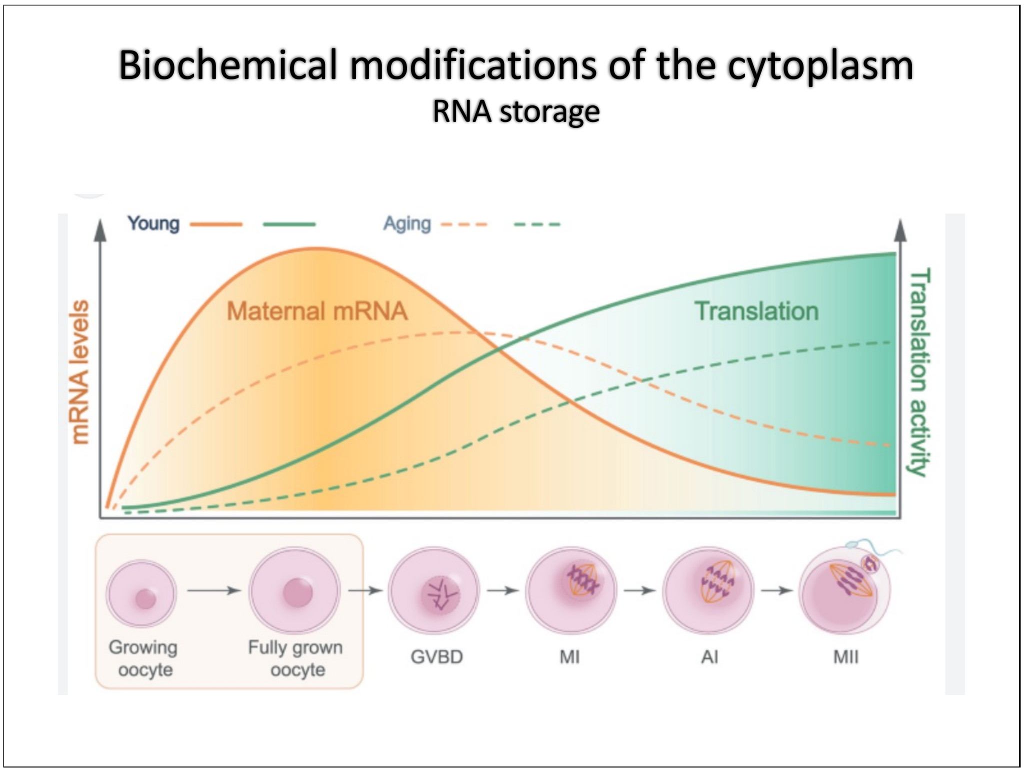
Growth Phase:
CYTOPLASM

Biochemical modifications

RNA and Protein accumulation



Biochemical modifications of the cytoplasm

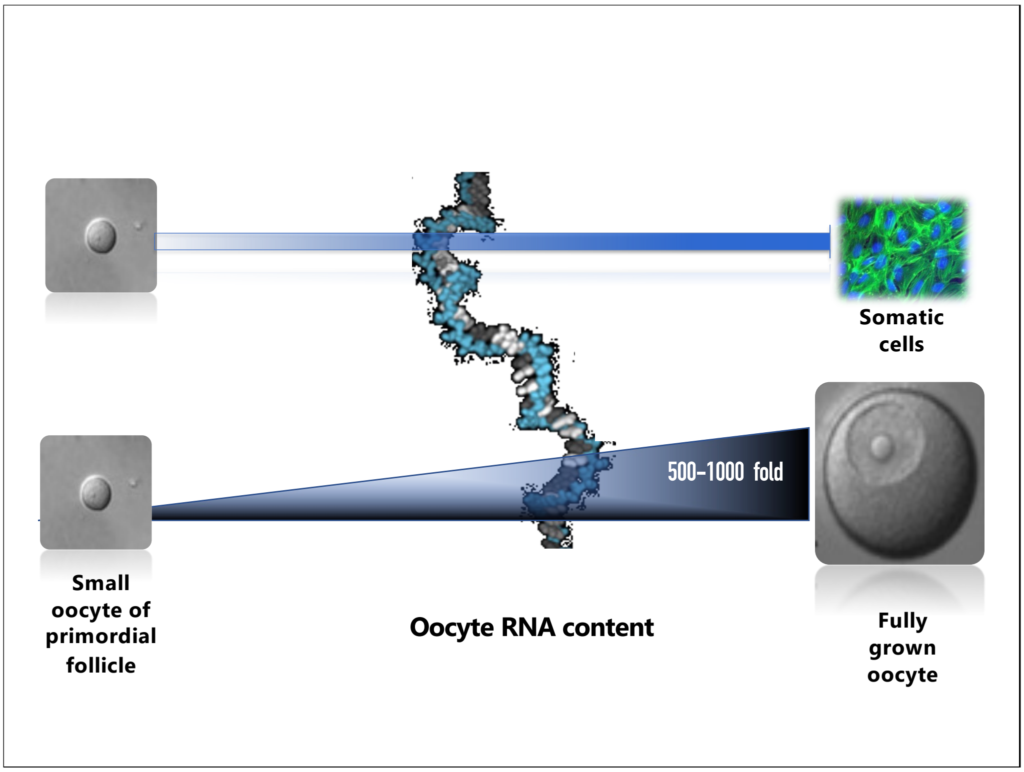
RNA storage

The first biochemical modification involving the growth phase of oogenesis is the accumulation of the products of transcription (RNA) and translation (proteins).

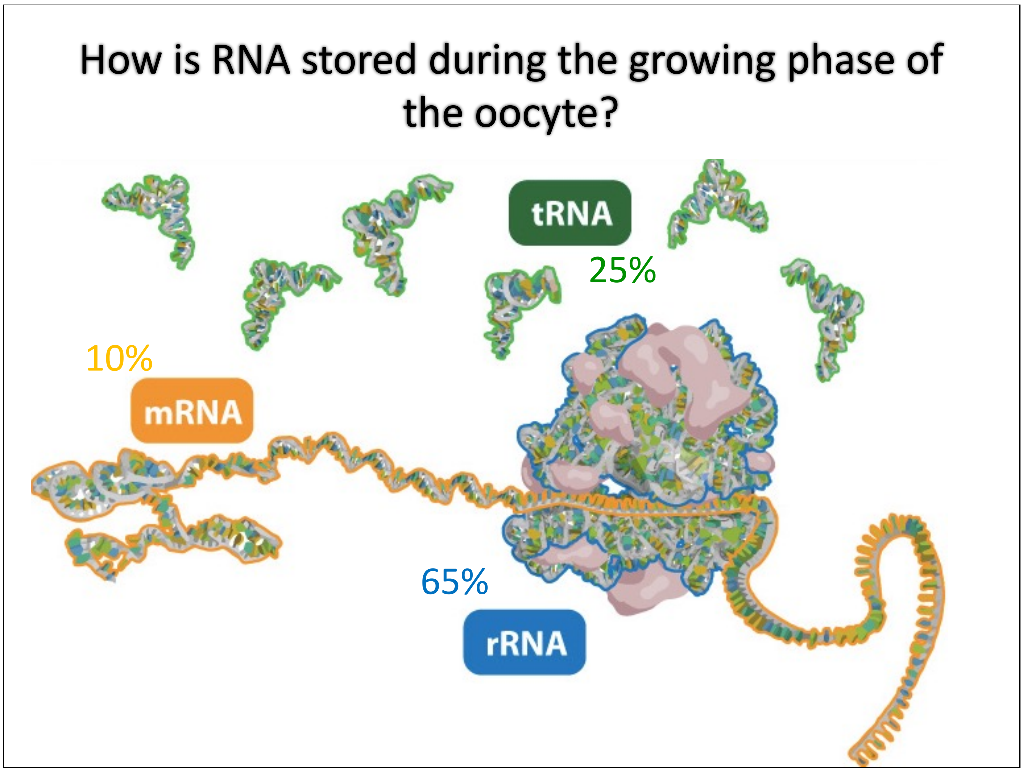
The stored RNA will represent a crucial molecular component for the success of the oocyte in the acquisition of the developmental competence.

Indeed, the stored RNA within the oocyte serves as a molecular toolkit that orchestrates activities during (I) the activation phase (II) the initial stages of embryo development and (III) the maternal-to-zygotic transition which enables zygotic gene products to replace the maternal ones (in other words: the initiation of active transcription from the embryo genome).

This significant RNA reservoir is established through an active transcription process that engages the post-natal oocyte until the conclusion of the growth phase.



To comprehend the magnitude of this phenomenon, it is essential to note that the mRNA content of a fully grown mouse oocyte is roughly 500-1000 times higher than that of somatic cells. This represents an active and gradual process, considering that, at the primordial stage of the oocyte, which marks the initiation of post-natal oogenesis, the RNA content is identical to that of a somatic cell.



How is RNA stored during the growing phase of the oocyte?

How is RNA stored during the growing phase of the oocyte?

Mostly rRNA accounting for the 65%

Then tRNA accounting for 25%

And finally the mRNA acccount for 10%

This distribution also reflets the relative amount of the RNA category used by the cells to accomplish their functions.

Focus on mRNA

Polyadenilation of mRNA ocours during the growth phase.
The main roles of polyadenylation in the oocyte growing phase include:

1. **Stability:** The addition of a poly(A) tail protects mRNA from degradation, ensuring that the stored RNA in the oocyte remains intact and functional over an extended period.

2. **Transport and Localization:** It helps in the proper subcellular localization of mRNA molecules, ensuring that they are positioned in the appropriate regions where they will be needed during subsequent stages of development.

3. **Translation Initiation**. The poly(A) tail plays a role in recruiting translation initiation factors and ribosomes, facilitating the efficient translation of mRNA into proteins when needed.

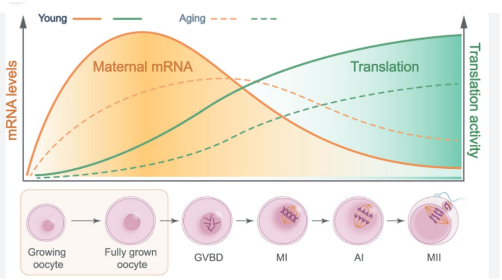
4. **Regulation of Gene Expression:** It influences the timing and efficiency of mRNA translation, contributing to the fine-tuning of gene expression patterns during oocyte growth and subsequent developmental stages.

When the stored RNA is used?

mRNA
It will be used immediately after fertilization to sustain the early phases of embryo development before the transcription of the embryo genome is activated.

tRNA and rRNA
They will support the process of protein synthesis at the time of the use of stored mRNA

RNA SYNTHESIS CONCLUDES AT THE END OF THE GROWTH PHASE when OOCYTE REACH ITS FULLY GROWN SIZE



The oocyte now is a fully specialized gamete, any additional genomic information is needed to support its function.

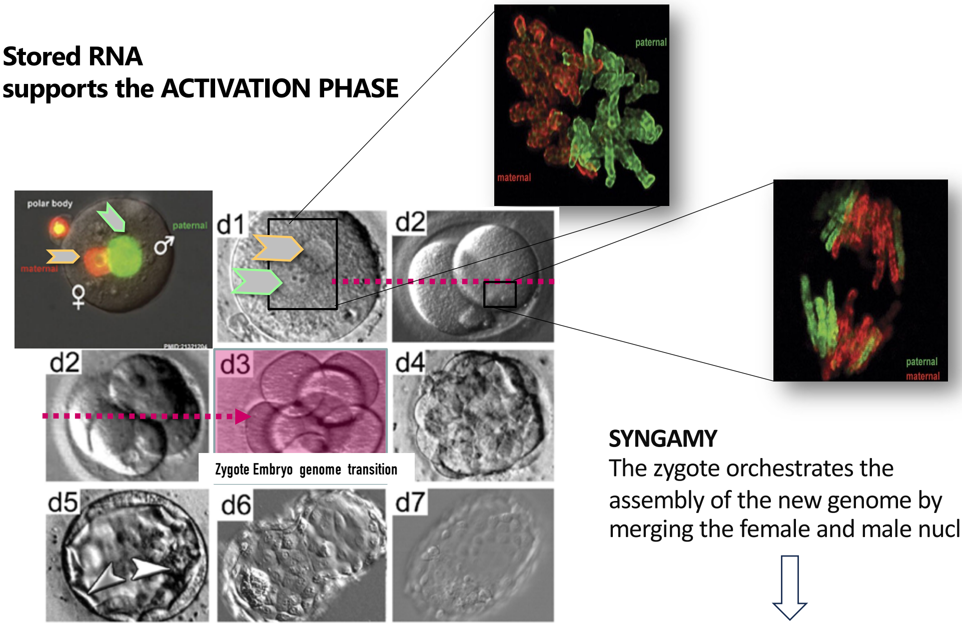
At this point, the cell is poised to potentially transform into another cell (ZYGOTE) by expressing its totipotency. The female gamete is ready!

When the transition occurs the oocyte genome must be deactivated

WHY?

A new genome needs to be arranged!

Stored RNA
supports the ACTIVATION PHASE



SYNGAMY
The zygote orchestrates the assembly of the new genome by merging the female and male nuclei

⇩

A new cell is designed
EMBRYO

During the activation phase (from fertilization to syngamy), the zygote orchestrates the assembly of the new genome by merging the female (oocyte) and male (spermatozoon) nuclei in a process known as syngamy.

Subsequently, a new cell is designated, namely the embryo. The new formed embryo genome is not <u>immediately</u> accessible for transcription but only after 2-5 mitotic divisions, depending on the species.

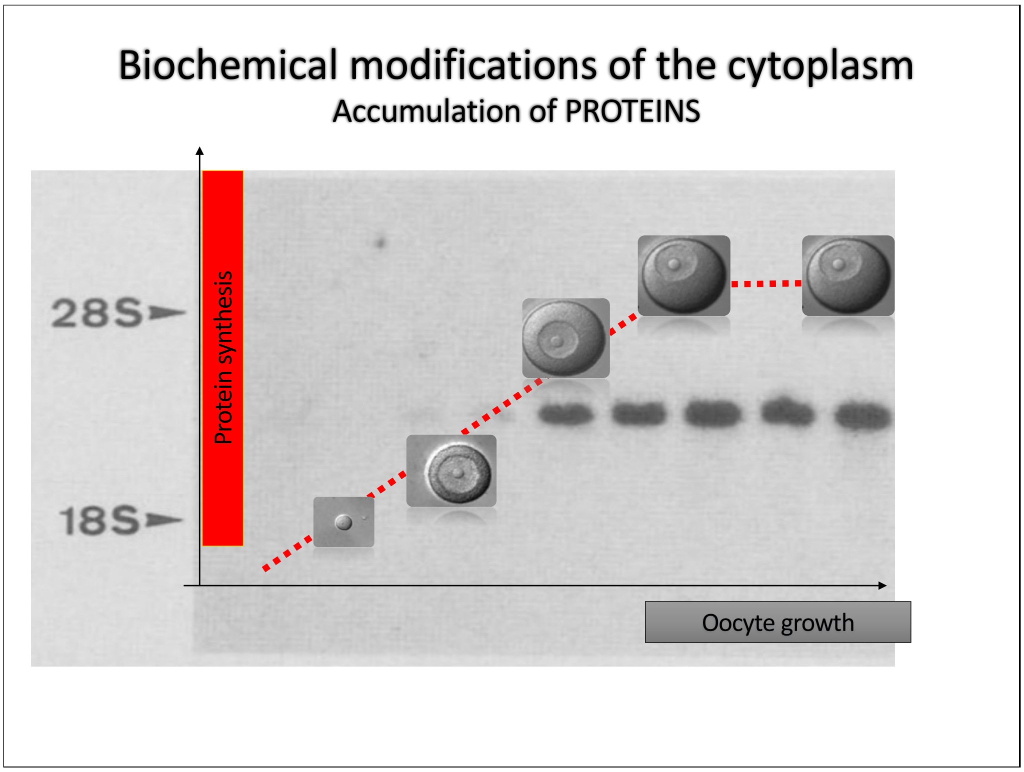
What have we learned?

The progression of embryo divisions during the early stages of development occurs **without embryo genomic activation**

WHY?

⇩

Given the possibility to use the accumulated RNA and proteins

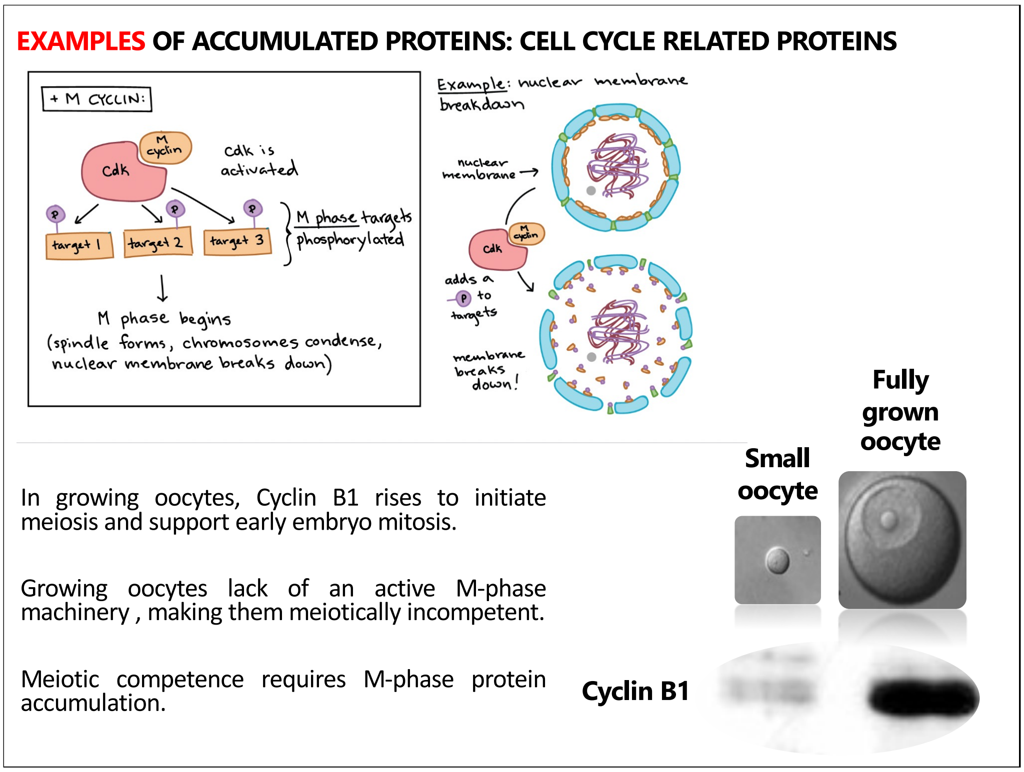


Biochemical modifications of the cytoplasm

Accumulation of PROTEINS

The growth phase is, in parallel, characterized by an active protein synthesis.

The protein synthesis increases proportionally during the growth phase reaching a plateau phase when the fully grown size has been reached.



EXAMPLES OF ACCUMULATED PROTEINS: CELL CYCLE RELATED PROTEINS

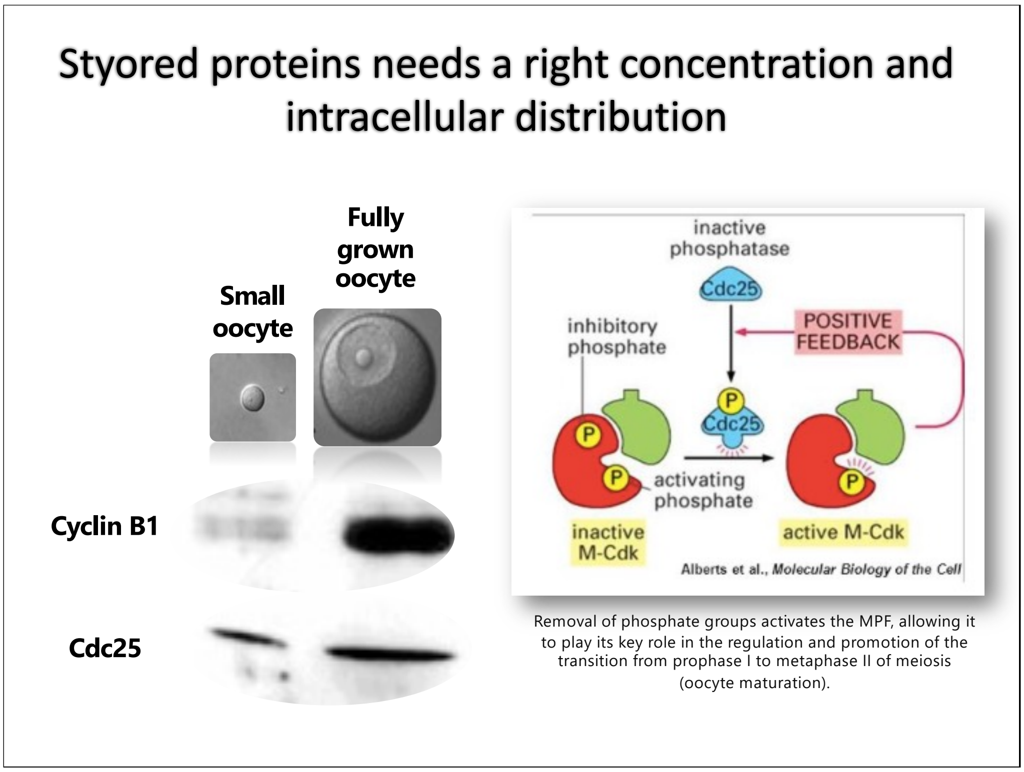
In growing oocytes, Cyclin B1 rises to initiate meiosis and support early embryo mitosis.

Growing oocytes lack of an active M-phase machinery , making them meiotically incompetent.

Meiotic competence requires M-phase protein accumulation.

During the growth phase, the oocyte undergoes an increase in the storage of cell cycle-related proteins, which accumulate at high levels within the nucleus and cytoplasm. Specifically, in growing oocytes, the M cyclin (Cyclin B1) reaches elevated levels, enabling the oocyte to initiate and complete the meiotic cell cycle, and subsequently support the early mitotic division during embryo development.

It's worth noting that the M cell cycle machinery is deficient in growing oocytes to the extent that they are meiotically incompetent.The attainment of meiotic competence primarily involves the accumulation of M cell cycle proteins.



Styored proteins needs a right concentration and intracellular distribution

Alberts et al., *Molecular Biology of the Cell*

Removal of phosphate groups activates the MPF, allowing it to play its key role in the regulation and promotion of the transition from prophase I to metaphase II of meiosis (oocyte maturation).

Achieving the correct concentration of cell cycle proteins is just one aspect of meiotic competence, as the proper distribution within the nucleus is equally crucial.

Indeed, another significant mechanism defyning the transition from the growing phase to the phase of maturation is the translocation of cell cycle proteins to.

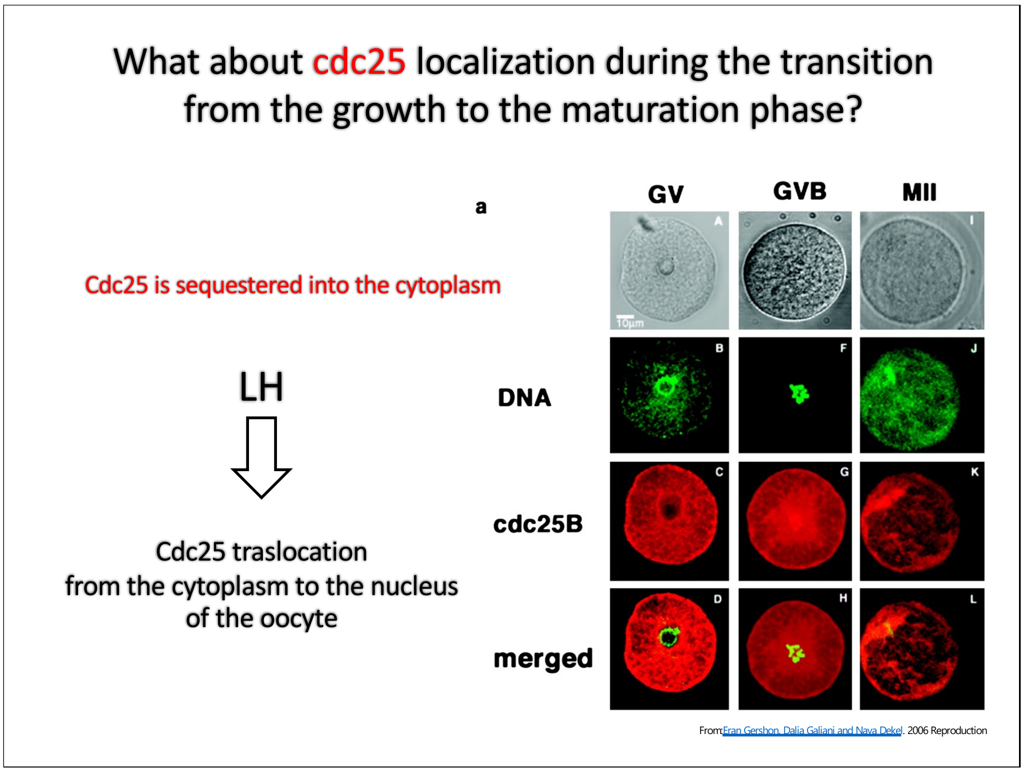
the cellular compartments where they need to exert their activities.

One example is the localization of the ***CDC25 (Cyclin-Dependent Kinase Phosphatase 25)***

*CDC25 regulates the Maturation Promoting Factor (MPF; a macromolecular protein comples supporting oocyte maturation) by acting on its activation. The CDC25 enzyme removes phosphate groups from the Cyclin-Dependent Kinase (**CDK1**), which, together with **Cyclin B1**, forms the Maturation Promoting Factor (MPF) complex. The removal of phosphate groups activates the MPF, allowing it to play its key role in the regulation and promotion of cell cycle phases, especially during the transition from prophase I to metaphase II of meiosis (oocyte maturation).*

This protein if injected into an immature oocyte is able promote maturation.

During the growth phase, both the levels of cyclin B (the regulatory subunit of M-CdK) and cdc25 (M-CdK activating phosphatase) increase dramatically.



What about cdc25 localization during the transition from the growth to the maturation phase?

Cdc25 is sequestered into the cytoplasm

LH

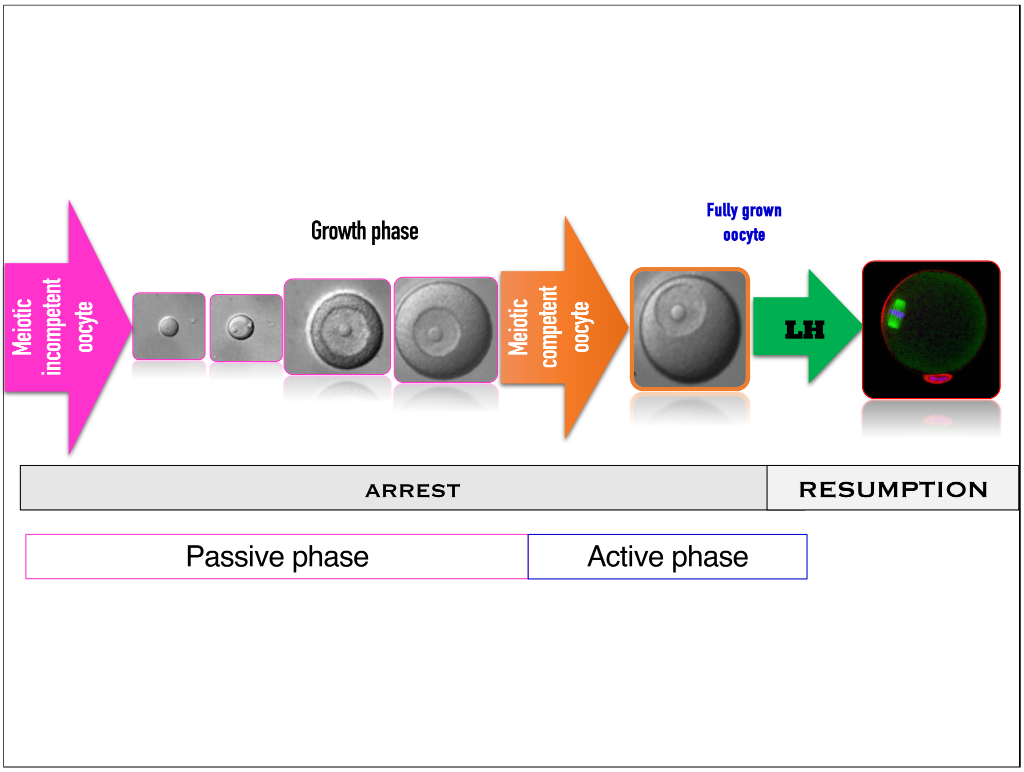
Cdc25 traslocation
from the cytoplasm to the nucleus
of the oocyte

From:Eran Gershon, Dalia Galiani and Nava Dekel. 2006 Reproduction

However, cdc25 remains sequestered in the immature oocytes mainly on the oolemma and in the cytoplasm, so far away from its target molecules: the M CdK that is localized in the nucleus.

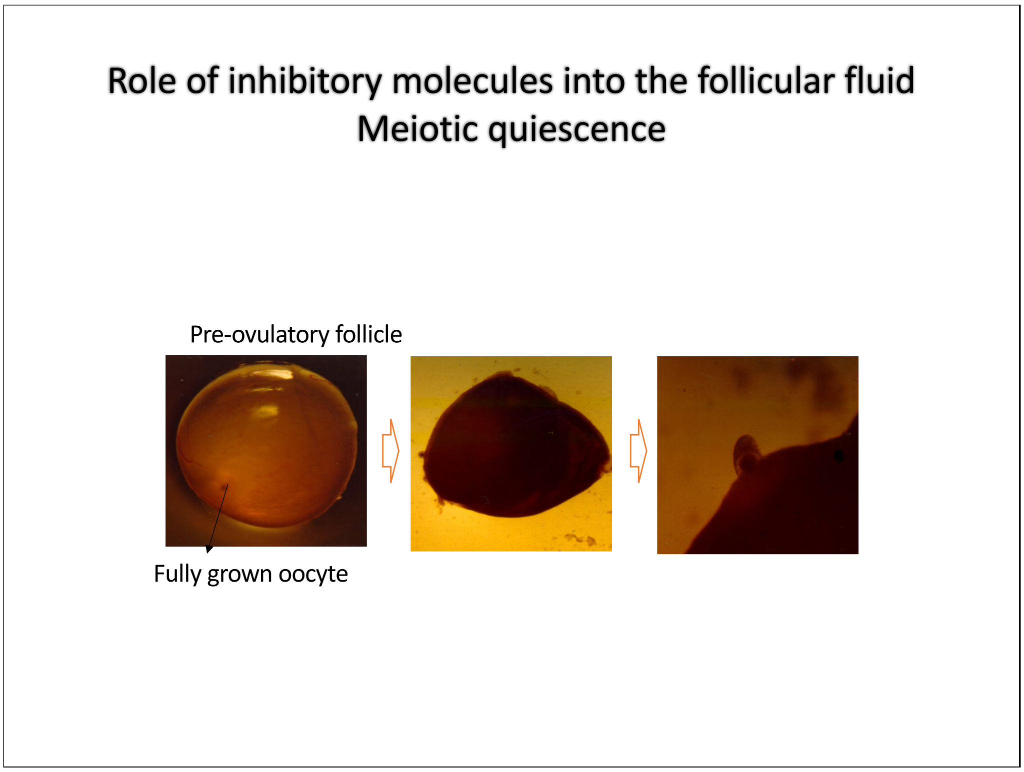
Until cdc25 remains sequestered into the cytoplasm, the meiotic cell cycle cannot be resumed.

Physiologically, the translocation and activation of cdc25 is stimulated by the LH surge, the activating meiosis hormone.



Based on this premise, it appears clear that once the fully grown oocyte acquires the meiotic competence (at the end of the growth phase) it does not express immediately this functional ability because of the cytoplasmic localization of some cell cycle proteins.

This is the reason why the condition of meiotic quiescence (block of the meiotic cell cycle at the diplotene stage of prophase I) is actively maintained by avoiding the nuclear import of cell cycle protein such as cdc25.



Role of inhibitory molecules into the follicular fluid
Meiotic quiescence

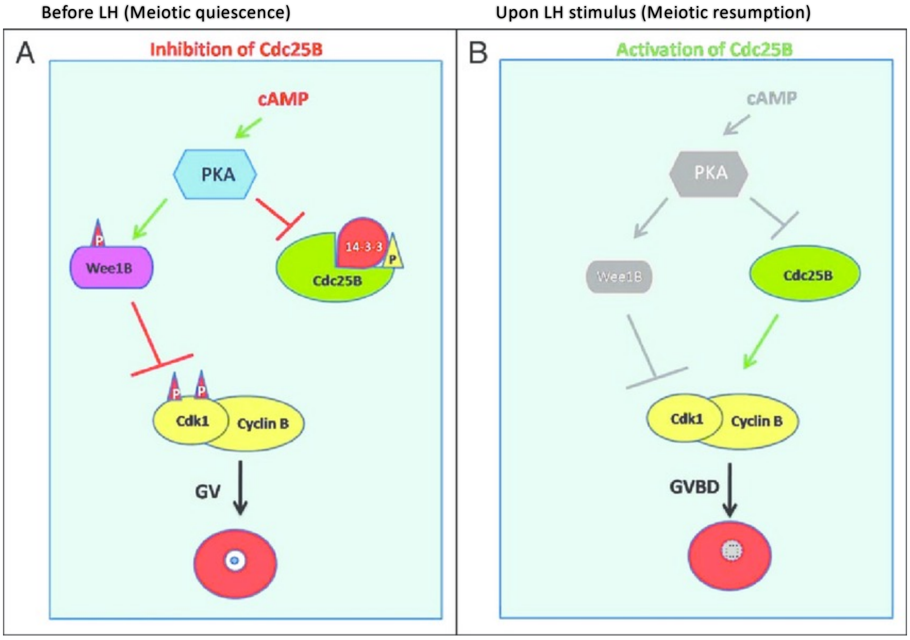
Pre-ovulatory follicle

Fully grown oocyte

Physiologically, only a fully grown oocyte encloses inside a pre-ovulatory follicle is enabled to resume meiosis after the LH stimulation. The condition of meiotic quiescence (block of the meiotic cell cycle at the diplotene stage of prophase I) is maintained once the oocyte become competent at the end of growth phase by the inhibitory action of several molecules* accumulated inside the follicular fluid (mostly bioactive lipids or small peptides).

**Inhibitory molecules role: They maintain high intracellular levels of cAMP required to segregated cdc25 into the cytoplasm and maintain hyperphosphorylated the MPF complex (inactive form).*

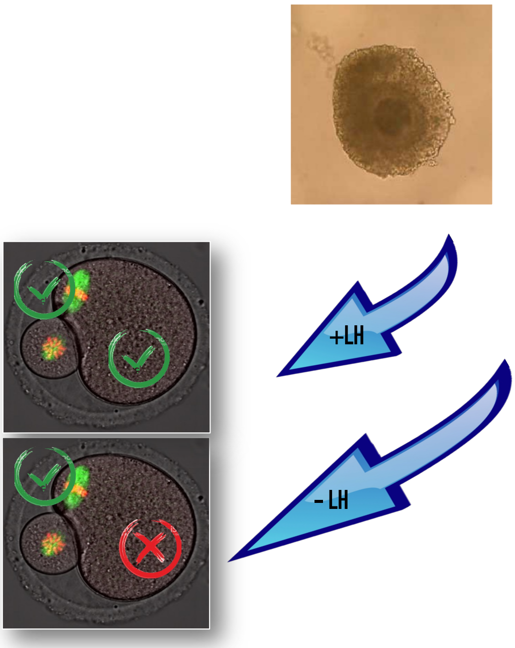
Molecularly….

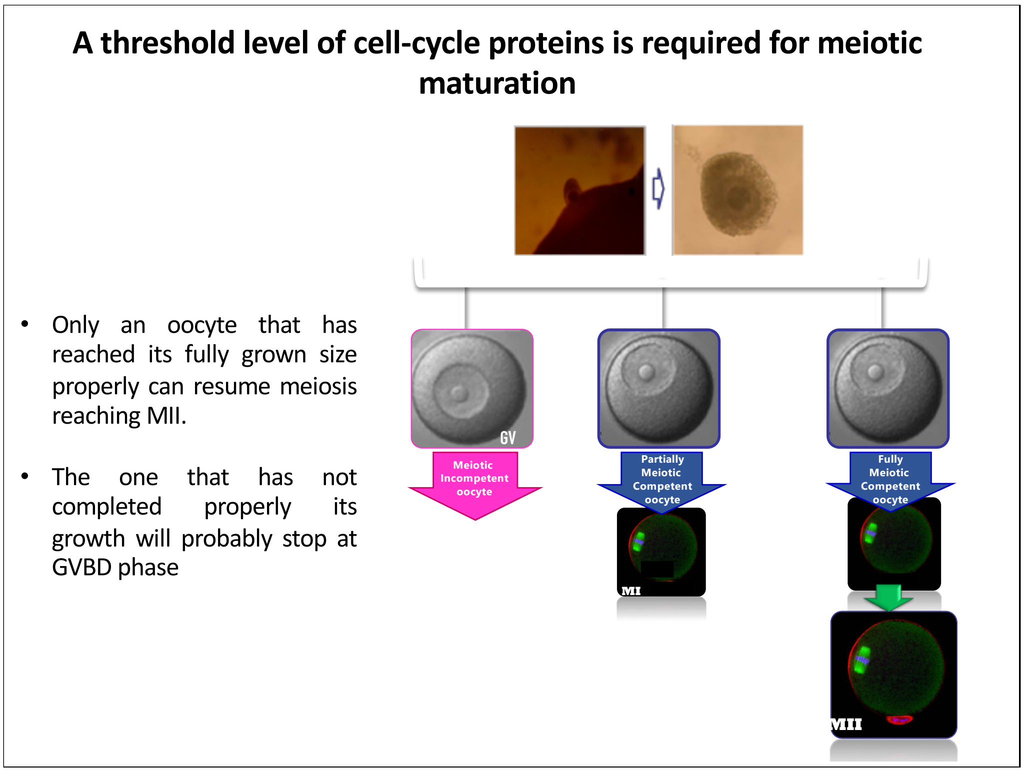


The inhibitory role exerted by these molecules becomes clearly evident once a fully grown oocyte is removed from the follicular environment. Immediately, the resumption of meiotic cell cycle takes place as a spontaneous event.

However, in the absence of LH, the efficiency of the maturation phase initiation is not high. Hormonal stimulation (such as LH in mammals) is the sole mechanism capable of synchronizing nuclear and cytoplasmic maturation.

Conversely, the removal of inhibitory signals is exclusively necessary to sustain the activation of the cell cycle.





A threshold level of cell-cycle proteins is required for meiotic maturation

- Only an oocyte that has reached its fully grown size properly can resume meiosis reaching MII.
- The one that has not completed properly its growth will probably stop at GVBD phase

However, if we are asked to evaluate the degree of meiotic competence of an oocyte we could simply remove the relative COC from its follicle and put it in culture to assess its ability to resume meiosis.

Using this experiment, we can test the meiotic competence and verify that this functional endpoint is reached in a stepwise manner. Indeed, the oocyte during the growth phase reaches, first, a partial meiotic competence to then complete the process once it achieved the fully-grown dimension at the end of the growth phase.

Indeed, the oocyte becomes first able to reach the GVBD/MI stage and only later when reaches its final size it becomes able to resume meiosis reaching the MII stage.

The stepwise process is expression of the progressive storage of cell cycle molecules.

So, a threshold level of cell-cycle proteins is required to trigger GVBD and a higher one to complete meiosis.

How can one identify the follicle housing a fully grown oocyte and, consequently, meiotically competent cell?

An essential question to address before employing oocytes in in vitro reproductive protocols is determining when the growth phase concludes, or more precisely, how to identify the follicle housing a fully grown and, consequently, meiotically competent cell.

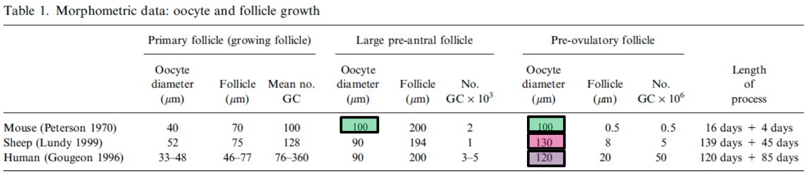


Table 1. Morphometric data: oocyte and follicle growth

	Primary follicle (growing follicle)			Large pre-antral follicle			Pre-ovulatory follicle			
	Oocyte diameter (μm)	Follicle (μm)	Mean no. GC	Oocyte diameter (μm)	Follicle (μm)	No. GC $\times 10^3$	Oocyte diameter (μm)	Follicle (μm)	No. GC $\times 10^6$	Length of process
Mouse (Peterson 1970)	40	70	100	100	200	2	100	0.5	0.5	16 days + 4 days
Sheep (Lundy 1999)	52	75	128	90	194	1	130	8	5	139 days + 45 days
Human (Gougeon 1996)	33–48	46–77	76–360	90	200	3–5	120	20	50	120 days + 85 days

***In vitro* Follicle Growth: Achievements in Mammalian Species**

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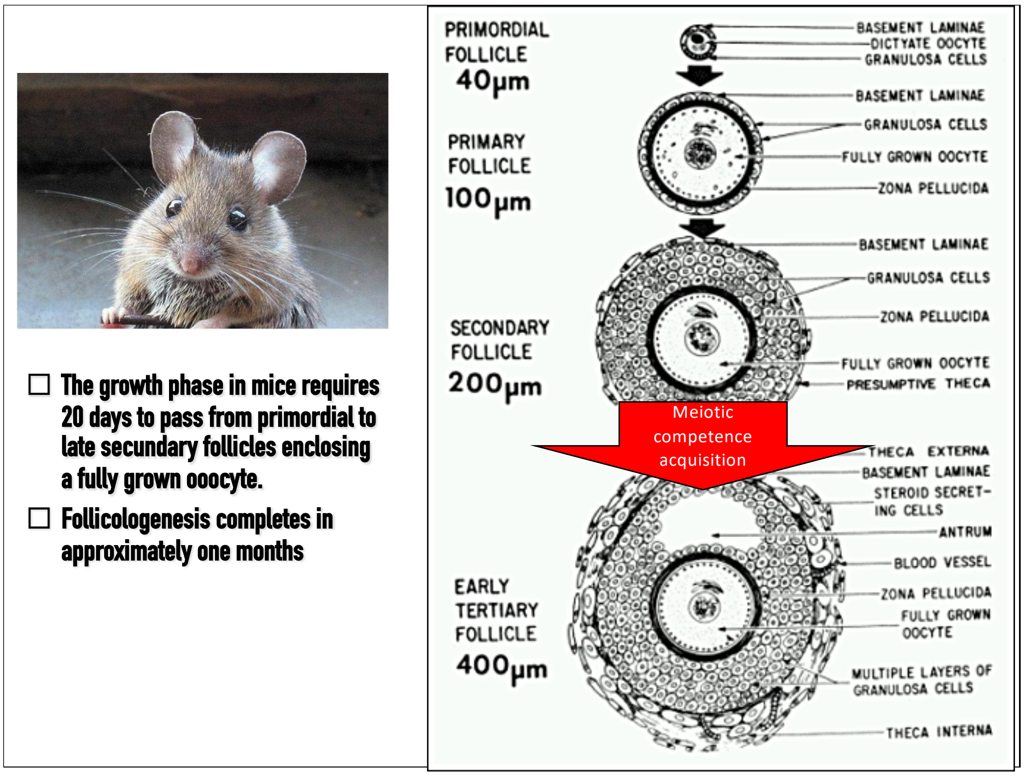
Unfortunately, knowledge about the female gamete biology is currently limited to specific mammalian species.

Primarily, the process of meiotic competence in mammals is highly species-specific and closely related to the body size. Smaller mammals, such as laboratory rodents, exhibit a high basal cellular metabolism, leading to accelerated body functions, including reproductive processes.

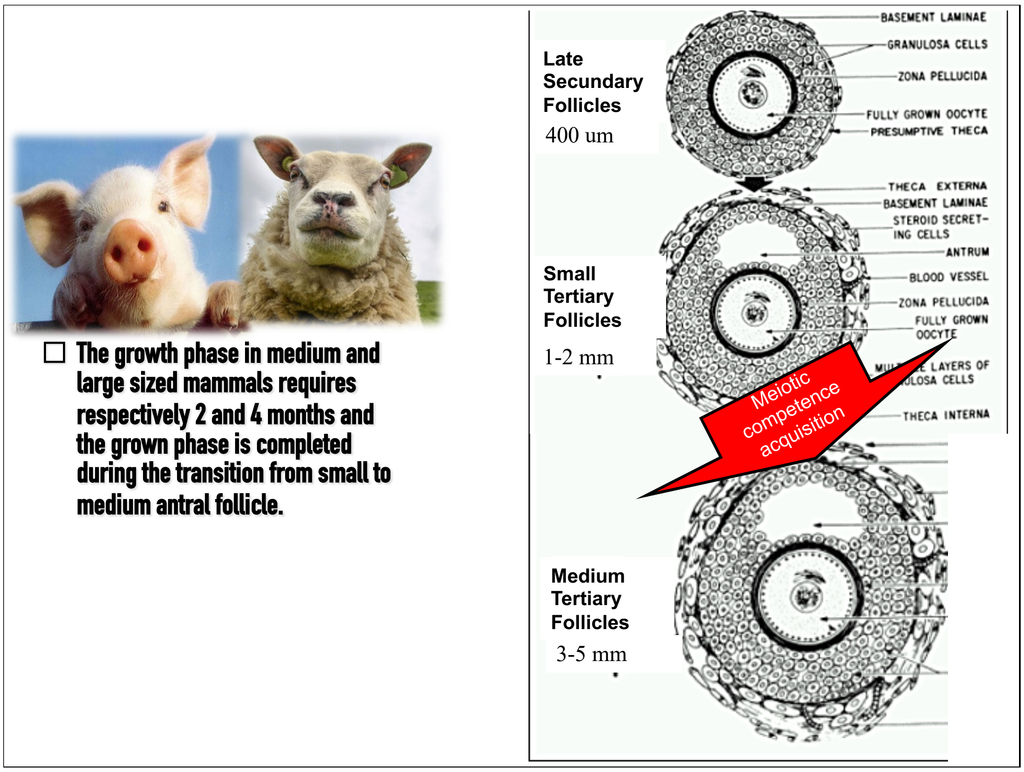
In the case of laboratory rodents, both folliculogenesis and oogenesis occur at an accelerated faster is compared with the ones of medium size mammals.



- ☐ **The growth phase in mice requires 20 days to pass from primordial to late secundary follicles enclosing a fully grown ooocyte.**
- ☐ **Follicologenesis completes in approximately one months**

More in detail, the mice complete the follicular phase (passing from a primordial to a preovulatory follicles) in a very short interval (approximately one months). Within less than 20 days, a primordial oocyte completes its growth phase reaching the final dimension. The growth phase ends in mice in the early stage of antral follicle differentiation. In particular, as summarized in this slide, the oocyte becomes meiotically competent during the transition from pre-antral to antral follicle.

Consequently, a substantial population of gametes can be utilized in mice for in vitro maturation (IVM) protocols, where fully grown and meiotically competent oocytes can be isolated from early antral to medium antral follicles.



- ☐ **The growth phase in medium and large sized mammals requires respectively 2 and 4 months and the grown phase is completed during the transition from small to medium antral follicle.**

Late Secundary Follicles
400 um

Small Tertiary Follicles
1-2 mm

Medium Tertiary Follicles
3-5 mm

Meiotic competence acquisition

Conversely, the process of follicular and oocyte growth is slower in medium-large sized mammals.

In this cases, several months are required to complete folliculogenesis: more in detail, 2 months and 4 months in medium and large mammals, respectively.

In addition, also oocyte growth phase is longer: the fully grown dimension is reached in medium-large mammals at later stage of folliculogenesis.

Indeed, just as an example let’s tell you about the timing of oogenesis in pig:

The oocyte becomes fully grown and fully meiotic competent at the antral stage and more precisely during the transition from small to medium antral follicles.

Medium antral follicles are retrieved for in vitro maturation (IVM) protocols in the porcine specie.

This is the reason why in typical in vitro maturation (IVM) protocols, porcine oocytes are retrieved from medium antral follicles distinguished by their follicle diameter, which ranges from 4 to 6 mm.

These follicles can be isolated either from pre-pubertal or pubertal dioestrus ovaries.