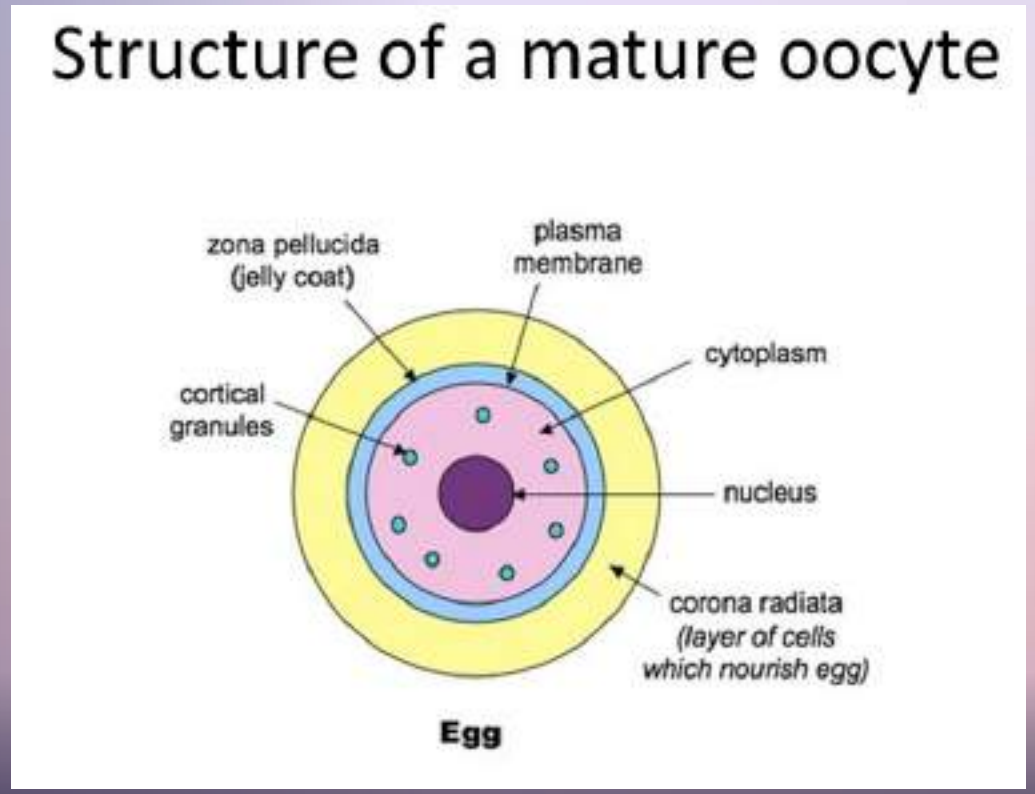
What is MEIOSIS?

Meiosis is a special type of cell division of germ cells in sexually-reproducing organisms used to produce the gametes, such as sperm or egg cells.

- **Cytoplasm** - oocytes are rich in cytoplasm, which contains many types of molecules to nourish the cell early in development.
Nucleus - during the primary oocyte stage of oogenesis, the nucleus is called a germinal vesicle.
- **Cortical granules** are regulatory secretory organelles found within oocytes and are most associated with polyspermy prevention after the event of fertilization.
- **Zona Pellucida**: glycoprotein layer surrounding the plasma membrane of mammalian oocytes. The zona pellucida first appears in preantral primary oocytes. This structure binds spermatozoa, and is required to initiate the acrosome reaction
- **Corona radiata**: is the innermost layer of the cells of the cumulus oophorus and is directly adjacent to the zona pellucida. Its main purpose in many animals is to supply vital proteins to the cell.



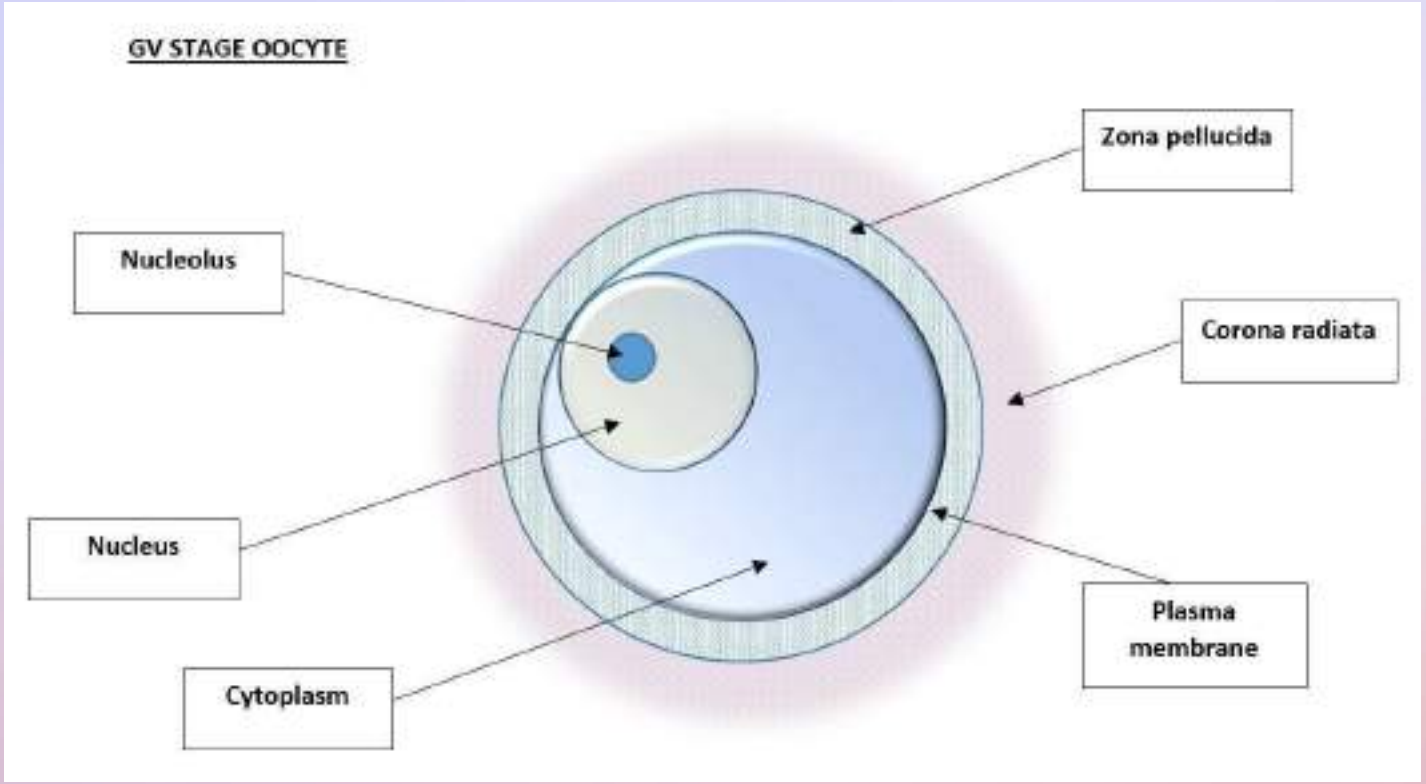
What are the main differences between **MEIOSIS I and MEIOSIS II?**

- **Meiosis I** segregates homologous chromosomes, producing two haploid cells (n chromosomes, 23 in humans) which each contain chromatid pairs. Because the ploidy is reduced from diploid to haploid, meiosis I is referred to as a *reductional division*.
- **Meiosis II** is an *equational division* analogous to mitosis, in which the sister chromatids are segregated, creating four haploid daughter cells.

NB: At the germinal vesicle (GV) stage, the oocyte has not yet resumed meiosis (oocyte blocked in Profase I)

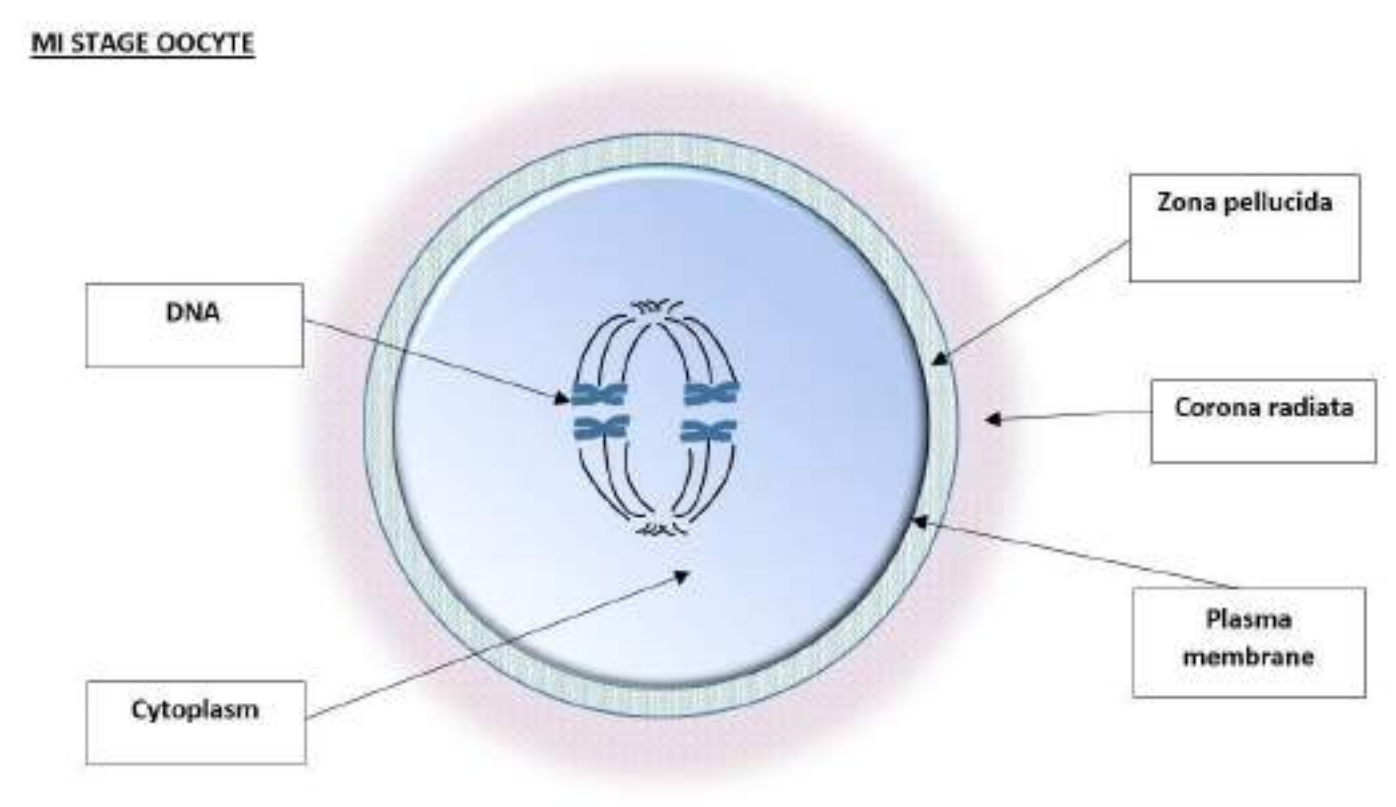
Only after the ovulatory surge of LH, the meiotic process resumes!

- Nucleus - during the primary oocyte stage of oogenesis, the nucleus is called a germinal vesicle.
- Nucleolus - It is a region of the cell nucleus responsible for the synthesis of ribosomal RNA (rRNA). It is the site of ribosome biogenesis.

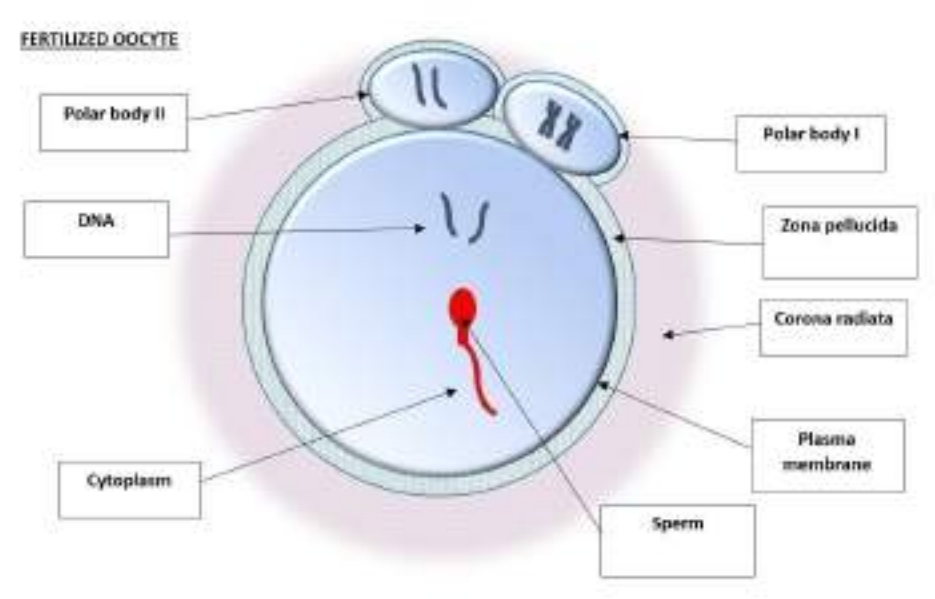


After the ovulatory surge of LH, the meiotic process resumes! The breakdown of the germinal vesicle (GVBD, equivalent to nuclear envelope breakdown in somatic cells) indicates a resumption of meiosis and the extrusion of the first polar body (1 PB) indicates completion of the first meiotic division in oocytes

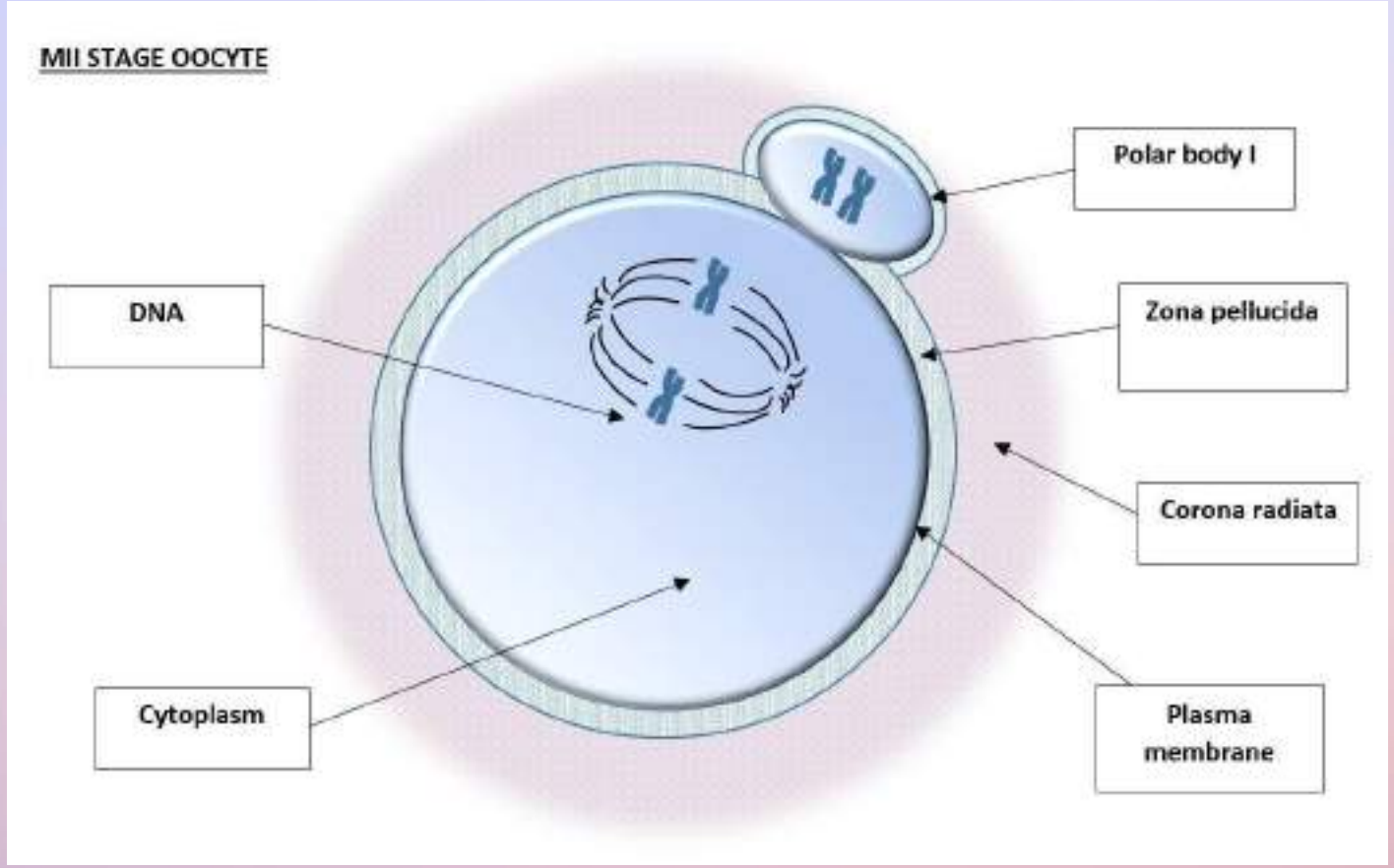
METAPHASE I: Homologous pairs move together along the metaphase plate. Homologous chromosomes separate.



Meiosis of a secondary oocyte is completed only if a sperm succeeds in penetrating its barriers. Meiosis II then resumes, producing one haploid ovum that, at the instant of fertilization by a (haploid) sperm, becomes the first diploid cell of the new offspring (a zygote)



METAPHASE II: the sister chromatids of each chromosome are separated.



Oocytes are ovulated in metaphase II. An oocyte in metaphase two is defined as **meiotically competent**

Why evaluate the oocyte nuclear stage?

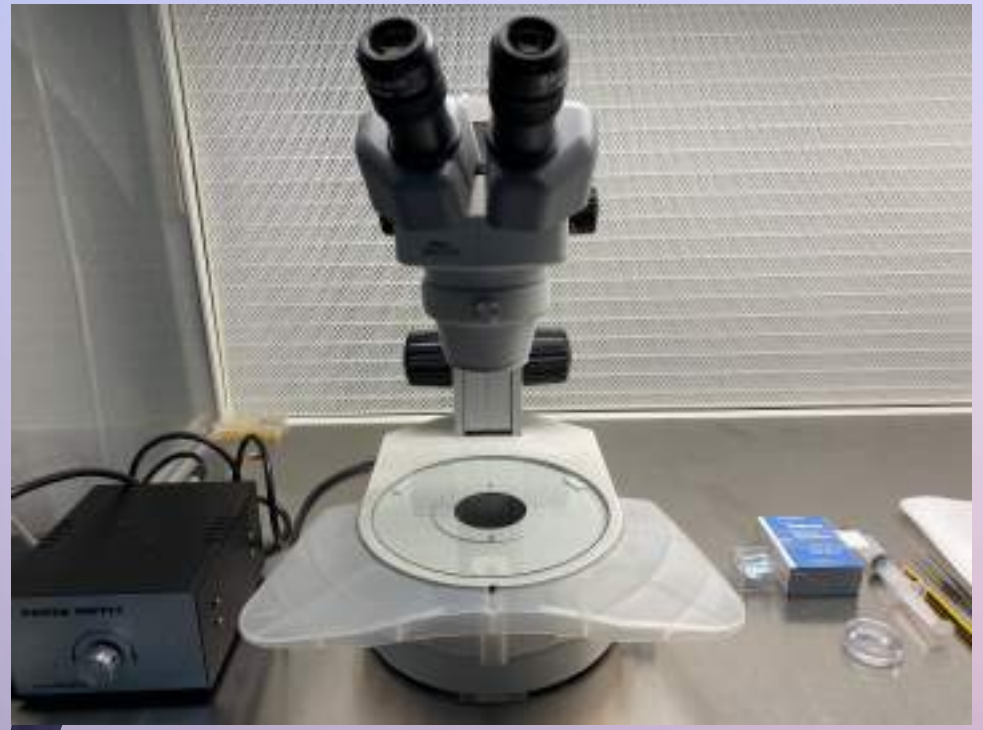
- **For IVF Lab procedures**: The oocyte is considered mature (from a nuclear point of view), and therefore usable for in vitro insemination, if it has reached the metaphase II stage.
- **For research purposes**: the meiotic competence needs to be evaluated to assess proper cultural method protocols or specific treatments.

What types of dyes can we use to evaluate the nuclear stage of oocytes?

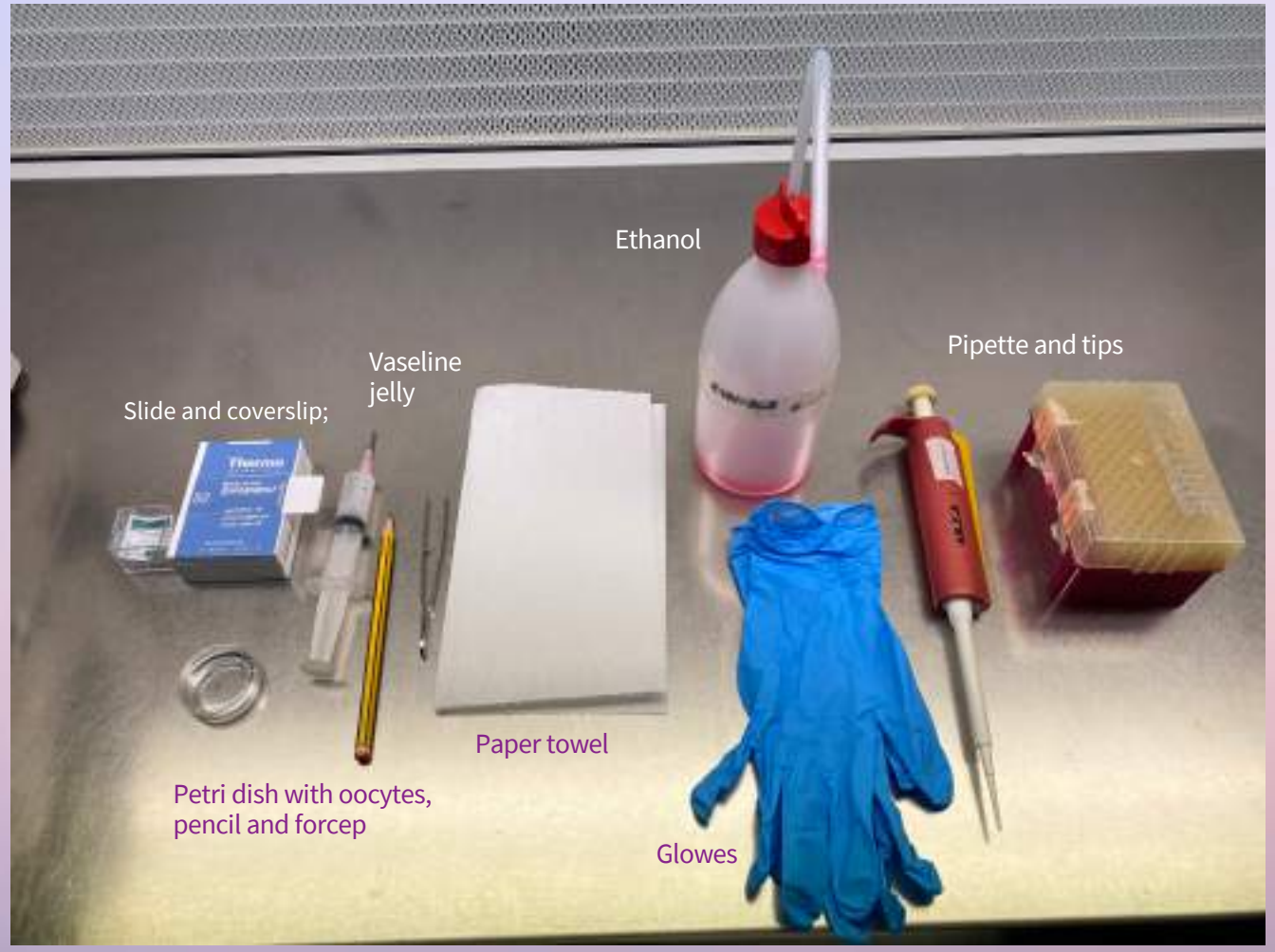
- **DAPI:** is a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA. It is used extensively in fluorescence microscopy. As DAPI can pass through an intact cell membrane, it can be used to stain both live and fixed cells, though it passes through the membrane less efficiently in live cells and therefore provides a marker for membrane viability.
- **Hoechst**: it may be used on live or fixed cells, and are often used as a substitute for another nucleic acid stain, DAPI. The key difference between them is that the additional ethyl group of Hoechst 33342 makes it more lipophilic, and thus more readily to cross intact cell membranes
- **Lacmoid:** specific dye for chromatin. The substantial difference with the first two dyes is that with Lacmoid we can see not only the nuclear stage, but also the quality of the cytoplasm

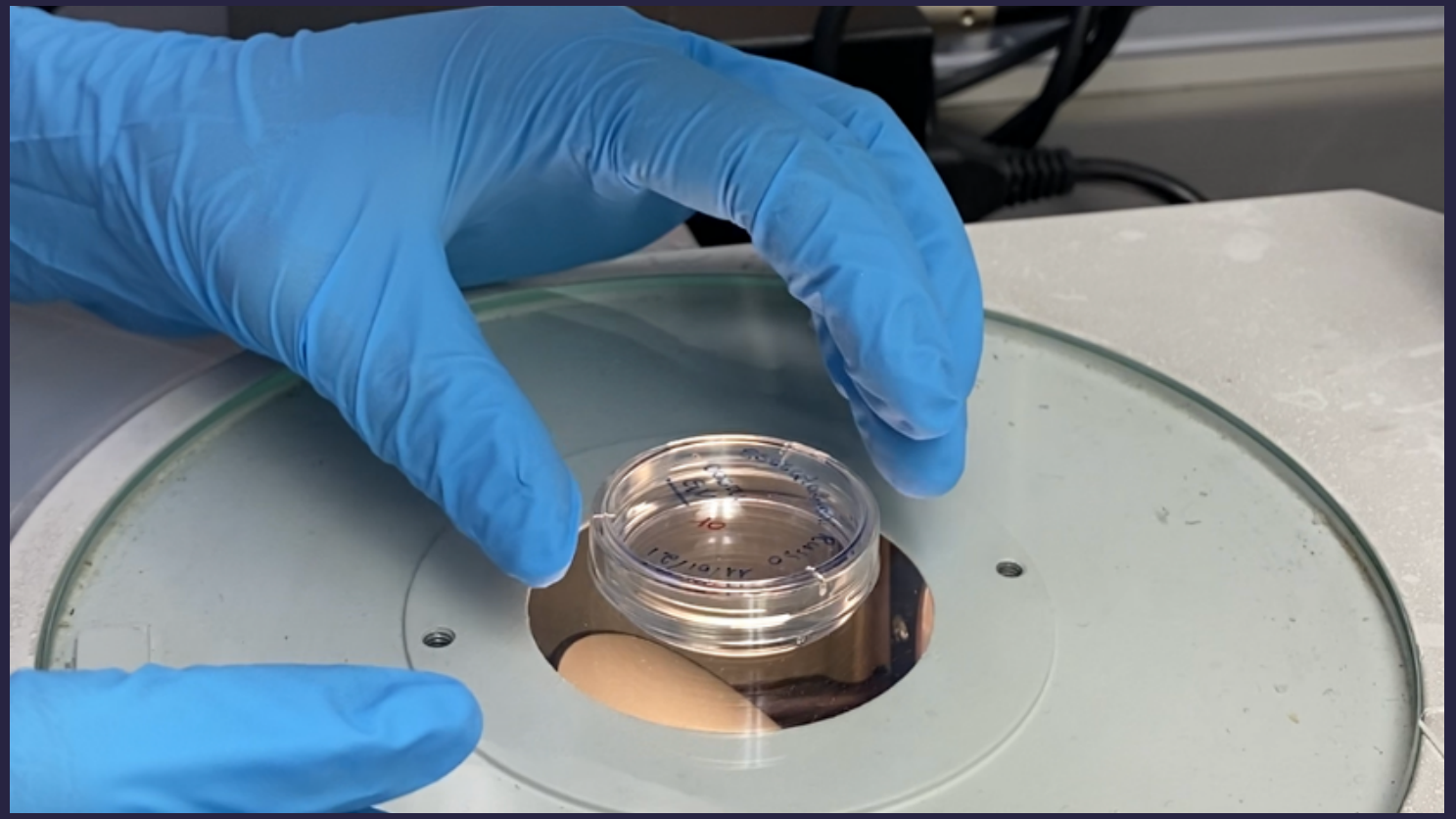
How can we evaluate the nuclear stage after having stained the oocytes for research purposes?

It is possible to mount the oocytes on a slide!

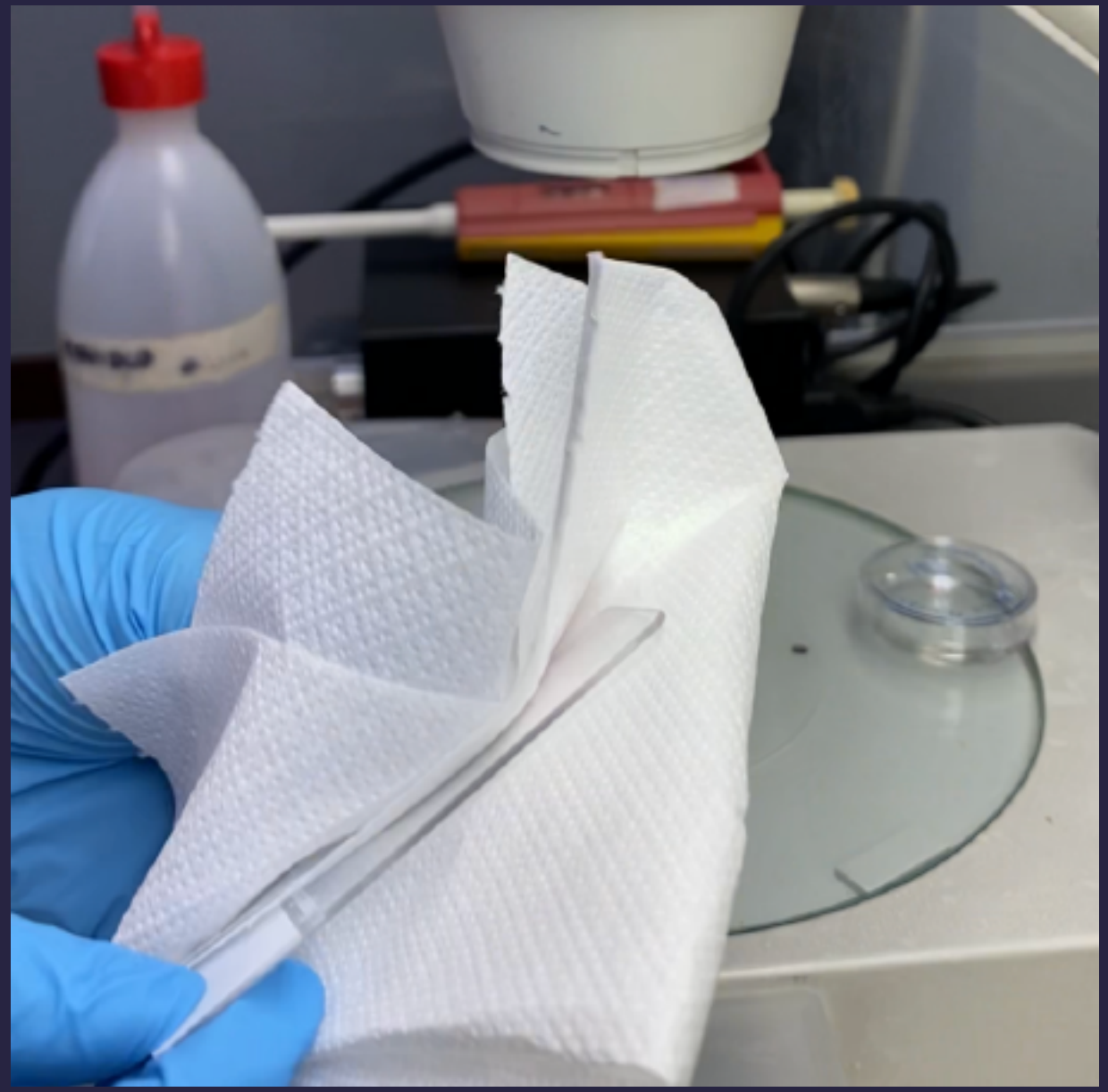


Stereomicroscope



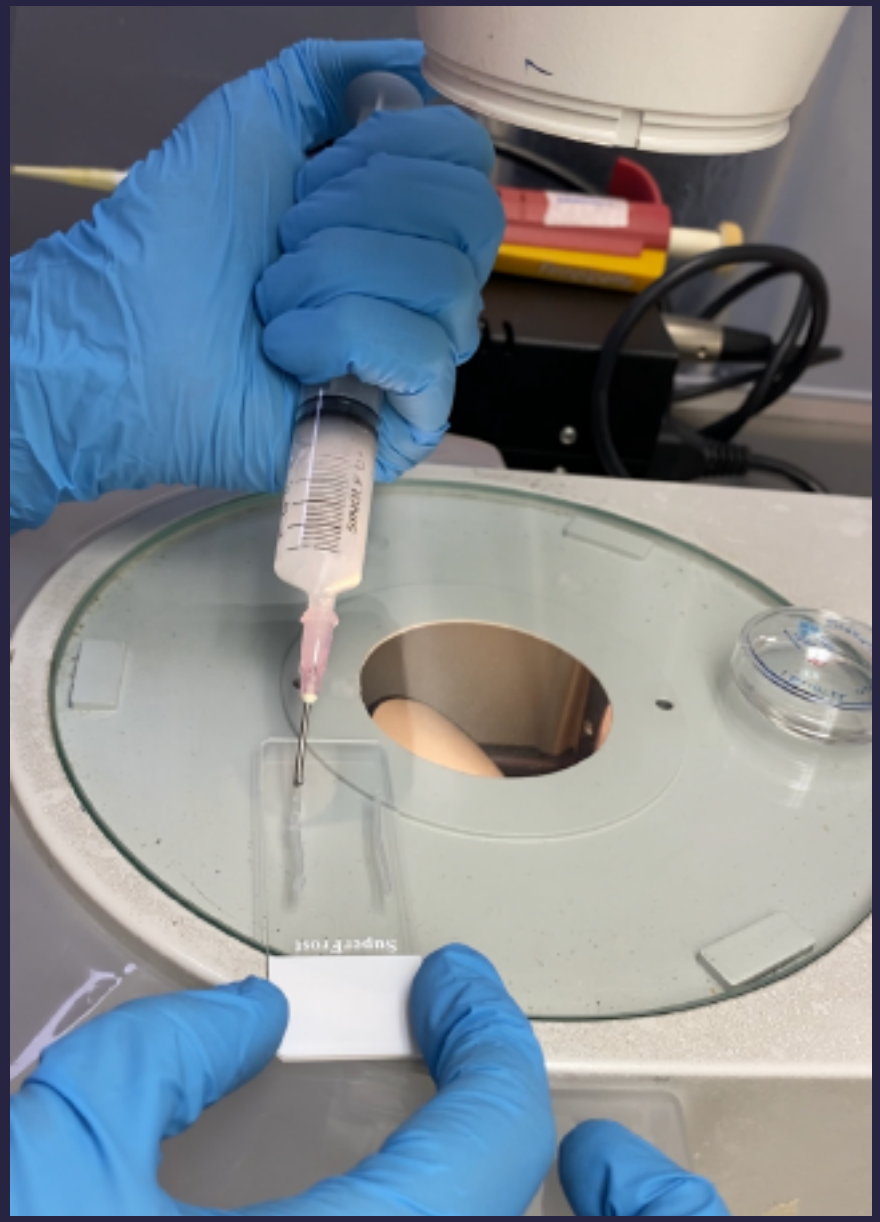


- **STEPS:**
- 1. Visualize the integrity of the oocytes under a stereomicroscope and count how many oocytes are present in the petri dish.
- 2

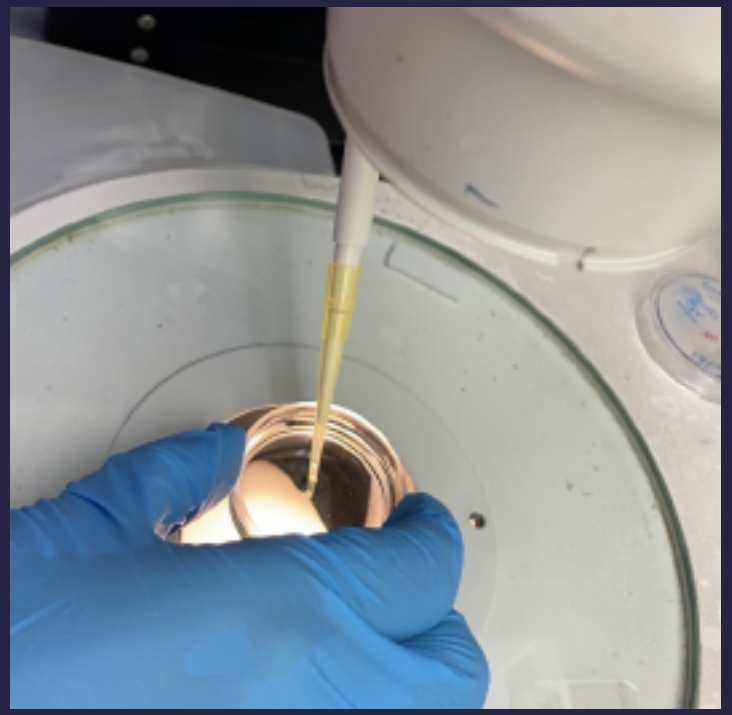


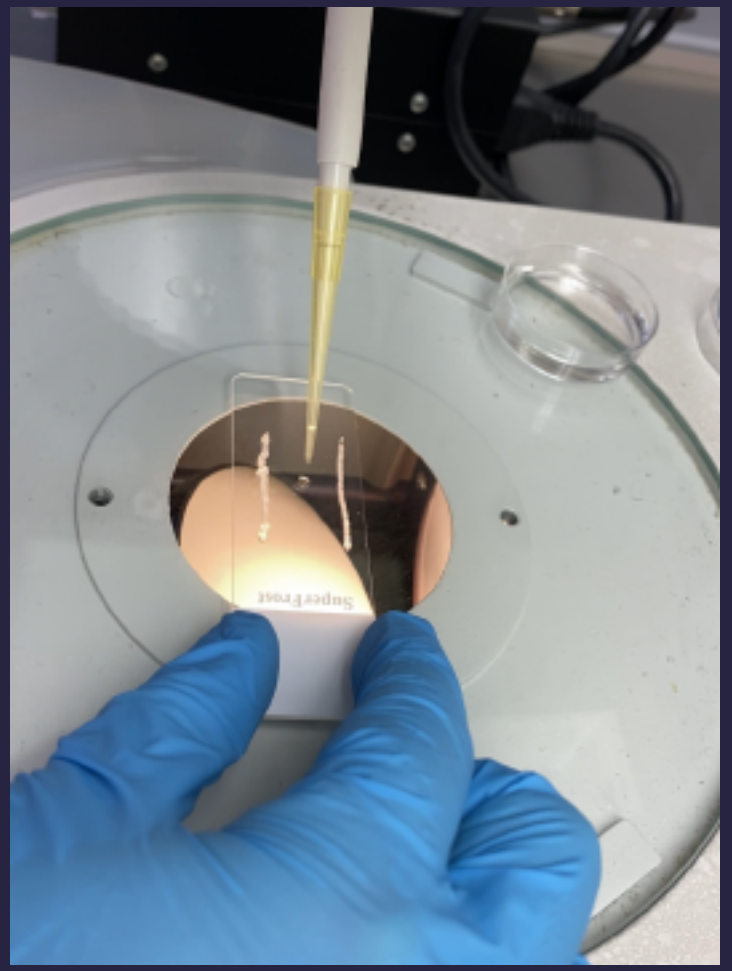
STEPS:

2. Clean the slide: this will facilitate the assembly of the oocytes.

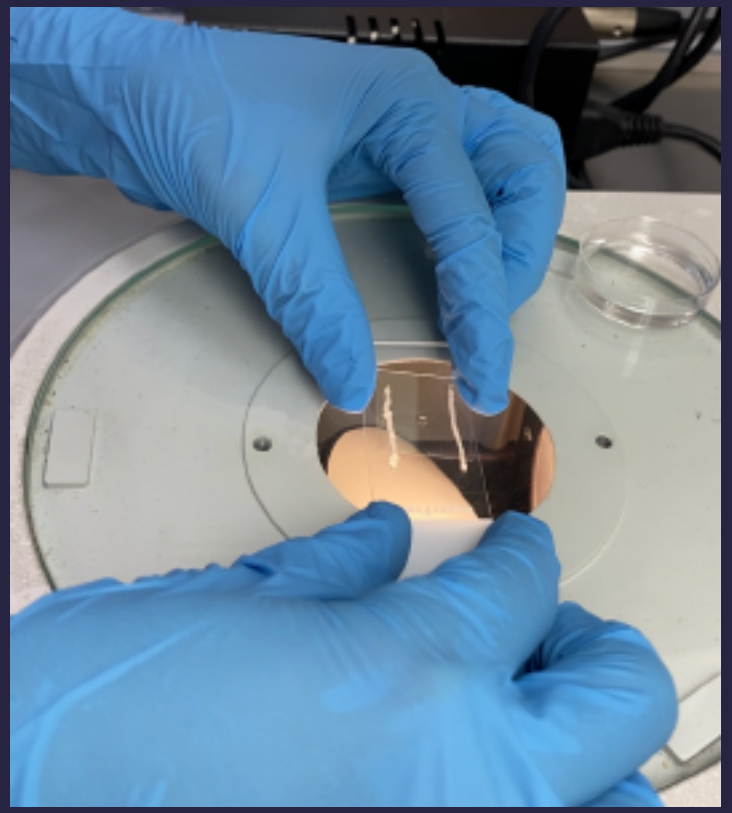


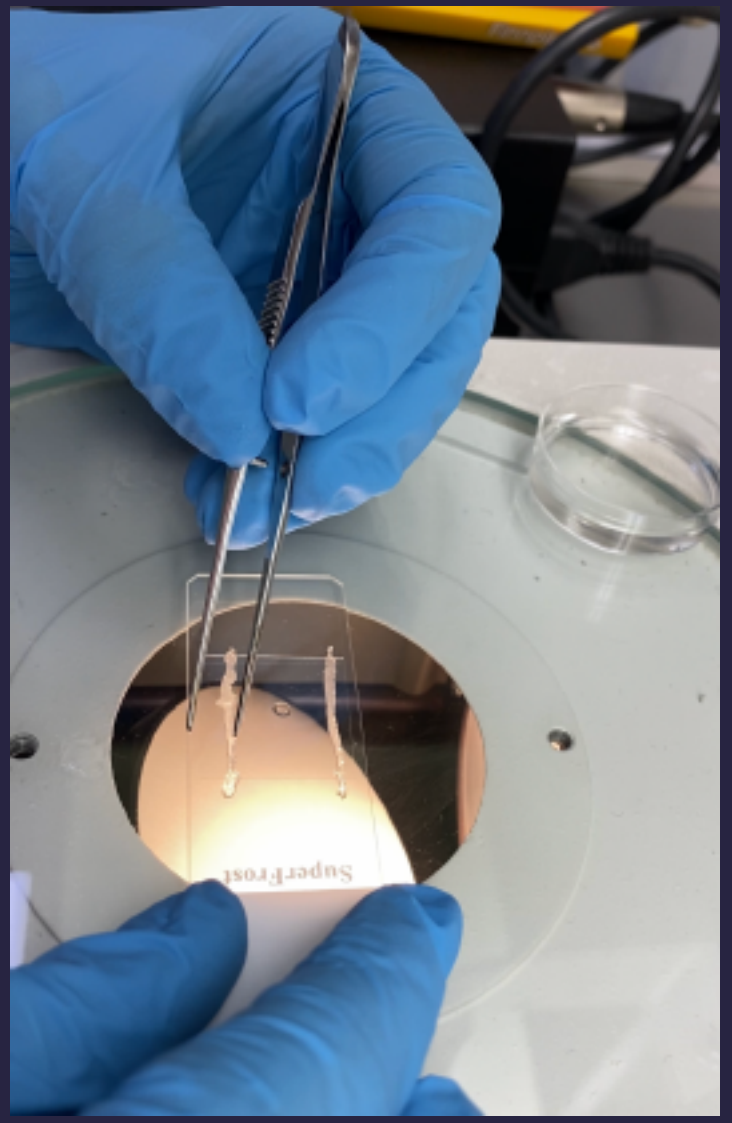
- **STEPS:**
- 3. Put the vaseline jelly on the slide, making two horizontal lines; in this way, the cover slide will not crush the oocytes.



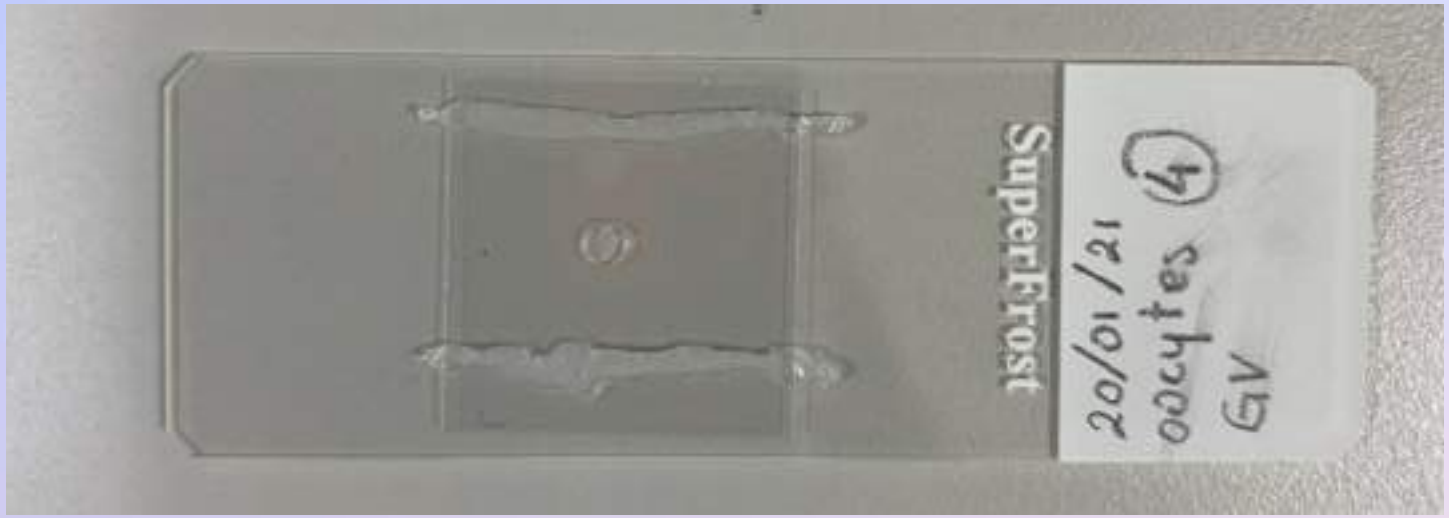


- **STEPS:**
- 4. Take the oocytes with some liquid (in this case, DPBS) and release the drop with the oocytes on the slide, between the two horizontal lines of vaseline.





- **STEPS:**
- 5. Then, place the coverslip on top and press lightly with the help of a forcep. A potential indicator of good oocytes mounting on the slide is the formation of a "bubble"



last, but not least ...
REMEMBER TO MARK THE SLIDE!