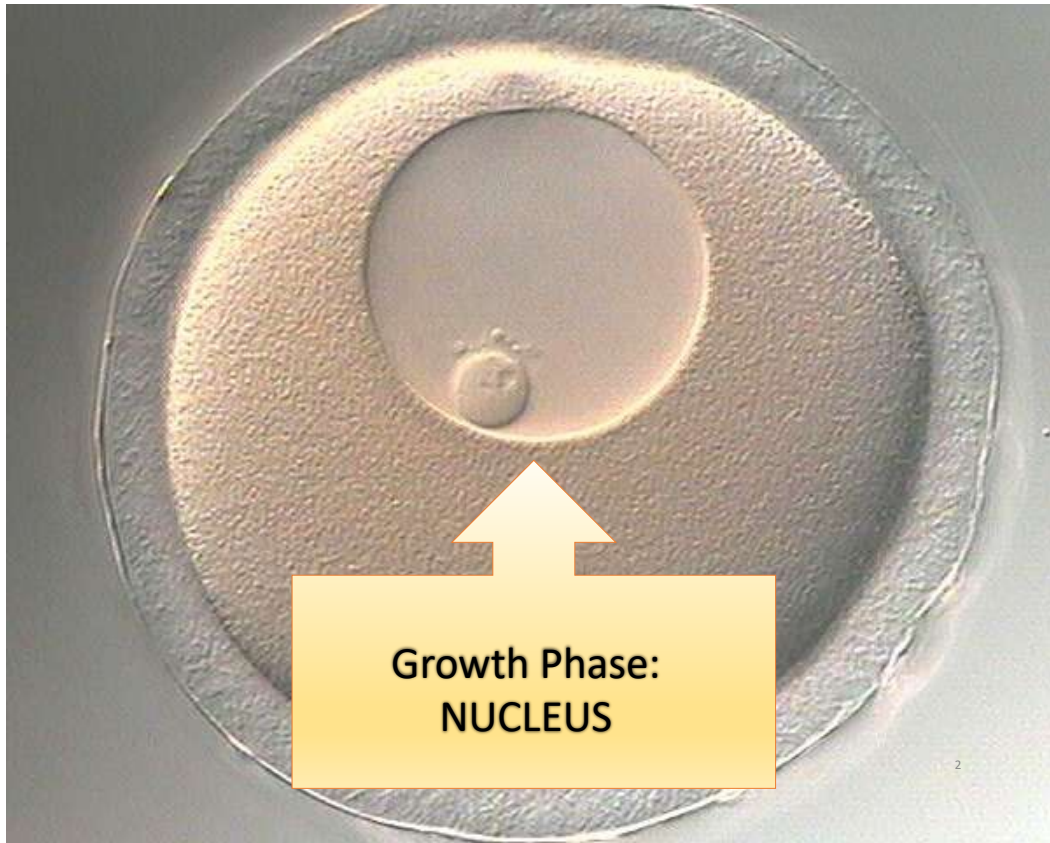


LESSON III

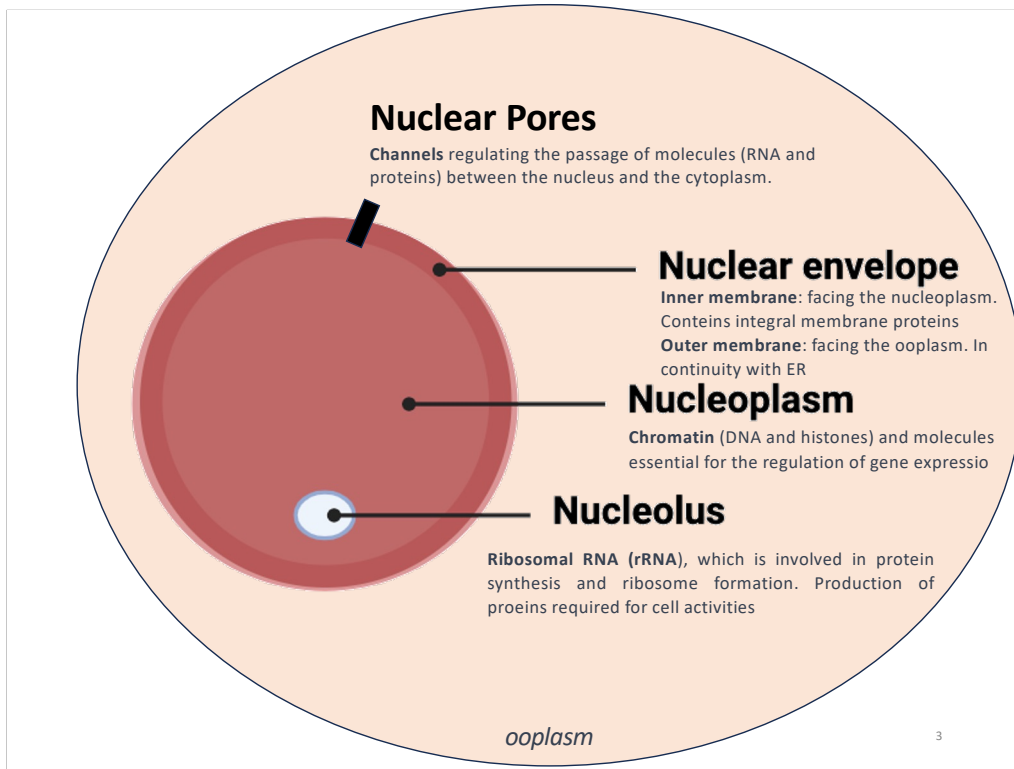
Post-natal oogenesis

Structural modifications of the nucleus



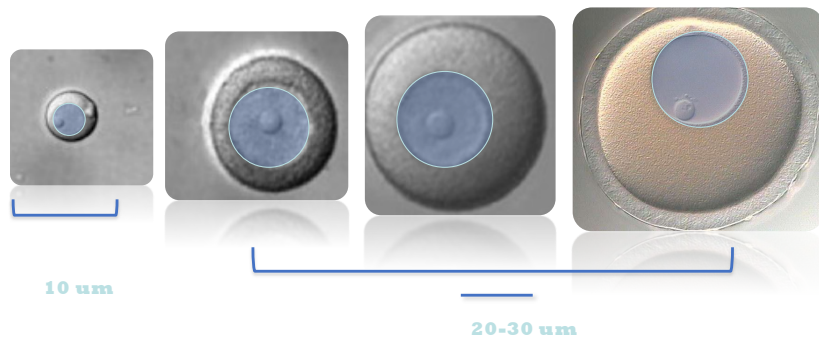
Structural modification during the growth phase also involve the nucleus of the oocyte, a structure that at this stage is known as Germinal Vesicle (GV).

Whereas the nucleus of the oocytes remains quiescent for years after birth in terms of meiotic point of view (until puberty occurs), its dimension and the molecular content change dramatically.



Structural modifications of the nucleus

A Mouse oocyte during its growth phase



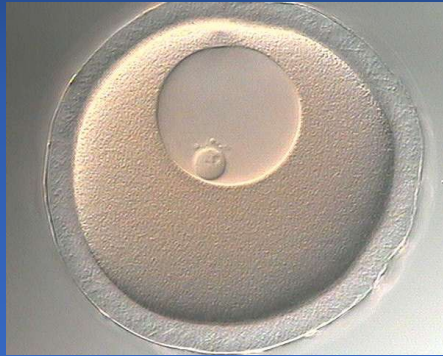
- ✓ increase in diameter
- ✓ undergoes translocation toward the periphery of the ooplasm

4

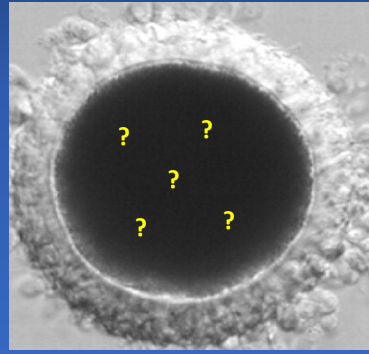
Initially, it experiences an increase in diameter, expanding from 10 to 20-30 μm . Additionally, it undergoes translocation toward the periphery of the ooplasm. Initially positioned centrally during the early stages of oogenesis, the GV subsequently transitions to a distinctly peripheral location.

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?



Mouse oocyte



Porcine oocyte

To make structural changes visible under an optical microscope 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.

KEEP THIS CONCEPT:

Extensive chromatin remodelling during the growth phase



**NUCLEI OF
IMMATURE
OOCYTES**

Interdispersed chromatin
within the nucleus



**NUCLEI OF
MATURE
OOCYTES**

Chromatin is condensed
within the nucleus



**The configuration of chromatin can be studied using
DNA-sensitive dyes**

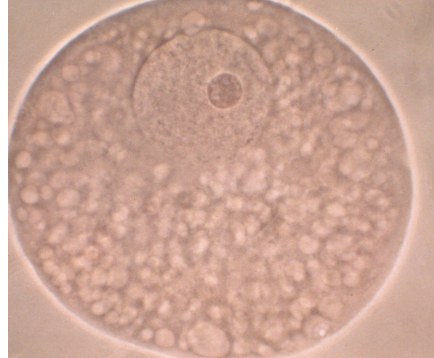
OPTICAL

E.g. Lacmoid

FLUORESCENT

E.g. Propidium
DAPI
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

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LACMOID STAINING

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 an 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

9

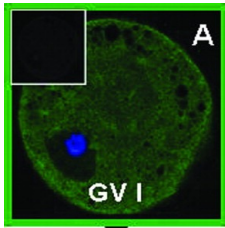
FLUORESCENT STAINING

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

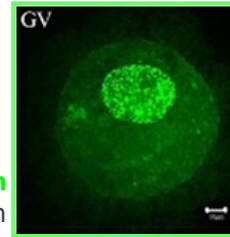
Although they come with a high cost and necessitate advanced microscopy, these dyes exhibit a stronger affinity for DNA.

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.

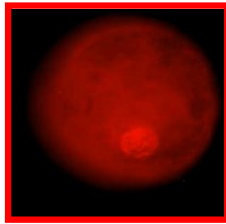
Chromatin sensitive dyes (fluorophores)



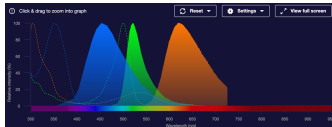
DAPI or Hoescht 33342
cell-permeant nuclear counterstain



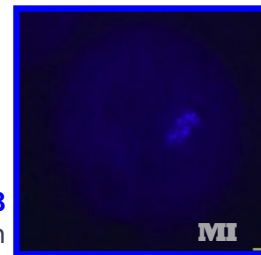
Sybr green
cell-permeant nuclear counterstain



Propidium iodide
Supravital nuclear counterstain



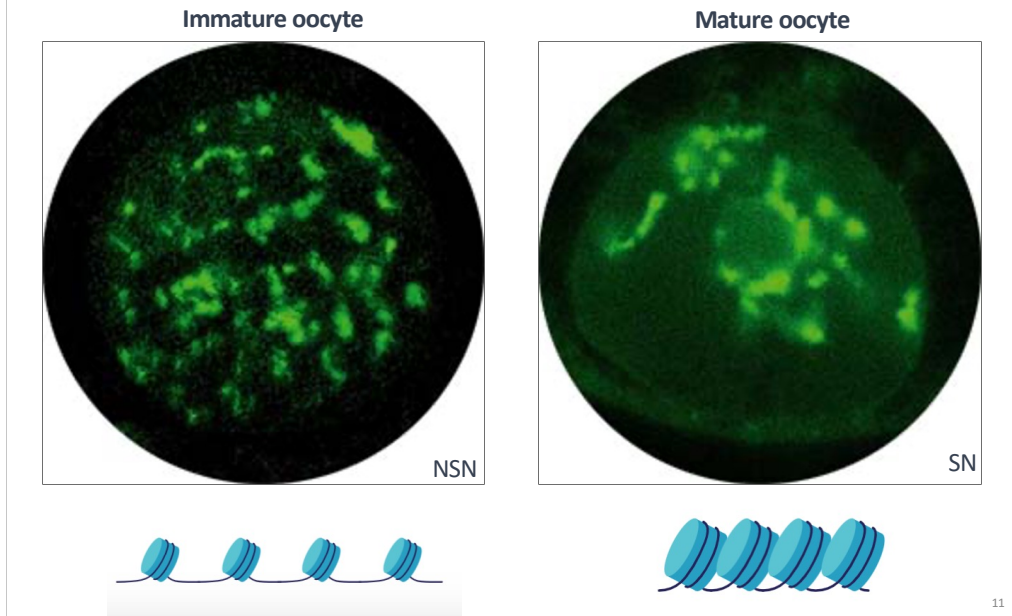
Hoescht 33258
Supravital nuclear counterstain



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

With a chromatin sensitive dye
e.g. Sybr green



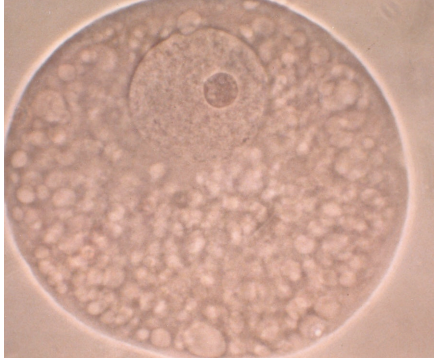
Example of staining with the fluorescent dye SYBR Green, allowing the chromatin remodeling state to be appreciated.

NSN: Non surrounding nucleolus

SN: Surrounding nucleolus

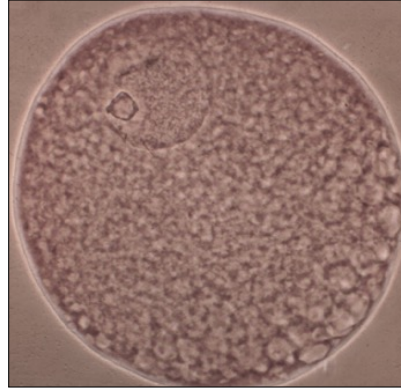
Resolution: Optical vs. Fluorescent dyes

Oocyte of a preantral follicle



Early stage oocyte
Immature growing gamete

Oocyte of a antral follicle

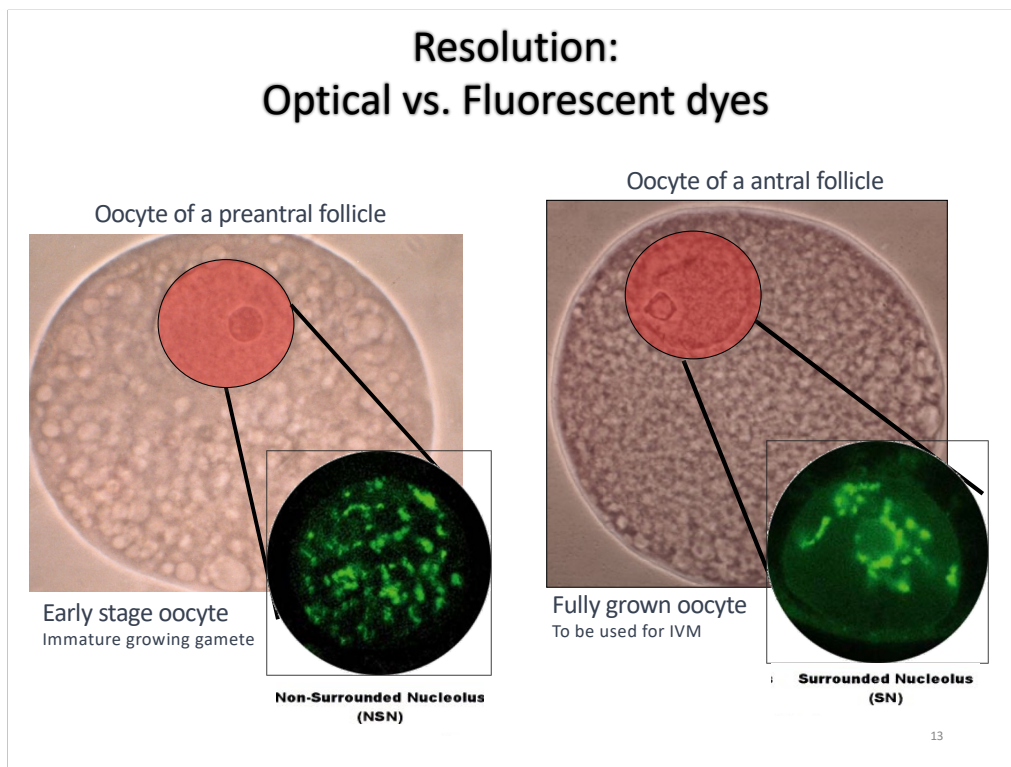


Fully grown oocyte
To be used for IVF

With an optical staining it is hard to discriminate the chromatin status of the nucleus due to the low affinity of the dye to DNA; in that case Lacmoid.

Left panel: immature pig oocytes isolated from a preantral follicle

Right panel: mature pig oocyte isolated from an antral follicle



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

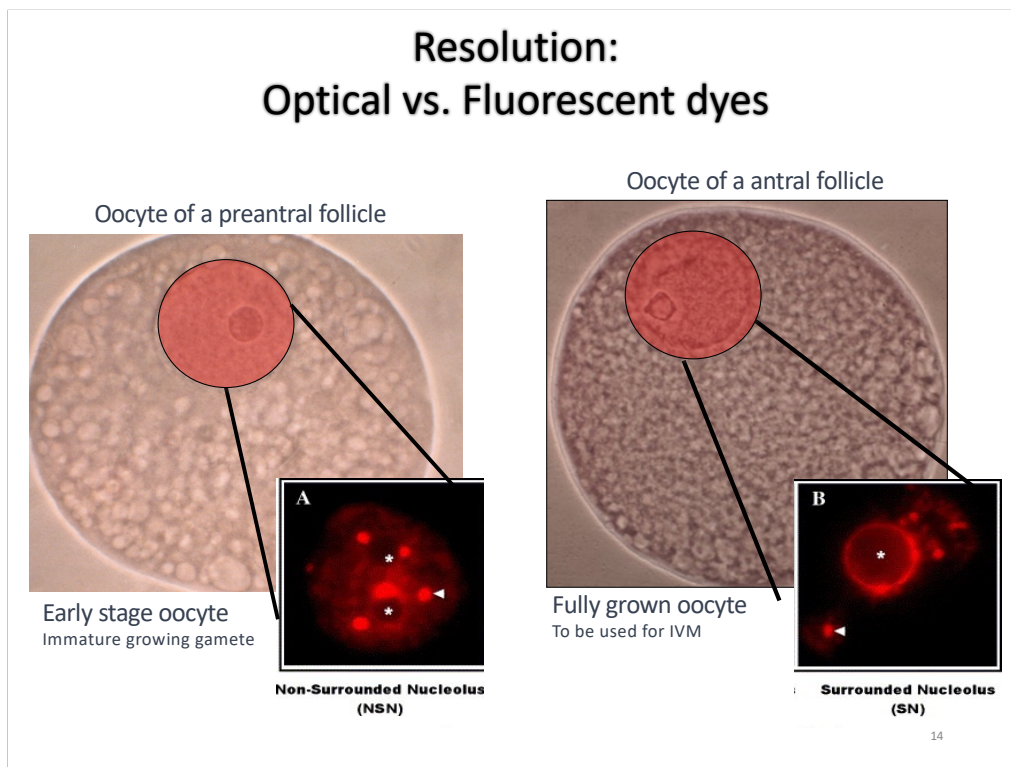
Example of fluorescent staining with a Sybr green dye.

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 surrounding 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.



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

Example of fluorescent staining with a Propidium iodide dye.

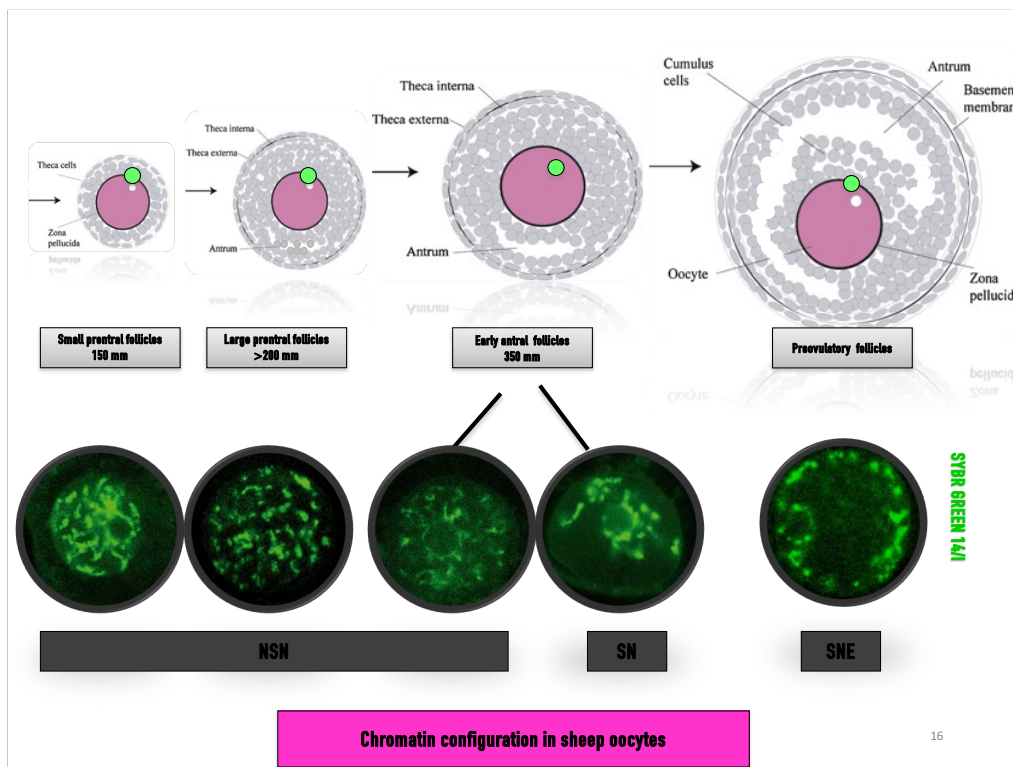
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		Oocyte category			
		Early Preantral follicles	Late Preantral follicles	Medium antral follicles	Preovulatory follicles
SYBR Green PA A in vitro EA NSN B in vitro EA SN C Preovulatory SNE D Sheep	Chromatin configuration				
	NSN	Mice, Pig, Sheep, cow, woman	Pig, Sheep, cow, woman		
	SN		Mice, Sheep	Mice, pig, cow, woman	Mice, pig, sheep, cow, woman
	SNE			Sheep	Sheep



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 species, 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.

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.

Extensive chromatin remodelling is linked to oocyte genome transcription inhibition!!!

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

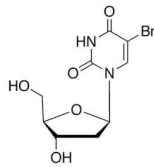
Specifically, chromatin remodelling experienced at the end of the growing phase within the oocyte's nucleus is associated with a transcriptional blockade.

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).

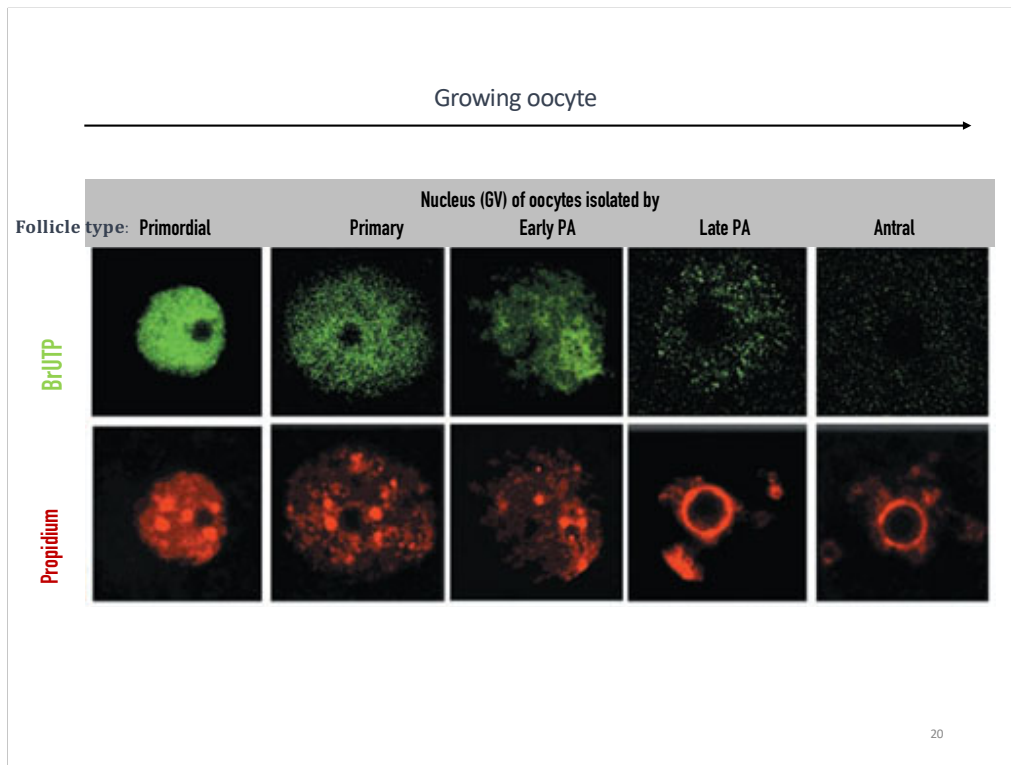
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The main reason behind the transcriptional block is to provide the oocyte with a time window to accumulate the necessary factors and resources to support subsequent cell division and embryonic development.

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.