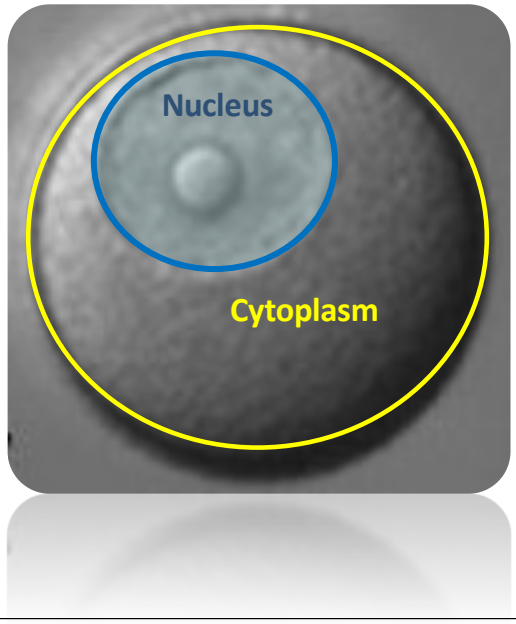
LESSON II

Post-natal oogenesis

Structural modifications of the cytoplasm

Growth Phase



The oocyte during the growth phase undergoes **structural** and **biochemical** modifications, whether within the cytoplasm or the nucleus

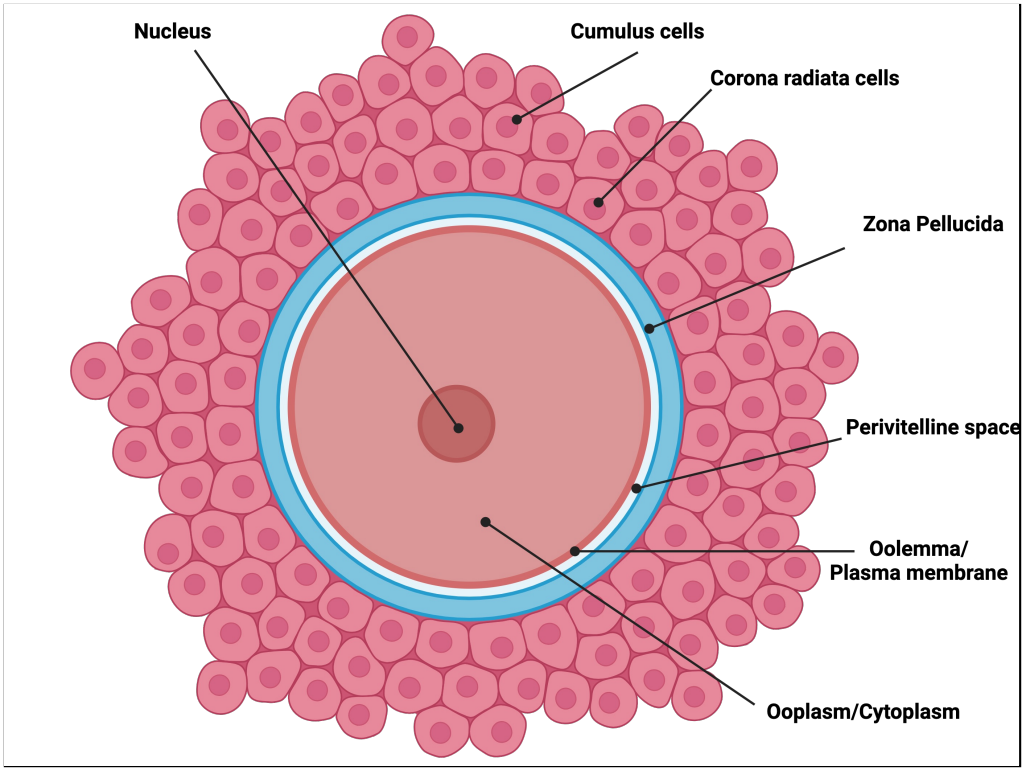
During the post-natal life, the pool of inherited oocytes has to become fertilizable by passing through two sequential phases of specialization:

- **Growth phase** and
- **Maturation phase**

The oocyte during the growth phase undergoes profound modifications that involve both cytoplasm and nucleus .

These modifications may be classified as

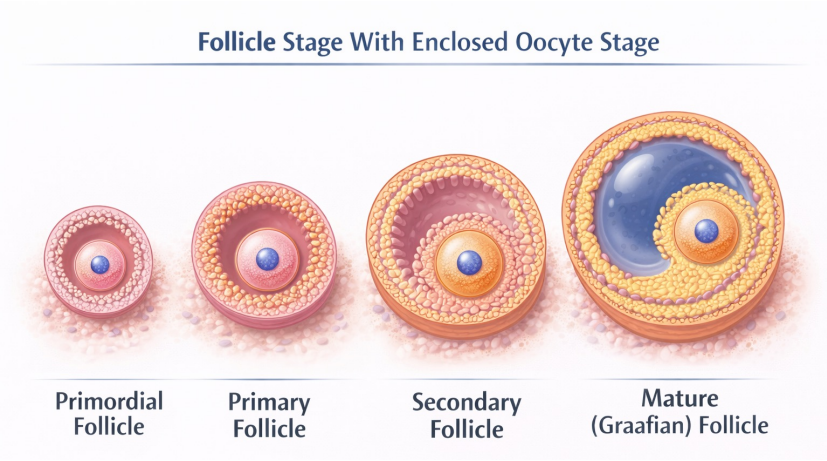
1. Structural and
2. Biochemical



Go to this link for further details:

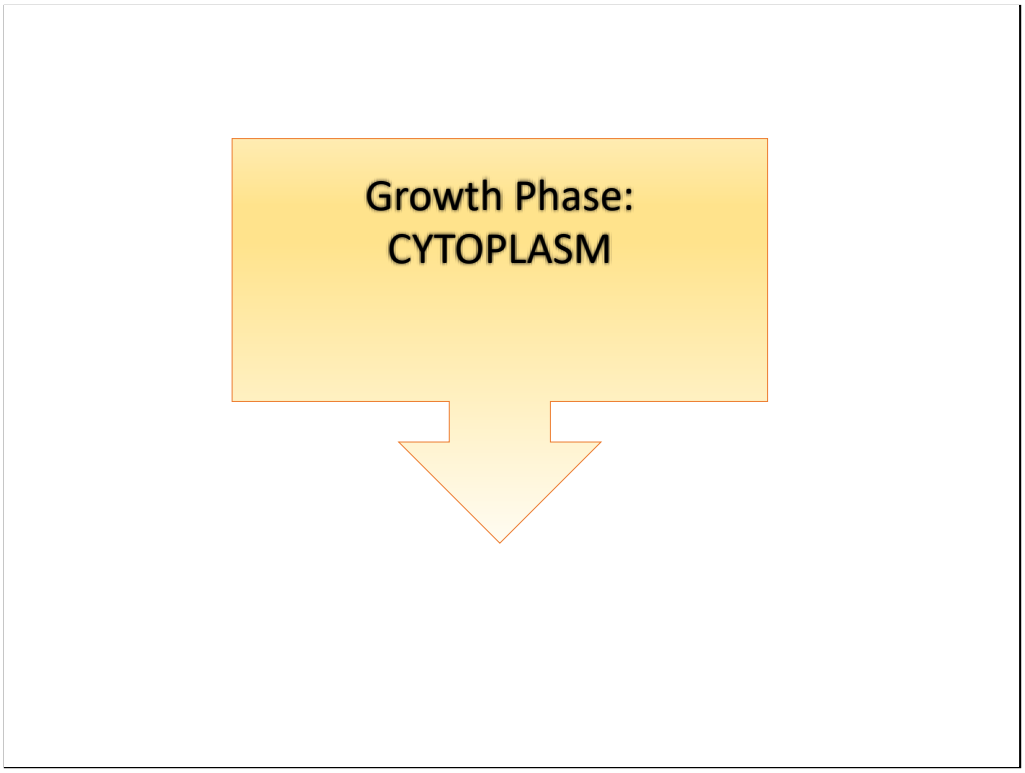
https://www.youtube.com/watch?v=psqnVgzsH7c

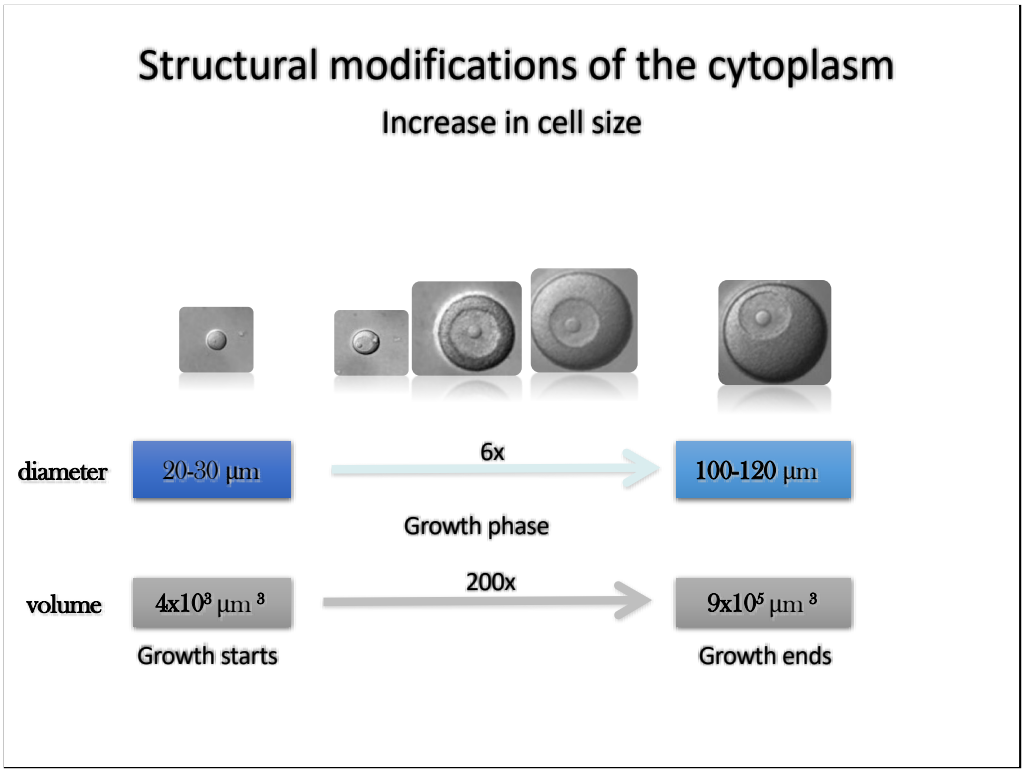
Follicle Stage With Enclosed Oocyte Stage



OOCYTE ⟸ **Arrested in meiosis I (prophase I)** ⟹

Small	**Growing**	**Growing**	**Fully grown**
• Surrounded by a single layer of flattened cells	• cuboidal granulosa cells, • beginning of zona pellucida formation	• multiple granulosa cell layers, • appearance of theca interna and externa, • early antrum formation	• surrounded by corona radiata and cumulus oophorus • large antrum • ready for ovulation


Growth Phase:
CYTOPLASM



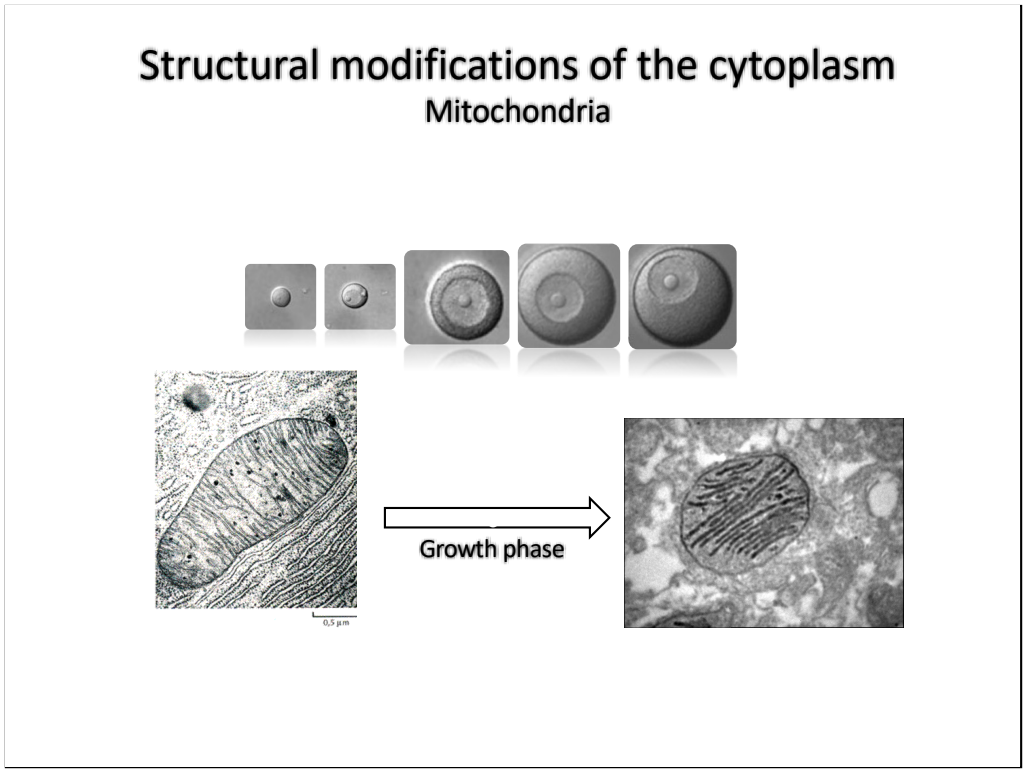
The major structural modification involving the oocyte during the growth phase is the increase in cell size.

Indeed, the cell diameter increases about 6-fold, passing from the 20 µm size of the oocyte enclosed into a primordial follicle

to the 100-120 µm of a fully grown oocyte at the end of the growth phase.

This increase is even more relevant if we express it in term of volume.

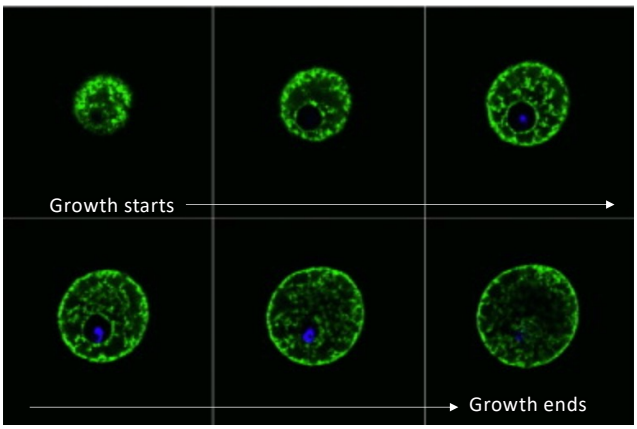
The volume enlarge about 200fold!!!!



Within the cytoplasm, various changes occur involving organelles (e.g.mitochondria). They increase both in number and activity.

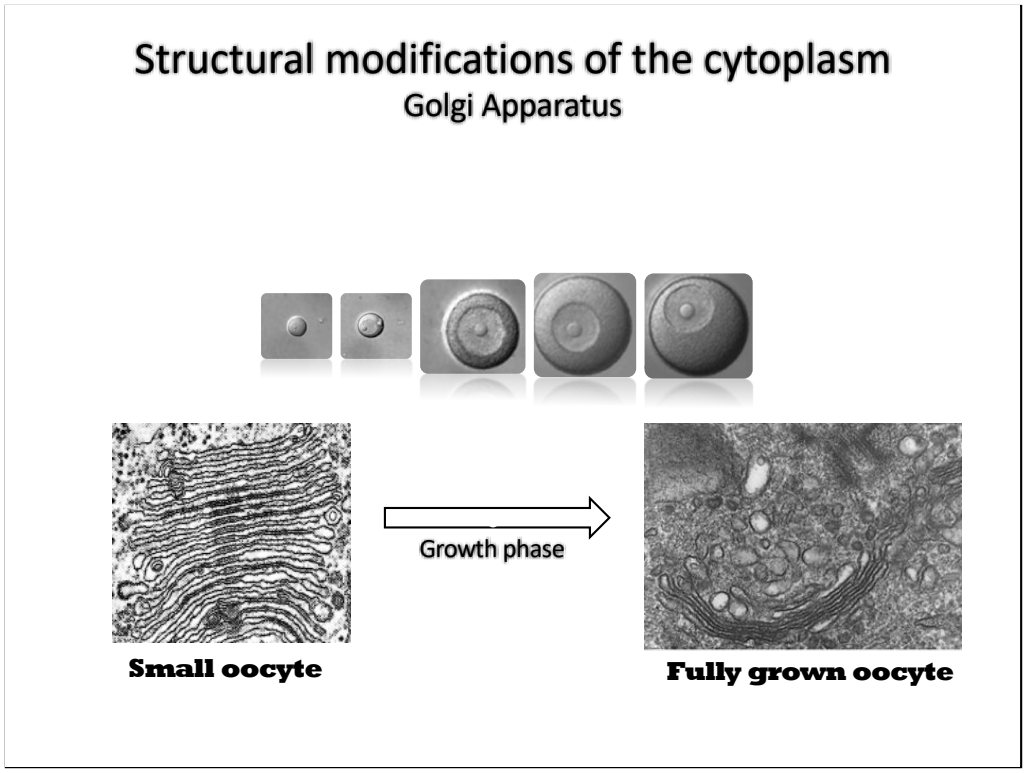
Structural modifications of the cytoplasm

Mitochondria



GREEN: *mitotracker staining of Mitochondria*

They increase in number, progressively trans-located at the periphery of the ooplasm and increases its function as revealed by the ultra-structural morphology.



An other organelle encountering profound structural modification is the Golgi apparatus.

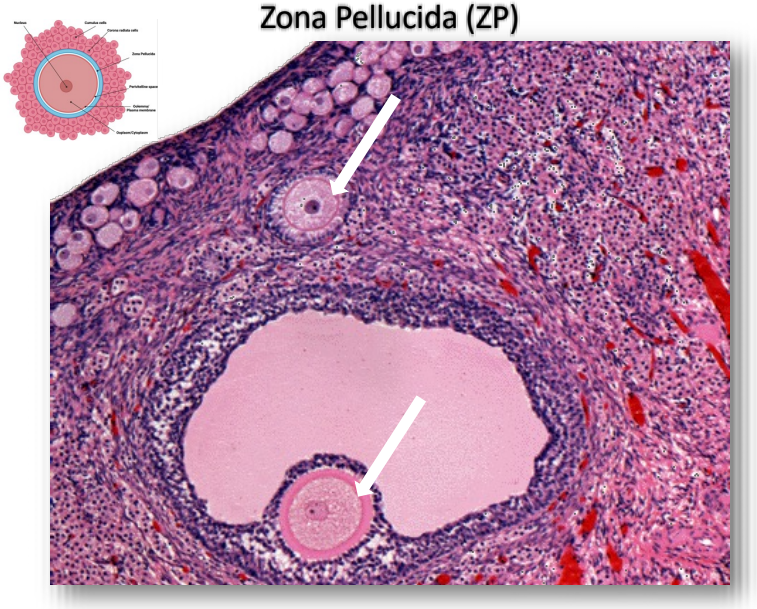
Importantly, its structural modification determines important changes in its activity, which is going to increase during growth.

In small oocytes, it appears compact with a few vacuoles and granules.

In the later stages of oogenesis, it acquires a three-dimensional, active structure with many vacuoles and granules and lipid vesicles.

Structural modifications of the cytoplasm

Zona Pellucida (ZP)

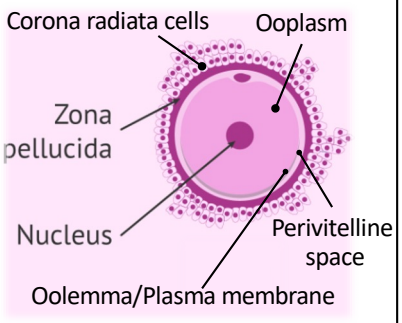


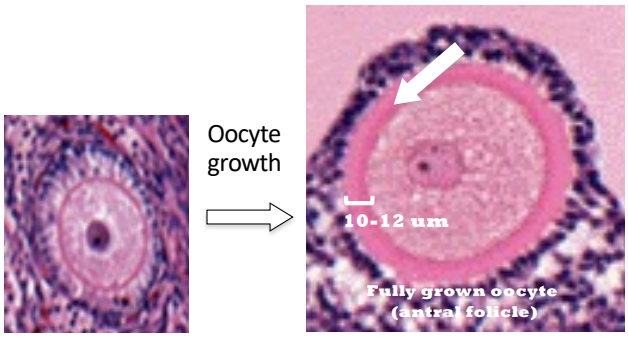
ZP is an addtional component deserving attention!

The Pellucida zone (ZP) is secreted and assembled externally during post-natal oogenesis.

Zona Pellucida

Composition: Glycoprotein envelope externally covering the oocyte.
Location: between peri-vitellinin space and follicular cells
Secreted during post-natal phase.
Appears late in post-natal oogenesis as a dense and thick acellular structure.





The ZP is a glycoprotein envelope that externally covers the oocyte.

it is an envelope located between peri-vitelline space and the follicular cells.

It is gradually secreted during the growth phase by the oocyte itself.

It appears late in post-natal oogenesis as a dense and thick acellular structure.

The thickness mostly increases toward the end of oogenesis, reaching a final dimension of 10 to 12 µm in oocytes enclosed within antral follicles.

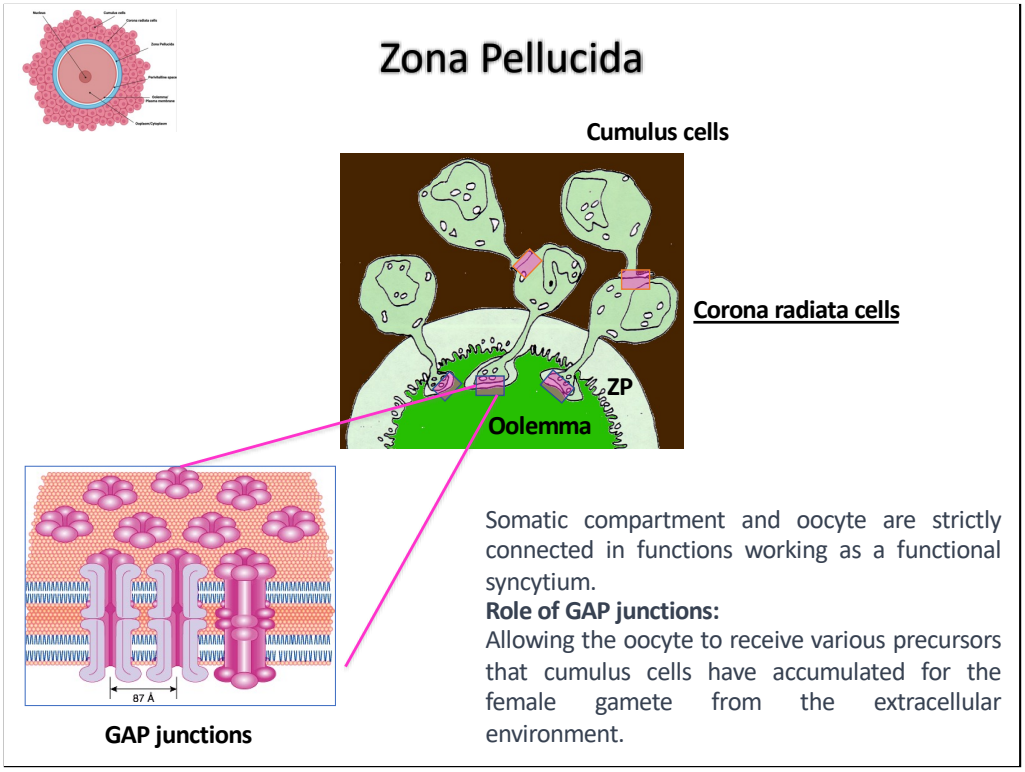
Zona Pellucida

ROLE I:

- ✓ serves as an extracellular matrix that protects the oocyte from external physical attacks.
- ✓ works as a barrier to the diffusion of molecules.

BUT

The ZP also constitutes a connection zone linking the follicular (somatic) micronevironment with the germinal one.

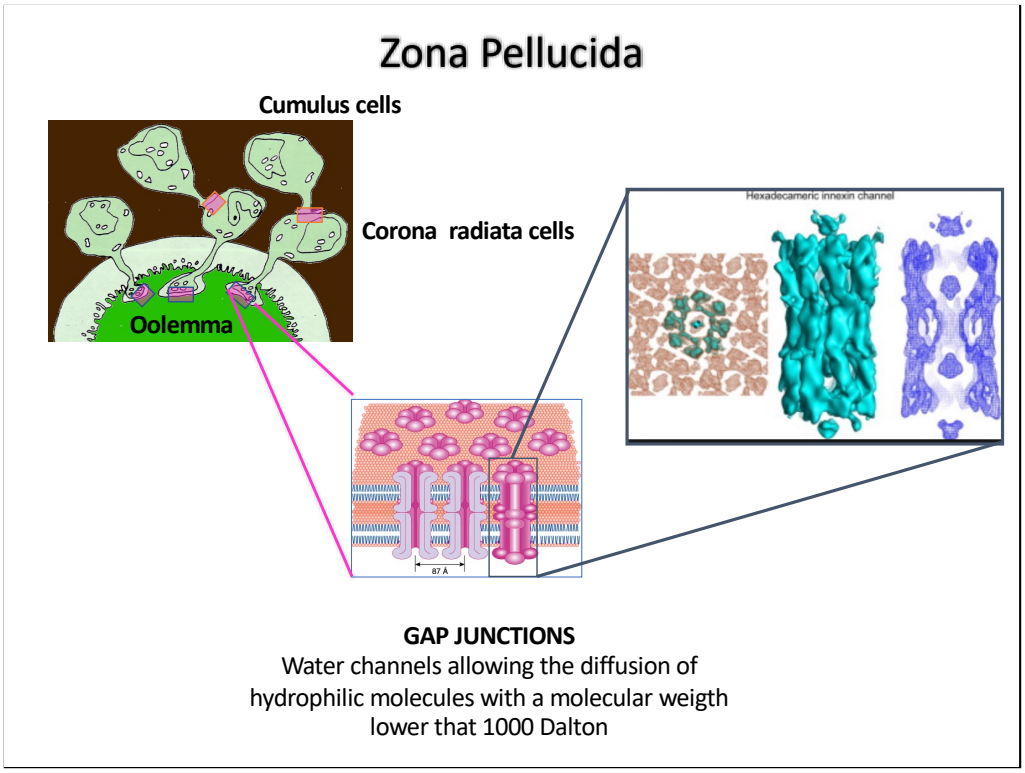


Indeed, corona radiata cells (the first layer of the somatic folliclular cells, CC) pass through it, coming into contact with the oolemma.

Within the region of interaction between corona radiata cells and the oolemma, diverse gap junction structures are active, allowing a dynamic metabolic coupling between the somatic compartment and the germinal one.

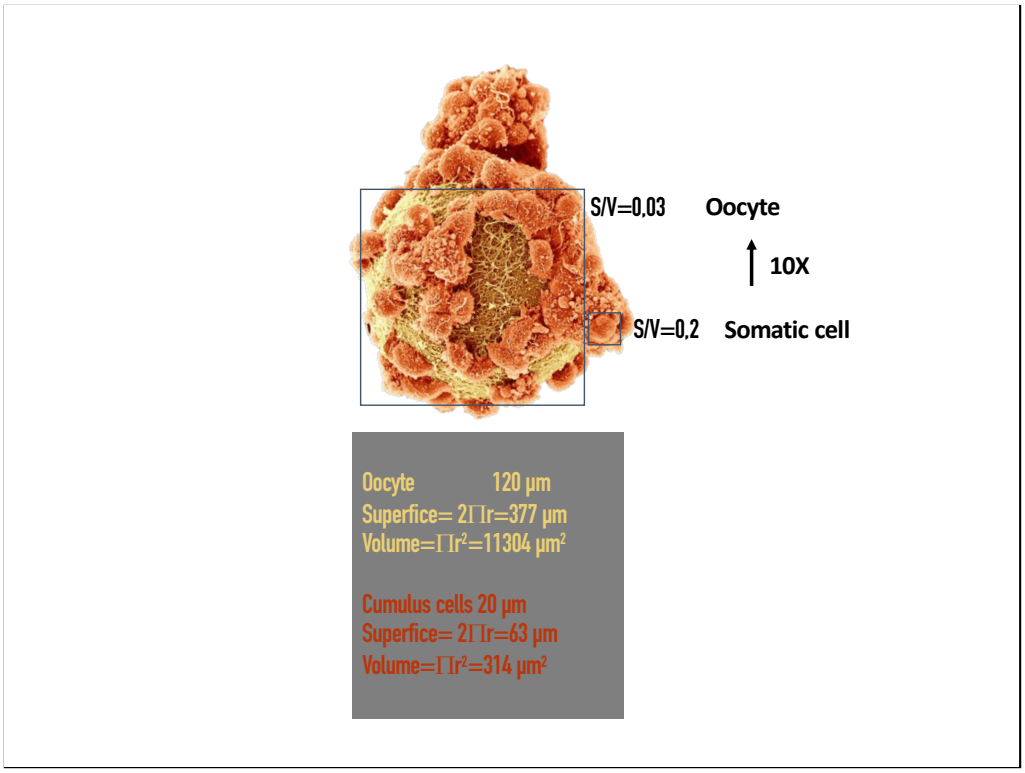
Furthermore, this metabolic connection is further enhanced by the fact that gap junctions also operate between cumulus cells (neighboring cells of corona one) and corona radiata cells.

This implies that the somatic compartment and oocyte are strictly connected in functions working as a functional syncytium. Thanks to the presence of gap junctions, the oocyte can receive various precursors that cumulus cells have accumulated for the female gamete from the extracellular environment.



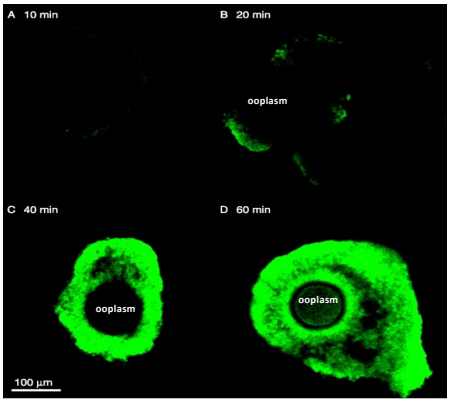
Gap junctions are specialized water channels that facilitate the diffusion of hydrophilic molecules with a molecular weight lower than 1000 Dalton, ensuring functional coupling between the oocyte and somatic compartments.

Gap junction ensure a continuous diffusion of precursors between corona radiata cells and the oocyte. Simultaneously, corona radiata cells receive molecules from the other layers of cumulus cells, thereby extending metabolic support from the oocyte to the entire cumulus complex.



In this manner, although the surface-to-volume (S/V) ratio, which regulates the ability of cell to manage molecules diffusion, is approximately 10 fold lower in the oocyte compared to somatic cells, the oocyte receives sufficient trophic support by utilizing the entire surface of cumulus cells. These cells are metabolically coupled to the female gamete via gap junctions, not the oolemma, as the latter alone would be insufficient to meet the high metabolic demands of a growing oocyte.

EXP: A cumulus-oocyte complex is exposed to green fluorescently labeled glucose.

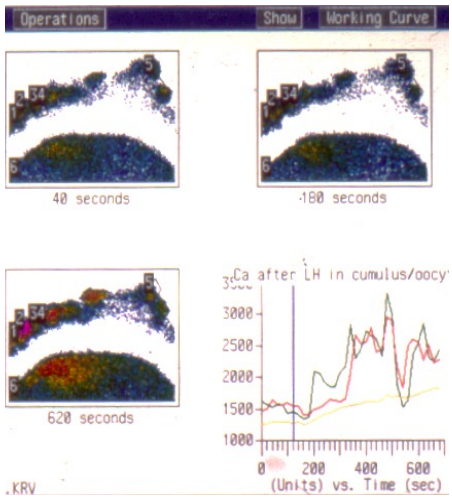


THE MAIN CONCEPT:
The METABOLIC COUPLING is crucial for all phases of oogenesis, as it ensures the passage of soluble molecules to meet the metabolic needs of the oocyte.

This concept becomes evident in this experiment, where a cumulus-oocyte complex is exposed to fluorescently labeled glucose. The glucose reaches the ooplasm by diffusing from the external to the innermost layers of cumulus cells, becoming evident once glucose is entrapped inside the cells. This diffusion process creates a clear gradient that involves the oocyte within approximately one hour of incubation.

To summarize: The described metabolic coupling is crucial for all phases of oogenesis, ensuring the passage of soluble molecules to meet the oocyte's metabolic needs. However, the metabolic coupling goes beyond that by allowing the oocyte to receive inhibiting and activating stimuli from cumulus cells.

EXP: LH stimulus



Cumulus cells play a crucial role in mediating this important message.

However, the metabolic coupling goes beyond that by allowing the oocyte to receive inhibiting and activating stimuli from cumulus cells.

An example is the LH stimulus.

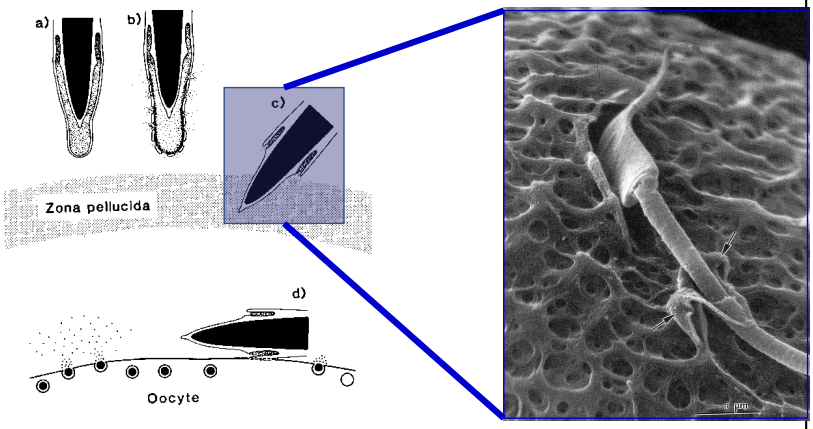
The oocyte lacks receptors for gonadotropins, including LH, even though, LH can trigger meiotic maturation. Cumulus cells play a crucial role in mediating this important message. Specifically, cumulus cells of preovulatory follicles begin to express high LH receptors at the preovulatory stage. When the LH surge occurs, these cumulus cells exclusively in preovulatory follicles receive and transmit this signal by intracellular Ca elevation and reduction of cAMP.

TAKE HOME MESSAGE:

The metabolic coupling mediated by gap junctions between cumulus cells and oocyte is essential for providing both trophic support and signaling control to the oocyte.

Any events disrupting this communication immediately compromise the survival of the oocyte, which is entirely dependent on the presence of coupled somatic cells.

Zona Pellucida-mediated sperm egg recognition



ROLE II:

✓ serves as a crucial structure facilitating the recognition of the oocyte by spermatozoa.

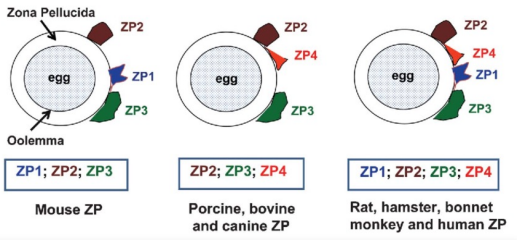
Capacitated spermatozoa possess specific receptors on the acrosomal region that bind to the glycoproteins constituting the PZ.

In most species ZP serves as a crucial structure facilitating the recognition of the oocyte by spermatozoa.

Specifically, capacitated spermatozoa possess specific receptors on the acrosomal region that bind to the glycoproteins constituting the ZP.

The glycoproteins mostly involved, (ZP1, ZP2, ZP3, and ZP4), have been characterized and classified based on their amino acid composition.

Zona Pellucida-mediated sperm egg recognition



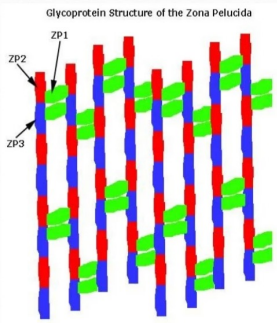
The homology between species never exceeds 70%

Wassermann and co-workers (1980, 1985, 1987, 1988)

Found that zona pellucida is composed of 3 glycoproteins

ZP1, ZP2, ZP3

Repeating subunits of ZP2 and ZP3 form filaments that are bound together by ZP1



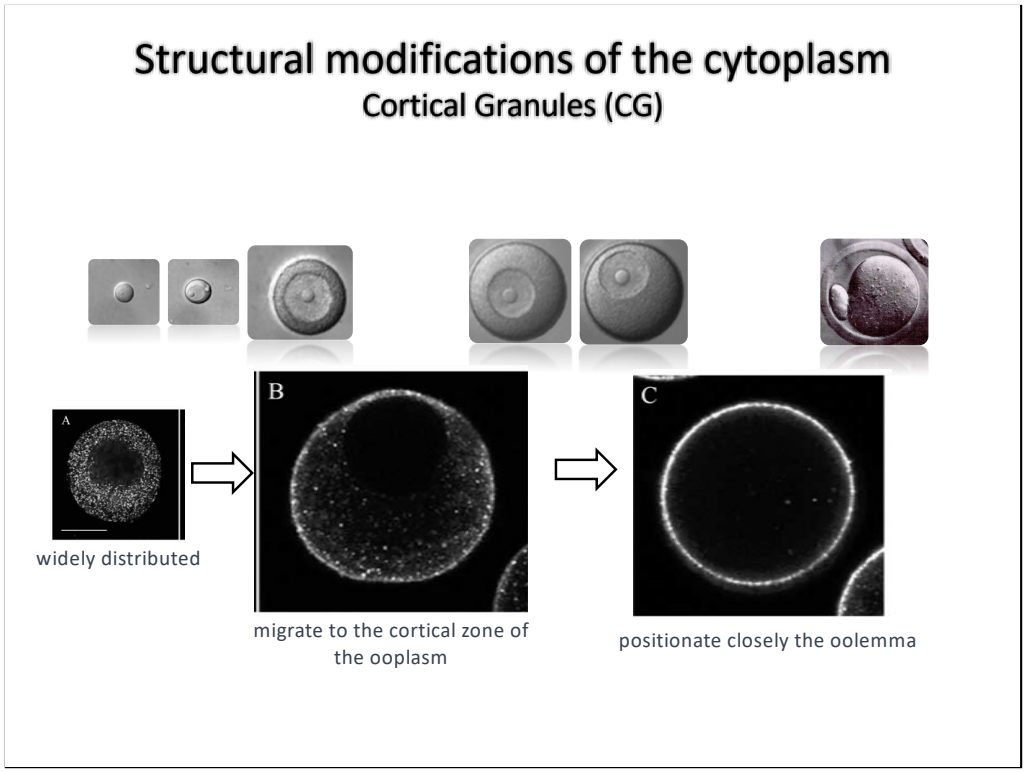
Mouse as a mammalian model to study ZP

ZP and SPERM RECEPTOR binding is a recognized mechanism capable of specifically activating the acrosome reaction

Var
differ in number and molecular structure.

Mice have been used as study model for ZP characterization and function.

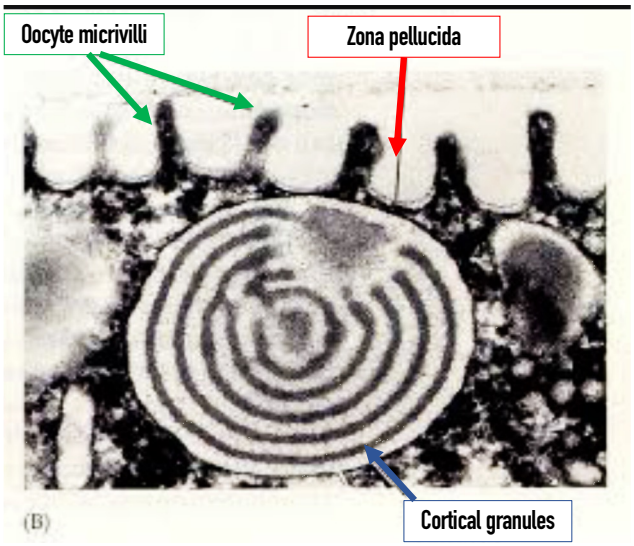
Of note, In mice, the reciprocal recognition between sperm and oocyte predominantly utilizes the glycan residues of ZP3. The binding between the ZP and sperm receptor is a recognized mechanism capable of specifically activating the acrosome reaction (AR), serving as a preliminary event leading to fertilization.



CG, like other intracellular organelles, undergo significant changes in concentration and location during the growth phase, as illustrated in these slides where they have been labeled with a fluorescent probe for their enzymatic content. Initially, they are widely distributed in the cytoplasm of primordial and primary oocytes. Over time, the CG progressively migrate to the cortical zone of the ooplasm, ultimately positioning closely the oolemma in fully grown oocytes.

Structural modifications of the cytoplasm

Cortical Granules (CG)



Composition:
CG are lysosomes enriched in several enzymes involved in:

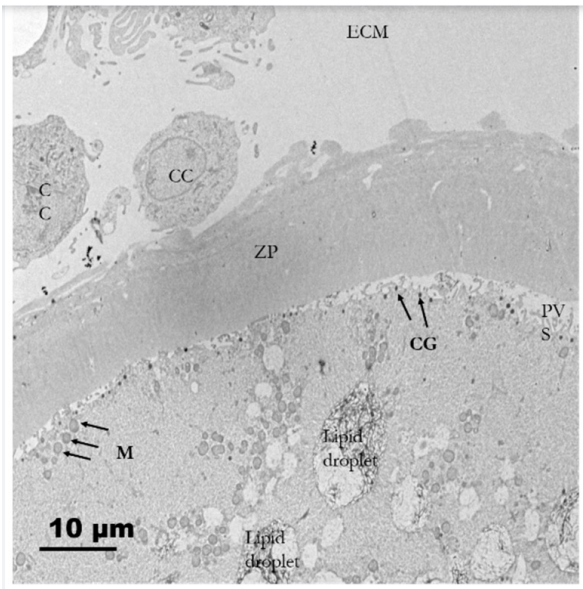
- **digestion** of molecular component of ZP
- ZP 3D architecture **remodelling**.

As you can see in this ultra-structural image, these organelles are lysosomes enriched in several enzymes involved in the digestion of diverse molecular component of ZP and in ZP 3D architecture remodelling.

They appear as intracellular vescicles displaying a round shape close to the oolemma.

Structural modifications of the cytoplasm

Cortical Granules (CG)



Electron micrograph of matured porcine oocytes

CG:cortical granules
M: mitochondria
CC: cumulus cell
ZP: Zona pellucida
ECM: extracellular matrix

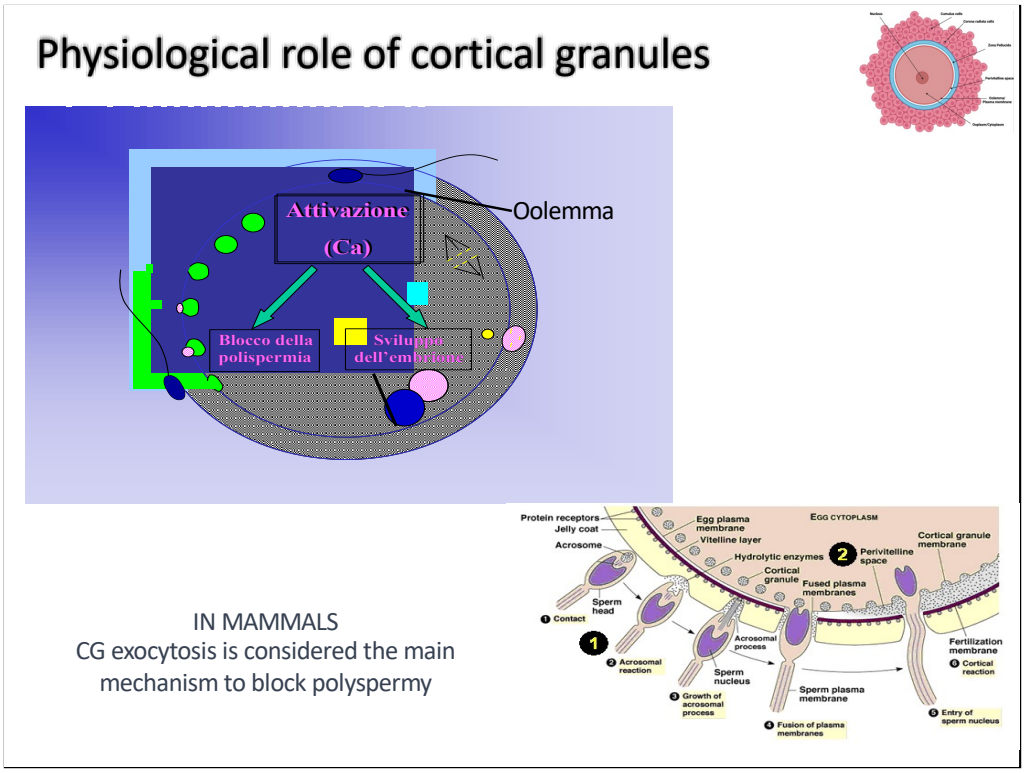
Distance between CG and Oolemma
=
Degree of specialization

CG get closer to the oolemma during the growth phase, reaching the minimal distance (in nanometers) at the time of fertilization.

The distance between the cortical granules and oolemma is dependent of the degree of specialization of the oocyte.

By the end of the growth phase, all cortical granules have migrated a few microns away from the oolemma. Subsequently, they move closer to the oocyte membrane, stopping just a few nanometers away before fertilization.

Once the cortical granules reach this final distribution, any event leading to an increase in intracellular calcium levels is responsible for the fusion between the granule and oocyte membranes, resulting in the exocytosis of enzymatic content.

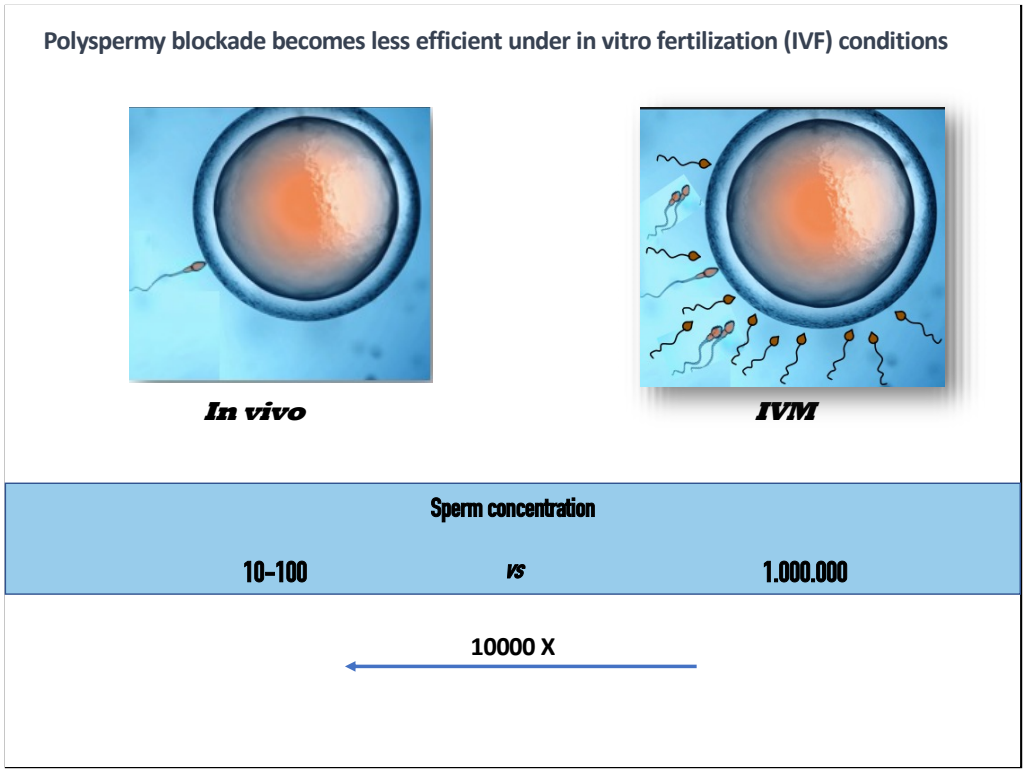


Physiologically, this intracellular Calcium elevations occurs after the fusion between the oolemma and sperm membrane at the moment of fertilization.

As a consequence, the membranes of CG fuses with the oolemma thus allowing cortical granules enzymes to be exocyted.

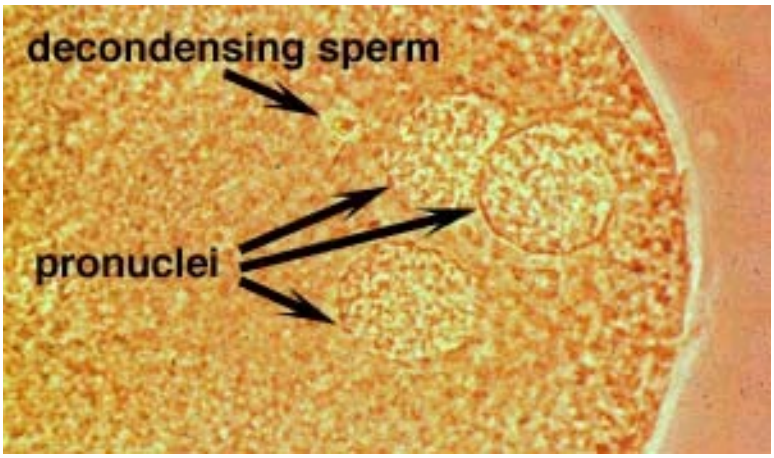
Immediately, the enzymatic content is released into the peri-vitelline space (the space between ZP and oolemma).

Their enzymatic influence extends to both oolemma, where they determine the detachment of fusogenic molecules, and ZP, where they are responsible for the 3D and chemical modification of ZP glycoproteins. This modification prevents the mechanism of sperm-egg recognition and fusion from taking place. For this reason, cortical granule exocytosis is considered the main mechanism in mammals to block polyspermy.



Unfortunately, the block of polyspermy becomes less efficient under in vitro fertilization (IVF) conditions. The high concentration of spermatozoa in the IVF protocol, which is ten thousand times higher than that observed under physiological conditions, is one reason for this inefficiency.

Additionally, the block of polyspermy mediated by cortical granule exocytosis is a slow event, taking 40-60 seconds after fertilization. During this time window, the oocyte is completely unable to prevent fertilization under IVF conditions. It is challenging to counteract the significant pressure exerted by spermatozoa in vitro, especially in certain species like pigs, where this mechanism is less effective physiologically.



Polyspermy case in pig model

In this species IVF have to be manage with great attention to avoid that several spermatozoa may entry inside the oocyte thus compromising the success of IVF.

Premature cortical granules case

Attention needs to be deserved!!!

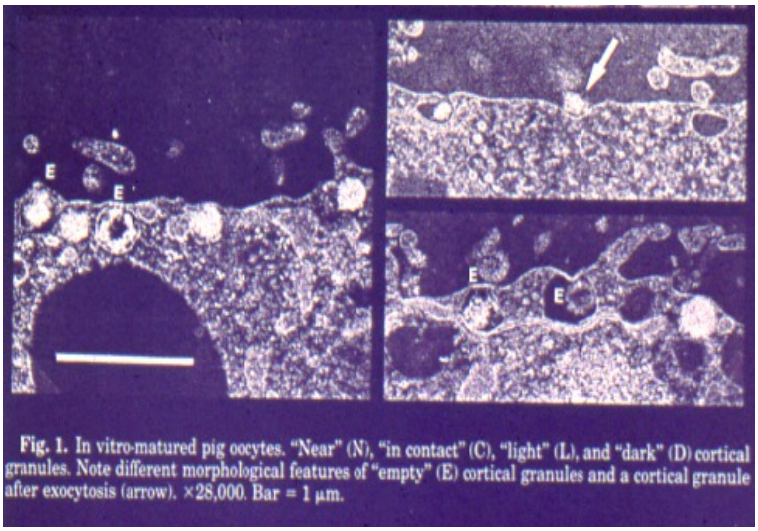


Fig. 1. In vitro-matured pig oocytes. "Near" (N), "in contact" (C), "light" (L), and "dark" (D) cortical granules. Note different morphological features of "empty" (E) cortical granules and a cortical granule after exocytosis (arrow). ×28,000. Bar = 1 μm.

In vitro manipulations of a mature oocyte can lead to intracellular alterations in ion concentrations risulting in premature cortial granules release

In some scenarios, there may be a need to induce the process of cortical granule exocytosis, while in other situations, it is crucial to prevent it. In the provided image, we observe a matured oocyte that has released cortical granules before fertilization.

A question arises: why does this occur?

It is now evident that various manipulations involving a mature oocyte can lead to intracellular alterations in ion concentrations, particularly an increase in intracellular calcium primarily originating from the extracellular environment. As a consequence of this rise in intracellular calcium, premature exocytosis of cortical granules may be triggered in a mature oocyte. This phenomenon occurs in cryopreservation protocols, as well as in inadequately designed in vitro maturation protocols.