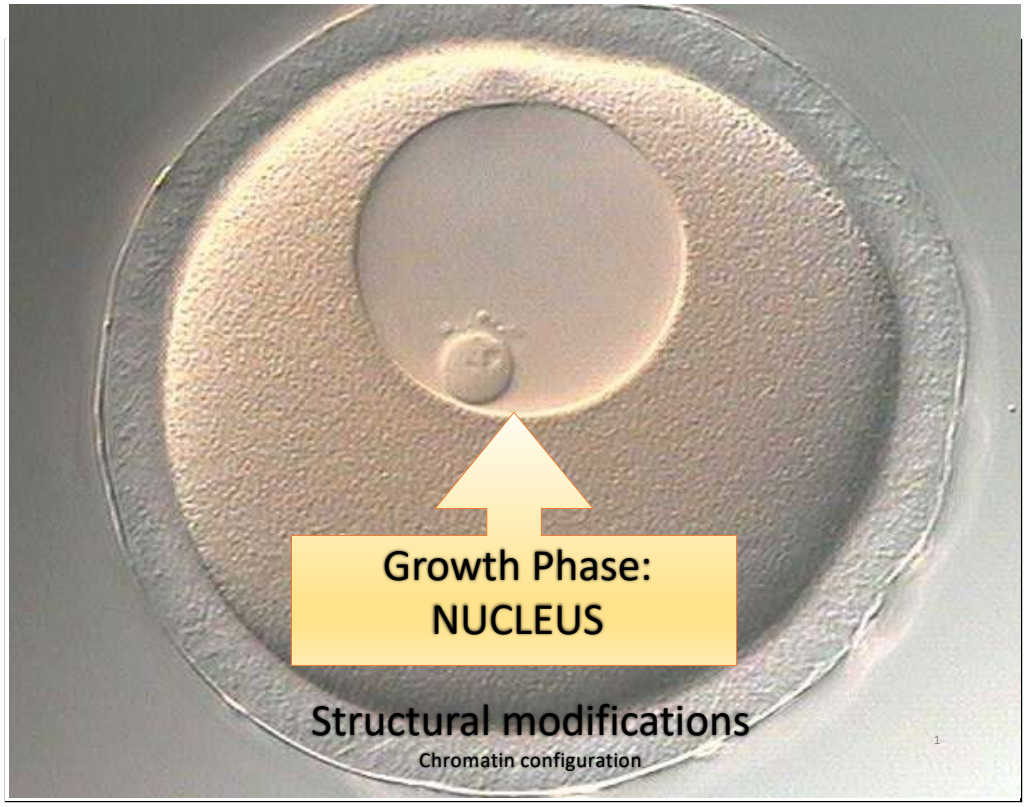
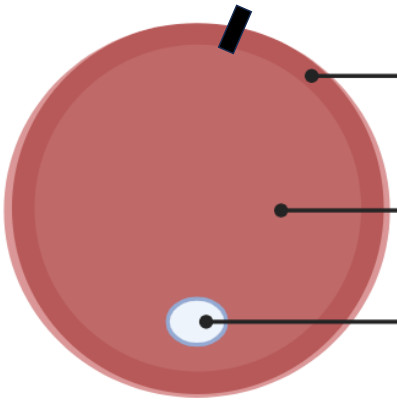


Structural modification during the growth phase also involve the nucleus of the oocyte, a structure that at this stage is known as Germinal Vescicle (GV).

Whereas the nucleus of the oocytes remains quiescent for years after birth, its dimension and the molecular content change dramatically.

Nuclear Pores

Channels regulating the passage of molecules (RNA and proteins) between the nucleus and the cytoplasm.



Nuclear envelope

Inner membrane: facing the nucleoplasm. Conteins integral membrane proteins
Outer membrane: facing the ooplasm. In continuity with ER

Nucleoplasm

Chromatin (DNA and histones) and molecules essential for the regulation of gene expression

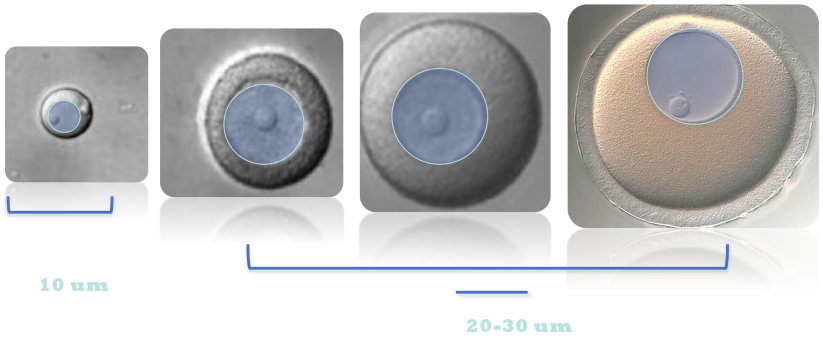
Nucleolus

Ribosomal RNA (rRNA), which is involved in protein synthesis and ribosome formation. Production of proeins required for cell activities

2

Structural modifications of the nucleus

A Mouse oocyte during its growth phase



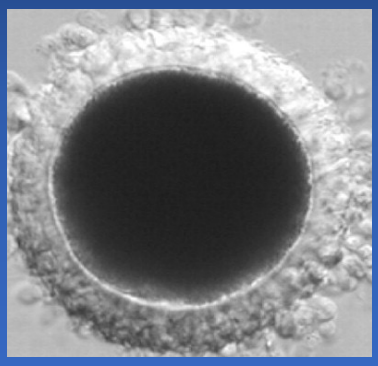
- ✓ increase in diameter
- ✓ undergoes translocation toward the periphery of the ooplasm

3

The germinal vesicle (GV), readily identifiable in mouse oocytes as a circular structure enclosed within its nuclear envelope, undergoes various structural modifications throughout the growth phase. Initially, it experiences an increase in diameter, expanding from 10 to 20-30 µm. Additionally, it undergoes translocation toward the periphery of the ooplasm. Initially positioned centrally during the early stages of oogenesis, the GV subsequently transitions to a distinctly peripheral location.

As you can see, in this image the GV (germinal vesicle) is clearly visible, even of this image has been captured with a phase-contrast microscope without any staining.

What about other species?

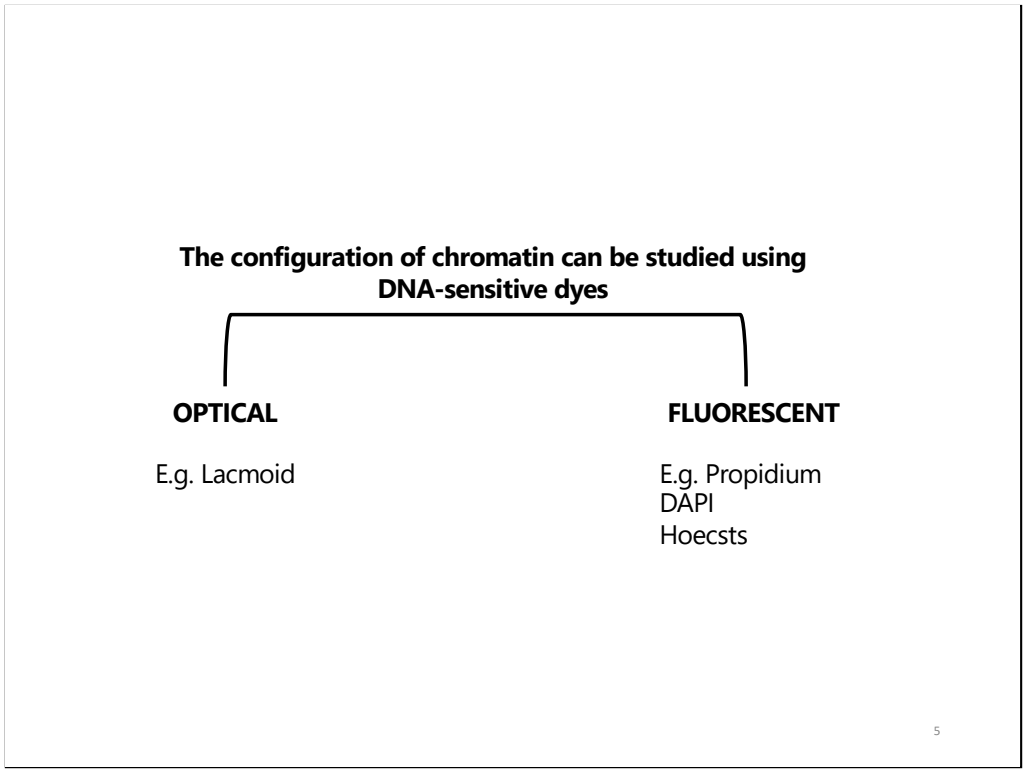


Mice oocyte

Porcine oocyte

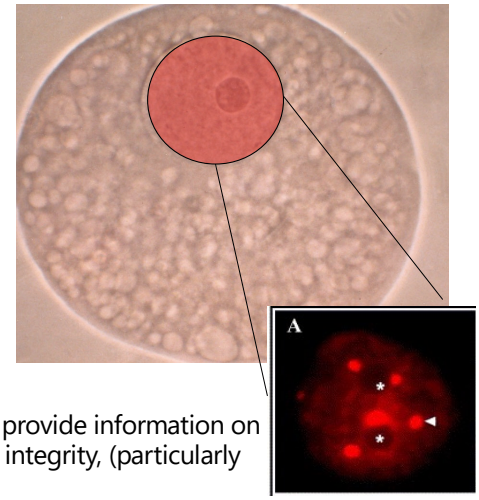
To make structural changes visible we need to use specific dyes!

Similar modifications occur in the oocyte nuclei of the other species. However, to identify the structure of the nucleus under an optical microscope a specific staining is required in several mammalian oocytes.



The configuration of chromatin can be studied using DNA-sensitive dyes such as Lacmoid and fluorescent based such as Propidium Iiodide, DAPI or Hoechst

Chromatin staining with Lacmoid



- Low cost
- It requires a simple optical microscope, provide information on cytoplasm organization and membrane integrity, (particularly the one of the nucleus).
- The affinity for DNA is lower compared to the fluorescent dyes

6

Lacmoid is a dye primarily used for the visualization of the cellular nucleus.

It has a low cost, requires a simple optical microscope, and provide information on cytoplasm organization and membrane integrity, particularly the one of the nucleus.

However, Lacmoid does not exhibit high affinity for DNA if compared to other dyes.

Hence, in case of immature oocyte, where the chromatin has a low degree of condensation, Lacmoid might fail to label it even though chromatin does exist.

So, to have information about chromatin visualization fluorescent dyes are preferred (e.g. in this image the Propidium staining is shown).

With this staining, the chromatin can be easily visualized, displaying a widespread distribution with small foci of condensation.

Chromatin sensitive dyes (fluorophores)

- High cost
- It requires a advanced optical microscope,
- Do not provide information on cytoplasm organization and membrane integrity.
- The affinity for DNA is higher compared to the lacmoid dye
- Are offered as vital dyes (supravital), enabling the analysis of the oocyte before proceeding with the culture
- serve to counterstain DNA alongside other fluorescent dyes that target diverse molecular structures

7

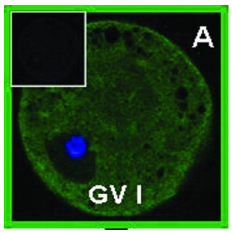
More information about chromatin can be gained through the use of fluorescent nuclear dyes.

Although they come with a high cost and necessitate advanced microscopy, these dyes exhibit a stronger affinity for DNA.

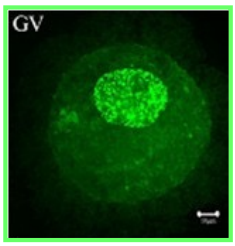
They are also offered as vital dyes (supravital), enabling the analysis of the oocyte before proceeding with the culture. Moreover, they can serve to counterstain DNA alongside other fluorescent dyes that target diverse molecular structures.

Supervital: Supravital staining is a method of staining used in microscopy to examine living cells that have been removed from an organism.

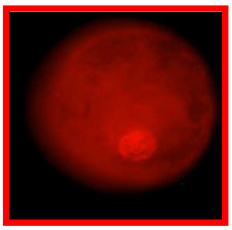
Chromatin sensitive dyes (fluorophores)



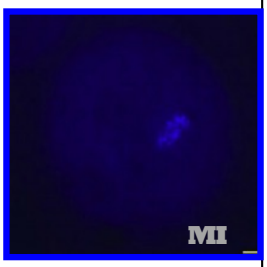
DAPI or Hoescht 33342
cell-permeant nuclear counterstain

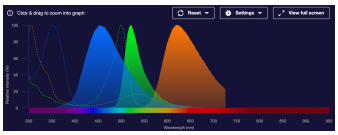


Sybr green
cell-permeant nuclear counterstain



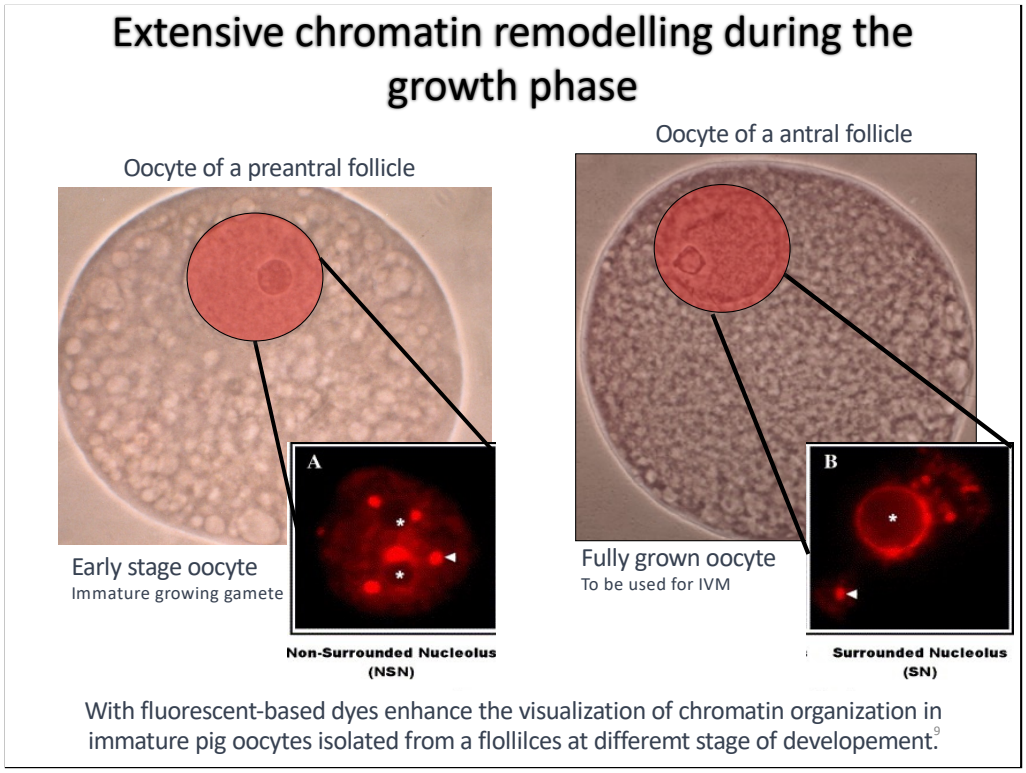
Propidium iodide
Supravital nuclear counterstain





Hoescht 33258
Supravital nuclear counterstain

8



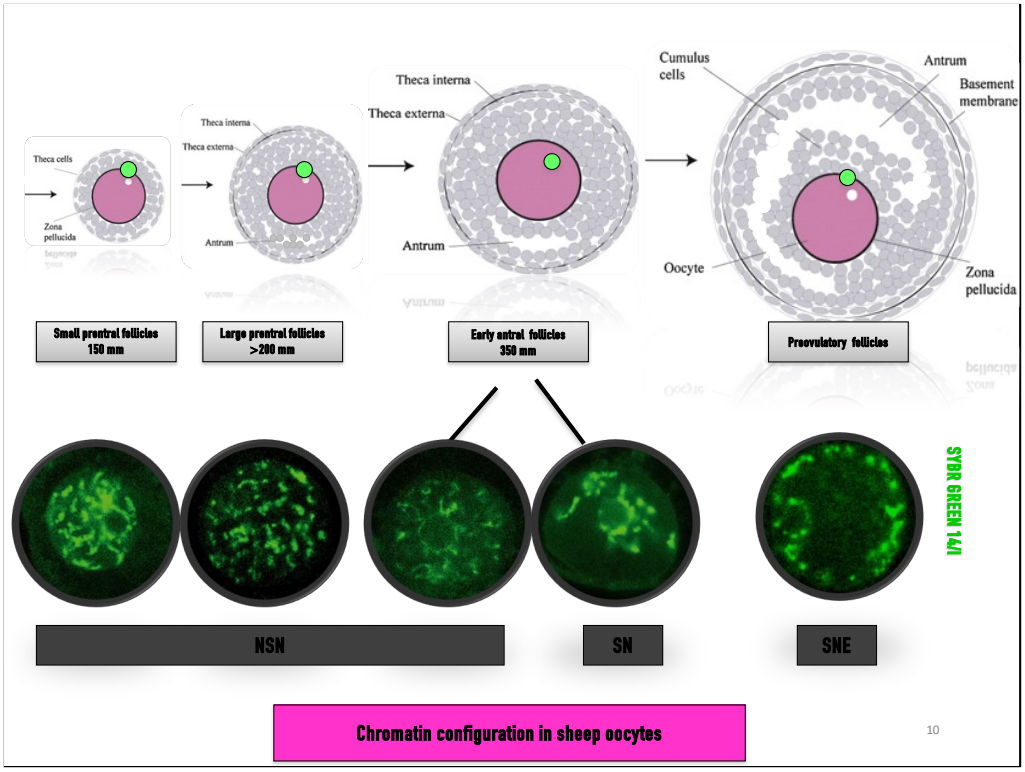
By utilizing a fluorescent-based dye as in this case the propidium, we can enhance the visualization of chromatin organization in immature pig oocytes isolated from a preantral follicle (left) and an antral follicle (right), respectively.

The chromatin within the nucleoplasm appears dispersed, with small foci of condensed chromatin. This chromatin arrangement characterizes oocytes in the early stages of the growth phase, such as those collected from primordial, primary, and pre-antral follicles.

Conversely, the chromatin of fully grown oocytes is more condensed and predominantly distributed around the nucleolus. This mature chromatin phenotype is conventionally defined as surrounded nucleolus (SN), representing the extensive chromatin organization in oocytes that have completed the growth phase.

On the other hand, the chromatin organization of early-stage oocytes is conventionally defined as NSN (chromatin not surrounding the nucleolus). This morphological aspect of chromatin indicates an immature, growing gamete.

Only oocytes that have attained the SN chromatin configuration can be utilized in in vitro maturation (IVM) protocols, as they have become highly competent.



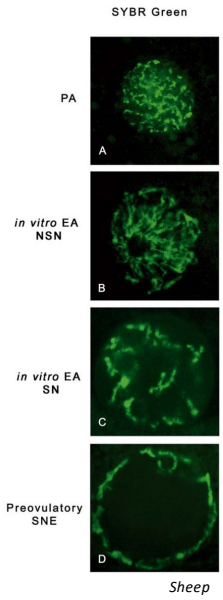
The chromatin configuration appears to be highly species-specific.

Let's focus on the sheep model.

These are ovine oocytes collected at different stages of oogenesis and evaluated for their chromatin organization using Sybr green dye. In this specie, the NSN configuration remains evident in oocytes collected from small preantral to early antral follicles. However, during this stage of folliculogenesis, some oocytes undergo a change in their chromatin configuration. A subpopulation of oocytes isolated from early antral follicles exhibits the SN configuration with condensed chromatin surrounding the nucleolus.

Yet, this chromatin aspect differs from the oocytes isolated from preovulatory follicles, representing the female gametes that will be fertilized. In these cases, all oocytes display highly condensed chromatin distributed close to the nuclear envelope. Consequently, the highly competent oocytes in sheep are considered as surrounding nuclear envelope (SNE) oocytes, indicating oocytes that exhibit a large chromatin configuration surrounding both the nucleolus and the nuclear envelope.

SNE configuration characterizes preovulatory oocytes of sheep.



Chromatin configuration	Oocyte category			
	Early Preantral follicles	Late Preantral follicles	Medium antral follicles	Preovulatory follicles
NSN	Mice, Pig, Sheep, cow, woman	Pig, Sheep, cow, woman		
SN		Mice, Sheep	Mice, pig, cow, woman	Mice, pig, sheep, cow, woman
SNE			Sheep	Sheep

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This table provides a summary of chromatin configurations observed in various species based on the developmental stage reached by the oocytes.

NSN: Non surrounded nucleolus

SN: Surrounded Nucleolus

SNE: Surrounded Nuclear Envelope

TAKE HOME MESSAGE:

We learn that chromatin organization can provide crucial insights into the developmental competence of an oocyte and help identify which oocytes are ideal for in vitro maturation (IVM) protocols.

12

TAKE HOME MESSAGE:

We learn that chromatin organization can provide crucial insights into the developmental competence of an oocyte and help identify which oocytes are ideal for in vitro maturation (IVM) protocols.

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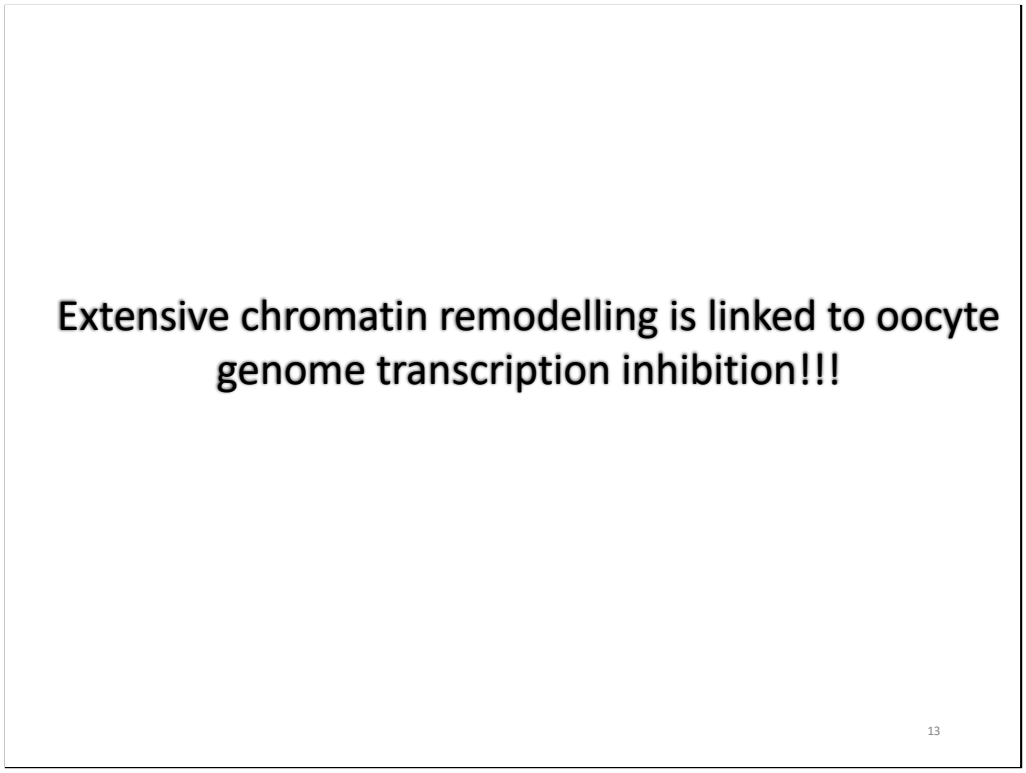


As in the case of structural modifications of the cytoplasm, also changes in chromatin structure reflect changes in nuclear functions.

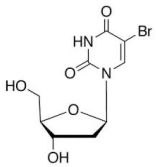
Specifically, chromatin remodeling experience at the end of the growing phase within the oocyte's nucleus is associated with a transcriptional blockade.

Why does the oocyte cease transcription?

- ✓ to provide the oocyte with a time window to accumulate the necessary **factors** and **resources** to support subsequent cell division and embryonic development.
- ✓ To maintain the **genetic stability** of the oocyte by preserving the integrity of genetic material during meiosis and reducing the risk of genetic errors.

TO SUM UP:

The transcriptional block during oocyte maturation is a key strategy to ensure proper embryonic development and the formation of a healthy organism



Strategies to monitor the transcriptional block: fluorescently labelled Bromodeoxyuridine (BrdU).

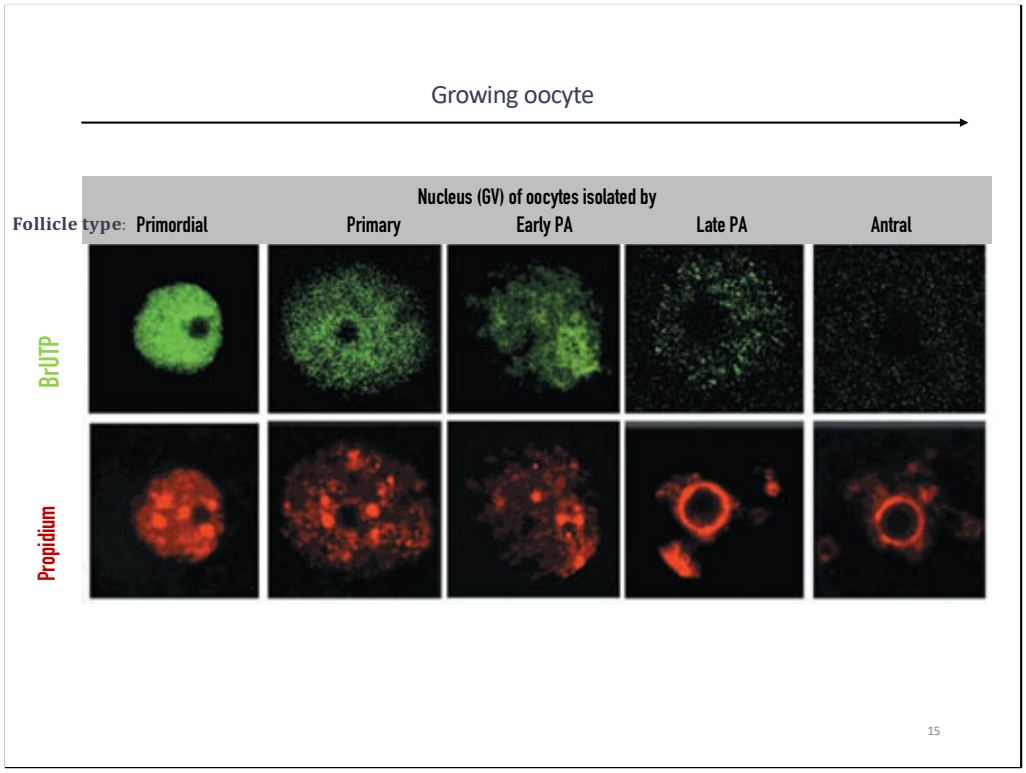
14

The main reason behind the transcriptional block is to provide the oocyte with a time window to accumulate the necessary factors and resources to support subsequent cell division and embryonic development.

Additionally, transcriptional block helps maintain the genetic stability of the oocyte by preserving the integrity of genetic material during meiosis and reducing the risk of genetic errors.

In summary, the transcriptional block during oocyte maturation is a key strategy to ensure proper embryonic development and the formation of a healthy organism.

There are strategies to monitor the transcriptional block, and these involve the use of nucleotide analogs marked with a fluorophore, such as Bromodeoxyuridine (BrdU). BrdU is an analog of thymidine, a nucleoside used as an indicator in DNA replication analyses. BrdU is incorporated into the DNA during the synthesis of the new strand during cell replication. Its chemical structure is similar to that of thymidine, but it contains a bromine atom instead of a hydrogen atom.



In the upper panels, you can observe staining for BrdU (in green), providing an indication of the transcriptional activity of the oocyte at different stages of growth. In the lower panels, staining for chromatin (in red) is visible, indicating the conformation of chromatin at various stages of growth.

This experiment clearly indicates that structural changes in chromatin (condensation) correspond to a stop in the transcriptional activity.