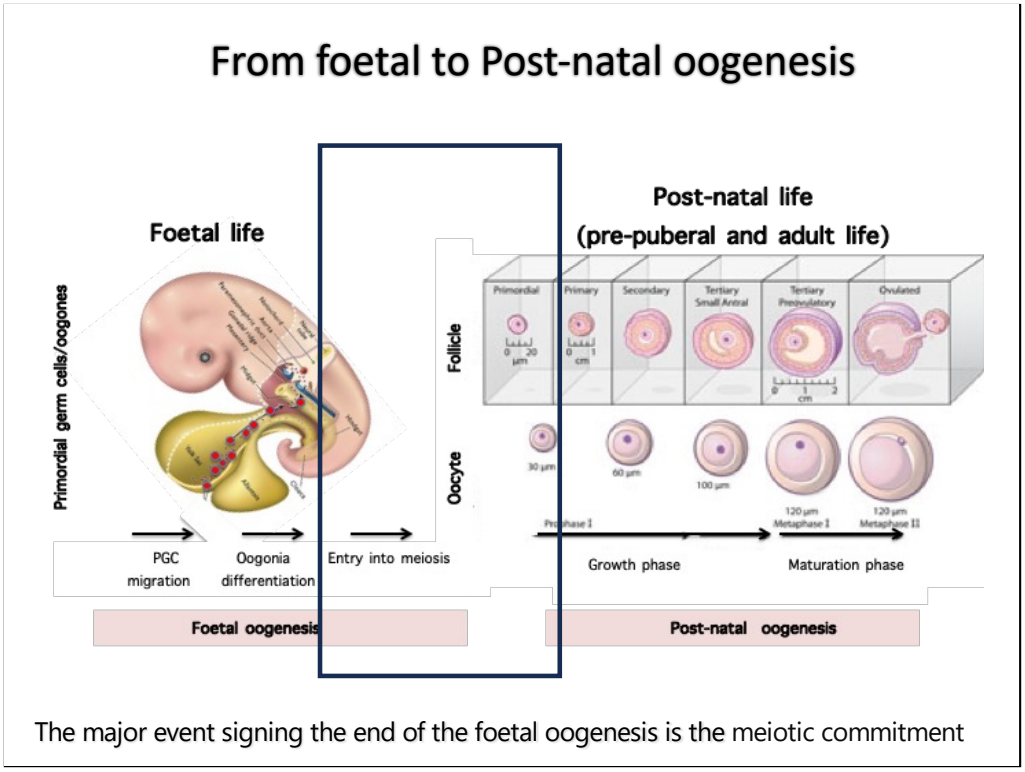


The major event signing the end of the foetal oogenesis is the meiotic commitment (THE ENTRY INTO MEIOSIS).
To be more precise, the transition from a mitotic female germ cells (oogonia) into a meiotic ones (oocyte).

1. **PGCs** migrate towards the developing gonads.
2. During their migration to the gonads, PGCs begin to proliferate through **mitosis.**
3. Mitotis continues until the PGCs enter a differentiation phase, becoming **oogonia**

WHAT ABOUT THE FOLLOWING STEP?

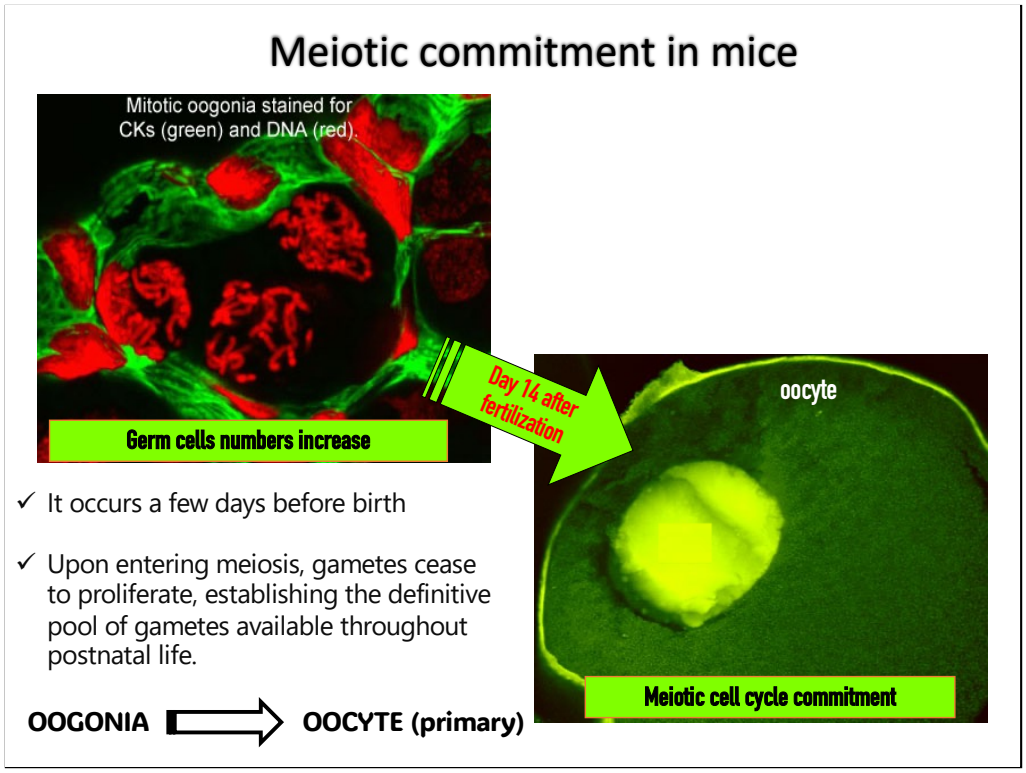
MEIOTIC COMMITMENT

Transition from oogonium to oocyte

We know that PGCs migrate towards the developing gonads. During their migration to the gonads, PGCs begin to proliferate through mitosis. Mitotis continues until the cells enter a differentiation phase, becoming oogonia.

The transition from oogonia to oocyte occurs during the G2 phase of the mitotic cell cycle and sign the occurrence of the meiotic commitment.

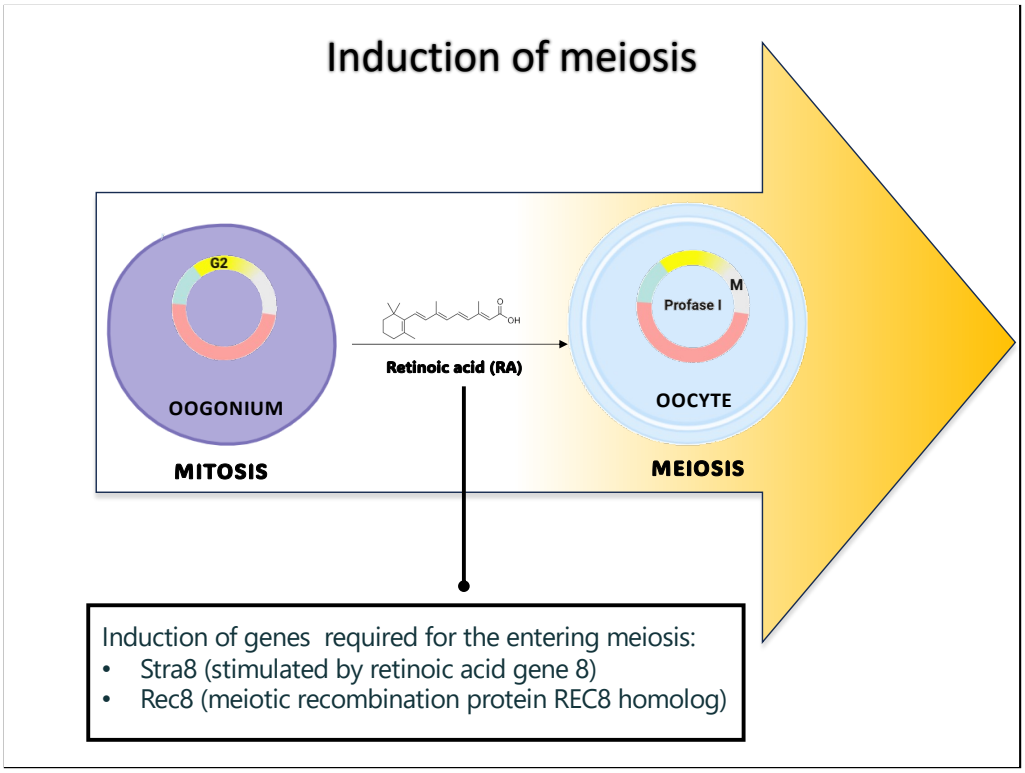
MEIOTIC COMMITMENT= ENTERING INTO MEIOSIS



In mice, this meiotic commitment occurs a few days before birth, while in medium and large mammals, it takes place a few days later.

Upon entering meiosis, gametes cease to proliferate, establishing the definitive pool of gametes available throughout postnatal life, as oocytes no longer undergo further proliferation.

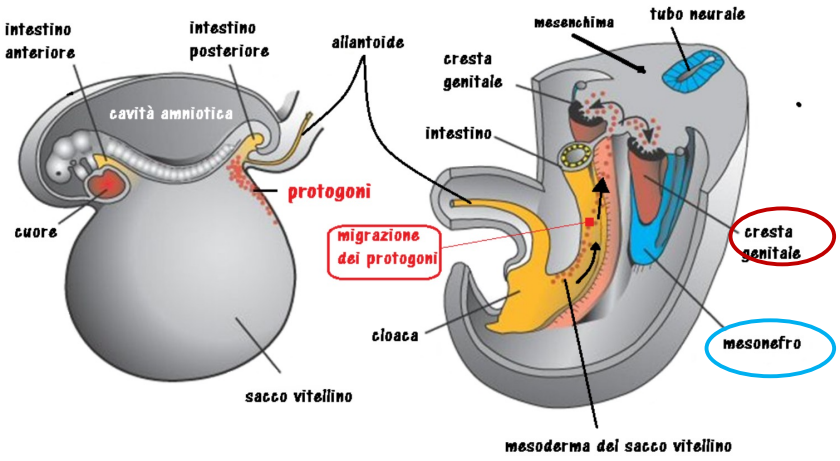
Going into more detail, in the mouse model, oogonia switch off the proliferative cell cycle approximately 14 days after fertilization. Subsequently, these gametes undergo specialization into meiotic cells, at which point they are referred to as oocytes.



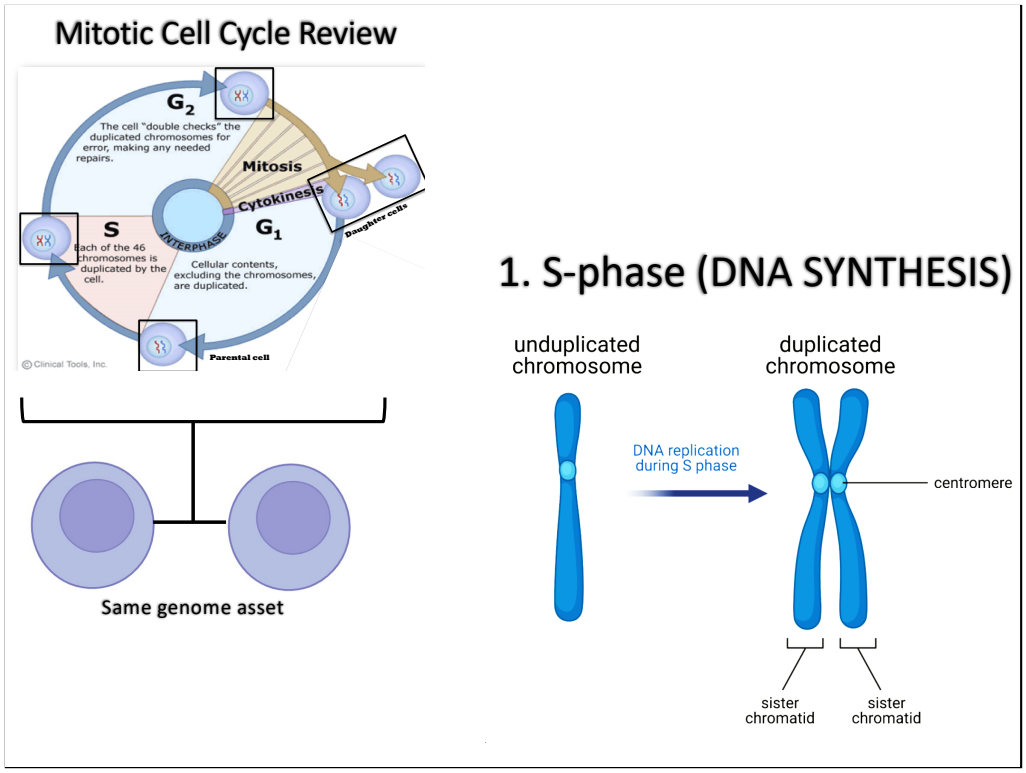
Induction of Meiosis: The induction of meiosis in oogonia is regulated by a combination of intrinsic and extrinsic molecular signals, including retinoic acid (RA), which plays a crucial role in inducing the expression of specific genes necessary for entering meiosis (Stra8 and Rec8). This signal can prompt oogonia, which are in the G2 phase, to bypass the typical progression of the mitotic cell cycle and initiate the meiotic process.

Arrest in Meiosis I:

Once oogonia have to begin meiosis, they exit to the G2 phase of the mitotic cycle and enter the prophase I stage, where they undergo a prolonged period of arrest, known as diplotene.



The transition from mitosis to meiosis in oogonia is primarily induced by somatic cells close to the germ cells, such as **mesonephric cells** and **stromal cells of the developing gonads**.



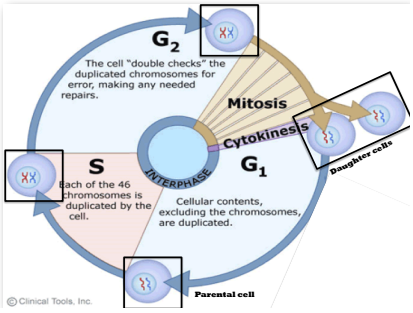
The mitotic cell cycle recognized 4 sequential phases aimed to obtain two cells with the same genome asset of the mother cell.

More in detail, the mitotic cell cycle recognizes two **active** phases:

S-phase for DNA replication (means SYNTHESIS)

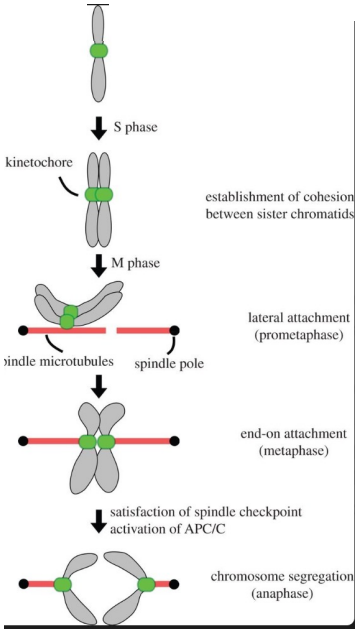
Each chromosome is replicated to produce two identical copies, known as sister chromatids, which are held together at a region called the centromere.

Cell cycle Review



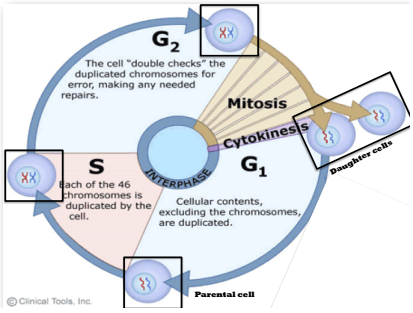
The chromosomes that were replicated during the S phase are segregated into two new nuclei.

M-phase (DNA SEGREGATION)



M-phase for DNA segregation, specifically sister chromatids segregation between the two daughter cells (means MITOSIS).

Cell cycle Review



G1-phase

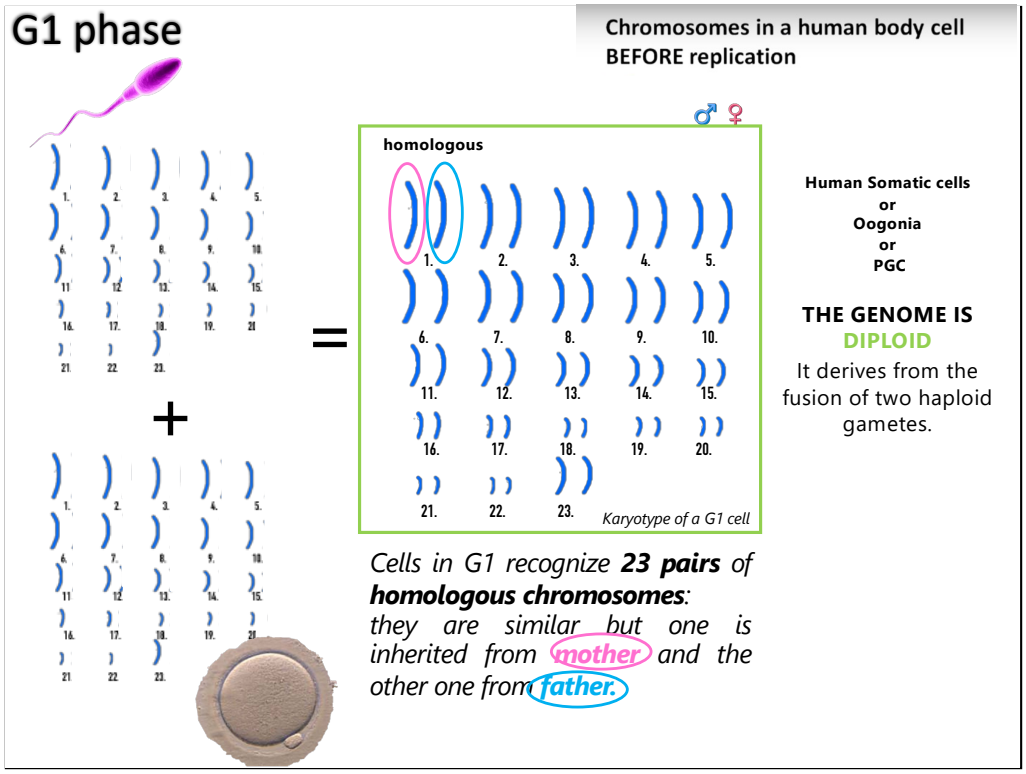
- Growth in size
- Production of RNA
- Synthesis of PROTEINS for DNA replication
- Check for DNA damage before proceeding to the S-phase

G2-phase

- Growth in size
- Production of RNA
- Synthesis of PROTEINS for Mitosis
- Check to ensure that DNA replication has been completed successfully
- Eventually repair of any DNA damage

During G1, the cell grows in size, produces RNA, and synthesizes proteins necessary for DNA replication. The cell also performs its normal functions and checks for any DNA damage before proceeding to the S phase.

During G2, the cell continues to grow and produce proteins, particularly those required for mitosis and cell division. The cell also checks to ensure that DNA replication has been completed successfully and repairs any DNA damage.

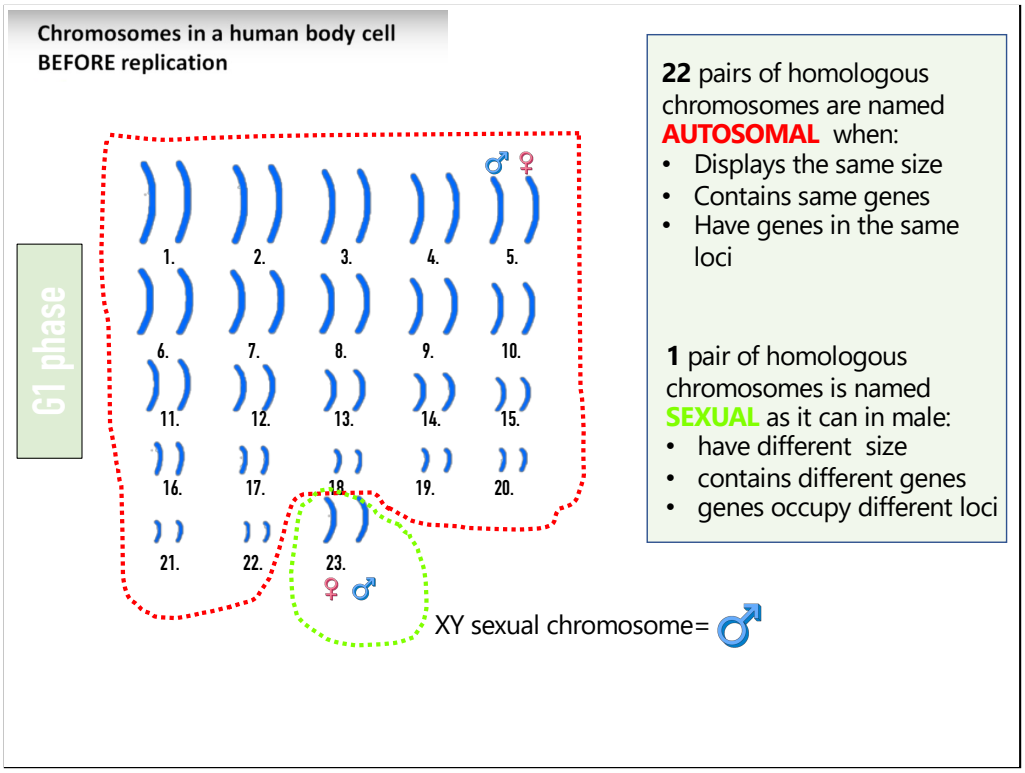


This is an example of a **karyotype of a G1 human cells** (somatic cells, oogonia or alternatively PGC).

The genome is diploid since it derives from the fusion of two haploid gametes (spermatozoon and oocyte).

In human organism both the gametes transfer to the embryo genome 23 chromosomes.

As a result, cells at G1 recognize 23 **pairs** of homologous chromosomes defined homologous since each of them is inherited from one parent (mother and father).



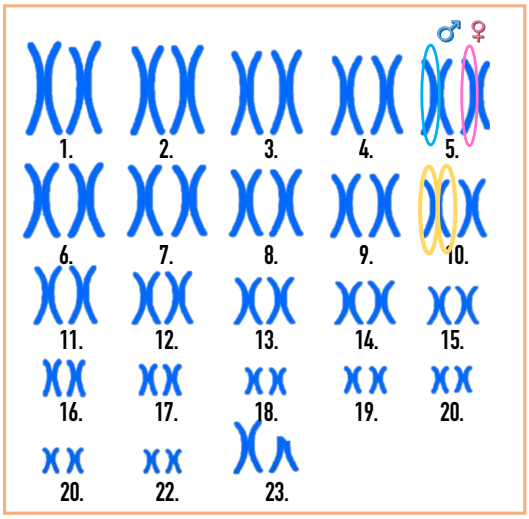
22 pairs of homologous chromosomes are named autosomal chromosome since they are similar in size, gene content and gene loci localization.

A pair of homologous chromosome is called sexual since they may be different in size, gene content and gene loci position in male organisms (XY).

Chromosomes in a human body cell
AFTER replication

DUPLICATED DIPLOID GENOME

S/G2 phases



When the cell entry in the S phase each homologous chromosome (the one from **father** and the one from mother) duplicated and becomes composed of **two sister chromatids**:

- Duplicated homologous chromosomes display two sister chromatids.
- Sister chromatids are maitained close each other

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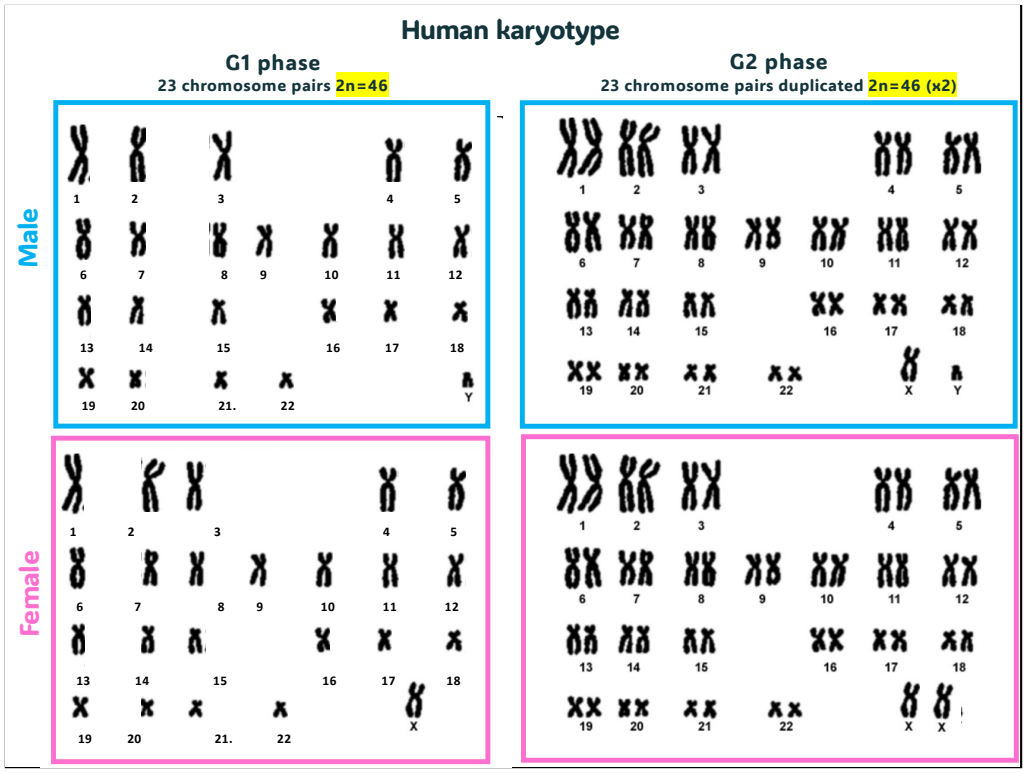
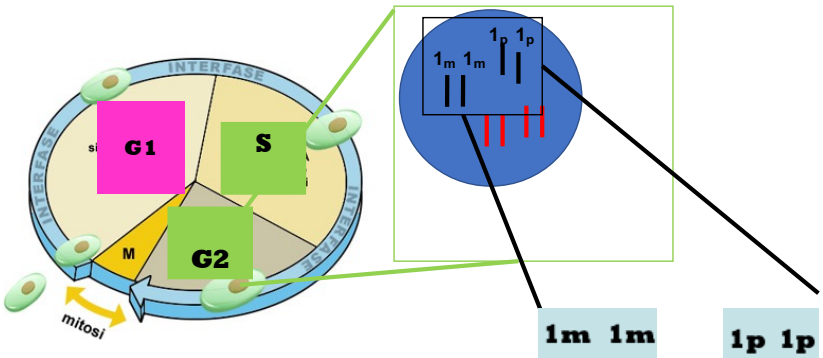
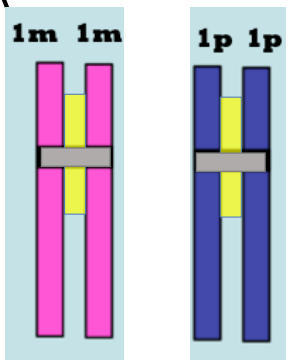

Human karyotype
G1 phase
23 chromosome pairs 2n=46
G2 phase
23 chromosome pairs duplicated 2n=46 (x2)
Male
Female



In G2, sister chromatids are closely associated along their length through two distinct molecular complexes.

Along the **longitudinal plane**, cohesins, specific proteins, bind the DNA of paired chromatids.
On the **equatorial level,** the centromere serves as another central strong point of adhesion.



n= 2 DNA =2

In G2, sister chromatids are closely associated along their length through two distinct molecular complexes.
Along **the horizontal plane**, cohesins, specific proteins, bind the DNA of paired chromatids.
On **the equatorial level,** the centromere serves as another central strong point of adhesion.

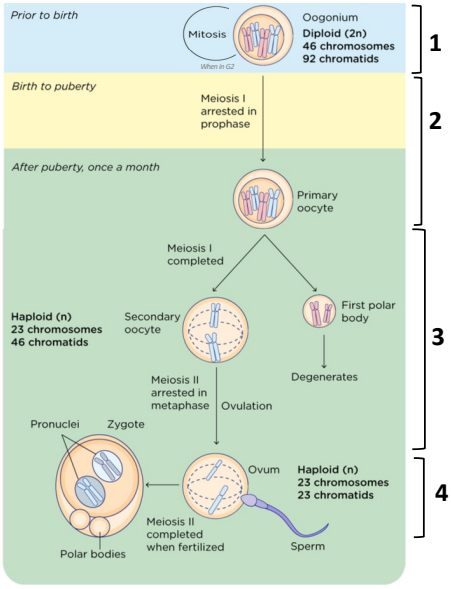
OOGONIUM
Before entering meiosis I, it is in G2 phase when exits from the mitotic cell cycle thank to the RA.
DIPLOID
46 chromosomes
92 cromatids

PRIMARY OOCYTE
After entering meiosis I, the primary oocyte remains arrested in prophase I. *Before LH occurs*
DIPLOID
46 chromosomes
92 chromatids (since meiosis I is not yet complete)

SECONDARY OOCYTE
After completion of the first meiotic division (*LH surge has occurred)*, it reduces to a haploid cell.
HAPLOID
23 chromosomes
46 chromatids (since the chromosomes are still composed of two sister chromatids)

MATURE OOCYTE (EGG):
After the second meiotic division (which occurs only if fertilization takes place), the mature egg is
HAPLOID
23 chromosomes
23 chromatids (since each chromosome now consists of a single chromatid)

LET's adapt these concepts to the oogenesis



1.

OOGONIA

before entering meiosis I divide trough mitosis. It is in G2 phase of the mitotic cell cycle when it exit from the cell cycle thank to the RA (DIPLOID, 46 chromososmes; 92 chromatids).

2.

PRIMARY OOCYTE

After entering meiosis, the primary oocyte remains arrested in prophase I

DIPLOID

46 chromosomes

92 chromatids (since meiosis is not yet complete)

3.

SECONDARY OOCYTE

After the first meiotic division (LH surge has occurred), it reduces to a haploid cell.

HAPLOID

23 chromosomes

46 chromatids (since the chromosomes are still composed of two sister chromatids)

4.

MATURE OOCYTE (EGG):

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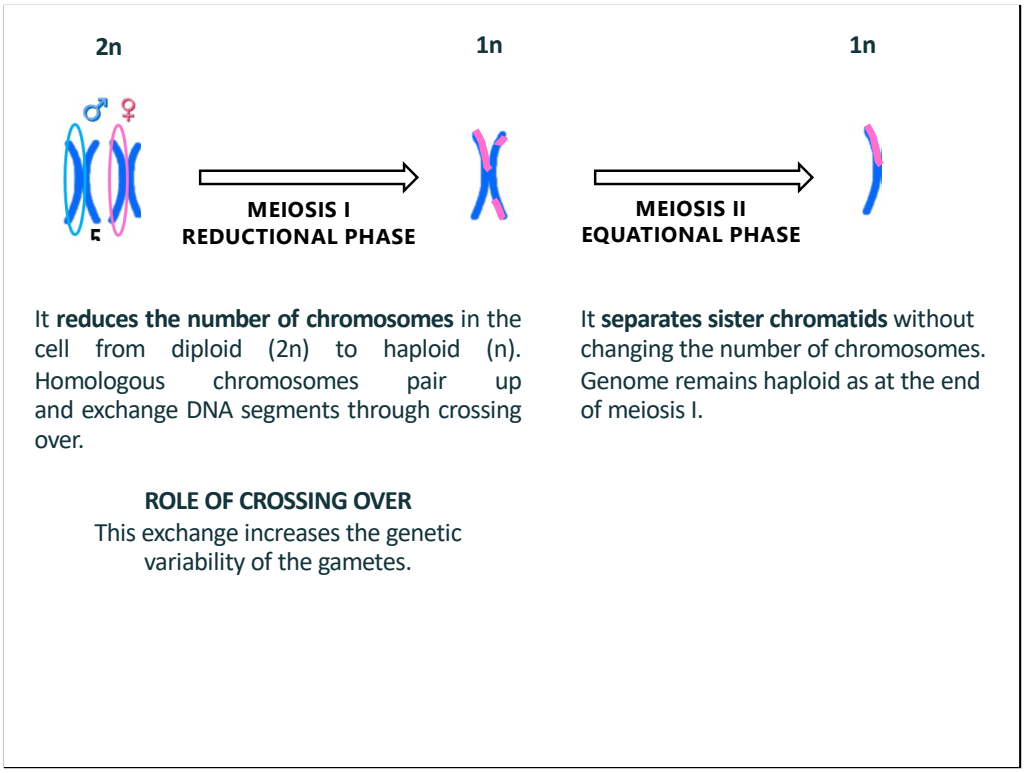
HAPLOID

23 chromosomes

23 chromatids (since each chromosome now consists of a single chromatid)



Meiosis I: Reductional Phase

Meiosis I is defined as reductional because it reduces the number of chromosomes in the cell from diploid (2n) to haploid (n). During this phase, homologous chromosomes, each of which has already been replicated during the S phase of the previous cell cycle and therefore consists of two chromatids, pair up and exchange DNA segments through crossing over. This exchange increases the genetic variability of the gametes. The main stages of meiosis I include:

•Prophase I, where the pairing of homologous chromosomes and crossing over occur.

•Metaphase I, homologous chromosomes align along the metaphase plate.

•Anaphase I, homologous chromosomes separate and migrate to opposite poles of the cell.

•Telophase I, concludes with the formation of two daughter cells, each with a haploid chromosome set but with chromosomes still composed of two chromatids.

Meiosis II: Equational Phase

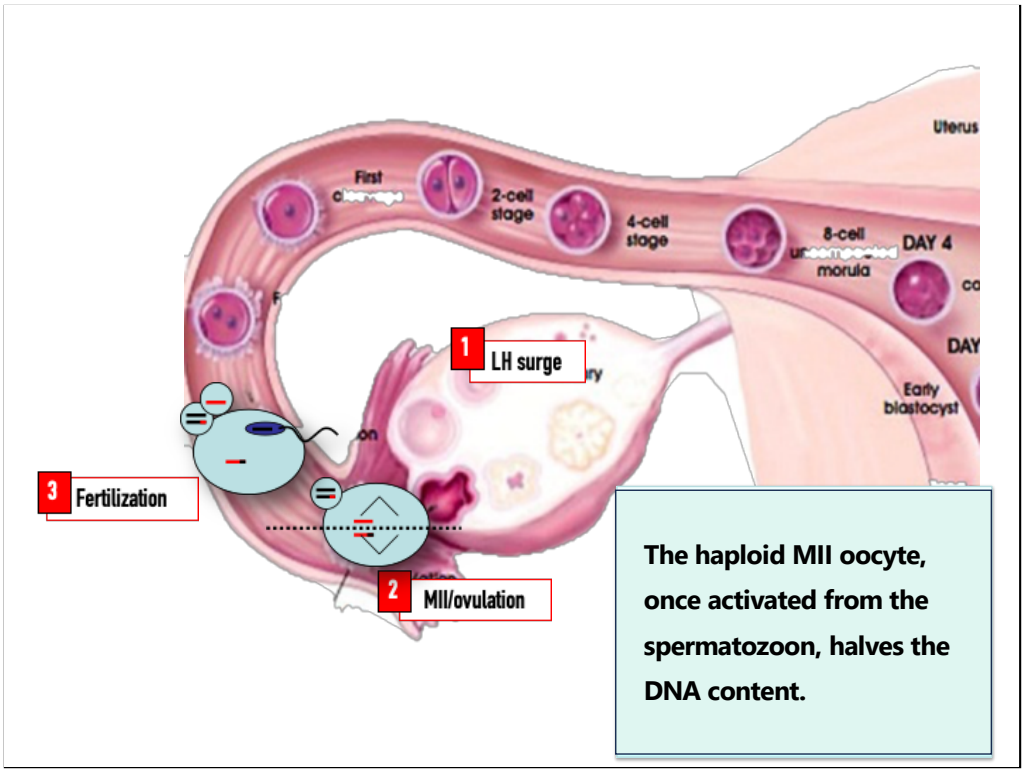
Meiosis II is defined as equational because the number of chromosomes in the daughter cells does not change; it remains haploid as at the end of meiosis I. This phase is very similar to mitosis, with the difference that it starts from a cell with a haploid chromosome set. The main stages of meiosis II include:

•Prophase II, where the chromosomes, still composed of two chromatids, begin to condense again.

•Metaphase II, chromosomes align at the center of the cell.

•Anaphase II, sister chromatids separate and migrate to opposite poles.

•Telophase II, concludes with the formation of four haploid daughter cells, each with single-chromatid chromosomes.



The haploid MII oocyte, once activated form the spermatozoon, halves the DNA content.

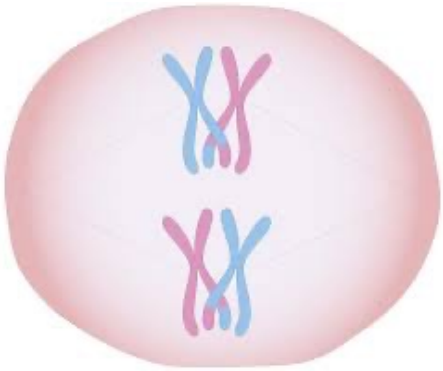
This process **ensures** that the union of two gametes (each with a haploid set) **restores a diploid chromosome set** (2n):

- keeping the number of chromosomes constant from one generation to the next
- contributing to genetic variability through crossing over and independent assortment of chromosomes.

Oocyte genetic variability

- crossing over (recombination)
  - independent assortment

Crossing Over
Crossing over occurs during **prophase I of meiosis I**,
where homologous chromosomes exchange DNA segments.
This recombination of genetic material creates new combinations of alleles on a single chromosome, which contributes to genetic diversity in the resulting gametes



Genetic variability is increased primarily through two mechanisms during meiosis: independent assortment and crossing over (recombination)

Independent Assortment

During metaphase I of meiosis I, homologous chromosome pairs align randomly at the metaphase plate. This random orientation means that the daughter cells resulting from meiosis can have numerous combinations of maternal and paternal chromosomes.

For humans, with 23 pairs of chromosomes, this can lead to about 8 million different.

combinations due to independent assortment alone.

Crossing Over

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Oocyte genetic variability

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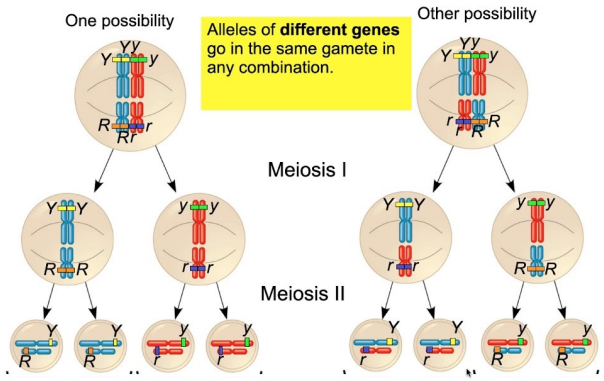


The genome variability induced by the first meiotic division is:

2^n pairs of chromosomes

For humans, with 23 pairs of chromosomes, this can lead to about
8 million different combinations

Genome variability in species with different karyotype

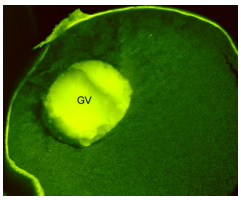
Specie		N° of different MII gametes
Drosophila Melanogaster	n=4	2^4=16
Apes mellifera	n=16	2^{16}=65.536
Mus musculus	n=20	2^{20}=1.048.576
Homo sapiens	n=23	2^{23}=8.000.000

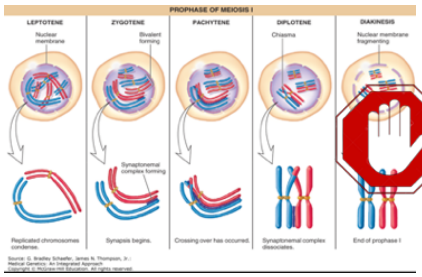
Here just some examples of genome variability in species with different karyotype.

OOGONIA enter MEIOSIS BUT STOP AT PROPHASE I

Now we know the reason of the meiotic blockade

Day 14 pc





Meiotic cell cycle commitment

The meiotic cell cycle block at the diplotene stage of Prophase I since:

1. IN EARLY OOGENESIS: Due to the meiotic incompetence (low RNA and proteins related to cell cycle)
2. IN LATE OOGENESIS: due to inhibitory signals into the FF

The oogonia undergo the transition into meiosis; however, once committed to the meiotic cell cycle, they do not complete it. The reason lies in the inadequate presence of cell cycle proteins in the early stages of oogenesis, a condition that persists until the oocyte attains its fully grown size, becoming meiotically competent.

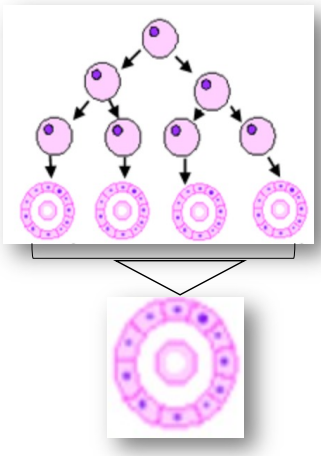
In more detail, initially, oocytes can progress through the first four stages of PROPHASE I, only to halt at the diplotene stage. This meiotic stage persists for an extended period, primarily because the oocytes are initially incompetent. Furthermore, in fully grown oocytes, inhibitory molecules present in the follicular fluid (FF) contribute to this persistence.

Physiologically, the resumption of the meiotic cell cycle occurs exclusively in response to a stimulatory signal, represented by the LH surge.

THE TRANSITION:
from Foetal to Post-natal oogenesis

The quiescence of meiotic cell cycle allows the oocytes to undergo a long process of **specialization involving nucleus** and **cytoplasm** during post-natal oogenesis.

When the **follicle and oocyte specialization** have **concluded** the meiosis cell cycle may resume under the inductive action of LH by obtaining a haploid cell that contains half of the original number of chromosomes (1N).

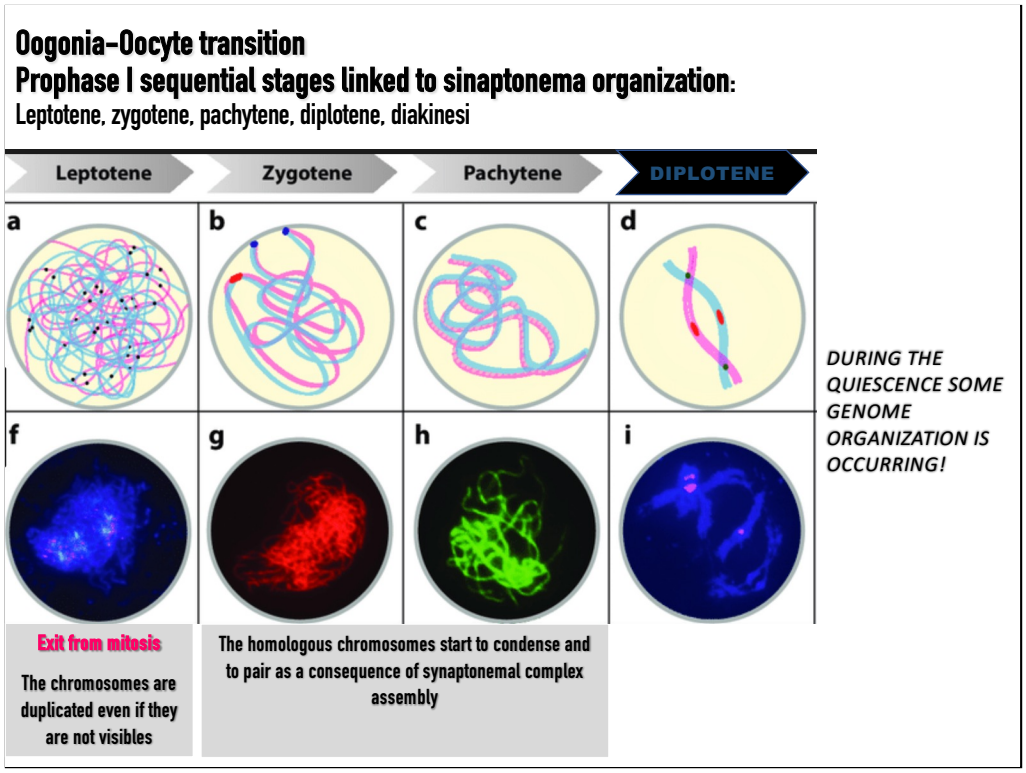


Oogonia

Oocytes

The long-term meiotic cell cycle quiescience allows that oocyte specialization may take place

The quiescence of meiotic cell cycle allows the oocytes to undergo a long process of specialization involving nucleus and cytoplasm during post-natal oogenesis. When the follicle and oocyte specialization have concluded the meiosis cell cycle may resume under the inductive action of LH by obtaining a haploid cell that contains half of the original number of chromosomes (1N).



The transition from oogonia to oocyte induces relevant transformation in gamete genome even if the female gamete undertakes only a small part of meiotic cell cycle.

The Prophase I is a long phase during which duplicated homologous chromosomes are assembled by a molecular complex called synaptonemal through sequential step-wise processes recognizing 4 phases: leptotene, zygotene, pachytene and diplotene.

These phases are classified on the basis of morphological changes involving the homologous chromosomes.

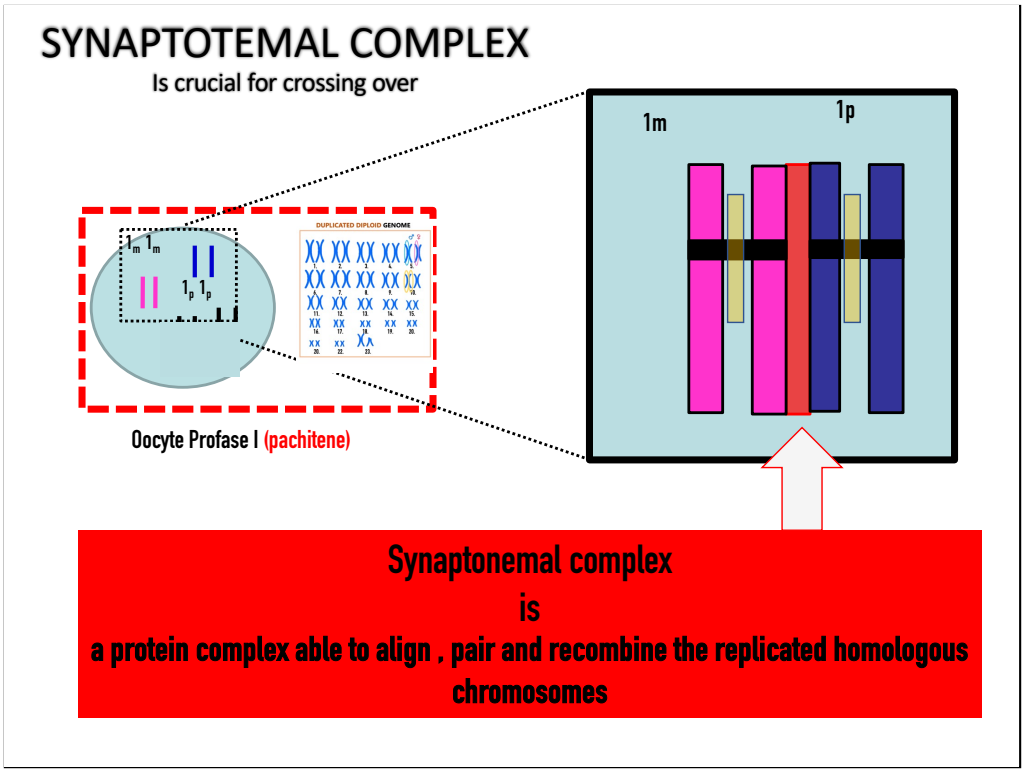
During the first phase, the leptotene, the duplicated chromosomes start to condense and become more visible as individual structures.

Immediately later, the cell entry into the zygotene phase, and the homologous chromosome start to become visible as parallel and elongated paired structures

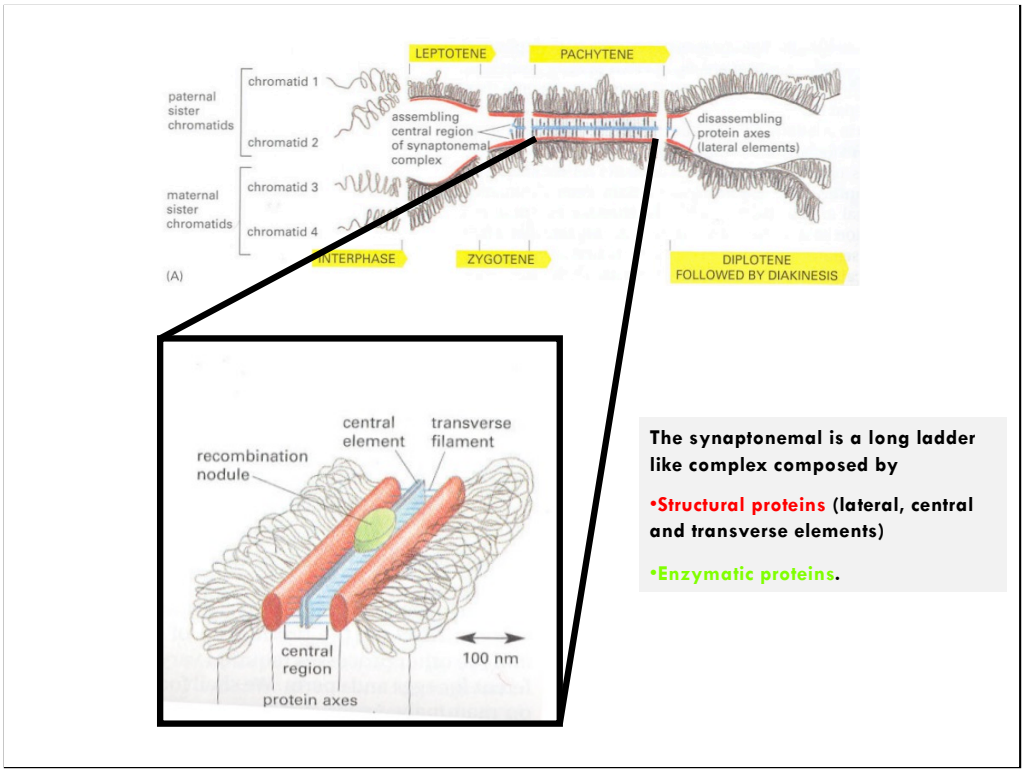
In pachytene, the homologous chromosomes are strictly in contact each other for days and highly condensed .

During the diplotene stage, the homologous chromosomes are less close each other even if they maintain point of contact.

Once the oocytes reach the diplotene stage, it arrests and the interphase nucleus remains in this quiescent status until the oocyte will become competent and it will be enclosed in a preovulatory follicle exposed to the LH surge.



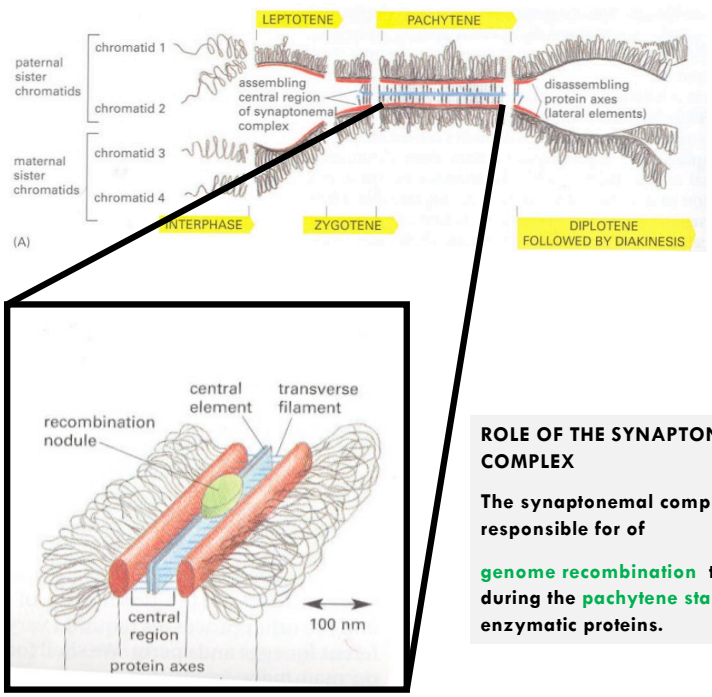
The synaptonemal is a macromolecular complex with a long ladder-like structure aimed to pair homologous duplicated chromosomes during meiosis.
The synaptonemal is represented in this slides from this red box aligning between two duplicated homologous chromosomes.



The synaptonemal is a long ladder like complex composed by

- **Structural proteins (lateral, central and transverse elements)**
- **Enzymatic proteins.**

In order to understand how synaptonemal work we have to remember that it is composed from different molecular structure the lateral proteins structure of synaptonemal complex colored in red links sister chromatids of each chromosome during the leptotene by inducing a progressive condensation of them
the transverse filaments assemble the synaptonemal as 3D ladder by linking the central element to lateral ones. The transverse elements are operative during the zygotene when replicated homologous chromosomes start to be aligned each others.
At the pachytene stage ethe synapotonemal complex is completely assembled thus exposing the replicated pairs of homologous chromosome to the enzymatic action of the recombinant nodules that are localized in the central area.
In conclusion the synaptomenal complex recognized structural components required to pair the homologous chromosome and the enzymatic component to remodel the DNA inducing an event of genome mutagenesis at the end of fetal oogenesis.



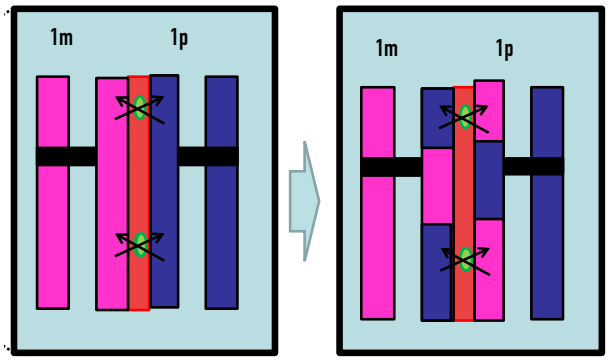
ROLE OF THE SYNAPTONEMAL COMPLEX

The synaptonemal complex is responsible for of

genome recombination taking place during the pachytene stage using the enzymatic proteins.

The enzymatic proteins of synaptonemal are responsible for the crossing over, an event of **genome recombination**, ***that takes place during the pachytene stage***.
There is strong indirect evidence that these genome mutagenesis occurring during meiosis are catalyzed by recombination nodules, very large enzymatic complexes that are sit at regular intervals on the synaptonemal complex.
This enzyme complex is placed like basketballs on a ladder between the two homologous chromosomes.

The recombinant nodules are able to break the DNA double helix in close maternal and paternal chromatids thus allowing the exchange of DNA fragments (recombination) between the two non sister chromatids in a reciprocal fashion



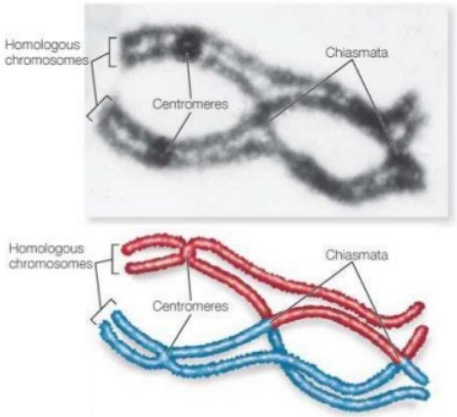
2-3 events of crossing over/chromosom guarantee a large geentic assortment

The recombinant nodules are able to break the DNA double helix acting on close maternal and paternal chromatids thus allowing DNA exchange between two non sister chromatids in a reciprocal fashion manner as illustrated in the slide.
The chromosomal crossing is responsible for a large genetic assortment.
In order to understand the dimension of this phenomenon an average of 2 or 3 crossover events may randomly occur on each pair of human chromosomes.

The consequence of crossing over events can be observed using TEM at the latest stage of prophase I (diplotene)
when the chromosome in the bivalent form are highly condensed and paired
(even if the synaptonemal complex disassembles)

Chiasmata: evidence of exchange between chromatids
This micrograph shows a pair of homologous chromosomes, each with two chromatids, during prophase I of meiosis in a salamander.

Two chiasmata are visible.

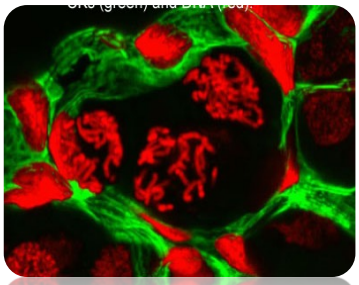


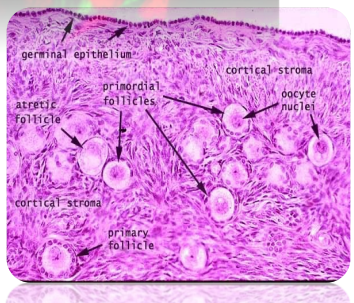
The DNA consequence of crossing over events can be observed using TEM at the latest stage of prophase I (diplotene) when the chromosome in the bivalent form are highly condensed and paired even if the synaptonemal complex disassembles
At this stage, the sister chromatids are tightly opposed along their entire length, and the two duplicated homologs (maternal and paternal) are seen to be physically connected from DNA bridges in specific points. This point, called chiasma corresponds to unsoved crossover event occurring between two close sister chromatids.
At this stage of meiosis, each pair of duplicated homologous is held together by at least one chiasma.
Many bivalent structures contain more than one chiasma, indicating that multiple crossovers may occur in pachitene oocytes.

A comprehensive summary of all the content covered in this course.

Fetal oogenesis

- The primordial gametes are differentiated early during embryo development (PGC).
- The somatic epigenetic asset is erased.
- The sex specific gamete lineage is defined (oogonia).
- The oogonia proliferate through mitosis by increasing the number of gametes.

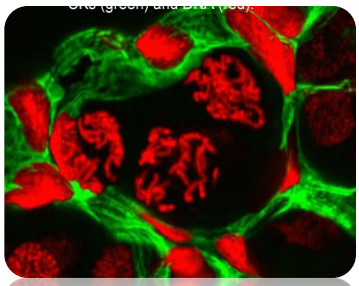


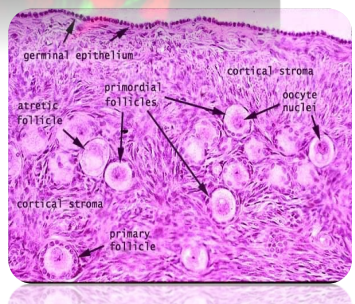


Transition from fetal to post-natal oogenesis

The differentiation of the oocyte few days before or after birth leads to:

1. Stop cell proliferation,
2. Entry into meiosis cell cycle
3. Increase genome variability
4. Block of gamete cell cycle at the diplotene stage of prophase I.





Post-natal oogenesis begins

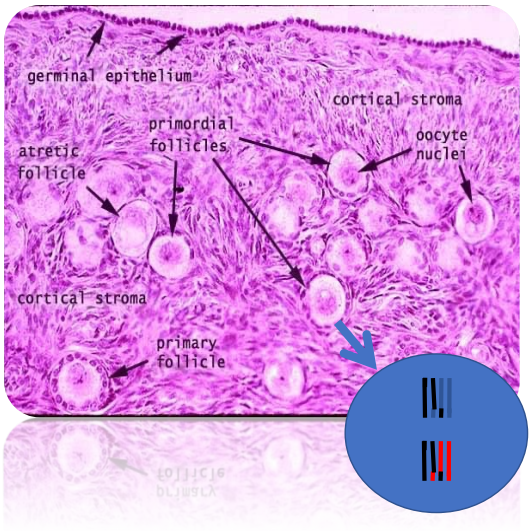
At birth the ovaries contain:

a) a fixed number of oocytes

b) with a highly variable genome.

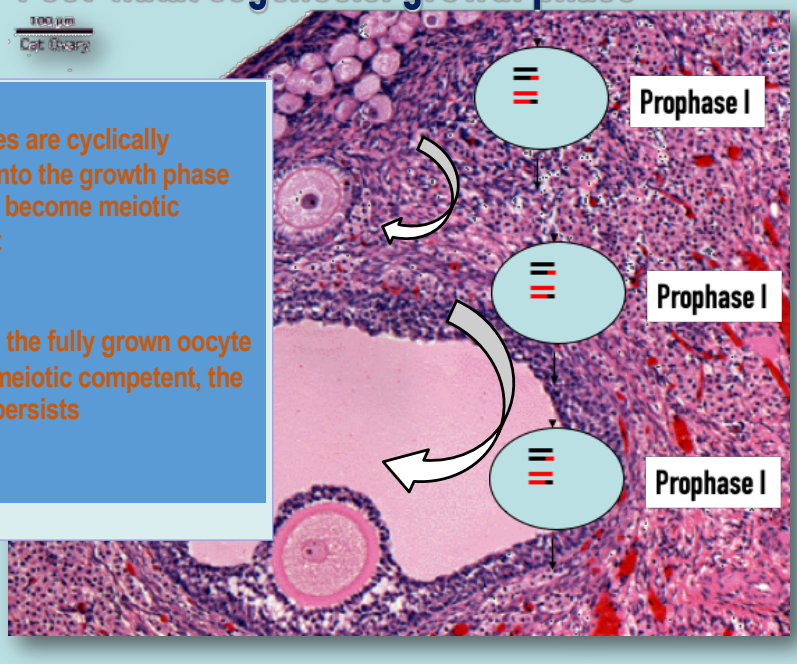
The oocytes are differentiated gametes even if they :

1. display small dimension,
2. are meiotically incompetent
3. have a high transcription activity with a genome without any epigenetic markers.



Post-natal oogenesis: growth phase

- The oocytes are cyclically recruited into the growth phase in order to become meiotic competent
- Even once the fully grown oocyte becomes meiotic competent, the GV stage persists
-



Oogenesi post-natal: maturation phase

- The meiotic cell cycle will be resumed when a fully grown oocyte will be enclosed within a pre-ovulatory follicles and exposed to LH surge.
- The resumption of meiosis is aimed to:

1. reach the developmental competence
2. complete epigenetic maturation
3. halve genome makeup
4. increase further genome variability