Role of Primary Imprinted Genes in Mammals

Genes regulating the synthesis of proteins involved in the process of

- embryo and fetal annexes growth
- tissue differentiation

What's the role of the imprinted genes?

In mammals most of the imprinted genes were identified as key genes **regulating** the synthesis of proteins involved in the process of

- embryo and fetal annexes growth,
- tissue differentiation.

A relationship between imprinted genes and parental behavior has been unveiled

- Lactation
- Mother-offspring interaction

Some imprinted genes are expressed in the brain where modulate behaviors related to

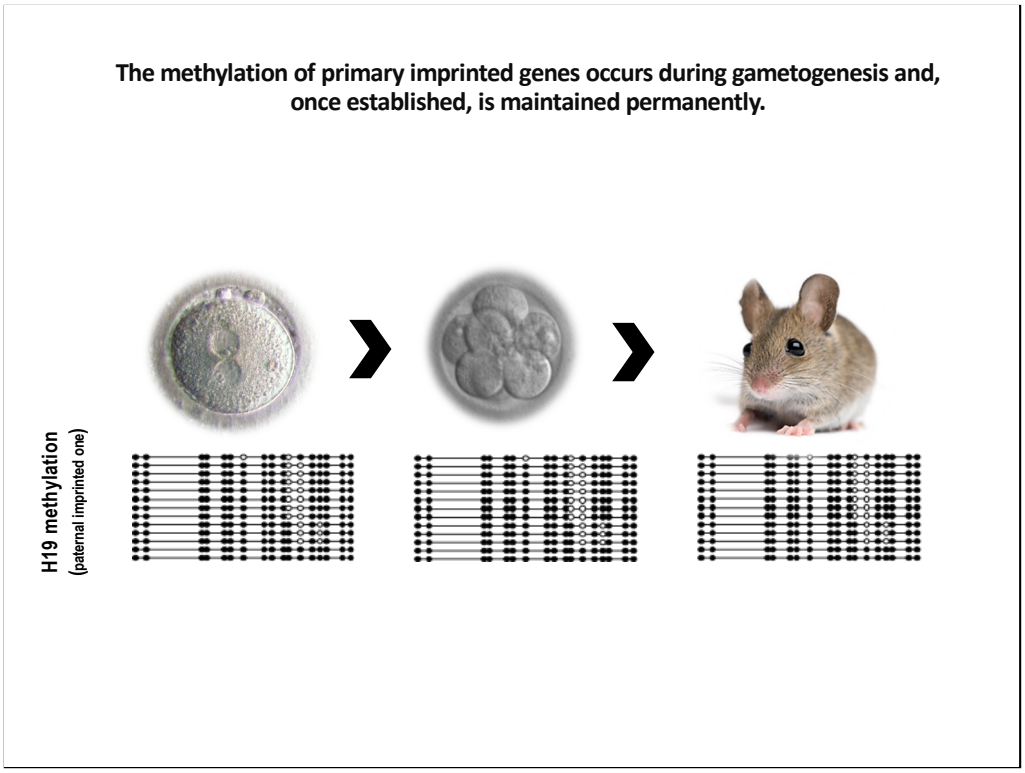
- Nurturing
- Feeding
- Emotional bonding

To date the exact relationship mechanisms must be disclosed!!!

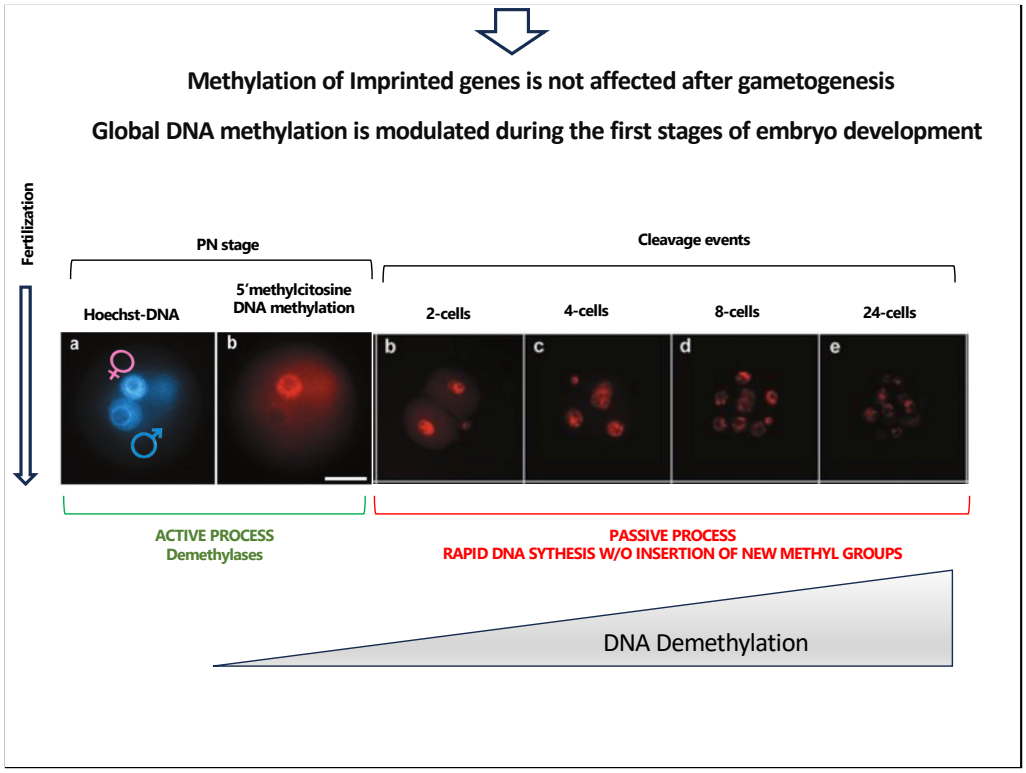
The relationship between **imprinted genes** and **parental behavior**, particularly in the context of **lactation** and **mother-offspring interactions**, is complex. Imprinted genes have been shown to play a role in the development of the brain and in behaviors that are crucial for the survival of offspring, such as maternal care.

It has been shown that certain imprinted genes are expressed in the brain and are implicated in modulating behaviors related to nurturing, feeding, and emotional bonding

However, the exact relationship mechanisms linking mother and offspring during lactation, mediated by imprinted genes, are complex and not fully detailed. Further research in this area would be necessary to fully understand the precise role of imprinted genes in modulating parental behavior throughout an adult's lifetime, especially during critical periods such as lactation.



The methylation of primary imprinted genes occurs during gametogenesis and, once established, is maintained permanently.



Conversely, global DNA methylation is modulated during the first stages of embryo development.

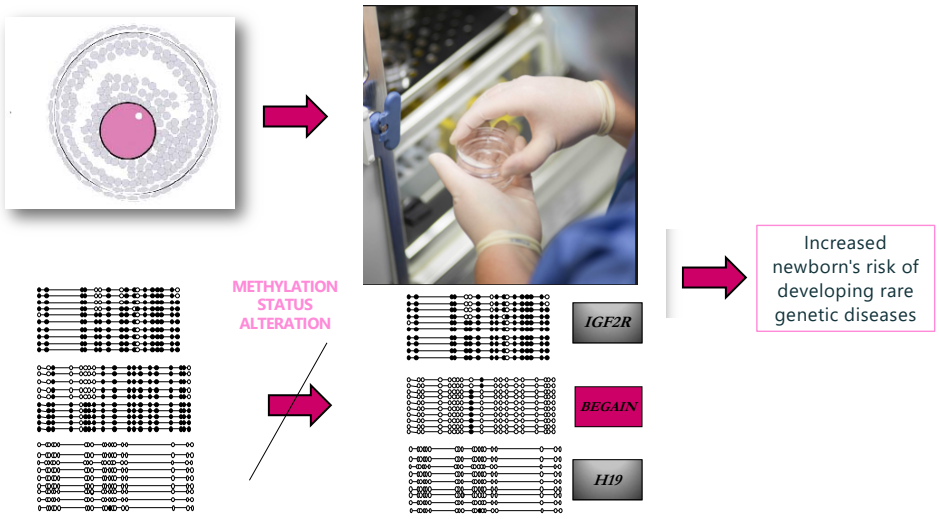
A consistent global DNA demethylation occurs in male pronucleus (PN) after fertilization. Subsequently**, in corrispondence** of each cleavage event (during the first stages of the embryo development) both male and female genomes are subjected to DNA demethylation.

Demethylation occurring in the male PN is dependent on the activity of demethylates (therefore it is defined as an active process).

Demethylation ocurring afterwards is a consequence of a rapid DNA synthesis that occurs without a corresponding insertion of new methyl groups.

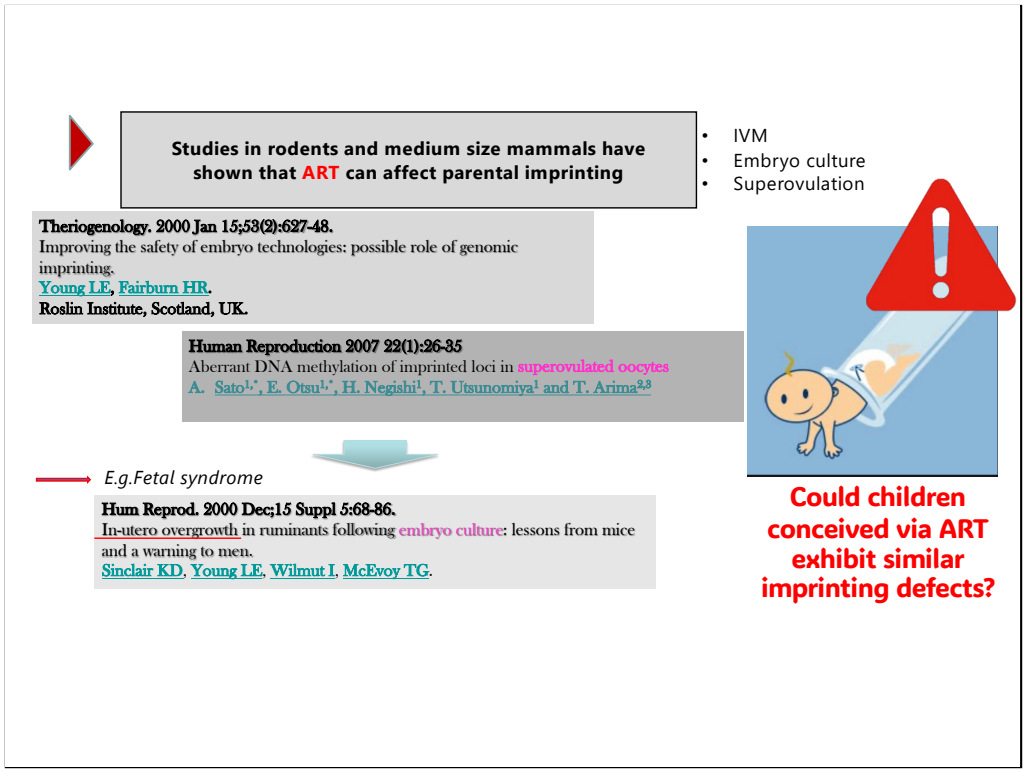
Imprinted genes are not involved by demethylation!!!

ART and defects of Primary Imprinting



Genes receiving imprinting later in oogenesis are more susceptible

Incorrèct artificial manipulation of gametes and embryos (Artificial Reproductive Technologies) might alter the methylation status of certain imprinted genes. These genes became abnormally methylated, hypo- or hypermethylated.
These uncontrolled methylation processes increase the newborn's risk of developing rare genetic diseases.
Not all imprinted genes are equally affected to this phenomenon, but those methylated later in oogenesis are more susceptible.



Studies in rodents and medium size mammals have shown that ART (IVM, embryo culture, superovulation) can affect parental imprinting status and consequently the expression of imprinted genes in embryos. As an example:

Defect of imprinting are responsable of fetal syndrome such the «in utero fetus overgrowth»

20 years ago, these findings raised concerns that children conceived via ART might also exhibit similar imprinting defects. Consequently, numerous meta-analyses have been conducted to evaluate the validity of this hypothesis.

Epidemiological studies have shown that children born following IVF and ICSI embryos, have a higher risk of developing genomic imprinting disorders.

DISEASE	Reference	No. of cases	Technology performed	Loss of imprinting	Country
Beckwith–Wiedemann	DeBaun et al. (2003)	7	IVF and ICSI	*KCNQ1OT1* and *H19*	USA
	Gicquel et al. (2003)	6	IVF and ICSI	*KCNQ1OT1*	France
	Maher et al. (2003)	6	IVF and ICSI	*KCNQ1OT1*	UK
	Bonduelle et al. (2002)	1	ICSI	ND	Belgium
	Boerrigter et al. (2002)	1	ICSI	ND	–
	Olivennes et al. (2001)	1	IVF and ICSI	ND	–
	Koudstaal et al. (2000)	1	IVF	ND	The Netherlands
	Sutcliffe et al. (1995a)	1	IVF	ND	UK
Angelman	Orstavik et al. (2003)	1	ICSI	*SNRPN*	Norway
	Cox et al. (2002)	2	ICSI	*SNRPN*	Germany
Silver–Russell	MRC Working Party (1990)	1	ICSI	ND	UK
Prader–Willi	Butler MG.(2009)	3	ICSI	NO	UK

Human Reproduction Update, Vol.10, No.1 pp.3–18, 2004 European Society of Human Reproduction and Embryology

Epidemiological studies have shown that children born following IVF and ICSI embryos, have a higher risk of developing genomic imprinting disorders such as the Beckwith-Wiedemann, Angelman, Silver-Russel and Prader-Willi.

However, since these findings are based on indirect epidemiological analysis rather than direct experimental studies—which cannot be conducted on humans—the results are not definitive.

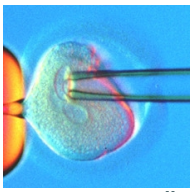
The increased incidence of imprinting syndromes may be due to a variety of factors, including inadequate reproductive protocols or pre-existing epigenetic defects in the gametes of infertile couples.

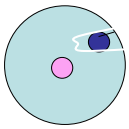
Concerns raised by these epidemiological findings have heightened attention to ART protocols.

Research efforts are now focused on optimizing in vitro techniques and implementing safeguards to minimize the epigenetic risks during in vitro manipulation of oocytes and embryos.

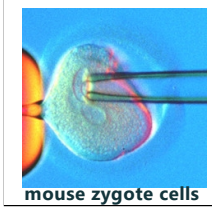
FIRST EXPERIMENT CONFIRMING THE ROLE OF GENE IMPRINTING
Surani created uniparental embryos

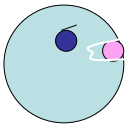
- impact on embryo development.
- Impact on fetal annexes development.





mouse zygote cells





mouse zygote cells

> Science. 1983 Dec 2;222(4627):1034-6. doi: 10.1126/science.6648518.

Development of gynogenetic eggs in the mouse: implications for parthenogenetic embryos

M A Surani, S C Barton

PMID: 6648518 DOI: 10.1126/science.6648518

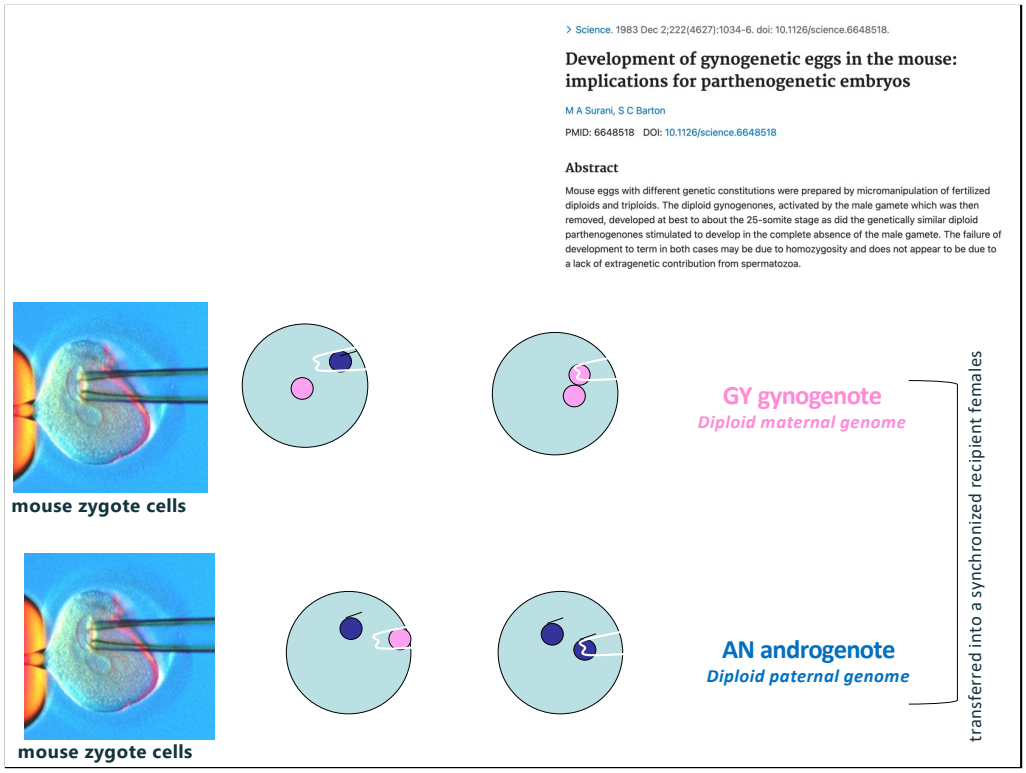
Abstract

Mouse eggs with different genetic constitutions were prepared by micromanipulation of fertilized diploids and triploids. The diploid gynogenones, activated by the male gamete which was then removed, developed at best to about the 25-somite stage as did the genetically similar diploid parthenogenones stimulated to develop in the complete absence of the male gamete. The failure of development to term in both cases may be due to homozygosity and does not appear to be due to a lack of extragenetic contribution from spermatozoa.

In 1983, Surani and colleagues successfully created uniparental embryos to explore their impact on embryo and fetal annex development.

Surani used mouse zygote cells, where the two genomes are separate and visibly distinct within the cytoplasm and where male and female pronuclei (PN) show different dimorphism. By employing a micromanipulator, Surani was able to **extract** one PN and then **reconstitute t**he zygote's genome with an **external PN** of the same **sex**.

The resulting reconstituted zygotes, displaying either a diploid paternal (AN androgenote) or maternal (GY gynogenote) genome, were then **transferred** into a **synchronized recipient female**.



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Two weeks after embryo transfer, both types of fetuses perished

Different were biological causes:

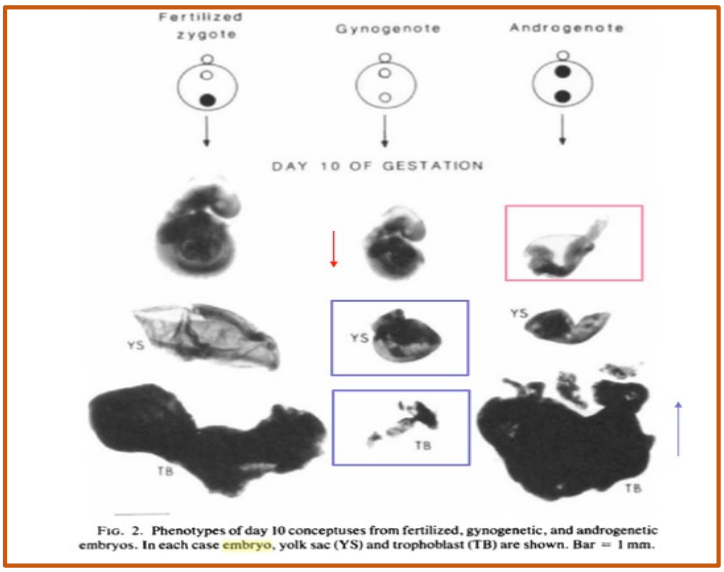


FIG. 2. Phenotypes of day 10 conceptuses from fertilized, gynogenetic, and androgenetic embryos. In each case embryo, yolk sac (YS) and trophoblast (TB) are shown. Bar = 1 mm.

Little fetal annexes
Delay in embryo growth

Excessive Trophoblast growth
Limited embryo growth

Unlike normal embryos, androgenote (AN) and gynogenote (GY) fetuses ceased development at early stages. Roughly two weeks after embryo transfer, both types of fetuses perished. Notably, AN and GY fetuses died due to different and seemingly opposite biological causes.

Gynogenote fetuses exhibited underdeveloped fetal annexes, such as the vitelline yolk sac and trophoblast. This underdevelopment led to a delay in embryonic growth, resulting in premature death due to insufficient nutritional support.

In contrast, **androgenote fetuses** died for the opposite reason. These embryos experienced limited embryo development in the face of excessive trophoblast growth, which ultimately compressed and suffocated the fetus.

Surani lacked **molecular evidence** for the existence of imprinting genes at the time

THE HYPOTHESIS
normal mammalian embryonic development requires the presence of both parental genomes, which contribute in a complementary manner to gene expression.

The expression of certain parental genes, later identified as imprinted genes, is functionally synergistic.

NOW WE CAN INTERPRETATE THE SURANI EXP
WHAT DOES IT TELL?

maternal genes appear to be crucial for normal embryo development	paternal genes are more vital for the growth of fetal annexes

Althought Surani lacked **molecular evidence** for the existence of imprinting genes at the time

these biological observations led him to hypothesize that normal mammalian embryonic development requires the presence of both parental genomes, which contribute in a complementary manner to gene expression.

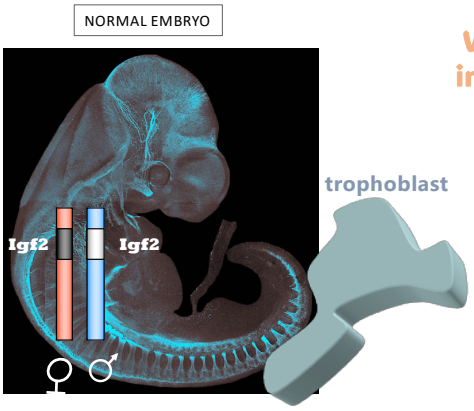
The expression of certain parental genes, later identified as imprinted genes, is functionally synergistic. Specifically, maternal genes appear to be crucial for normal embryo development, while paternal genes are more vital for the growth of fetal annexes.

Let's now look at the Insulin-like Growth Factor 2 gene

UNIT-II
Lesson work 1

Maternal imprinted gene

Expressed only by the paternal allele in normal embryos where regulates fetus annexes growth

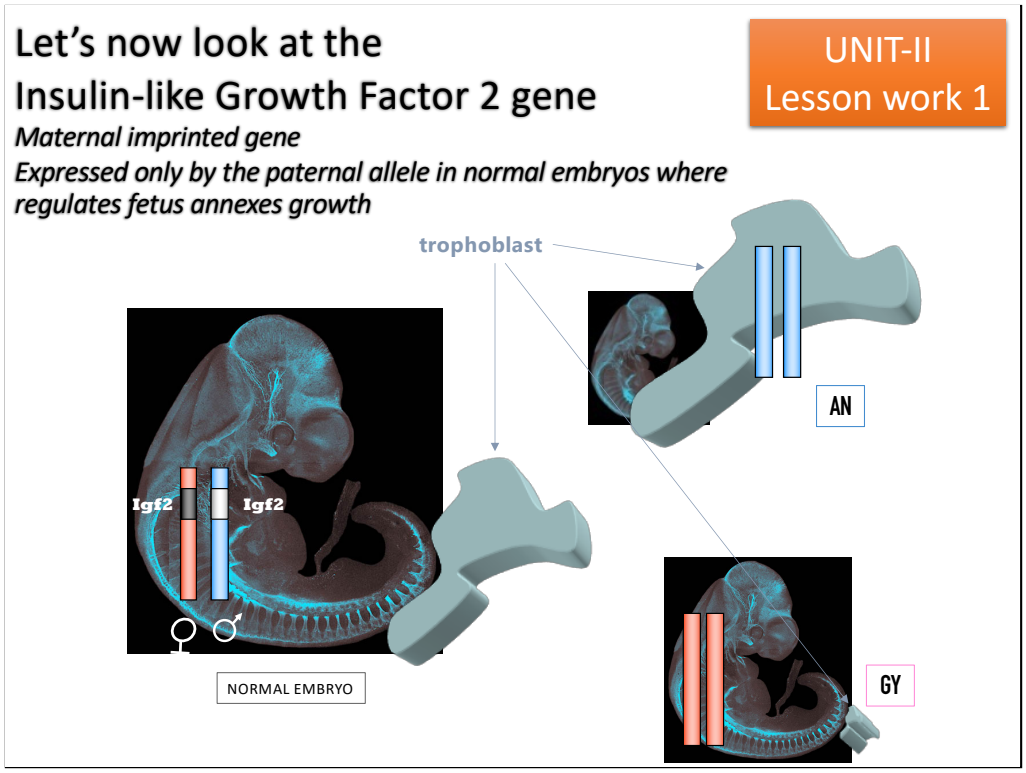


What would you expect to happen in an androgenetic embryo and in a gynogenetic embryo?

One such genes is Insulin-like Growth Factor 2 (IGF2), crucial for the development of **placental annexes**.

IGF2 is normally expressed solely from the paternal allele. It is imprinted (silenced) in the oocyte.

In androgenote (AN) embryos, IGF2 levels are doubled, leading to excessive growth of placental annexes. Conversely, in gynogenote (GY) embryos, IGF2 is not expressed, hindering the growth of fetal annexes. In both scenarios, the embryos perish due to the lack of synchronized development between the fetus and its annexes, highlighting the importance of mono-allelic expression of this gene for successful development.



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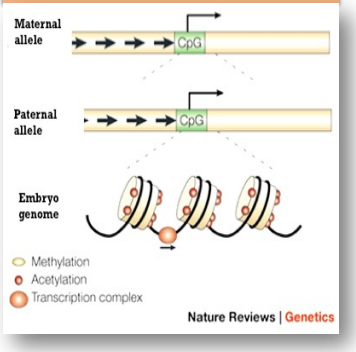
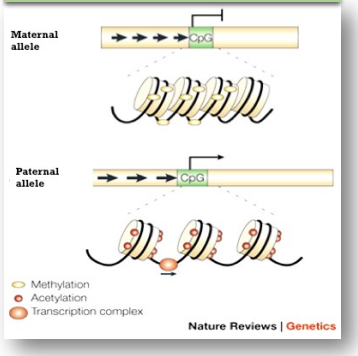
TAKE HOME MESSAGE

While somatic genes typically exhibit biallelic transcription, meaning both maternal and paternal alleles are expressed**, imprinted genes are characterized by monoallelic expression,** where only one allele—either maternal or paternal—is expressed**.**
This imprinting process is essential in mammals, as it ensures that both maternal and paternal genomes contribute in a complementary and necessary manner to development.

Transcription of somatic genes



Transcription of imprinted genes



Telomerase activity during the growth phase

Definition

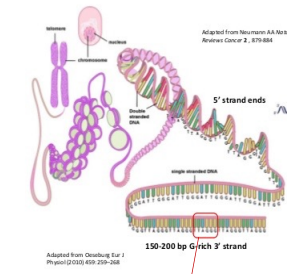
Telomerase is a ribonucleoprotein.

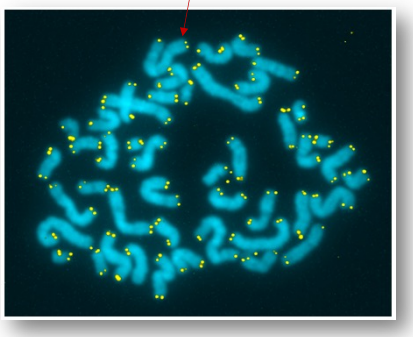
Activity

Telomerase is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes.

Role

- Maintaining the length of telomeres, thereby ensuring the **stability** and **integrity** of the **chromosomes**. Genetic stability is required for ensuring that mitosis goes well durng the first cleavage events upon fertilization.
- Telomeres **protect chromosomes** from deterioration or fusion with neighboring chromosomes, which is crucial for cellular aging and cancer prevention.





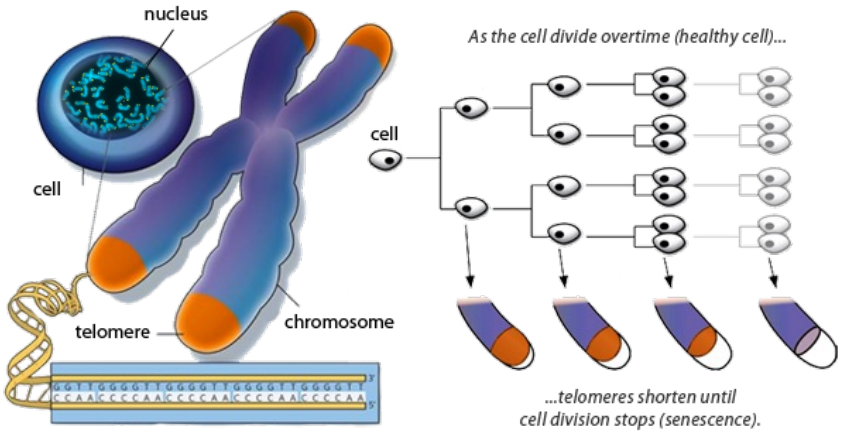
Telomerase is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes.

This process is essential for maintaining the length of telomeres, thereby ensuring the stability and integrity of the chromosome. Telomeres protect chromosomes from deterioration or fusion with neighboring chromosomes, which is crucial for cellular aging and cancer prevention.

Telomerase is a ribonucleoprotein, meaning it contains both RNA and protein components; the RNA component serves as a template for the telomere repeat sequence.

In most somatic cells, telomerase activity is not detectable, leading to a gradual shortening of telomeres with each cell division, which eventually triggers cellular senescence or apoptosis. However, in stem cells, germ cells, and cancer cells, telomerase activity is present, allowing these cells to divide indefinitely without the typical chromosomal degradation associated with aging.

Crucial for preventing the loss of genomic information, which can occur during each round of DNA replication.

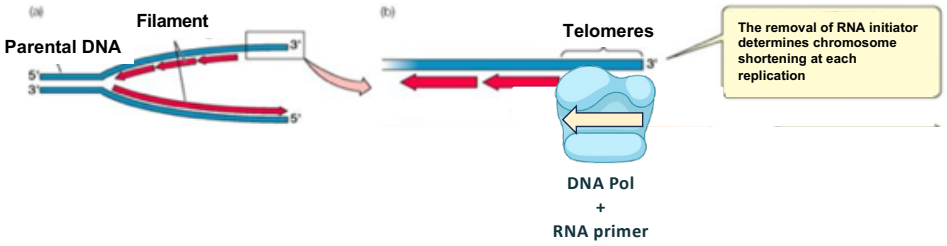


More in detail. They are crucial for preventing the loss of genomic information, depicted here as the orange segment of the chromosome, which can occur during each round of DNA replication.

Molecular basis of telomere shortening

It is related to the way DNA polymerase replicates DNA !

During DNA replication, DNA polymerase cannot completely copy the ends of chromosomes, due to the "**end-replication problem**»

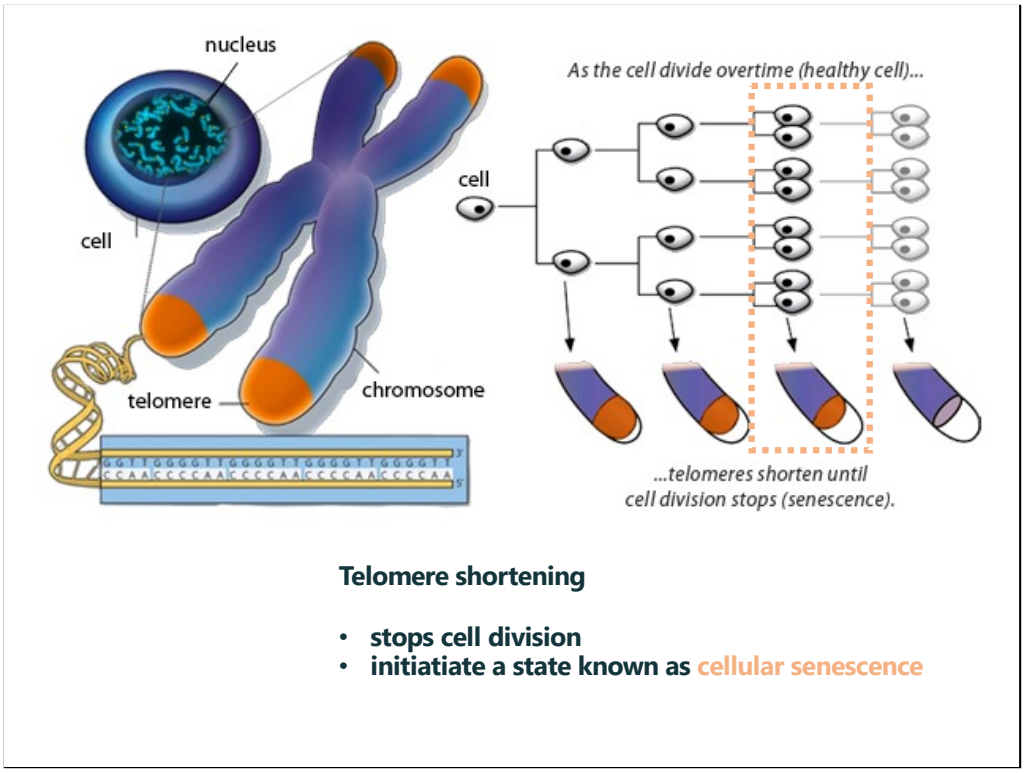


With each cell division, telomeres progressively shorten

The molecular cause of telomere shortening is related to the way DNA polymerase replicates DNA.

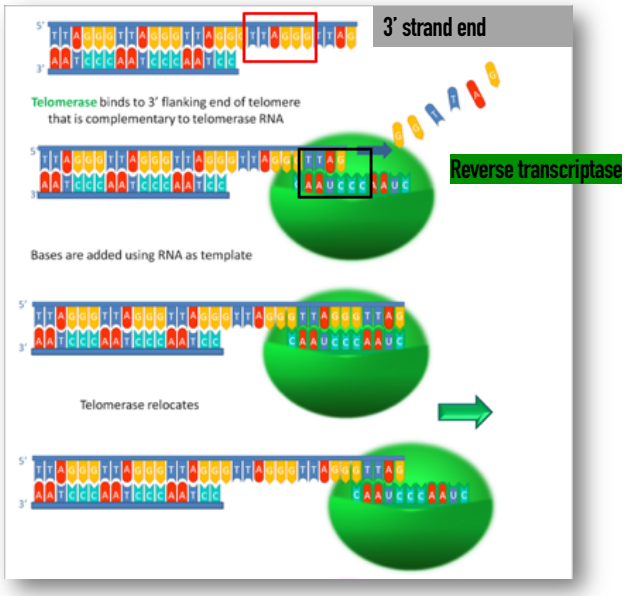
During DNA replication, DNA polymerase cannot completely copy the ends of chromosomes, known as telomeres, due to the "end-replication problem."

This issue arises because DNA polymerase requires an RNA primer to initiate DNA synthesis, and after this primer is removed, there remains an unreplicated DNA segment at the 3' end of the lagging strand. Consequently, with each cell division, telomeres progressively shorten.



This protective mechanism impacts cellular proliferation, as cells can only divide as long as their telomeres are sufficiently long. Once **telomeres reach a critical threshold length**, indicated by the blue box, an internal checkpoint activates a protective process, leading to senescence (a permanent cell cycle arrest in which cells remain metabolically active but do not divide) and ultimately to apoptosis.

Thus, the length of telomeres determines the lifespan of a cell.



The phenomenon of telomere shortening is inhibited by the presence of enzymes such as telomerase.

The biological consequence: cells with an active telomerase may proliferate without limits

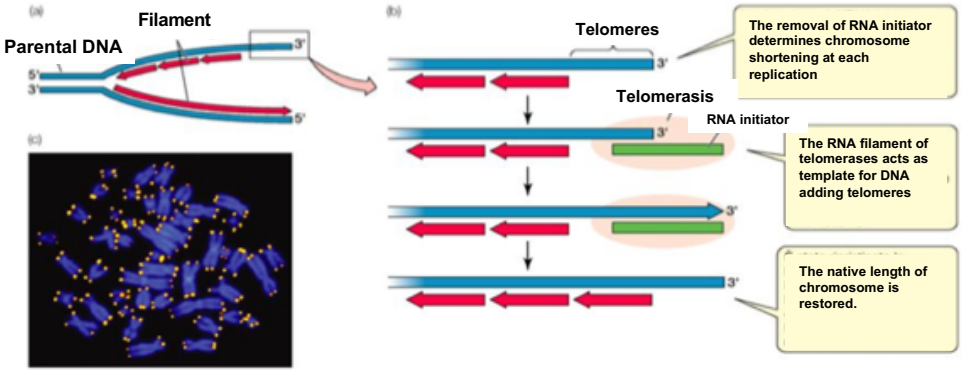
However, fortunately, the phenomenon of telomere shortening is inhibited by the presence of enzymes such as telomerase.

The biological consequence of that is that such as cells with an active telemorares may proliferate without limit and so they may be considered immortal.

A limited number of cells of the adult organism exibits active telomerase, such as:

1.Embryo stem cells
2.Adult stem cells
3.Cancer stem cells
4.Early stage gametes

RESULT ⇨ these cells can proliferate without entering senescence, as their telomeres are actively elongated with each round of DNA replication **SELF-RENEWAL**



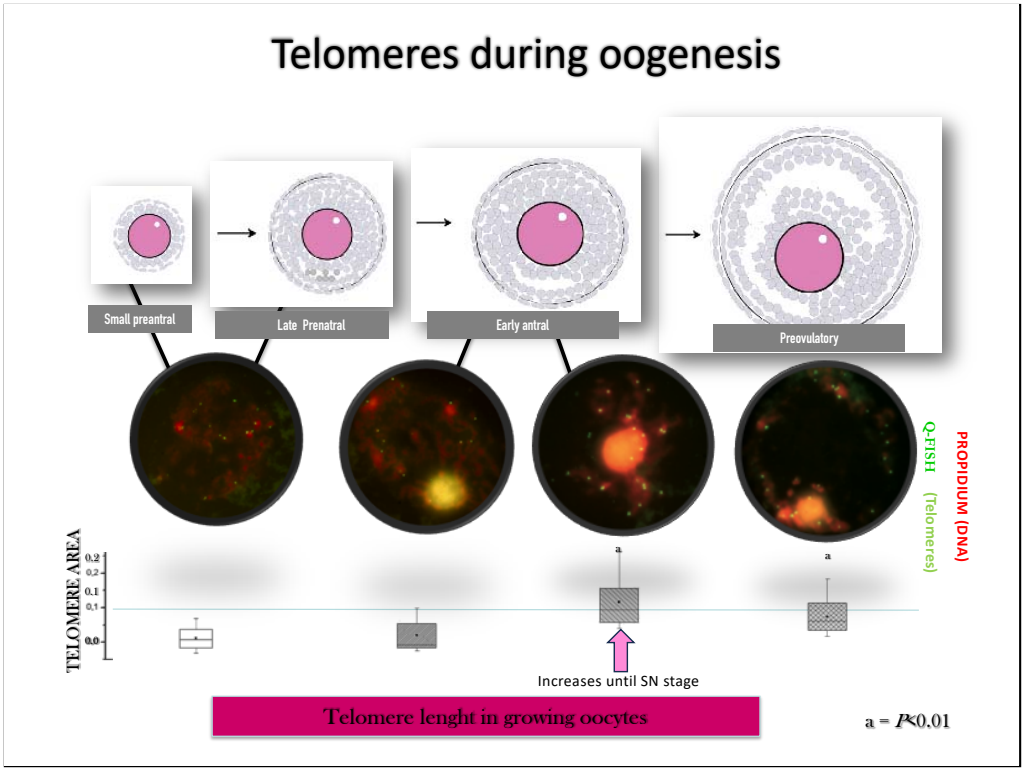
A very limited number of cells exhibits active telomerase, including:

- Embryonic stem cells,
- Fetal and adult stem cells,
- Cancer stem cells.

As a result, these cells can proliferate without entering senescence, as their telomeres are actively elongated with each round of DNA replication.

This capacity for indefinite replication is commonly referred to as self-renewal. Additionally, oocytes in the early stages of development also possess active telomerase.

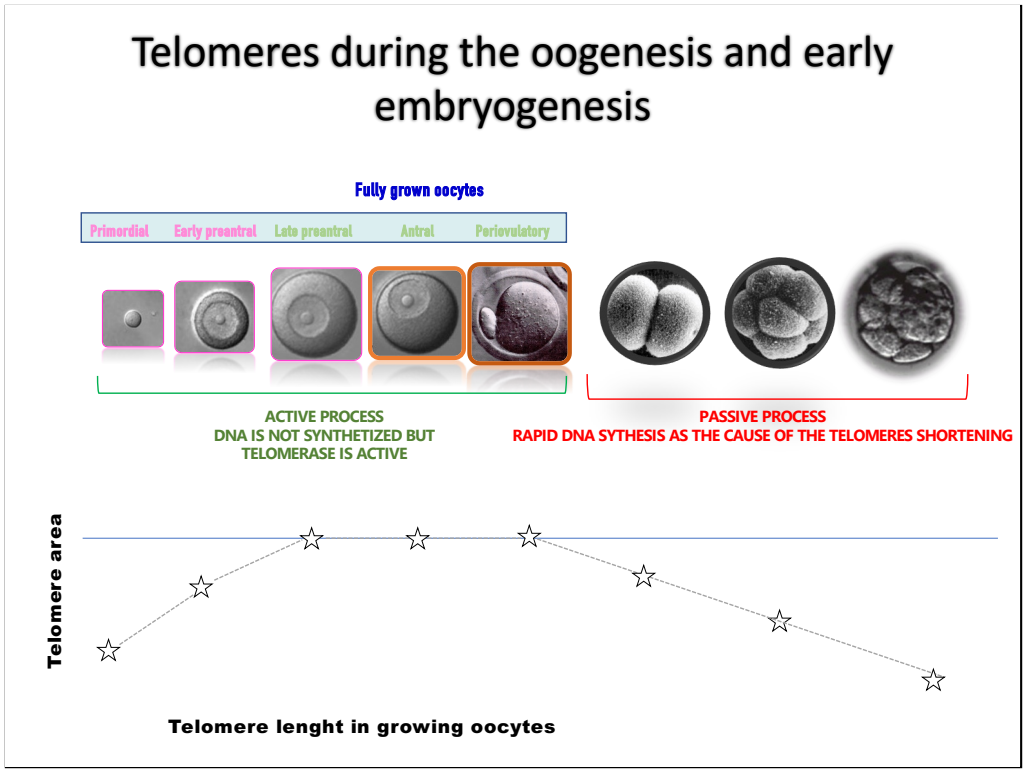
- ✓ How telomeres are managed during the early stages of oogenesis?
- ✓ What about the role of telomerase in cells like the oocyte, which are not in a replicative state?



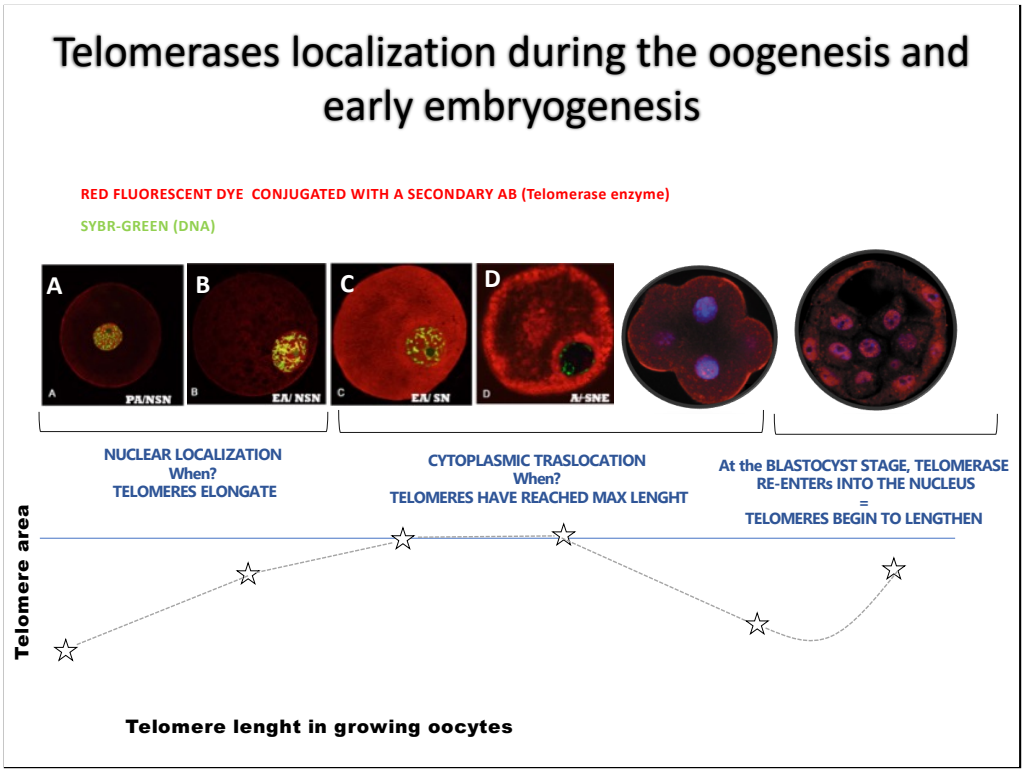
We must now turn our attention to understanding how telomeres are managed during the early stages of oogenesis and the role of telomerase in cells like the oocyte, which are not in a replicative state.

We have collected the oocyte at different stage of follicologenesis and then we can evaluate the telomeres size using genomic primers designed for telomeres sequences.

Focus on experiement depicted in the slide representing a sheep oocyte at different growth phases:
- Telomeres are measured using the FISH (fluorescence in situ hybridization) technique with a probe that recognizes the TTAGGG sequence of the human genome. The green fluorescent spots within the nuclei represent the telomeres.
Their lengths are measured to compare their sizes throughout oogenesis. The box plots indicate the average telomere area in oocyte nuclei, showing that these structures lengthen as the oocyte progresses from early to advanced stages of follicle development. Quantitative analysis reveals that telomeres are actively elongated in sheep oocytes until the chromatin configuration reaches the SN (surrounded nucleolus) stage. The active elongation of telomeres ceases subseuqntly to SN stage.



Since the oocyte's DNA is not replicated throughout oogenesis, this process results in a significant increase in telomere length. The telomeres then maintain their size until the mature oocyte is fertilized and segmentation begins.
As illustrated in this slide, once the zygote enters the cleavage process, the telomeres gradually decrease in size with each mitotic division.
This observation indicates that during the rapid DNA replication active in the early stages of embryonic development, the telomeres are passively shortened.



Telomerase is always present in oocytes, although it is not continuously active in the nucleus during oogenesis.

The enzyme's nuclear presence is present only in growing oocytes from preantral (A) and early antral follicles (B) with a non-surrounded nucleolus (NSN) chromatin configuration. Subsequently, telomerase relocates from the nucleus to the ooplasm in oocytes with a surrounded nucleolus (SN) chromatin configuration, which are collected from early antral follicles (C). This translocation occurs when telomeres have reached their maximum length and cease to elongate (C). Later, the enzyme is found exclusively in the cytoplasm, as seen in oocytes from pre-ovulatory antral follicles (D).

During the early stages of embryogenesis, telomerase remains outside the blastomere nuclei, leading to a progressive reduction in telomere length during the initial divisions. This shortening continues until the embryo reaches the blastocyst stage, at which point telomeres begin to lengthen. As shown here, it is precisely at this stage that telomerase re-enters the nucleus, correlating with the resumption of telomere elongation in relation to the enzyme's nuclear import.

Telomeres lenght influences embryo development

UNIT-II
Lesson work 2

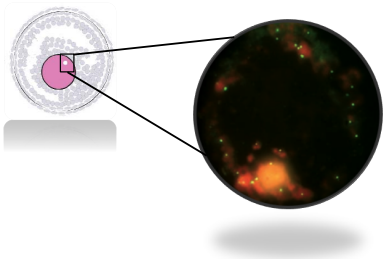
Premise
The group of the Prof. Blasco demonstrated for the first time that the oocyte used in ART have telomeres with sizes strongly related to the age of the patient

Oocyte derived from women of <30 years old have normal sized telomeres

Oocytes derived from women of >45 years old have significantly smaller telomeres structures

Question
1) Could you discuss which potential effects may take place when oocytes displaying a limited elengation of telomeres are enrolled in fertilization and embryo development processes?

2) Could you speculate about the mechanims involved in the smaller dimension of telomeres in women in advanced stage of reproduction (>45 years old)?



Am J Obstet Gynecol. 2005 Apr;192(4):1256-60; discussion 1260-1.
Telomere length predicts embryo fragmentation after in vitro fertilization in women--toward a telomere theory of reproductive aging in women.
Keefe DL1, Franco S, Liu L, Trimarchi J, Cao B, Weitzen S, Agarwal S, Blasco MA.

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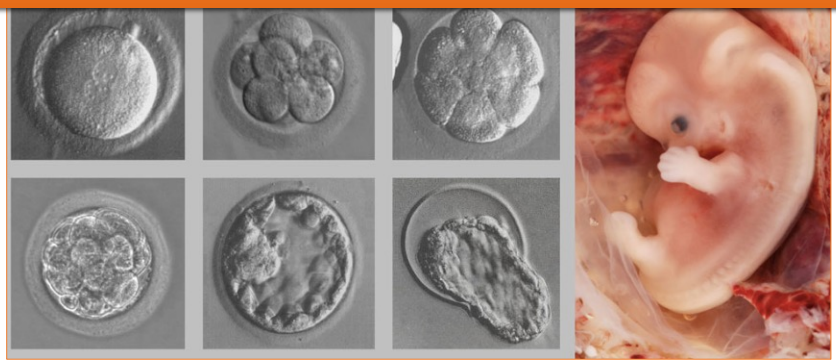
They failed due to the genetic instability generated by the telomere shortening. This influences negatively the mitoic division that a zygote encounters in the early stage of development. The embryo failed before blastocyst state, so before it can be transferred into the recipient female

2) Could you speculate about the mechanims involved in the smaller dimension of telomeres in women in advanced stage of reproduction (>45 years old)?

Even though oocytes have levels of telomerase, the length of telomeres shortens with advancing age due to various factors. Additionally, oocytes from reproductively aged females show higher levels of reactive oxygen species (ROS), which can cause DNA damage, including to telomeres, accelerating their shortening.

What does the zygote ultimately receive from the female gamete?



The zygote inherits from the grown oocyte:

1. **Macromolecules**
2. **Organelles**
3. **Large remodelled chromatin**
4. **Epigenetic arranged eu and eterochromatin**

that are all required to transform the zygote in a totipotent cells.

The general concept is that the succes of embryogenesis is strongly related with the cytoplasmic and nuclear modifications which occur during the oogenesis.