**Lab practical session IVM**

*(session 2)*

## Assessment of IVM

The right functional assessment of IVM is fertilization. It is the sole method to evaluate the nuclear and cytoplasmic quality of the IVM process by examining the oocyte's ability to undergo activation and initiate early embryo development post-fertilization.

To this aim ideally the process of IVM should be evaluated through a three steps procedure:

### Assessment of cumulus expansion

This process is initiated by the LH surge in cumulus cells. In response to LH, somatic cells begin to accumulate hyaluronic acid and proteins in the extracellular environment, enlarging the cumulus mass by detaching cells from each other. It's important to note that the corona radiata cells are not physiologically involved in this process.

- Hyaluronic acid production during maturation helps to stabilize the egg and facilitate interaction with spermatozoa.

**M & M**

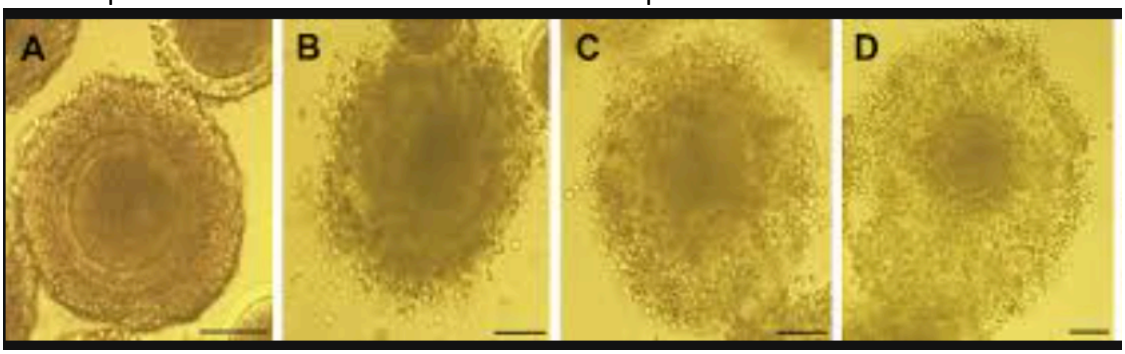
To assess the process of expansion we can use a conventional score:

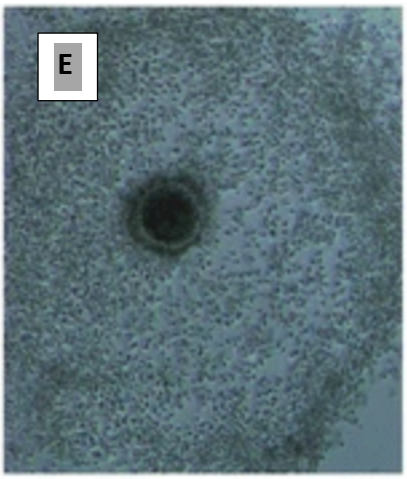
**0** any expansion

**1** the expansion involves exclusively the periphery of COC.

**2** The expansion involves only a part of the COC and several compact layer of cumulus cells persisted surrounding the ZP.

3 the expansion involves all the cumulus cell except corona radiata



A before LH
B-E after LH
B score 0
C score 1
D score 2
E score 3

**QA**
The percentage of MII oocyte have to be similar the incidence of oocytes scored as 2-3

## Nuclear stage evaluation

The IVM must induce the resumption of meiosis up to the Metaphase II stage.
To verify the achievement of this biological target cumulus cells, must be removed through a method defined as “oocyte denudation”.

**M &M**

The process of matured oocyte denudation takes advantage.

1) enzymatic digestion of expanded cumulus with hyaluronidase
2) Mechanical removal of cumulus cells still attached to the ZP.

Then the oocyte can be checked:

1) Under a stereomicroscope. It could be visible the first polar body (This is not easy to perform in our animal model as the first polar body is a very small structure).
2) With an optical or fluorescence microscope to visualize the chromatin. In the first case the chromatin needs to be stained with a visible spectrum dye, conversely in the second case the stainig should be performed with a fluorescent dye on a fixed and permeabilized oocyte*.

**Quality assessment**

The percentage of MII oocyte have to be close to percentage of fetilized oocyte after IVF

*The configuration of chromatin can be studied using specific DNA-sensitive dyes that can be visualized through either an optical or fluorescence approach.

**LACMOID STAINING (visible spectrum)**

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 nuclei.
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.

**DAPI or HOECHST or PROPIDIUM STAINING (fluorescent spectrum)**

So, to have information about chromatin visualization fluorescent dyes are preferred.
With this staining, the chromatin can be easily visualized, displaying a widespread distribution with small foci of condensation.

## IVF

The M & M of IVF will be discussed and performed under the supervision of Prof. Gioia.