Embryology I
OOGENESIS

autumn 2024

Development of reproductive system

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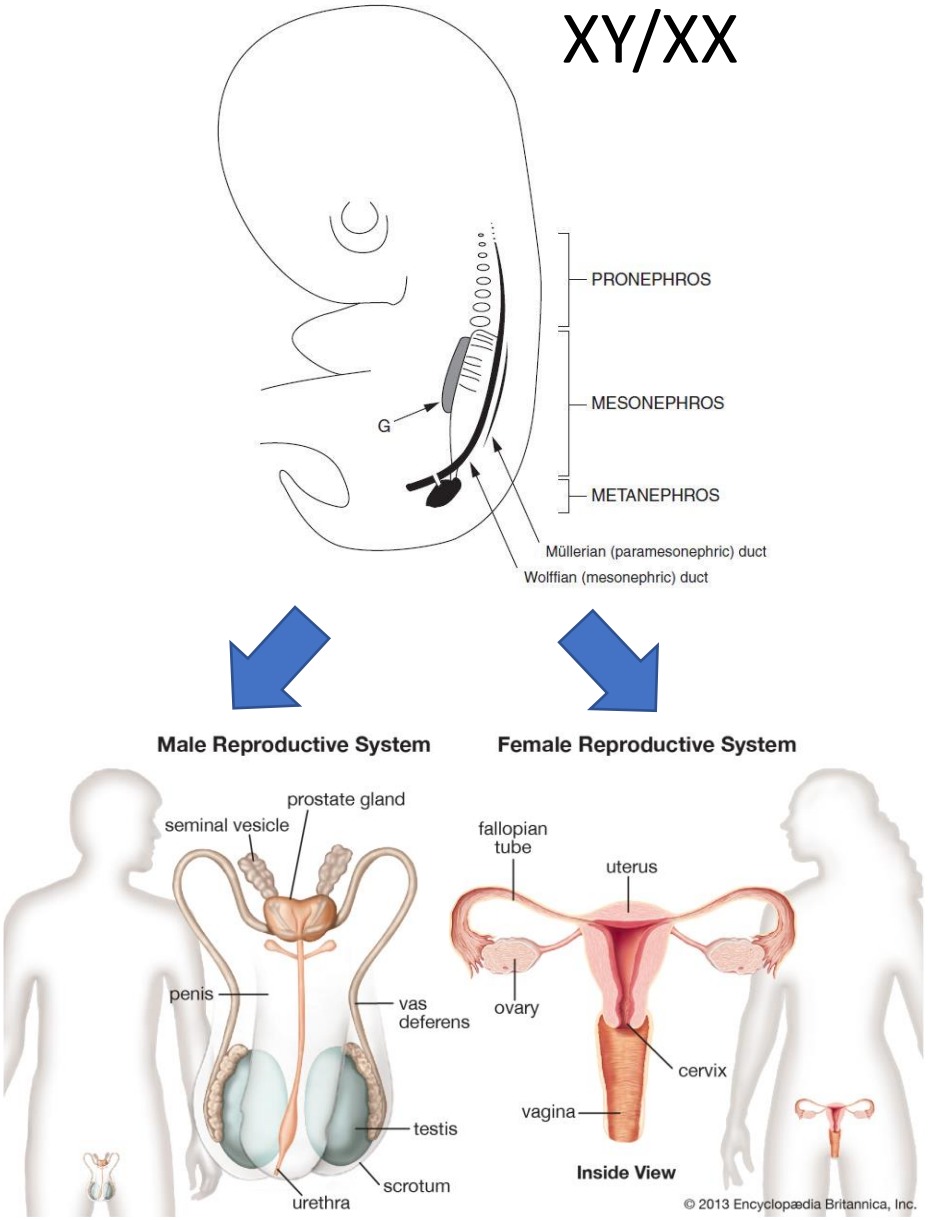
Development of reproductive system

- 0-7 week - **indiferent stage**
- from week 7 sexual differentiation
- mammalian gonads develop as integral part of **mesoderm**-derived urogenital system
- formation of gonads due to **interaction of germ cells with mesonefric mesenchyme**
- genital portion of reproductive system develops 1-2 weeks later
  - XX - Müller (paramesonefric) duct
  - XY - Wolff (mesonefric) duct



Primordial germ cells timeline

= primordial germ cells (PGCs)

- sex cells progenitors

week 3 ➢ Specification

week 4

week 5 ➢ Migration

week 6

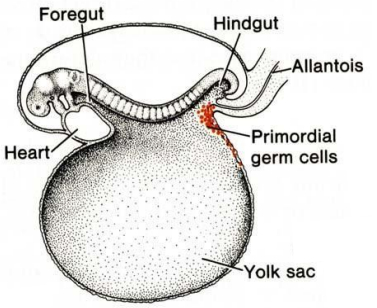
week 7 ➢ Colonization of genital ridges

week 8

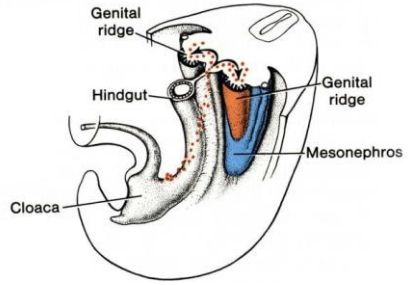
week 9 ➢ Sexual diferentiation

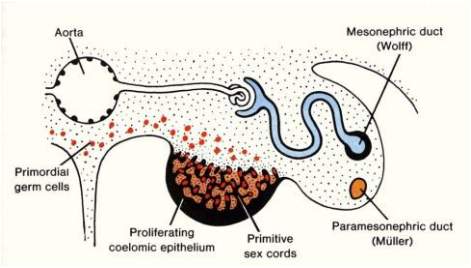
week 10 → oogonia (ovary); → gonocytes (testis)

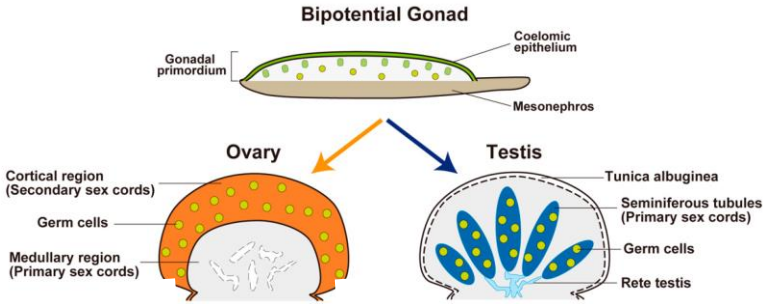
post conception (pc)



PROLIFERATION



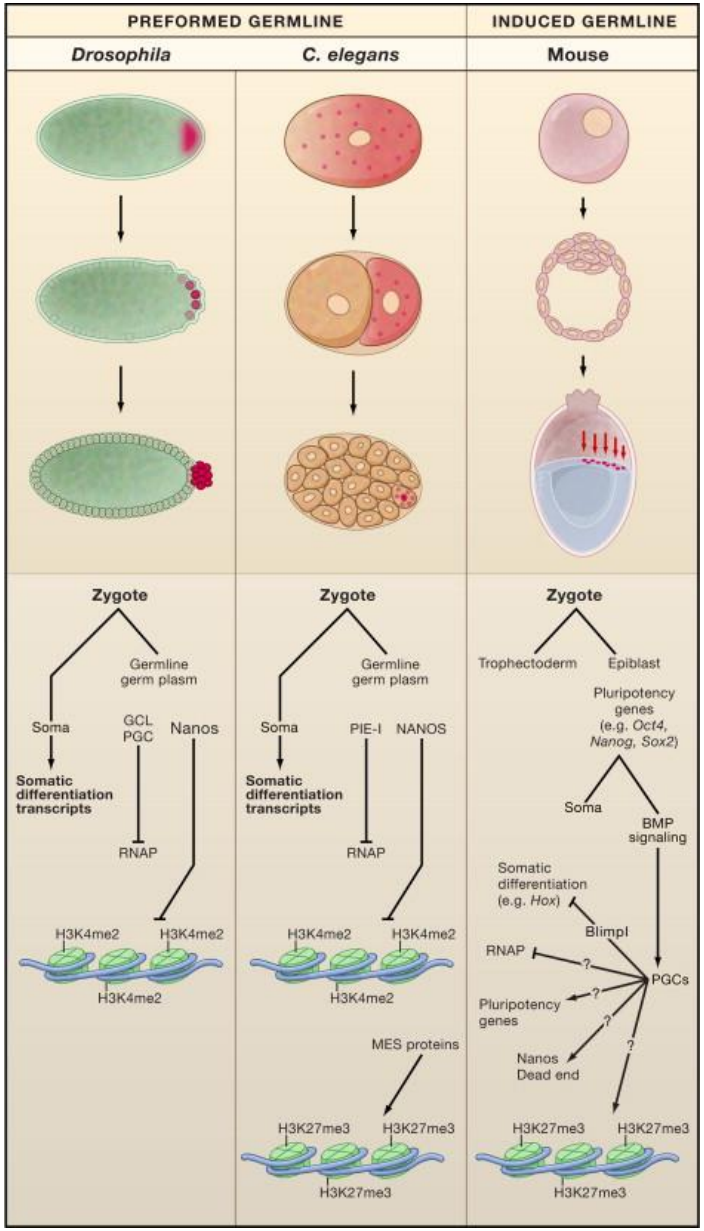




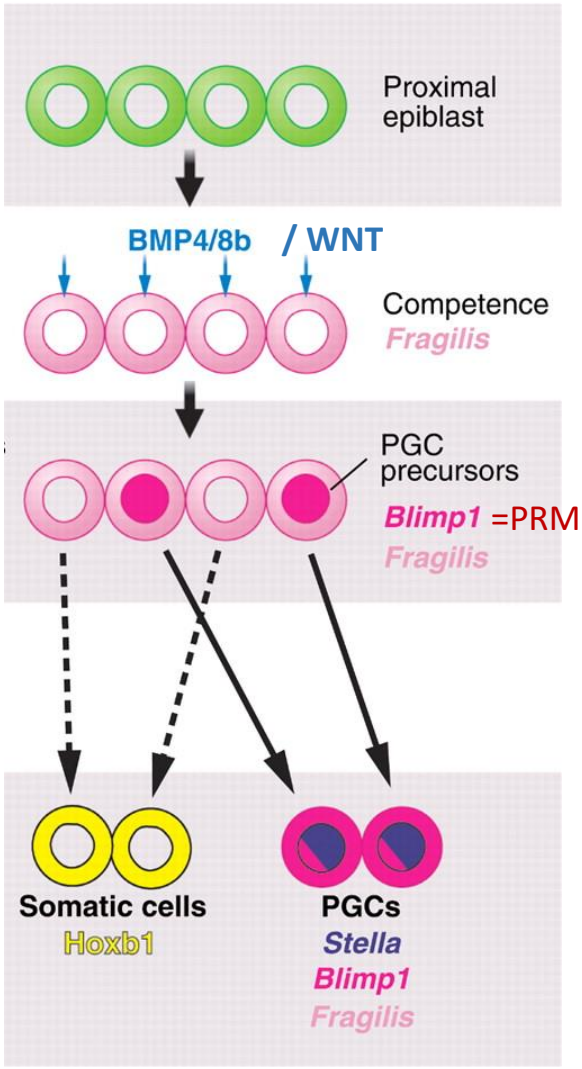
PGCs specification

- In lower species germ line specified based on inheritance of specific area of cytoplasm (**germplasm**) containing maternally encoded proteins and RNAs (maternal factors)

- In mammals, specification occurs within posterior epiblast of postimplantation embryos before gastrulation onset based on **inductive cell-cell signalling**



PGCs specification



WNT
BMP
FGF?

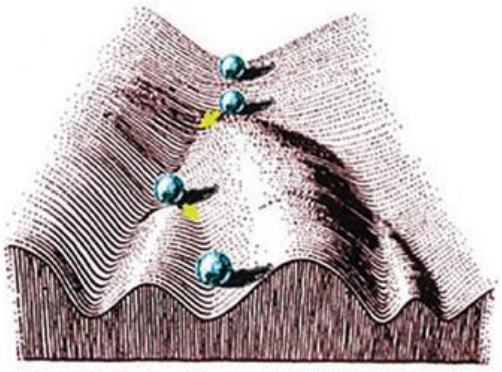
BLIMP1
Fragilis

BLIMP1
Fragilis
Stella

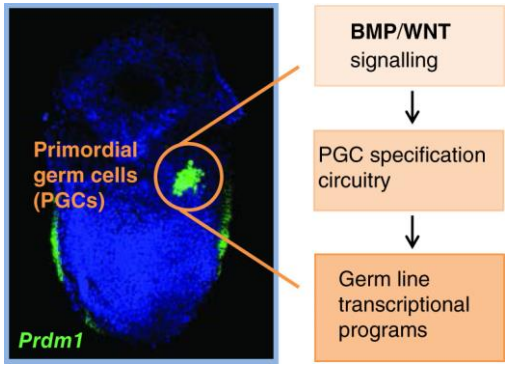
Posterior epiblast

„PGCs-competent" epiblast

Move out trough developing primitive streak



Waddington´s landscape



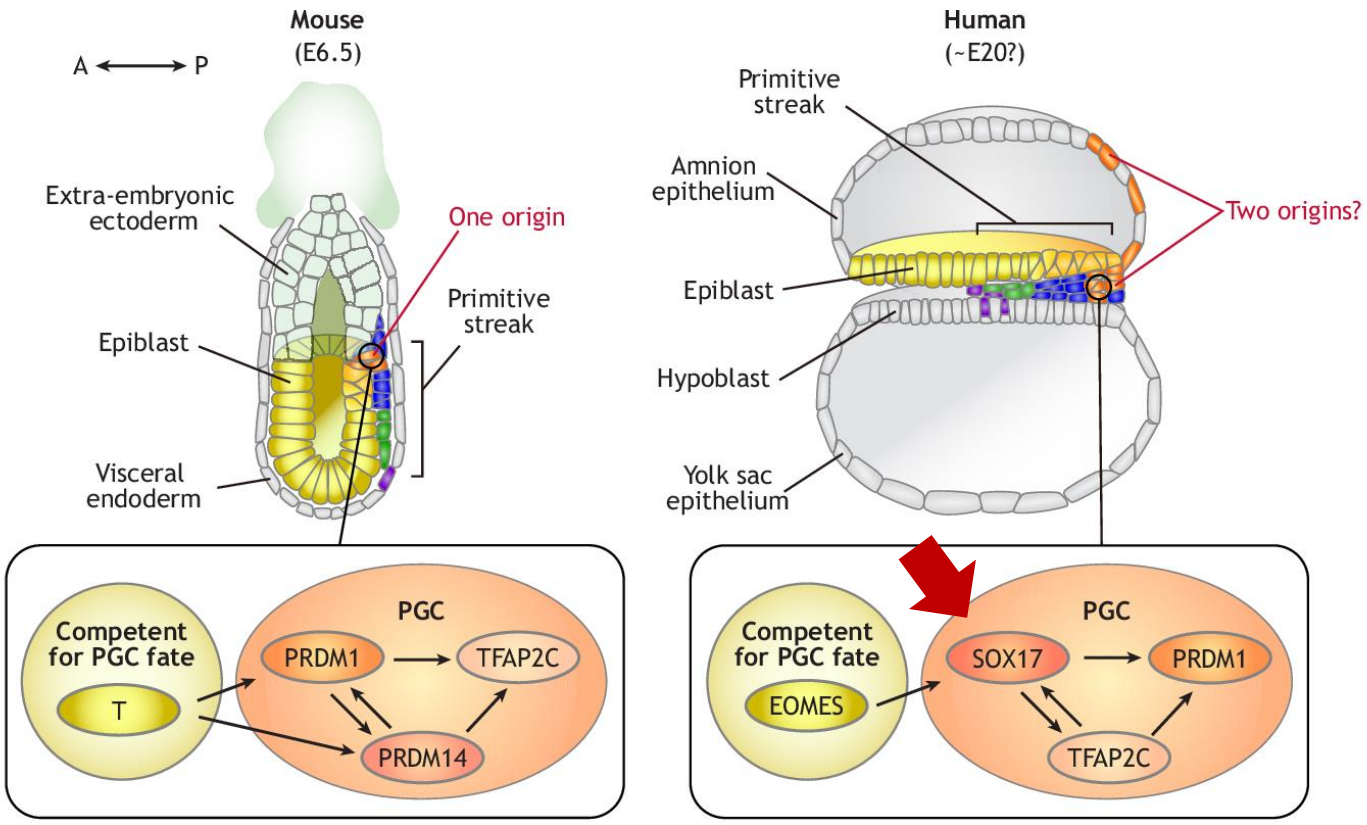
priming → commitment → licencing → specification

PGCs specification

Pregastrulation embryo

cylindr

bilaminární disk



Azim Surani

T=TBXT=brachyury

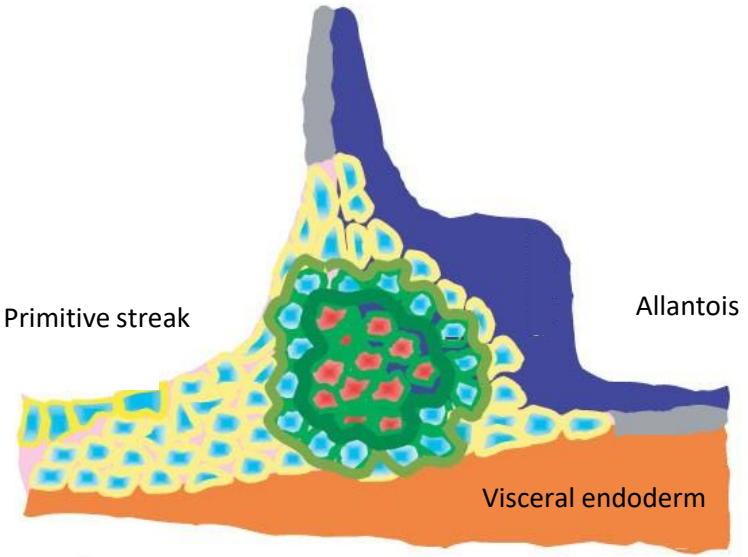
PRDM1=BLIMP1

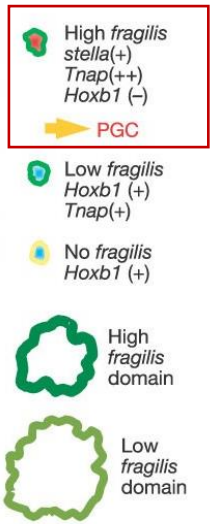
TFAP2C = AP2gamma

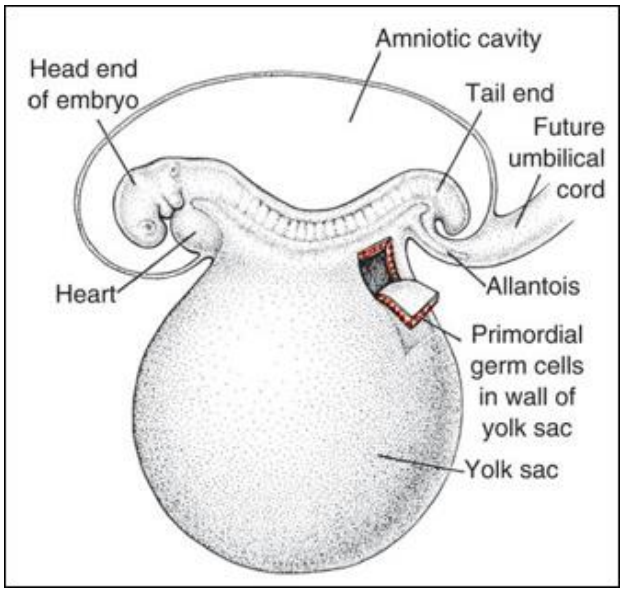
Kobayashi and Surani, 2018.

PGCs specification

- Human PGCs first detected in endodermal epithelium of yolk sack wall close to allantois ~21-22 days pc
- ~50 cells expressing ***BLIMP1, Fragilis, Stella*** and tissue non-specific alkalic phosphatase ***(TNAP)***
- Transient extraembryonic deposition allows PGCs to escape from molecular signals inducing somatic differentiation into 3 germ layers (ectoderm, mesoderm, endoderm) and thus prevent their pluripotency marked by expression of *Oct3/4 and Nanog (in mice also Klf4 and Sox2)*

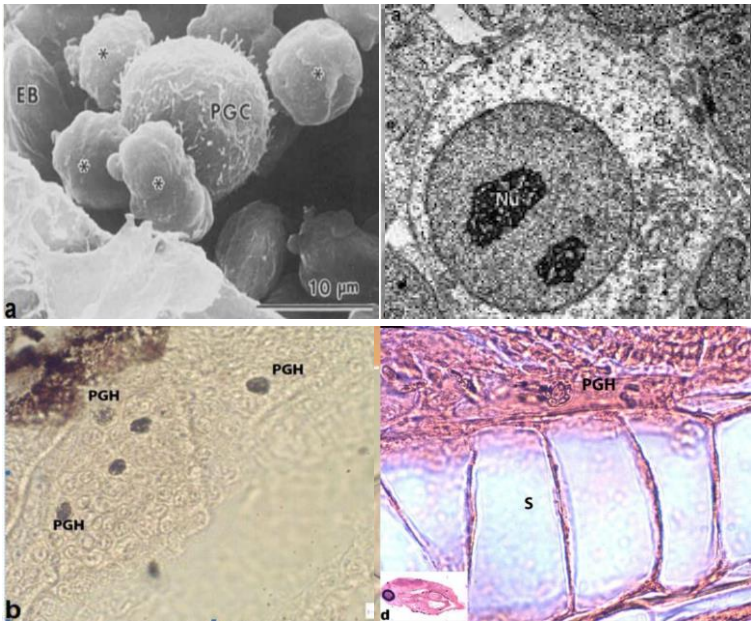


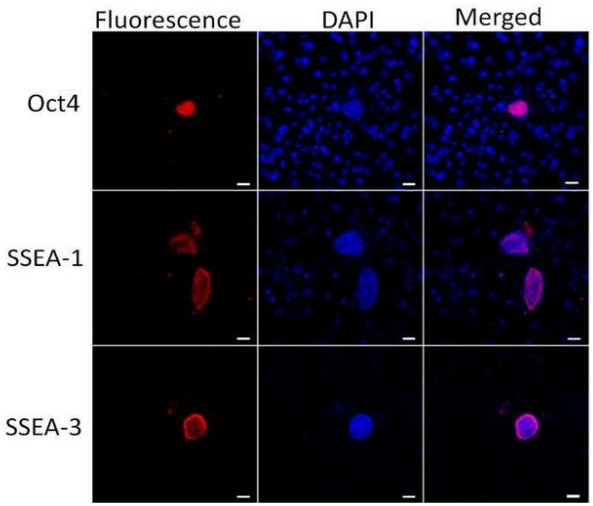




PGCs morphology

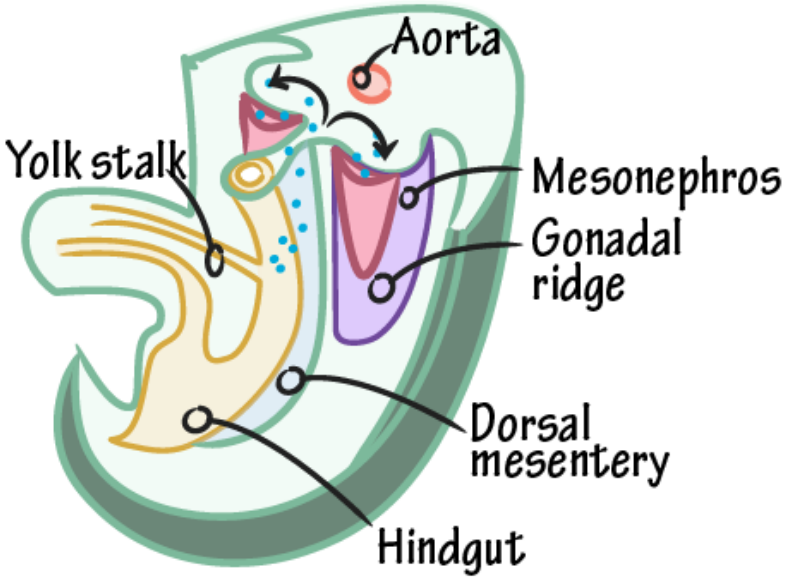
- large round/oval cells (10-20 μm)
- large excentric nucleus with prominent membrane
- dense a granular cytoplasm
- cytoplasmic deposits of glycogen a lipid droplets, round pale mitochondria, abundant ribosomes, ER and GA underdeveloped
- glycogen and lipids consumed during PGCs development and the number of mitochondria increases
- „nuage“ material – electron-dense granules lokalized on cytoplasmatic side of nuclear membrane
- histologically detectable alkalic phosphatase and PAS staining (periodic-acid-Schifft)
- imunofluorescence detection of transcriptional factors Vasa (DDX4), Nanos, Oct3/4, Stella, Fragilis, BLIMP1 a glycoprotein surface antigens SSEA-1/SSEA-3/SSEA-4



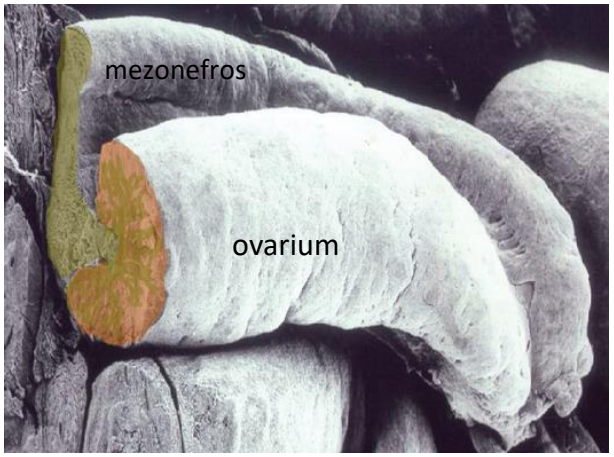


PGCs migration

- passive – ~week 4 pc
- embryonic disc is bending and a portion of the yolk sack is incorporated into the embryo body
- PGCs are translocated to endodermal epithelium of the hindgut

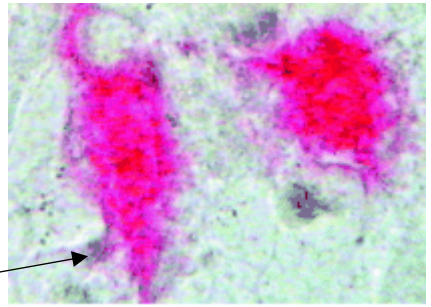


- active – 5-6th week pc
- PGCs penetrate the mesenchyme of the hindgut in the 16th somite region and migrate through dorsal mesentery in the lateral direction towards nesonefros
- Here, PCGS colonize mesenchymal ground of urogenital ridge in L1-L3 area → genital ridge → basis of paired **gonads**

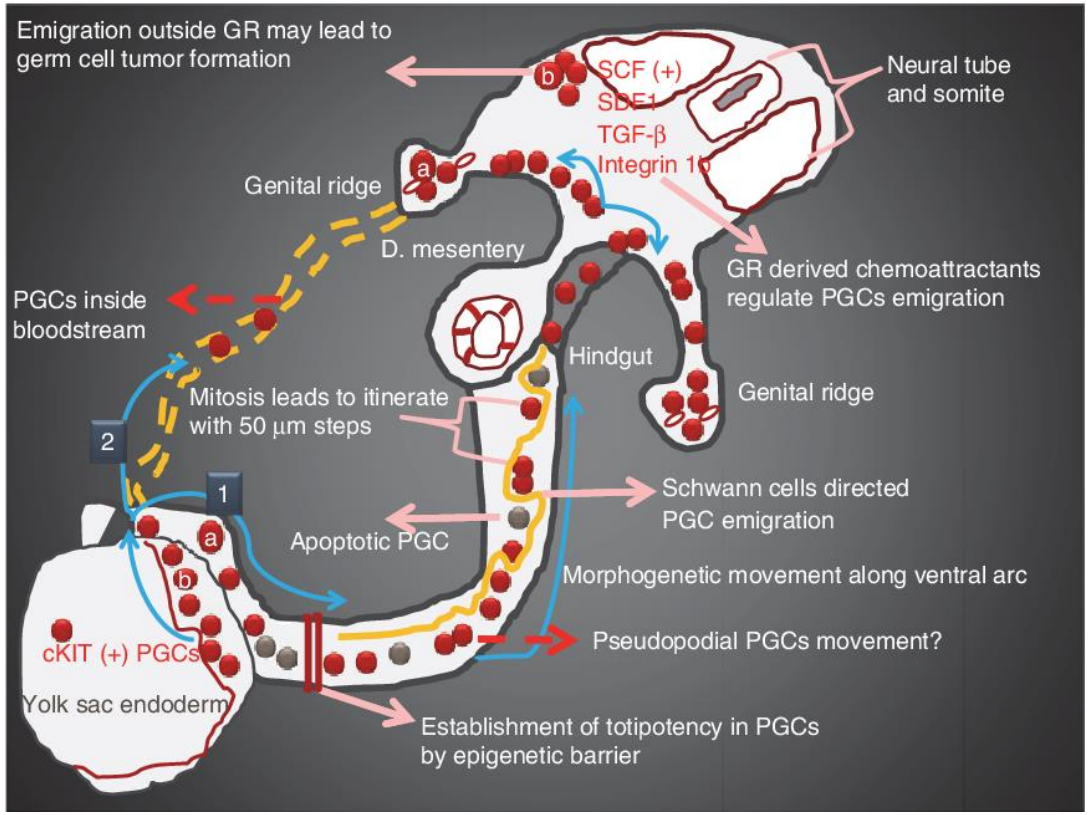


PGCs migration

- Active migration towards urogenital ridge
  - Ligand-receptor chemotaxis
    - atraction/repulsion signals from coelomic epithelium of gonadal ridge
      SCF/c-Kit ligand --- c-Kit receptor PGCs
      SDF1---- CXR4 receptor of PGCs

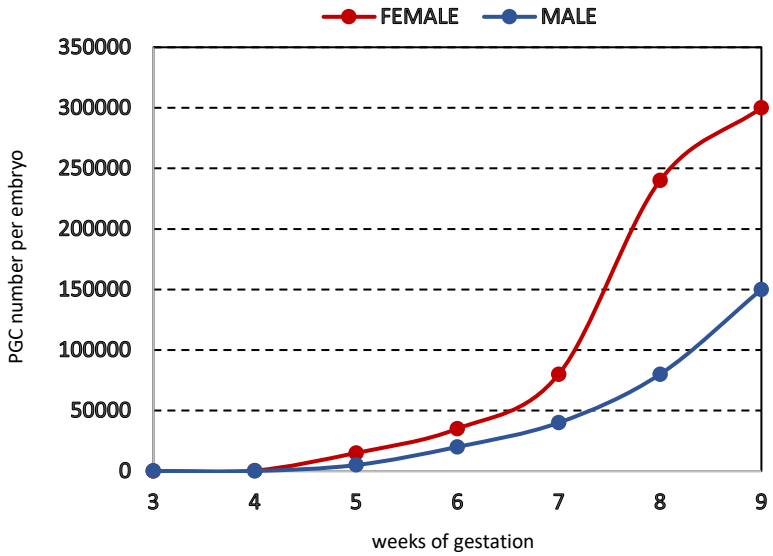


  - Migration along autonomous nerve fibres a Schwan cells
  - Interaction with ECM
    (β1 integrins)
  - ameboid movement

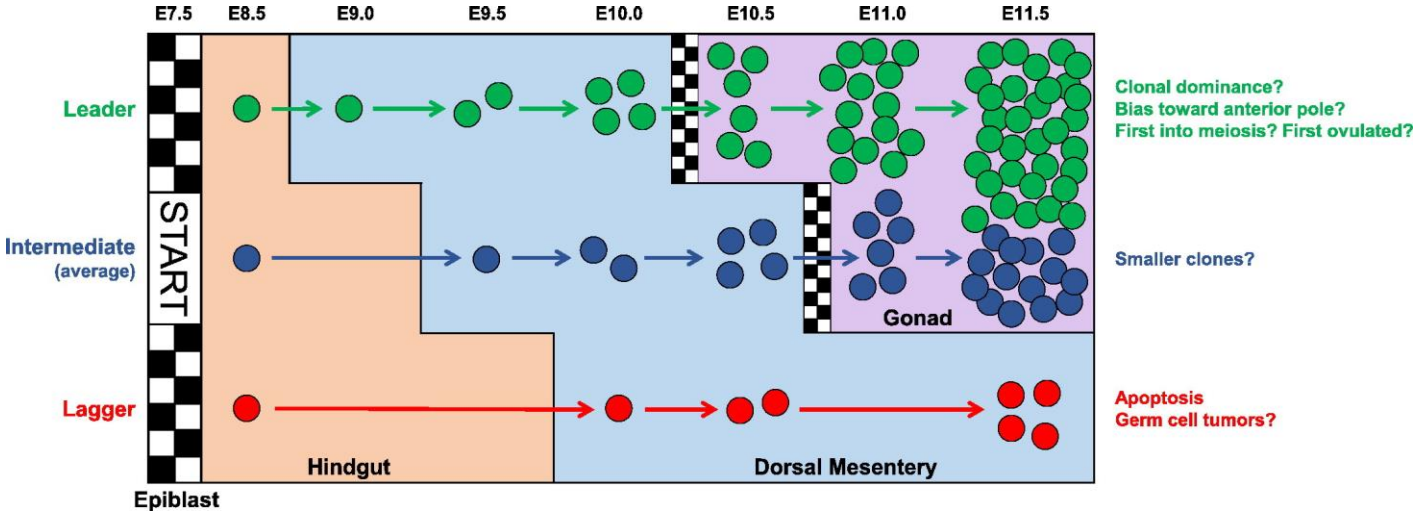


PGCs proliferation

- occurrs during migration and intensifies after arrival to genital ridges
- mitogen signalling from microenvironment
- selective mechanisms → survival vs. apoptosis

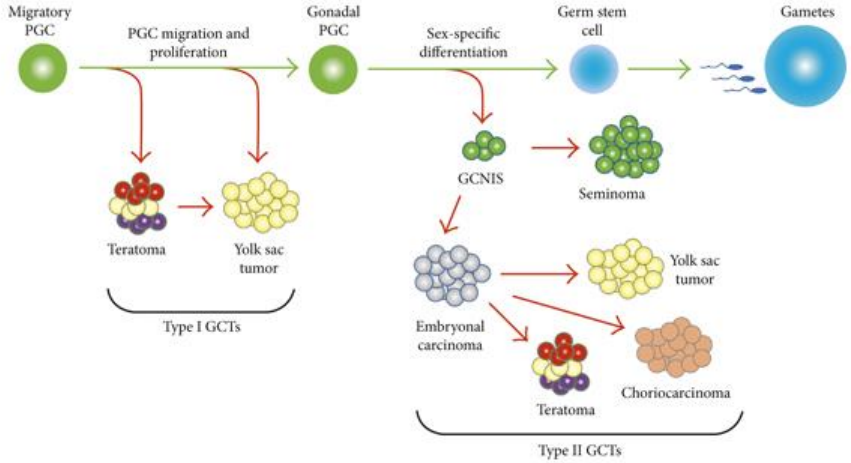


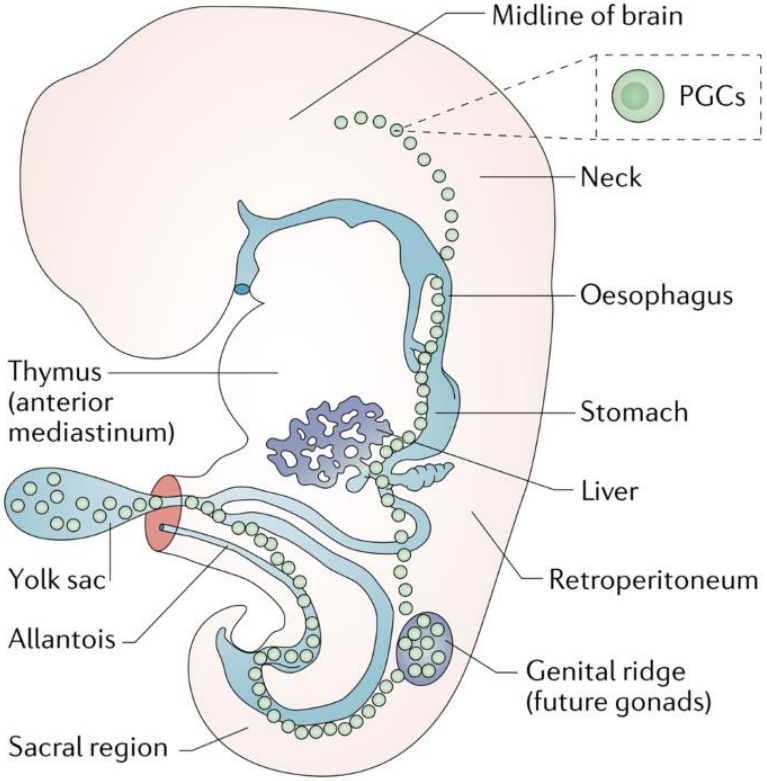
Cotticchio et al. Oogenesis. Springer 2013

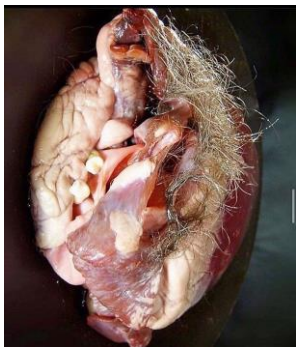


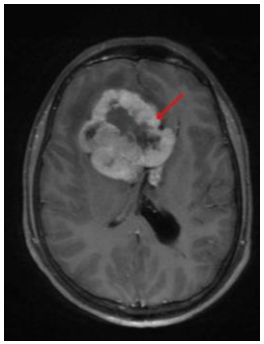
Ectopic localisation of PGCs

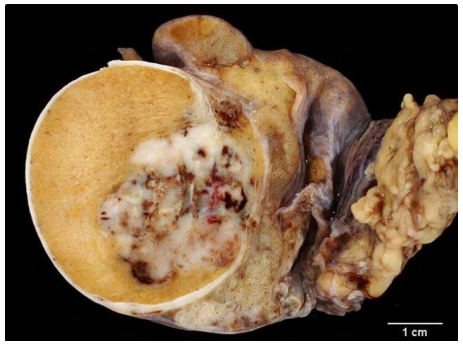
- defects of migration/colonisation/apoptic process can lead to **germ cells tumors**





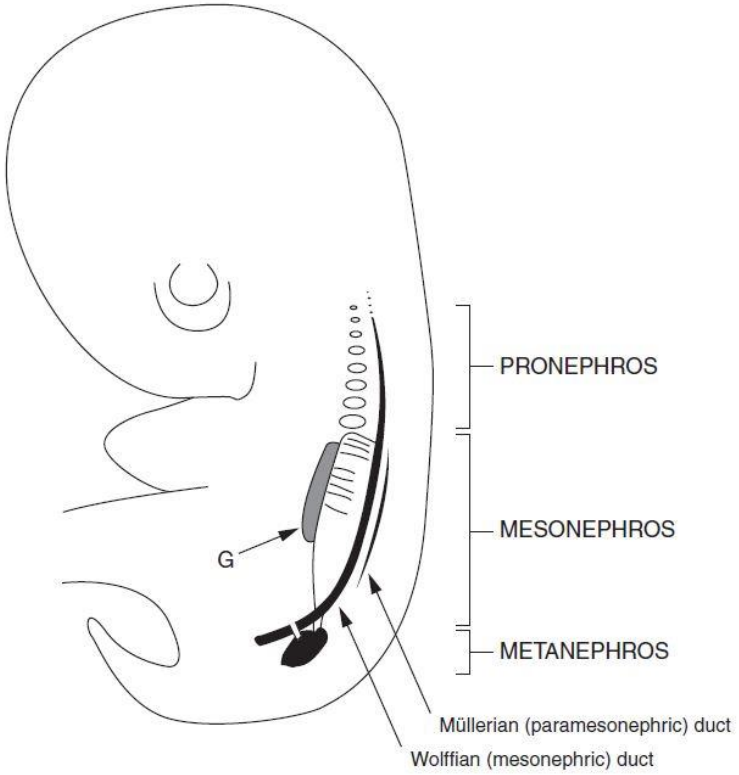


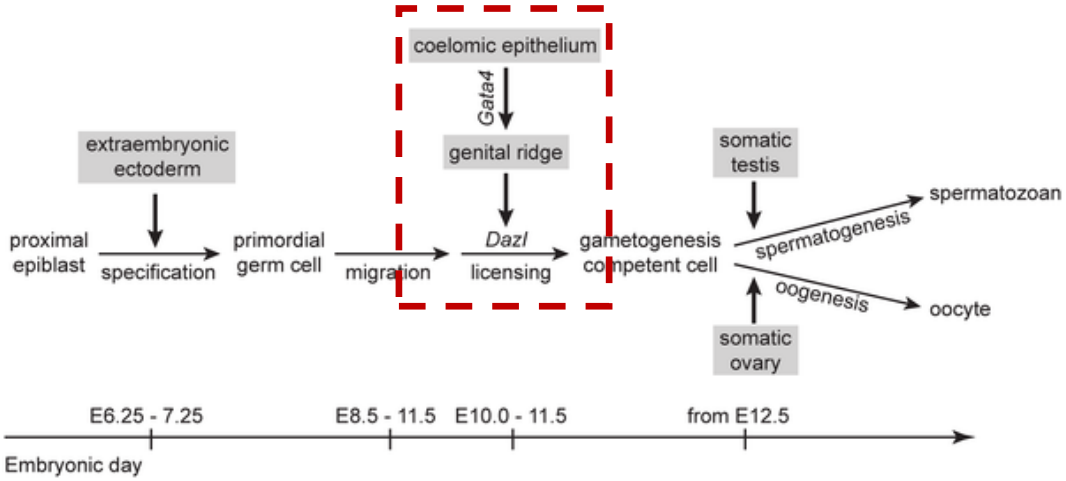




PGCs colonisation of gonads

- (uro)genital ridge
  - in Th6-S3 area
  - derived from mesoderm
  - formed by mesenchyme and covered by coelomic epithelium
- gonads develop in L1-L3 region
- epithelial cells from degenerating structures of mesonefros undergo epithelial-mesenchymal transition (EMT) and contributes to formation of sexually indiferent gonads
- Gata4 expresion in somatic tissue of gonadal ridge is critical for PGCs „ licencing“ for sexual differentiation





in Gata4 KO mice PGCs arrive to genital ridge but do not differentiate

Hu et al 2015

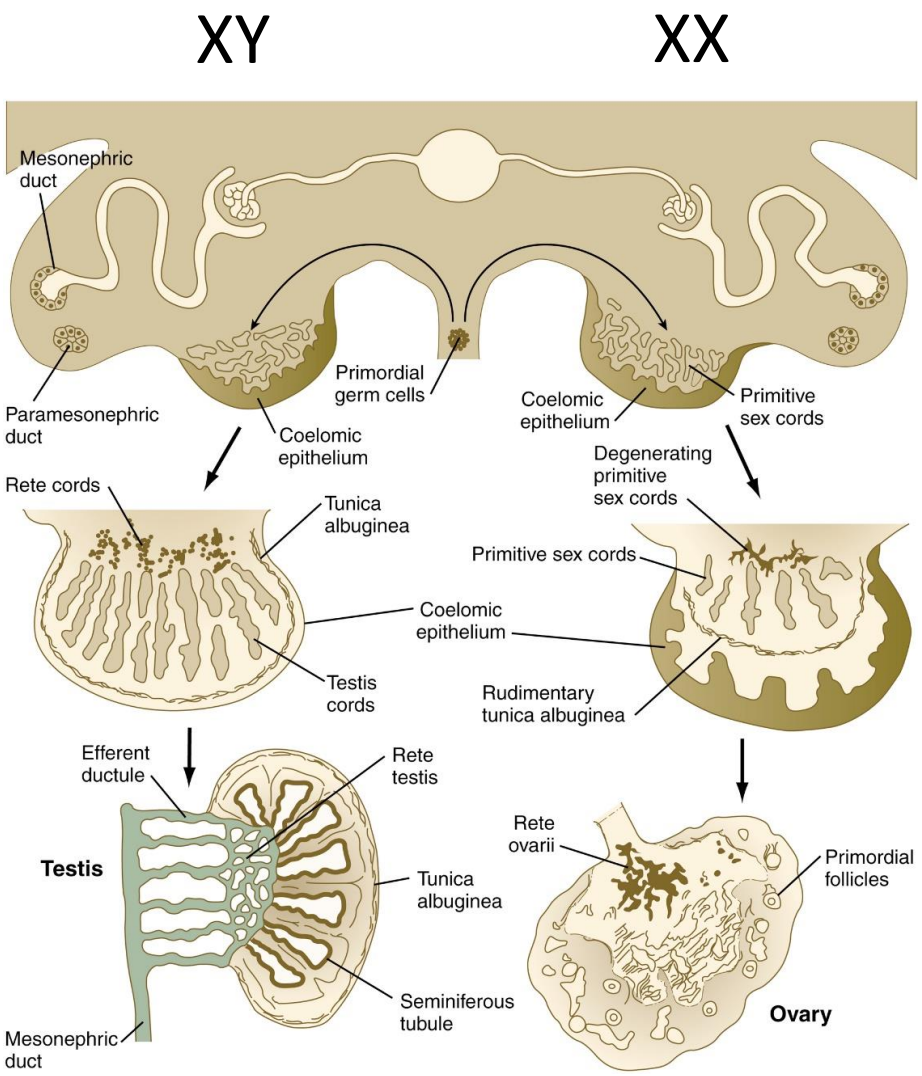
PGCs colonisation of gonads

- somatic portion of gonads is derived from
  - mezonefric mesenchyme
  - mezonefric epithelium
  - coelomic epithelium mesonefros

  + nerves, vessels, blood elements,...

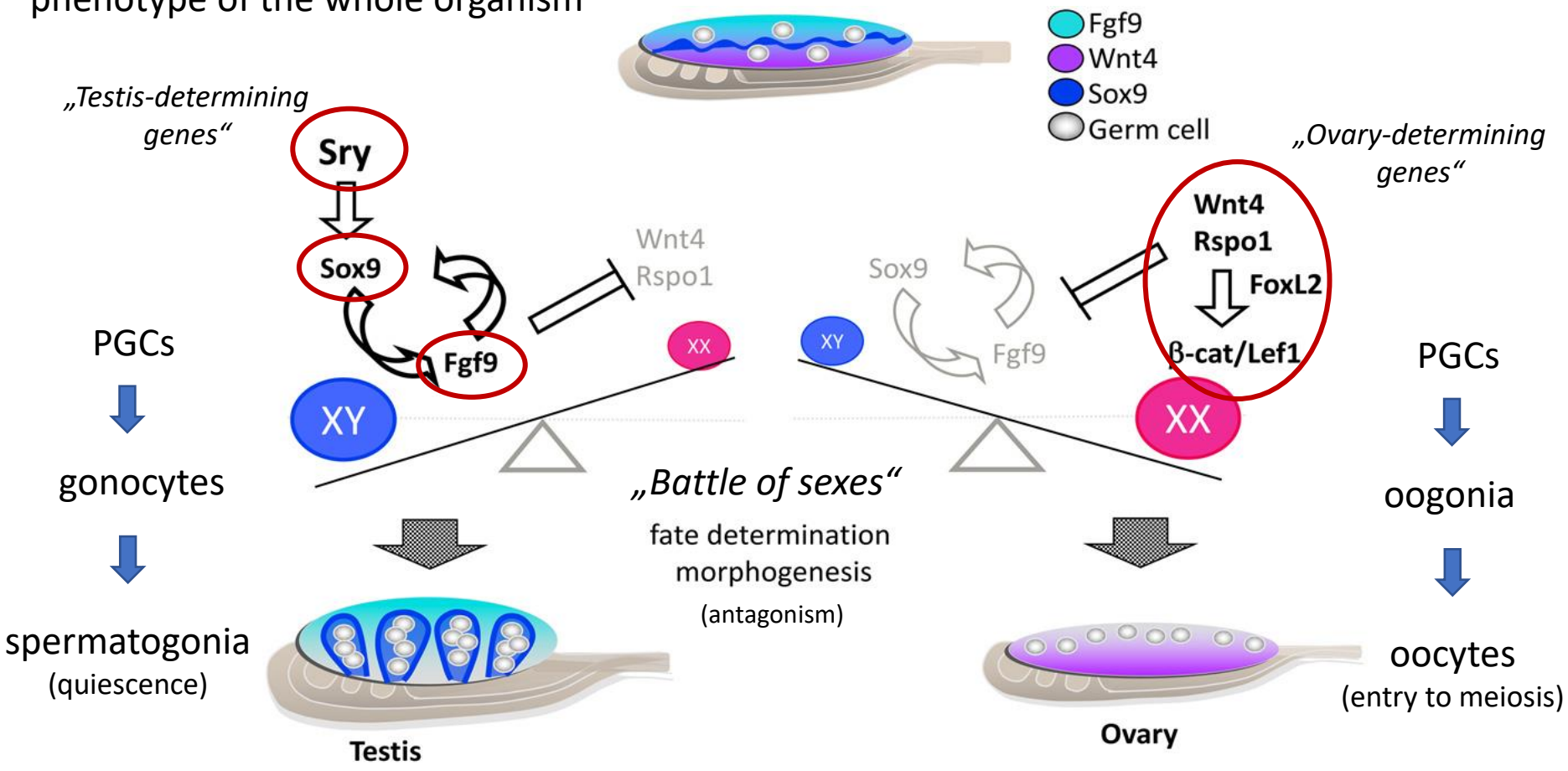
- **first PGCs arrive to sexually indiferrent primary sex cords ~30 day pc and colonisation of gonadal mesenchyme continues in following weeks (6-7th week pc)**

- PGCs expression of pluripotency markers decreases and so does PGCs´capability to generate pluripotent cells *in vitro*



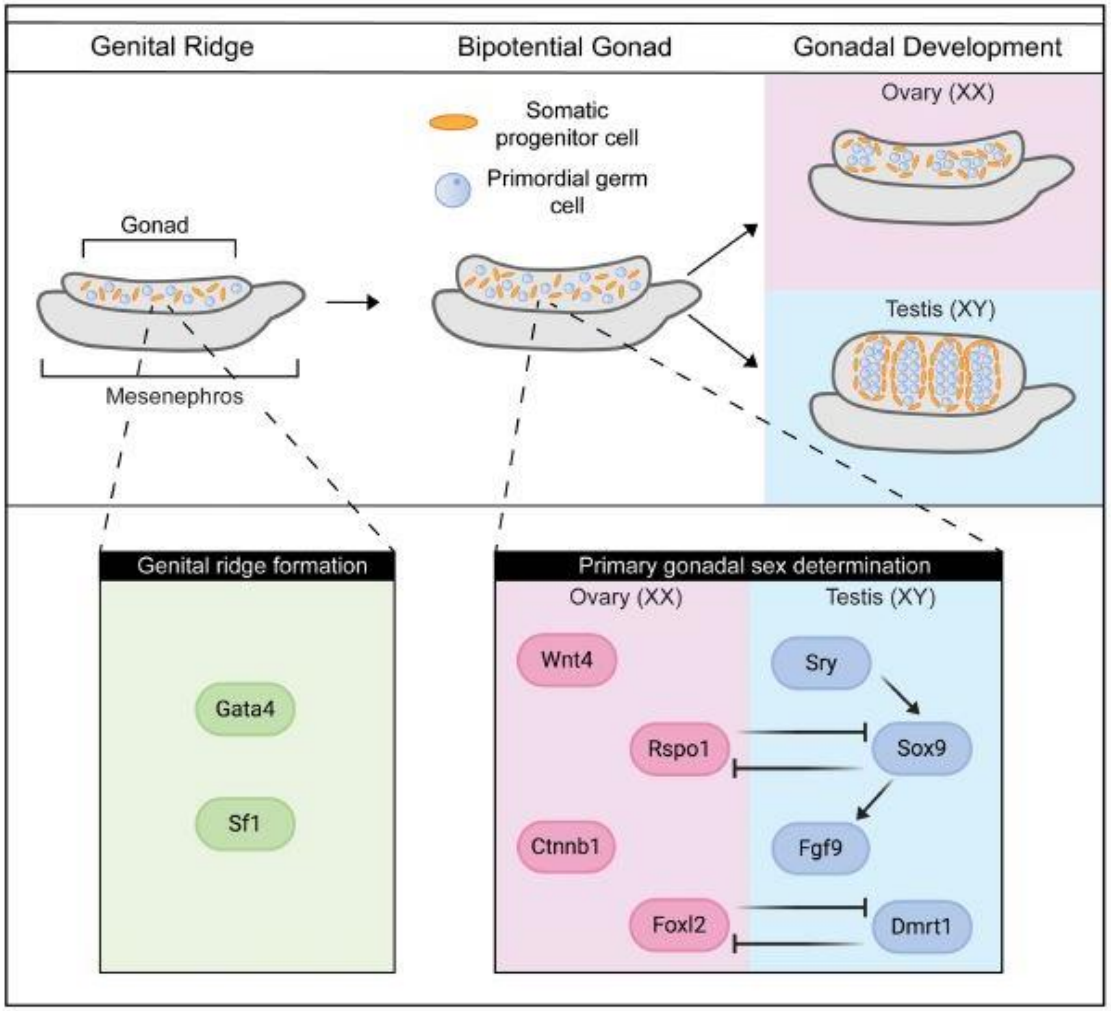
Sexual differentiation

- genetically encoded by *Sry* and Sry-box (*Sox9*) genes localized on chromosome Y
- expression of testis-/ovary-determing genes in somatic compartment of future gonads is critical for PGCs entry to either male or female gametogensis, sex of germ cells and phenotype of the whole organism



Sexual differentiation

The sex determining switch



Anne McLaren

- PGCs have a potential to enter either spermatogenesis or oogenesis

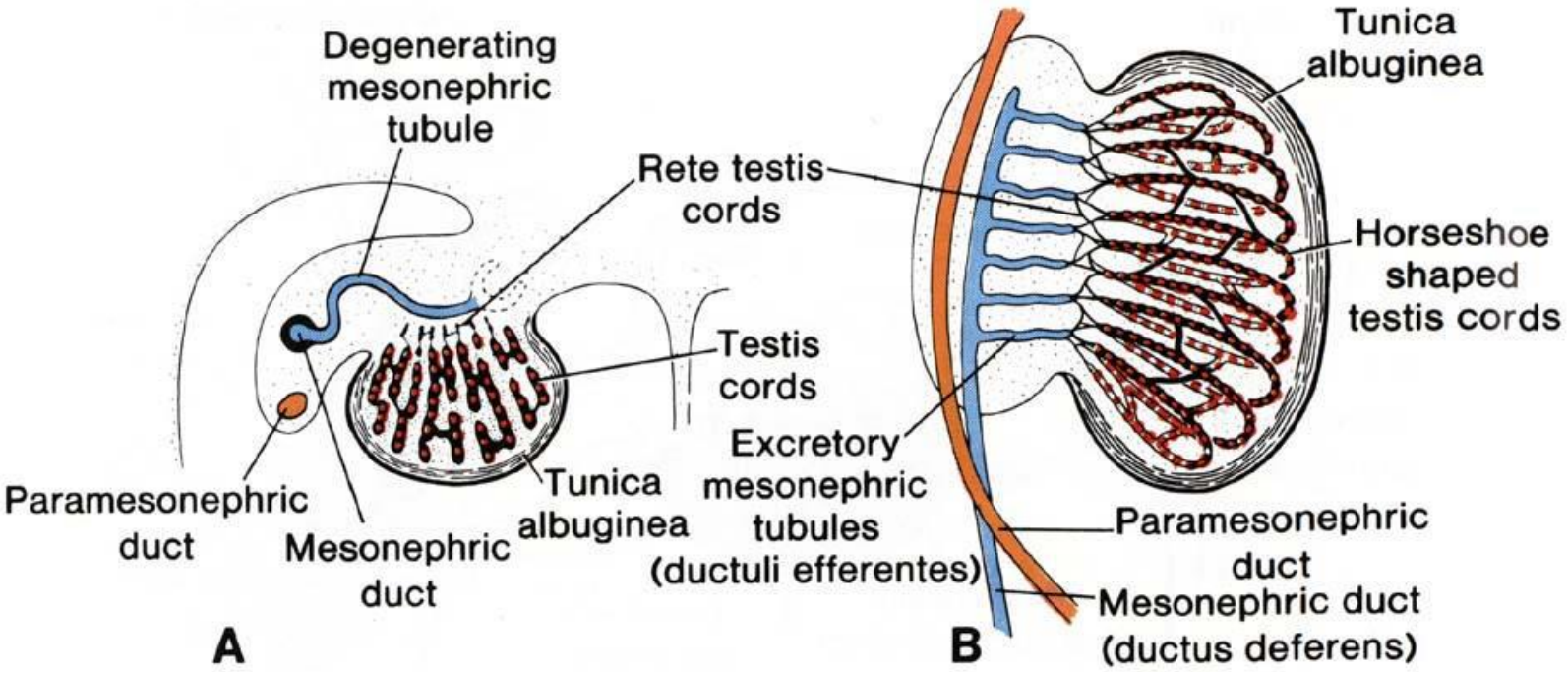
- *„the phenotypic sex is not determined by chromosomal constitution of germ cells (XX/XY) but cellular environment PGCs are exposed to during embryonic development“*

McLaren 1988

- germ cells are dispensable for organogesis of reproductive system!

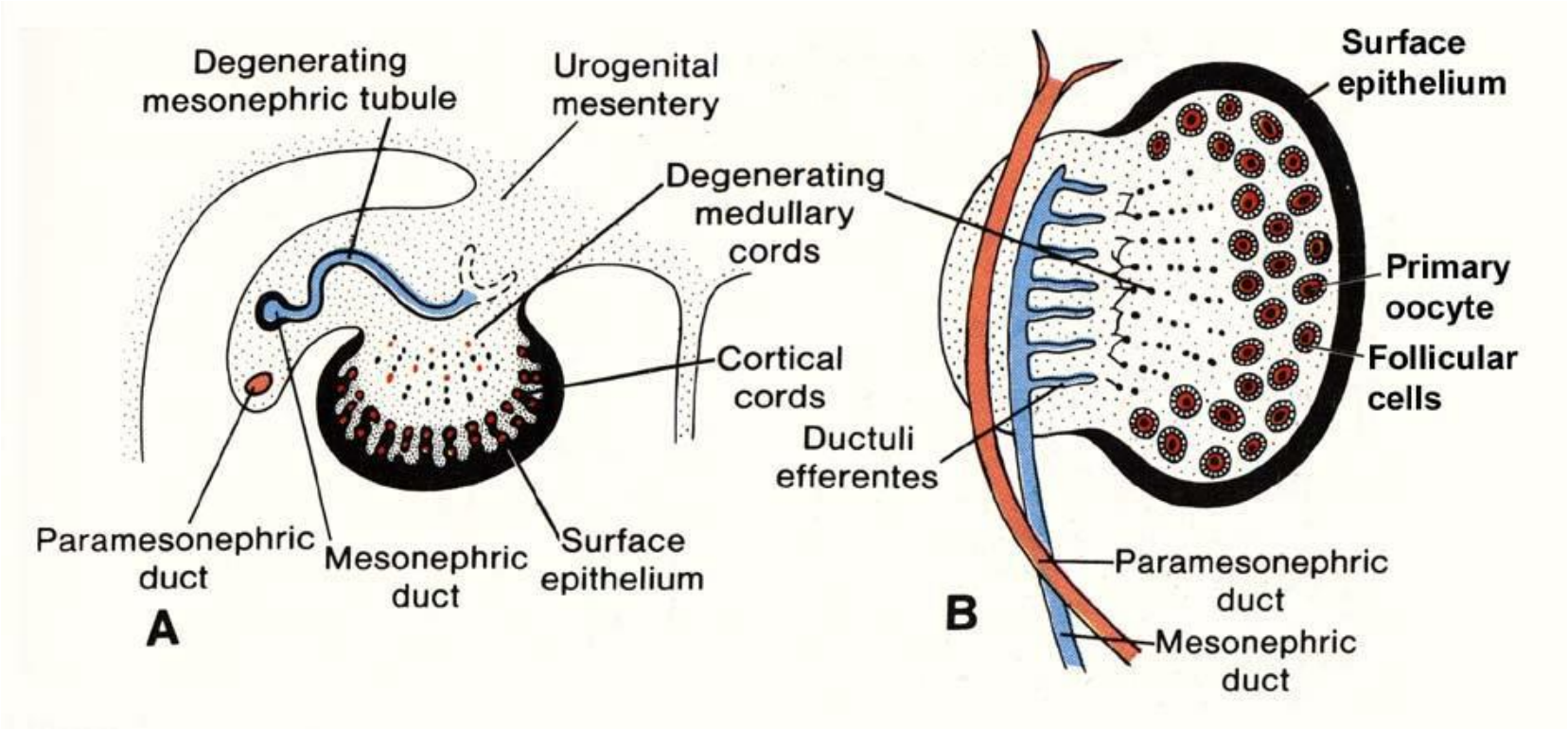
Testis development

- ***Sry-Sox9-FGF9*** expresion in somatic cells of indiferent gonadal region induces growth and sprouting of primitive sex cords in medullary region and their differentiation in seminiferous tubules which are colonized by PGCs (gonocytes)
- connecting primitive seminiferous tubules to mesonefros forms anastomotic network (***rete testis***)
- **Sertoli cells** arise from sex cord endothelium and start to produce **antimüllerian hormone (AMH)**
- **Leydig cells** originate from intermediate mesenchyme and produce **testosteron**
- PGCs immigrated into a developing testis (gonocyty) intensively proliferate, and during 2nd trimestr differentiate to mitotically-inactive **prospermatogonia**



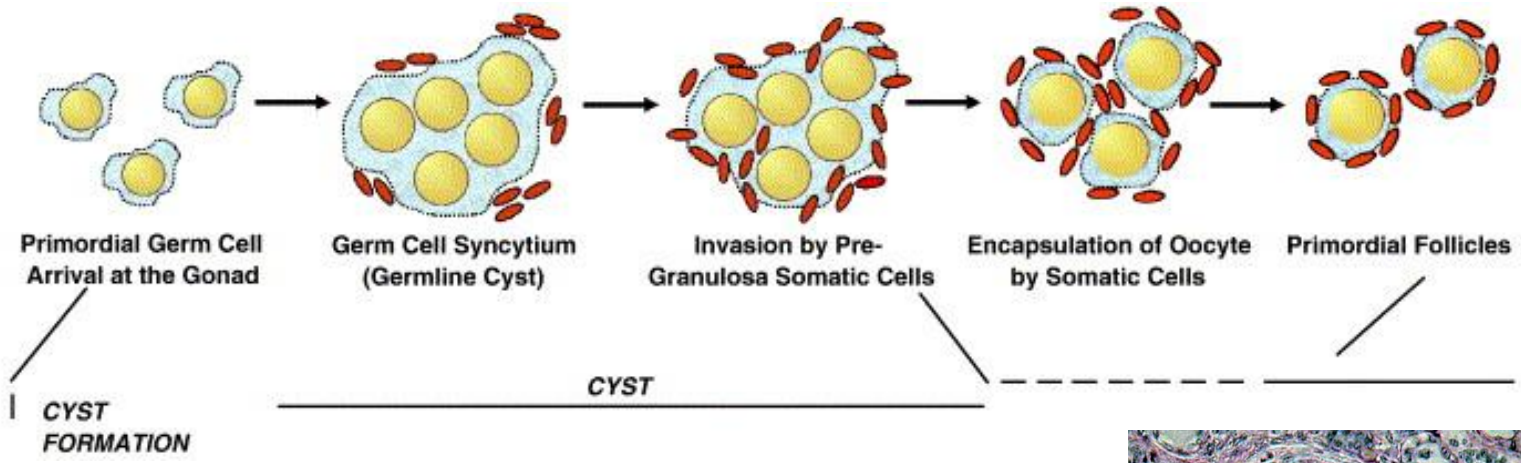
Ovary development

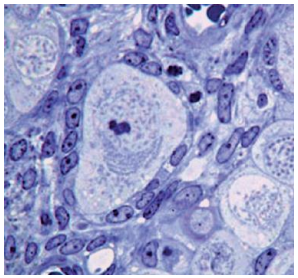
- **No *Sry-Sox9-FGF9 expression***
- primary sex cords in medullar region undergo **fragmentation** and are replaced by vascular fibrous tissue
- in cortical part of ovary, primary sex cord cells undego secondary diferentiation and surround immigrated PGCs



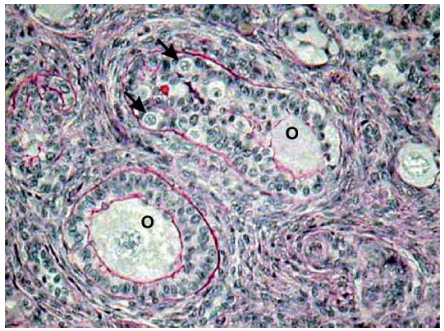
Ovary development

- Immigrated PGCs diferentiate to **oogonia**, which first divide by mitosis and later enter to meiosis but arrest in prophase
- clusters of oogonia are surrounded by somatic cells which later invade syncytium
- individual meiotically arrested oocytes enclosed by single layer of follicular (pre-granulosa) cells form **primordial follicle**





Asociation with somatic cells is critical for oocyte survival!



Germ cell entry to meiosis

- **Retinoic acid (RA)**

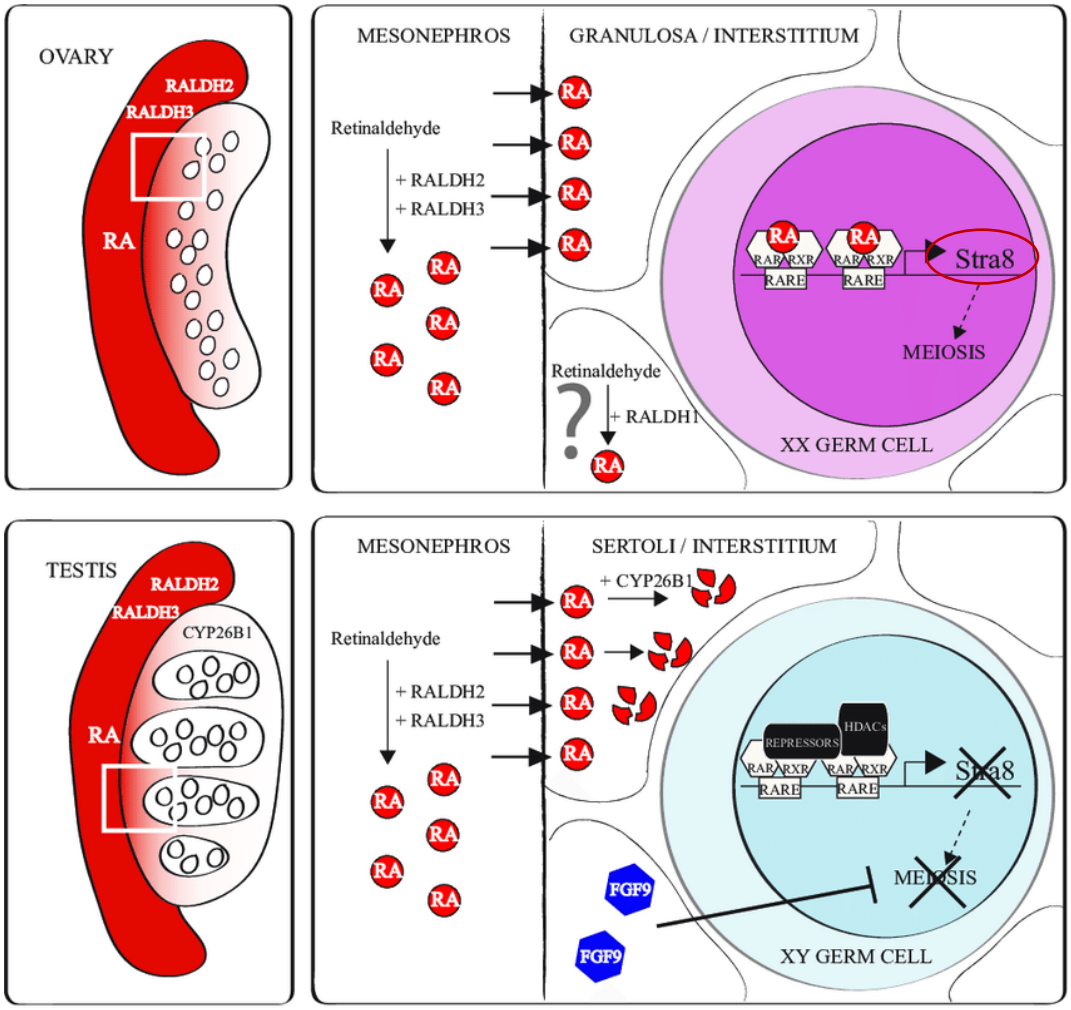
= meiosis inducing factor

- derived from retinaldehyde oxidation by dehydrogenase produced in mesonefric tissue surrounding the gonads

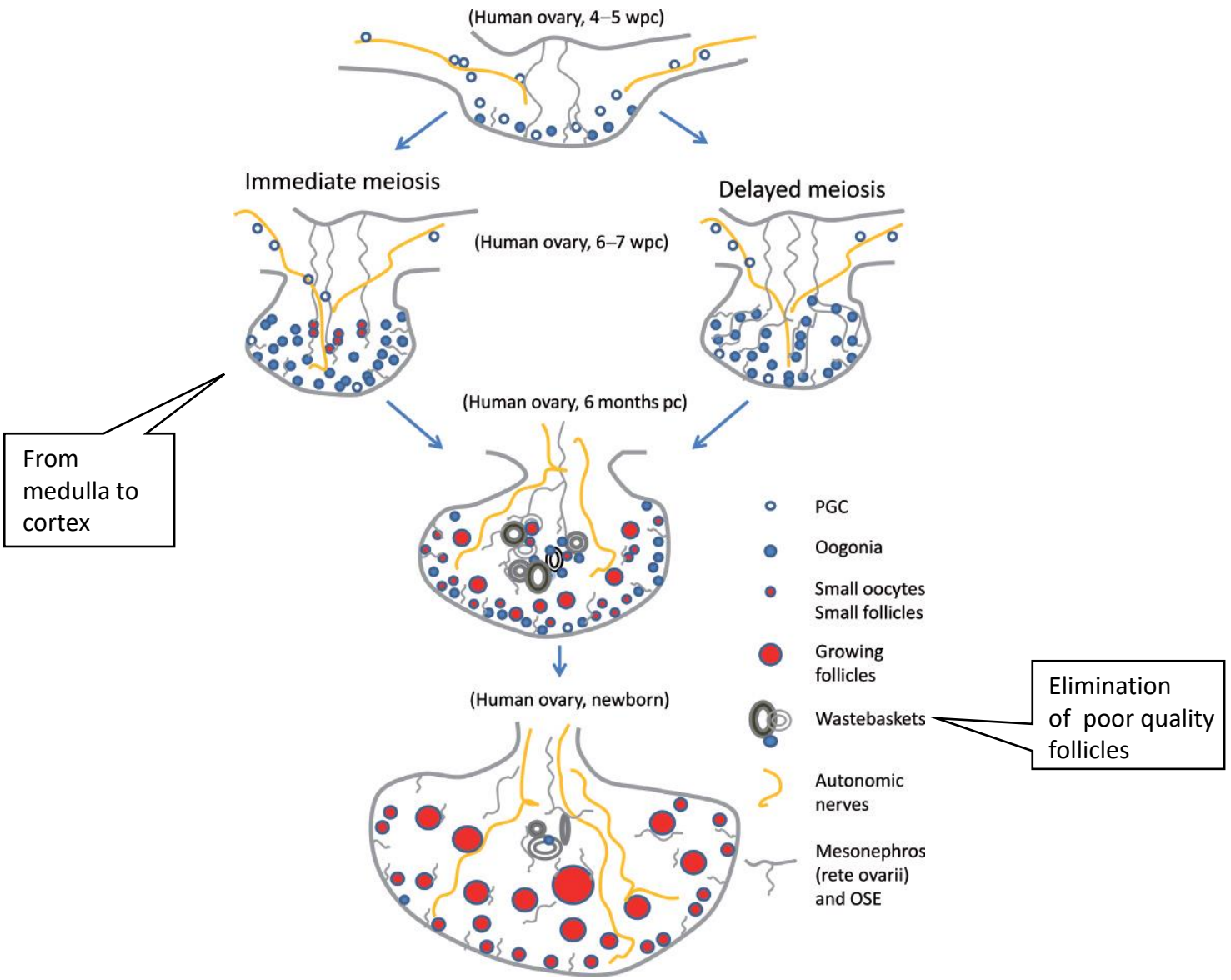
- causes activation of nuclear receptors RAR/RXR which modulate transcription of RA-responsive elements

- in the ovary RA stimulates oogonia to enter meiosis

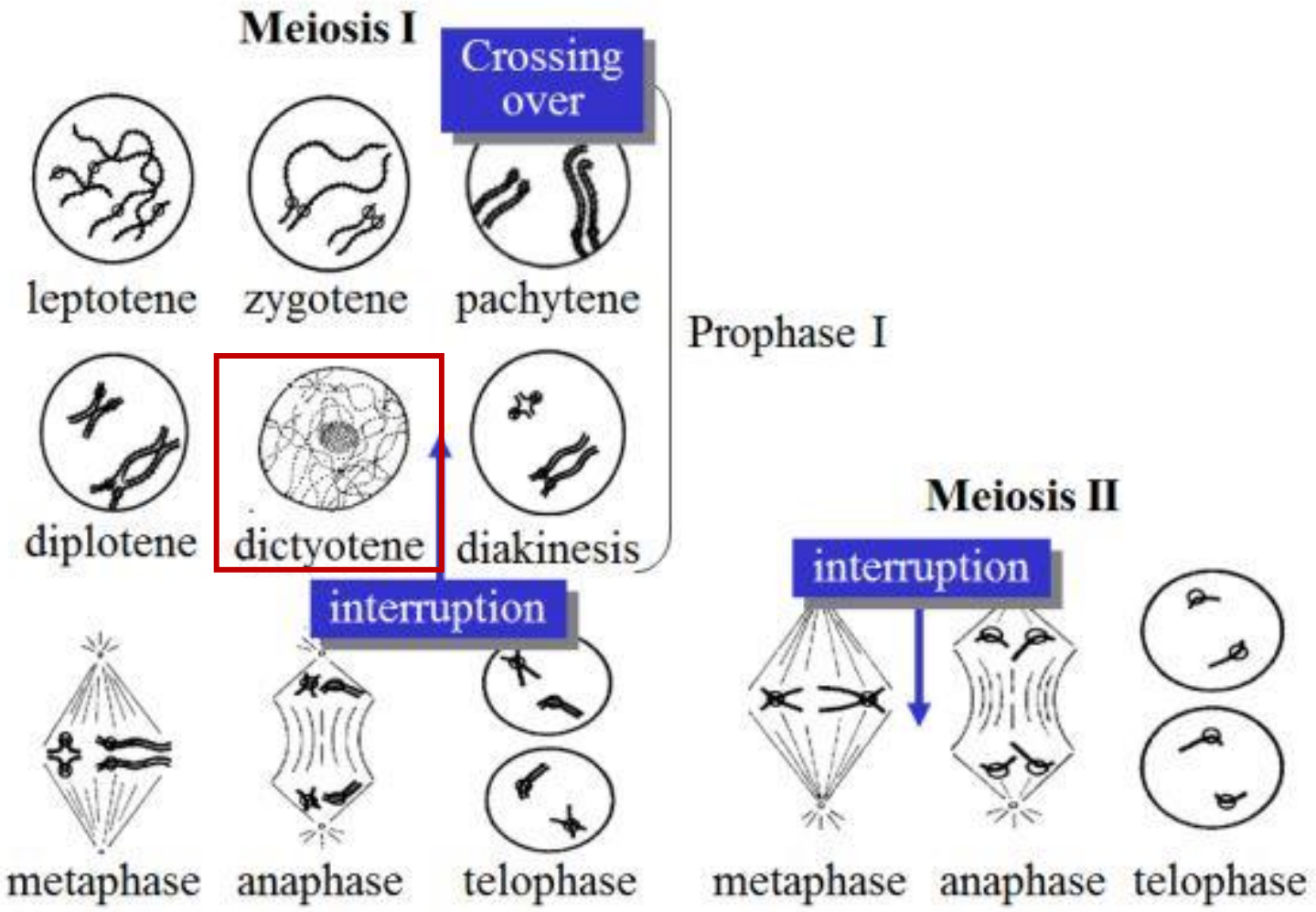
- in fetal testis RA actively degraded by cytP450 (CYP26B1)



Meiosis in the ovary



Female meiosis timeline



Ovarian stem cells

- Report about presence of mitotically active **ovarian stem cells (OSC)** in adult mouse and human ovary
- *de novo* oogenesis in adults?

Johnson et al, Nature 2004.

articles

Germline stem cells and follicular renewal in the postnatal mammalian ovary

Joshua Johnson*, Jacqueline Canning*, Tomoko Kaneko, James K. Pru & Jonathan L. Tilly

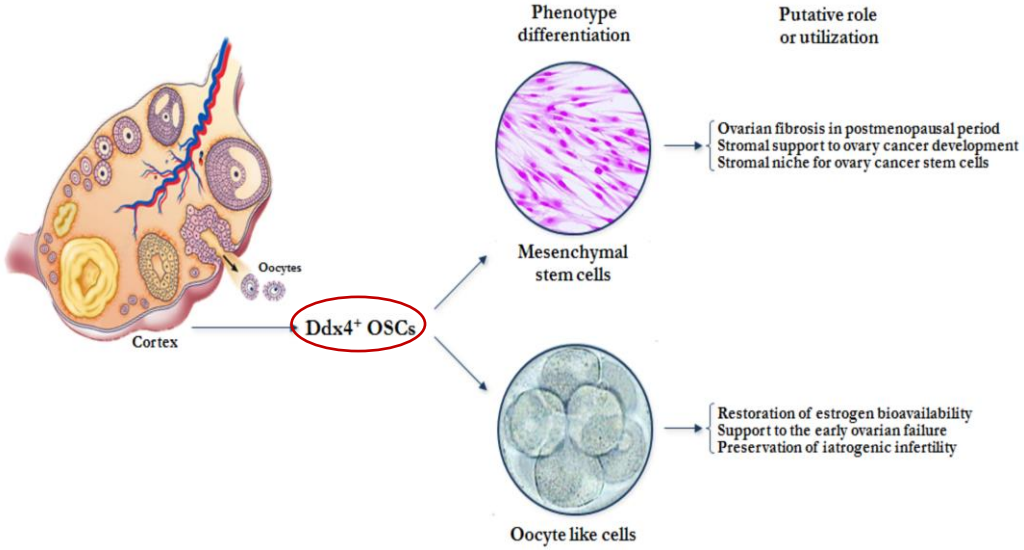
Vincent Center for Reproductive Biology, Vincent Obstetrics and Gynecology Service, Massachusetts General Hospital, and Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, Massachusetts 02114, USA

*These authors contributed equally to this work

A basic doctrine of reproductive biology is that most mammalian females lose the capacity for germ-cell renewal during fetal life, such that a fixed reserve of germ cells (oocytes) enclosed within follicles is endowed at birth. Here we show that juvenile and adult mouse ovaries possess mitotically active germ cells that, based on rates of oocyte degeneration (atresia) and clearance, are needed to continuously replenish the follicle pool. Consistent with this, treatment of prepubertal female mice with the mitotic germ-cell toxicant busulphan eliminates the primordial follicle reserve by early adulthood without inducing atresia. Furthermore, we demonstrate cells expressing the meiotic entry marker synaptonemal complex protein 3 in juvenile and adult mouse ovaries. Wild-type ovaries grafted into transgenic female mice with ubiquitous expression of green fluorescent protein (GFP) become infiltrated with GFP-positive germ cells that form follicles. Collectively, these data establish the existence of proliferative germ cells that sustain oocyte and follicle production in the postnatal mammalian ovary.

Jonathan Tilly

CONTROVERSIAL



ARTICLES

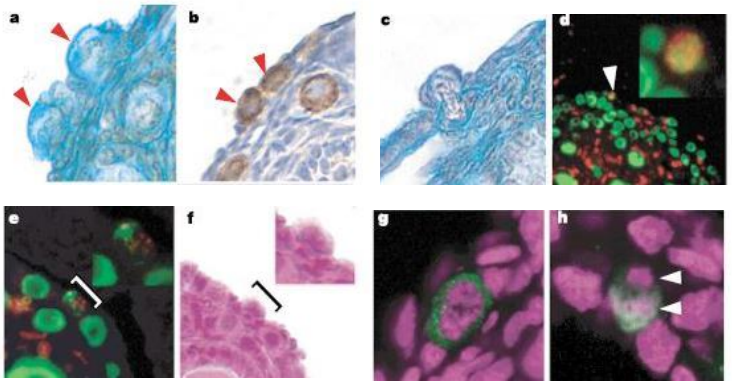
nature medicine

White et al, Nature Medicine 2012.

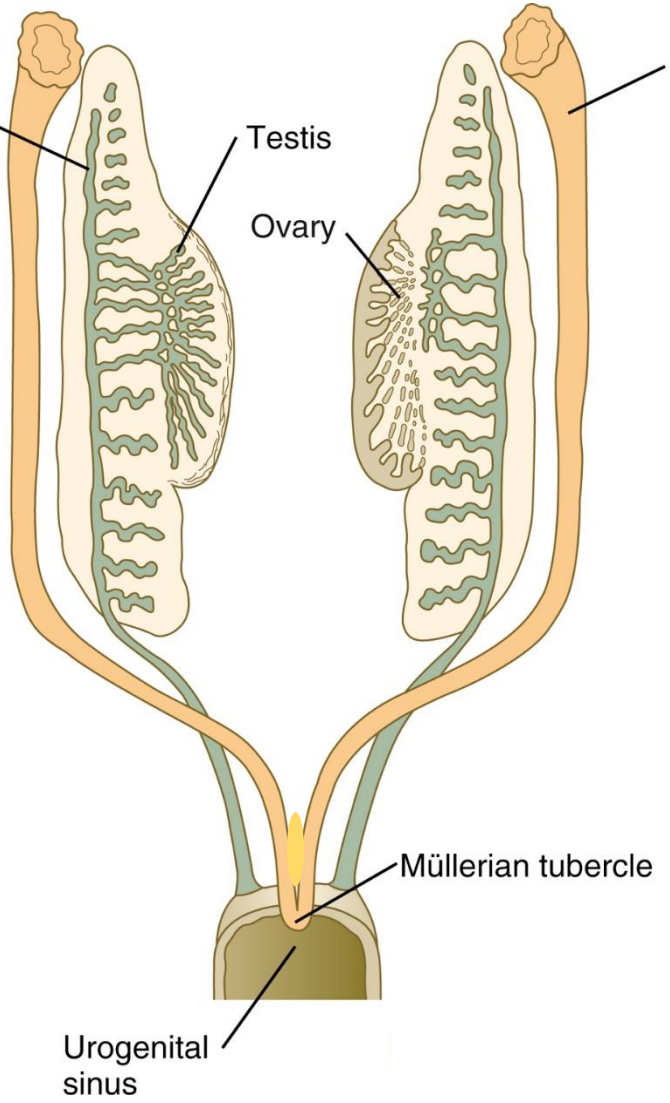
Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women

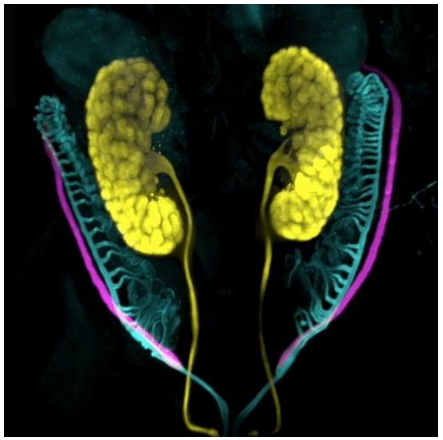
Yvonne A R White[1,2,4], Dori C Woods[1,2,4], Yasushi Takai[3], Osamu Ishihara[3], Hiroyuki Seki[3] & Jonathan L Tilly[1,2]

Germline stem cells that produce oocytes *in vitro* and fertilization-competent eggs *in vivo* have been identified in and isolated from adult mouse ovaries. Here we describe and validate a fluorescence-activated cell sorting-based protocol that can be used with adult mouse ovaries and human ovarian cortical tissue to purify rare mitotically active cells that have a gene expression profile that is consistent with primitive germ cells. Once established *in vitro*, these cells can be expanded for months and can spontaneously generate 35- to 50-μm oocytes, as determined by morphology, gene expression and haploid (1*n*) status. Injection of the human germline cells, engineered to stably express GFP, into human ovarian cortical biopsies leads to formation of follicles containing GFP-positive oocytes 1–2 weeks after xenotransplantation into immunodeficient female mice. Thus, ovaries of reproductive-age women, similar to adult mice, possess rare mitotically active germ cells that can be propagated *in vitro* as well as generate oocytes *in vitro* and *in vivo*.

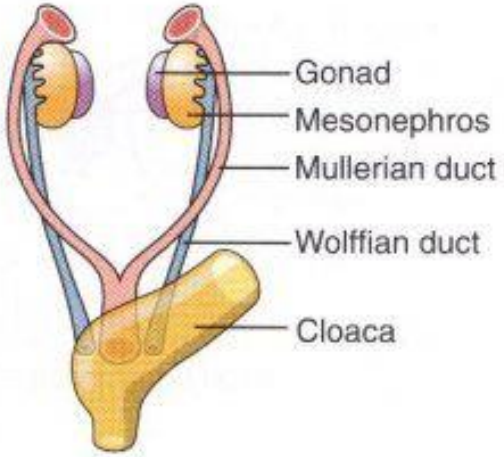


Development of reproductive tract





Development of reproductive tract



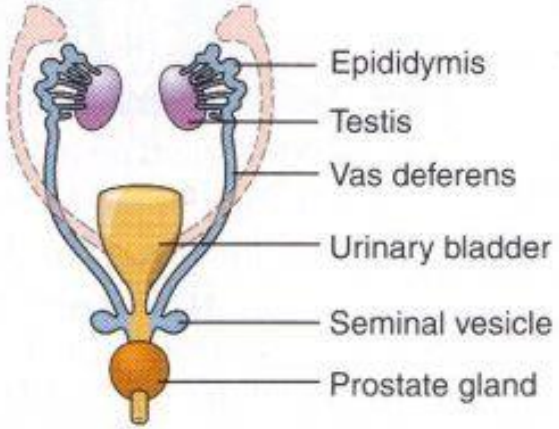
INDIFFERENT STAGE

Leydig cells ⇩ testosterone ⇩ **development of Wolffian duct**

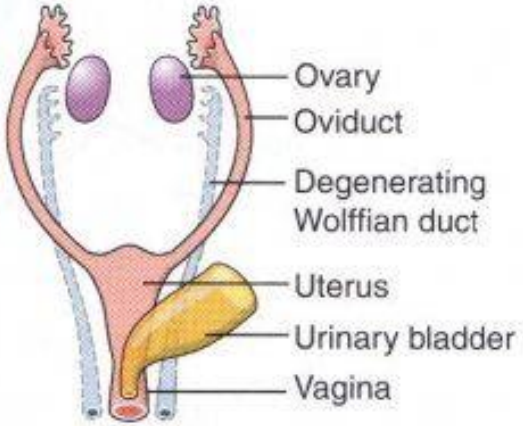
Sertoli cells ⇩ AMH ⇩ **regression of Müllerian duct**

~~Leydig cells~~ ⇩ ~~testosterone~~ ⇩ **regression of Wolffian duct**

~~Sertoli cells~~ ⇩ ~~AMH~~ ⇩ **development of Müllerian duct**



MALE



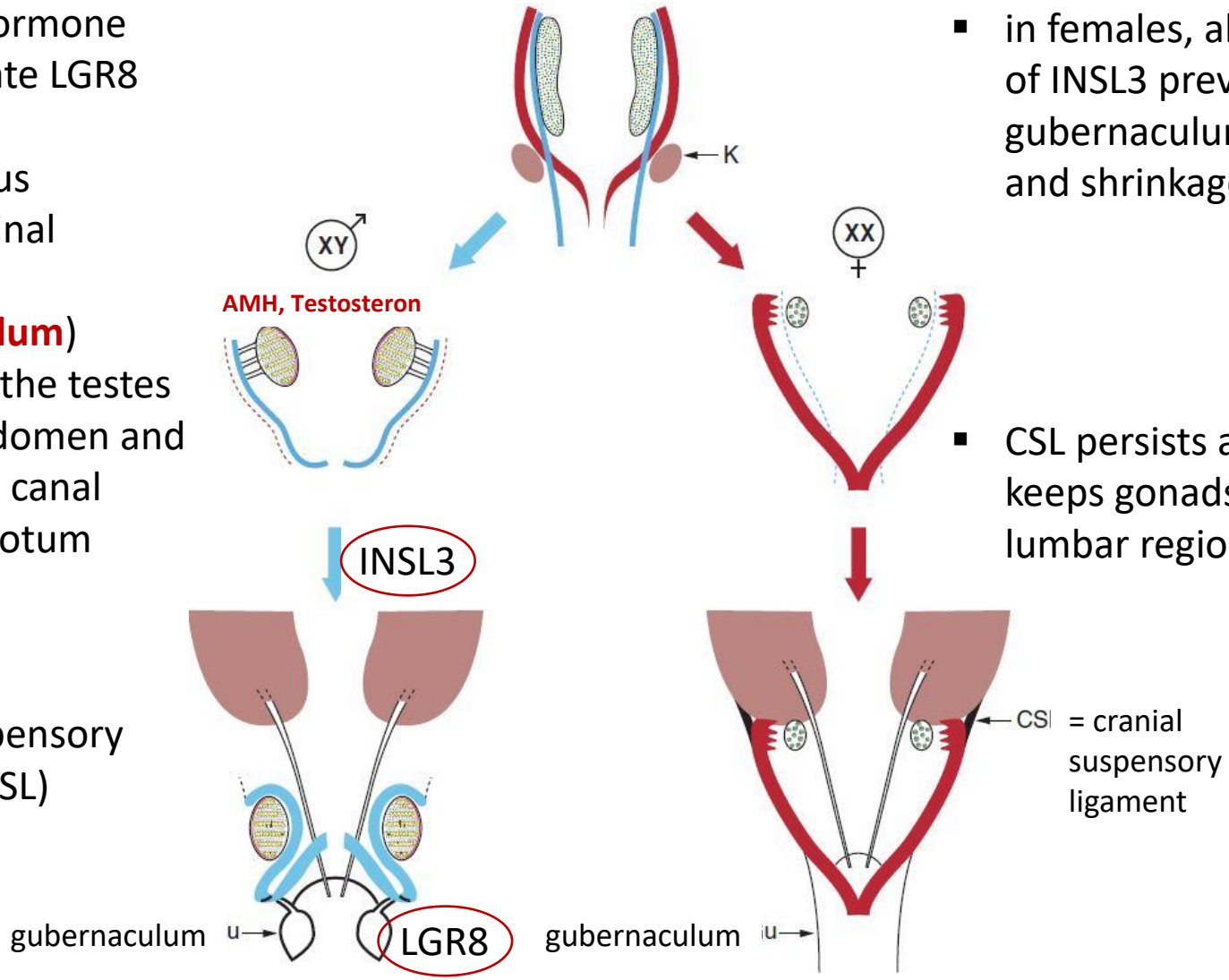
FEMALE

8-9th week pc

Development of reproductive tract

- testicular hormone INSL3 activate LGR8 receptor in mucofibrosus genito-inguinal ligament (**gubernaculum**) which pulls the testes through abdomen and the inguinal canal down to scrotum

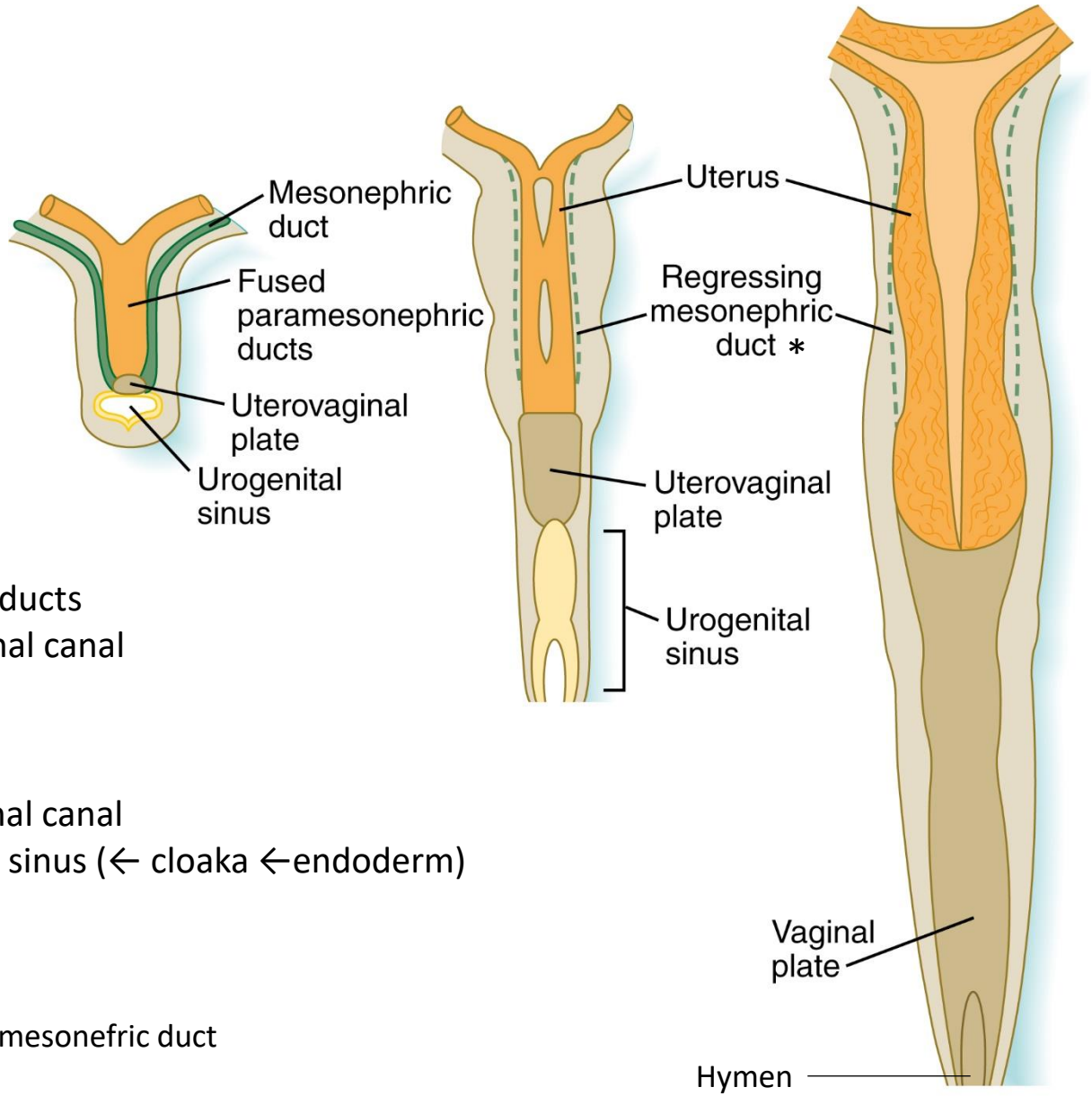
- cranial suspensory ligament (CSL) regresses



- in females, absence of INSL3 prevents gubernaculum grown and shrinkage

- CSL persists and keeps gonads in lumbar region

Development of female reproductive tract

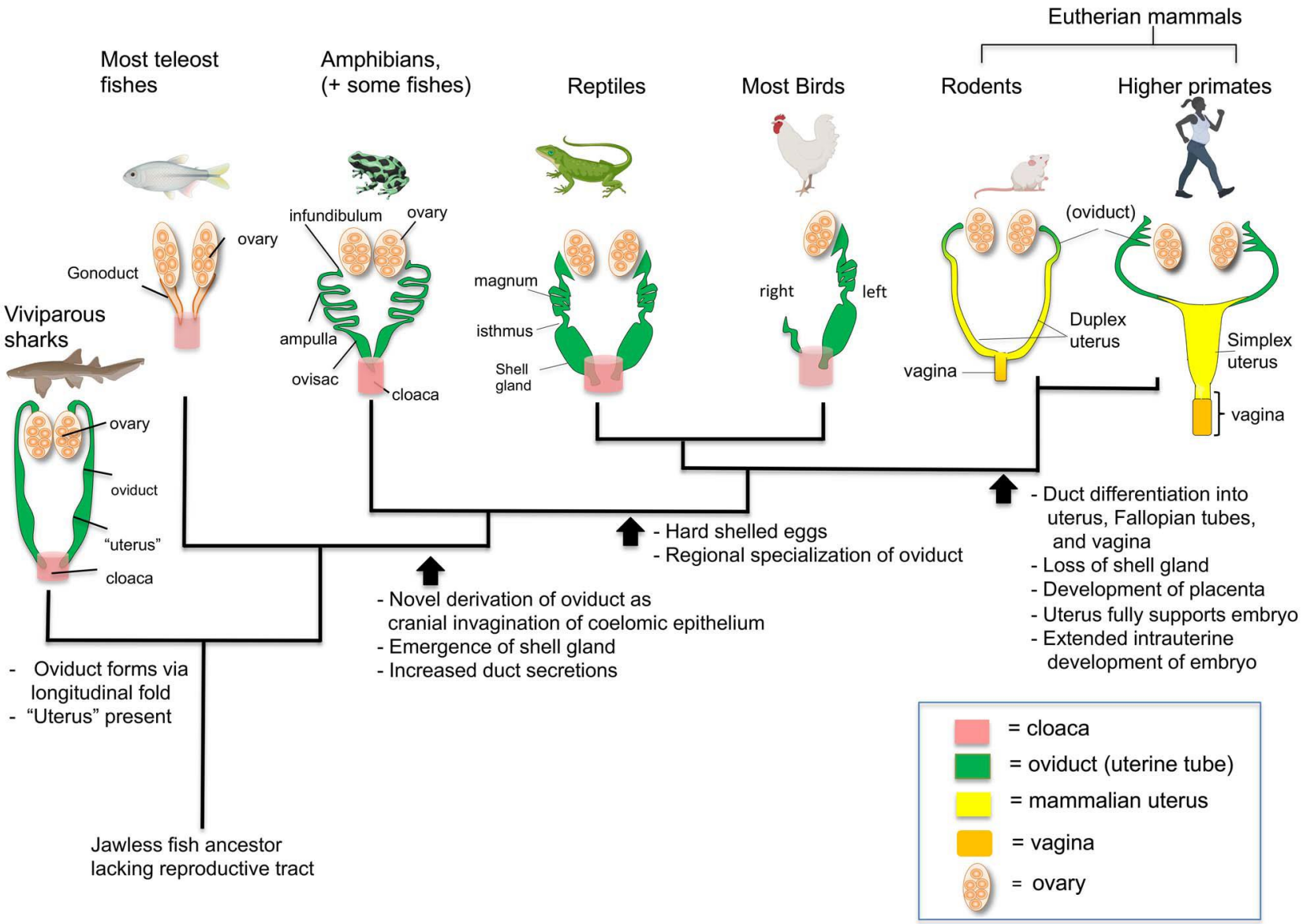


UTERUS
← caudal part of Müllerian ducts
← cranial part of uterovaginal canal

VAGINA
← caudal part of uterovaginal canal
← cranial part of urogenital sinus (← cloaka ←endoderm)

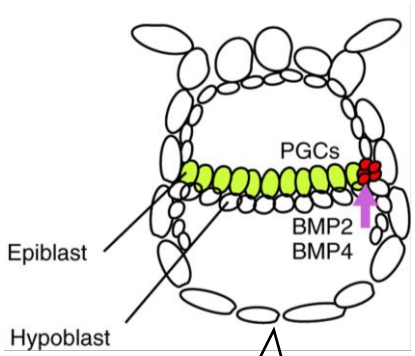
* Gartner´s canal – remnant of mesonefric duct

Evolution of female reproductive tract



Major et al, 2022

Molecular regulation of PGCs development

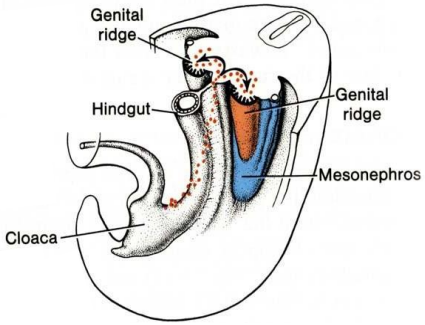


- **PGCs specification**

BMP4/BMP8
WNT
FGF?

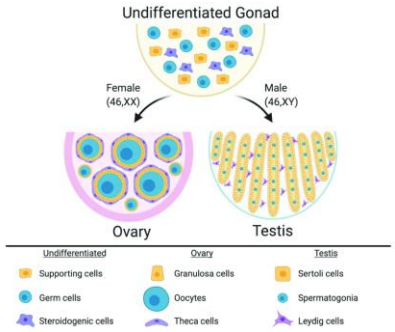
BLIMP1/PRDM1
Sox17
Fragilis
Stella
TNAP

Oct3/4
Nanog



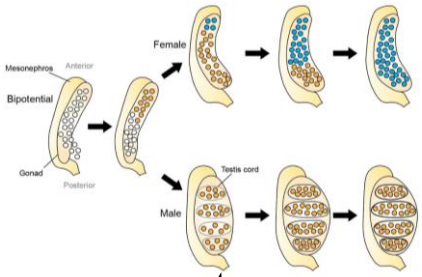
- **PGCs migration**

SCF/c-Kit ligand - c-Kit receptor
SDF1- CXR4 recptor
ADAM
PECAM1



- **Genital ridge colonization**

Dazl
Ddx4
Wt1
Gcna1
Gata4



- **Sexual diferentiation**

Sry
Sox9
FGF9
Wnt4
Rspo1
FoxL2
β-catenin

- **Meiosis entry**

RARLDH (RA)
Stra8
Cyt26B1

- **PGCs proliferation**

PTEN
WNT
Nanos3
Pog

Reproductive system development overview