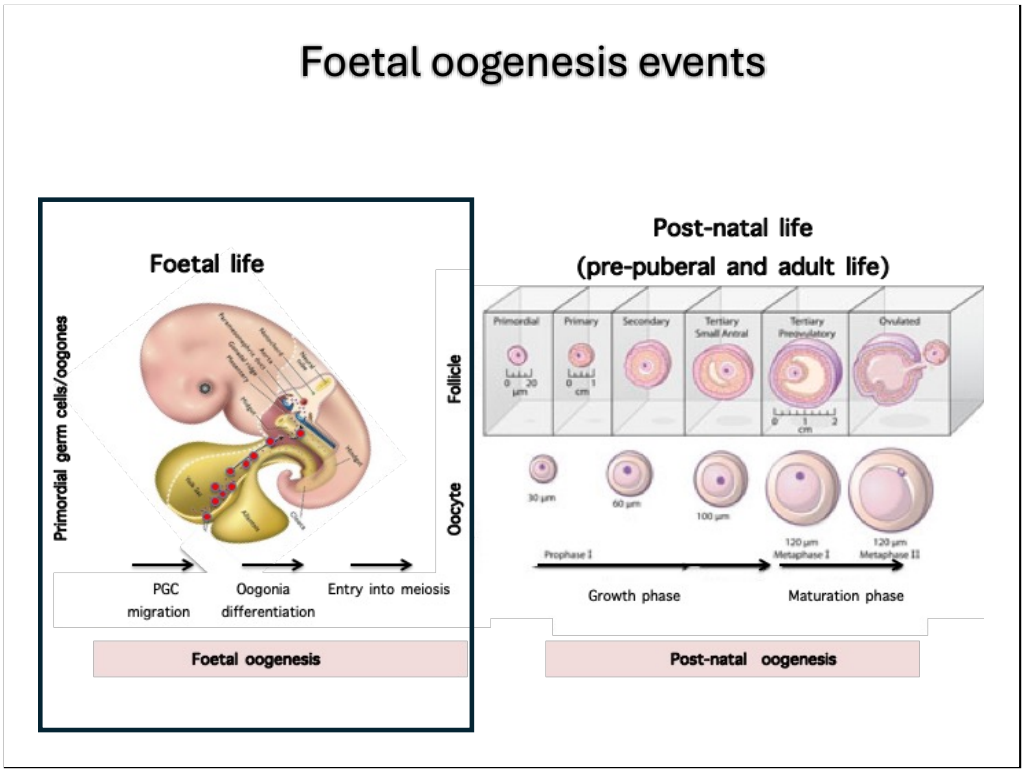
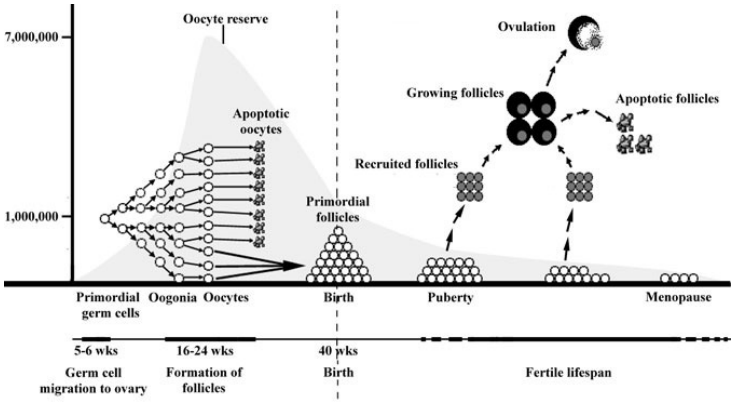


Unit III focus on the foetal oogenesis and mechanisms driving the transition between the foetal and the Post-natal oogenesis.

What is the origin of the gametes?



1.Differentiation of primordial germ cells (PGC)

2.Differentiation of a sex-specific gamete lineage (transition from PGC to oogonia)

3.Increase in the total number of female gametes

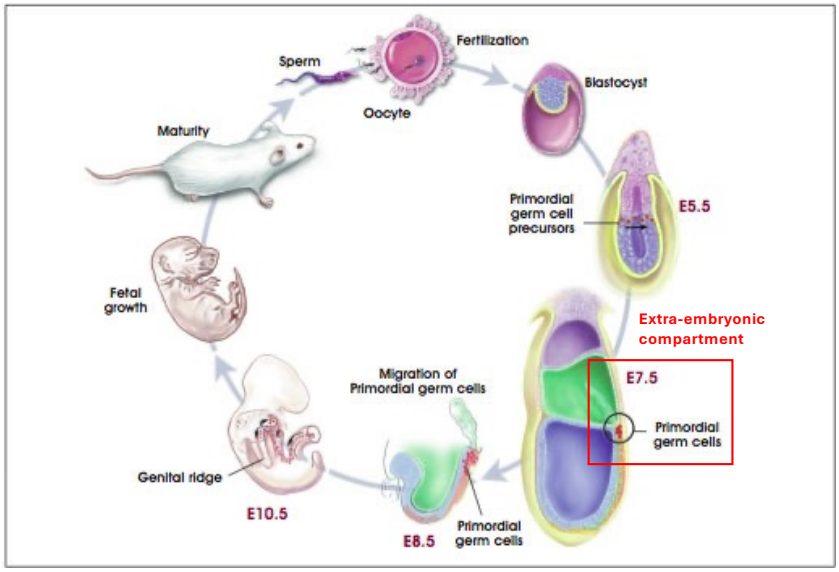
4. Transition from mitotic female germ cells (oogonia) to meiotic ones (oocytes)

5. Definition of the individual final pool of gametes available during postnatal life

6. Increase in genomic variability through the crossing over mechanism (mutagenesis) occurring during the first phases of the meiotic cell cycle

Several relevant events occuring during fetal oogenesis are summarized in this slide.

1. Differentiation of Primordial Germ Cells (PGCs)



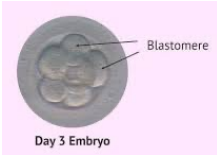
The primordial germ cells origin very early during fetal life.
The presence of PGC is documented in mice approximately at 7 days after fertilization when 20-50 PGC are visualized for the first time in extra-embryonic compartment (red box).

How do PGCs form?

After fertilization, the zygote forms and encounters many cleavage events creating cells that are called as blastomeres.

Blastomers

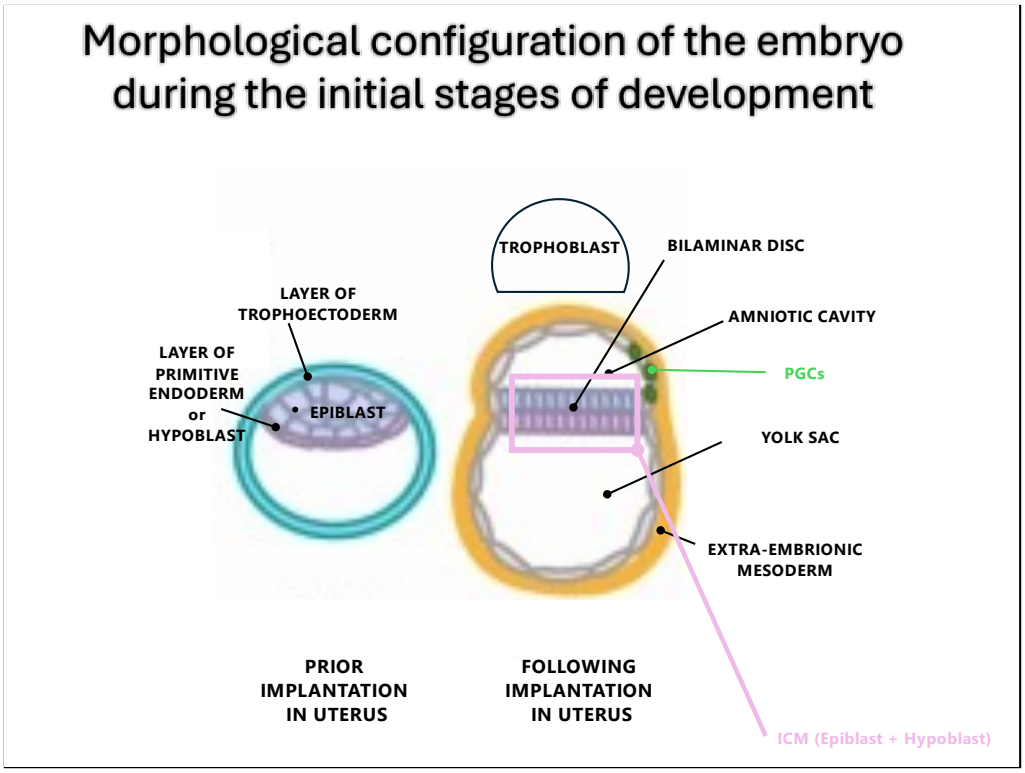
are totipotent
in the early stages of development



PGCs

are specified from blastomers and they are exclusively committed to the germ cell lineage.

- pluripotent cells
- sexual undifferentiated progenitor cells since they are more similar to stem cells than to gametes.



To accurately understand the **timing** and **differentiation of PGCs**, it is essential to have a clear understanding of the morphological configuration of the embryo during the initial stages of development.

Prior to implantation, the embryo consists of a segregated pluripotent epiblast, between a layer of hypoblast or primitive endoderm and a layer of trophectoderm.

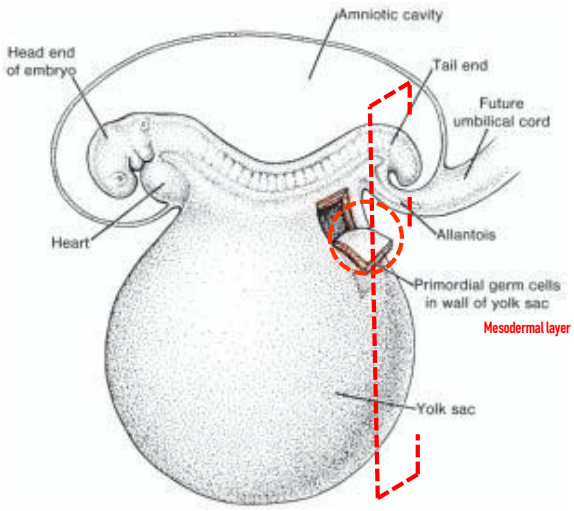
Following implantation, the embryo undergoes morphological changes **that lead to the formation** of the bilaminar disc, but also the formation of the extraembryonic structures that support embryonic development, such as the amnion, the visceral yolk sac and trophoblast.

(Epiblast + Hypoblast = ICM)

For further detail about the conformation changes please have a look at:

https://www.youtube.com/watch?v=WcDgXAwp4rg

PGCs origin

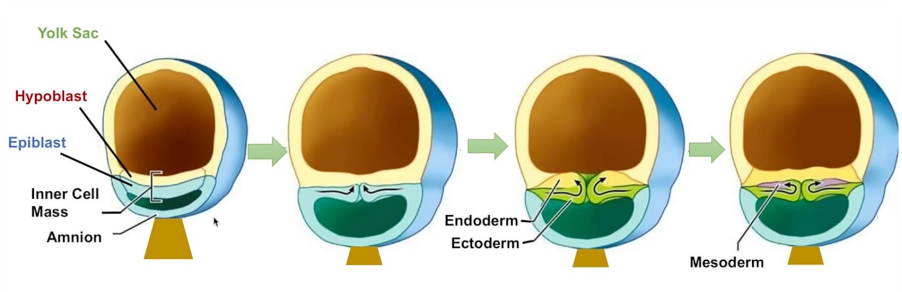


20-50 PGCs when they become visible

PGCs originate **in** the mesodermal layer of the yolk sac wall in response to growth factors released from ectodermal cells.

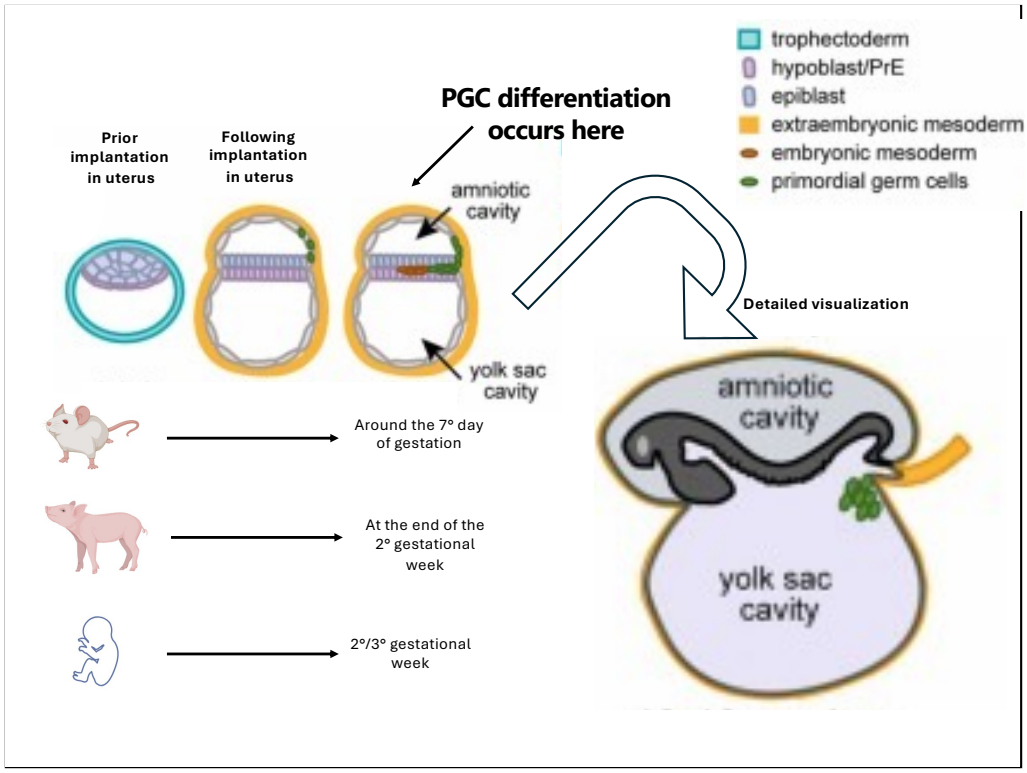
The ectoderm is one of the three primary germ layers that make up the embryo

It derives from the epiblast

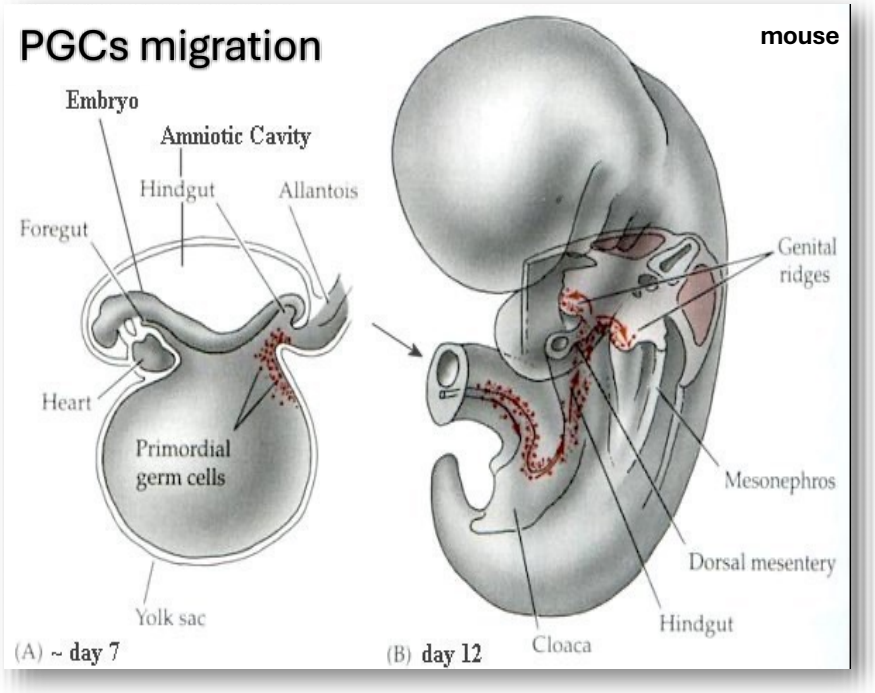


The ectoderm is one of the three primary germ layers that make up the embryo. It derives from the epiblast. The ectoderm gives rise to multiple tissues and structures of the body, including the nervous system, skin, hair, nails, sweat and mammary glands, as well as the lenses of the eyes and the epithelial lining of the nose, mouth, and rectum.

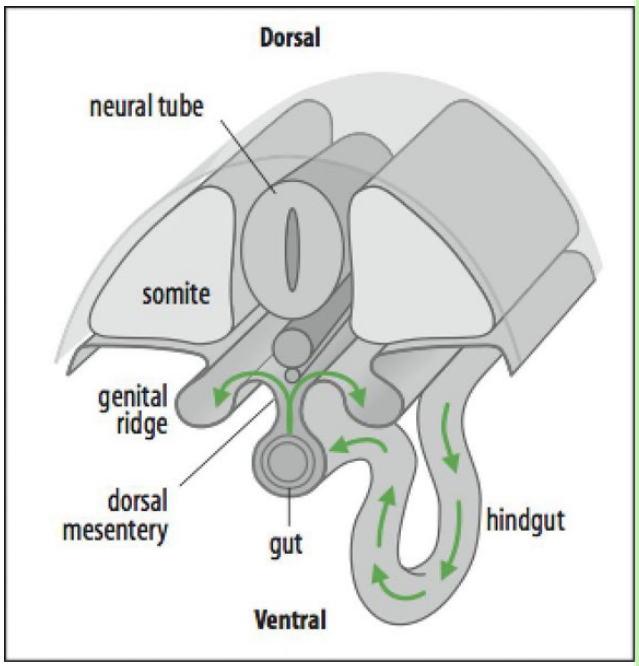
Extraembryonic structures, such as the yolk sac and placenta, develop from other tissues, such as the endoderm and extraembryonic mesoderm.



Since the kinetic of oogenesis is closely related to the body size, the appearance of PGCs during fetal life varies among different mammalian species. In mice, pigs, and humans, PGCs differentiate at approximately day 7, 13, and 21, respectively.



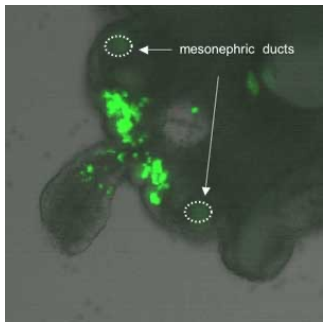
Immediately after differentiation, PGCs proliferate and, in response to local chemotactic stimuli, migrate from the extraembryonic compartment towards the embryo.



DAY 12 in MICE

PCGs leave the yolk sack to reach the primitive genital ridge travelling through the wall of intestinal tract.

They proliferate during their journey. When they reach the primitive genital ridge they are apporximatively **10-50.000**.

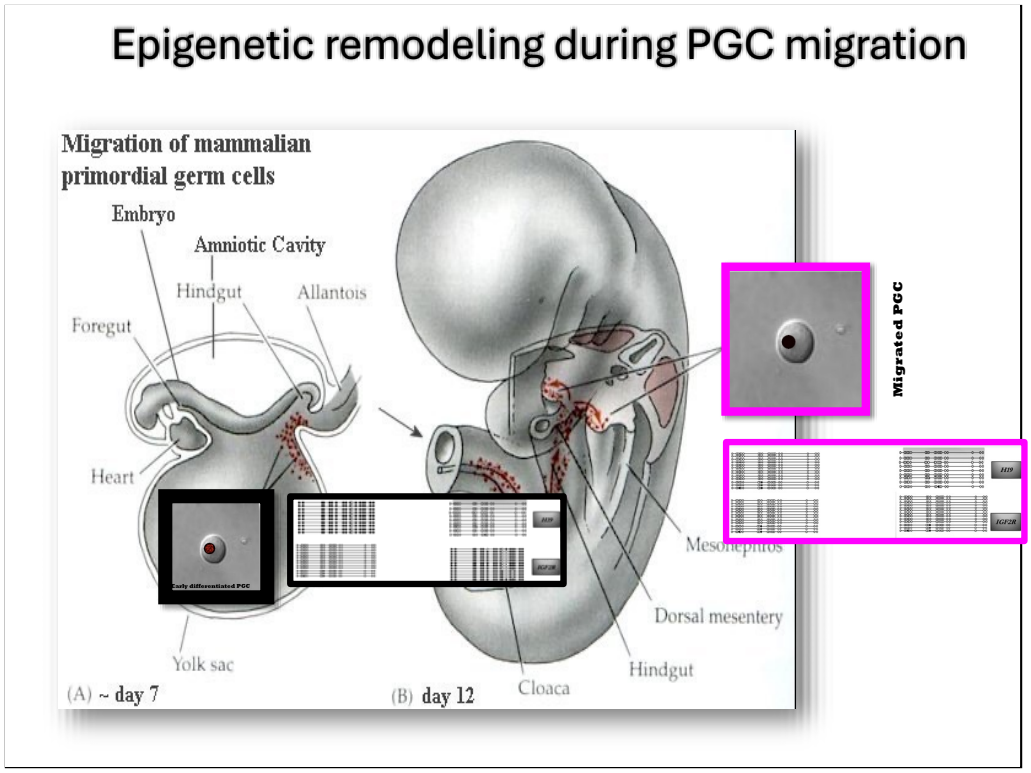


The PGC leave the yolk sac to reach the primitive genital ridge at day 12 in mice.

They undertake this long journey travelling along the wall of the hindgut.

During the journey the PGC continue to proliferate by increasing further in number.

The PGC that reach the primitive gonads at day 12 are approximately 10-50 thousand cells, so from 200 to 1000 times more than the native ones (about 50 cells).



Additionally, during migration, primordial germ cells (PGCs) undergo profound epigenetic remodeling. The chromatin in these cells experiences dramatic active demethylation, affecting all DNA and gene sequences, including those that are imprinted. By the time the cells reach the genital ridges around day 12, all DNA methylation markers have been completely erased, resulting in PGCs with entirely demethylated DNA. No other cell types in the organism exhibit a similar epigenetic status.

Methylation of Imprinted genes is not affected,
only global demethylation occurs in all somatic cells of the organism

PGCs do not follow the general rule!!!

WHY?

PGCs have to differentiate oogonia or spermatogonia.

OOGONIA or SPERMATOGOINA
Gametes that need to acquire a complementary epigenetic asset related to imprinted genes

They will receive imprinting during gametogenesis
and will then remain unchanged.

During the first stages of the embryo development in all somatic cells of the newly generated organism the methylation asset of the imprinted genes is not affected. Only global DNA demethylation occurs.

PGCs do not follow this general rule!

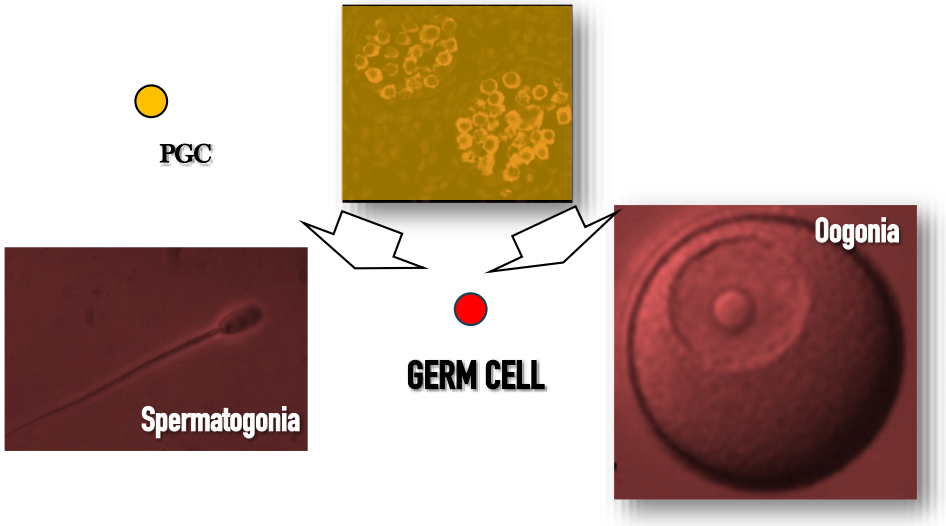
Their somatic epigenetic asset can not be maintained for long time since the PGC have di differentiate oogonia or spermatogonia so two typology of gametes with a complementary epigenetic asset for what concern imprinted genes

In conclusion, PGC have to become sexually differentiated gamete and, for this reason, the somatic genome makeup would not be adequate with their future reproductive function.

The gametes, indeed, have to develop a complementary parental genome before fertilization occurs through the active process of primary imprinting.

2. Differentiation of a sex-specific gamete lineage

transition from PGCs to oogonia or spermatogonia

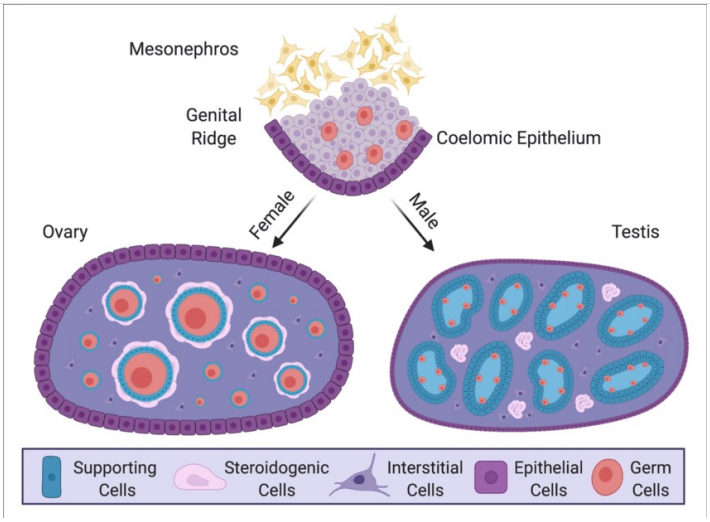


When?

When have completed their migration to the genital ridge.

When PGC reach the genital ridge are sexual undifferentiated progenitor cells. PGC will receive signals form the tissue microenvironment, this signal will allow the sex specification.

Who is giving the signal for the induction of sex specification (commitment)?



- During early embryonic development, the gonads are initially bipotential
- Sex differentiation will be influenced by the genetic sex of the embryo
- The somatic cells within the gonads play a pivotal role in this differentiation process.

Somatic Cells Origin:
The somatic cells of the gonads originate from the coelomic epithelium, a layer of cells that covers the developing gonad.

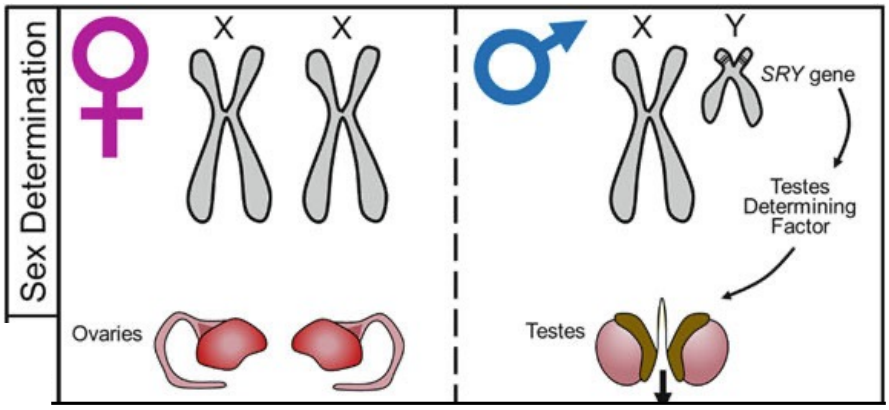
Somatic cells composing the primitive gonads will be responsible for the induction of this sex lineage gamete specification.

The somatic cells, indeed, are responsible of PGC commitment towards spermatogonia or oogonia.

More in detail:

During early embryonic development, the gonads are initially bipotential, meaning they have the potential to develop into either male or female reproductive organs. The differentiation path they take is influenced by the genetic sex of the embryo, determined at fertilization by the presence or absence of the Y chromosome. The somatic cells within the gonads play a pivotal role in this differentiation process.

Somatic Cells Origin: The somatic cells of the gonads originate from the coelomic epithelium, a layer of cells that covers the developing gonad. These cells are crucial for the formation and function of the gonads.



- The presence of the SRY gene on the Y chromosome is a key factor in determining the development of **testes**.

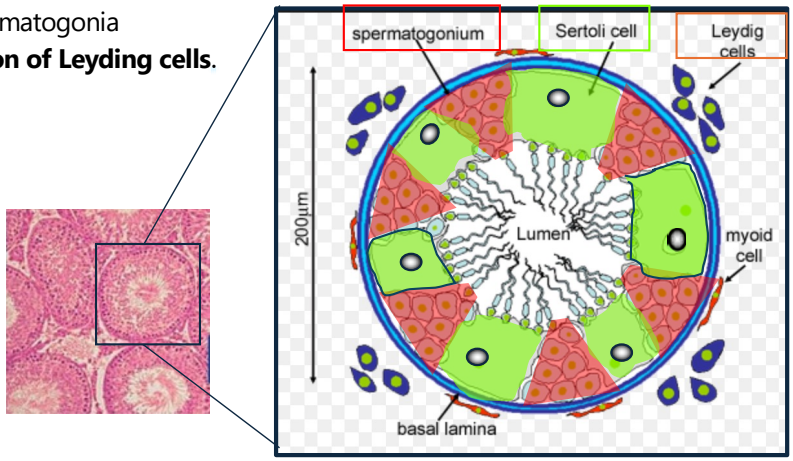
WHAT IS HAPPENING?

- Somatic cells in the gonads expressing the SRY gene (as in XY embryos) differentiate into **Sertoli cells**, which support the development of male germ cells (spermatogonia).
- In the absence of SRY gene (as in XX embryos), the somatic cells differentiate into pregranulosa cells, which support the development of female germ cells (oogonia).

Sertoli cells orchestrate the specification of the male reproductive system

How → **Taking advantage of their paracrine activity (*in situ* releases of soluble factors)**

- **Induce PGCs differentiation** toward spermatogonia
- **Specification of Leyding cells**.



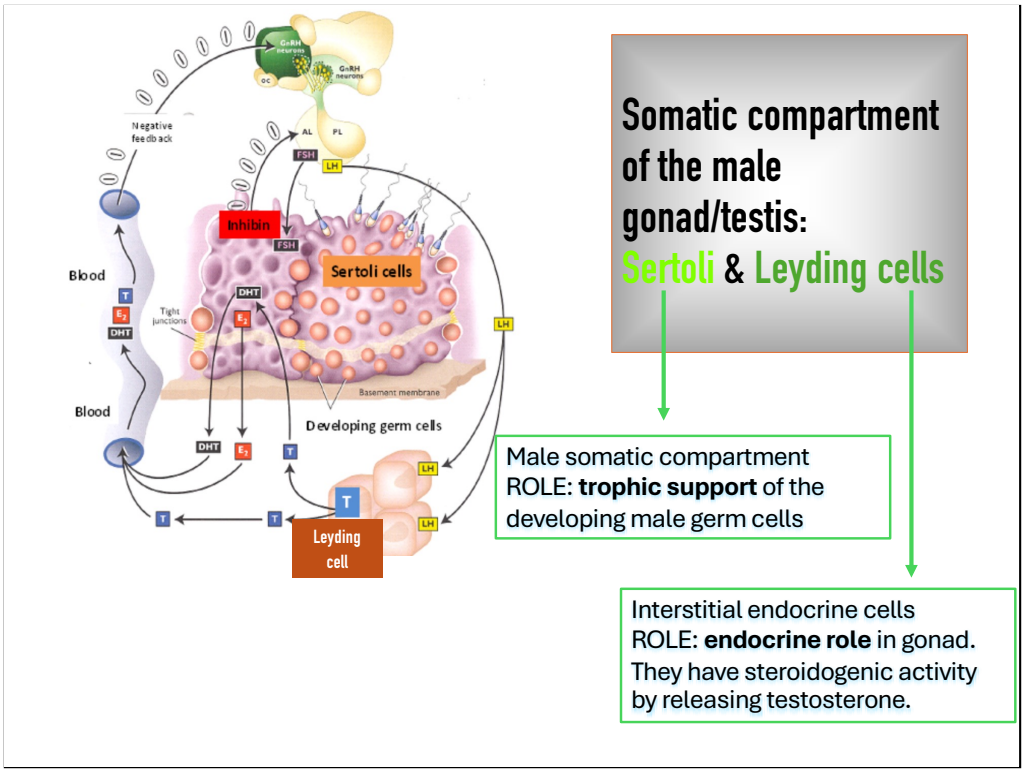
More in detail:

The Sertoli cells orchestrate the sexual commitment of the PGCs involving the gonads such as:

1. Differentiation of PGC in spermatogonia
2. The specification of another category of somatic cells
3. and definition of the male reproductive system.

Taking advantage of their paracrine activity (*in situ* releases of soluble factors), Sertoli cells are responsable of the differentiation fate of the gonad:

First of all they induce PGC differentiation toward spermatogonia and, in addition, of the specification of Leyding cells, another important male reproductive cell lineage.



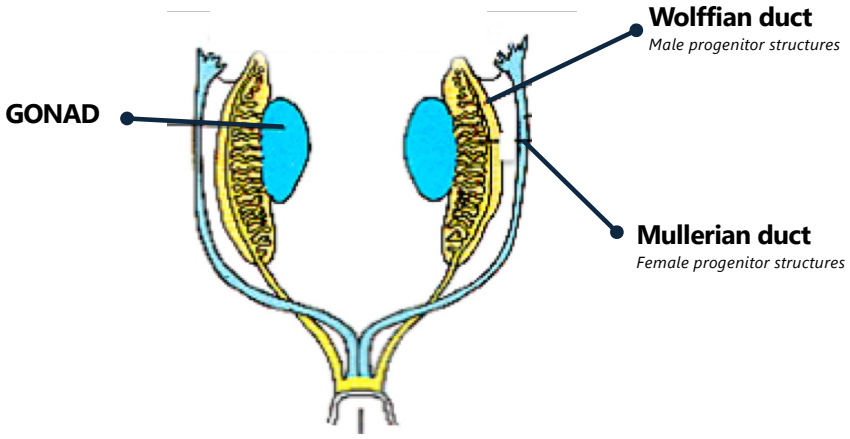
Indeed, while the Sertoli cells represent the male somatic compartement involved in the trophic support of the developing male gem cells, the Leyding cell, also known as interstitial endocrine cells, have an active endocrine role in gonad.

They are actively involved in steroidogenesis by releasing high level androgenes (mainly testosterone).

Testosterone is the male steroid reproductive hormone that control spermatogenesis in post natal life by acting, at the same time, as an ipothalamus-hypofisis axis modulator.

Sertoli cells stimulate the specification of male genital organs

Before sexual differentiation the genital tract is an undifferentiated structure



The **Sertoli cells** are also responsable for the differentiation of male reproductive system by stimulating the specification male genital organs.

Before sexual differentitation the genital tract is an undifferentiated structure recognizing either male or female ducts. More in detail, it displays the wolffian tubuls that are male progenitor structures and mullerian tubuls that on the contrary are female progenitor structures.

IN MALE...

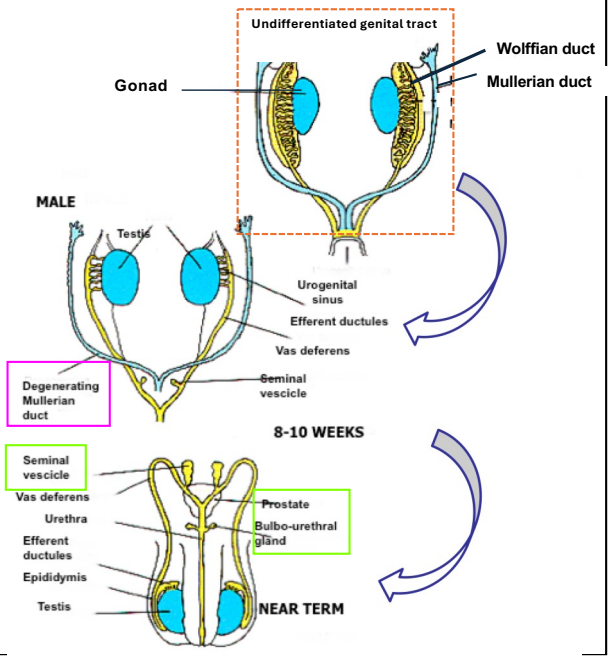
1) Sertoli cells through anti-Mullerian hormone

inhibites the development of mullerian ducts into oviduct and uterus

1) Leyding cells through testosterone

stimulates the differentiation of male genital glands

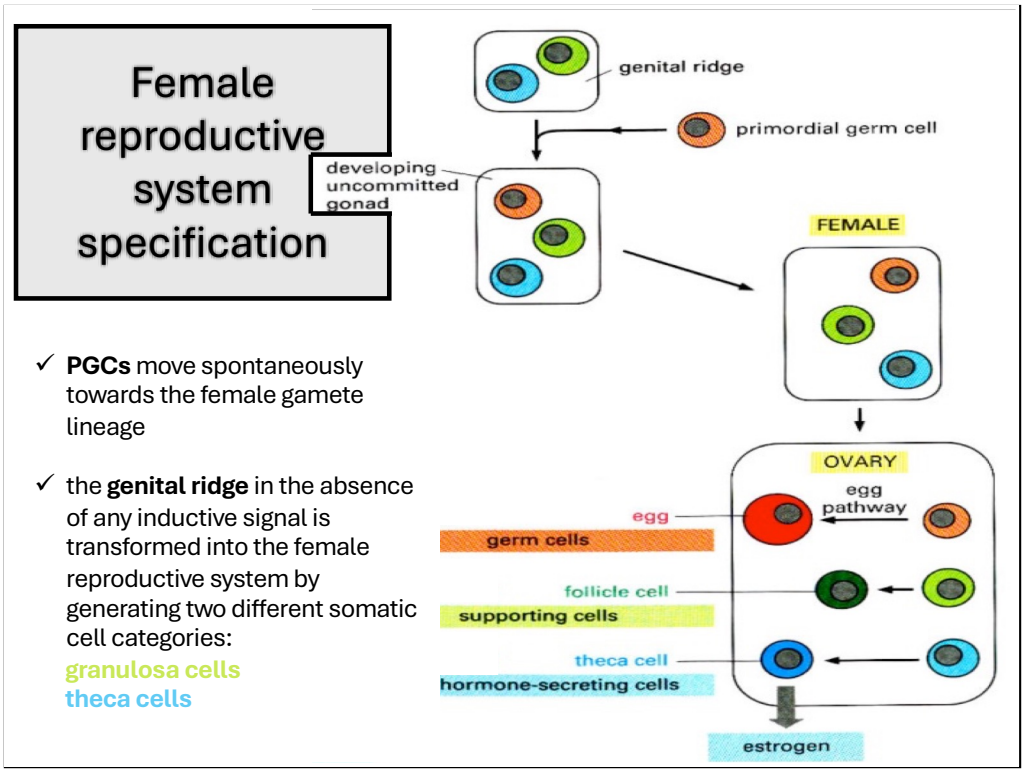
inhibits the degeneration of seminiferous tubules



In male, specified Sertoli cells **immediately started to** actively secrete a factors, the anti-Mullerian hormone, that inhibits the development of female related ducts (from which oviduct and uterus could differentiate).

In addition, as we stated before, the growth factor released by Sertoli cells are responsable for the specialization of Leyding cells thus activating male steroidogenesis.

Indeed, immediately after the Leydig cells commitment, the level of testosterone increases and this is a key event able to stimulate the differentiation of glands supporting the male genital system. Finally, testosterone levels also prevents, in parallel, that the degeneration of seminufourus tubules takes place.



In a female organism that does not have the Y sexual chromosome,

the PGCs move spontaneously towards the female gamete lineage

and the genital ridge in the absence of any inductive signal is, in parallel, transformed into the female reproductive system by generating two different somatic cell categories the granulosa and theca cells.

The granulosa cells may be considered as throphic cells supporting oogenesis, while the theca cells are essential in controlling female steroidogenesis.

1) Sertoli cells

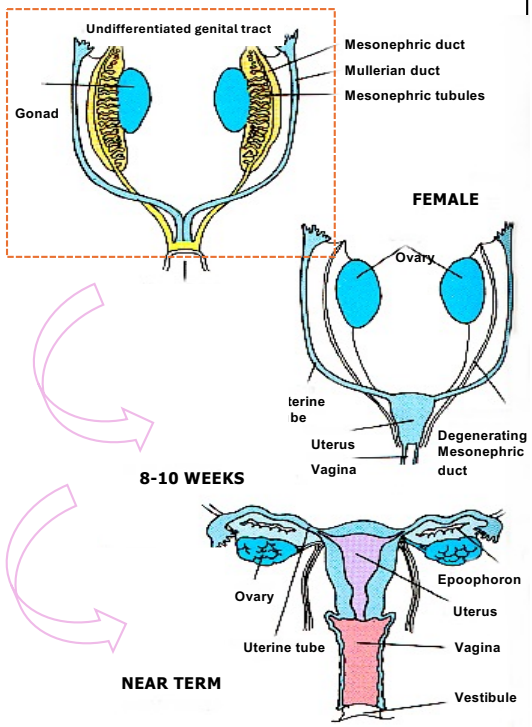
AMH is not released at high level.

Mullerian ducts develop

1) Leydig cells

Testosterone is not produced.

Degeneration of seminiferous tubules



In a similar manner, in the absence of Sertoli cells, AMH is not released, leading to the persistence of Müllerian ducts, which subsequently develop into the oviduct and uterus structures.

The second phase involves the degeneration of seminiferous tubules (indicated by yellow ducts), which occurs due to the lack of paracrine support from Leydig cells, represented by testosterone.

Consequently, the female genital tract develops in a simplified form, specializing into two gonads (ovaries) and two ducts that culminate in an enlarged, communicating structure known as the uterus body.

Role of SRY was confirmed experimentally by *Koopman* in 1990

Generation of a transgenic mouse by the insertion of the SRY gene into the nucleus of a female zygote.

Transgenic mouse undertook a male sexual specification.

FIRST SCIENTIFIC EVIDENCE !!!

Only the SRY portion of theY

is enough

to determine the sex male differentiation

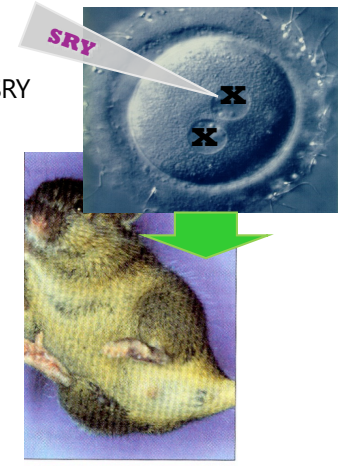


Figure 20–17 *Sry*-induced reprogramming of a female mouse embryo to develop into a male. The *Sry* gene, injected into the nucleus of an XX female zygote, caused the transgenic embryo produced to develop into a male. The external genitalia of the transgenic mouse are indistinguishable from those of a normal XY male mouse. (From P. Koopman et al., *Nature* 351:117–121, 1991. © Macmillan Magazines Ltd.)

The key role of SRY in inducing male reproductive system specification has been clarified adopting this experiment.

In 1991 Koopman and colleagues generated a transgenic mouse by inserting the SRY gene into the nucleus of a female zygote.

The resulting transgenic embryo expressed the SRY genes in a female chromosome asset (XX chromosomes).

What did the researchers observe?

The transgenic mouse undertook a male sexual specification. (as you can appreciate by looking the specification of the external genitalia)

This serves as robust scientific evidence supporting the notion that the Y chromosome is responsible for male differentiation. Moreover, the findings indicate that not the entire Y chromosome is necessary for this process; rather, only the SRY portion is required as it encompasses all the genes essential for male sexual specification.