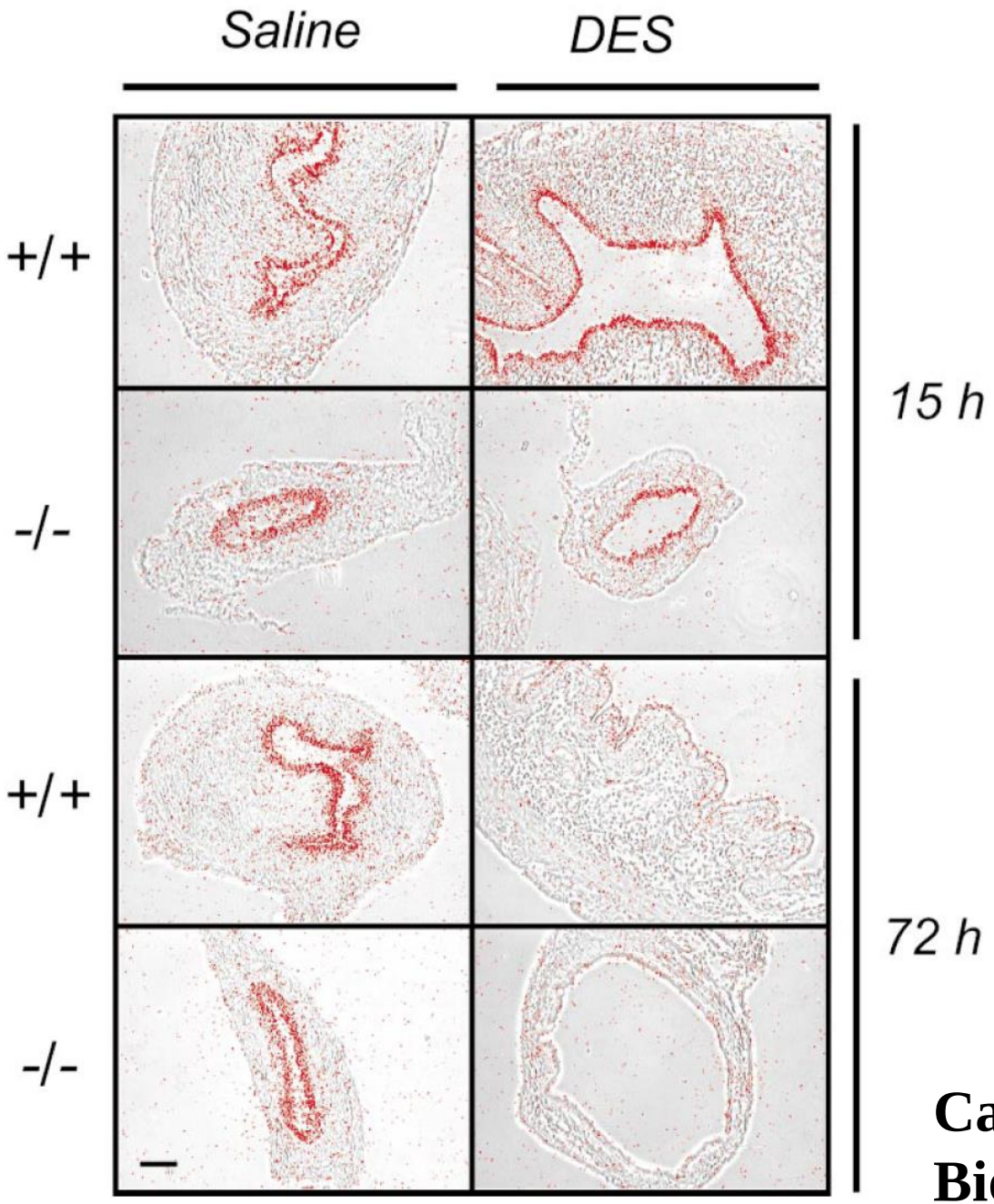
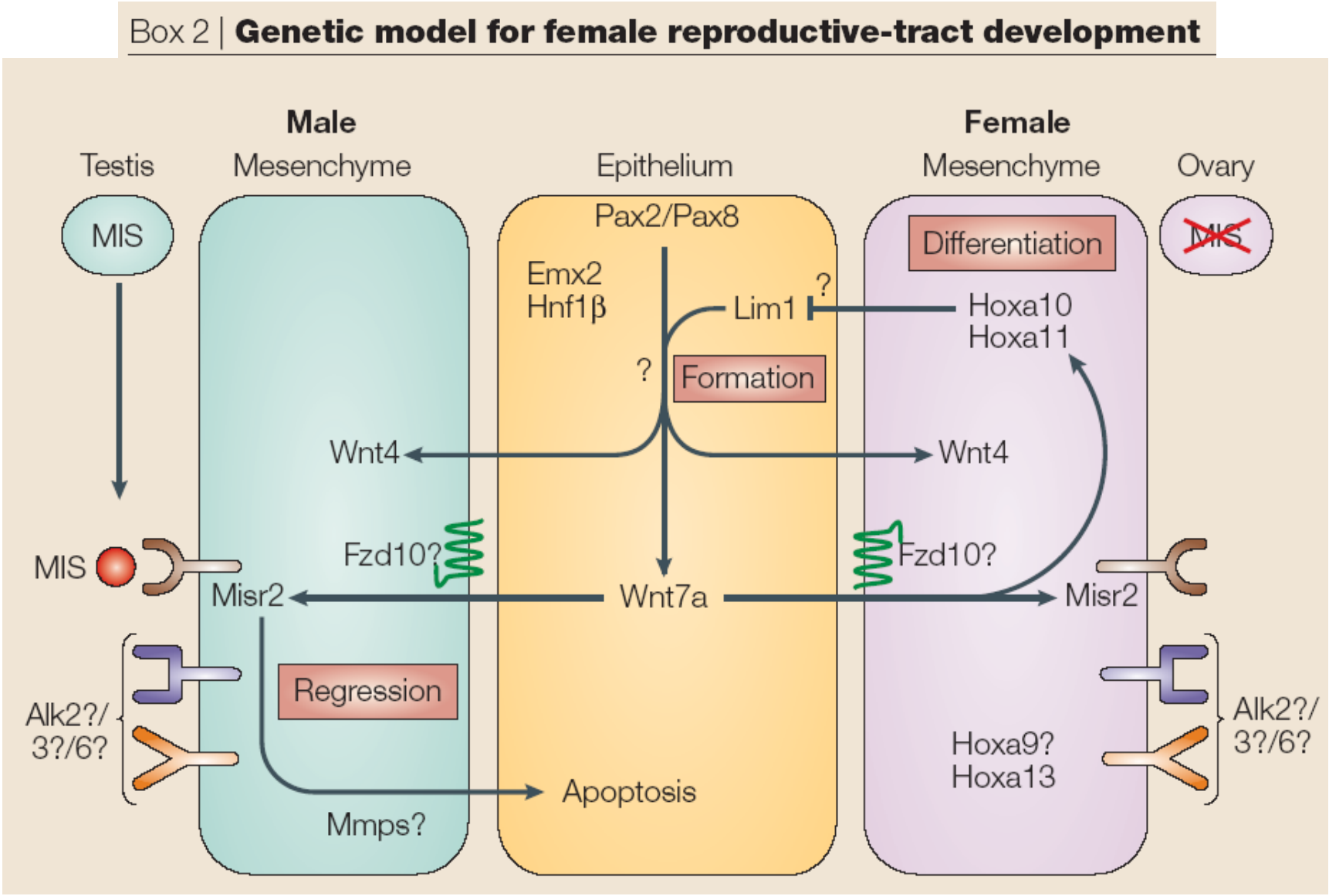
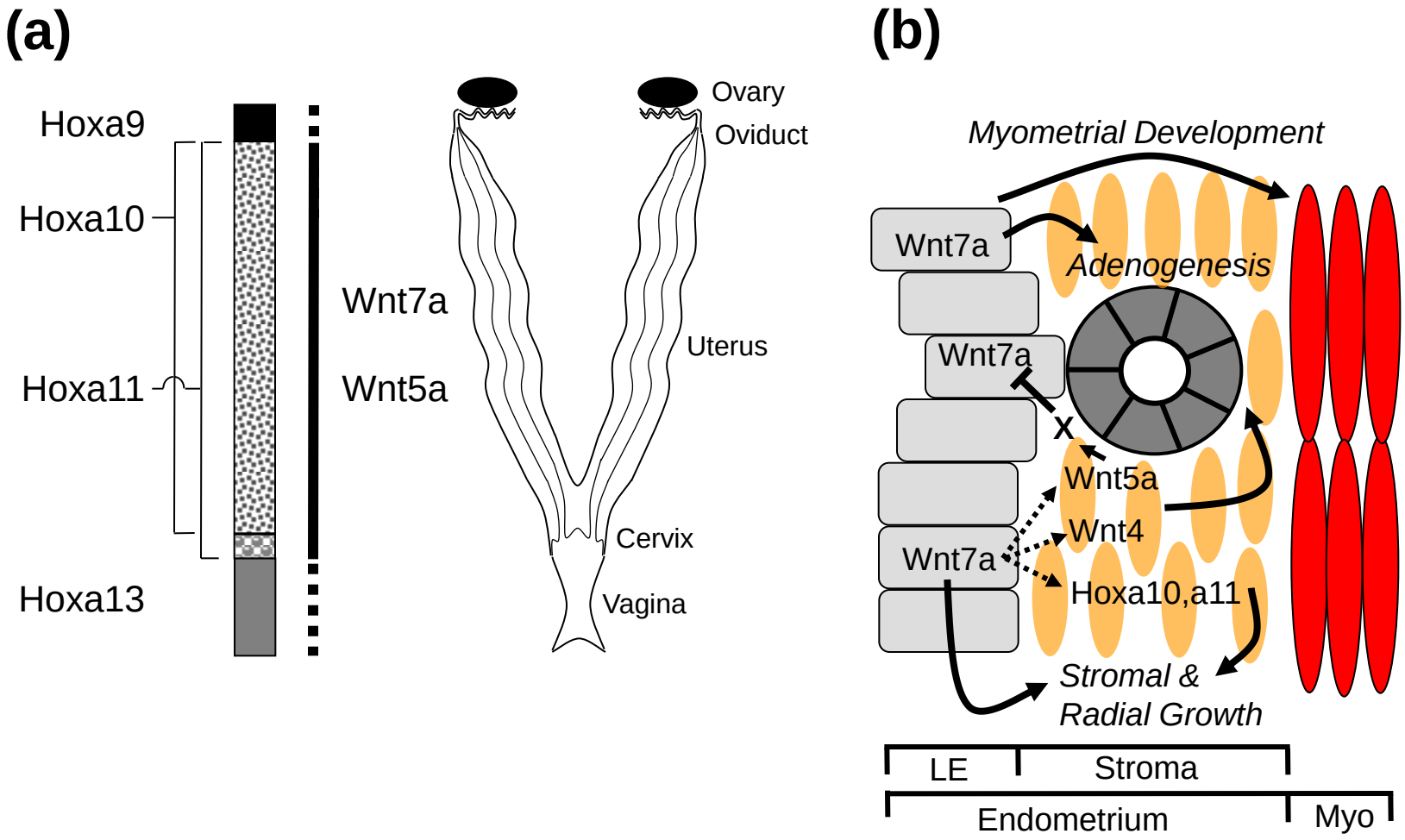


FIG. 6. *Wnt7a* transcripts are detectable during the first 15 h following DES exposure and down-regulated by 72 h. Tissue sections of wild-type and mutant uteri were hybridized for *Wnt7a* at 15 h following a single injection of saline or DES or at 72 h following a series of saline or DES injections. Photomicrographs are composites of phase and dark-field (red) for direct comparison of in situ signal with tissue. The cRNA probe used recognizes both normal and recombined (mutant) transcript. Bar = 100 μm.

Carta and Sassoon
Biol Reprod 2004; 71:444

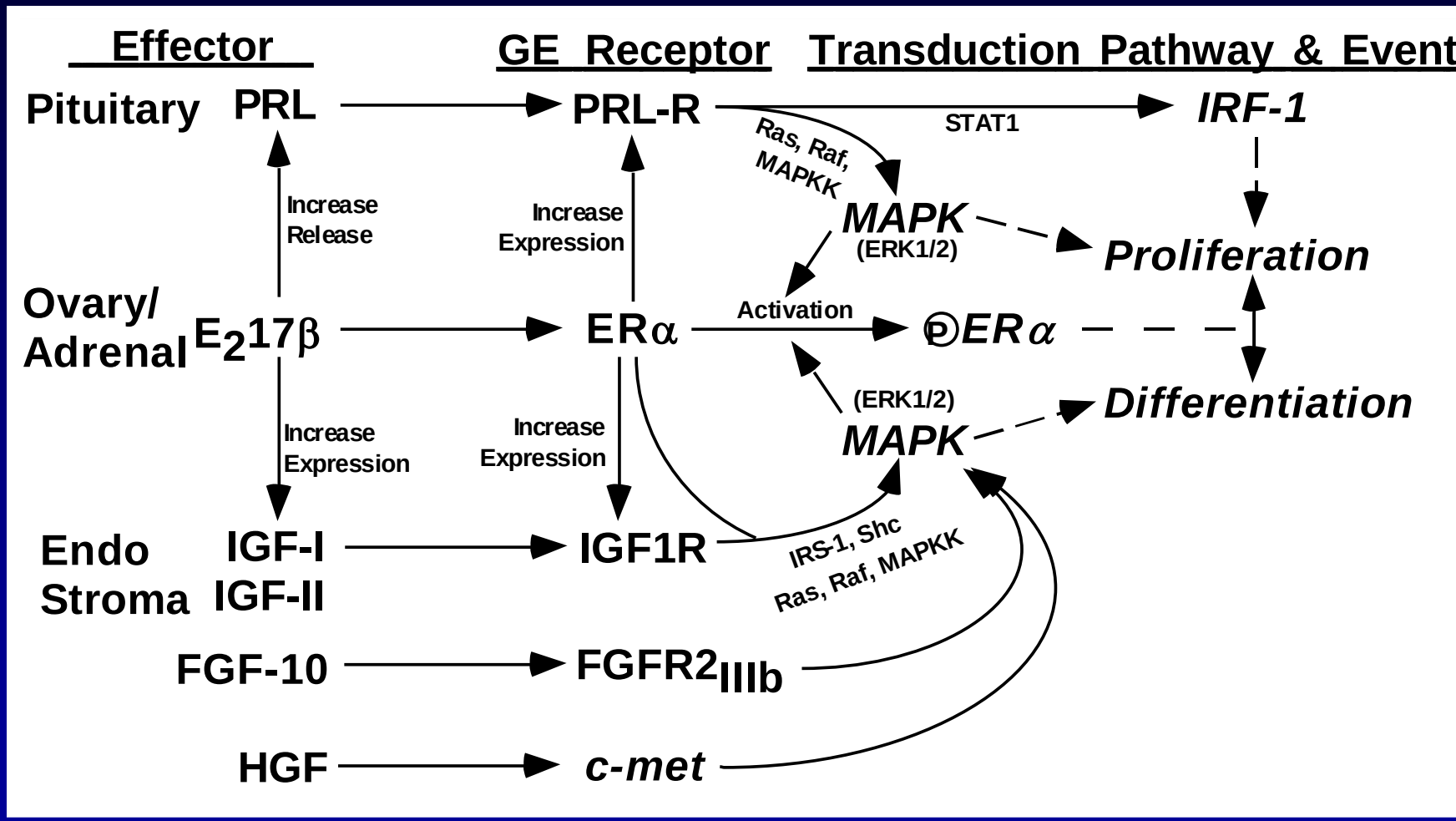


Kobayashi and Behringer, Nature Rev Genetics 2003; 4:969



Mericksay et al., Development 2004; 131:2061

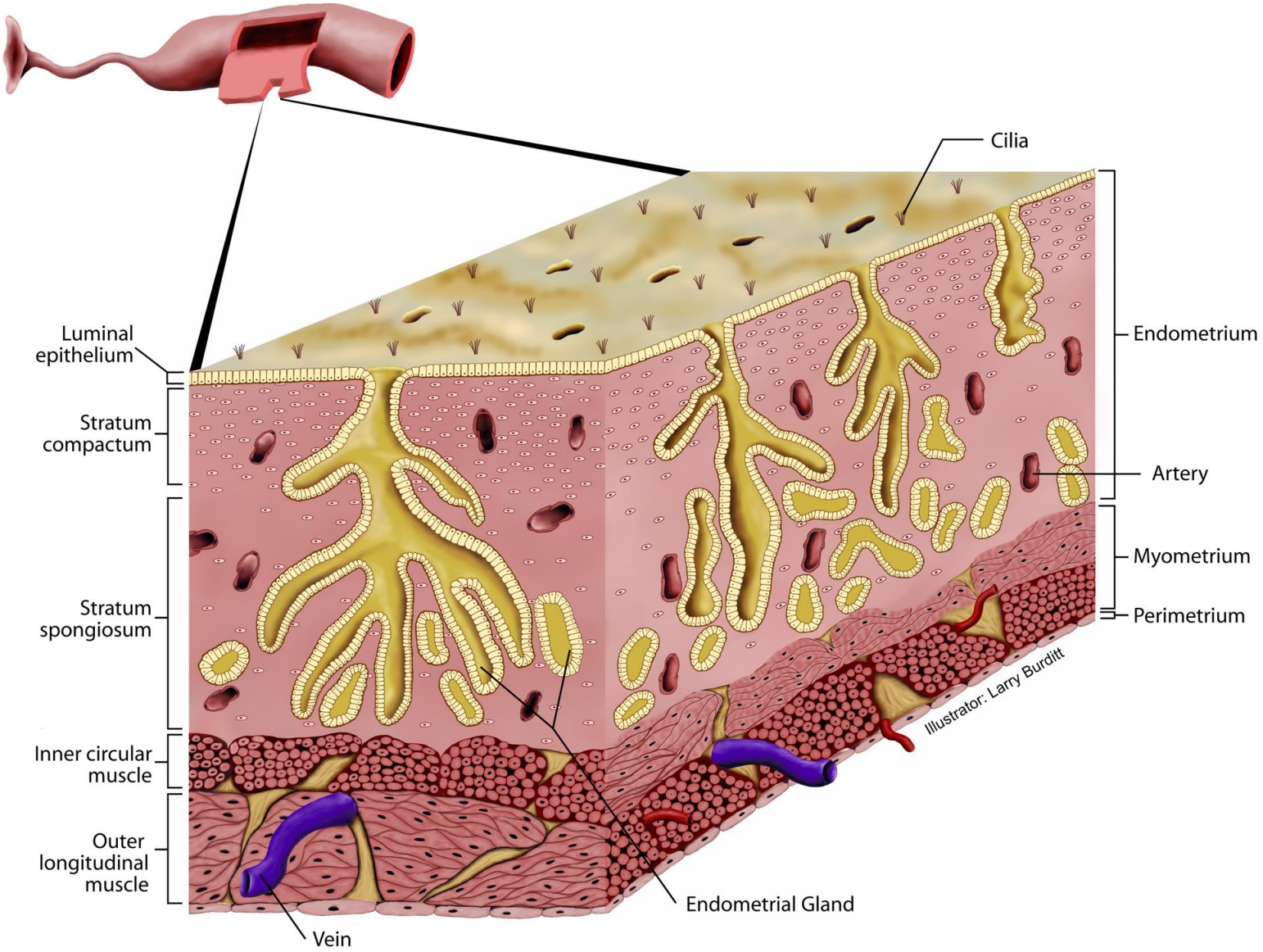
Hormone and Growth Factor Network Regulating Endometrial Adenogenesis



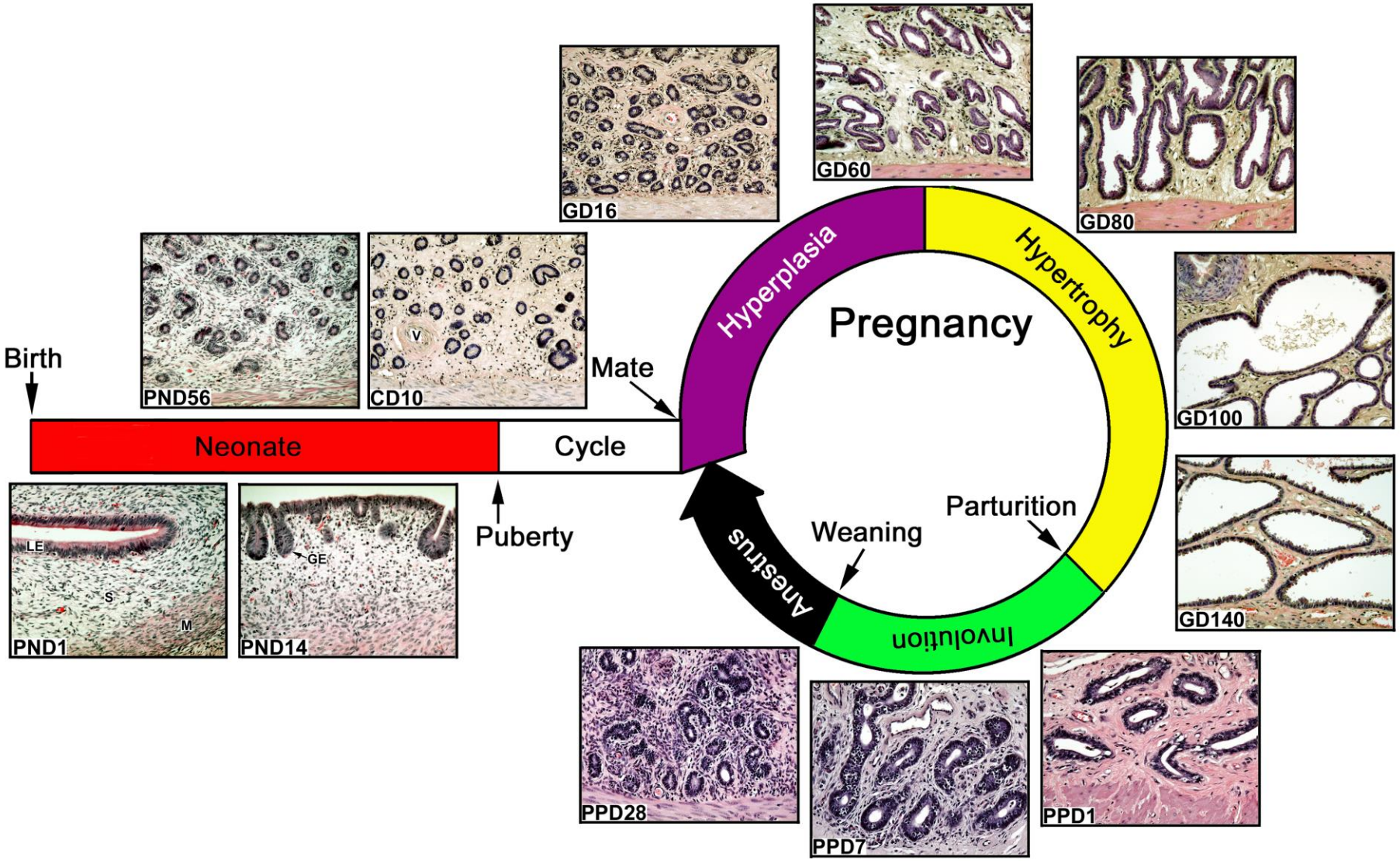
Postnatal Development of Reproductive Tract

- The mammalian uterus is not completely differentiated at birth
- Postnatal development:
  - Mice: vagina, cervix, uterus
  - Domestic animals and human: primarily uterus
- Critical period that is exquisitely sensitive to detrimental effects of endocrine disruptors, particularly disruption of ESR1 (estrogen receptor alpha) by progestins or estrogen agonists
- Nonligand activation of ESR1 important

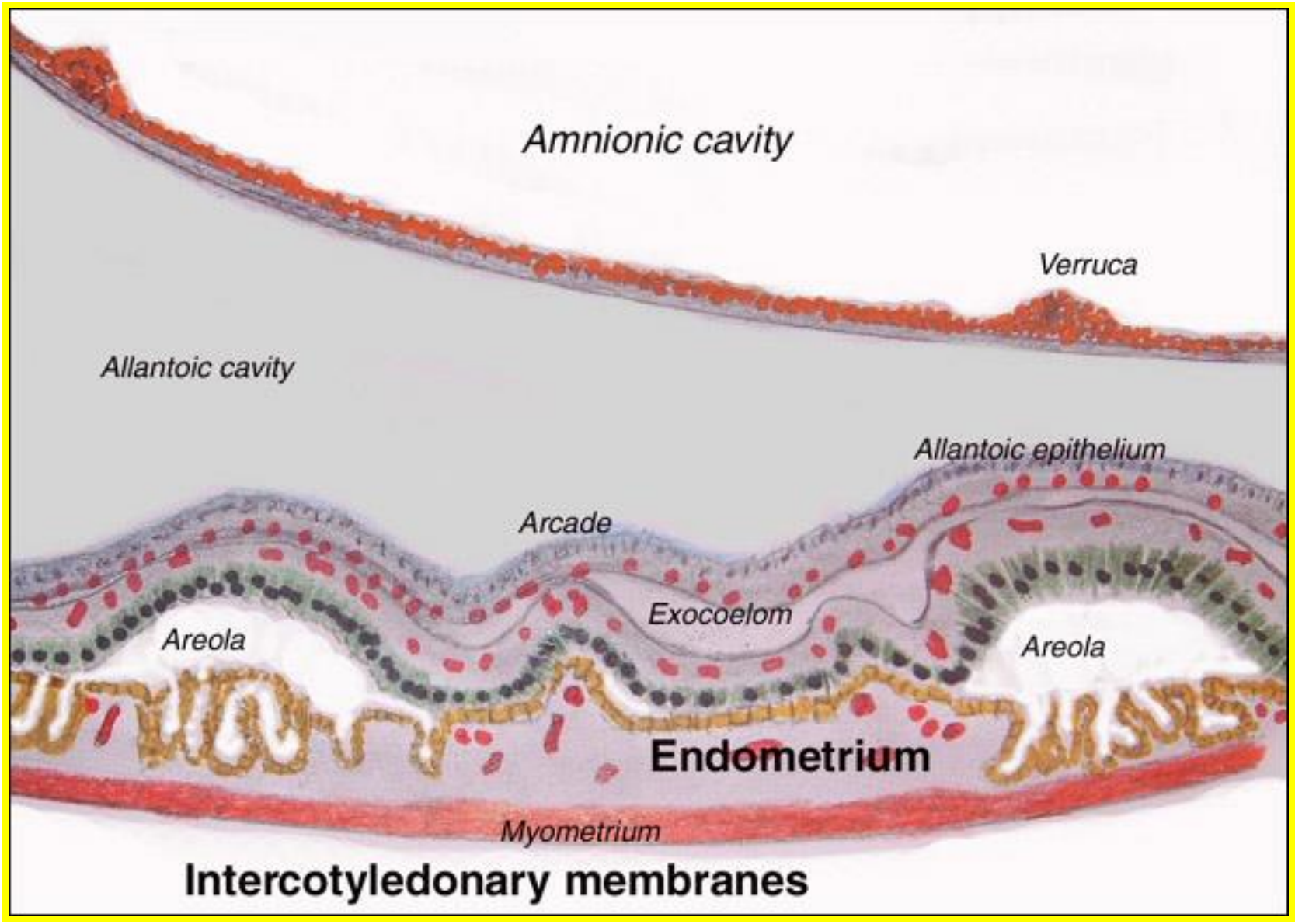
Uterus


Cilia
Luminal epithelium
Stratum compactum
Stratum spongiosum
Inner circular muscle
Outer longitudinal muscle
Endometrium
Artery
Myometrium
Perimetrium
Illustrator: Larry Burditt
Endometrial Gland
Vein

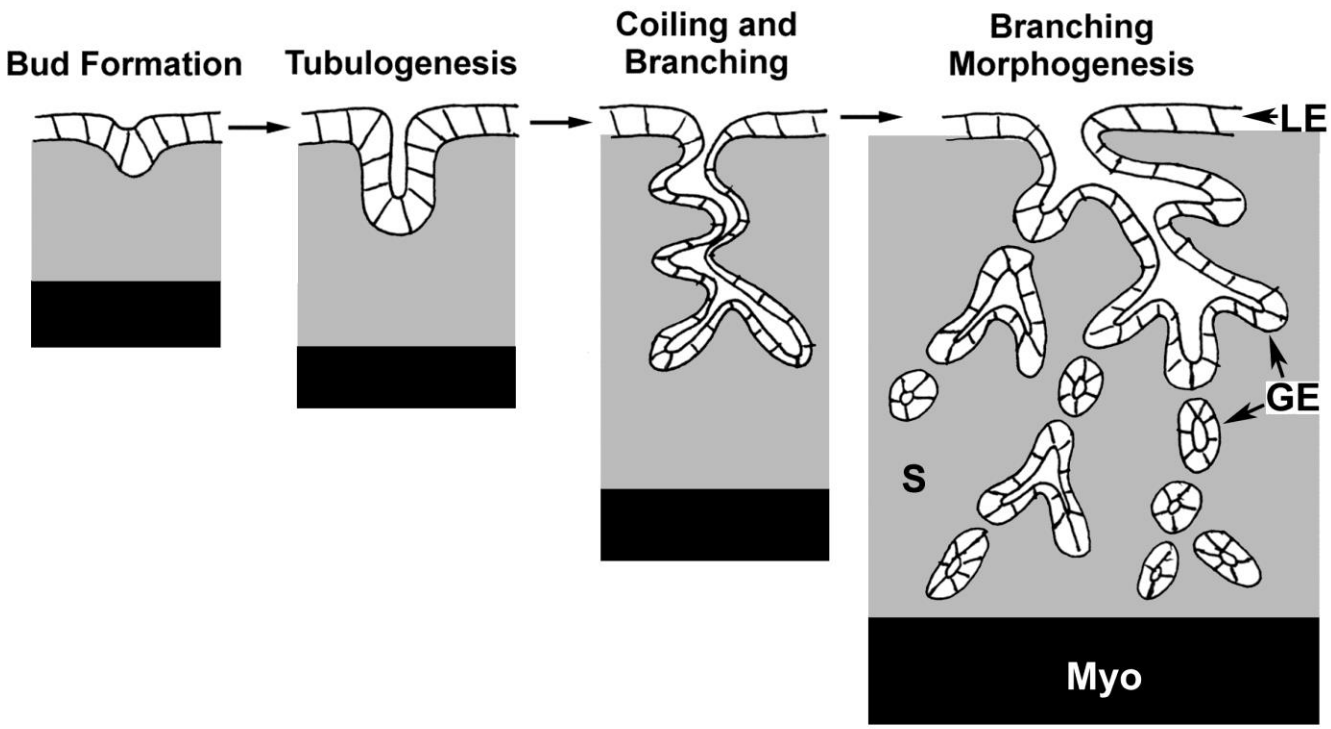
Uterine Gland Life Cycle



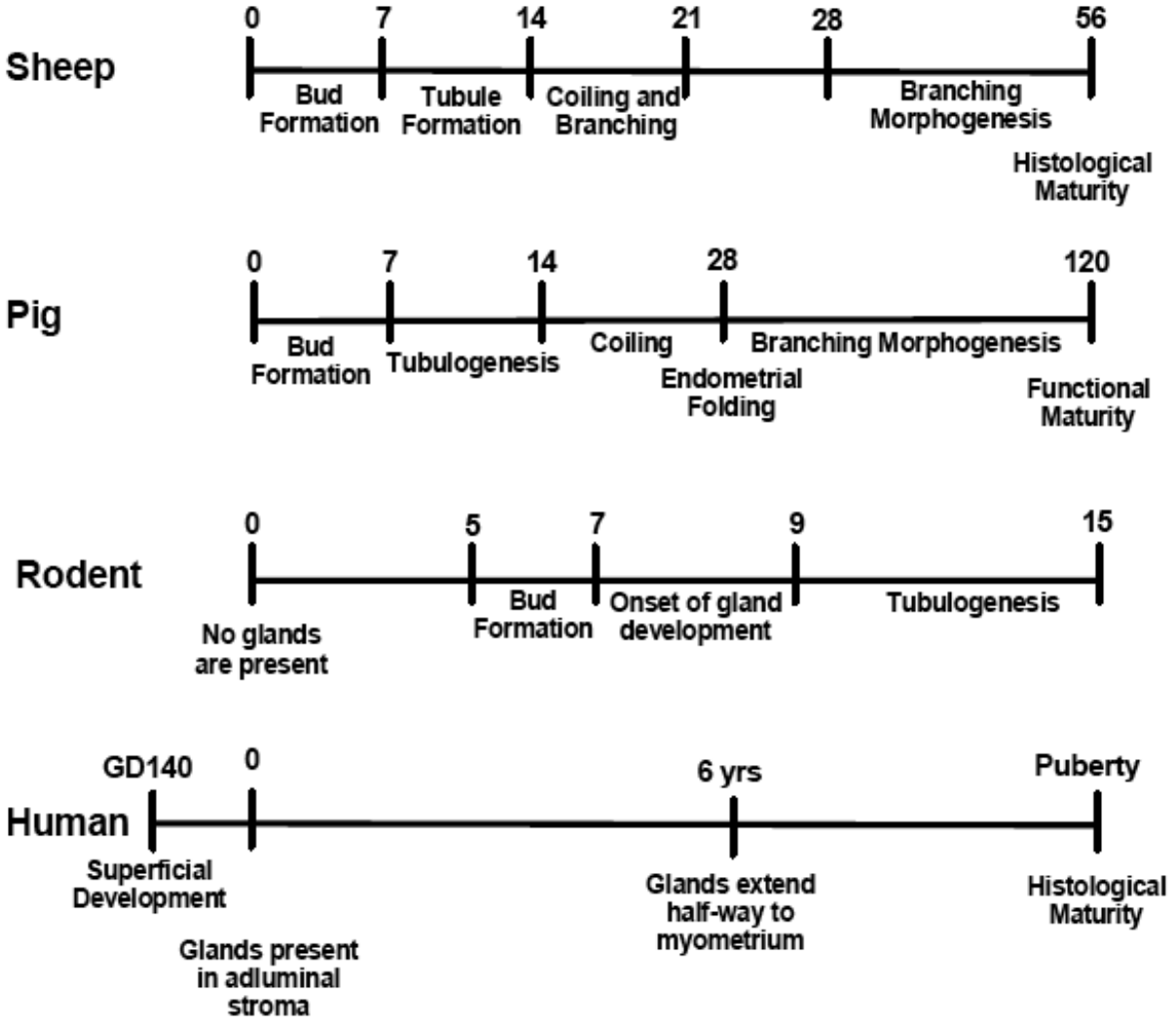
Gray et al., Biol Reprod 2001; 65:1311

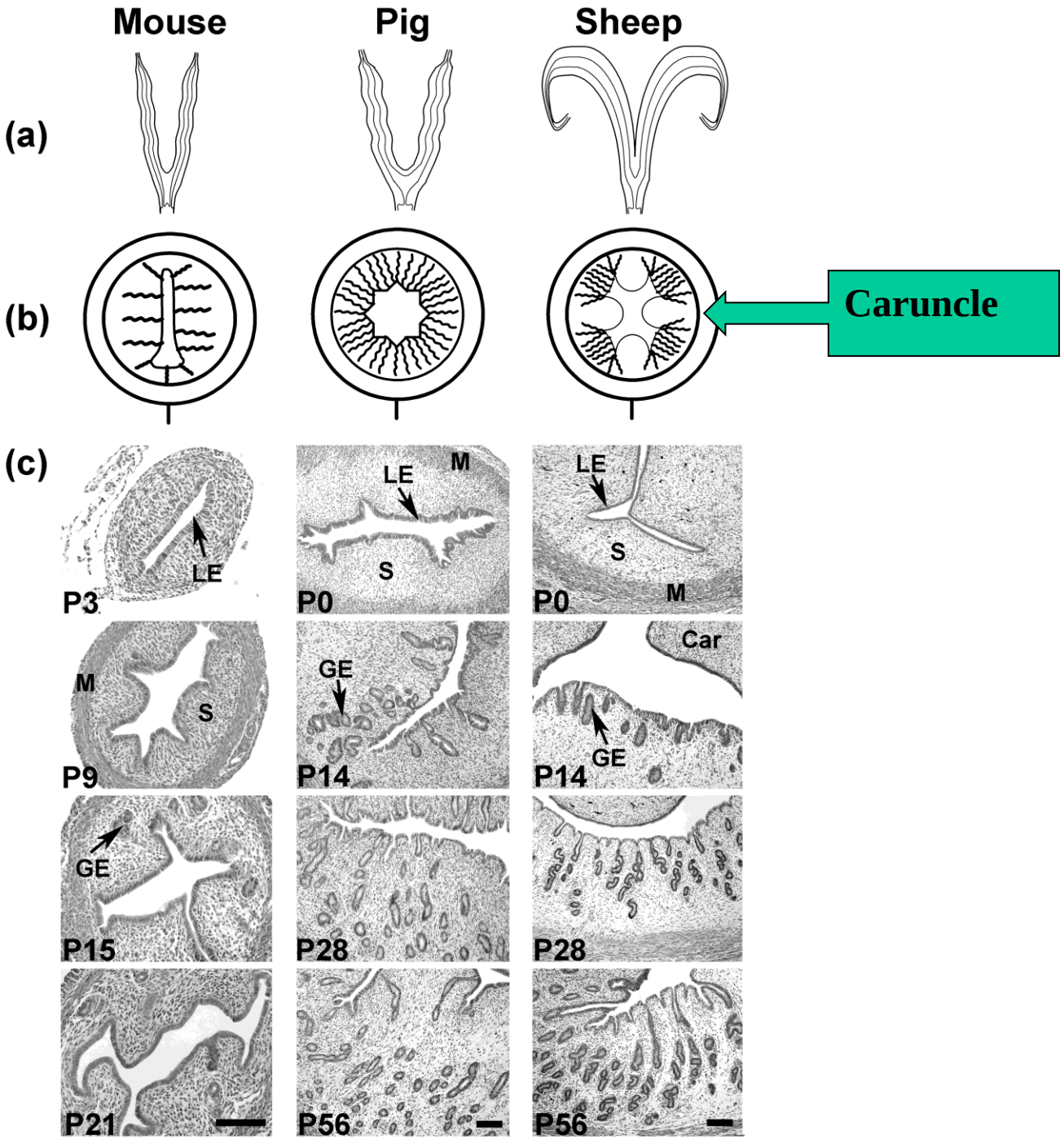

Amnionic cavity
Verruca
Allantoic cavity
Allantoic epithelium
Arcade
Exocoelom
Areola
Areola
Endometrium
Myometrium
Intercotyledonary membranes

Branching Morphogenesis



Comparative Developmental Timeline




Mouse
Pig
Sheep
(a)
(b)
Caruncle
(c)
LE
P3
LE
M
S
P0
LE
S
M
P0
M
S
P9
GE
P14
Car
GE
P14
GE
P15
P28
P28
P21
P56
P56

Prolactin Regulation of Neonatal Ovine Uterine Gland Morphogenesis

KAREN D. CARPENTER, C. ALLISON GRAY, SEKONI NOEL, ARIEH GERTLER, FULLER W. BAZER, AND THOMAS E. SPENCER

Center for Animal Biotechnology and Genomics (K.D.C., C.A.G., S.N., F.W.B., T.E.S.), Department of Animal Science, Texas A&M University, College Station, Texas 77843-2471; Institute of Biochemistry, Food Science and Nutrition (A.G.), The Hebrew University of Jerusalem, Rehovot 76100, Israel

Uterine gland development or adenogenesis in the neonatal ovine uterus involves budding, proliferation, and branching morphogenesis of the glandular epithelium (GE) from the luminal epithelium (LE) between birth (postnatal day or PND 0) and PND 56. This critical developmental event is coincident with increases in serum PRL and expression of long and short PRL receptors specifically in the nascent and proliferating GE. In study one, ewes were treated with a placebo pellet as a control (CX) or a bromocryptine mesylate pellet from PNDs 0–56. On PND 56, the endometrium of bromocryptine mesylate ewes contained fewer glands, particularly in the stratum spongiosum that contained numerous coiled and branched glands in CX uteri. In study two, ewes were treated with saline as a CX or recombinant ovine PRL from PNDs 0–56. Treatment with PRL increased gland number and density on PND 14 and PND 56. In study three, expression of signal transducers and activators of transcription (STAT) 1, 3, and 5 proteins was detected in the developing glands from PNDs 7–56. In study four, Western blot analyses indicated that PRL increased levels of phosphorylated STATs 1 and 5, but not STAT 3, and phosphorylated ERK 1 and 2 MAPKs and c-*Jun* N-terminal kinase/stress-activated protein kinase proteins in explanted PND 28 ovine uteri. Collectively, results indicate that PRL regulates endometrial adenogenesis in the neonatal ovine uterus. (*Endocrinology* 144: 110–120, 2003)

Ovarian Regulation of Endometrial Gland Morphogenesis and Activin-Follistatin System in the Neonatal Ovine Uterus[1]

Karen D. Carpenter,[3] Kanako Hayashi,[3] and Thomas E. Spencer[2]

Center for Animal Biotechnology and Genomics, Department of Animal Science, Texas A&M University, College Station, Texas 77843-2471

TABLE 1. Weights and histomorphometrical measurements of uteri from CX and OVX ewes on PND 56.

Measurement	CX	OVX	SEM	*P*
Uterine wet weight (g)	3.8	2.0	0.4	0.007
Ductal gland invaginations (no./section)	71	67	3	0.30
Endometrial glands (total no./section)	789	439	80	0.0001
Stratum compactum gland density (no./200 μm²)	5.0	5.0	0.2	1.0
Stratum spongiosum gland density (no./200 μm²)	10.6	8.3	0.3	<0.0001
Endometrial thickness (μm)	600	467	21	<0.0001
Myometrial thickness (μm)	506	428	22	0.014

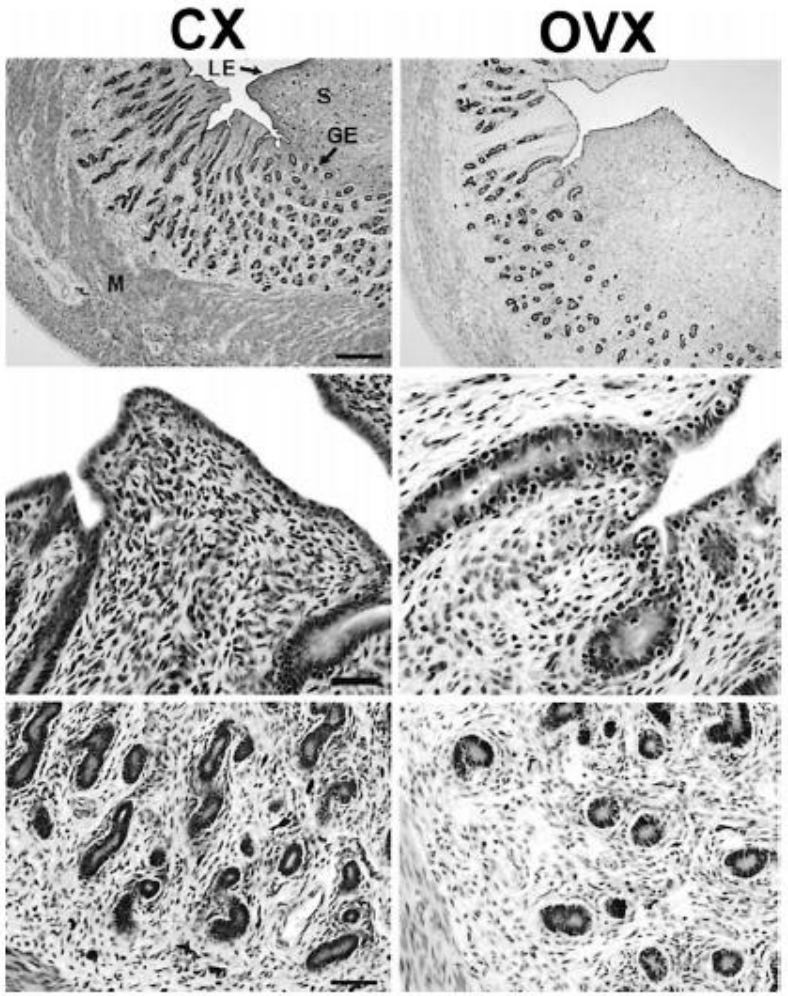


FIG. 1. Representative photomicrographs illustrating the histoarchitecture of uteri from CX and OVX ewes on PND 56. Tissues were prepared and stained using hematoxylin and eosin. M, Myometrium; S, stroma. Bar = 500 μm at low magnification and 50 μm at high magnification.

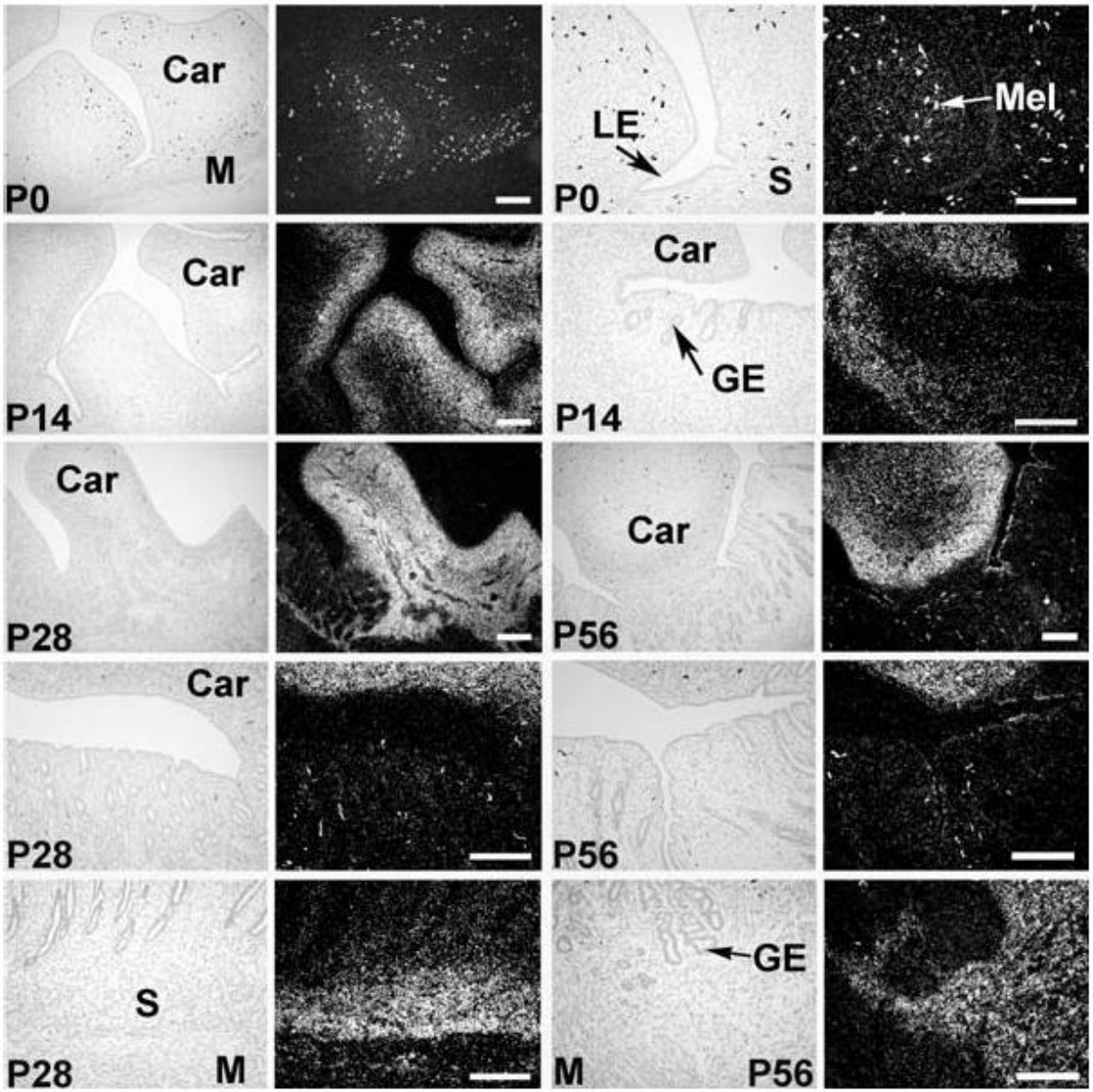


FIG. 2. In situ hybridization analysis of *SFRP2* mRNA in the neonatal ovine uterus. In each panel portion, representative photomicrographs of in situ hybridization results are presented in darkfield illumination. The black melanocytes (Mel) in the uterus appear white under darkfield illumination. Car, Caruncular endometrium; GE, glandular epithelium; LE, luminal epithelium; M, myometrium; S, stroma. Bar = 100 μm.

SFRP2 – secreted Frizzle-related Protein – interferes With Wnt signaling

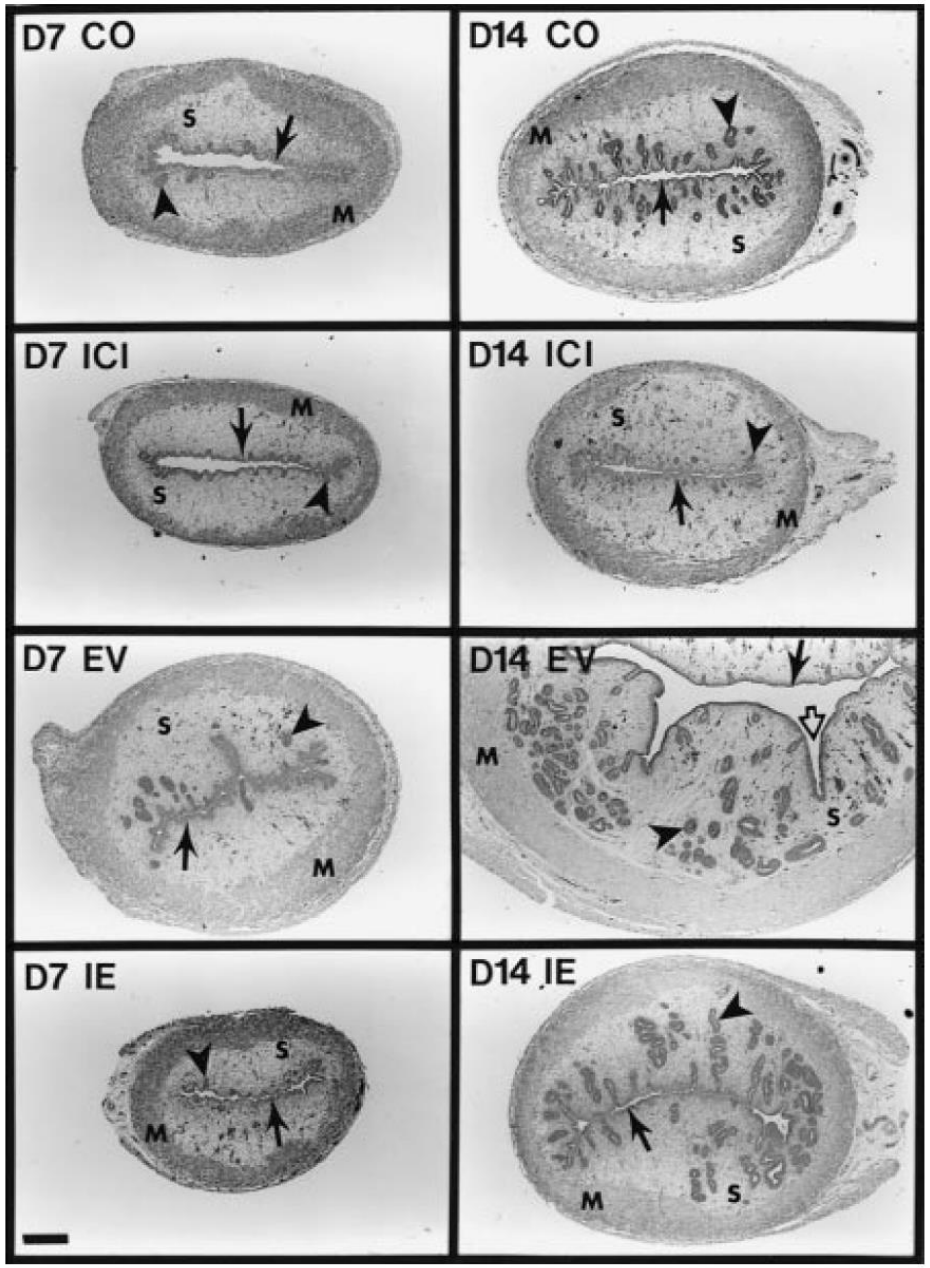


FIG. 2. Histology of the porcine uterus on PND 7 (D7; left) and PND 14 (D14; right) following treatment of gilts from birth with CO, ICI, EV, or IE as described for study one. Sections were stained with buffered azure eosin. Arrow, LE; arrowhead, GE; S, stroma; M, myometrium; open arrow, endometrial fold. Bar = 300 μm.

Neonatal Pig Uterine Development

- Ovary- and estrogen-independent
- ESR1 (estrogen receptor alpha) dependent

ICI = antiestrogen (ESR1 antagonist)

EV = estradiol valerate

IE- EV and ICI

Cycle of Ovulation and Menstruation in Humans

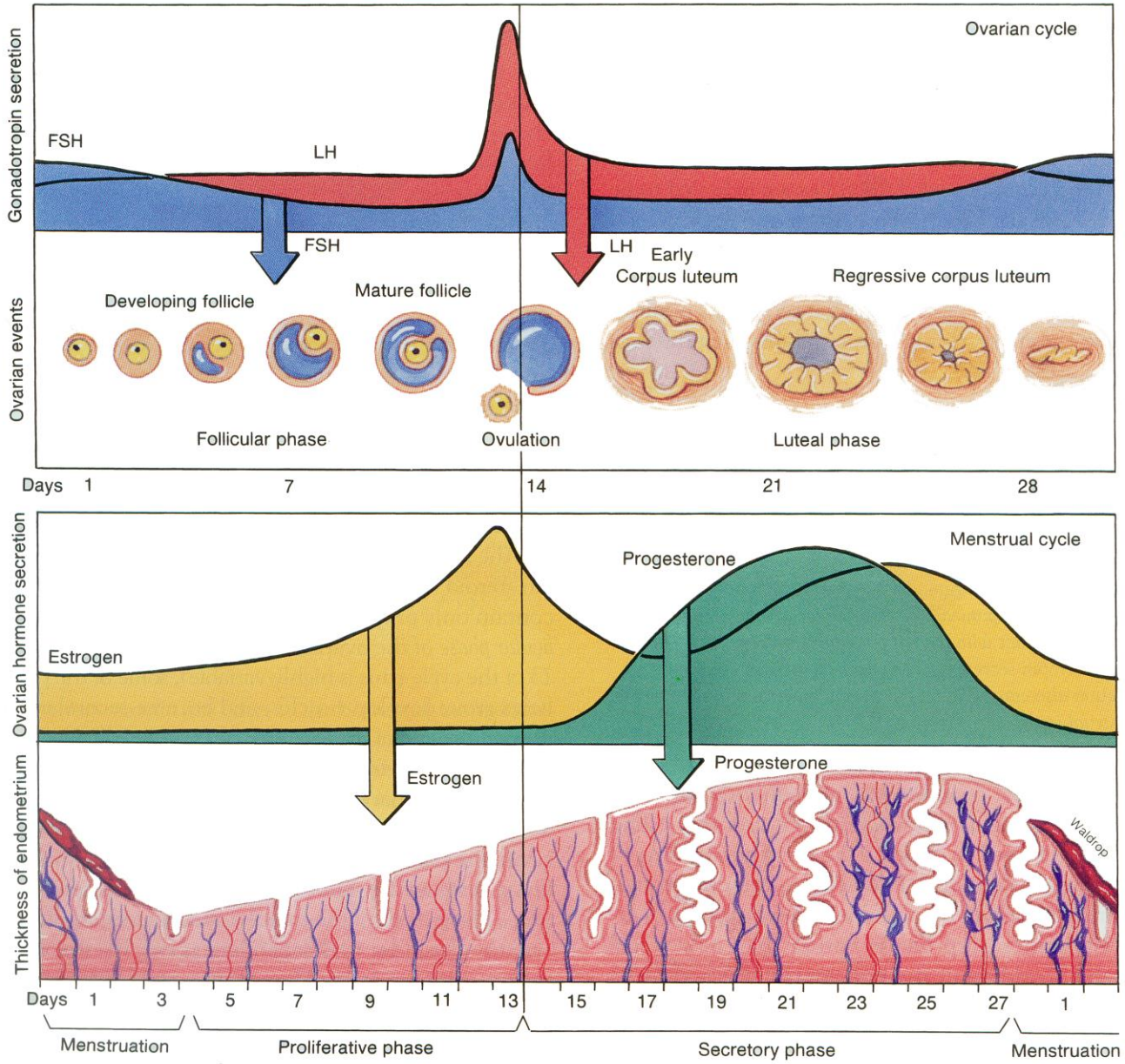
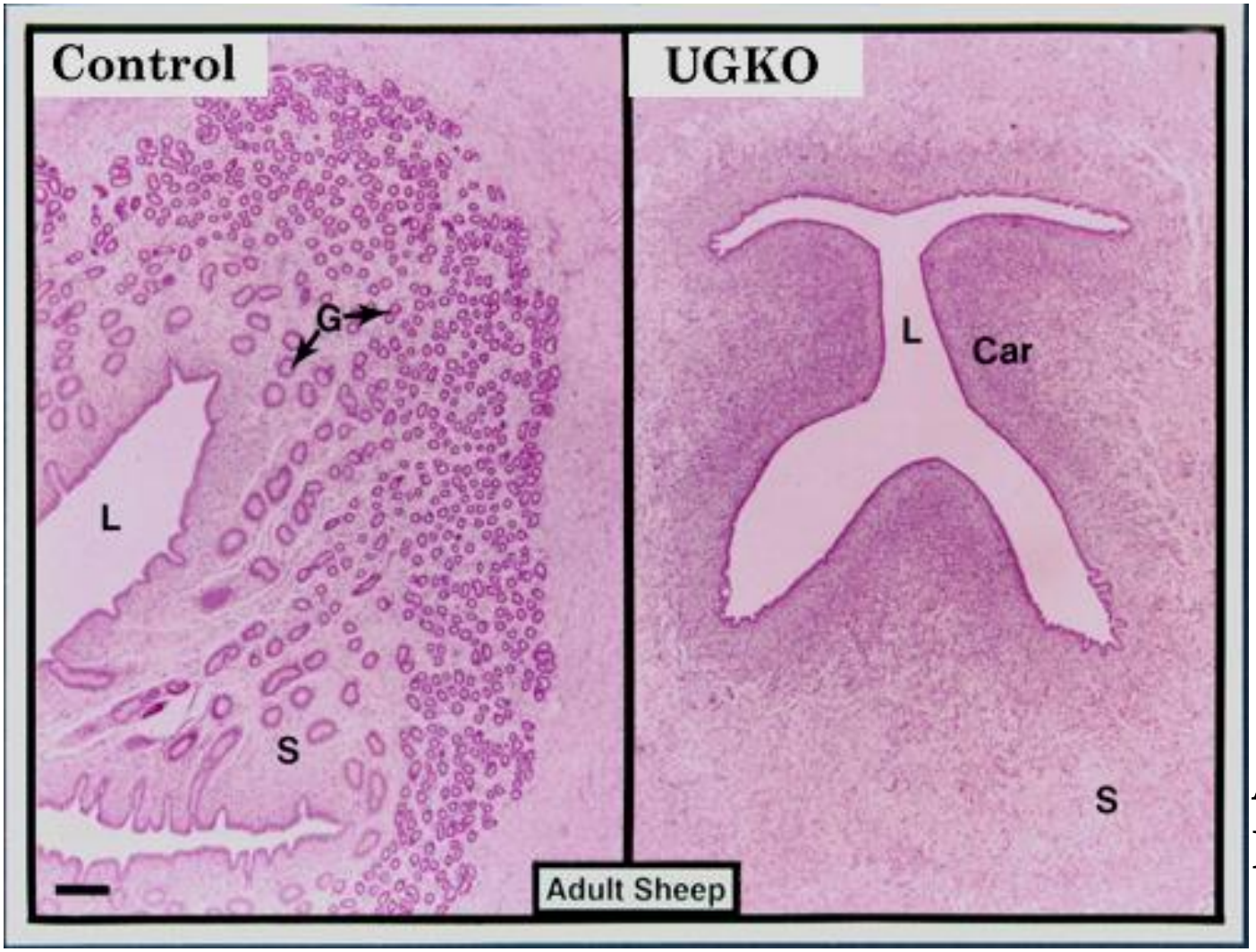


FIGURE 29.14
The cycle of ovulation and menstruation.

Steroids and Uterine Development

- **Exposure to ovarian steroid-based compounds during critical developmental windows permanently affects adult uterine function**
  - **Mouse, Rat, Sheep, Pig, Cow and Human**
- **Perturbations include endometrial dysgenesis or dysplasia leading to infertility**
- **Associated with development of cancer**

Progestins Inhibit Endometrial Adenogenesis



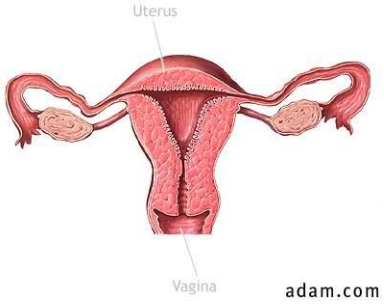
Cyclic Fertile

Acyclic Infertile

Neonatal ewes treated with progestin from birth to postnatal day 56

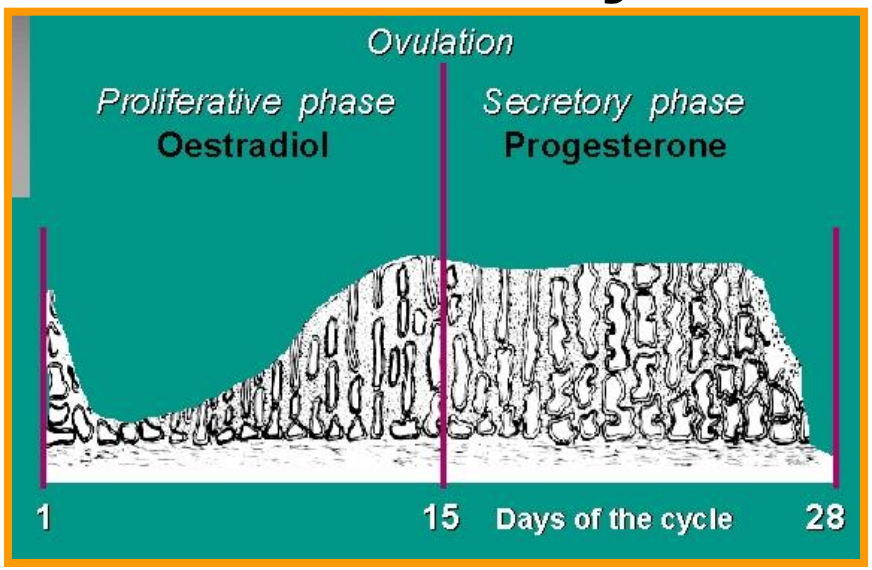
Gray et al., Biol Reprod 2000; 62:448

IMPLICATIONS



- Unexplained infertility may be caused by insufficient endometrial development
- Perturbations in pre-pubertal or follicular phase endometrial development may be unrecognized causes of unexplained infertility

Menstrual Cycle



- Endometrial development may be affected by therapies which act directly on the uterus, ovary, and/or hypothalamus and pituitary

Wolffian Ducts

- Two paired Wolffian ducts ultimately develop into or form part of the structures of the male reproductive tract.
- The structures involved include the **epidymis, vas deferens, seminal vesicle, and prostate**.

Freemartinism: Role of Testosterone

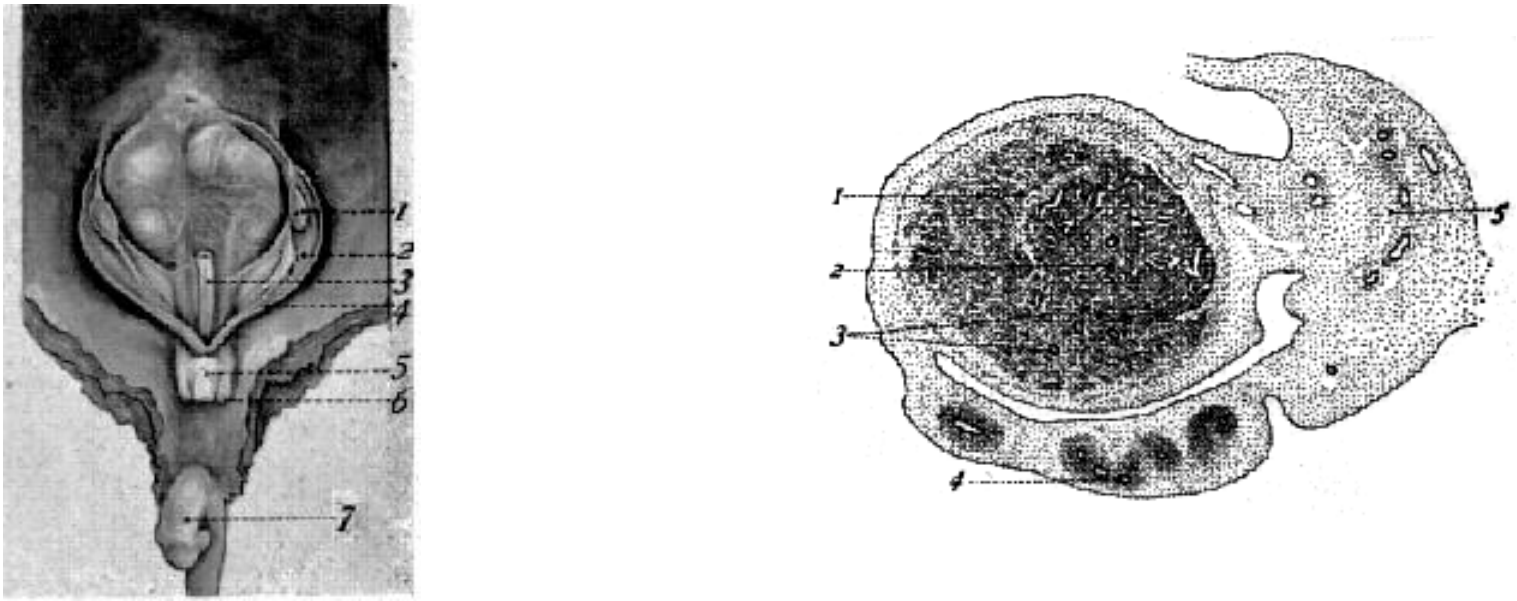


Figure 1. Masculinization of the XX bovine gonads and genital tract in freemartins. (A) Urogenital system of a sterile freemartin (7.5 cm long). 1. gonad; 2. Wolffian body; 3. rectum; 4. genital duct; 5. allantois; 6. umbilical artery; 7. enlarged clitoris. (B) Cross-section through gonadal region of a freemartin (21.5 cm long). 1, sex cord resembling medullary (male) cord; 2, rete; 3, sex cords resembling seminiferous subules; 4, Wolffian duct in fold of peritoneum overhanging the gonad; 5, rudiment of the Wolffian body. (A after Lillie, 1917; B after Chapin, 1917.)

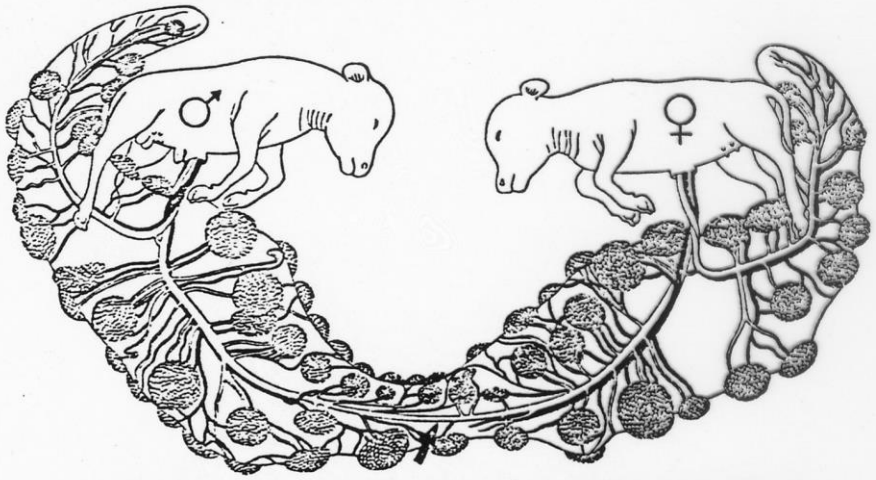


Fig. 2-4. The cause of the freemartin condition. When twin fetuses of opposite sex share a common circulation as a result of fusion of placental blood vessels (arrowed), some substance passes from the male to the female, causing partial sex-reversal of her ovaries. This interferes with the normal development of the female reproductive tract, so that the animal is sterile. (From F. R. Lillie. *J. Exp. Zool.* 23, 271 (1917).)

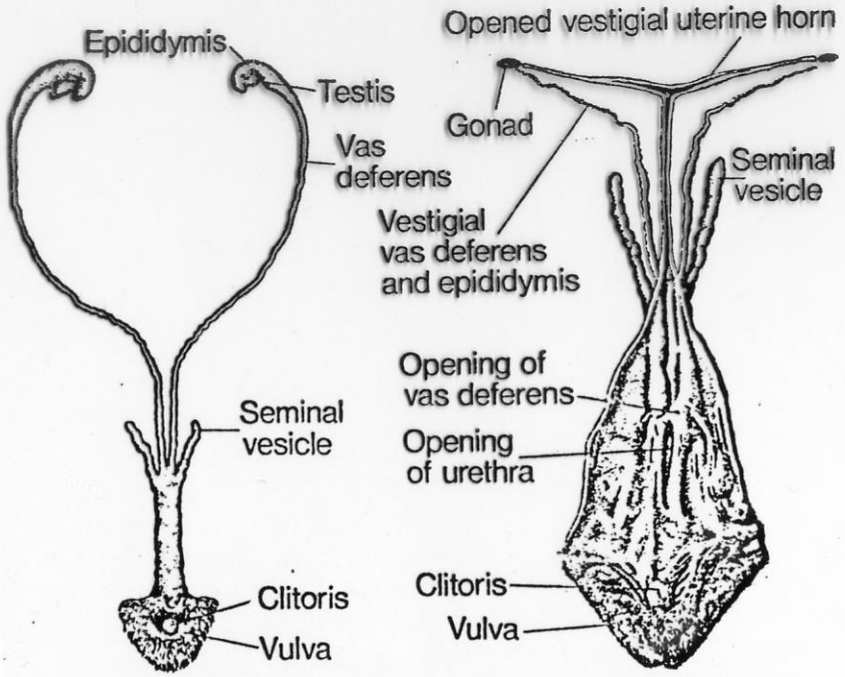


Fig. 2-5. The reproductive tracts of two bovine freemartins, showing the great variability that exists. On the left is the tract of a newborn animal described by Lillie with complete masculinization of the internal genitalia. The gonads, although very small, have been completely transformed into testes and have descended into the inguinal canals. The epididymi, vasa deferentia and seminal vesicles are well developed, and all remnants of the Mullerian duct have been completely suppressed. However, the external genitalia are still female in appearance, apart from slight hypertrophy of the clitoris. On the right is the reproductive tract of an adult freemartin, dissected and drawn for John Hunter 200 years ago. The gonads are extremely small, and both male and female duct systems are present, but incompletely developed. The external genitalia are those of a normal cow, although the clitoris is slightly hypertrophied.

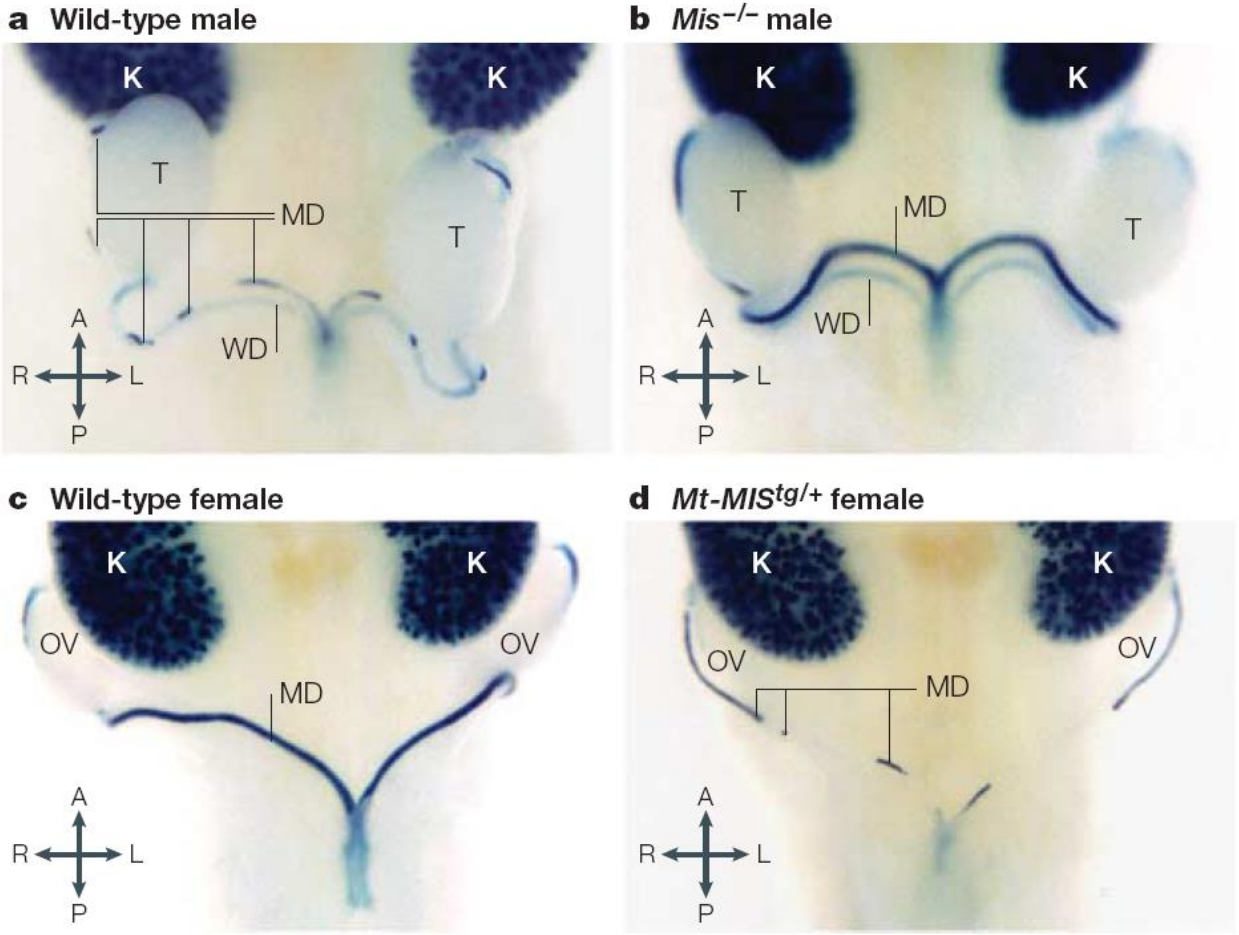


Figure 3 | **Müllerian duct regression.** The developing Müllerian ducts are visualized by *Lim1–lacZ* expression[11] in the mouse embryo at embryonic day (E) 15. **a** | In XY male mice, Müllerian-inhibiting substance (MIS) is produced by the testes and eliminates the Müllerian ducts. The regressing 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 REF. 11 © (2003) The Company of Biologists Ltd.

Male Reproductive Tract Development

- Dependent on intrinsic and extrinsic factors
  - Epithelial-mesenchymal interactions
  - Transcription factors (Hox genes)
  - Growth factors (FGF10)
  - Steroids (T and DHT)
  - Steroid Receptors (AR)

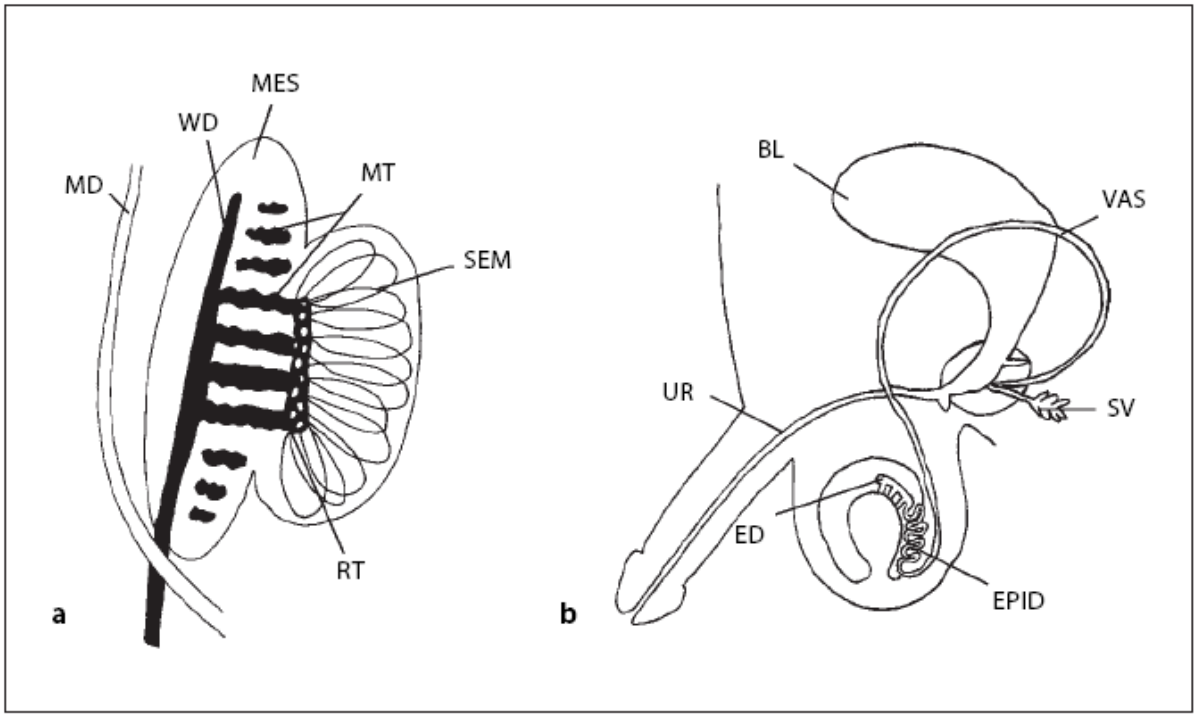


Fig. 1. Schematic drawing of the development of the male genital tract in humans. **a** Schematic drawing showing the excretory system of the gonad and MES at 8 weeks gestation. SEM anastomose to form the RT, which is connected to the MT that drain into the WD. Mesonephric tubules that are not connected to the testis degenerate. **b** Male urogenital tract in a newborn. The mesonephric tubules and WDs have developed into ED, EPID, VAS and SV. BL = Bladder; ED = efferent ducts; EPID = epididymis; MD = Müllerian duct; MES = mesonephros; MT = mesonephric tubules; RT = rete testis; SEM = seminiferous cords; SV = seminal vesicle; UR = urethra; VAS = vas deferens; WD = Wolffian duct.

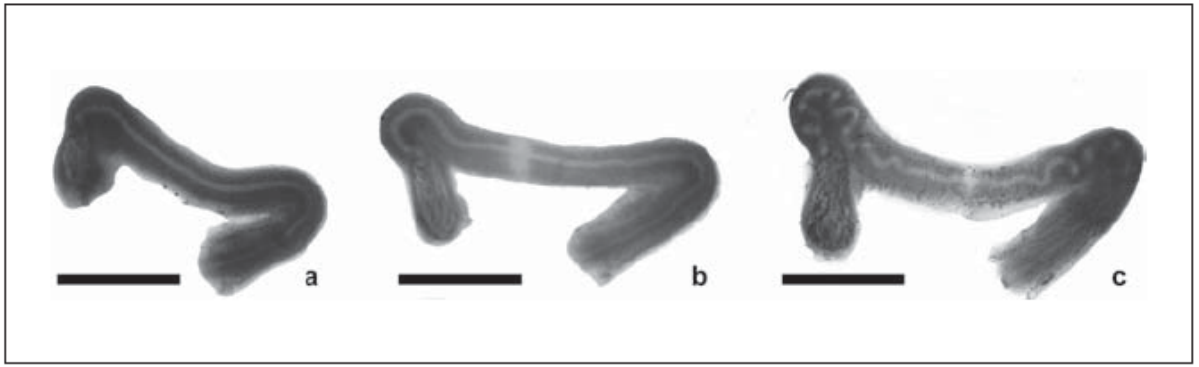


Fig. 2. Development of the foetal rat WD. Macroscopic photos of the WD at E18.5 (**a**), E19.5 (**b**) and E20.5 (**c**) showing growth and coiling of the duct. Scale bars = 1 mm.

Horm Res 2007;67:142–151

Wolffian Duct Differentiation

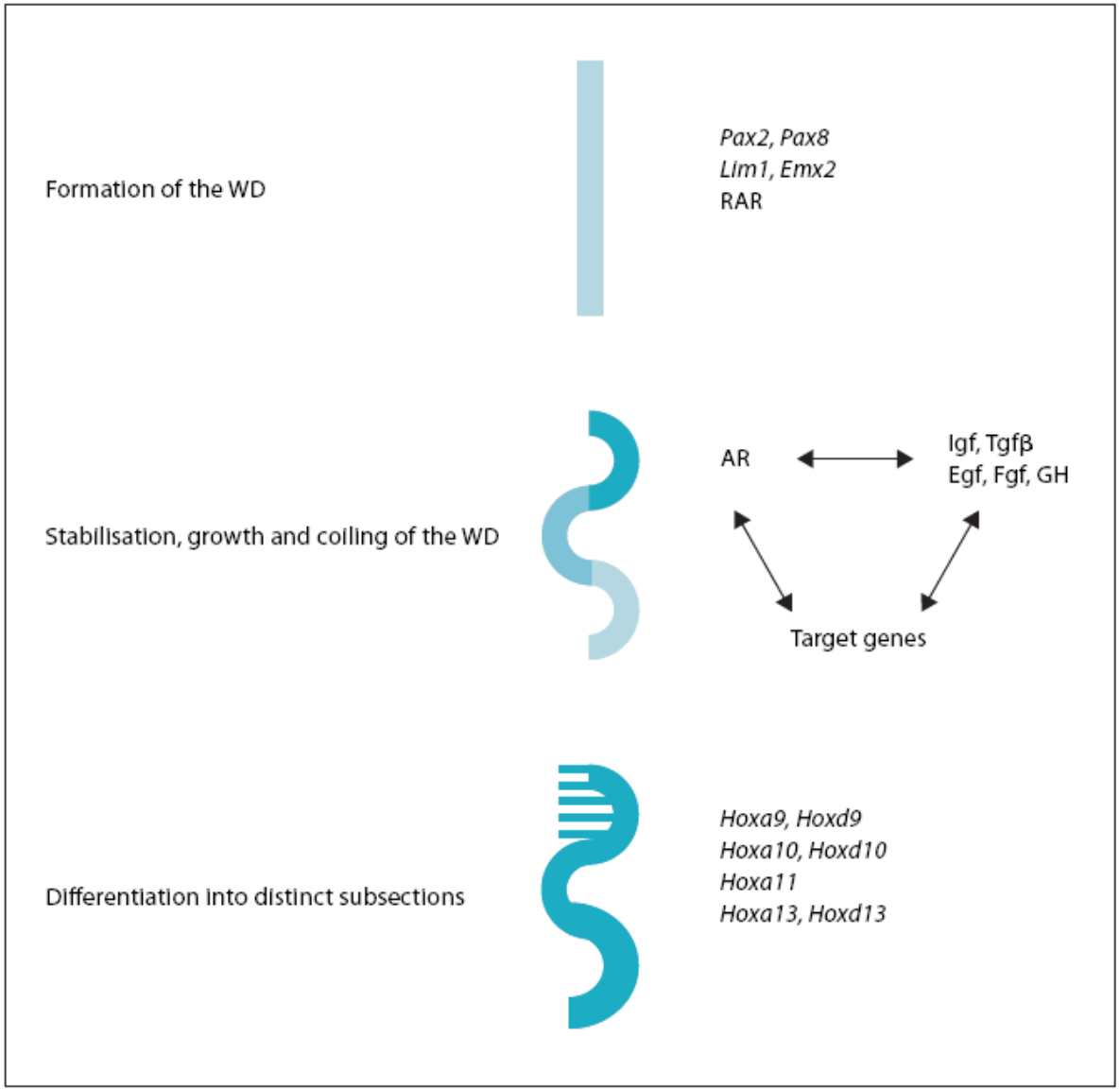


Fig. 3. Genes involved in WD development. Overview of genes implicated in early formation of the WD (occurring in both sexes), androgen-dependent stabilisation of the WD and differentiation of the WD into distinct subsections. RAR = Retinoic acid receptor.

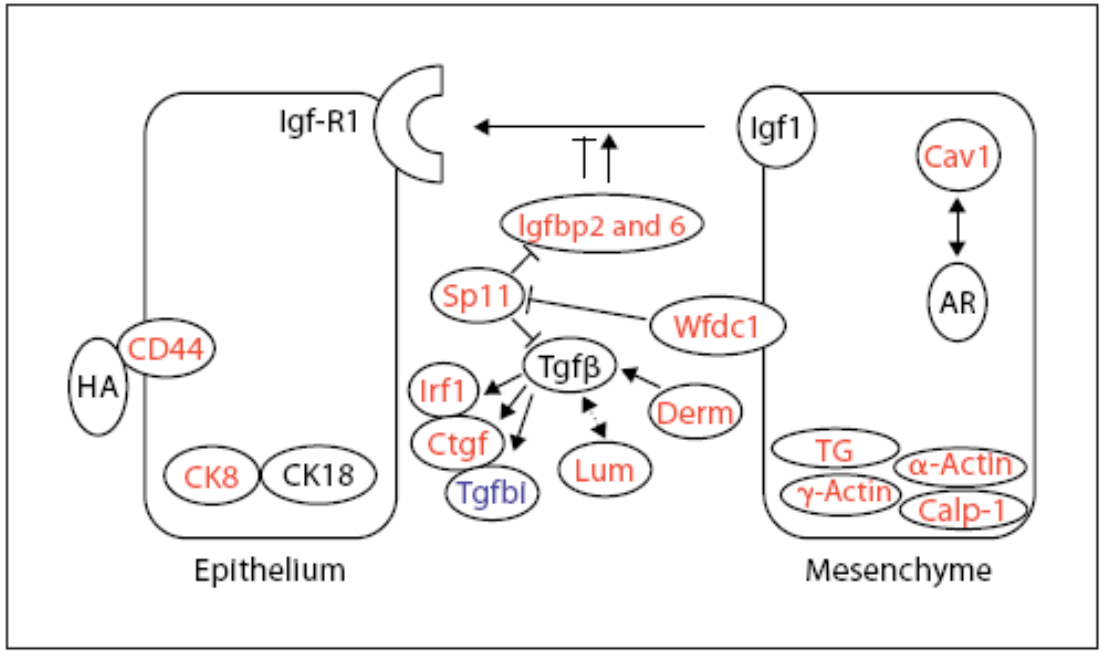
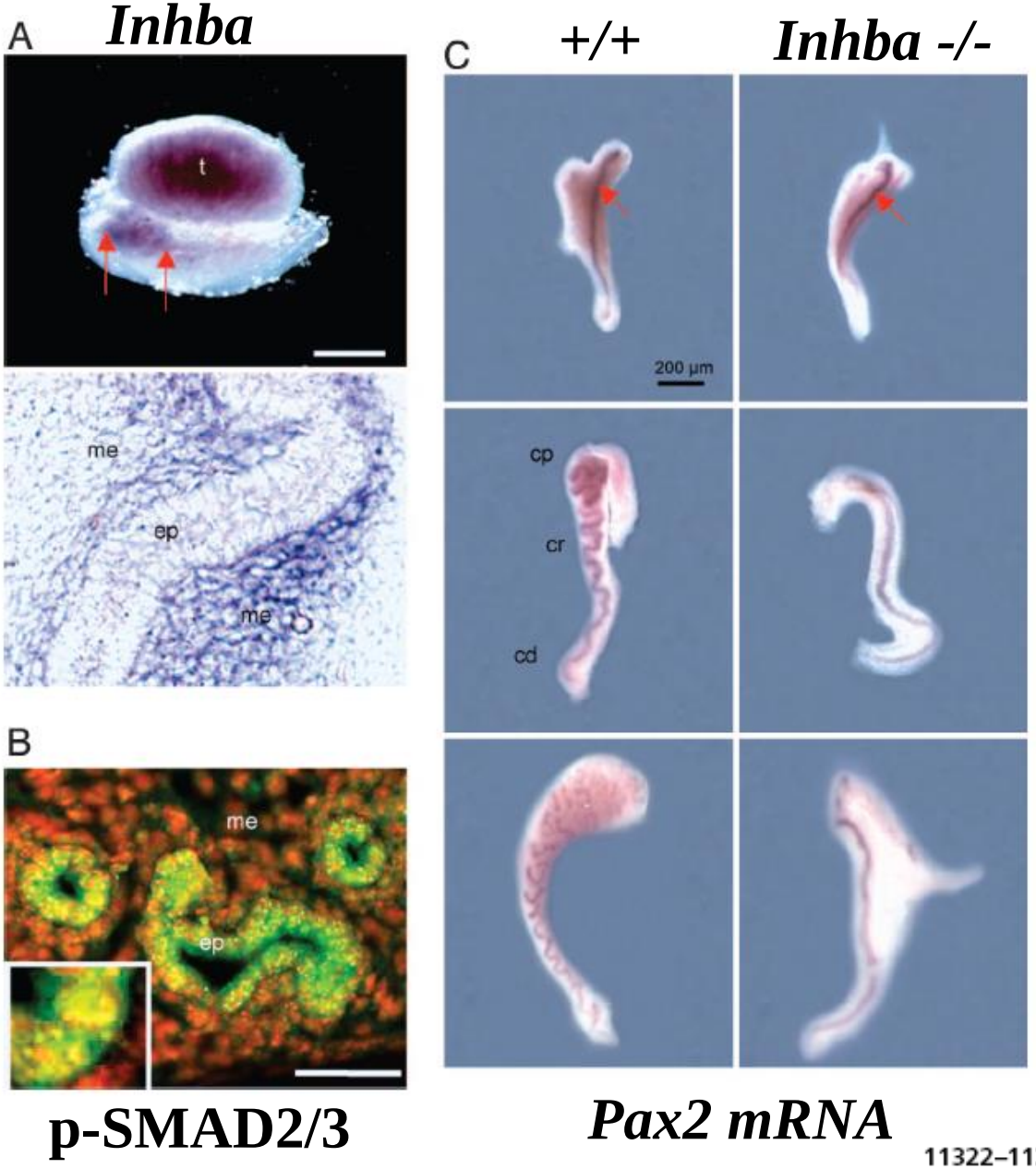


Fig. 4. Model of the interaction of several genes in the developing WD, previously shown to be upregulated (red) and downregulated (blue) between E17.5 and E20.5 [39]. Calp1 = Calponin-1; Cav1 = caveolin-1; CK8 = cytokeratin-8; Ctgf = connective tissue growth factor; Derm = dermatopontin; HA = hyaluronan; Irf1 = interferon regulatory factor-1; Lum = lumican; Sp11 = serine protease 11; TG = transgelin; Tgfbi = Tgfβ-induced; Wfdc1 = WAP four disulfide core domain-1.

Inhba Regulates Epididymal Coiling



Tomaszweski et al.,
PNAS 2007; 104:11322

Fig. 1. Functional roles of *Inhba* in mouse epididymal coiling. (*A*) Expression of *Inhba* mRNA in whole-mount (*Upper*) and sectioned (*Lower*) mesonephroi at E15.5. Positive staining (red arrows) appears as dark purple. Anterior of the embryo is on the left. (*B*) Immunocytochemistry for phospho-SMAD2/3 (green) counterstained with nuclear marker DAPI (red) in the Wolffian duct at E15.5. (*Inset*) A higher magnification (×40) of a portion of the epithelium is shown to demonstrate the speckled nuclear staining of the phospho-SMAD2/3. (*C*) Whole-mount *in situ* hybridization for *Pax2*, an epithelial cell marker for the Wolffian duct, on mesonephroi without testes attached on wild-type (*Left*) and *Inhba−/−* (*Right*) embryos at E15.5 (*Top*), E17.5 (*Middle*), and E19.5 (*Bottom*). Specific staining appears as dark purple (red arrows and magnification was ×4). cp, caput; cr, corpus; cd, cauda; ep, epithelium; me, mesenchyme; t, testis. (Scale bars: 500 μm, *A*; 100 μm, *B*.)

Prostate Development

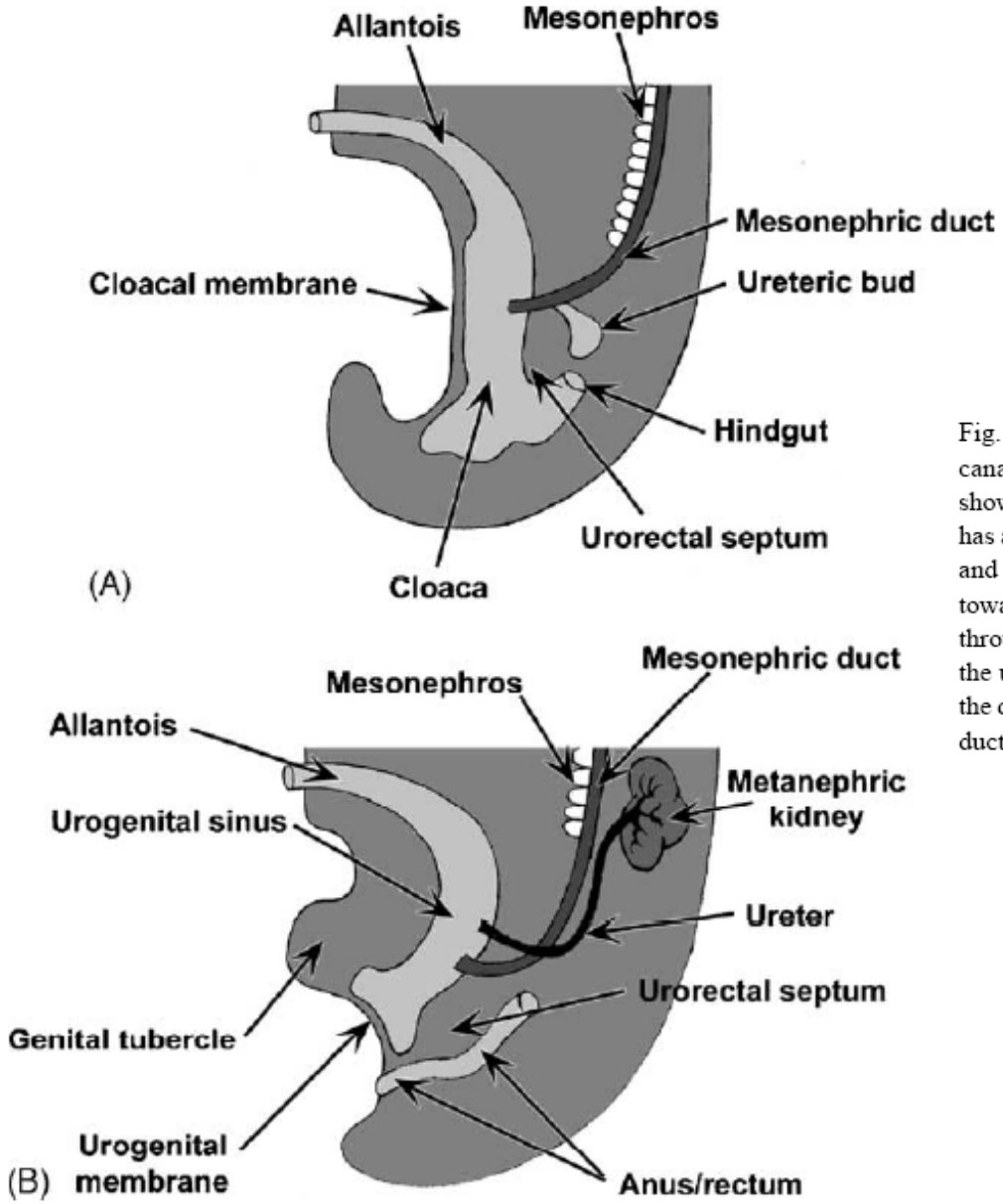
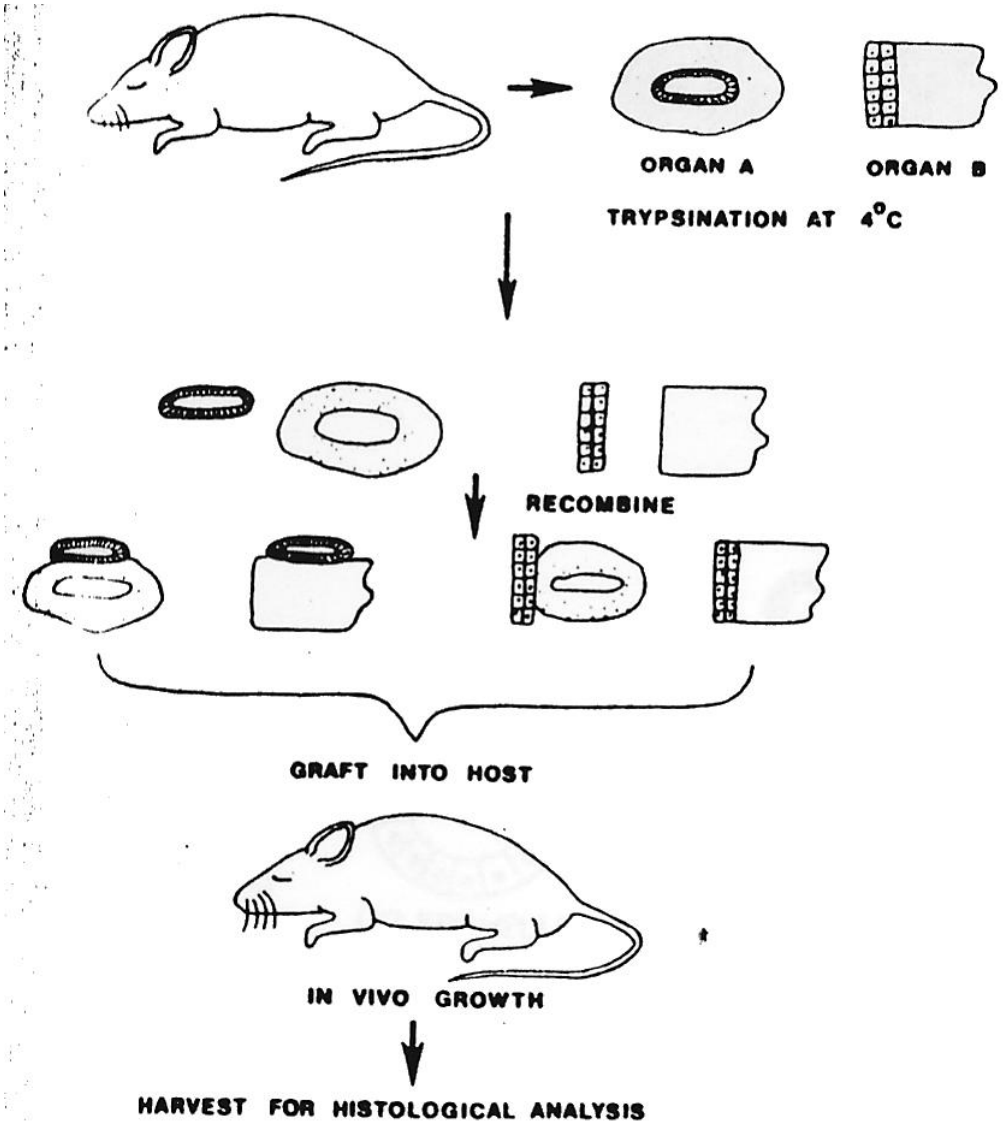


Fig. 1. Division of the cloaca into the urogenital sinus, rectum and anal canal. (A) Mid-sagittal view through the pelvis of a 4 week human fetus showing the cloaca, the blind caudal terminus of the hindgut. The cloaca has a ventral diverticulum, the allantois, extending up the anterior body wall and terminating in the umbilical cord. The urorectal septum grows caudally towards cloacal membrane to subdivide the cloaca. (B) Mid-sagittal view through the pelvis of a 7 week human fetus showing division of the cloaca into the urogenital sinus ventrally and rectum and anal canal dorsally. Note that the division of the cloaca has occurred in a manner in which the mesonephric duct and ureter empty into the urogenital sinus.

Urogenital mesenchyme express AR

G.R. Cunha et al. / Journal of Steroid Biochemistry & Molecular Biology 92 (2004) 221–236

Epithelial-Mesenchymal Interactions



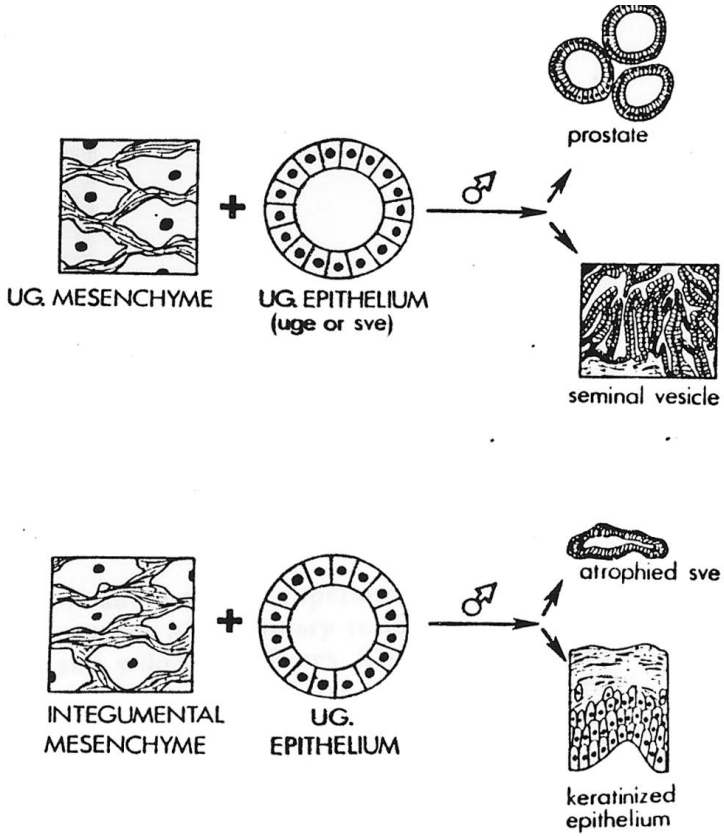


FIG. 2. When epithelium of the urogenital sinus (UGE) is associated with integumental mesenchyme and grown in intact adult male hosts, the UGE differentiates as a stratified squamous vagina-like epithelium, instead of expressing androgen-induced prostatic morphogenesis. Likewise, epithelium of the seminal vesicle (SVE) forms narrow tubules of atrophied epithelium when grown in association with integumental mesenchyme. Conversely, if these urogenital epithelia (UGE or SVE) are associated with urogenital mesenchyme and grown under identical conditions, typical androgen-induced glandular morphogenesis proceeds and after 4 weeks of growth, secretory activity is expressed.

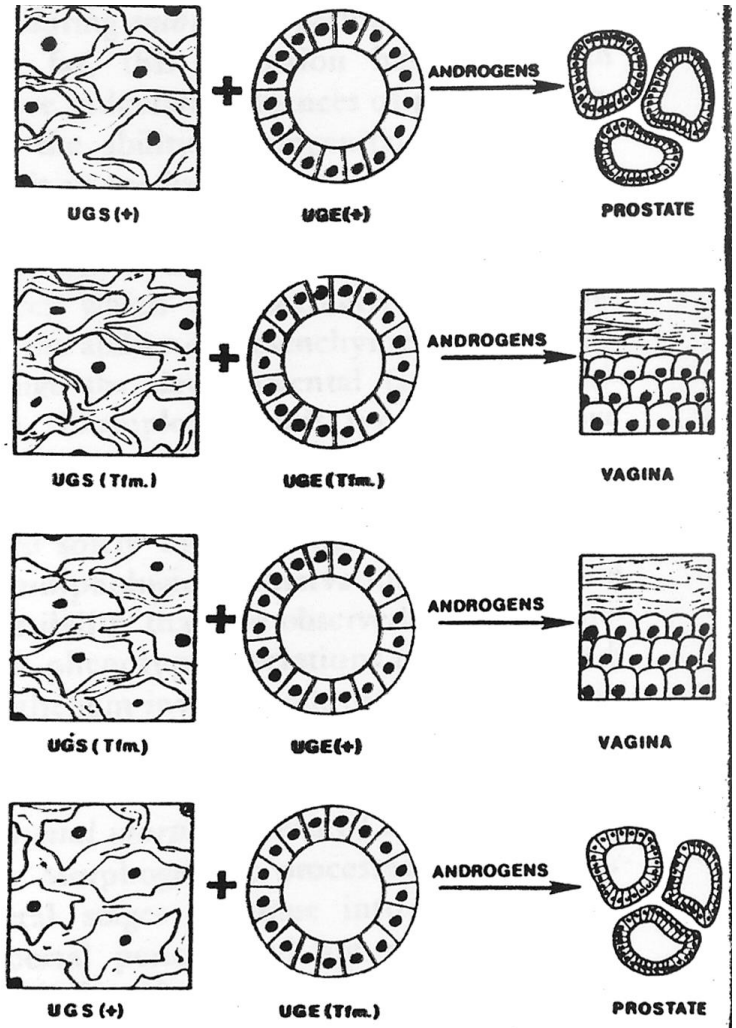


FIG. 3. A summary of recombination experiments between urogenital sinus components from Tfm/Y and wild-type embryos. A positive androgenic response (prostatic morphogenesis) occurs when wild-type mesenchyme is grown in association with either wild-type or Tfm/Y epithelium. Conversely, vagina-like differentiation occurs when either wild-type or Tfm/Y epithelium is grown in association with Tfm/Y mesenchyme in male hosts.

TABLE 2. Mesenchyme (stroma)-induced alteration of epithelial differentiation in accessory sexual structures.

Mesenchymal inductor	Responding epithelium	Epithelial response	Reference
Uterus (neonatal)	Vagina (neonatal)	Uterine	Cunha (1976b)
Vagina (neonatal and adult)	Uterus	Vaginal	[illegible]
Cervix (neonatal and adult)	Uterus (neonatal)	Cervical	Cunha and Lung (1979)
Seminal vesicle (embryonic)	Epidermis (embryonic)	Glandular	Cunha (1972a)
Seminal vesicle (neonatal)	Bladder (neonatal and adult)	Glandular	Fujii and Cunha (unpublished)
Urogenital sinus (embryonic)	Bladder (neonatal and adult)	Prostatic	Cunha and Lung (1978) Cunha et al. (1980)
Bulbourethral gland (neonatal)	Bladder (neonatal)	Bulbourethral gland	Fujii and Cunha (unpublished)
Urogenital sinus (embryonic)	Vagina (neonatal and adult)	Prostatic	Cunha (1975b)
Urogenital sinus (embryonic)	Skin (embryonic)	Glandular	Cunha (1972a)
Mammary (embryonic and adult)	Skin, salivary gland, lung (embryonic)	Glandular	Kratochwil (1969) Propper and Gomot (1977) Sakakura et al. (1979a)
Chick oviduct (embryonic)	Chick skin (embryonic)	Glandular	Moscona (1961)

***Tfm* = AR negative**

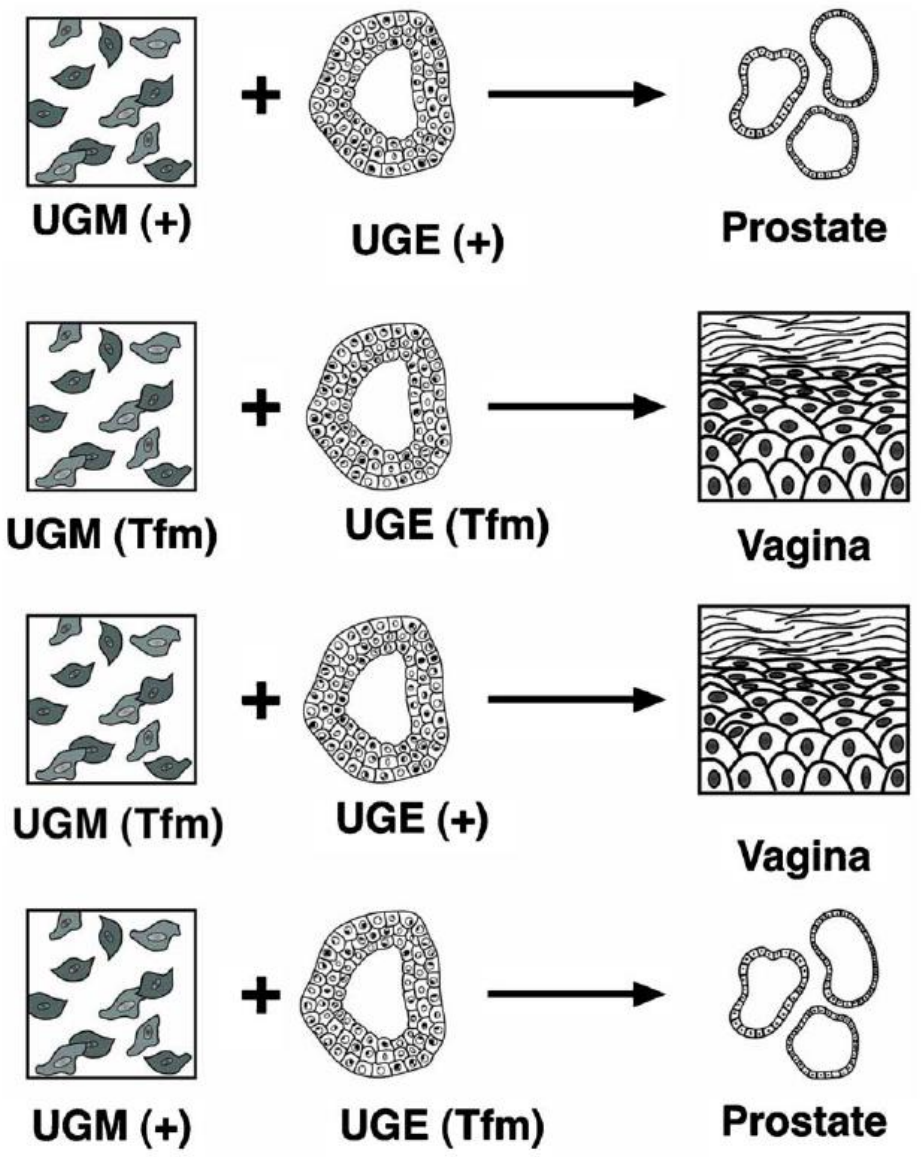


Fig. 2. Summary of tissue recombination experiments between urogenital sinus mesenchyme and epithelium from Tfm and wild-type embryos. A positive androgenic response (prostatic morphogenesis) occurs when wild-type mesenchyme is grown in association with either wild-type or Tfm epithelium. Conversely, vagina-like differentiation occurs when either wild-type or Tfm epithelium is grown in association with Tfm mesenchyme. These results demonstrate that androgens elicit many of their effects on epithelial development via mesenchymal androgen receptors (redrawn from [132]).

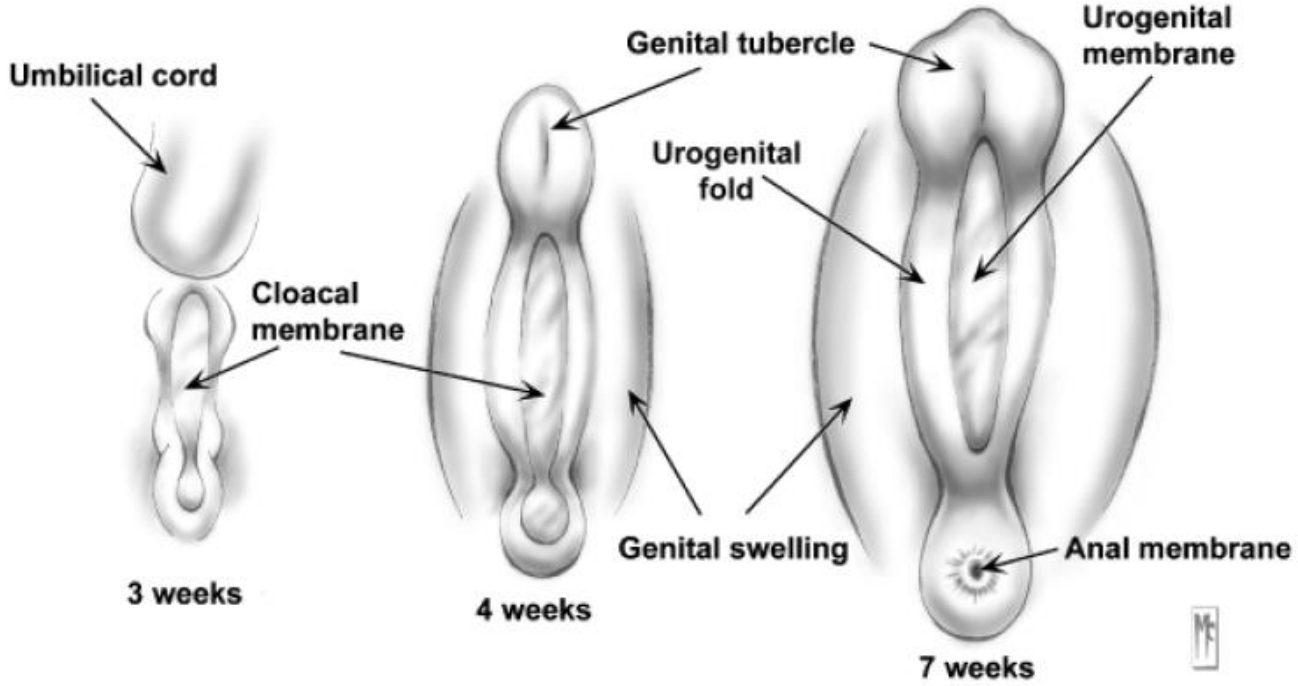


Fig. 2 Detail of the cloacal membrane, its division, and the development of the genital tubercle and genital swellings. At 3 weeks, the cloacal membrane extends to the base of the umbilicus. Mesenchymal cells migrate toward the midline to elicit "retraction" of the cloacal membrane. At 4 weeks, division of the cloacal membrane into the urogenital and anal membranes is nearly complete, and the genital tubercle and genital swellings are recognizable. At 7 weeks, the cloacal membrane is divided by the urorectal septum into the urogenital and anal membranes. The genital tubercle and genital swelling have formed, and the urogenital membrane is demarcated laterally by the urogenital folds.

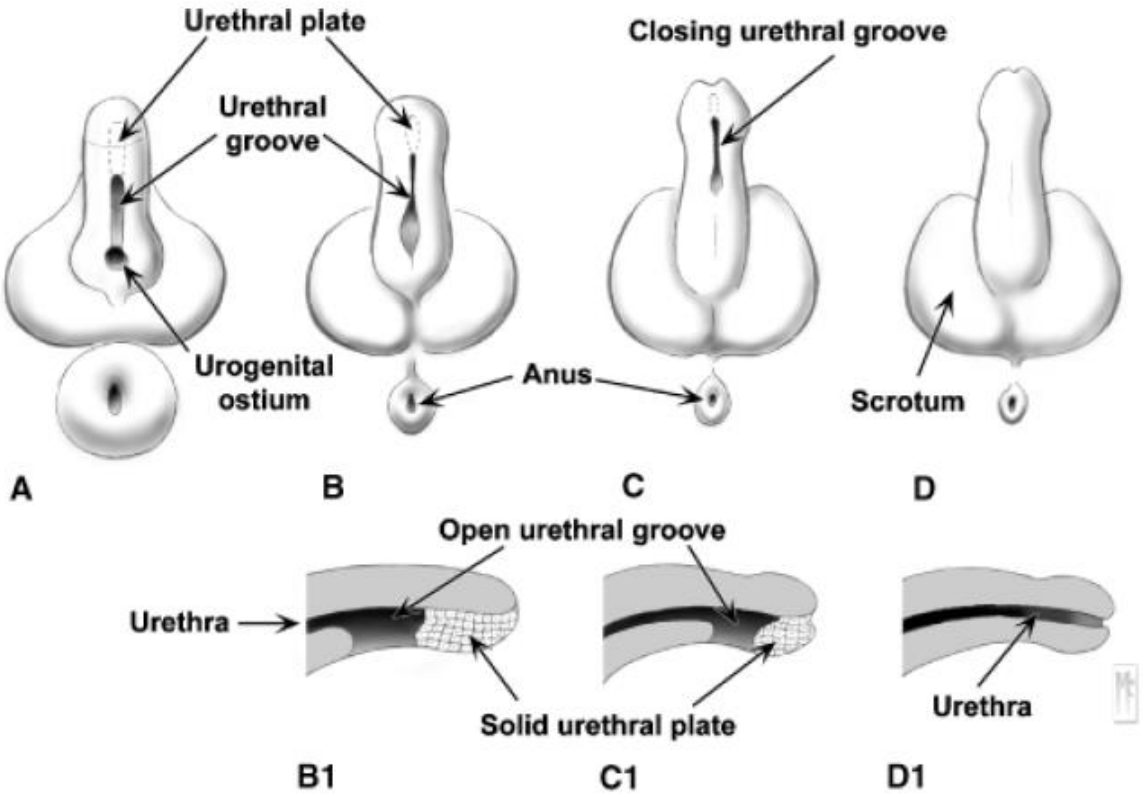


Fig. 4 Detail of the urethral groove, urethral folds, and urethral plate. (**A**) At the beginning of the ambisexual stage, the urethral groove bounded by the urethral folds extends about halfway distally along the ventral aspect of the elongating genital tubercle. At the distal aspect of the urethral groove is the solid urethral plate (dotted lines) that extends to the glans of the developing penis. The urethral groove extends distally to the glans by canalization of the urethral plate (**B**,**C**). The urethral folds grow to the midline where they fuse to extend the penile urethra distally to the glans (**B**–**D**). B1, C1, and D1 are mid-sagittal sections showing canalization of the urethral plate and formation of the penile urethra.

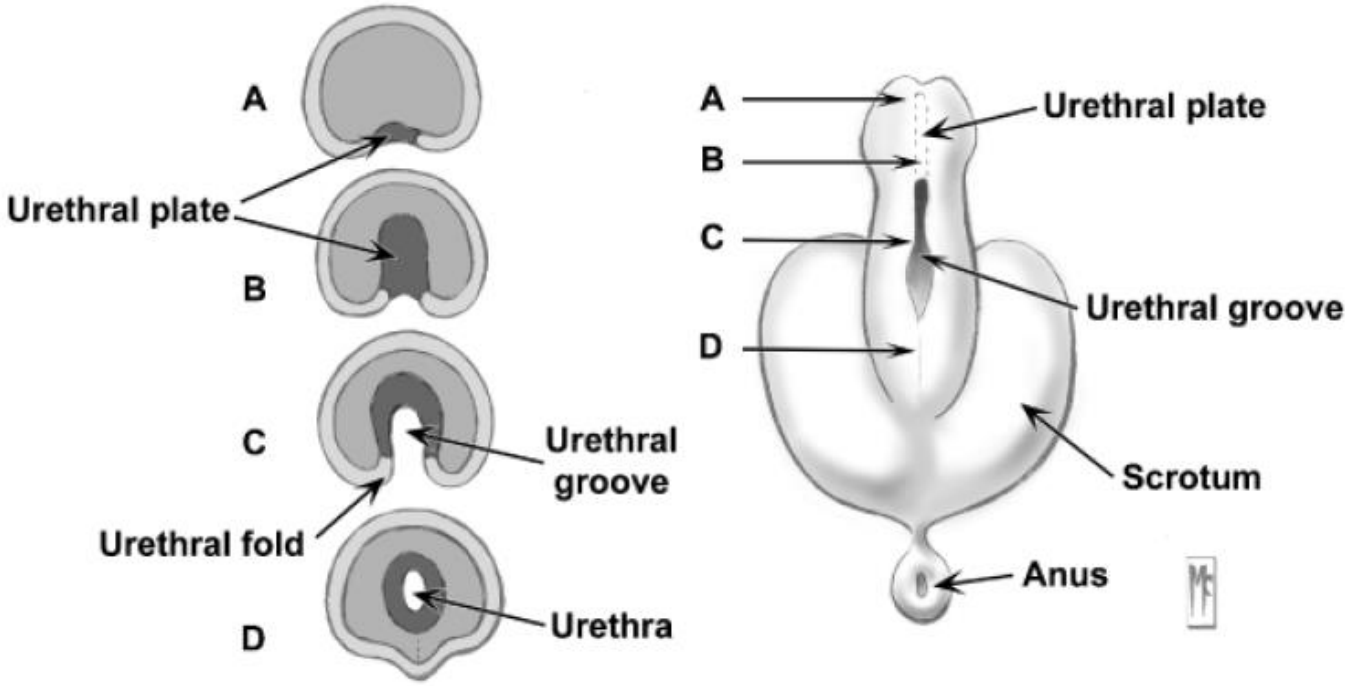


Fig. 5 Detail in transverse section of the urethral groove, urethral folds, and urethral plate. Transverse sections (**A**–**D**) are shown at the levels depicted (arrows). The solid urethral plate is present in the distal part of the phallus (**A**,**B**). Canalization of the solid urethral plate (**C**) forms the urethral groove bounded by urethral folds, which grow to the midline and fuse to form the tubular urethra (**D**). After fusion of the urethral folds, the resultant epithelial seam is removed (**D**).

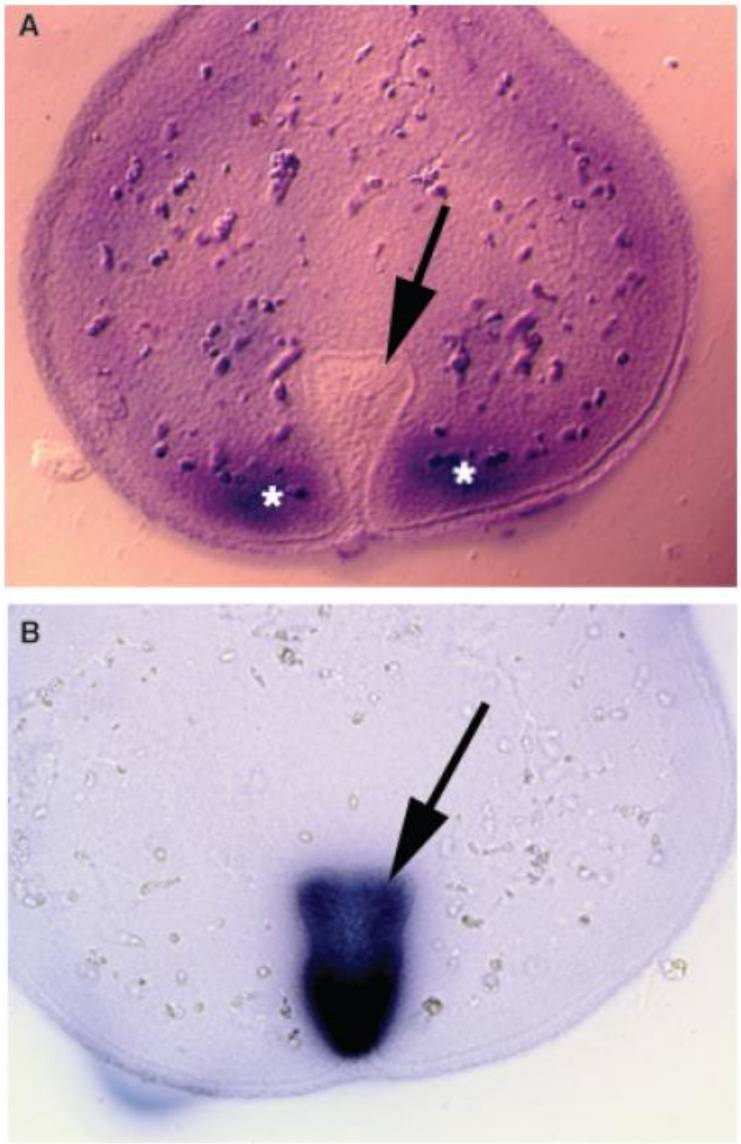


Fig. 11 Fgf10 (**A**) and Shh (**B**) transcript expression in the 13.5-day-old embryonic mouse GT. (**A**) The urethral plate (arrow) extends from the surface epithelium into the substance of the glans. *In situ* hybridization for Fgf10 transcripts (**A**) shows bilateral expression (*) in the mesenchyme surrounding the midline urethral plate. (**B**) *In situ* hybridization for Shh showing expression in the urethral plate epithelium. Arrows indicate the urethral plate.

Fgf10 and *Shh* Regulate Genital Tubercle Development

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