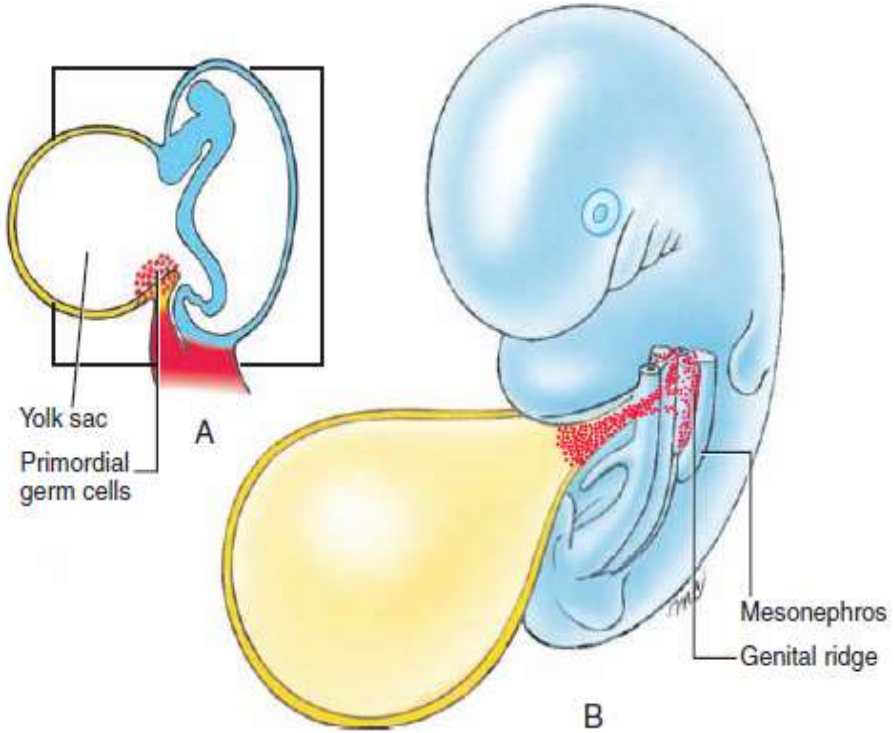

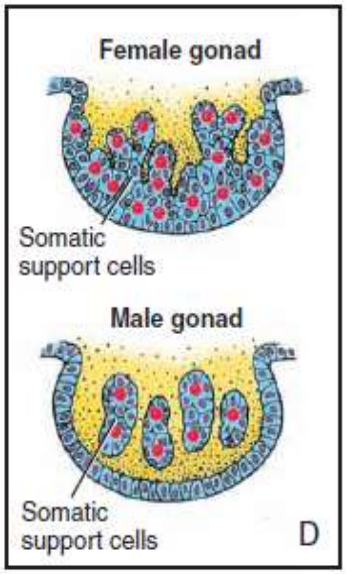

David Dora

Semmelweis University,
Department of Anatomy
2017

Developmental biology I.

Sex determination
Migration of germ cells
Development of gonads

Determination of sex

- **Formation and migration of PGCs**
- **Formation of gonad primordia, colonisation by PGCs**
- **Establisment of primary and secondary sexual characteristics**

I. Genetical sex, chromosomal sex

Genetic sex is determined during fertilization

In higher vertebrates the genetical sex determination dominates → homotherm animals

In heterotherm animals it can be temperature-dependent (TSD), or environmental-dependent SD, ESD)
E.G.: turtles, crocodiles

Sex determination: insights from the chicken

Craig A. Smith* and Andrew H. Sinclair

BioEssays 26:120–132, © 2004 Wiley Periodicals, Inc.

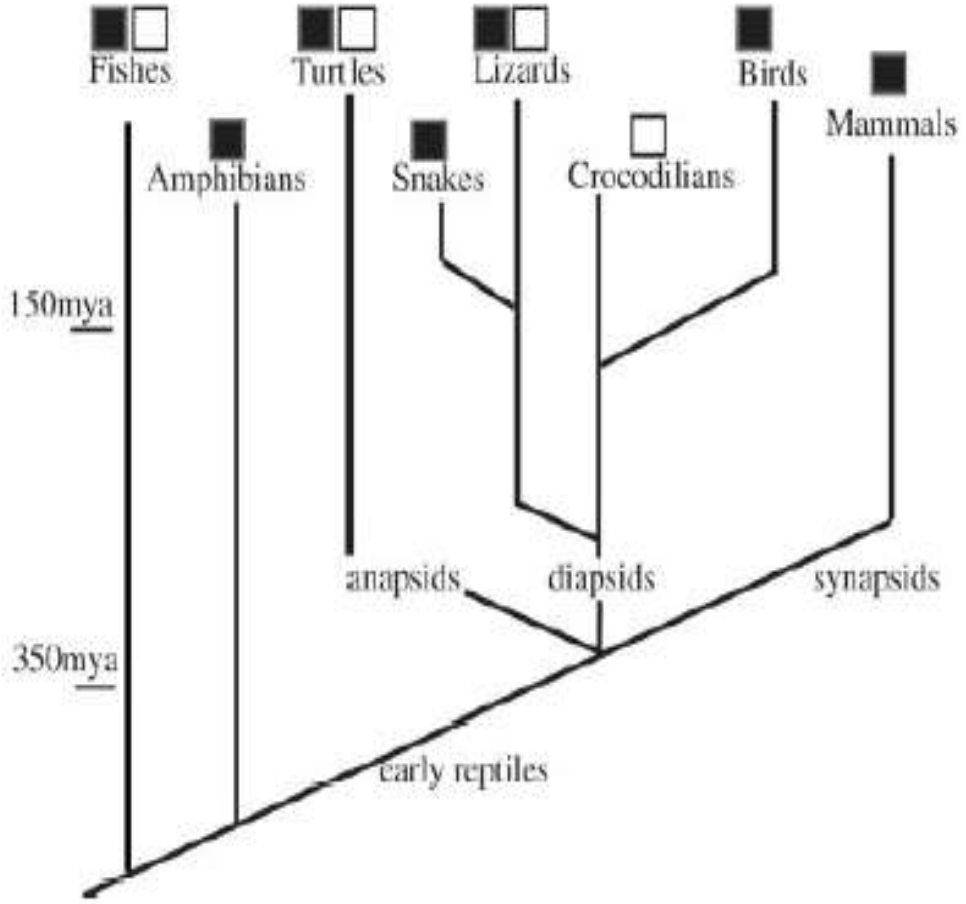

Figure 1. Phylogenetic distribution of sex-determining mechanisms among vertebrates. Mammals evolved from synapsid reptiles, while diapsid reptiles gave rise to the crocodilian/bird (archosaurian) and lizard/snake (lepidosaurian) lineages. Turtles are traditionally thought to have derived from a separate reptilian group, the anapsids, although recent molecular evidence suggests that turtles are close relatives of crocodilians. ■, Genetic sex determination; □, temperature-dependent sex determination.

The male sex depends on the presence of Y chromosome

<u>Gondadal sex</u>

→ **XY – testes develop**

→ **XX – ovaries develop**

The genotype will decide whether male (testis) or female gonads (ovaries) will develop.

→ When the gonadal and phenotypical sex differs → <u>pseudohermaphroditism</u>

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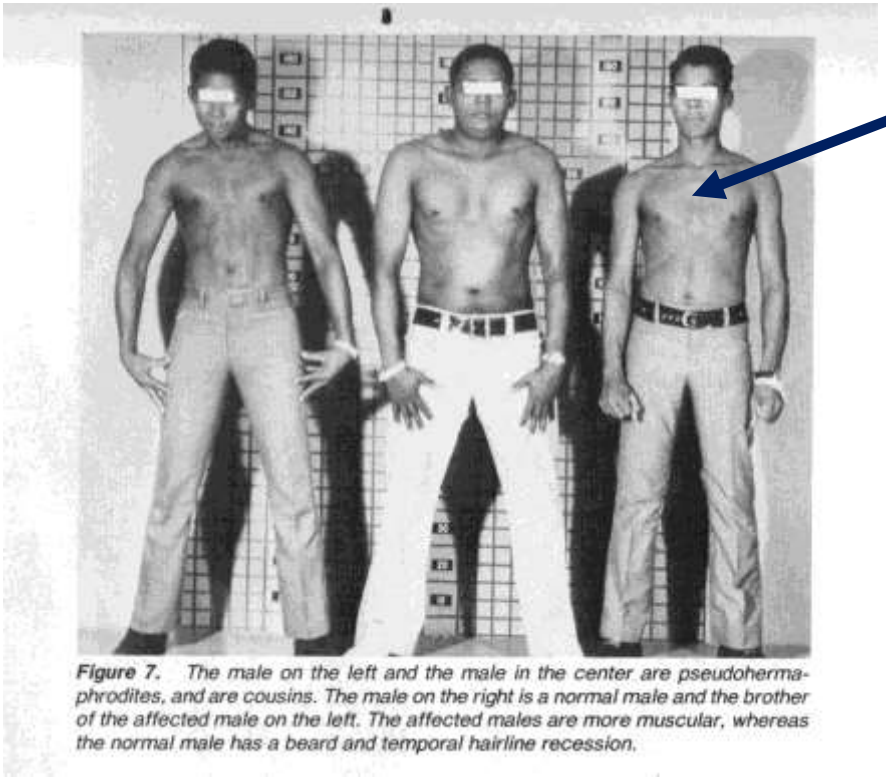


Figure 7. *The male on the left and the male in the center are pseudohermaphrodites, and are cousins. The male on the right is a normal male and the brother of the affected male on the left. The affected males are more muscular, whereas the normal male has a beard and temporal hairline recession.*

The only „normal" man

<u>Causes of male pseudohermaphroditism:</u>

-Development disorder of testes

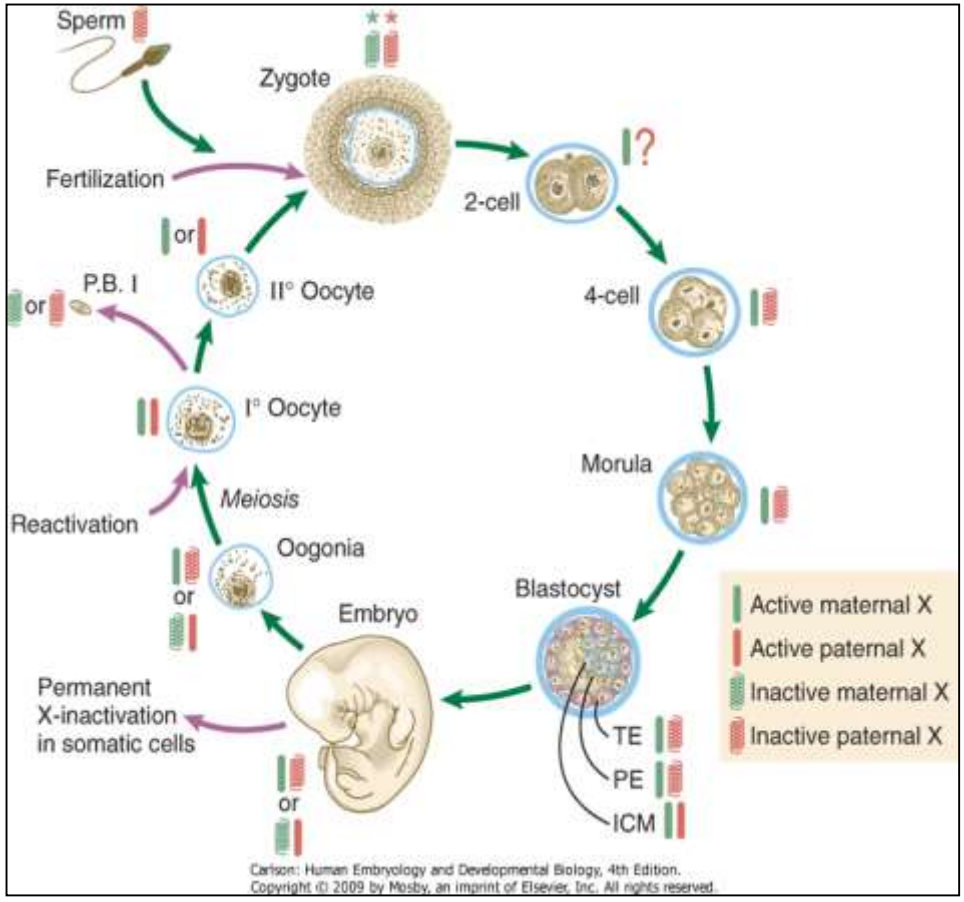
-5-alpha-reduktase deficiency → lack of testosteron

-Androgenic insensitivity (receptor defect) → Testicular feminisation

<u>Causes of female pseudohermaphroditism:</u>

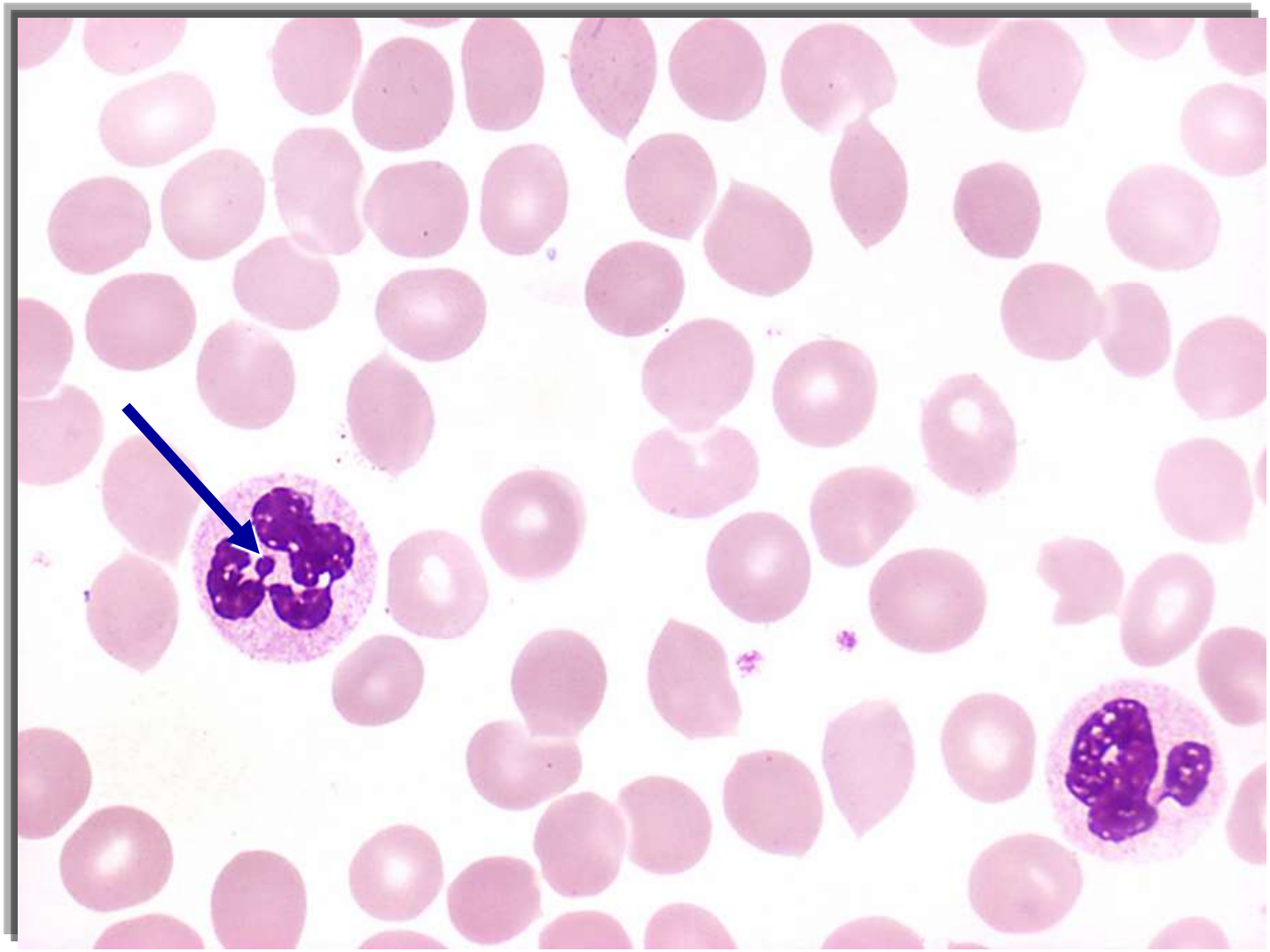
- Congenital adrogenital syndrome *Congenitális adrogenitalis hyperplasia (CAH)*
- Androgen producing tumor in the mother

Inactivation of X chromosome in females, „dosage compensation”

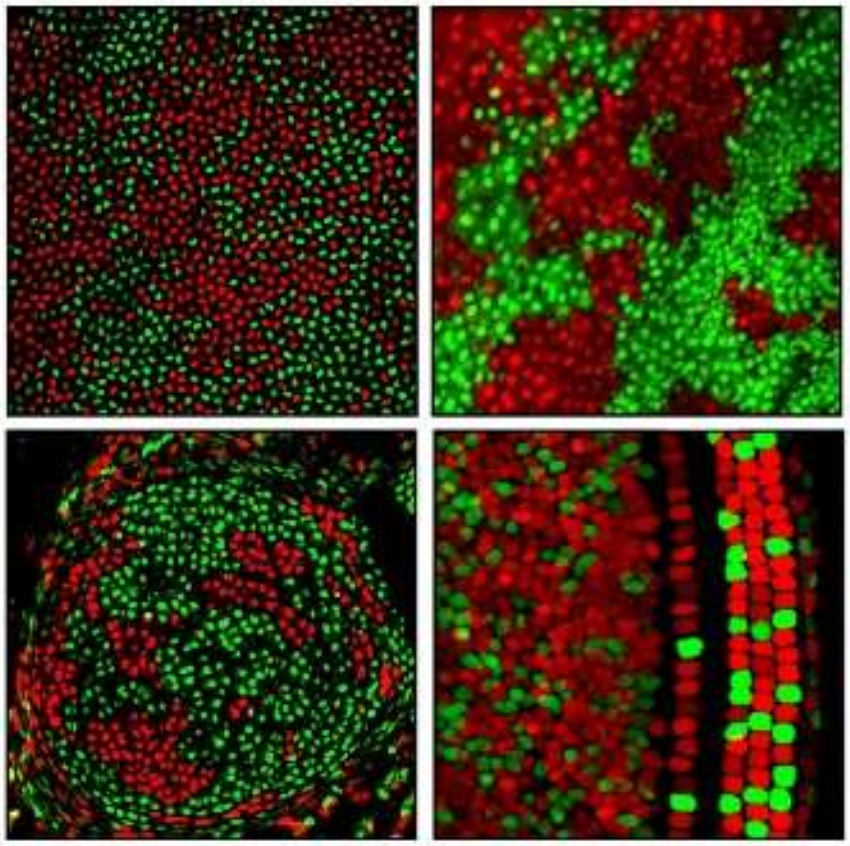


- Many genes on the X chromosome have nothing to do with sexual traits → they have no homologous area on the Y chromosome
- These genes would be present in „two doses” in females
- The solution is the inactivation of one X chromosome in somatic cells → X-Inactive Specific Transcript (XIST) gene is essential for inactivation
- The inactivated chromosome persists as a „Barr-body”
- Activating factor: RNF12
- Inhibitor: OCT4, SOX2, Nanog

Barr-body



Az X-inactivation is not uniformal in our somatic cells → mosaicism



Fluorescent X chromosomes in mouse
- paternal: red
- maternal: green

During early development every cell choose, which X chromose it will inactivate (maternal or paternal) → *undescribed random mechanism*

After the „choosing" the cell line derived from the progenitor cell will all express the same X chromosome and the genes localised in it.

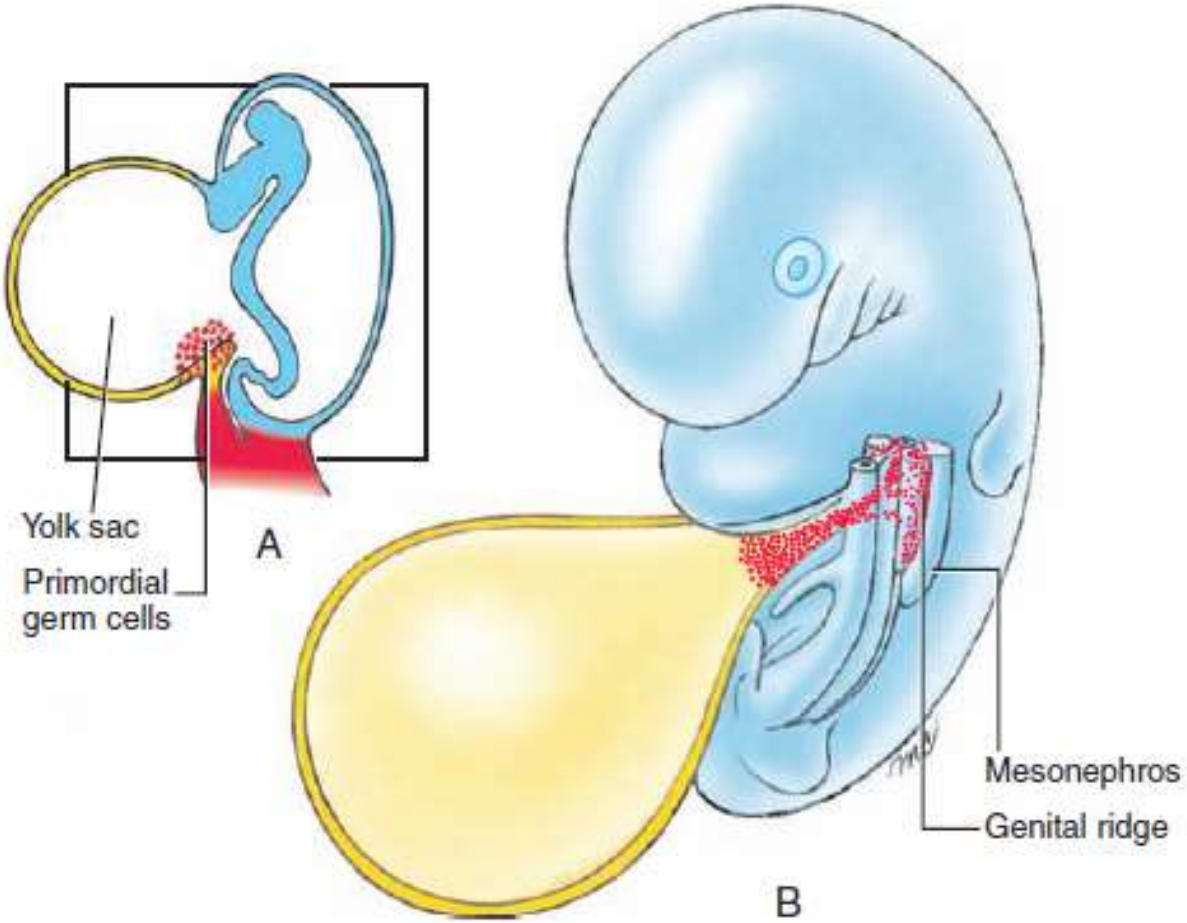
Neuron,
January 8, 2014

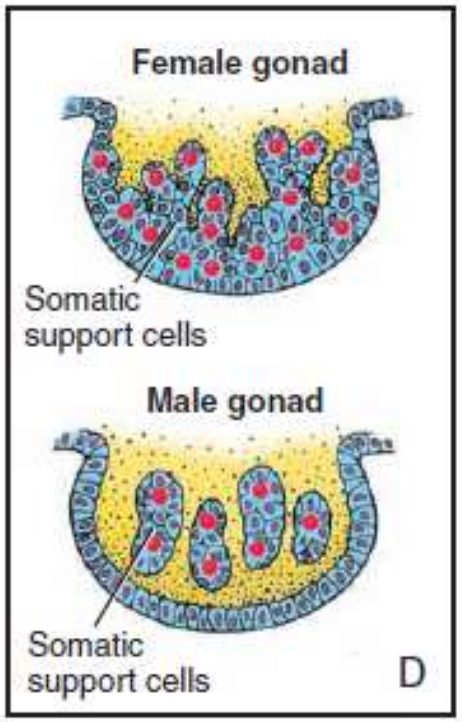
Genetical sex is decided at the moment of fertilization

The only morphological sign until the end of 7th gestational week:

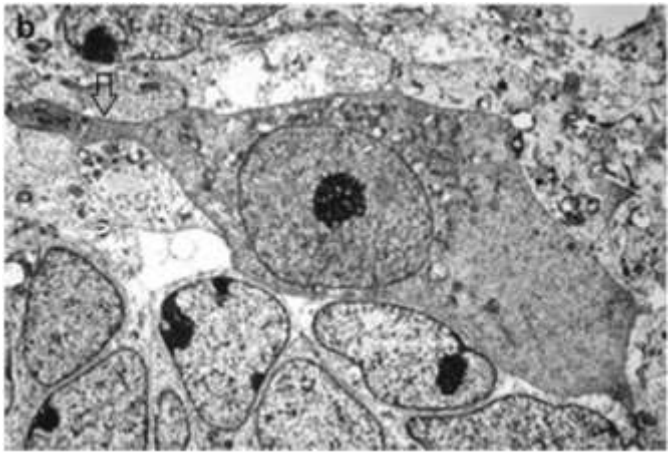
The presence of Barr-body

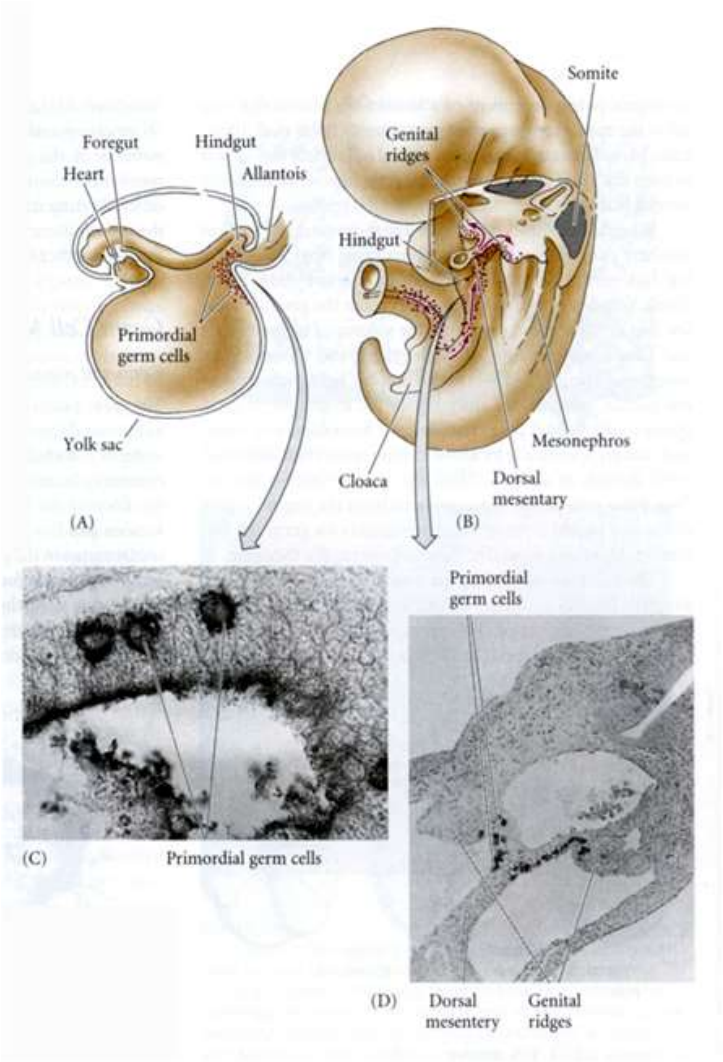
On the 6th week the PGCs residing in the wall of the yolk sac start to migrate to the dorsal mesogastrium and the dorsal body wall and will colonize the gonad primordia medially from the mesonephros (plica genitalis, genital ridge) at the level of the 10. thoracal segment. From the coeloma epithelium of the body wall will the somatic supporting cells develop from, that will assist their long maturation process





Primordial germ cells, PGCs

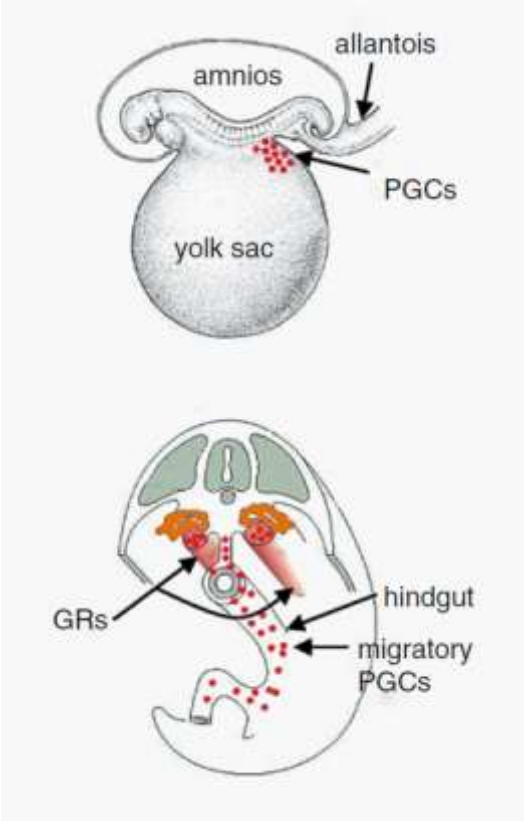




Migration of PGCs in mouse embryo.

PGCs express alkalic phosphatase

TNAP (tissue nonspecific alkaline phosphatase) - histochemistry



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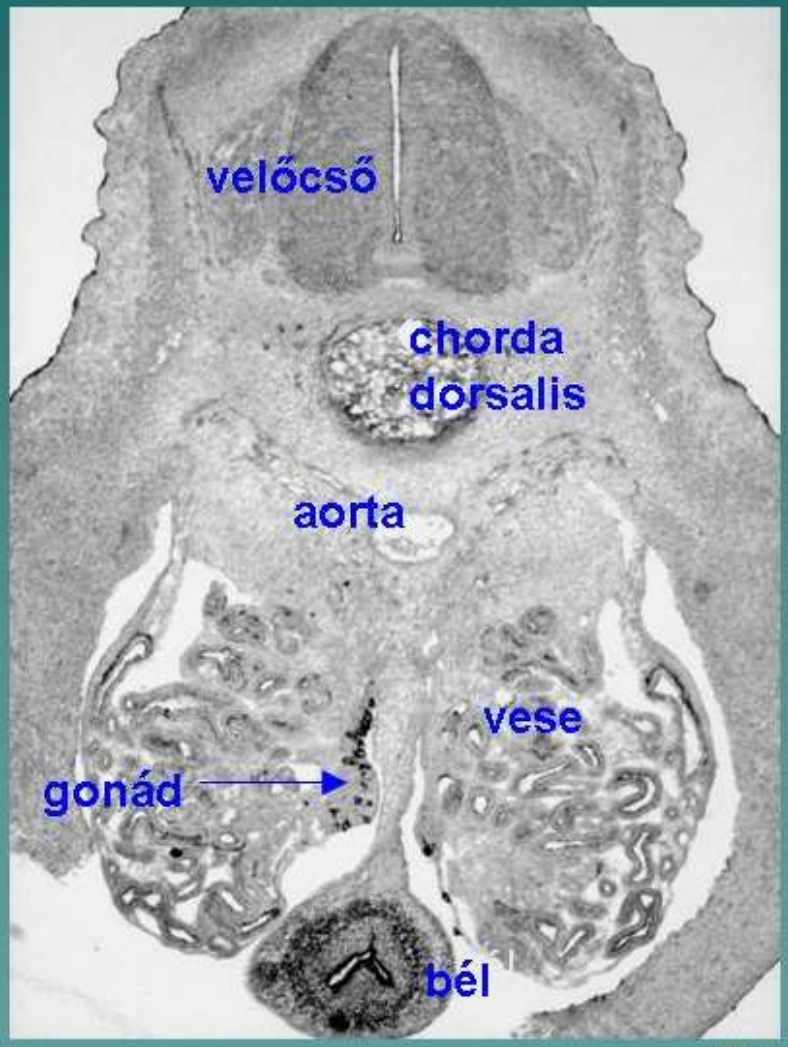
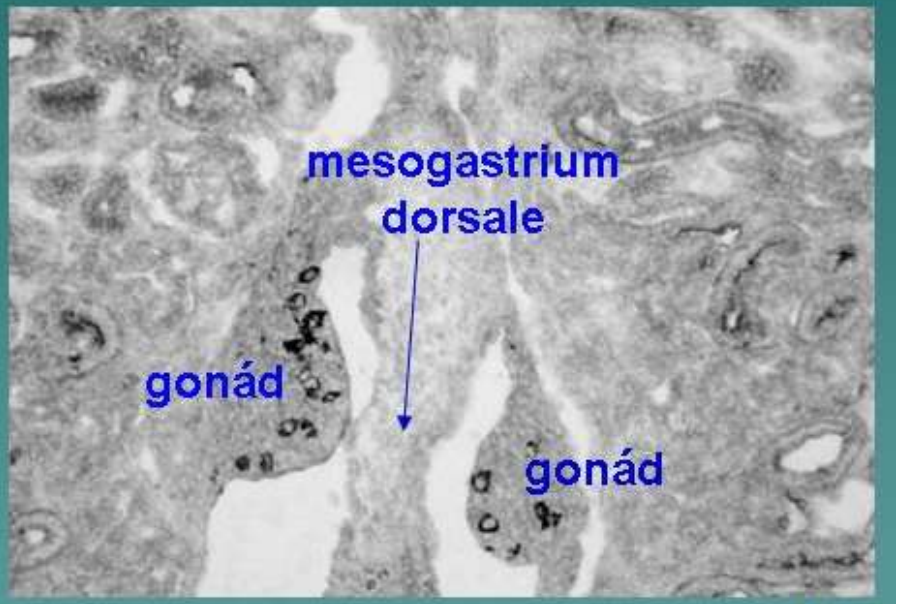
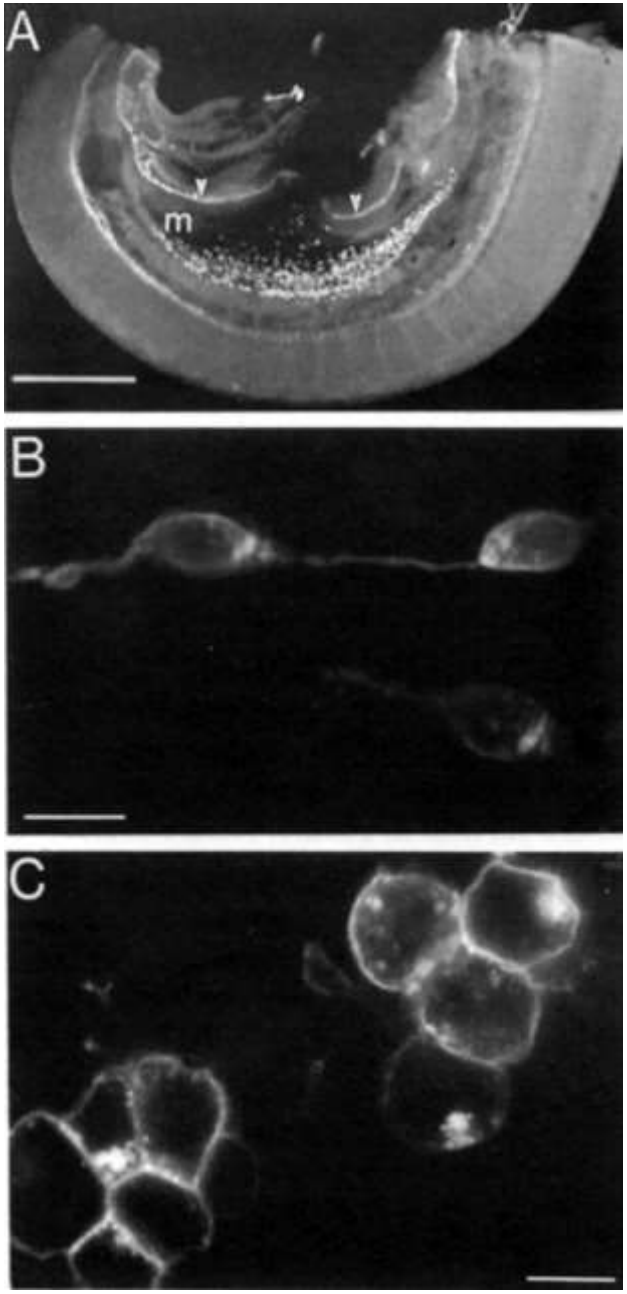

velőcső
chorda dorsalis
aorta
vese
gonád
bél

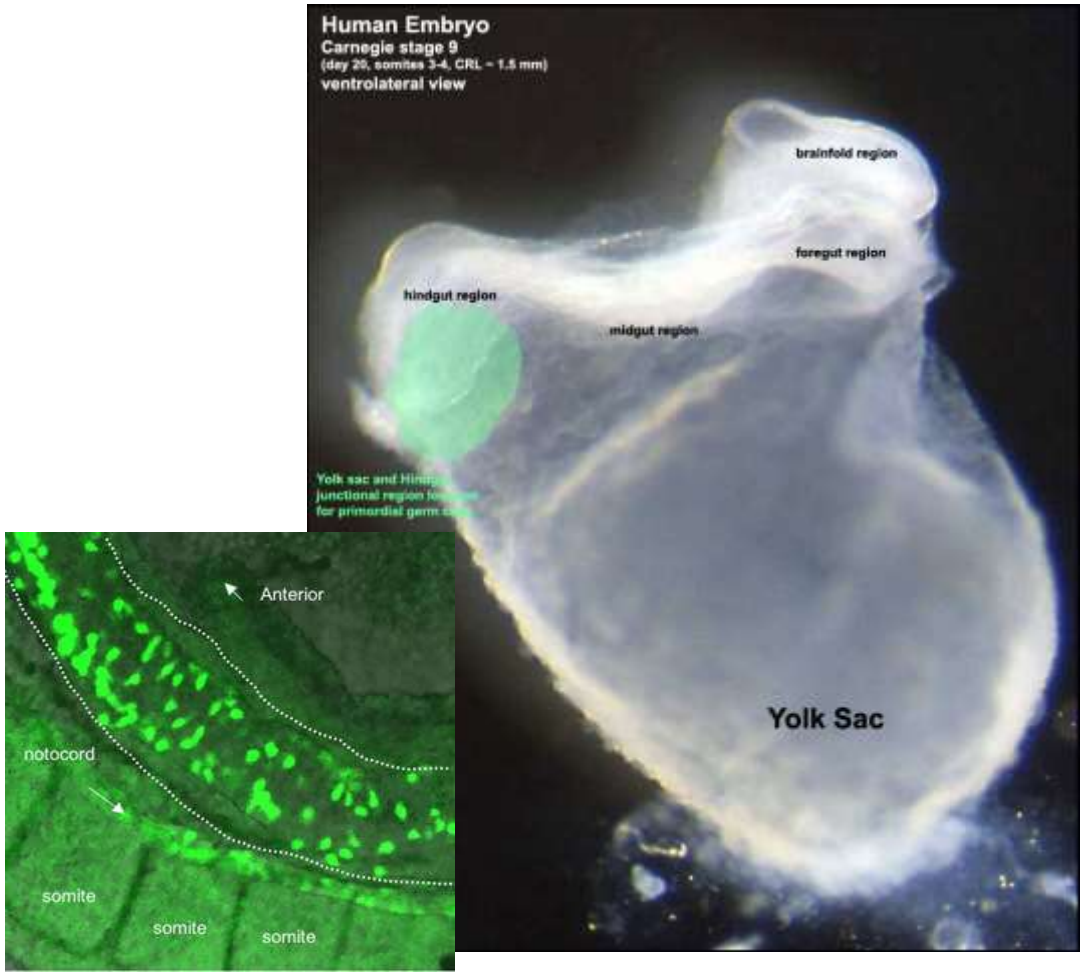
5x


mesogastrium dorsale
gonád
gonád

10x

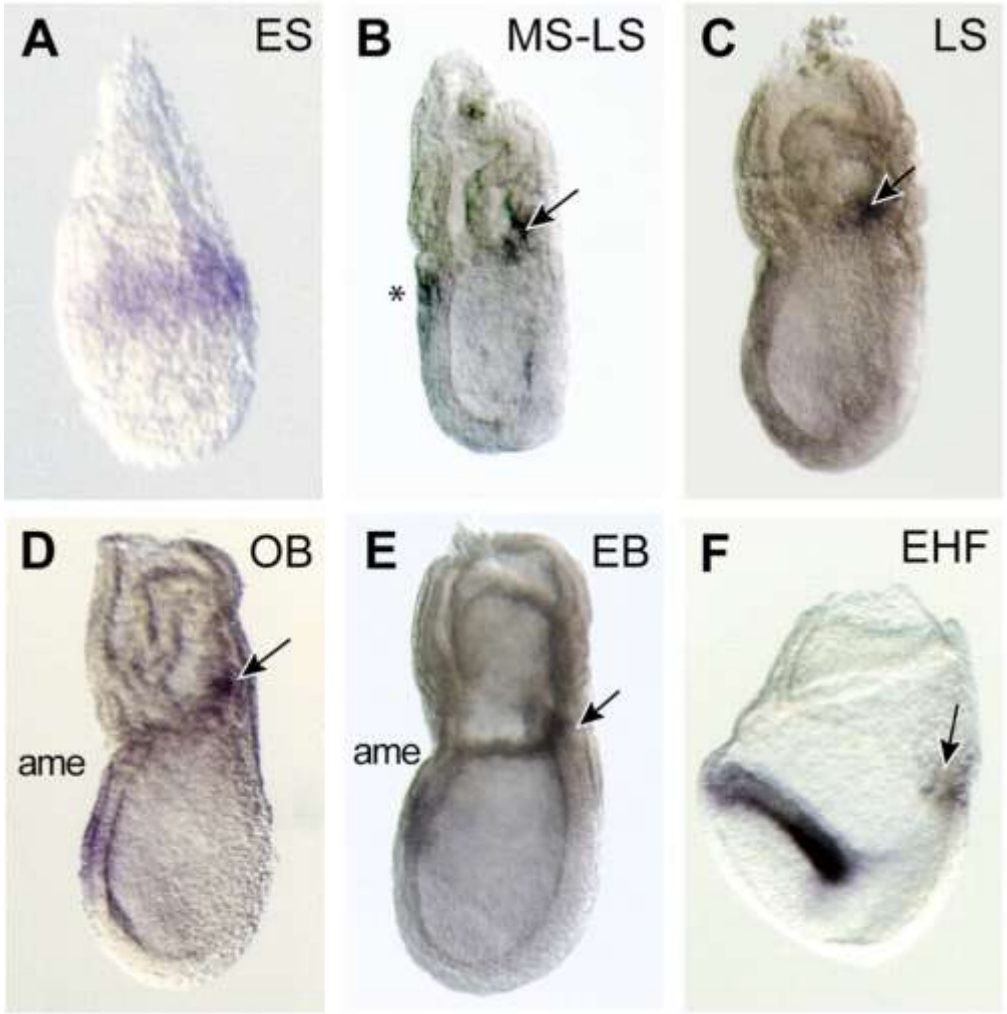


Migrating (A,B) and arrived (C) PGCs in mouse embryo (SSEA-1 IH)



http://embryology.med.unsw.edu.au/embryology/index.php?title=Primordial_Germ_Cell_Migration_Movie#Mouse_E9.0_Primordial_Germ_Cell_Migration

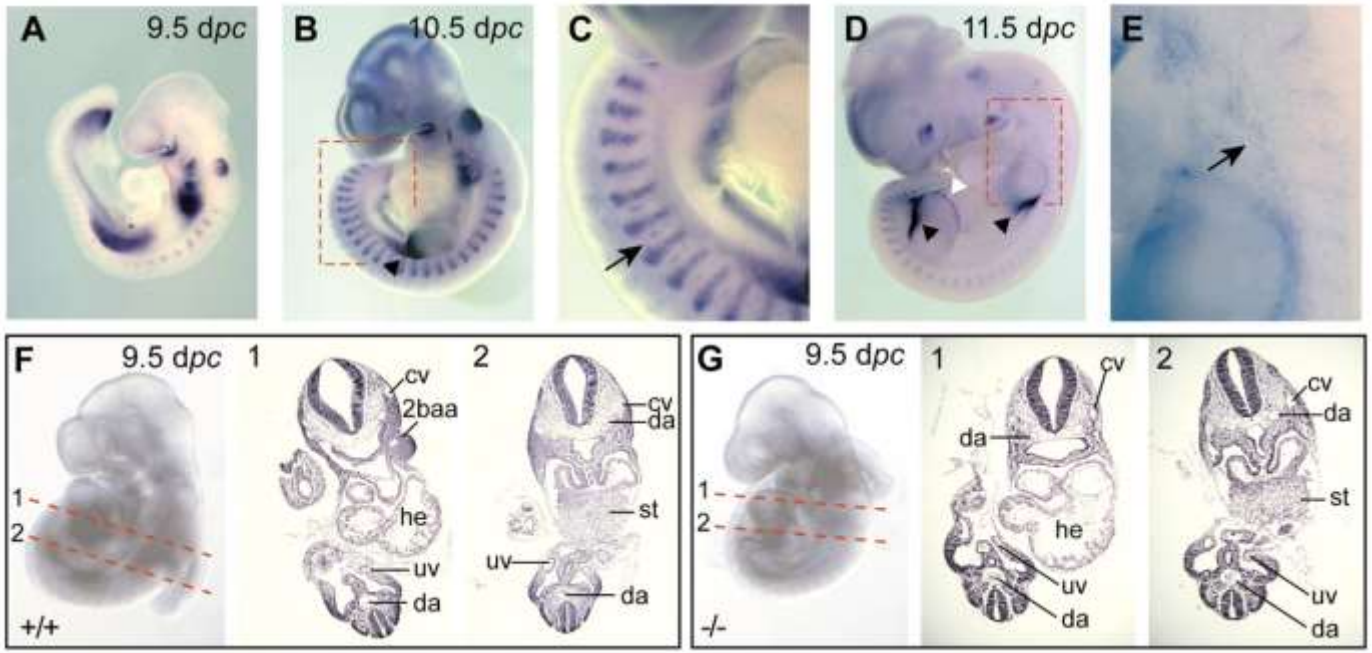
Expression of BLIMP1 in early mouse embryo



- Specific marker for PGC precursors
- Transcriptional repressor, product of PRDM1 gene
- In adult, it is a repressor of TGF-beta → promotes immunological response in viral infections → B-cell recruitement
- Important regulator protein in hematopoiesis
- Repressors of Hox genes, that is essential for PGC specification and differentiation

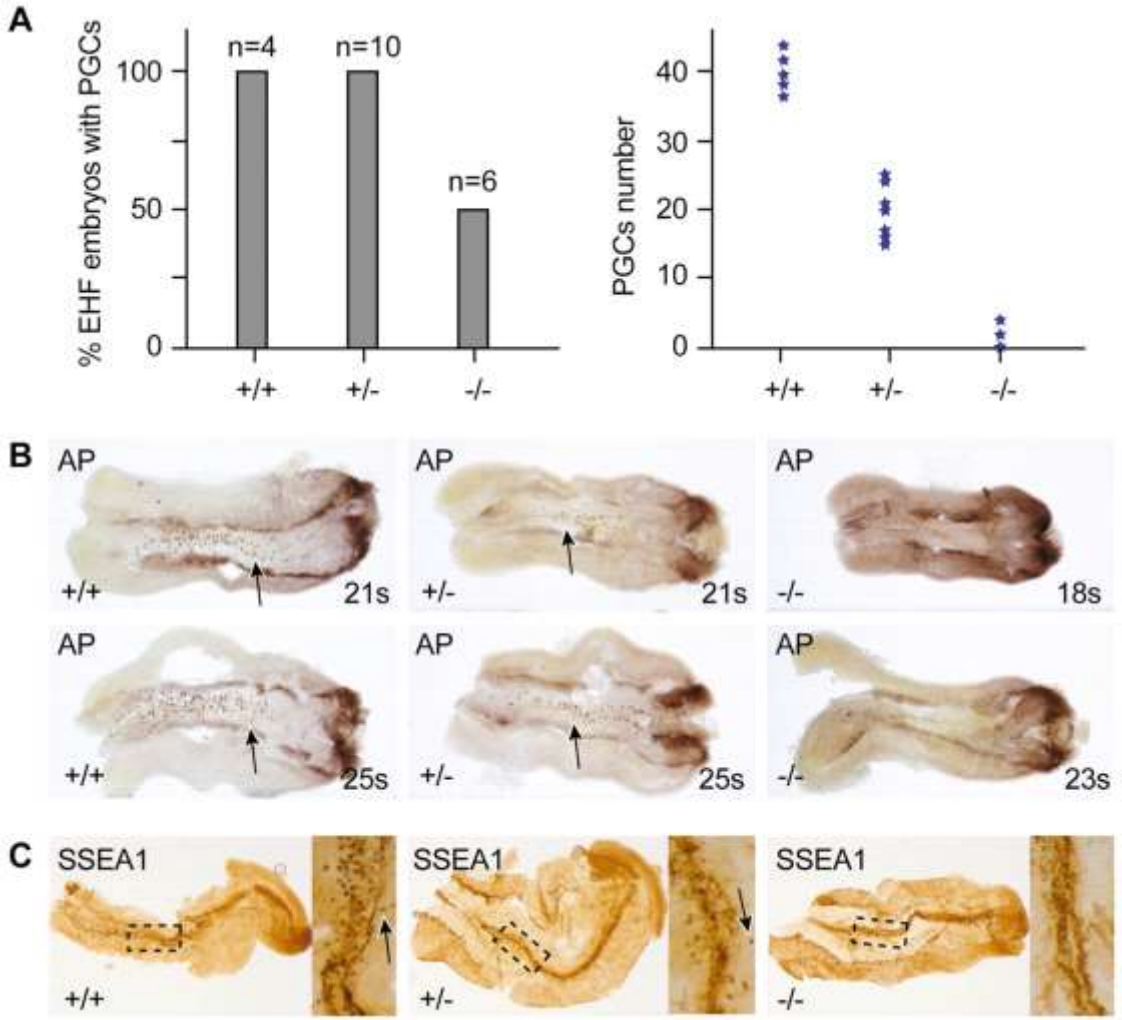
Vincent, S. D. et al. Development 2005;132:1315-1325

Morfogenesis and tissue patterning is intact in BLIMP-1 deficiency



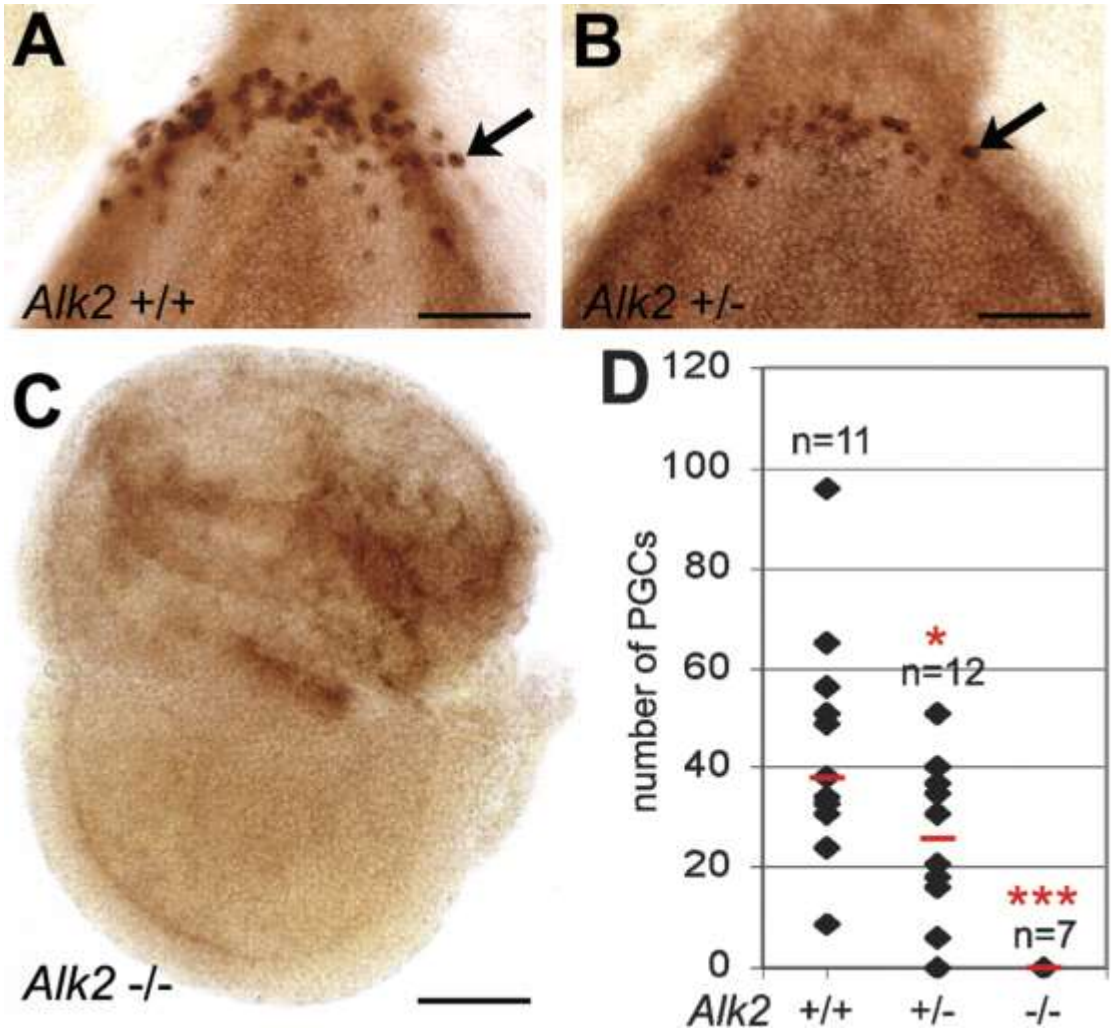
Vincent, S. D. et al. Development 2005;132:1315-1325

A Blimp 1 mutant mice's PGC development is impaired



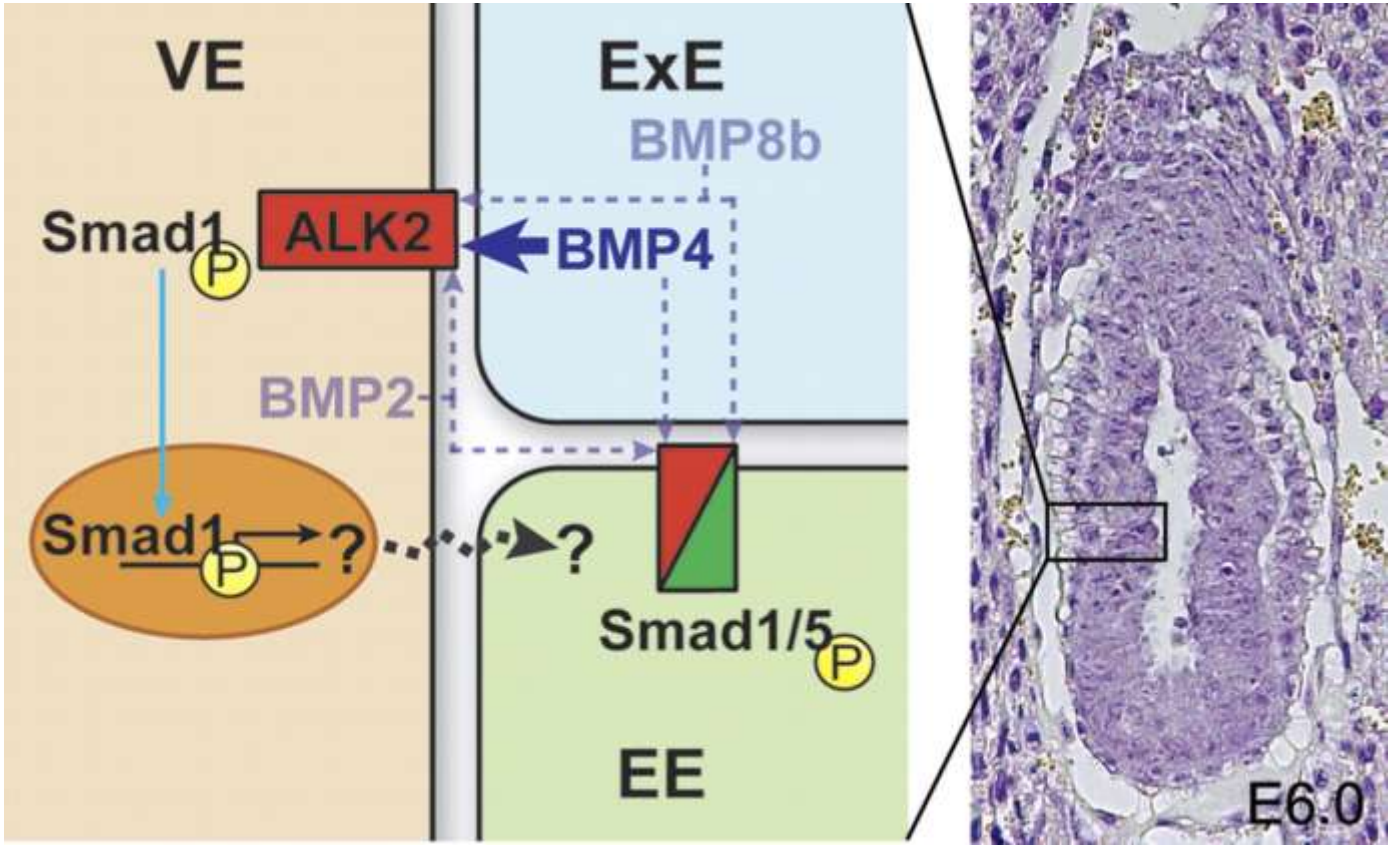
Vincent, S. D. et al. Development 2005;132:1315-1325

ALK2 is essential for PGC formation



activin receptor-**l**ike **k**inase-2

BMP signaling and PGC formation

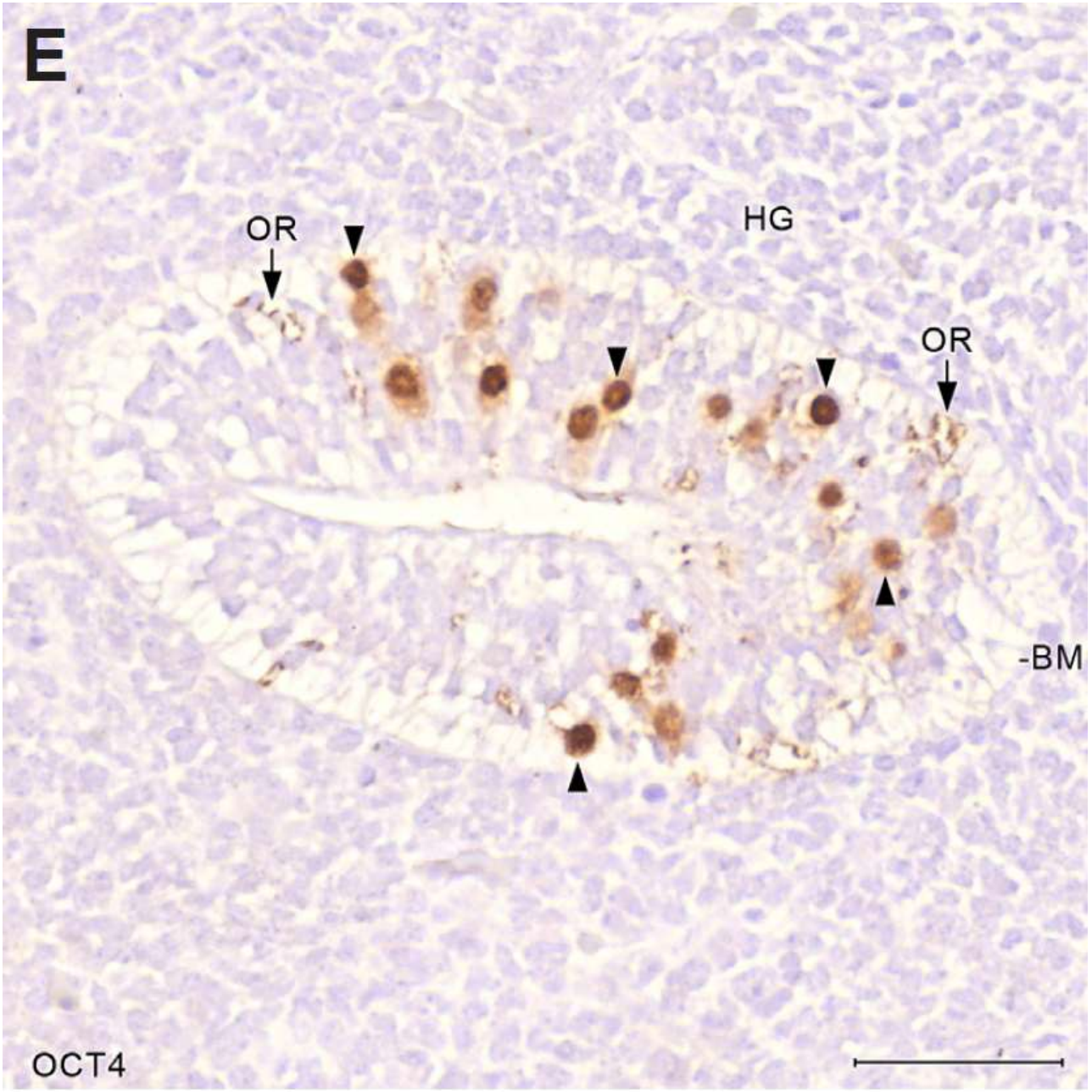


ExE → Extraembryonic ectoderm ALK2 → BMP receptor type I
VE → Visceral endoderm
EE → Proximal epiblast

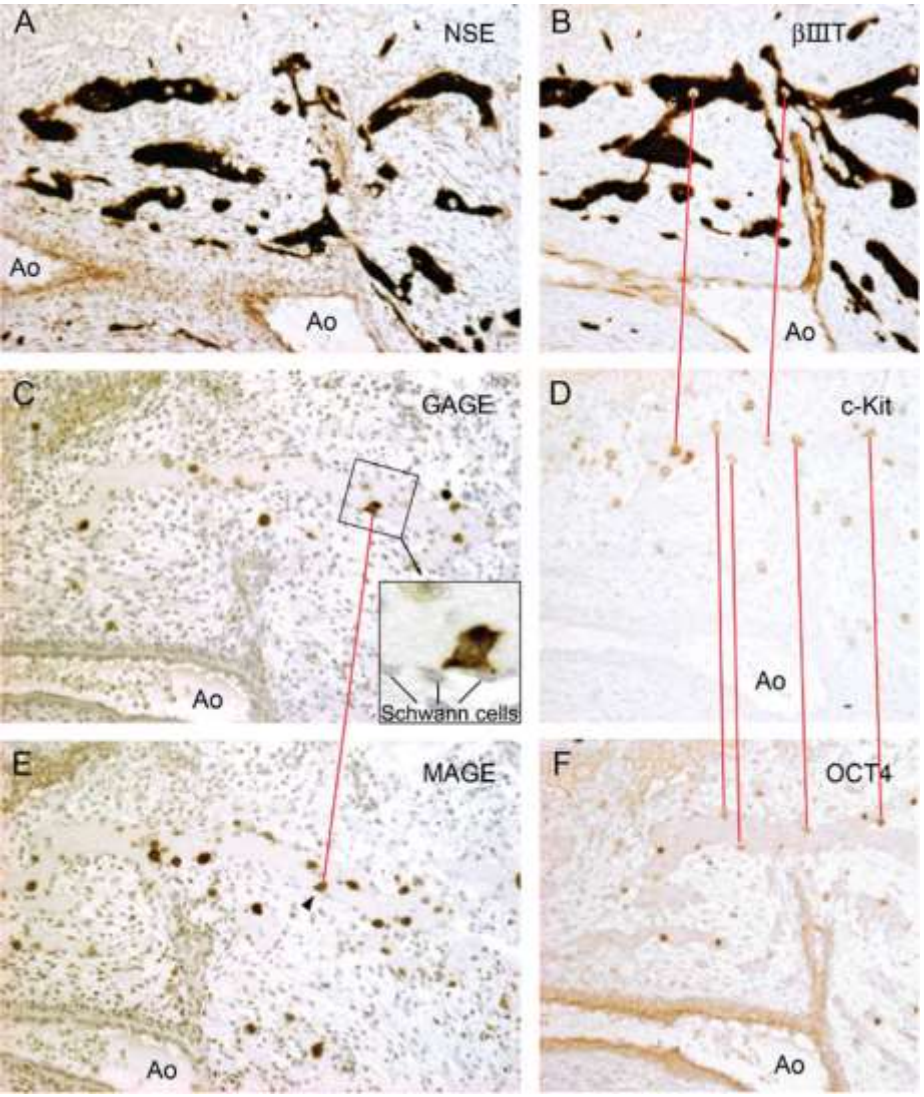
Int. J. Dev. Biol. 56: 771-778 (2012)

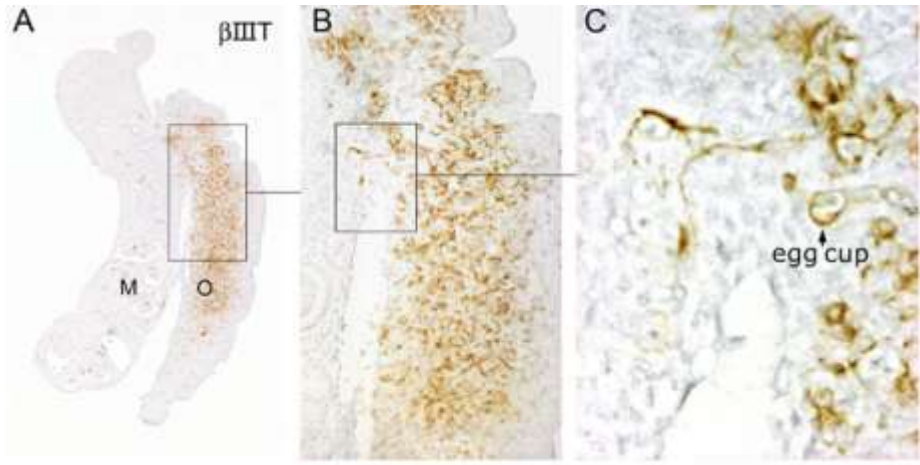
Human fetus 5,5 embryonic week

PGCs among hindgut cells (Oct4-immunohistochemistry)



PGCs migrate along autonomic nerve fibers





- **Recent studies suggest PGCs migrate along autonomic nerve fibers in dorsal mesentery**

- **Schwann cells have important role in the direction of PGC migration by chemotactic factors and growth homones**

Molecular Human Reproduction, Vol.16, pp. 621–631, 2010

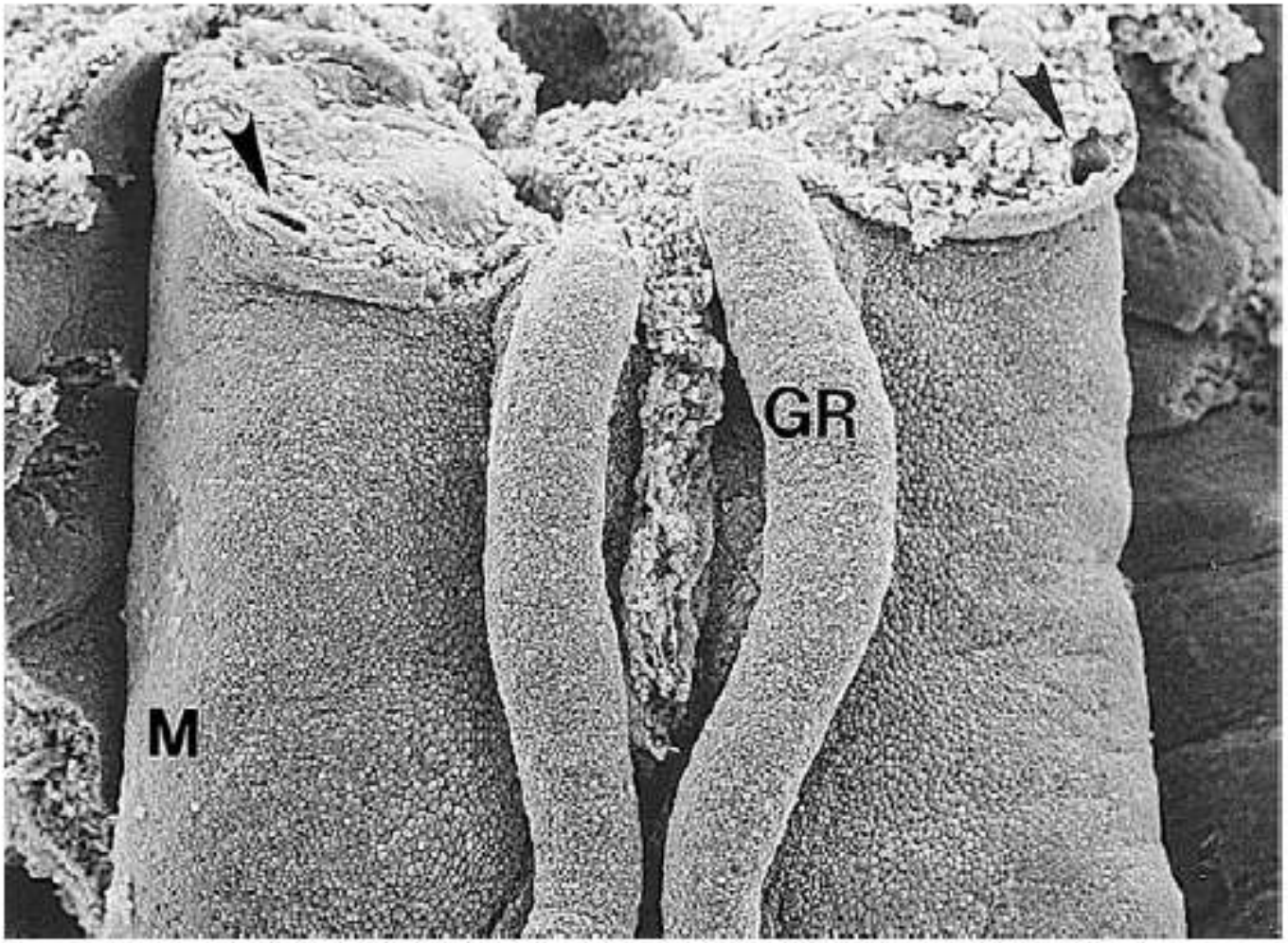


Figure 15-17. Scanning electron micrographs showing the relationship between the developing genital ridges (GR) and the mesonephroi (M). Arrowheads: Mesonephric ducts seen in cross section.

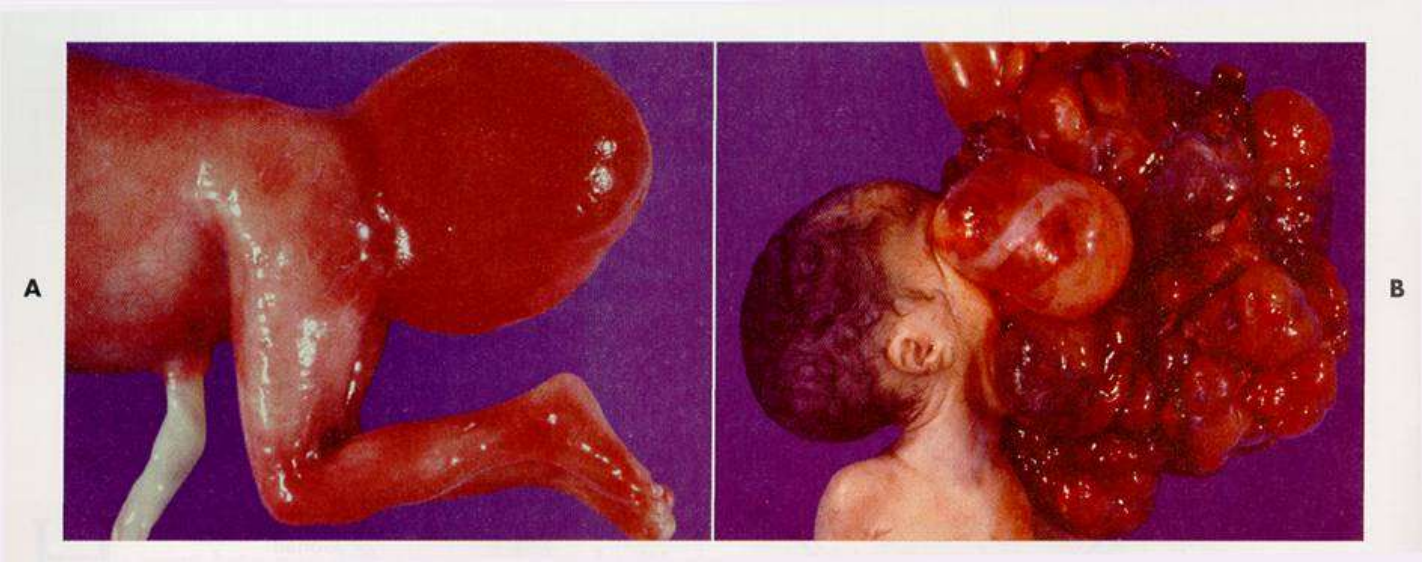
Disorders of PGC migration

Sacrococcygeal (A) és oropharyngeal (B) teratomas in fetuses

Migrating PGCs arrest early in migration, but continue to proliferate

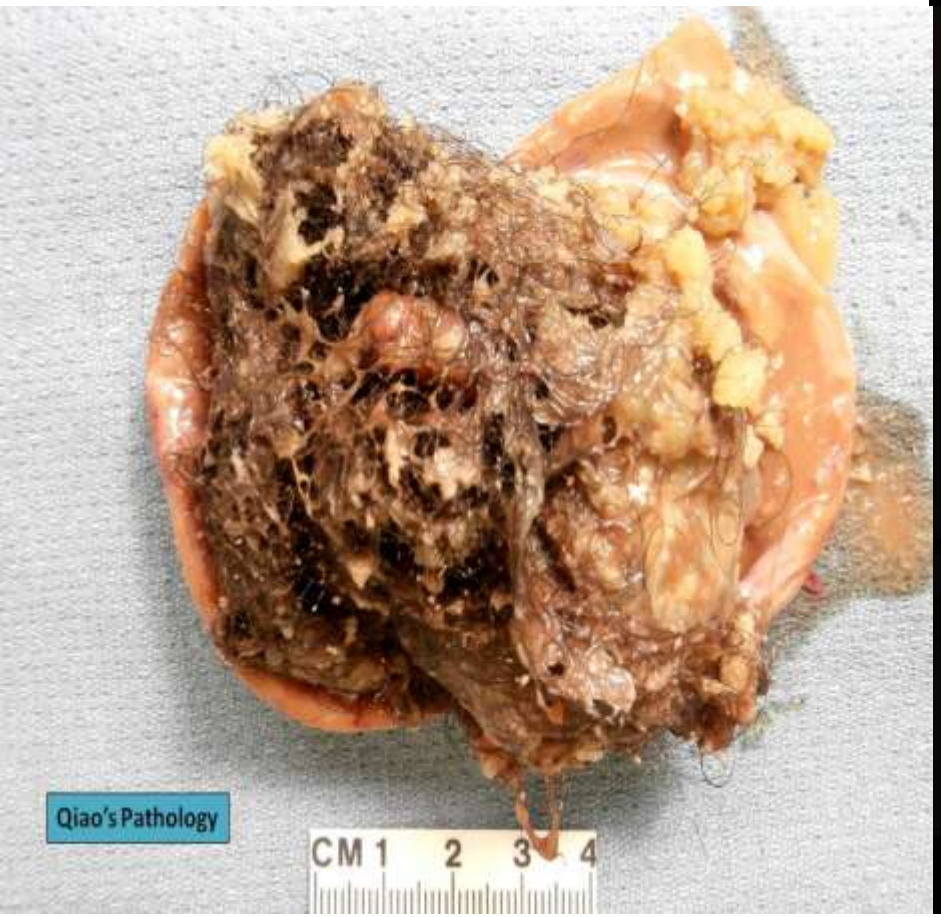
Table 2. Anatomical locations of extragonadal germ cell tumors (EGGCTs) [11]

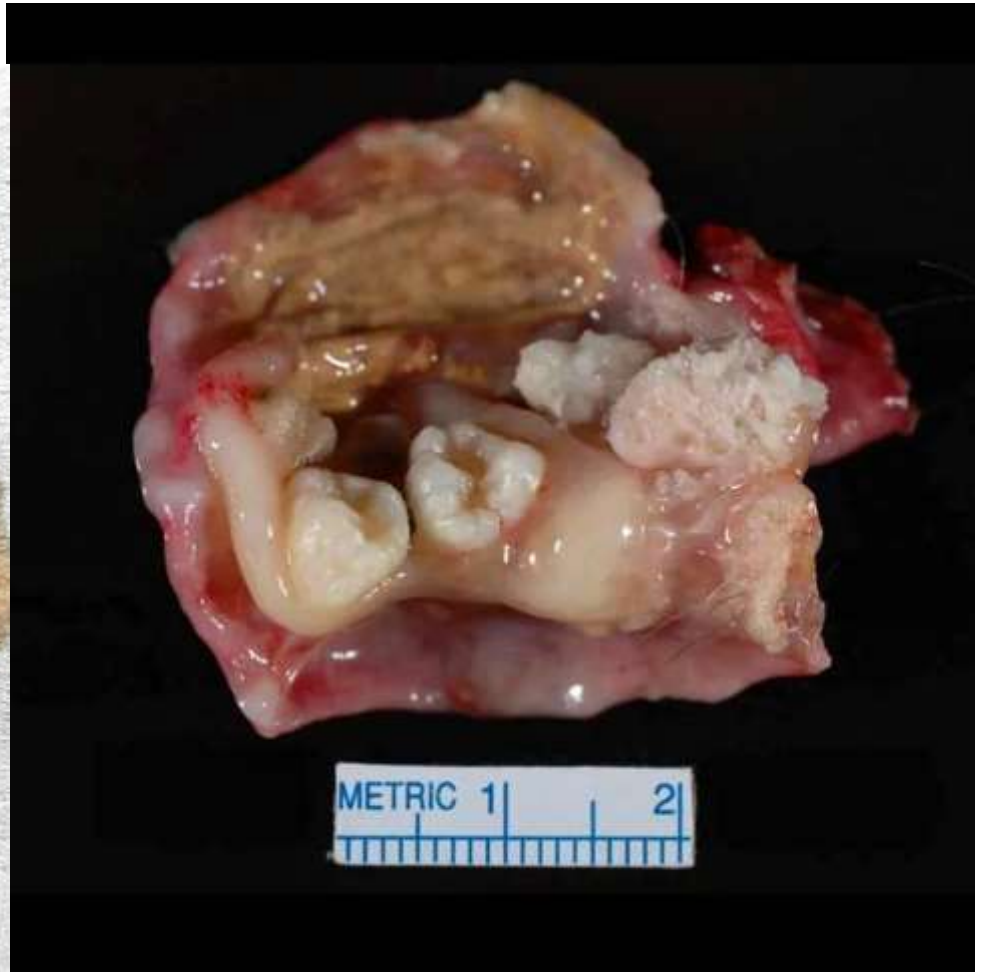
Common
• Mediastinum
• Pineal and suprasellar regions
• Sacrococcyx (infants and young children only)
Controversial
• Retroperitoneum (metastatic versus true EGGCT?)
Very rare
• Vagina
• Prostate
• Liver and gastrointestinal tract
• Orbita

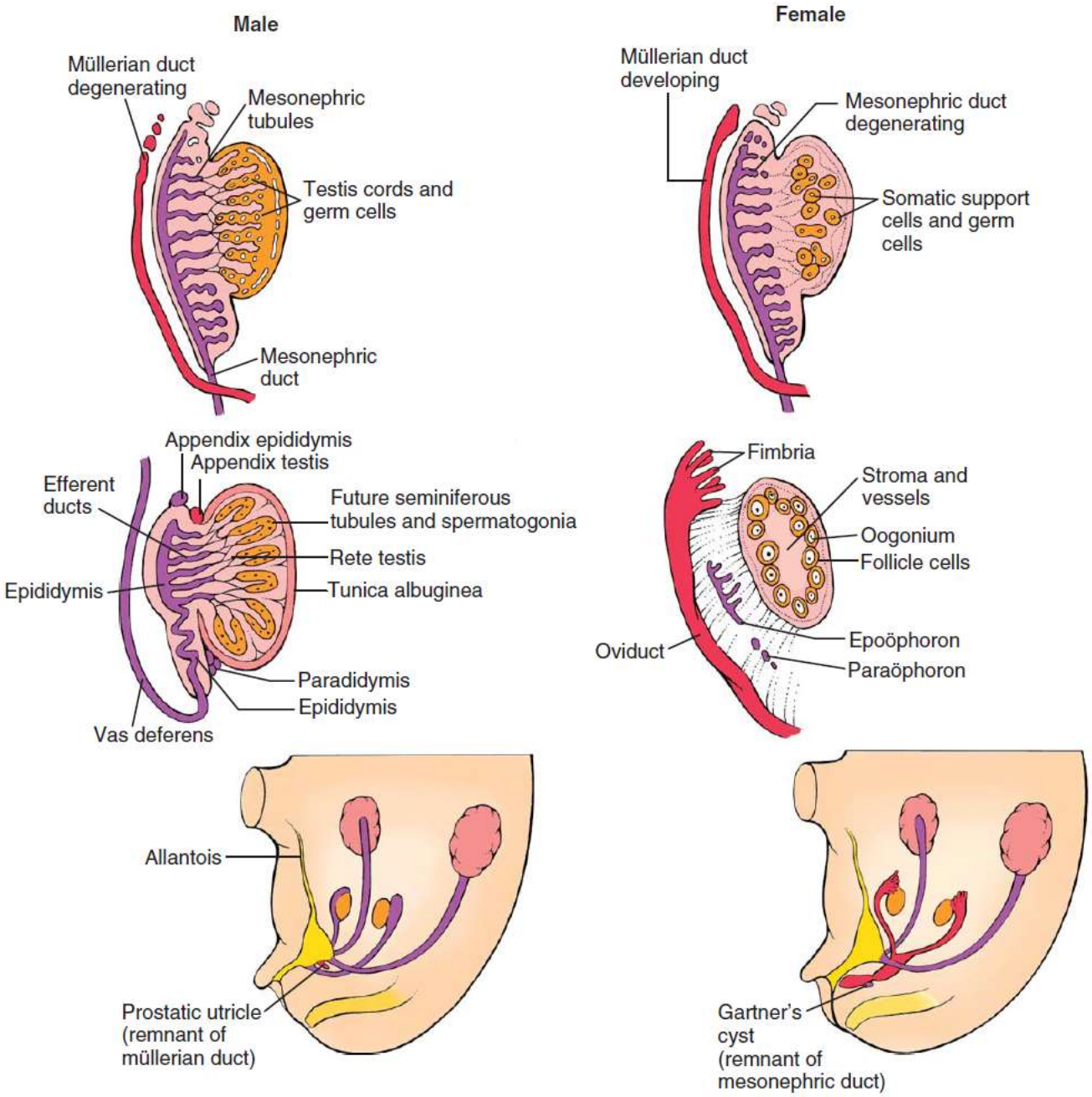


Schmoll 2002

Dermoid cyst






Male
Müllerian duct degenerating
Mesonephric tubules
Testis cords and germ cells
Mesonephric duct
Appendix epididymis
Appendix testis
Efferent ducts
Future seminiferous tubules and spermatogonia
Rete testis
Epididymis
Tunica albuginea
Paradidymis
Epididymis
Vas deferens
Allantois
Prostatic utricle (remnant of müllerian duct)
Female
Müllerian duct developing
Mesonephric duct degenerating
Somatic support cells and germ cells
Fimbria
Stroma and vessels
Oogonium
Follicle cells
Epoöphoron
Oviduct
Paraöphoron
Gartner's cyst (remnant of mesonephric duct)

II. Formation of phenotype
Gonadal sex

Gonads are derivatives of intermedier mesoderm

The gonadal primordia (anlage) are localised in the posterior wall of the embryo, medially from the mesonephros található. At the 5th week of development the posterior body wall thickens, altogether with the coeloma epithelium → genital ridge (plica genitalis).

The phoenomen happens simultaneusly in both sexes! The gonads here are still indifferent → no sex determination is possible

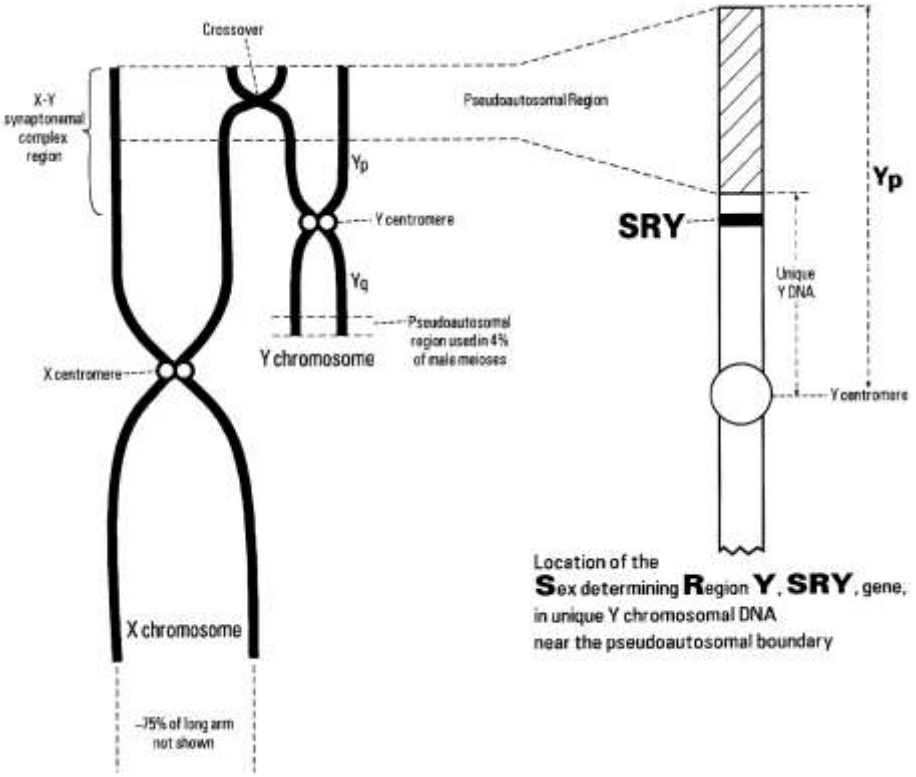
1. **Formation of the male gonad needs the presence of the Y chromosome (Sry gene) on the short arm of Y chromosome). The male phenotype is determined by the testosteron production of the differentiationg male gonad**

2. **Formation of the female gonad requires the presence of 1 X chromosome and the lack of Y chromosome, but the formation of a functionally sound ovarium needs both X chromosomes. → Turner syndrome (X0)→ infertility**

Molecular background

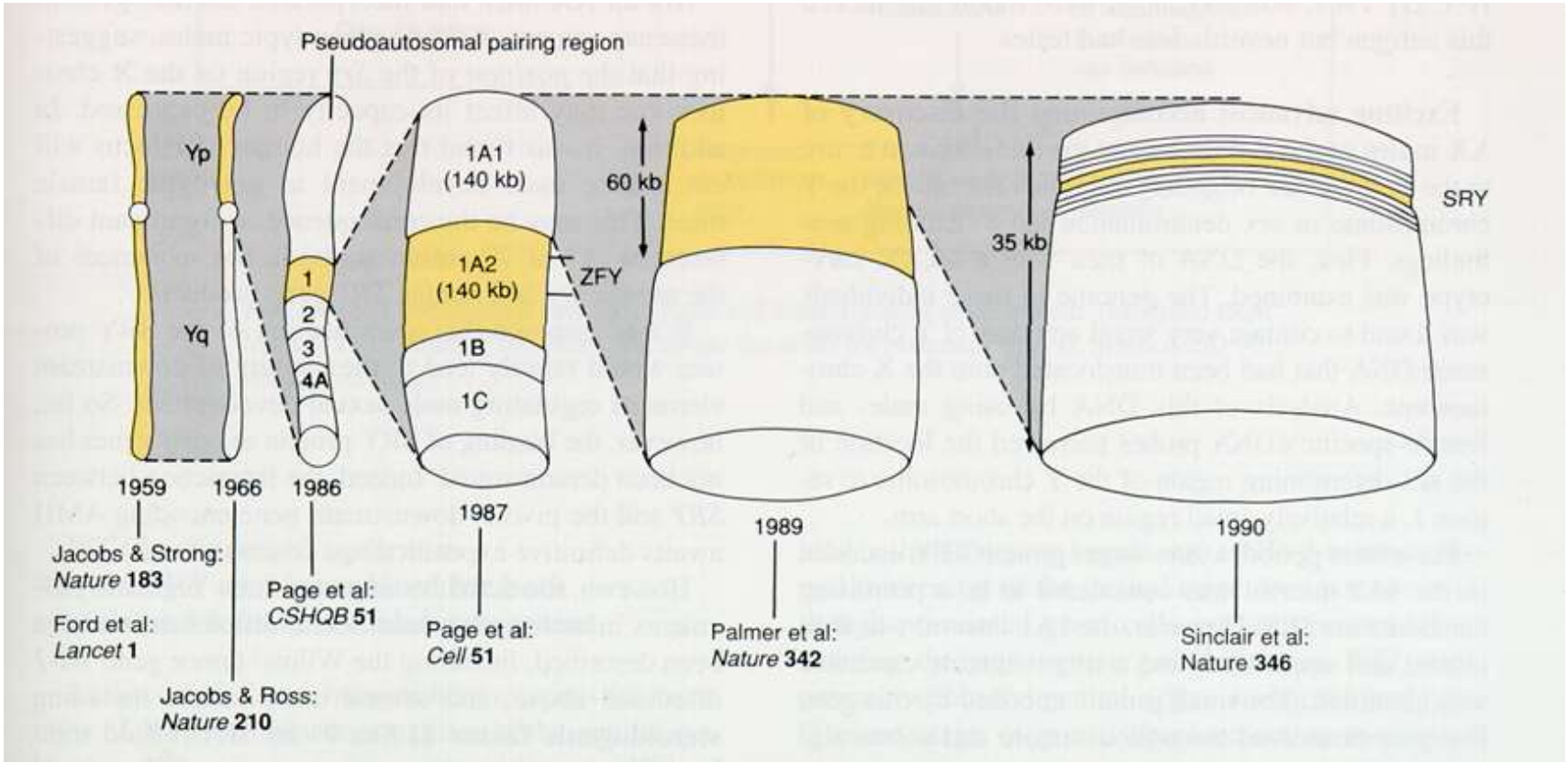
Short arm of Y chromose is essential for the formation of testes

The Sry gene codes for the 223 AA polypeptide SRY protein



http://physrev.physiology.org/content/78/1/1

- **Product of the SRY gene the SRY protein, or TDF (testis determining factor) is required for testis-formation**



Mechanism of action of Testis Determining Factor

The supporting cells (Sertoli, Leydig) are the primary target of TDF

- Testis Determining Factor has no effect on PGCs

-THE TESTIS STILL DEVELOPS WITHOUT MALE PGCs

- a **TDF (SRY)** 's main target is the **Dax1 gene**, on the X chromosome

-In the ovaries it acts as an anti-testis gene

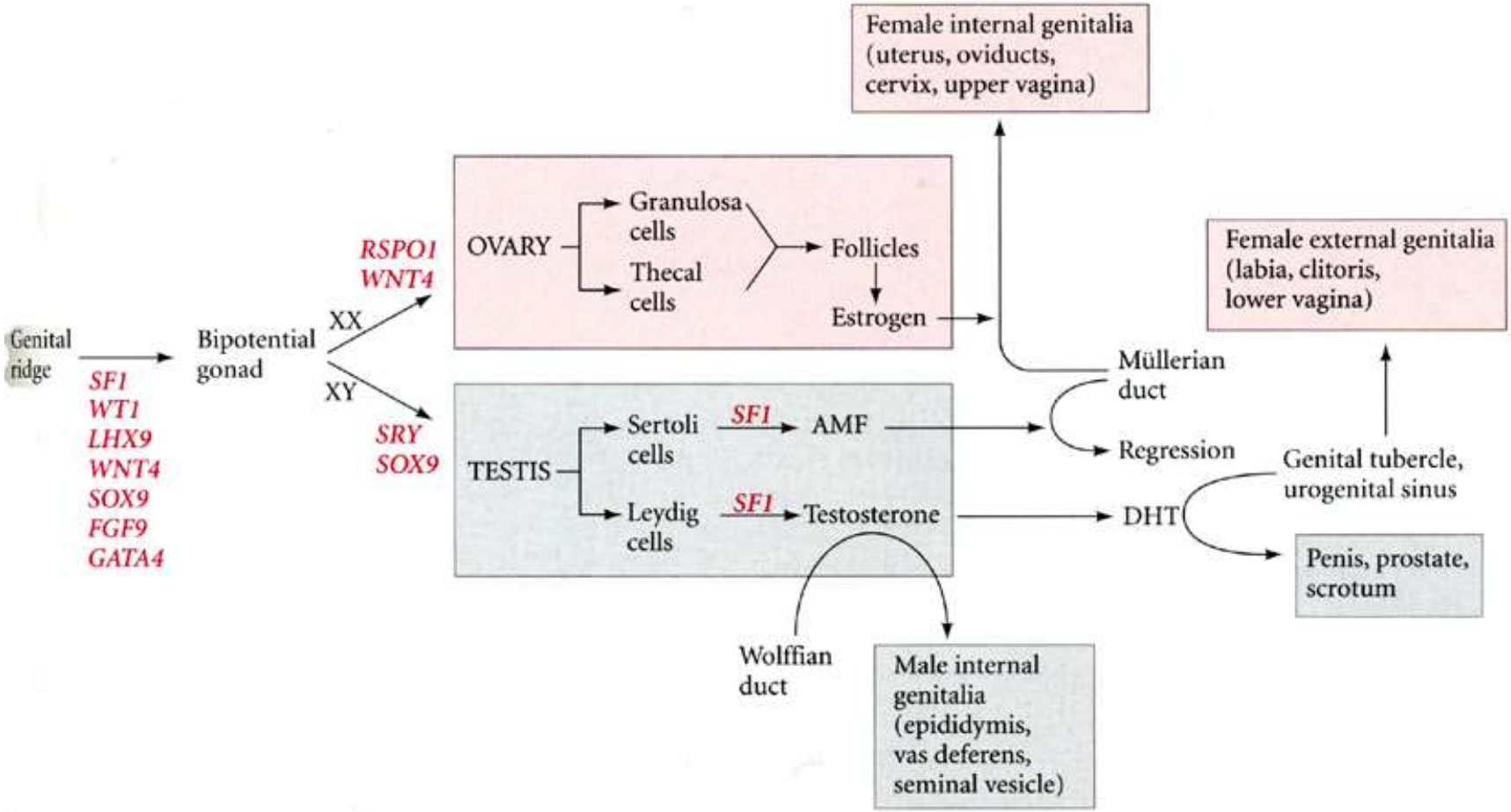
- During the formation of testis DAX1 downregulates

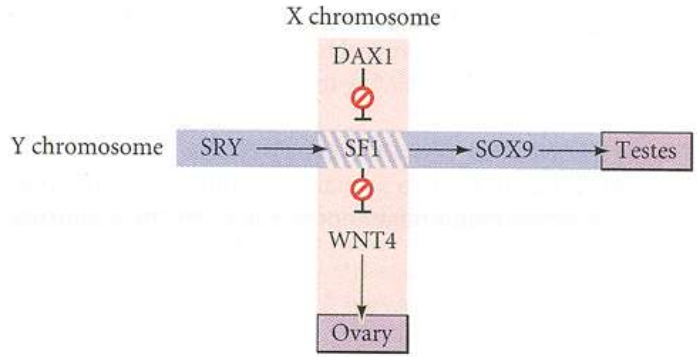
- DAX1 activity persists in the ovaries

DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1)

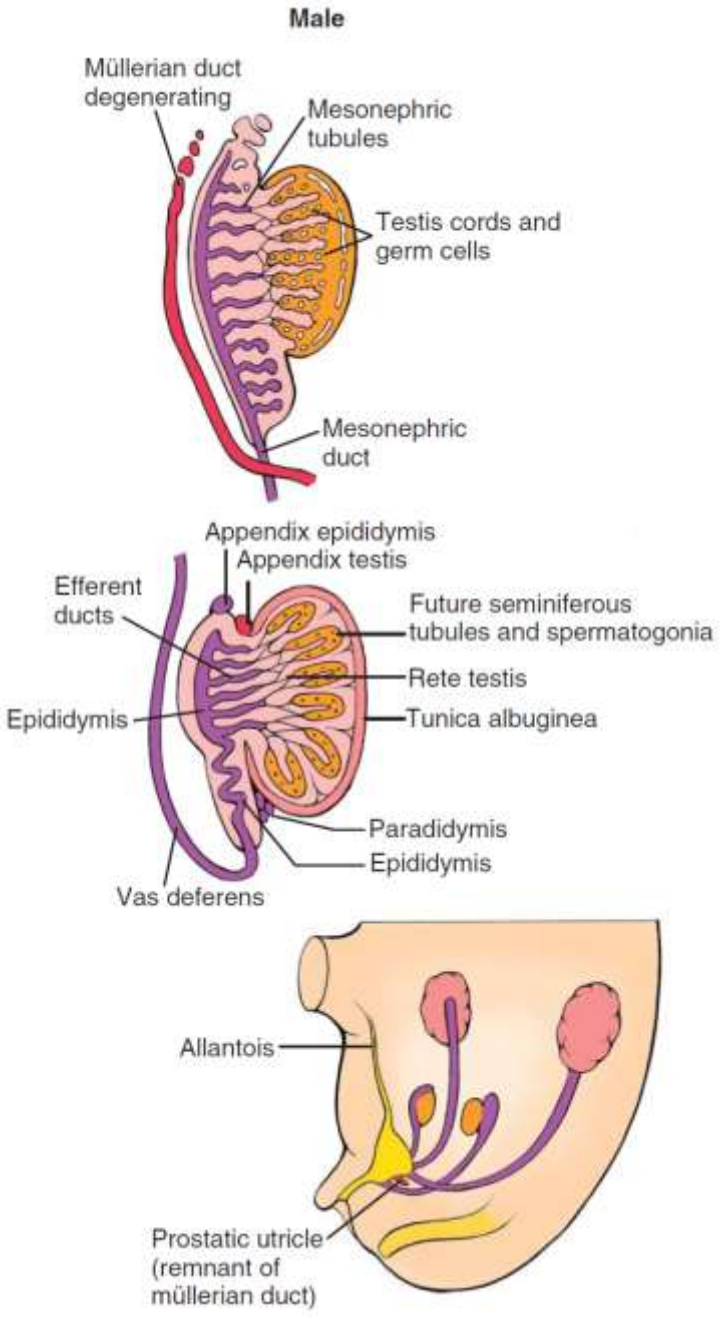
Autosomal genes in sex-determination: SOX-9

- A SOX9 transcription factor expression is continous in Sertoli cells → expression starts immediately after the production of SRY protein
- SOX-9 +/- human: growth impairment and ¾ of XY-patients are females
- Sex-determination cascade: **SRY** expression induces **SOX9** expression → New gene is activated → **SF-1**-et.
- Az SF-1 (steroidogenetic factor-1) is important in the synthesis of sexual steroid hormones, it also binds to the promoter of AMH (Anti-müller) hormone.





Sex determination, early steps



In genetic males, the Y-linked testis-determining gene (*SRY*) is expressed in the somatic support cells, resulting in the production of SRY protein. Genetic females lack this gene and do not produce SRY protein.

SRY Protein

In response to SRY protein, the somatic support cells of the gonad differentiate into Sertoli cells and secrete anti-müllerian hormone (AMH).

? **AMH**

Signals arising from Sertoli cells recruit mesenchymal cells into the gonadal ridge that differentiate into Leydig cells and secrete testosterone.

AMH induces degeneration of the müllerian ducts.

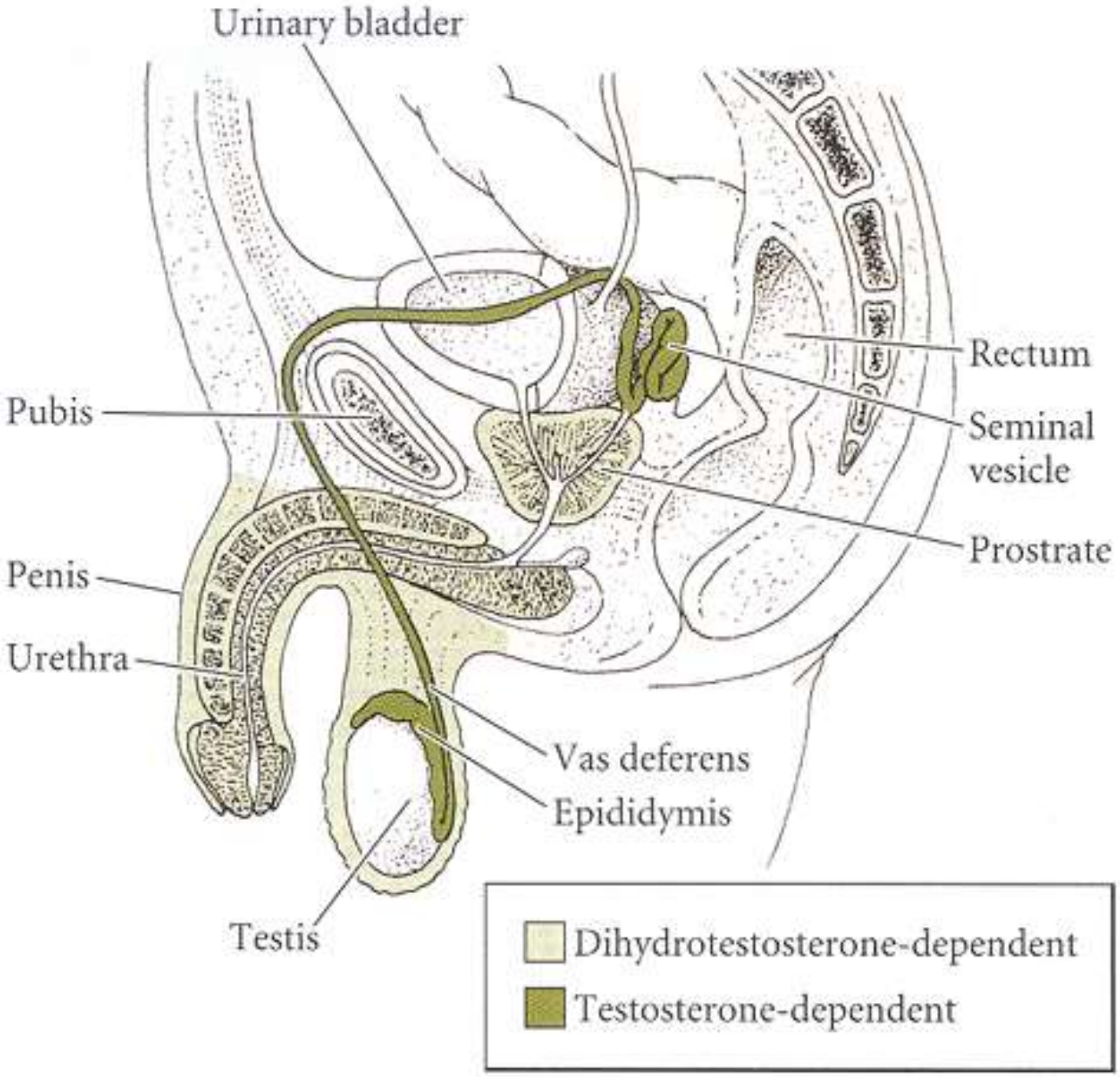
Testosterone → **Dihydrotestosterone**

During fetal life, testosterone induces differentiation of the epididymis, vas deferens, and seminal vesicles from the mesonephric duct and male differentiation of the brain.

The testosterone surge at puberty causes the seminiferous tubules to canalize, mature, and commence spermatogenesis, and induces the other pubertal changes in primary and secondary sexual characteristics.

During fetal life, conversion of testosterone to dihydrotestosterone within the relevant target tissues causes the indifferent external genitalia to differentiate into a penis and scrotum and also induces the development or differentiation of some other male structures, such as the prostate.

Figure 15-18. Summary of the differentiation cascade of the male genital system development.


Urinary bladder
Rectum
Pubis
Seminal vesicle
Prostrate
Penis
Urethra
Vas deferens
Epididymis
Testis
Dihydrotestosterone-dependent
Testosterone-dependent

Somatic cells of the gonads:

1) Supporting cell line

- Derivatives of primary sex chords of the coelomic epithelium:
  Sertoli cells in testes – AMH – Regression of Müller duct

- Derivatives of secondary sex chords of the coelomic epithelium:
  Granulosa cells (follicular cells) in the ovaries

2) Steroid – secreting cell line:

- From mesenchyme: Leydig cells in testes (8th week from: testosteron, androstendion production
Peak of testosteron production is on the 17-18th week: Differentiation of male genital tract and external genitalia
-After week 18th Leydig cells go through a relative regression

-Theca cells appear in ovaries during puberty

XY embryo

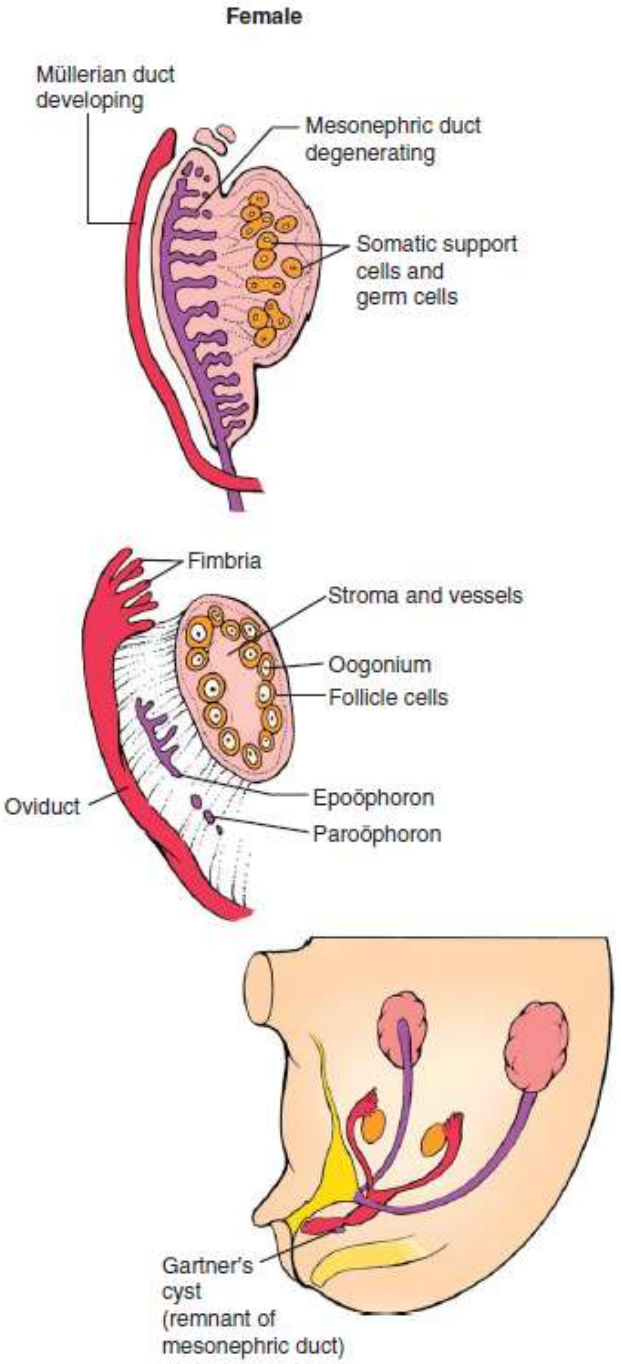
- **Primitive sex chords persist in the medulla – testis chords**
- **In the chords pre-Sertoli cells differentiate → first male specific cell type**
- **PGCs arrive and establish contact with pre-Sertoli cells → they not yet begin meiosis**
- **<u>They divide with mitosis slowly until puberty</u>**
- **From sex chords: tubuli seminiferi, rete testis, tubuli recti develop**
- **From the ducts of mesonephros: ductuli efferentes testis, ductus epididymis**

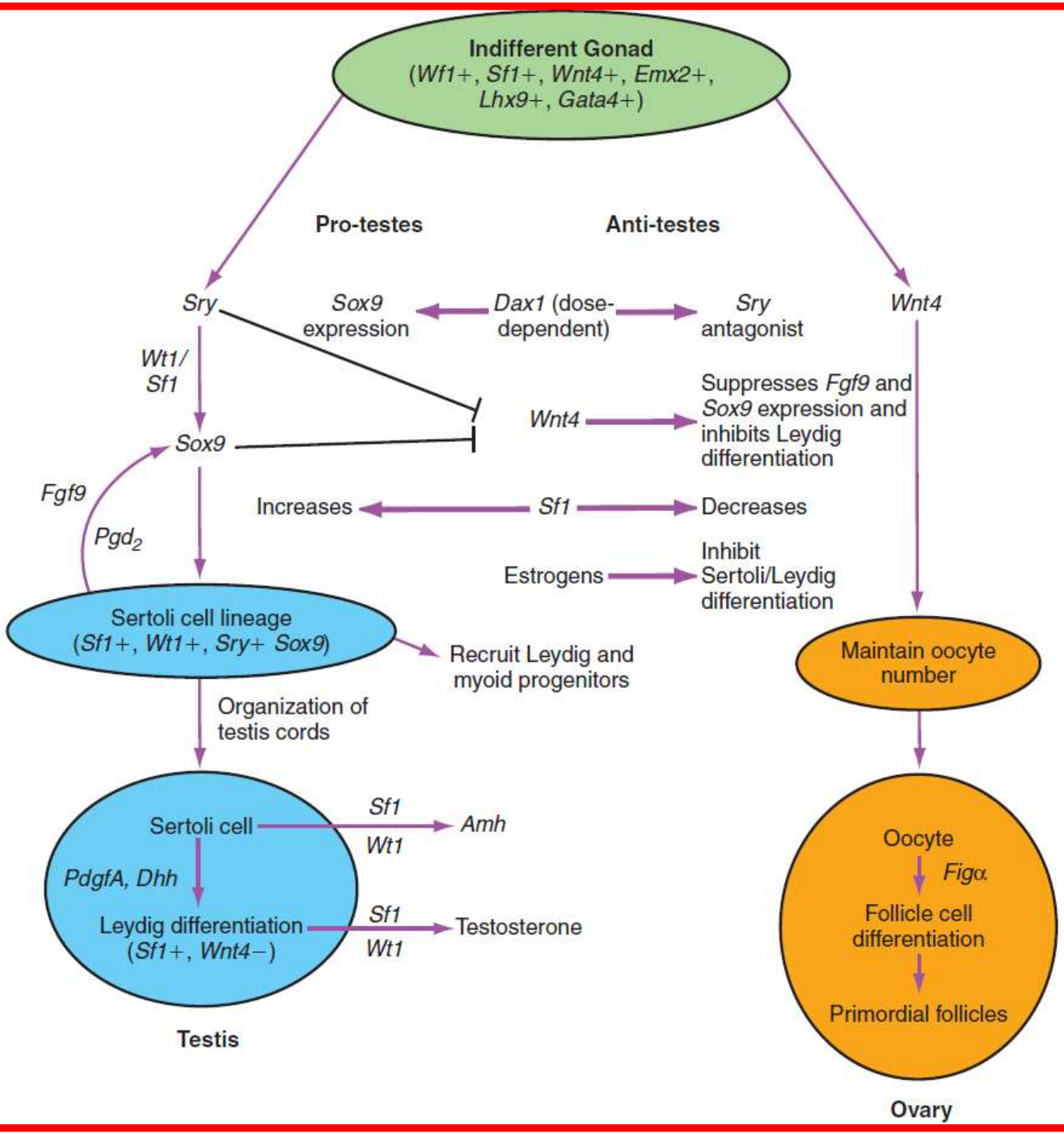
XX embryo

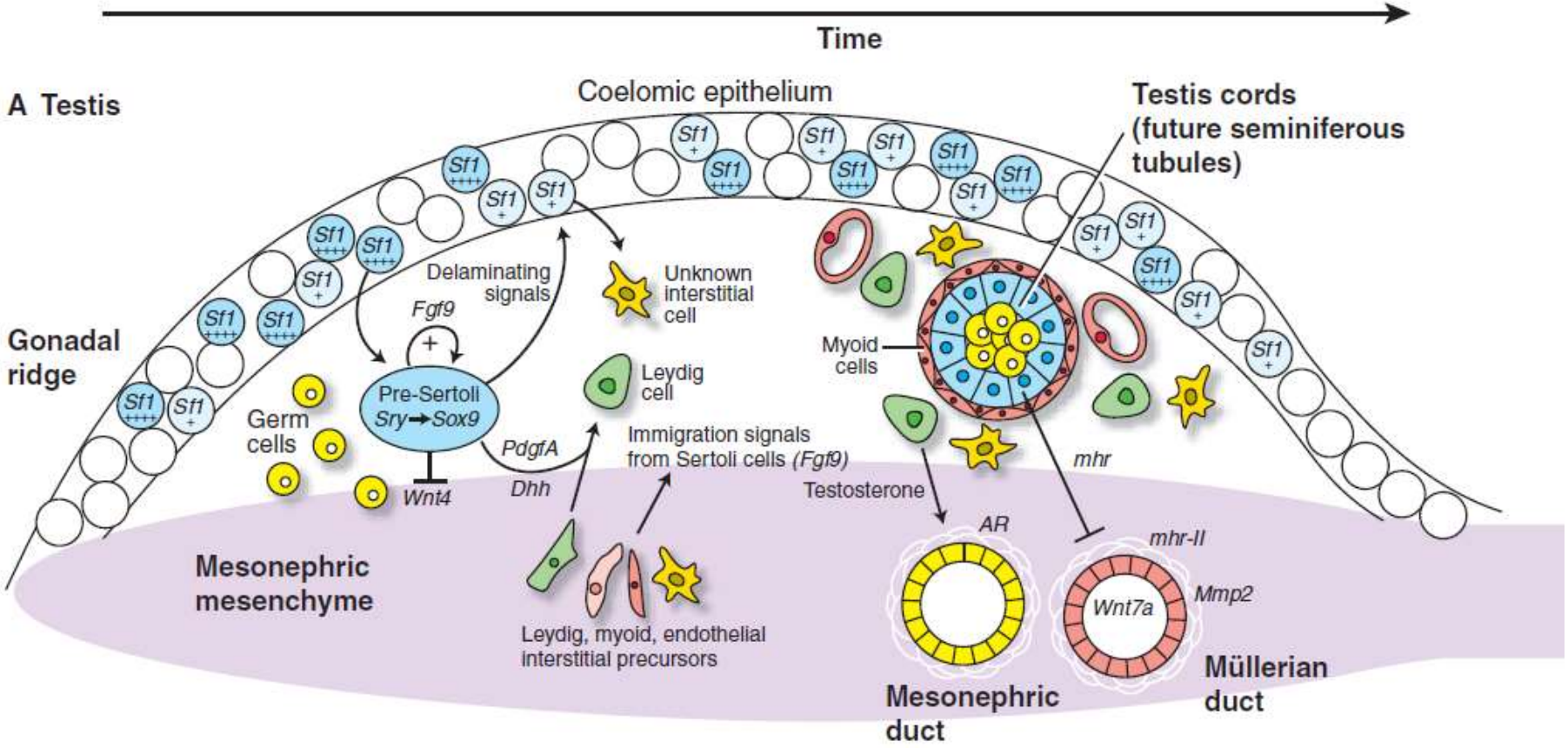
- **Primitive sex chords degenerate in the medulla (rete ovarii)**
- **Coelomic epithelium forms new sex chords in the mesenchyme → secondary sex chords**
- **Definitive ovaries form from the cortical part**
- **Oogonia proliferate with mitosis during fetal life**
- **The first meiotic division begins in some oogonia during the 12-16th week → PRIMARY OOCYTES – sorrounded by follicular cells: primordial follicle**
- **Meiosis of primary oocytes arrest in the diploten phase of prophase**
- **Coelomic epithelial cells invade the mesenchyme and prefollicular cells differentiate from the**

No more oogonia goes through mitosis after birth!

In the absence of Y chromose female development




Indifferent Gonad
(Wf1+, Sf1+, Wnt4+, Emx2+, Lhx9+, Gata4+)
Pro-testes
Anti-testes
Sry
Sox9 expression
Dax1 (dose-dependent)
Sry antagonist
Wnt4
Wt1/ Sf1
Sox9
Wnt4
Suppresses Fgf9 and Sox9 expression and inhibits Leydig differentiation
Fgf9
Pgd2
Increases
Sf1
Decreases
Estrogens
Inhibit Sertoli/Leydig differentiation
Sertoli cell lineage
(Sf1+, Wt1+, Sry+ Sox9)
Recruit Leydig and myoid progenitors
Maintain oocyte number
Organization of testis cords
Sertoli cell
Sf1
Wt1
Amh
PdgfA, Dhh
Leydig differentiation
(Sf1+, Wnt4−)
Sf1
Wt1
Testosterone
Testis
Oocyte
Figα
Follicle cell differentiation
Primordial follicles
Ovary


Time
A Testis
Coelomic epithelium
Testis cords
(future seminiferous
tubules)
Gonadal
ridge
Sf1
Delaminating
signals
Fgf9
+
Unknown
interstitial
cell
Leydig
cell
Myoid
cells
Germ
cells
Pre-Sertoli
Sry→Sox9
Wnt4
PdgfA
Dhh
Immigration signals
from Sertoli cells (Fgf9)
Testosterone
AR
mhr
mhr-II
Wnt7a
Mmp2
Mesonephric
mesenchyme
Leydig, myoid, endothelial
interstitial precursors
Mesonephric
duct
Müllerian
duct

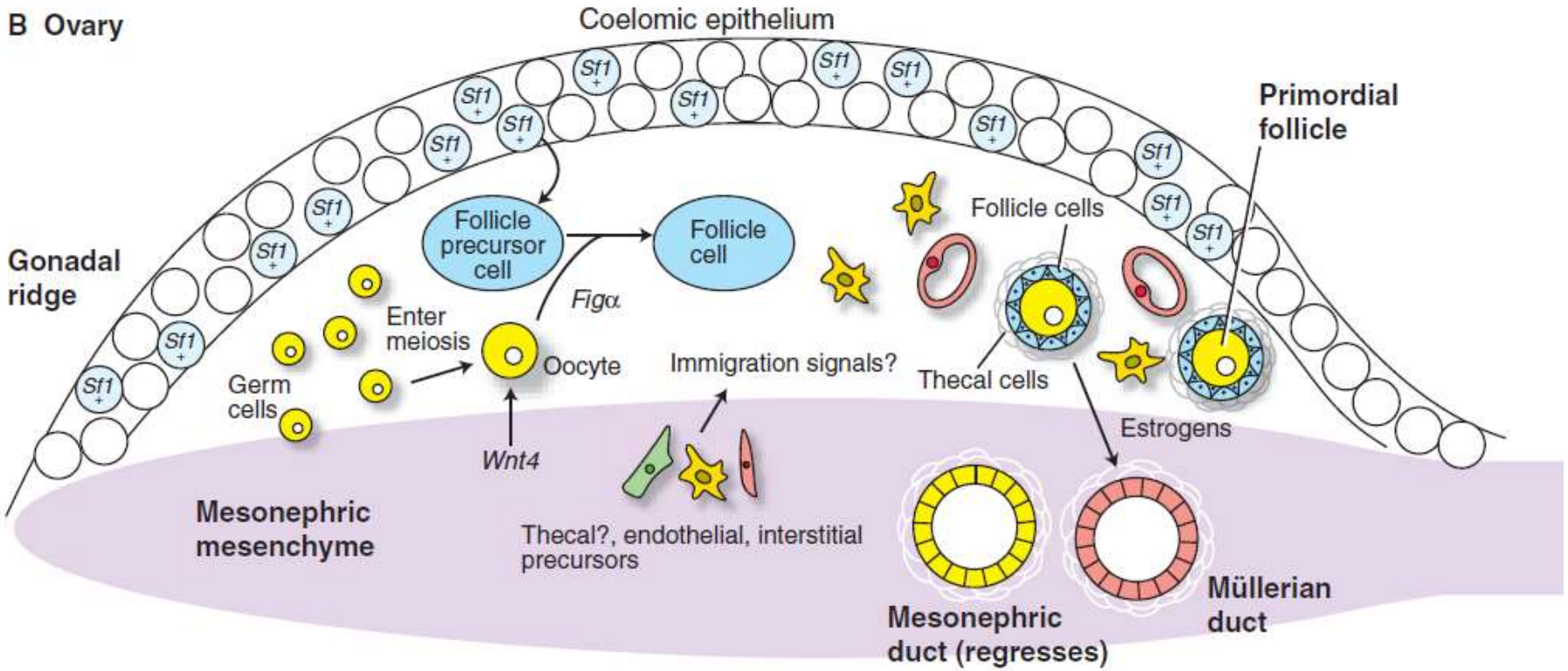

B Ovary
Coelomic epithelium
Sf1
Primordial follicle
Gonadal ridge
Follicle precursor cell
Follicle cell
Follicle cells
Figα
Enter meiosis
Oocyte
Germ cells
Immigration signals?
Thecal cells
Estrogens
Wnt4
Mesonephric mesenchyme
Thecal?, endothelial, interstitial precursors
Mesonephric duct (regresses)
Müllerian duct

Table 15-2 Adult Derivatives and Vestigial Remnants of Embryonic Male and Female Reproductive Structures

Presumptive Anlagen	Male Structure	Female Structure
Indifferent gonad	Testis	Ovary
Primordial germ cell	Spermatogonia	Oocytes
Somatic support cell	Sertoli cells	Follicle cells
Stromal cells	Leydig cells	Thecal cells
Gubernaculum	Gubernaculum testis	Round ligament of the ovary Round ligament of uterus
Mesonephric tubules	Efferent ducts of testis Paradidymis	Epoöphoron Paroöphoron
Mesonephric duct	Appendix of epididymis Epididymis Vas deferens Seminal vesicle Ejaculatory duct	Appendix vesiculosa Duct of epoöphoron Gartner's duct
Müllerian duct	Appendix of testis	Fallopian tubes Uterus
Urogenital sinus	Prostatic and membranous urethra Prostatic utricle Prostatic gland Bulbourethral glands	Membranous urethra Vagina Urethral/paraurethral glands Greater vestibular glands
Sinus tubercle	Seminal colliculus	Hymen
Genital tubercle	Glans penis Corpus cavernosa of penis Corpus spongiosum of penis	Glans clitoris Corpus cavernosa of clitoris Bulbospongiosum of vestibule
Urogenital folds and urethral plate	Penile urethra/ventral penis	Labia minora
Labioscrotal folds	Scrotum	Labia majora