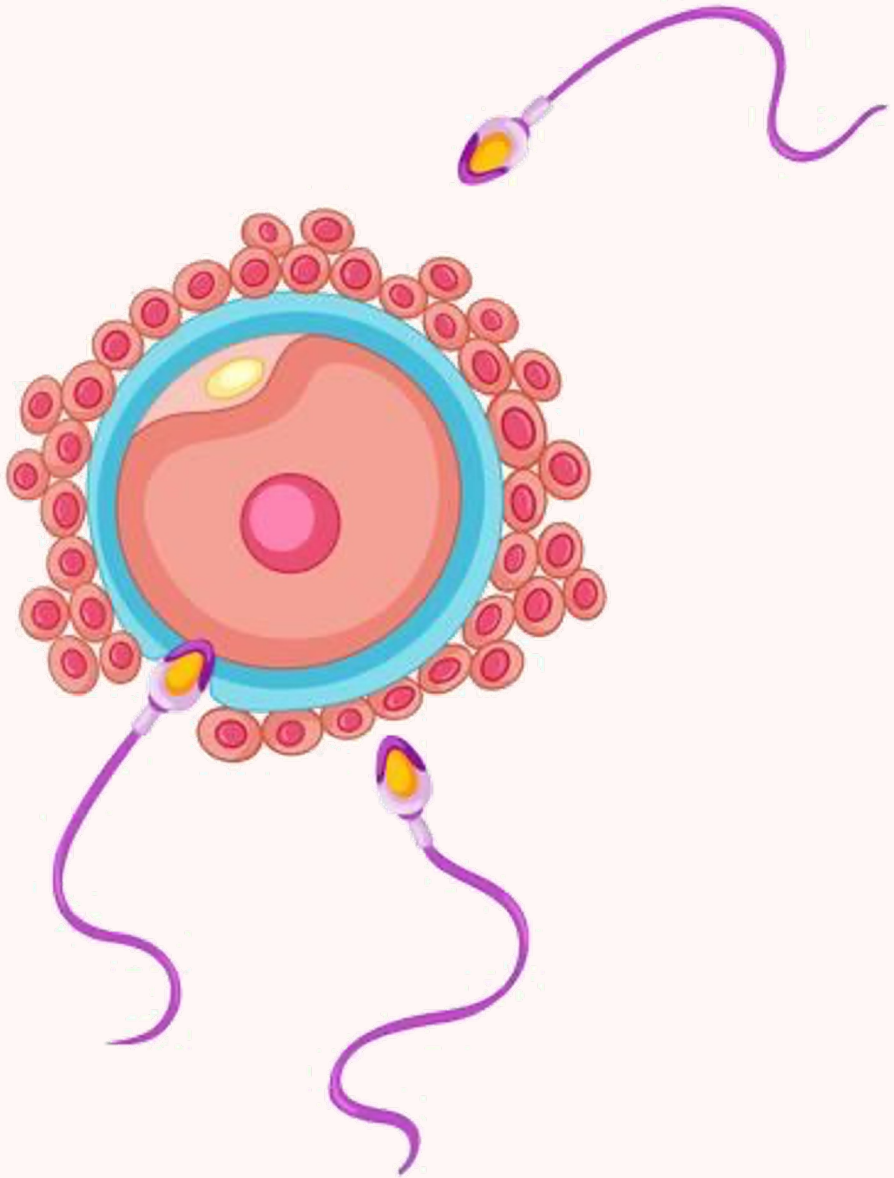
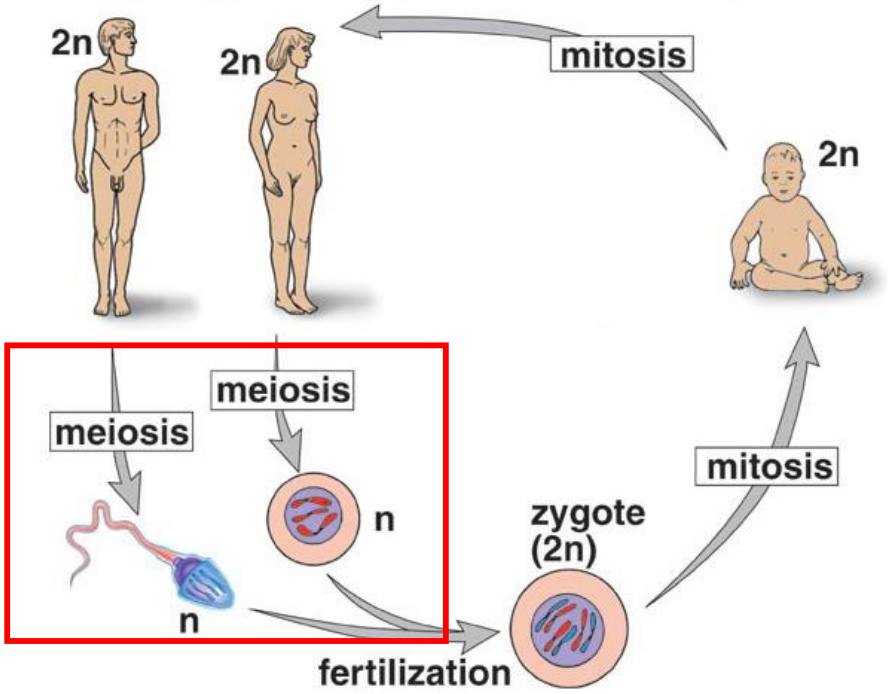
Meiosis and Fertilization



The Fertilization

The Fertilization is a complex process in which there is:

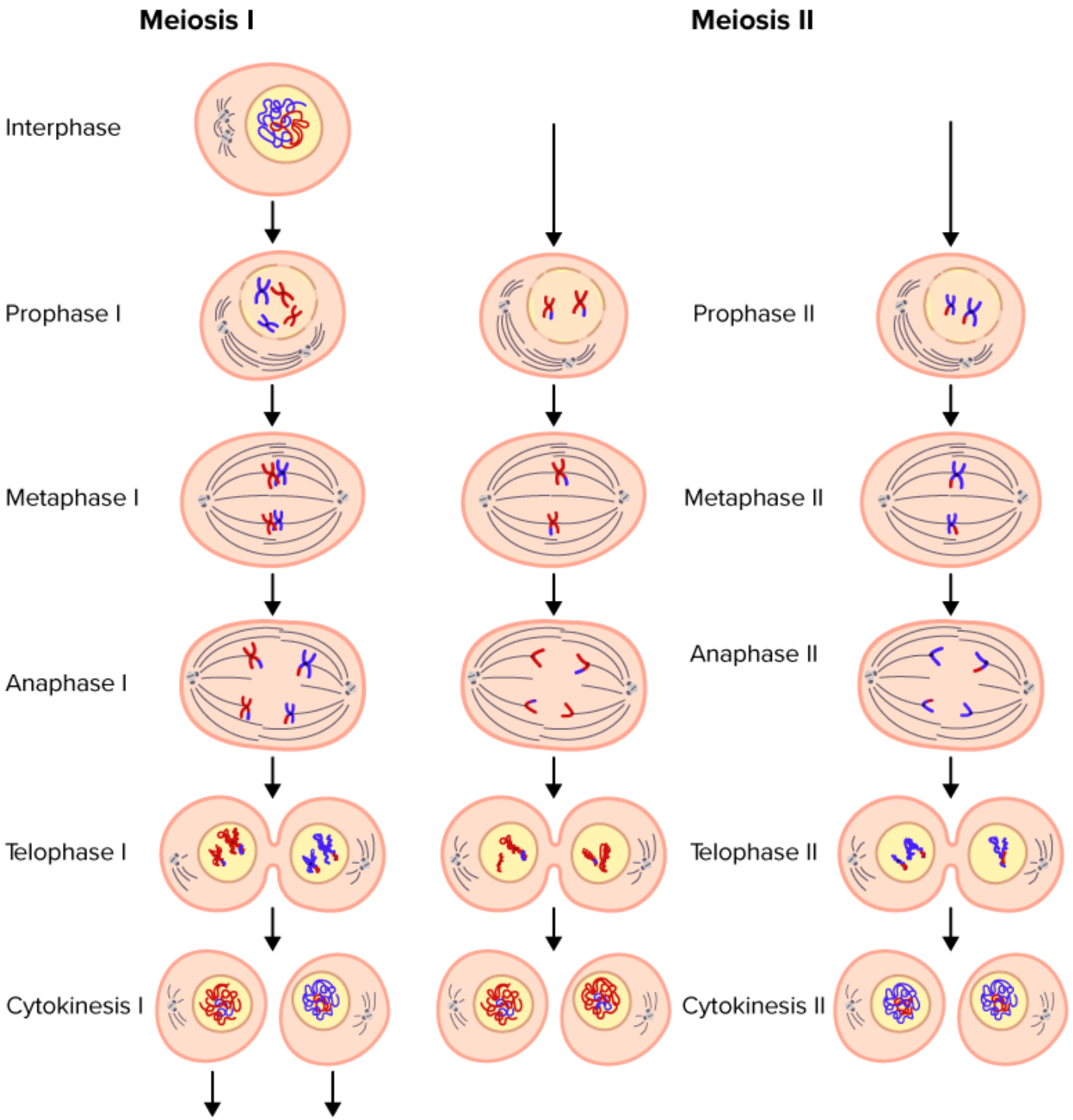
- the union between 2 gametes
- reset of diploid cromosomes
- formation of a new organism

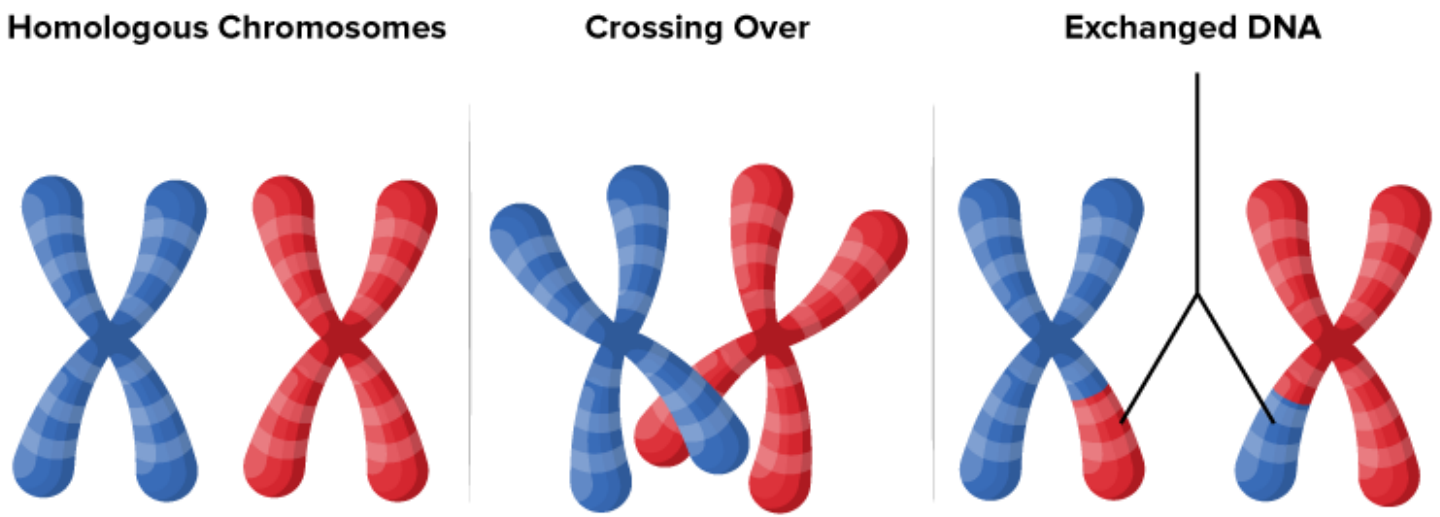


Meiosis

The Meiotic cell division occurs in the germ cells

During this division the genetic material of a diploid germ cell undergoes in 2 nuclear division obtaining 4 haploid daughter cells

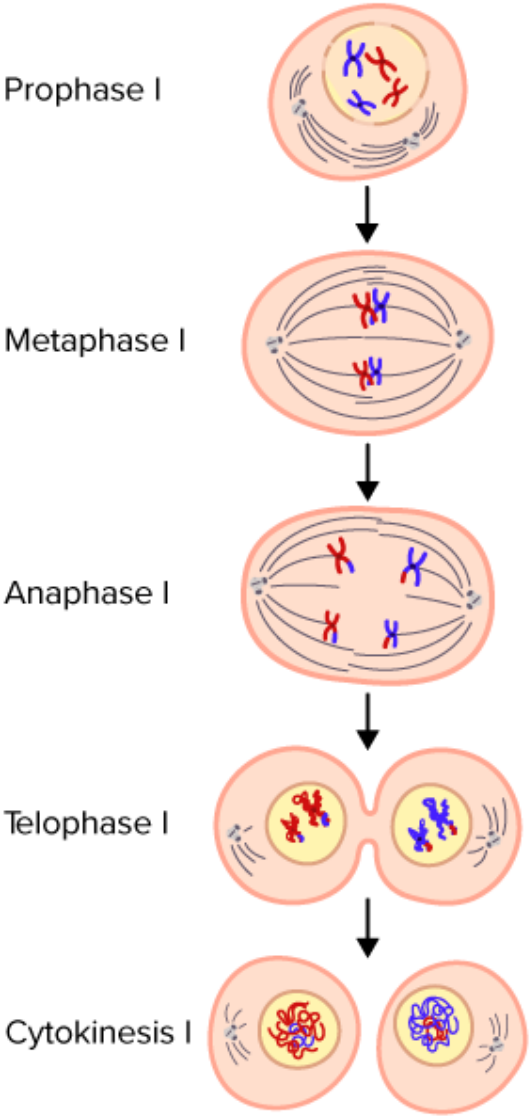

Meiosis I
Meiosis II
Interphase
Prophase I
Metaphase I
Anaphase I
Telophase I
Cytokinesis I
Prophase II
Metaphase II
Anaphase II
Telophase II
Cytokinesis II



Unique characteristic of Meiotic Division

Meiosis I

- **Prophase I:** Nuclear envelope breaks down, chromosomes condense, homologous chromosomes pair up, and crossing over occurs.
- **Metaphase I:** Paired homologous chromosomes align at the cell equator and spindle fibers attach.
- **Anaphase I:** Homologous chromosomes separate and move to opposite poles.
- **Telophase I + cytokinesis:** Nuclear membranes reform, the cell divides, and two haploid cells are produced. No DNA replication occurs before meiosis II.



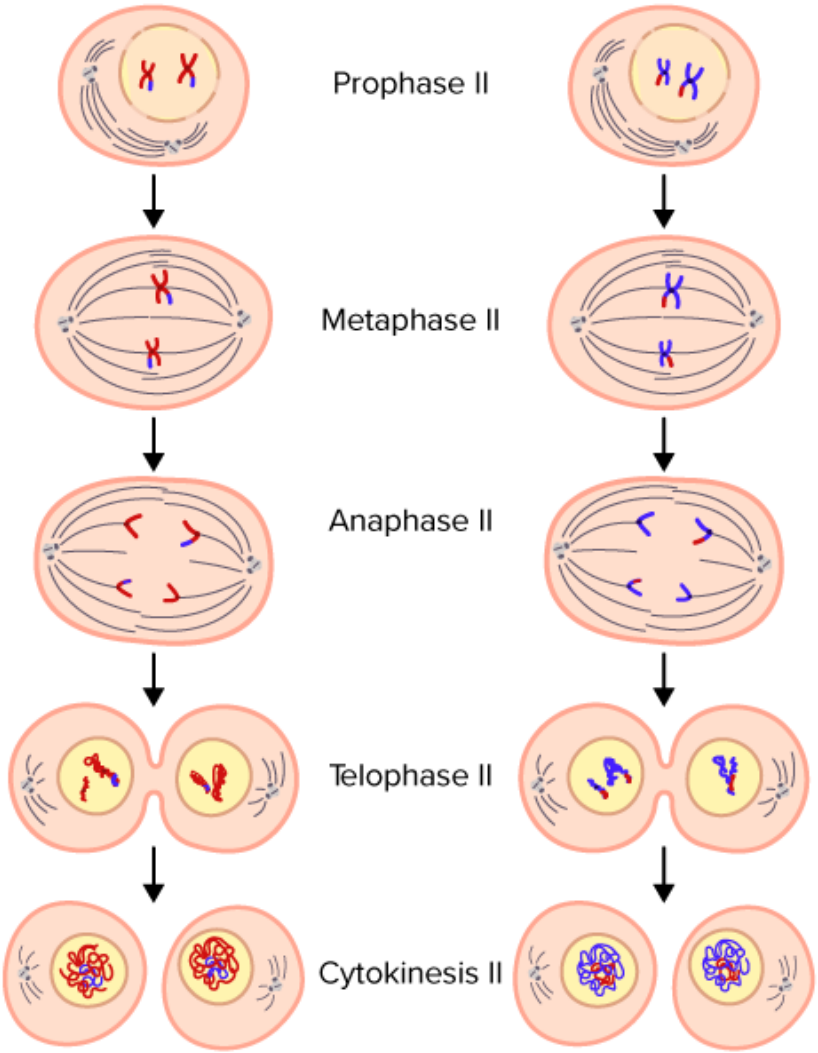
Meiosis II

Prophase II: Nuclear envelope breaks down and the spindle forms in each haploid cell.

Metaphase II: Single sister chromatids align at the equator.

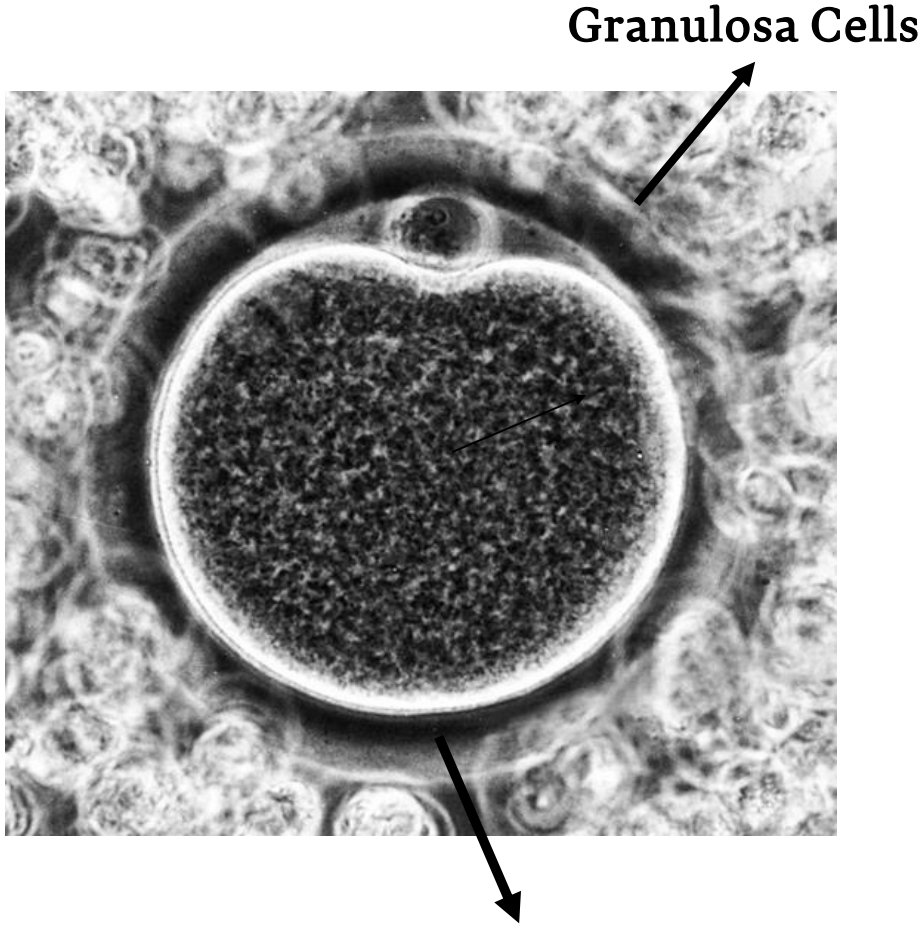
Anaphase II: Sister chromatids separate and move to opposite poles.

Telophase II + cytokinesis: Nuclear membranes reform, cells divide, and four unique haploid cells are produced.

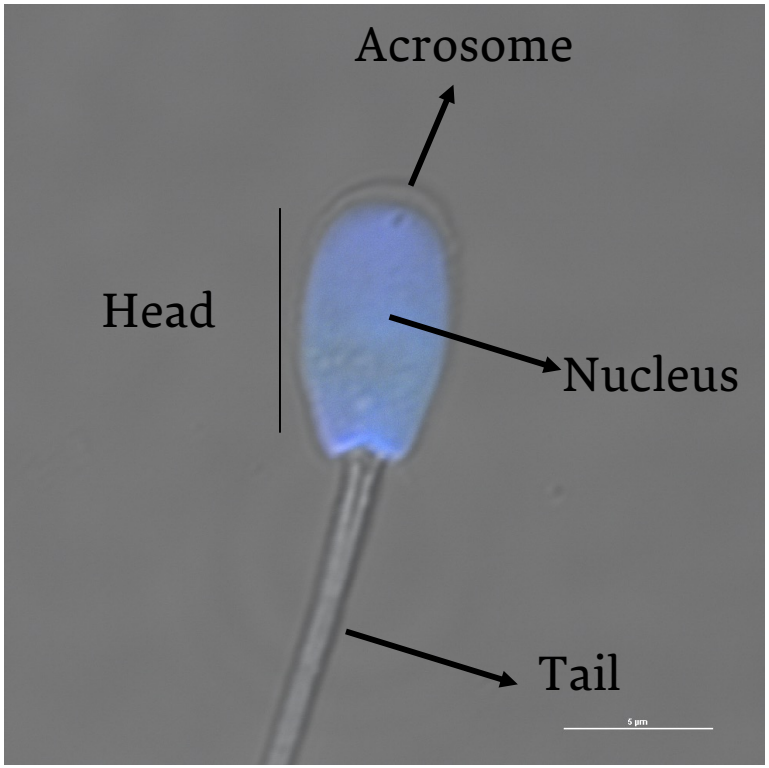


Gametes

Oocyte



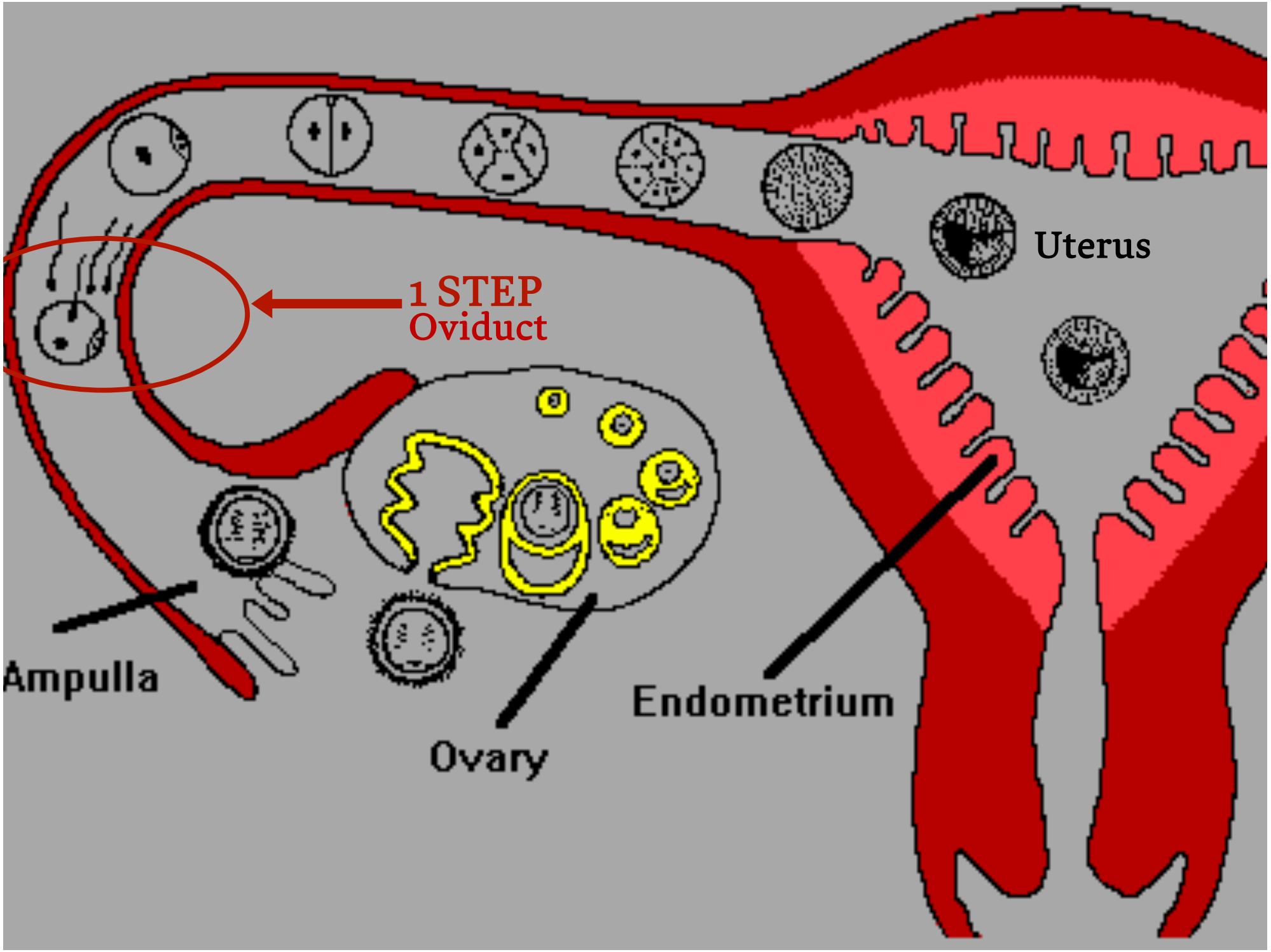
Spermatozoo



FERTILIZATION

3 Steps:

1. Gametes meeting
2. Spermatozoa penetration in oocytes membrane
3. Syngamy (fusion of genetic material)


1 STEP
Oviduct
Uterus
Ampulla
Ovary
Endometrium

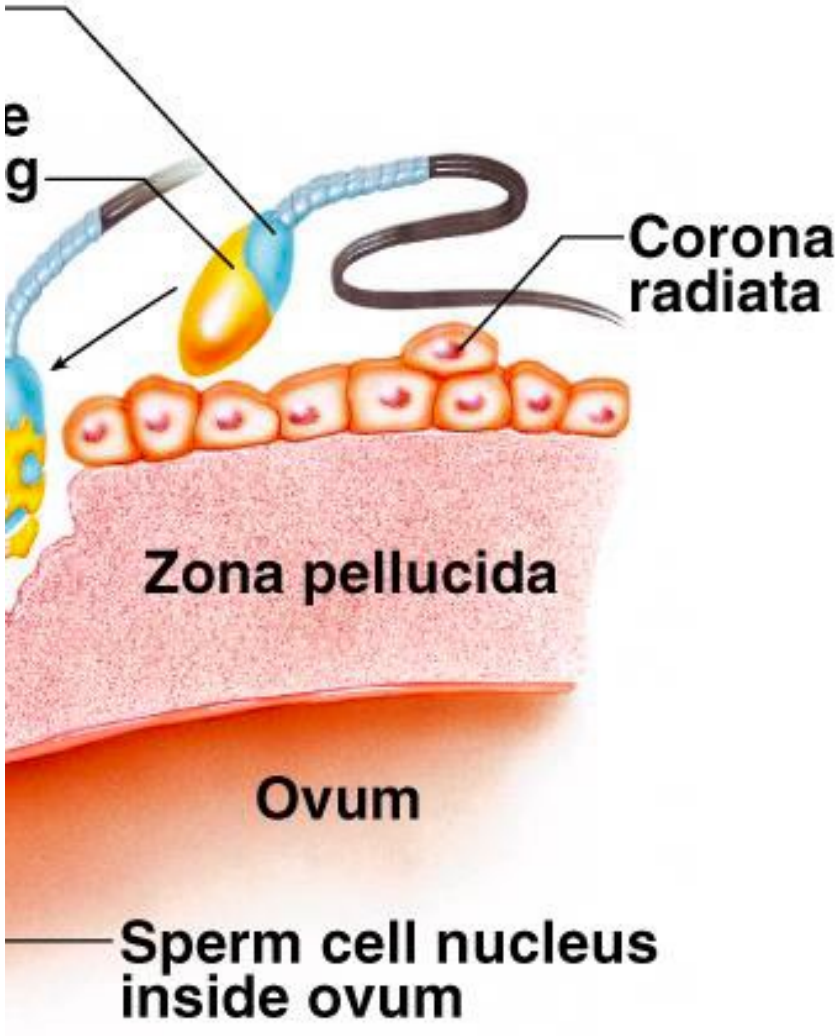
CAPACITATION

Ability of the Spermatozoa to fertilize the oocyte after a period in female genital track in which there's the production of Bicharbonate Ions (HCO_3^-) that activate cAMP Pathway.

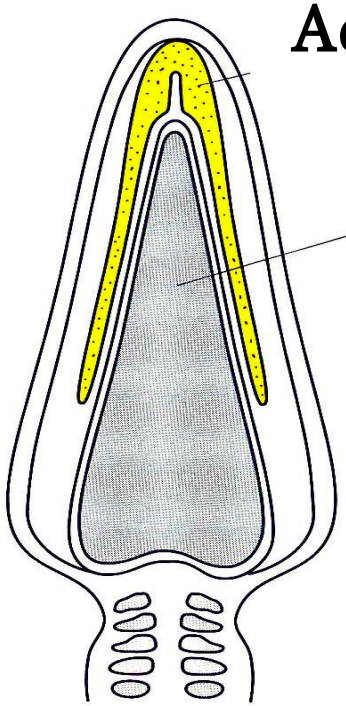
- Removal of Glycoprotein (cover protection of the spermatozoa) by the female enzymes

- Modifying the Acrosome that become more fluid by losing of the Cholesterole

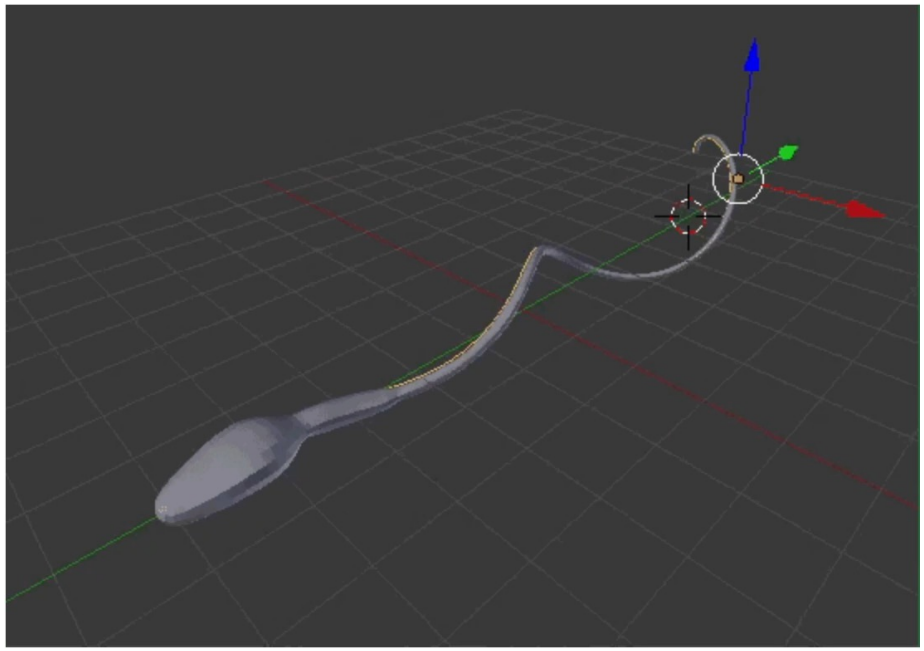
THE AIM OF THE CAPACITATION IS THE PENETRATE THE OOCYTES MEMBRANE



1. **ACROSOMAL REACTION:** modification of Acrosome to penetrate in the oocyte membrane



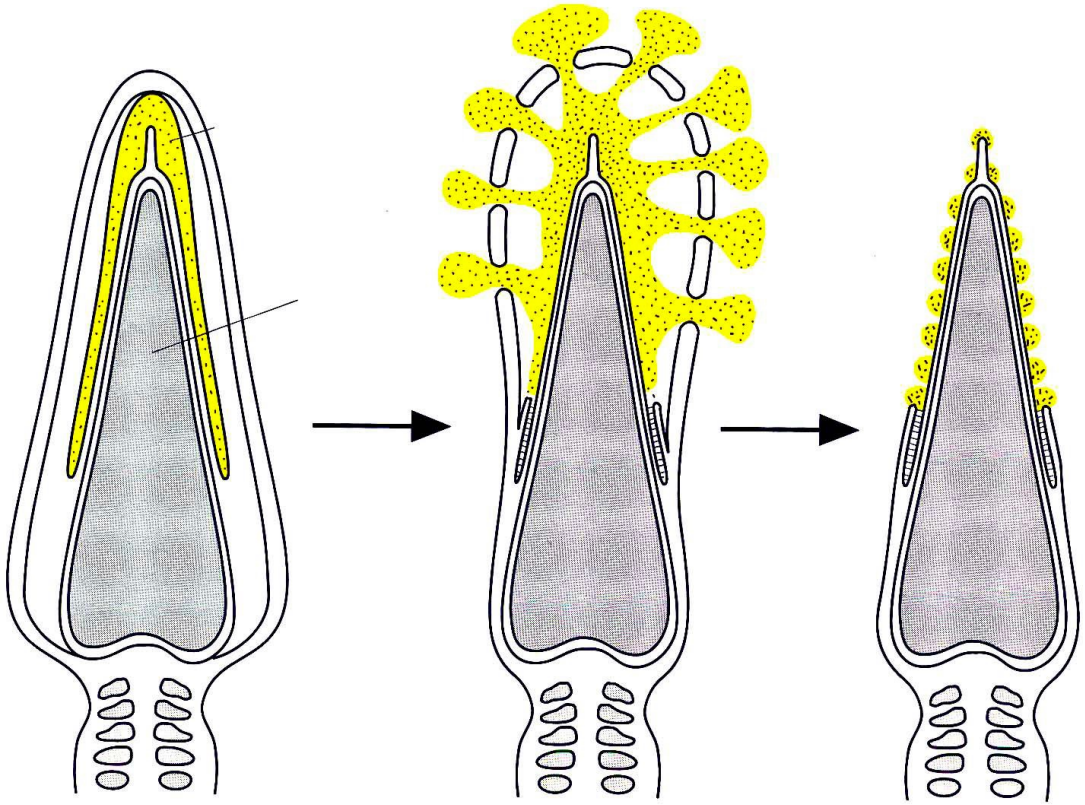
2. **TAIL MOVEMENT** as Whiplash



PENETRATION
OOCYTE MEMBRANE

When the Spermatozoa is near the cumulus cells the ACROSOMAL REACTION starts, releasing:

- **HYALURONIDASE:** hydrolyzing the Hyaluronic Acid that make thight juction within the follicular cells
- **ENZYME:** to penetrate the membrane that disrupt the cell junctions



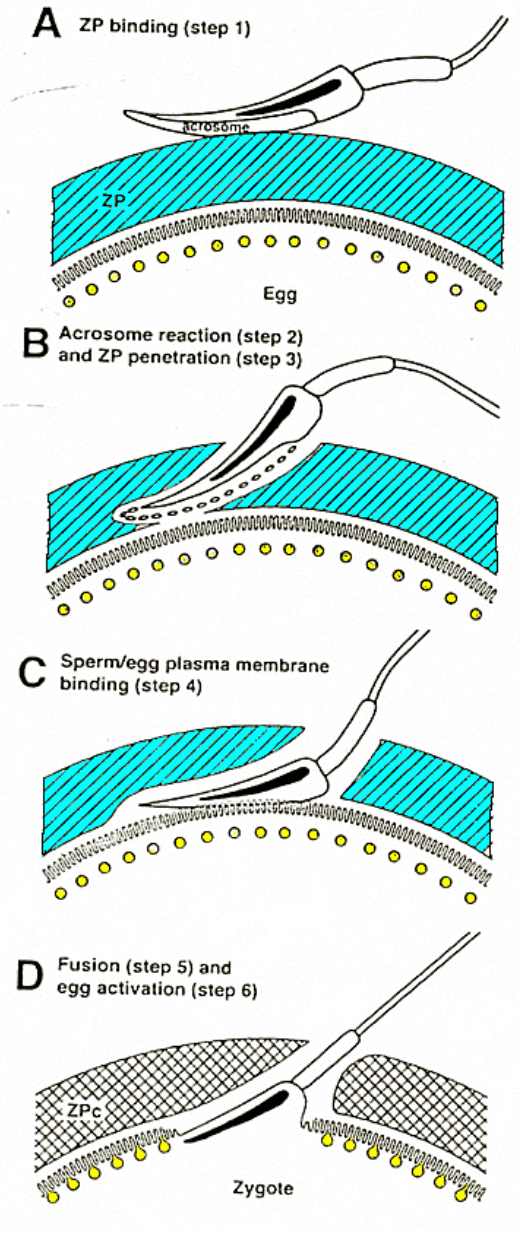
ACROSOME blows, its membrane fuses with the plasmatic membrane releasing lyses enzymes

ZONA PELLUCIDA (glycoproteines)

BINDING: between spermatozoa and Specific Zona Pellucida Receptors (ZP3, bindind species-specific)

ACROSOMAL REACTION: release of Hyaluronidase, Acrosin and glycosidase that for a channel for the spematozoo that penetrate with whiplash movement of the tail

FUSION of Plasmatic Membranes and formation of a fertilization channel



OOCITE REACTION

OOCYTE ACTIVATION is the reaction to the spermatozoo penetration

The fertilized oocyte activate the «POLISPERMIA BLOCK» through:

- a rapid plasmatic membrane DEPOLARIZATION (from −70 to +20 mV ca.)

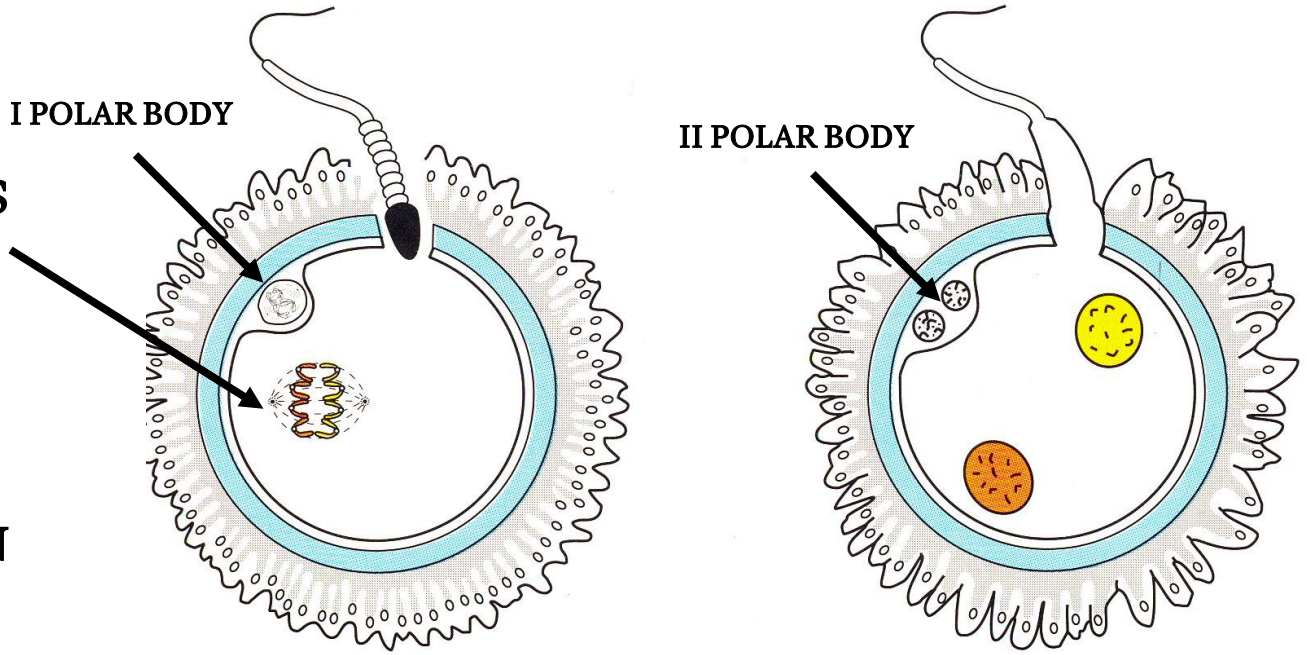
- CORTICAL and ZONAL REACTION in which the zona pellucida become refractory to spermatozoa penetration.

CROMOSOMES FUSION (SYNGAMY)

COMPLETE MEIOSIS

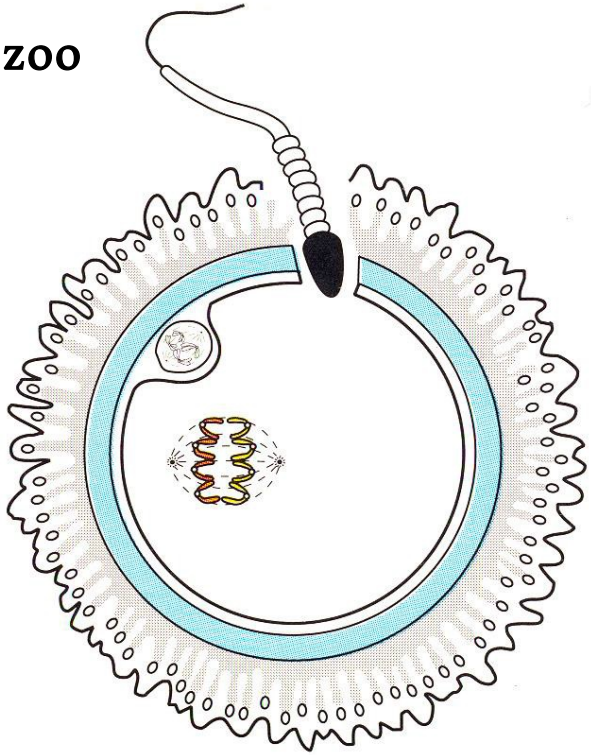
CHROMOSOMES FUSION

I MITOTIC DIVISION (ZYGOTE)

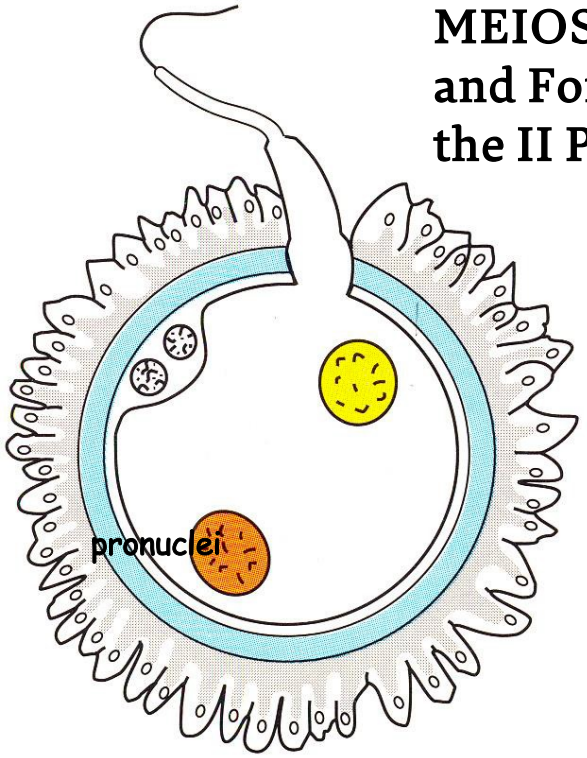


- Spermatozoo enters in the Oocyte -> Chromosomes decondensation and the protamines replaced by Maternal Hystones
- Complete the Meiosis in 4hs Post Fertilization and II Polar Body formation
- Male and Female Chromosomes are surronded by Nuclear Membranes -> Formation of 2 PRONUCLEI

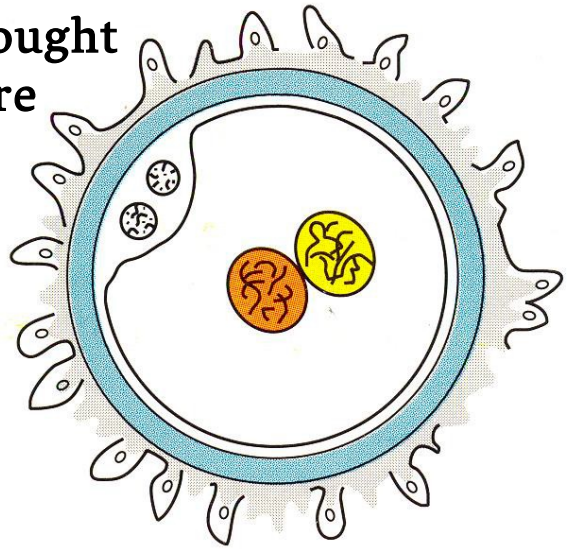
Spermatozoo enters



MEIOSI completed and Formation of the II Polar body



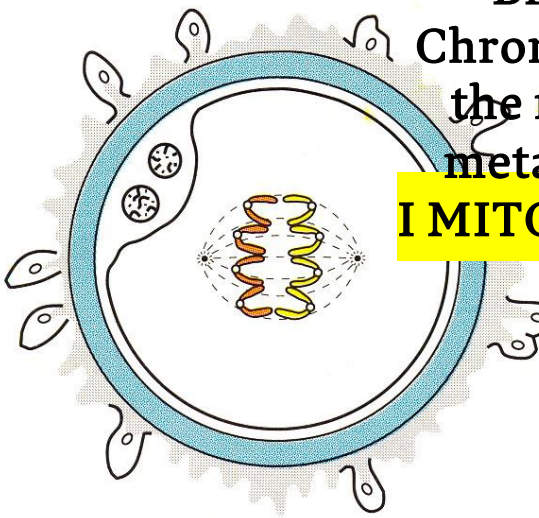
2 PRONUCLEI migrate throught the centre



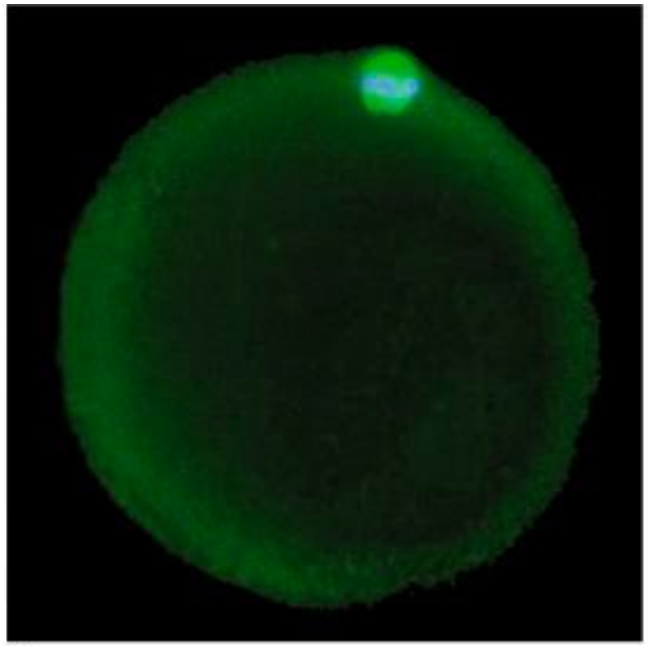
Pronuclei Membranes Break and the Chromosomes are in the middle on the metaphasic plate :

I MITOTIC DIVISION

24-30 hpf

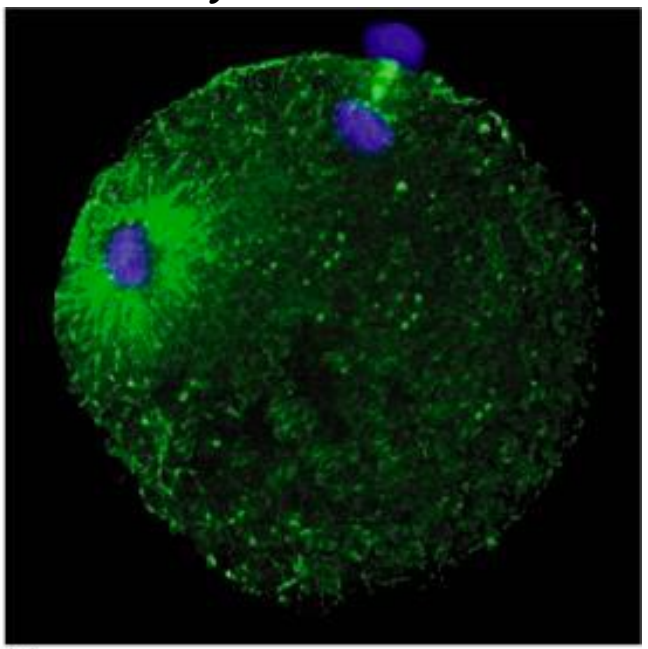


Metaphase II



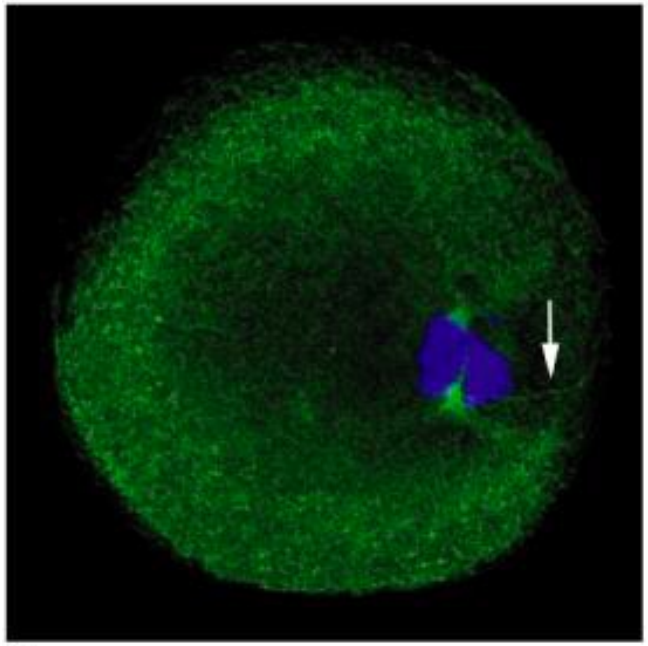
(A)

Oocite fertilized and II Polar Body Formation

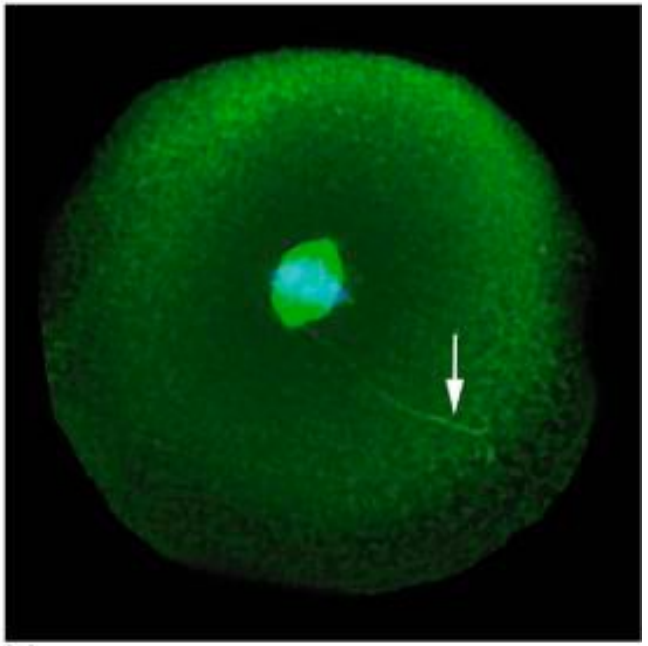


(B)

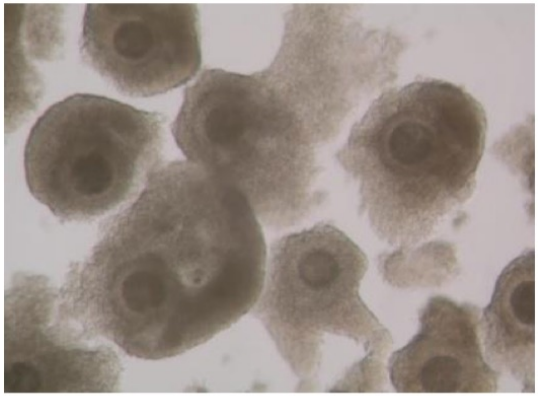
Pronuclei Fusion



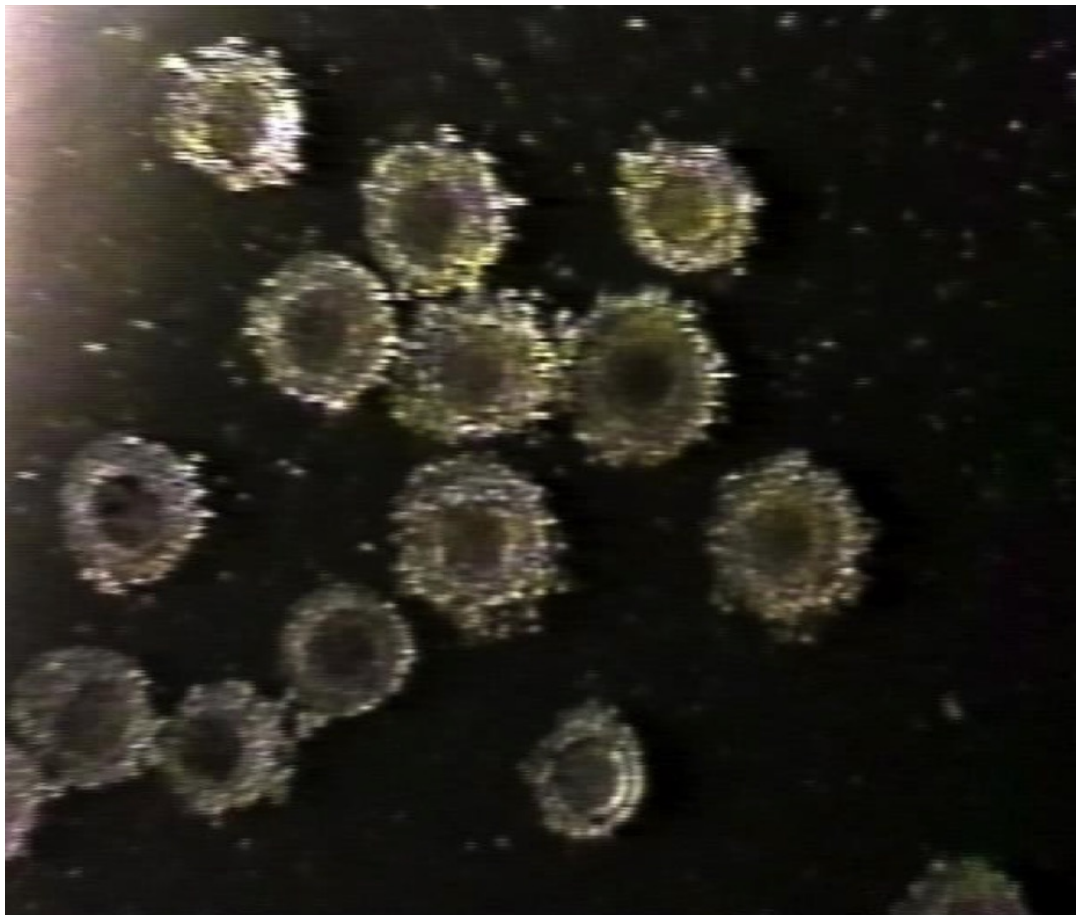
I MITOSIS



IVF



+



To fertilize oocytes *in vitro* we need:

Oocytes in MII stage (matured)

Seme (frozen)

Medium for oocytes

- Wash medium (H199 + BSA)
- Hyaluronidase (to remove cumulus cells)

Medium for sperm

- Sperm wash (to wash sperm from freezing medium)

Medium for IVF

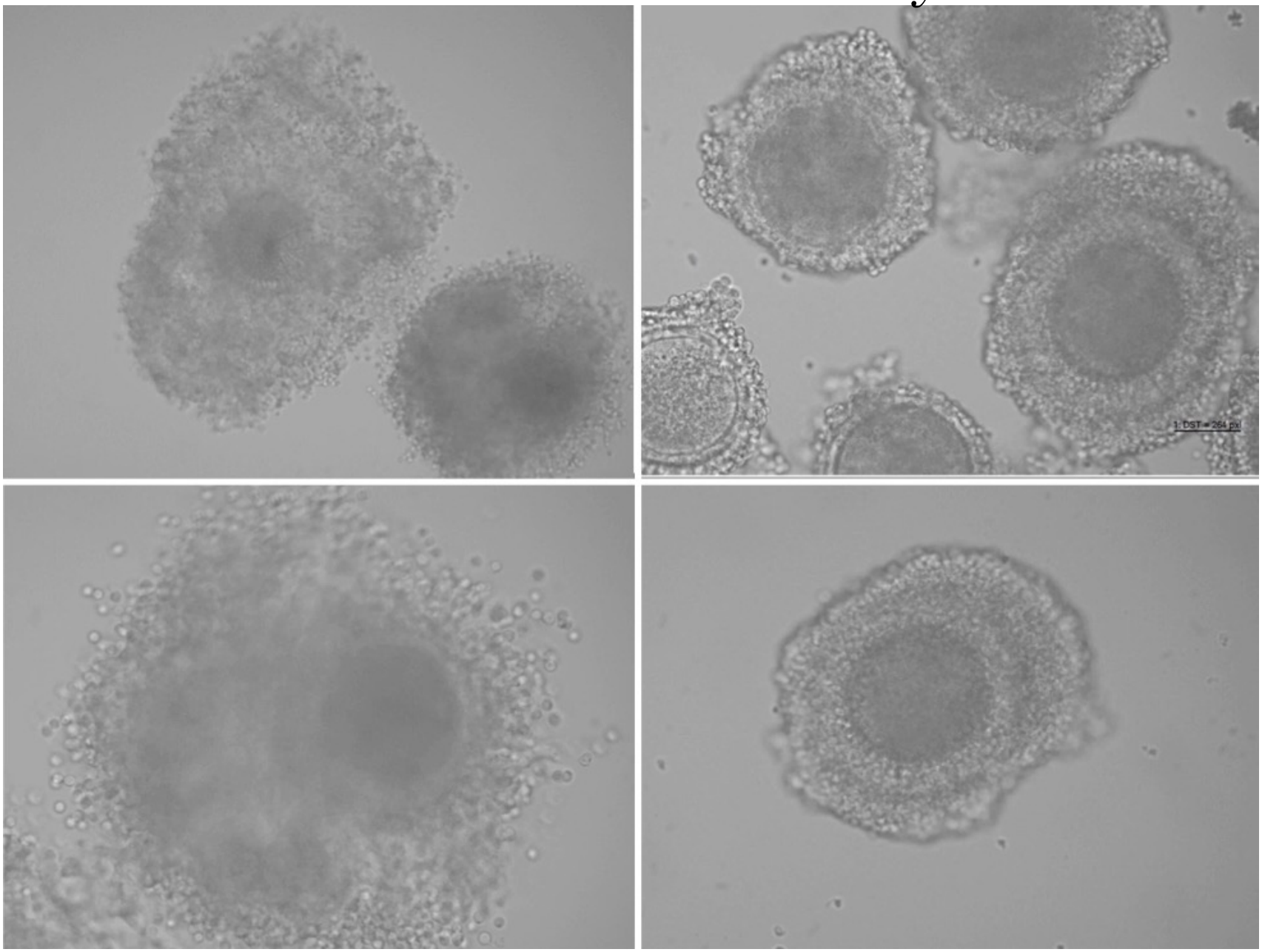
- IVF medium

Medium and dish for embryo culture (SOF-BSA)

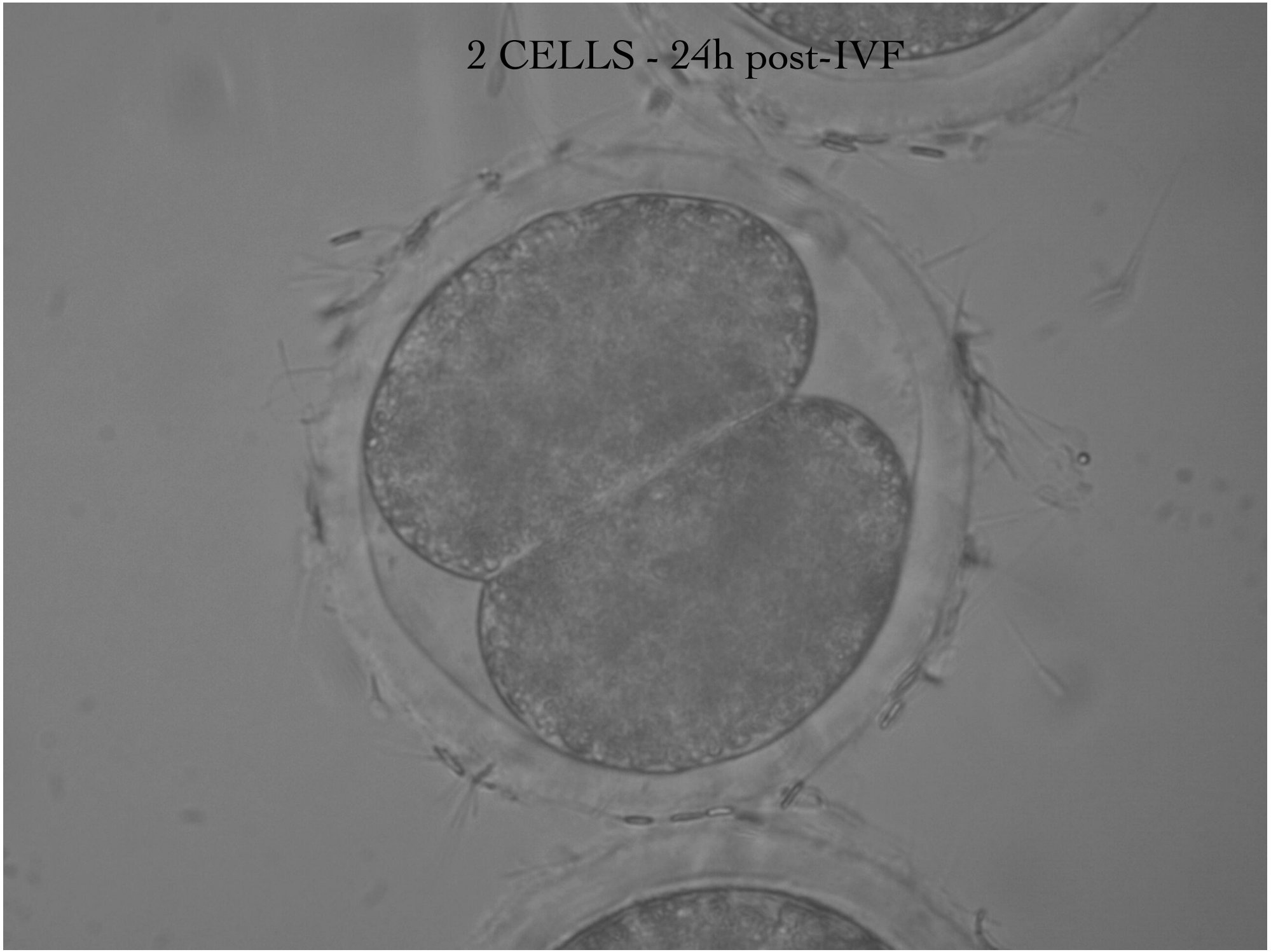
Capilari a bocca

After maturation

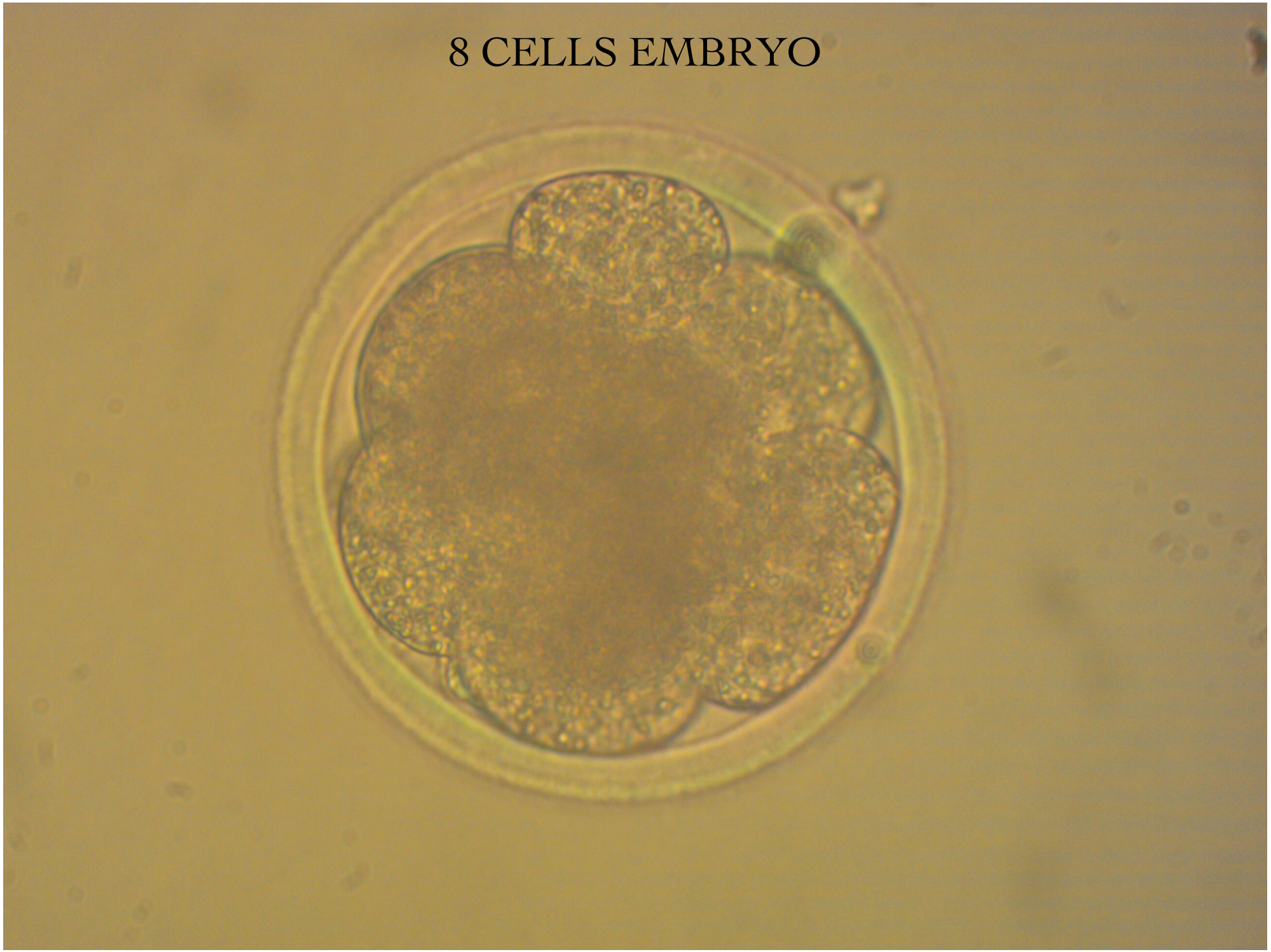
Ready for IVF



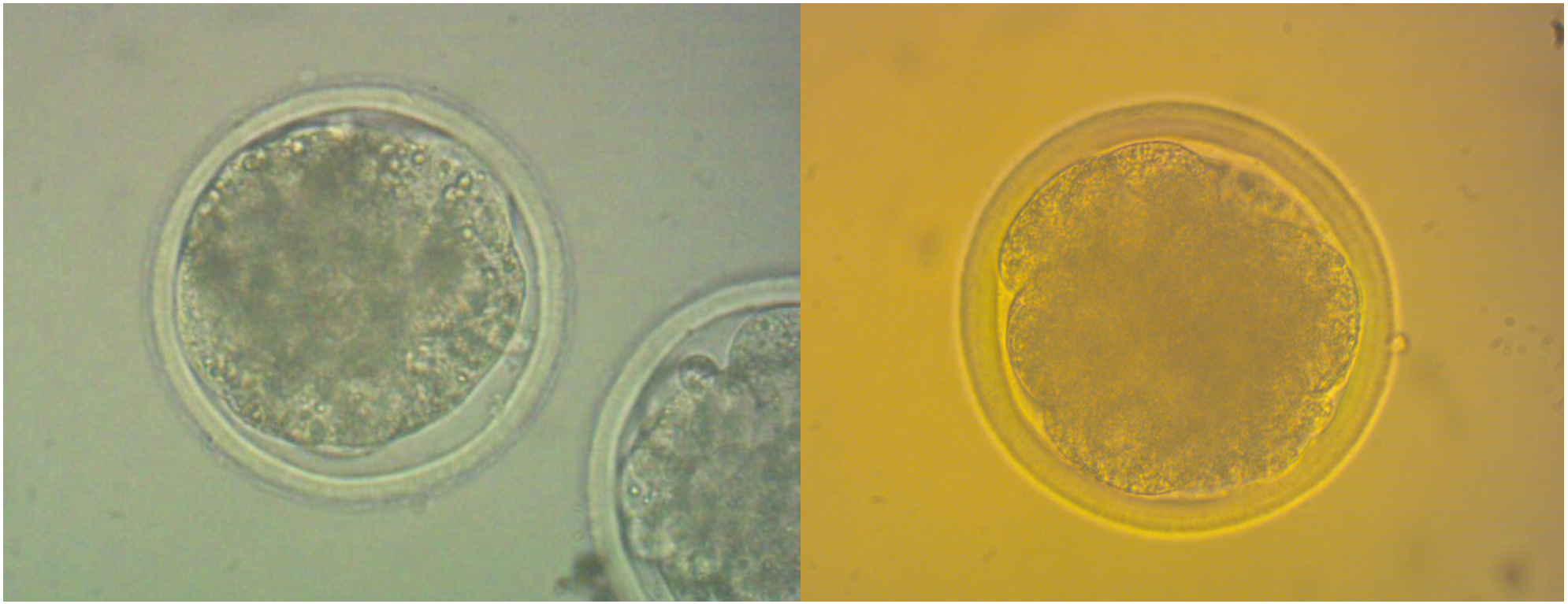

ZYGOTE - 10 ore post-IVF


2 CELLS - 24h post-IVF

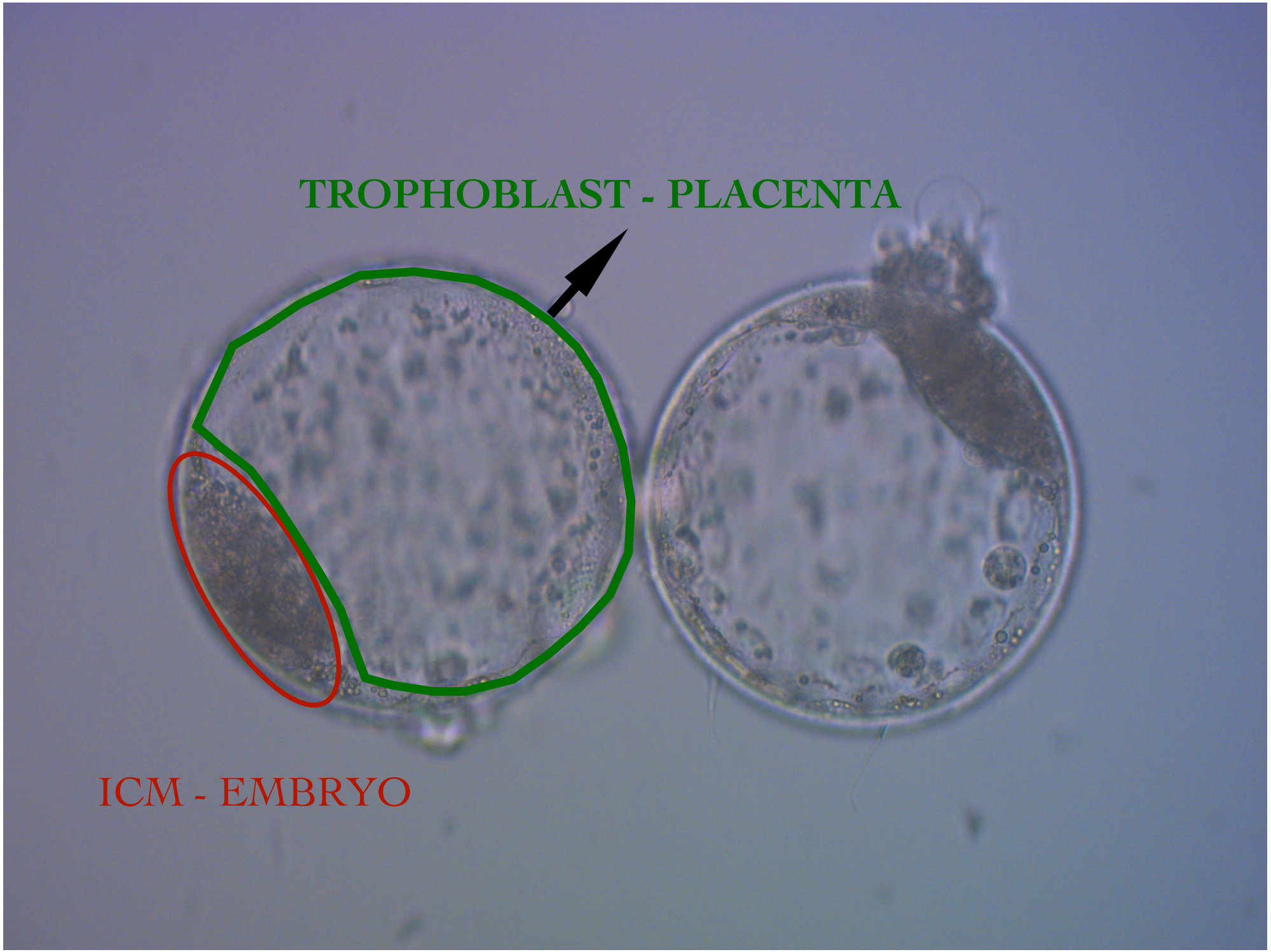
8 CELLS EMBRYO



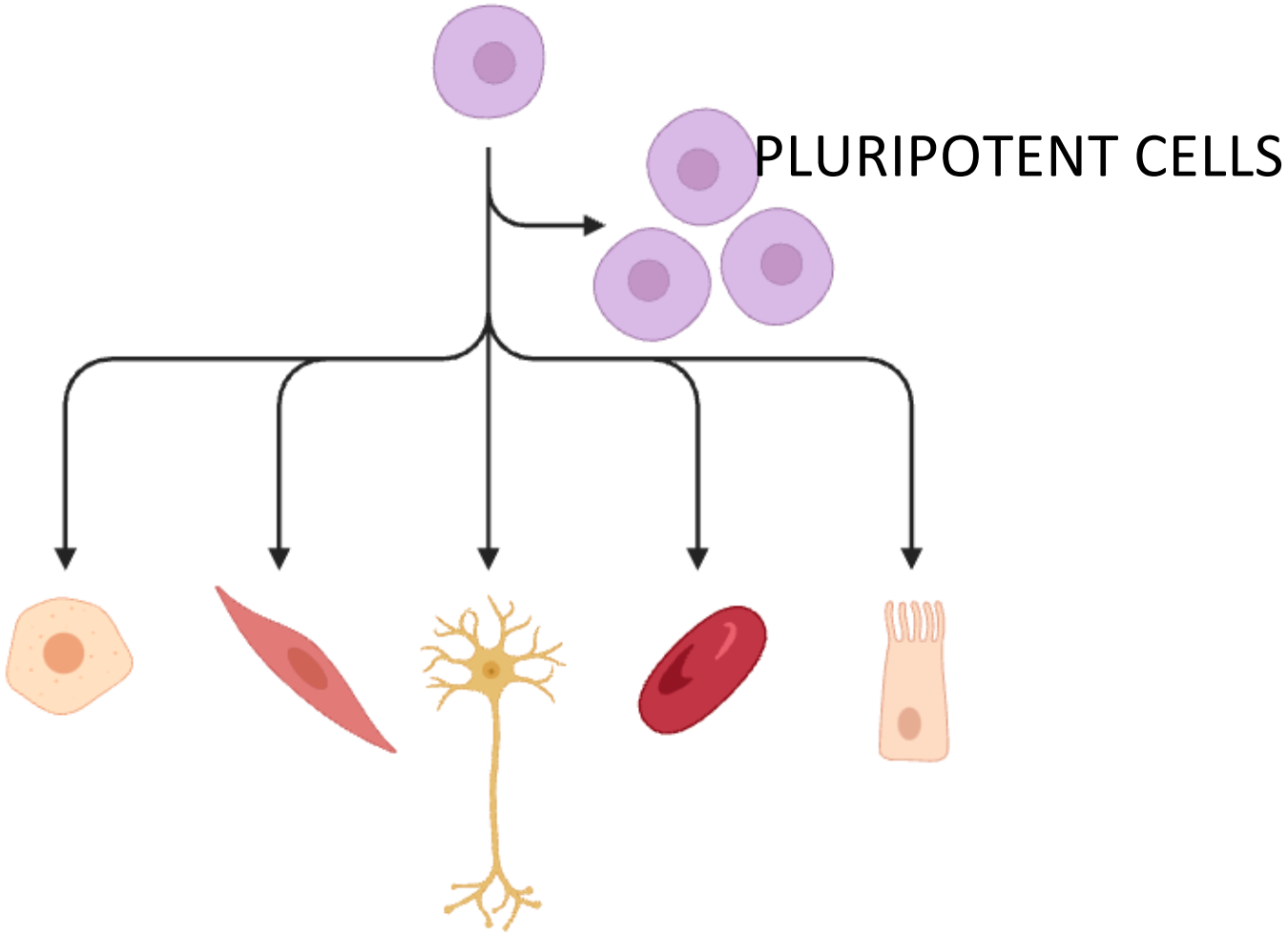
EARLY COMPACTION



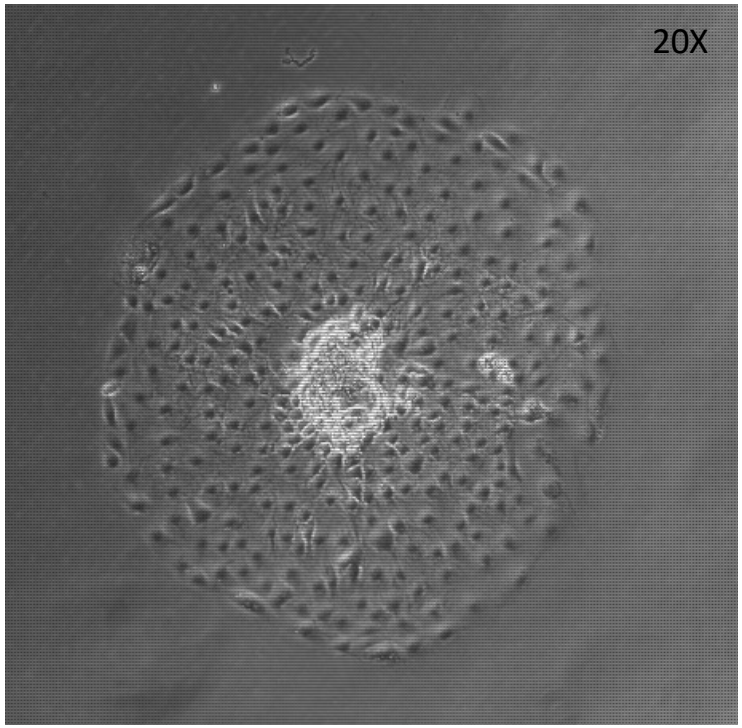
BLASTOCYST


TROPHOBLAST - PLACENTA
ICM - EMBRYO

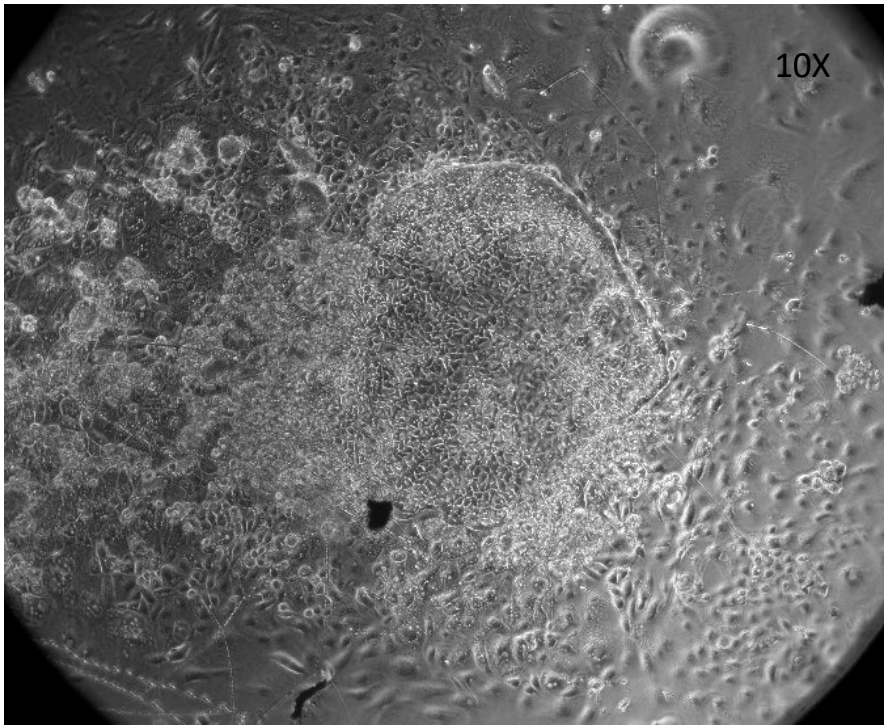
EMBRYONIC STEM CELLS



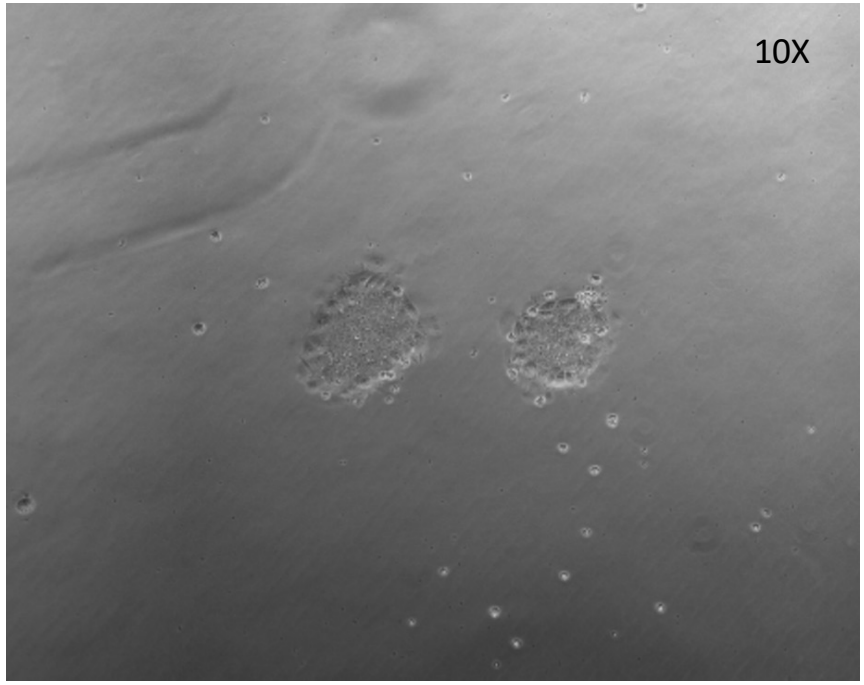
IMMUNOSURGERY



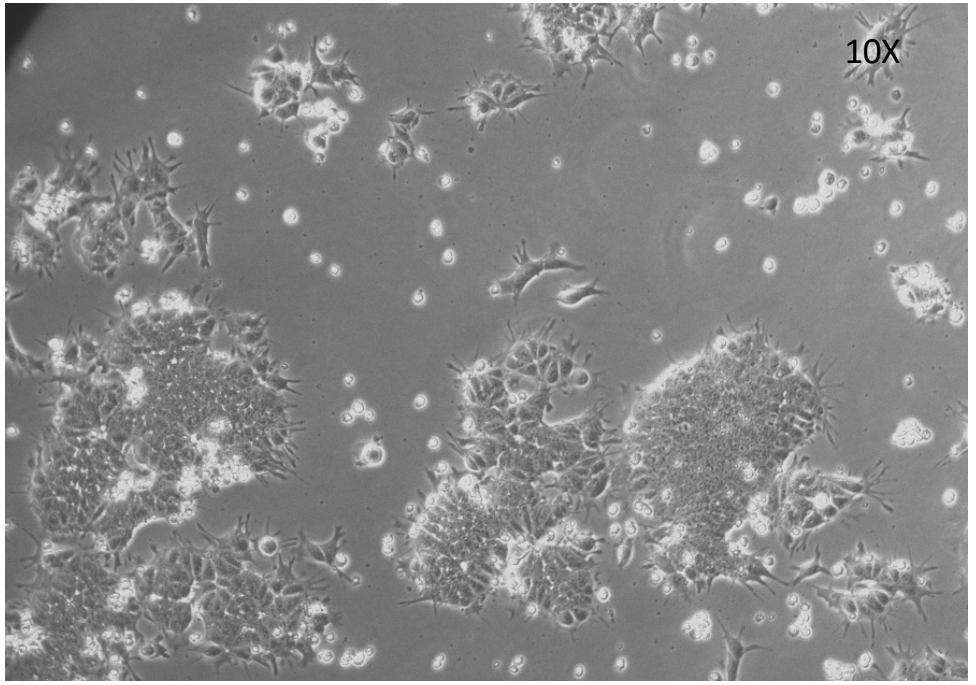
DAY2



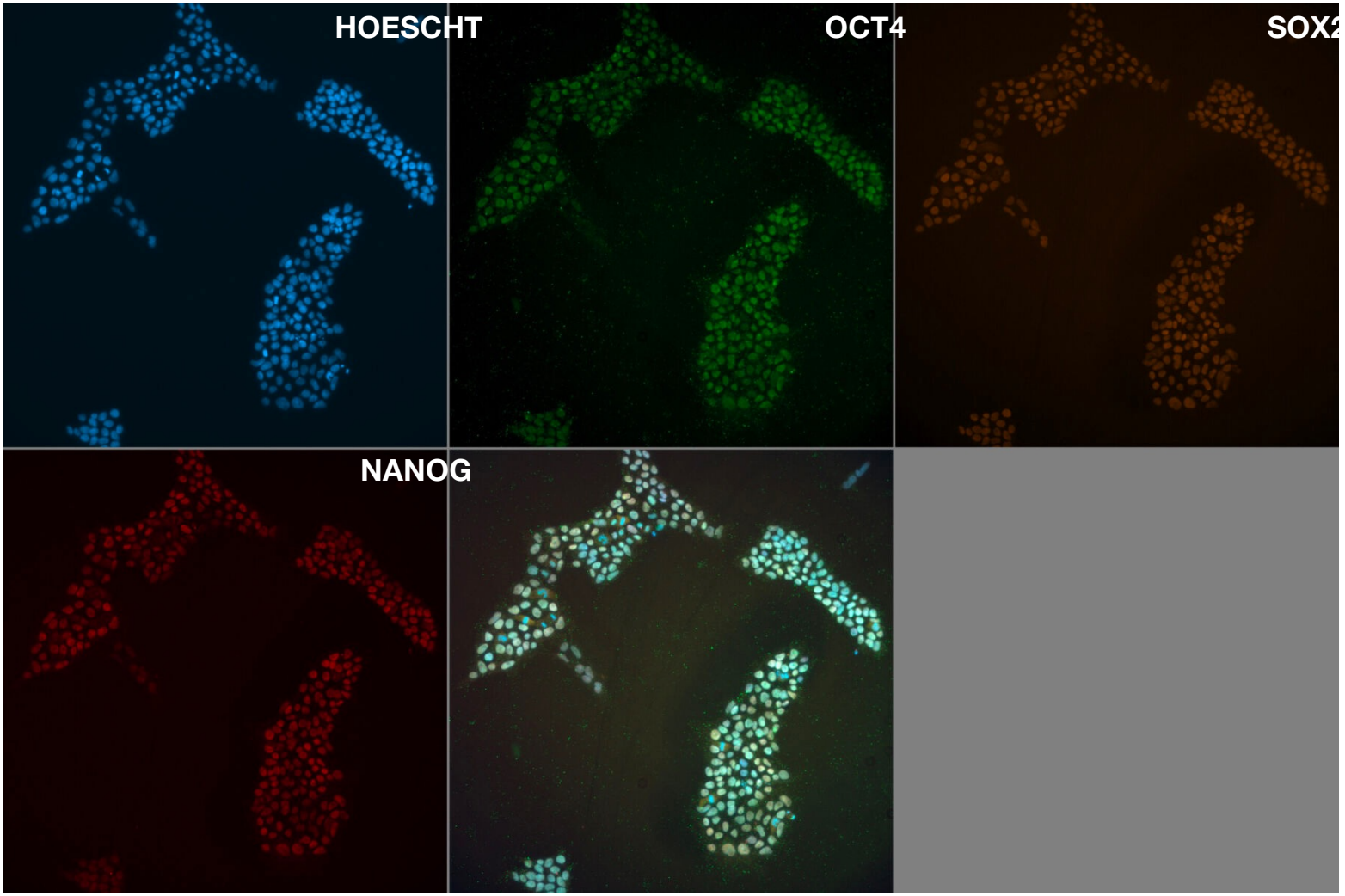
DAY8



DAY16



DAY21


HOESCHT
OCT4
SOX2
NANOG