«ICSI Procedure and advanced techniques in medically-assisted procreation»

Second-Cycle Degree Course in "REPRODUCTIVE BIOTECHNOLOGIES"

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MAIN TOPICS

- **ASSISTED REPRODUCTIVE TECHNIQUES**

•The oocyte retrieval (*Pick-Up*);
•*In-vitro* insemination;
•Intracytoplasmatic Sperm Injection (*ICSI*);
•Assisted zona hatching;
•Blastocyst biopsy;
•Pre-implantation genetic diagnosis and embryo screening;
•Vitrification of oocytes and embryos.

THEROICAL LESSON

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FOLLICOLAR GROWTH MONITORING → **INTRA-UTERINE INSEMINATION (IUI)**

OOCYTE RETRIEVAL

OOCYTES VITRIFICATION

SURGICAL SPERM RETRIEVAL *(EVENTUALLY CRYOPRESERVATION)*

IVF-ICSI

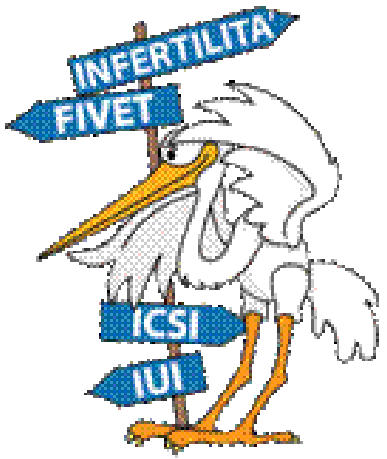
EMBRYO VITRIFICATION

OOCYTES THAWING

PREIMPLANTATION GENETIC SCREENING OR DIAGNOSIS

EMBRYO TRANSFER

EMBRYO THAWING



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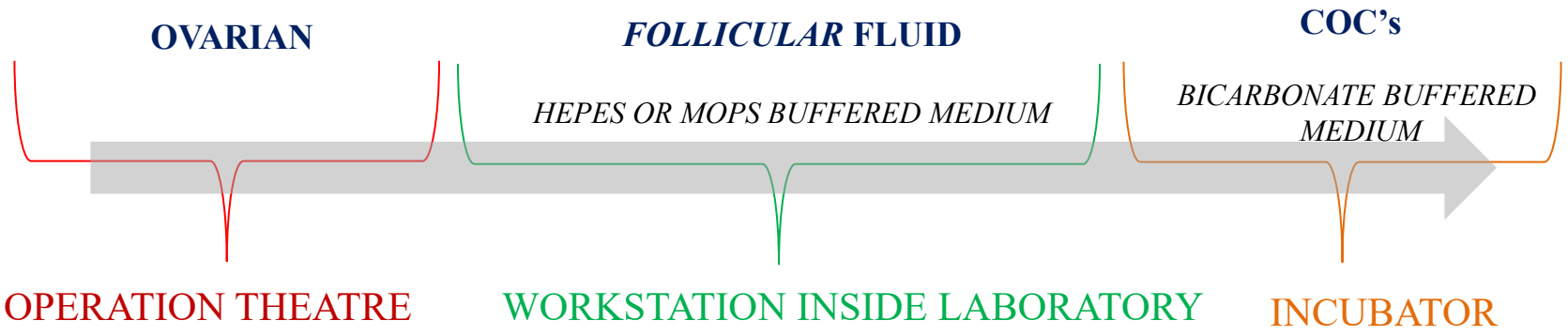
"PICK-UP": THE OOCYTE RETRIEVAL

- PERFORMED INSIDE THE THEATRE UNDER ANESTHESIA (PROPOFOL) IN PRESENCE OF ANESTHESIST, GYNECOLOGYST, NURSE AND BYOLOGYST

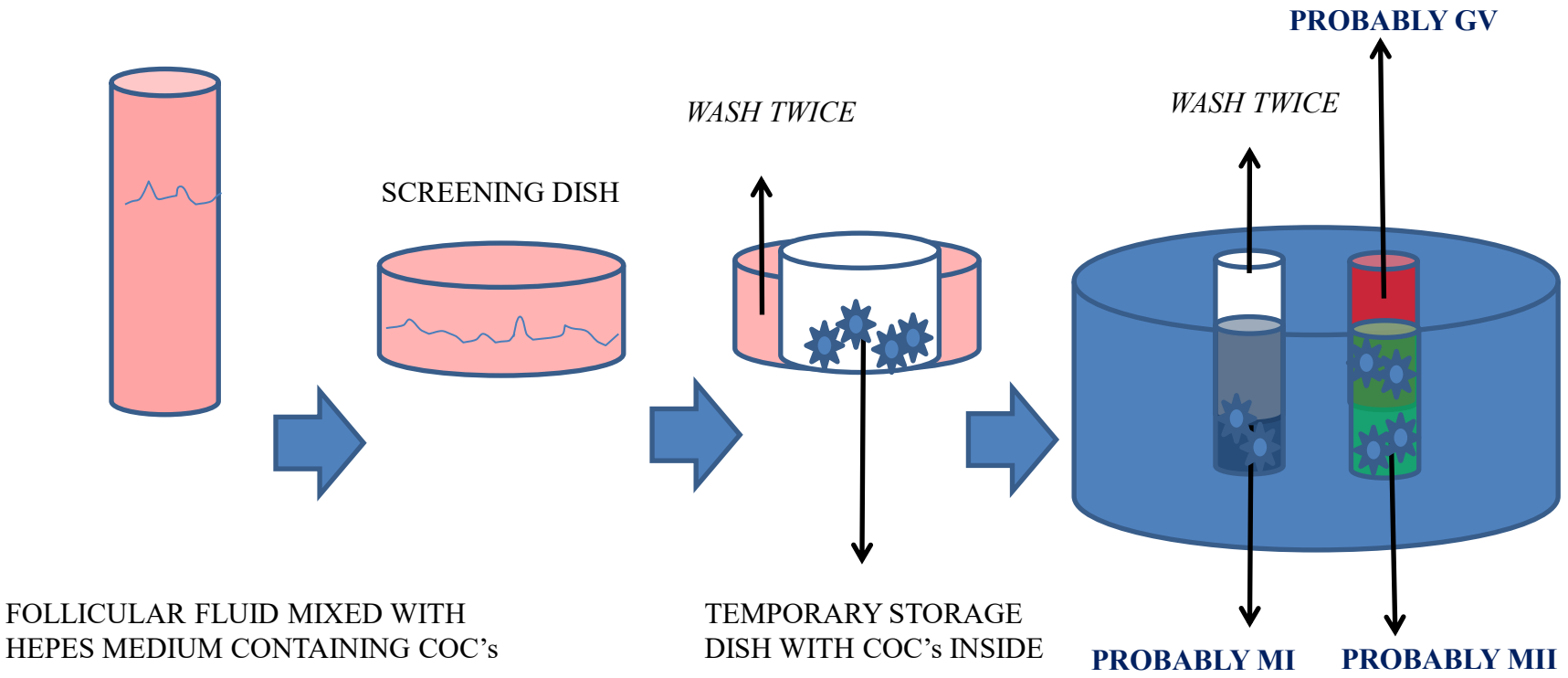
- ULTRASOUND-GUIDED WITH 17 GAUGE NEEDLE (FOLLICULAR PUNCTURE)

- PERFORMED 35 (URINARY HCG) OR 36-38 (RECOMBINANT HCG OR GNRH-AGONIST) HOURS AFTER HCG ADMINISTRATION



"PICK-UP": THE OOCYTE RETRIEVAL

The cumulus-oocyte-complexes are *retrieved from follicular* fluid, washed and *stored temporary in a clean dish* filled of Hepes Buffered Medium. At the end of pick-up, COC's are *sorted by nuclear stage maturity* in a new 4 well dish (bicarbonate buffer medium filled) and left in a CO_2/O_2 incubator for 3 hours.



Medium utilized: **HTF/wHEPES** **HTF**

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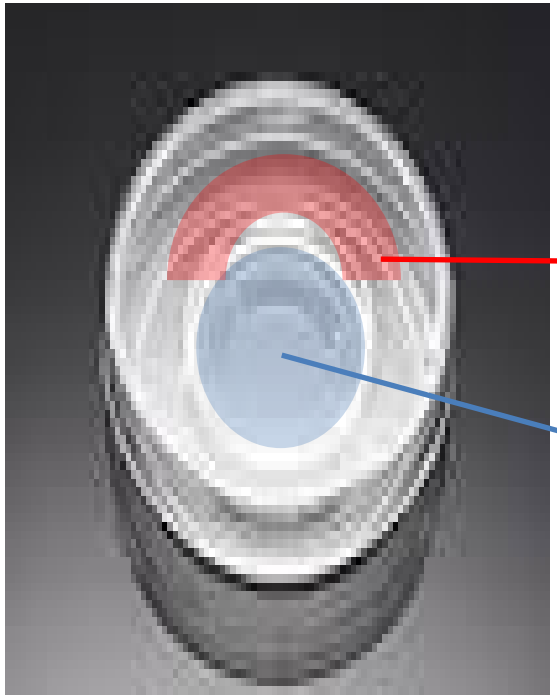
Environmental Condition	Incubator Condition
CO_2 0,04% O_2 21 %	CO_2 6% O_2 5%
HEPES/MOPS buffer medium	CHO_3^- buffer medium

In order to maintein the pH intracellular

“PICK-UP”: THE OOCYTE RETRIEVAL



COC’s WHASHING AREA

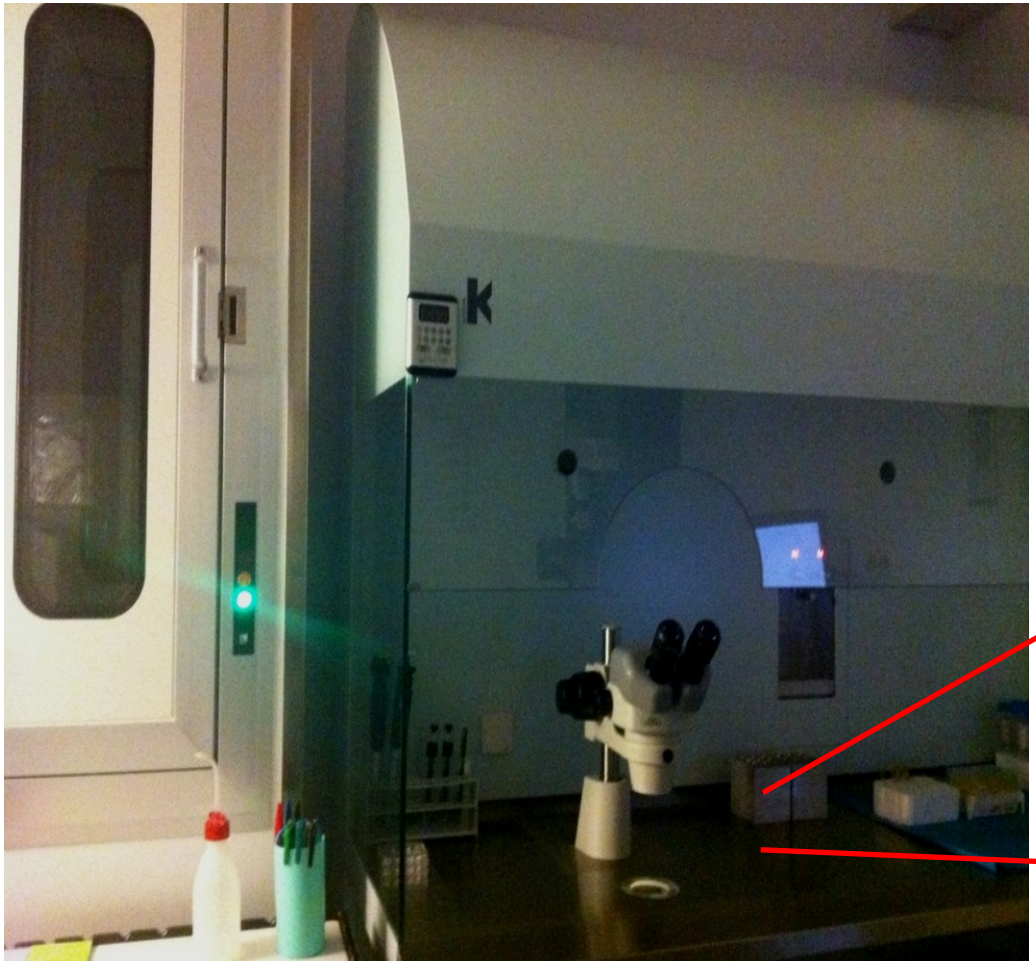
COC’s STORAGE AREA

TEMPORARY STORAGE CENTER WELL DISH FOR COC’s PRIOR TO INCUBATION

4 WELL DISH FOR COC’s INCUBATION AFTER OOCYTE RETRIEVAL

“PICK-UP”: THE OOCYTE RETRIEVAL

Keep attention: working temperature and timing of COC’s outside incubator.



“PASS BOX” ***(IN ORDER TO MAINTAIN ASEPTIC CONDITIONS)***

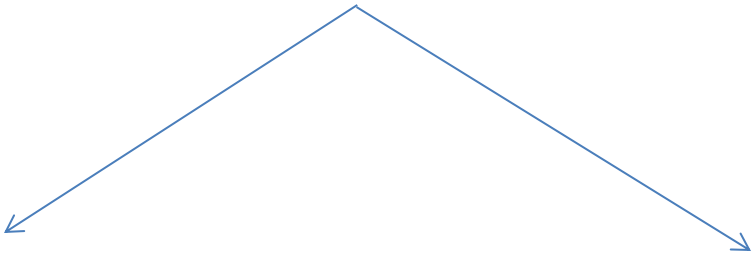
TERMOBLOCK

WORKSTATION HEATED STAGE

(..TO MANTAIN THE CORE BODY TEMPERATURE)

IN-VITRO INSEMINATION

38 hours after HCG administration : we have to choose the tecnique!



CONVENTIONAL *IN-VITRO* INSEMINATION (FIVET)

- **TUBAL OBSTRUCTION**
- **NORMAL TO MODERATE OAT SEMEN SAMPLE**
- **ENDOMETRIOSIS**
- **PREVIOUS FAILURE WITH I.U.I.**

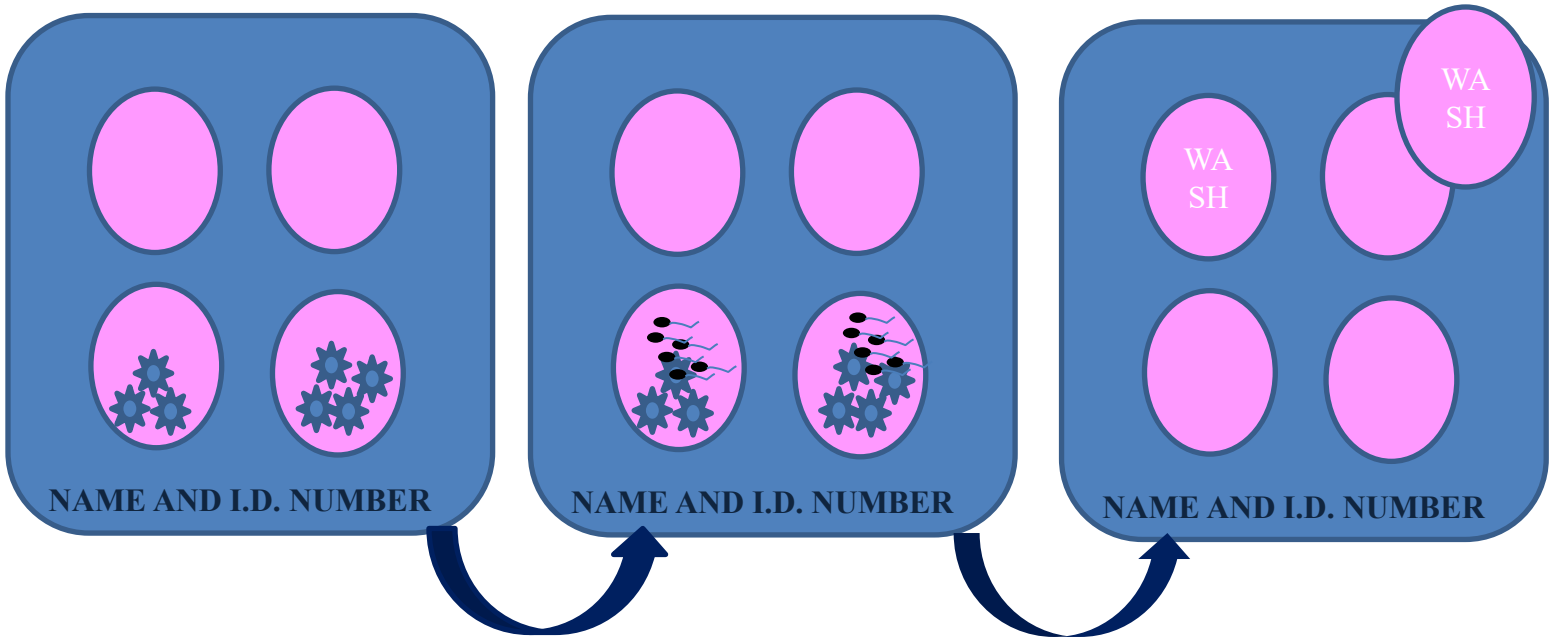
INTRACYTOPLASMATIC SPERM INJECTION (ICSI)

- **MODERATE TO SEVERE OAT SEMEN SAMPLE**
- **SURGICAL SPERM RETRIEVAL (TESE/TESA/MESA)**
- **OOCYTE THAWING**
- **NO TO LOW FERTILIZAION RATE AFTER FIVET**

CONVENTIONAL *IN-VITRO* INSEMINATION

After *38 hours from HCG, t*he COC's are able to reach the *cytoplasmic and nuclear maturity*.

Optimal sperm concentration inside each well is*100.000 motile spz/COC*.



Medium utilized: **HTF** ("Human Tubal Fluid") *(Quinn, P.)*

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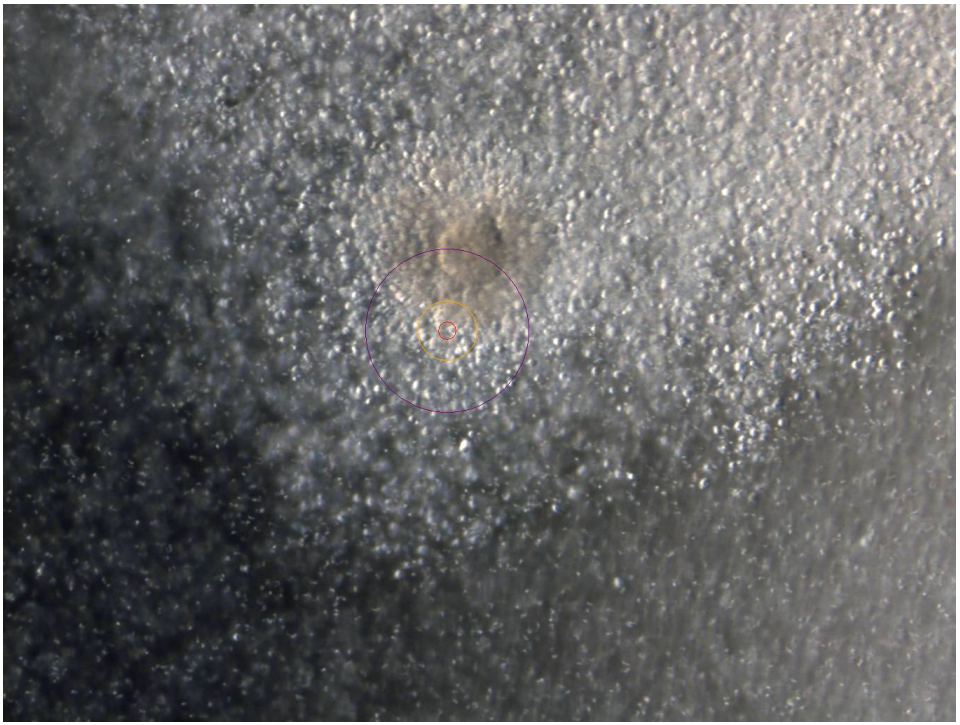
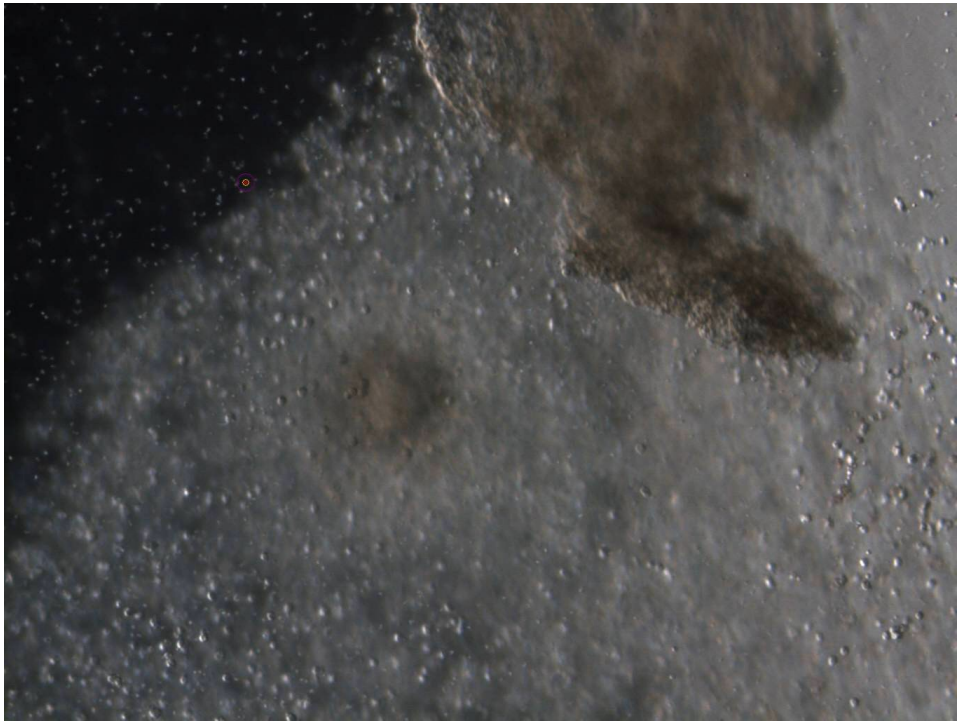
CONVENTIONAL IN-VITRO INSEMINATION





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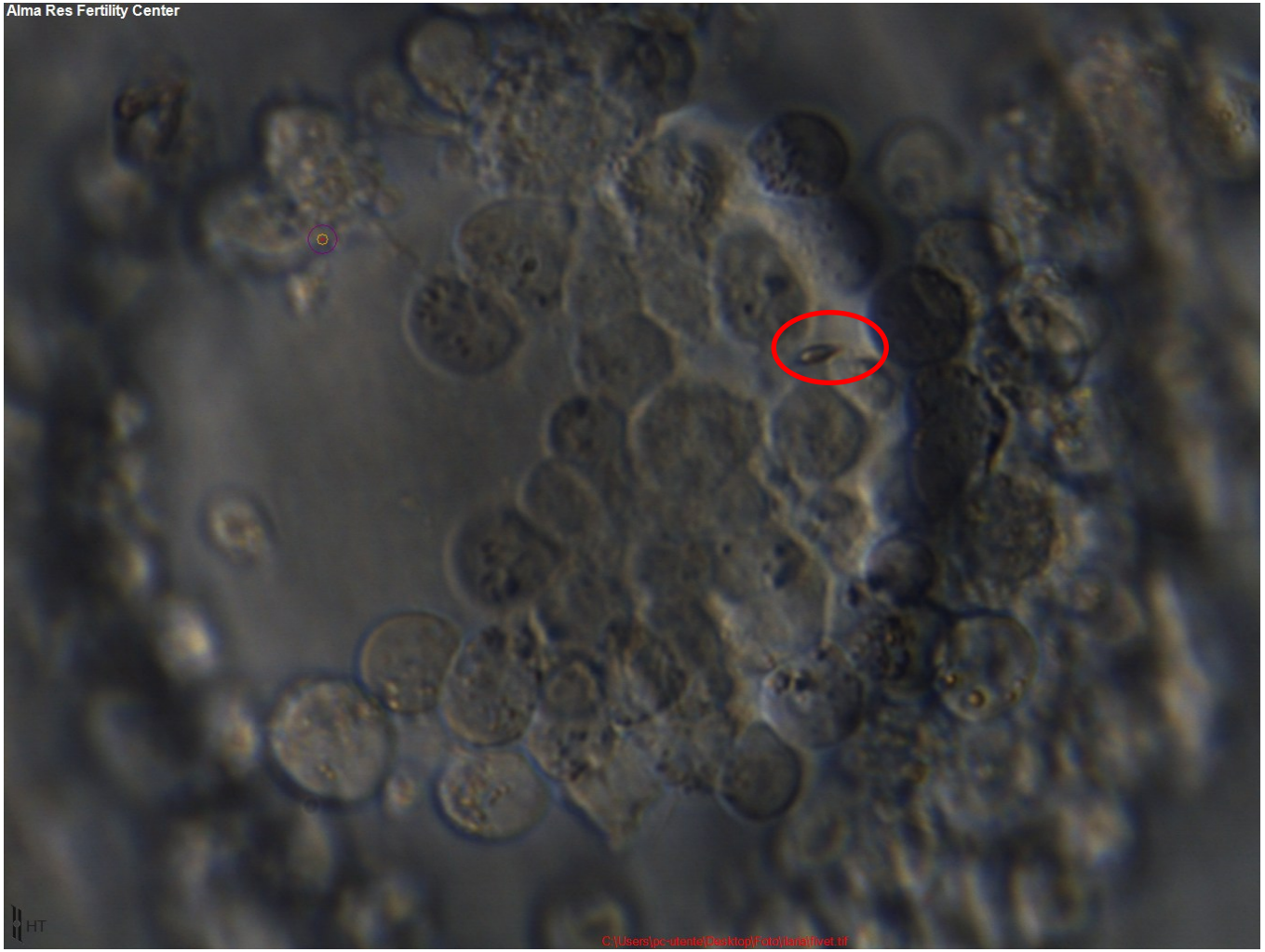
CONVENTIONAL IN-VITRO INSEMINATION



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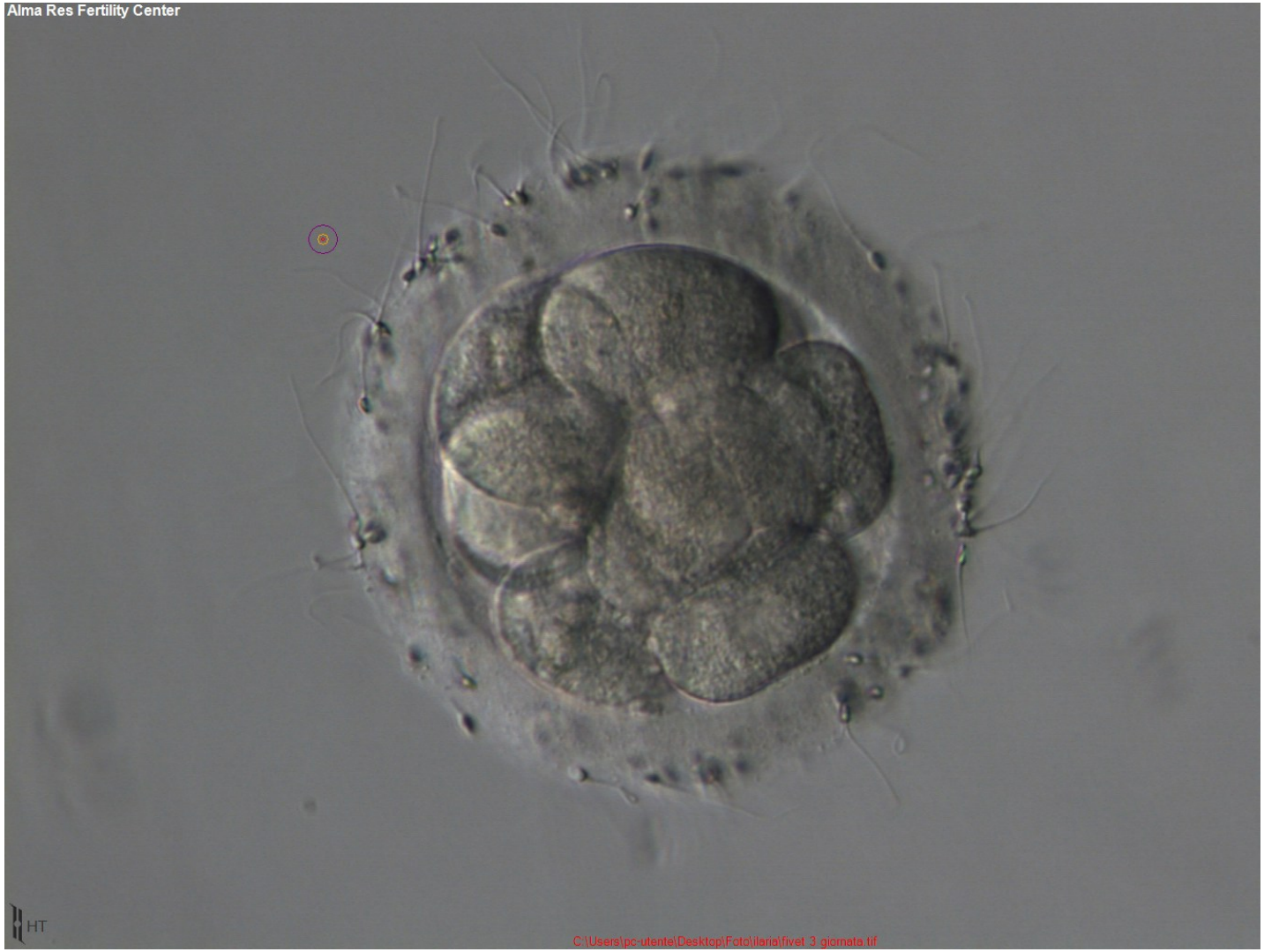
CONVENTIONAL IN-VITRO INSEMINATION



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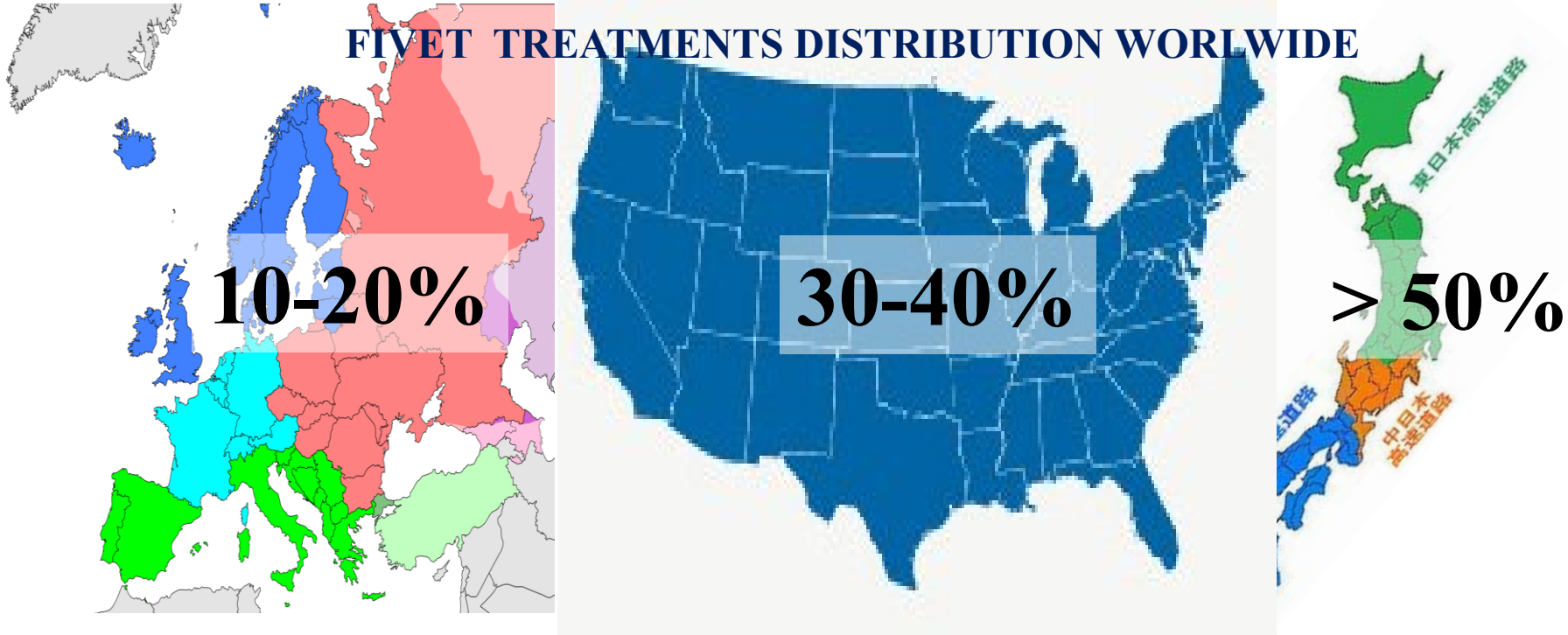
CONVENTIONAL IN-VITRO INSEMINATION



Due to the mean age of the patients and the mean percentage of oocytes harvested, in Europe biologist perform ICSI ***also when unnecessary****.*

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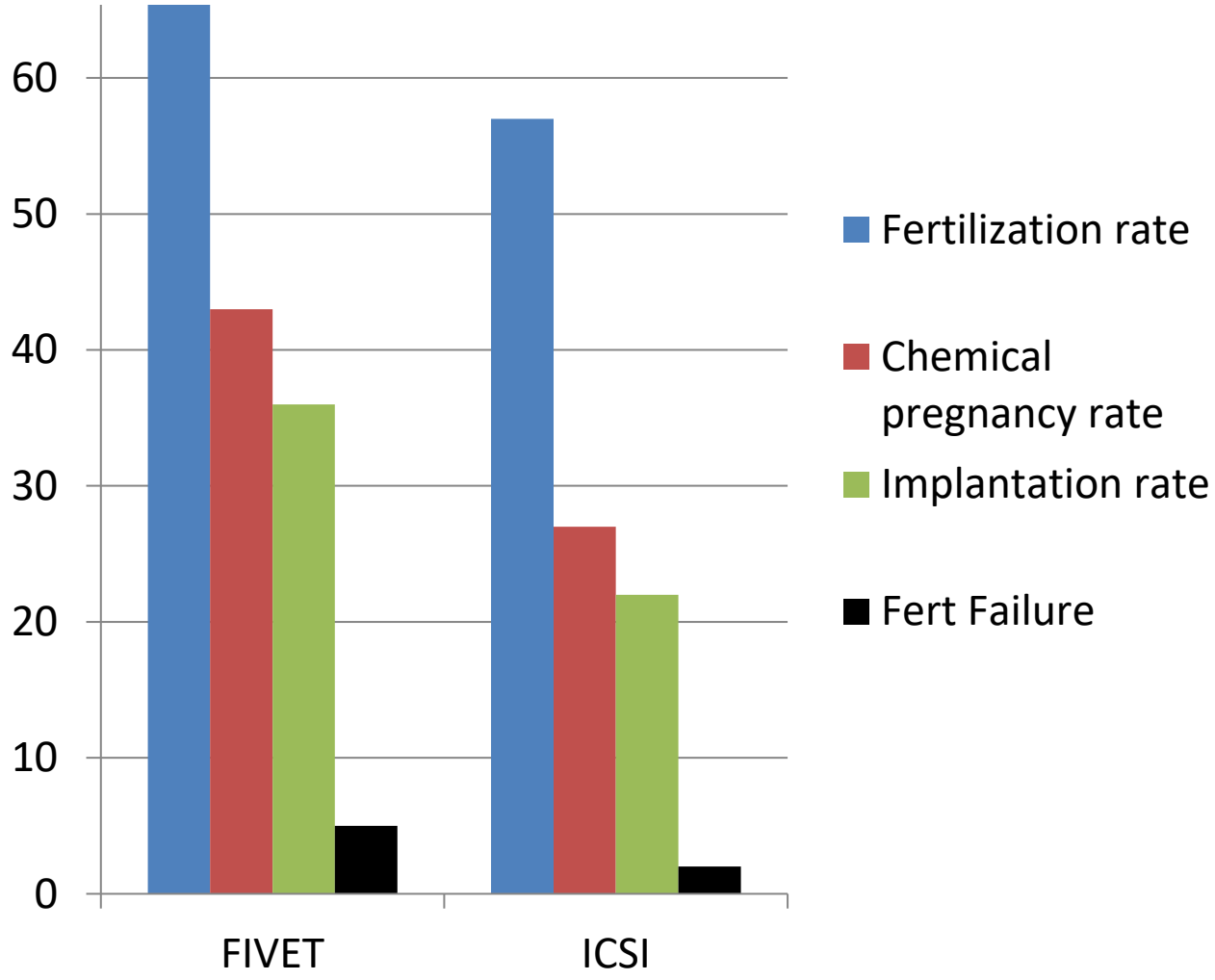
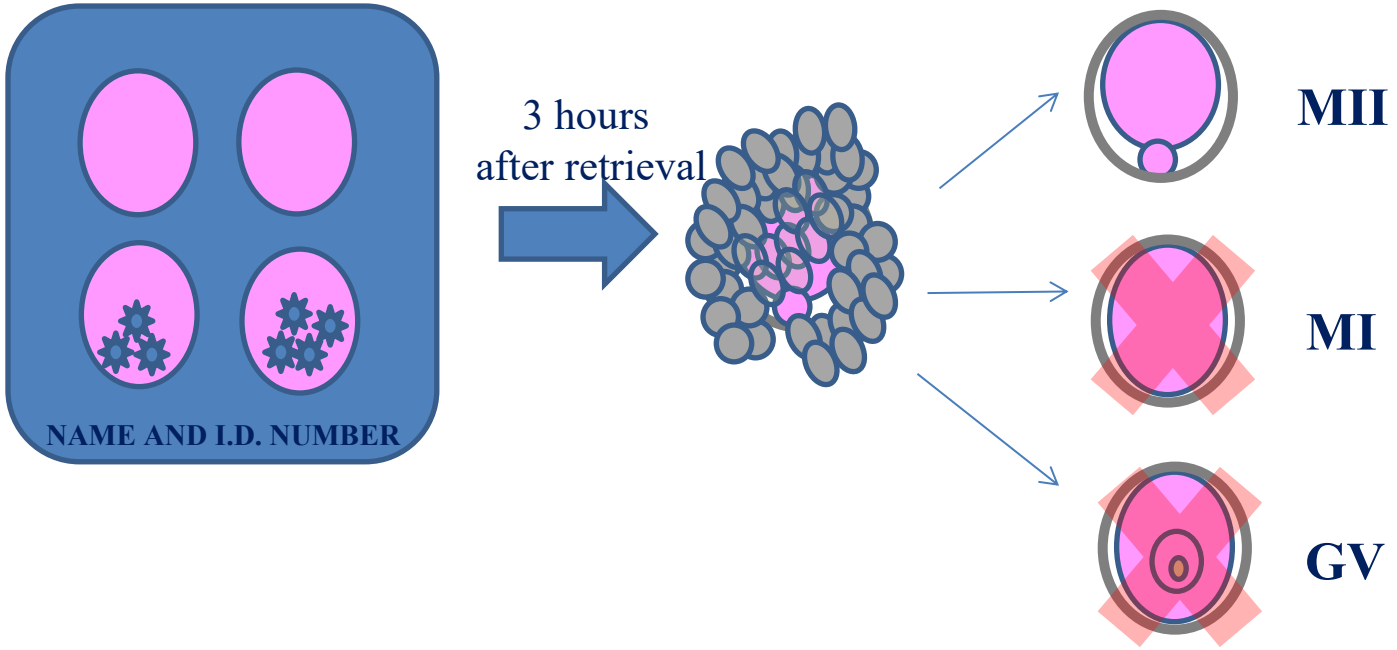
CONVENTIONAL IN-VITRO INSEMINATION



Eftekar et al. (2012). "Comparison of conventional IVF versus ICSI in nonmale factor, normoresponder patients". *J Reprod Med Vol. 10. 131-136*

INTRA- CYTOPLASMATIC SPERM INJECTION (ICSI)

ICSI must be performed after *38 hours from HCG administration.* The COC's are able to reach also the *cytoplasmic maturity*. But, differently to fivet, *we must to remove the cumulus cells* and check for the nuclear maturity of each oocytes harvested. *Immediately, we have to perform ICSI* only on MII oocytes.

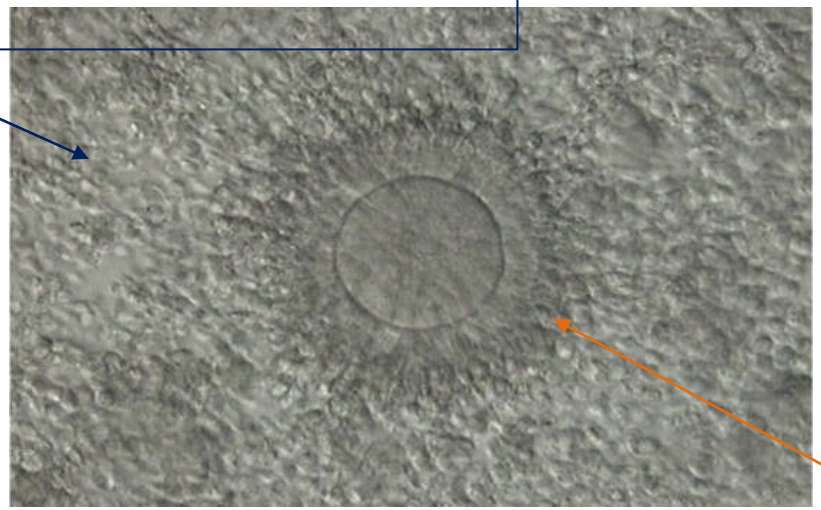


Keep attention: oocyte denuding can be performed in HTF without hepes for skilled embryologist. During training, it's better to use HTF with hepes in ordet to avoid the pH rise outside incubator.

CUMULUS-ENCLOSED OOCYTES

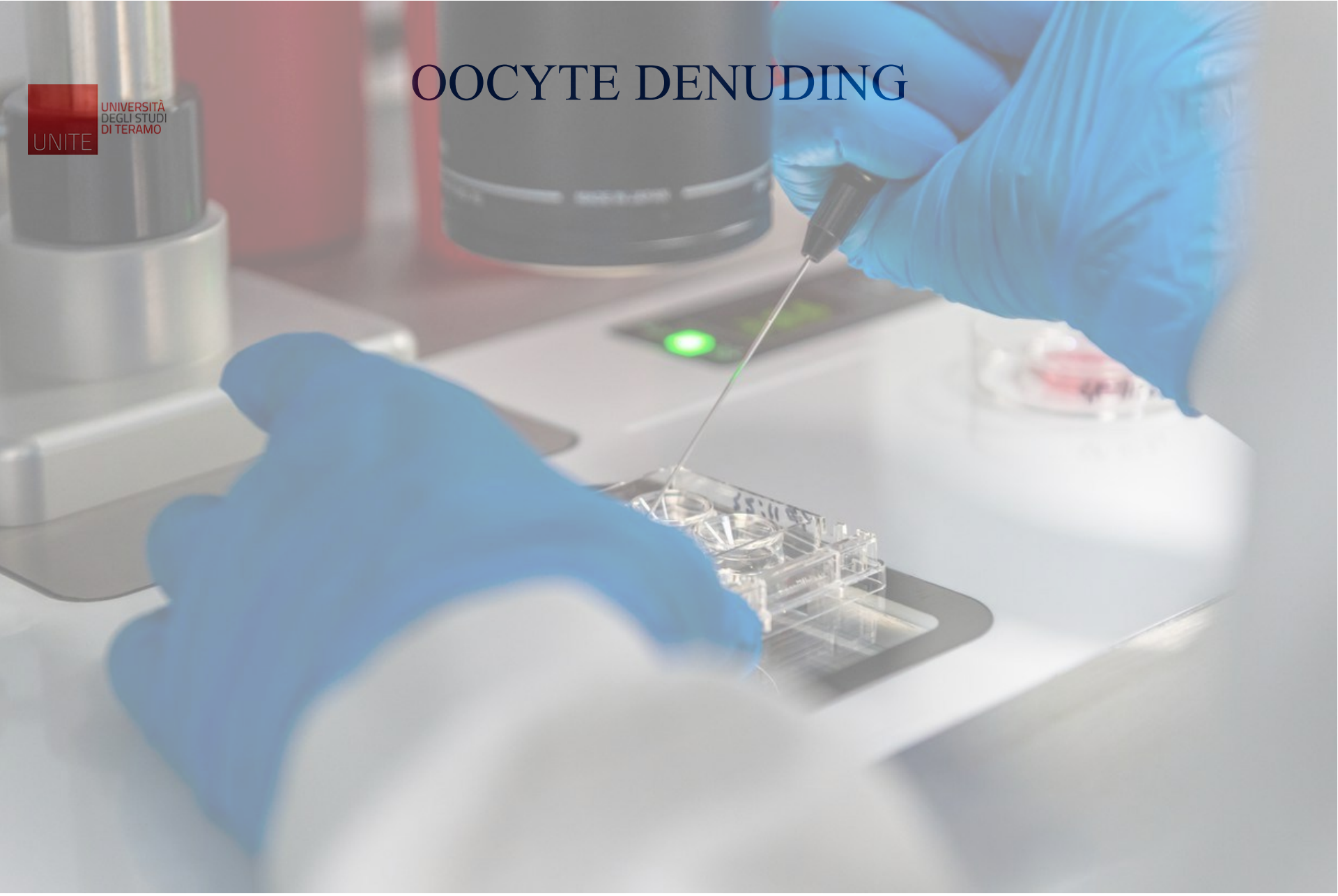
During the preovulatory growth, within the follicle, the oocyte is surrounded by two different somatic cell layers: *granulosa and thecal cells* that sustain oocyte nutrition and maturation providing essential metabolites, hormones, and growth factors.

The outer CCs separated from each other by extracellular matrix



The corona cells (granulosa) immediately adjacent to the oocyte becoming less compact and radiating away from the ZP.

OOCYTE DENUDING



https://www.youtube.com/watch?v=xI1Hd8CZxnM

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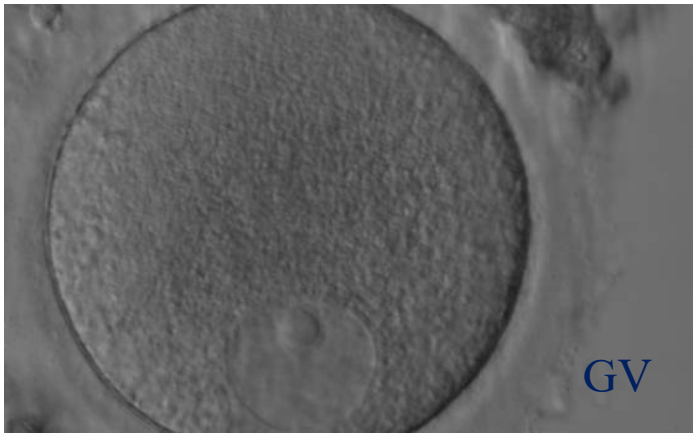
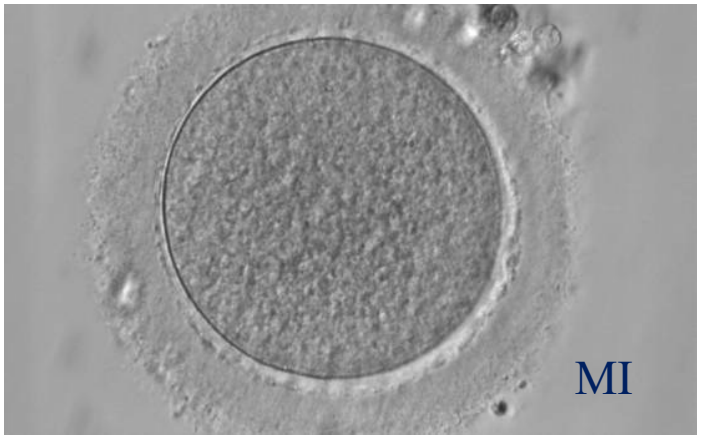
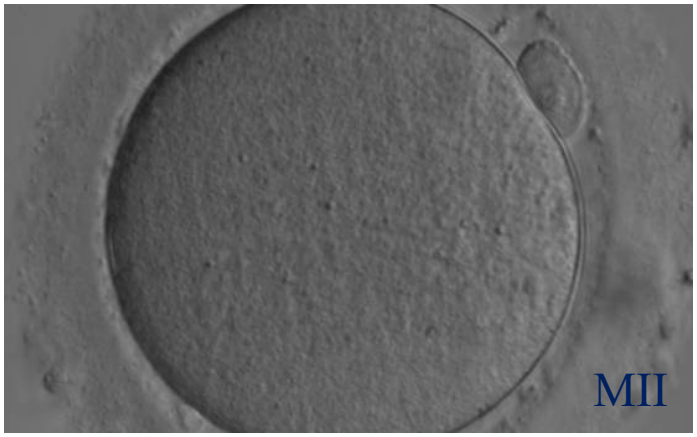
OOCYTE MATURATION STAGE



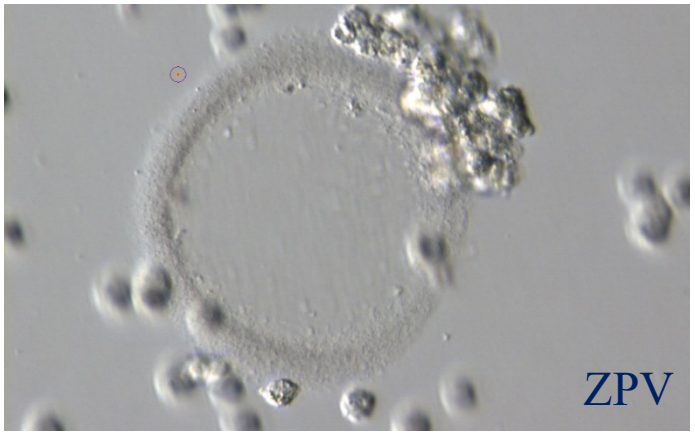
GV oocyte with an eccentrically placed nucleus and a prominent single nucleolus



MI oocyte. This oocyte has no visible nucleus and has not as yet extruded the PBI



Denuded MII oocyte; an intact PBI is clearly visible in the PVS



An empty zona pellucida

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OOCYTE SIZE AND SHAPE

Normal-sized oocyte next to giant oocyte (right)

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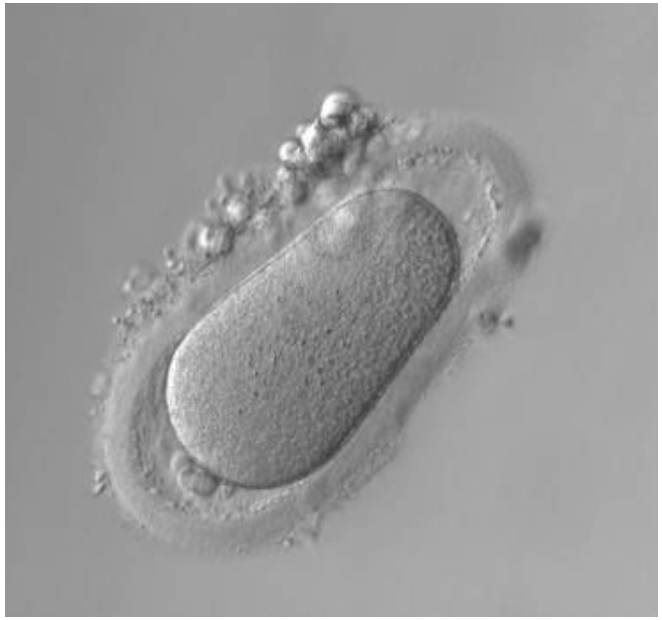
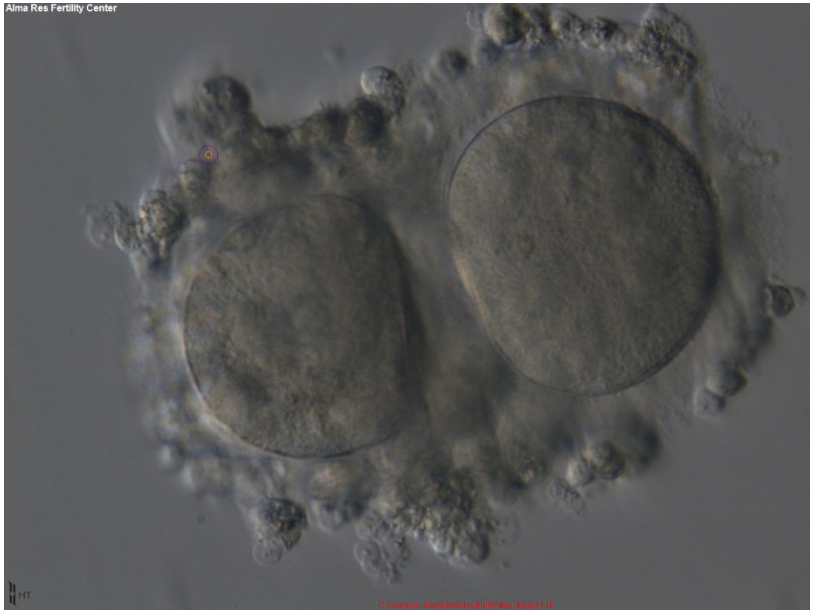
OOCYTE SIZE AND SHAPE

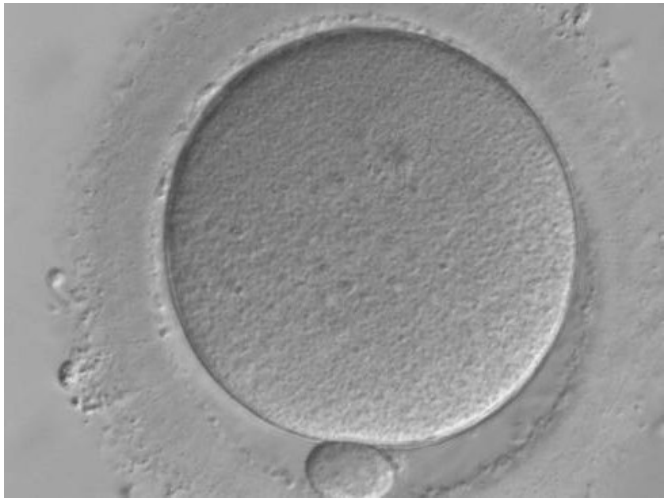


Note the ZP is ovoid in appearance and the PVS is enlarged at both poles

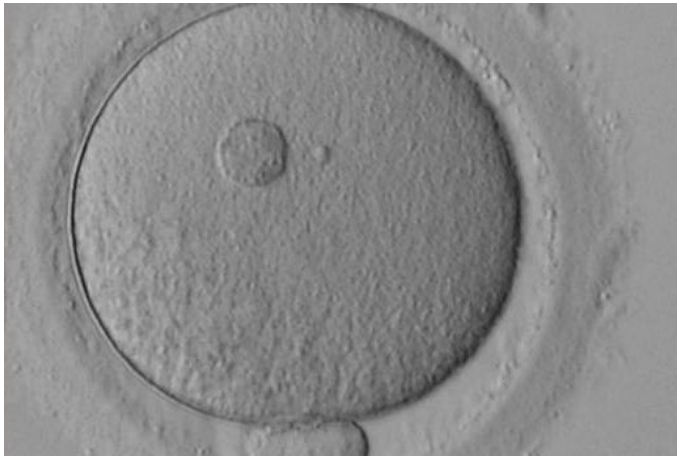


Twin oocytes in a single zona pellucida

CYTOPLASMIC FEATURES

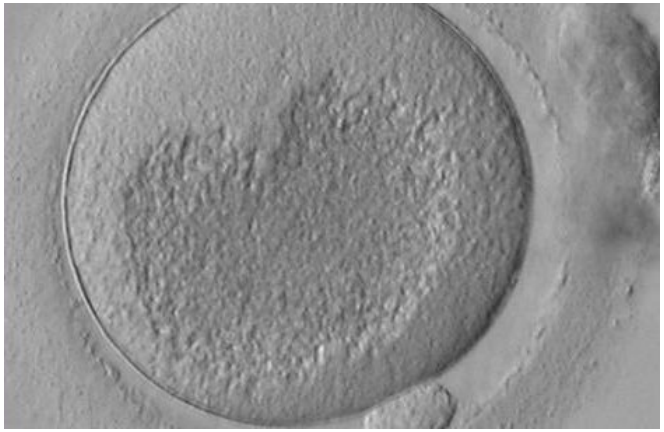


Normal homogenous cytoplasm in an MII oocyte

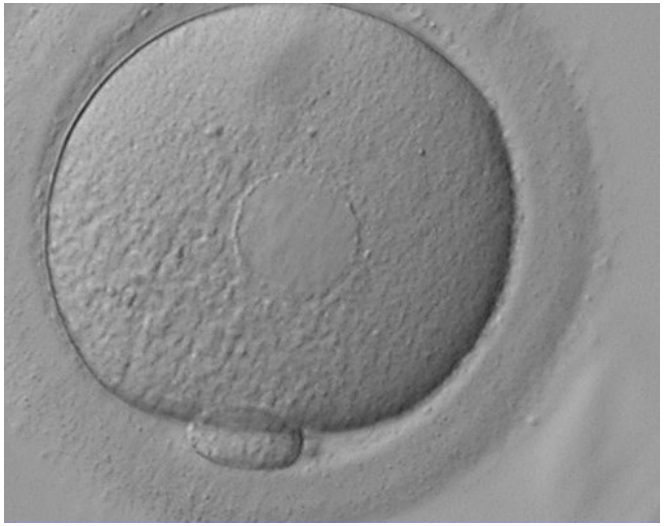


MII oocyte showing a large refractile body

CYTOPLASMIC FEATURES



MII oocyte showing a very large centrally located granular area



MII oocyte showing plaques of dilated SER discs in the cytoplasm

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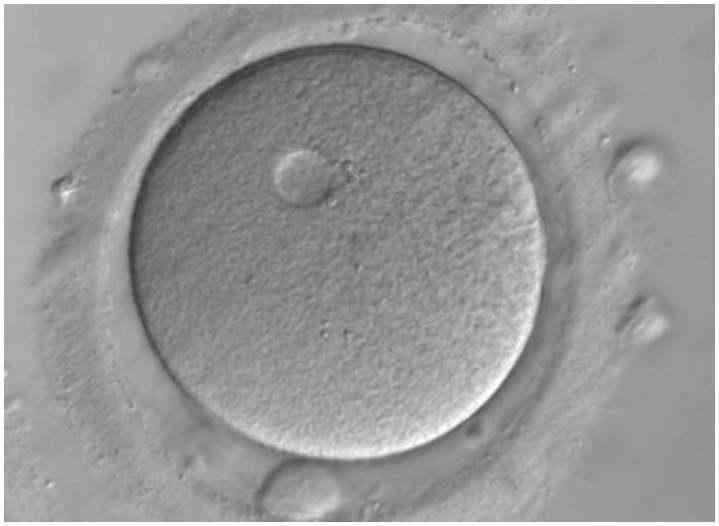
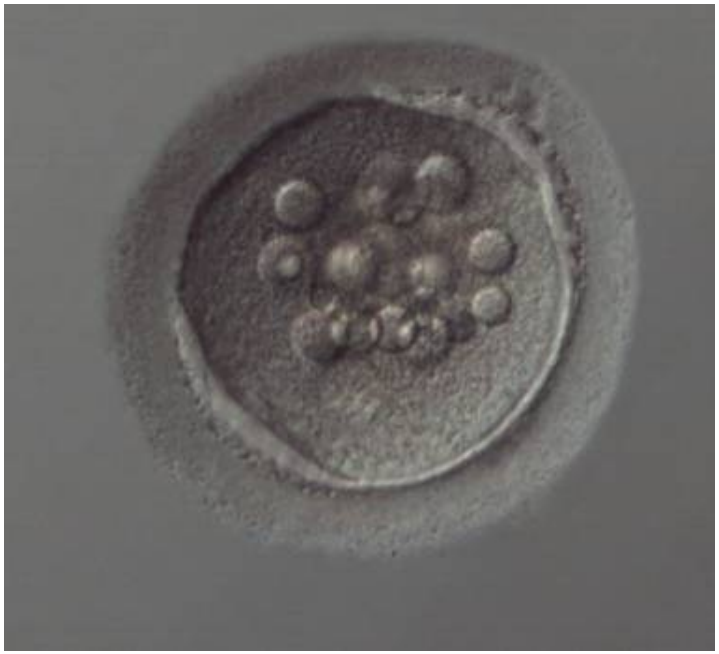
CYTOPLASMIC FEATURES

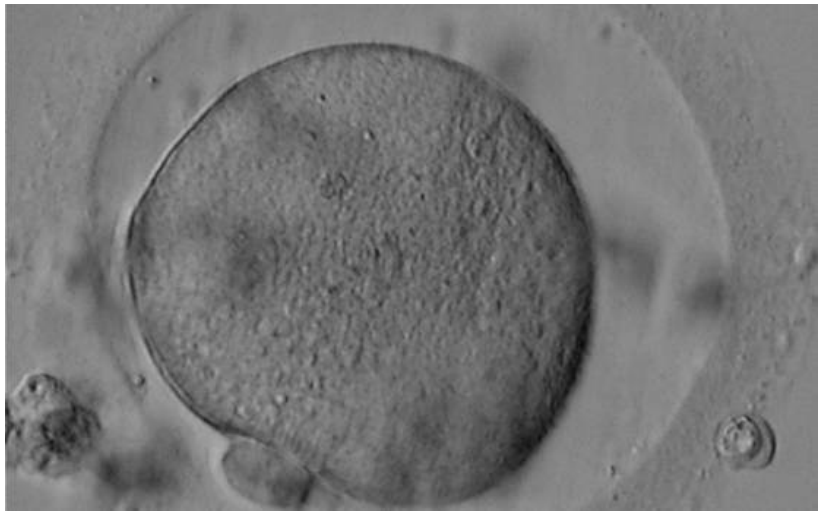


Vacuolated oocyte

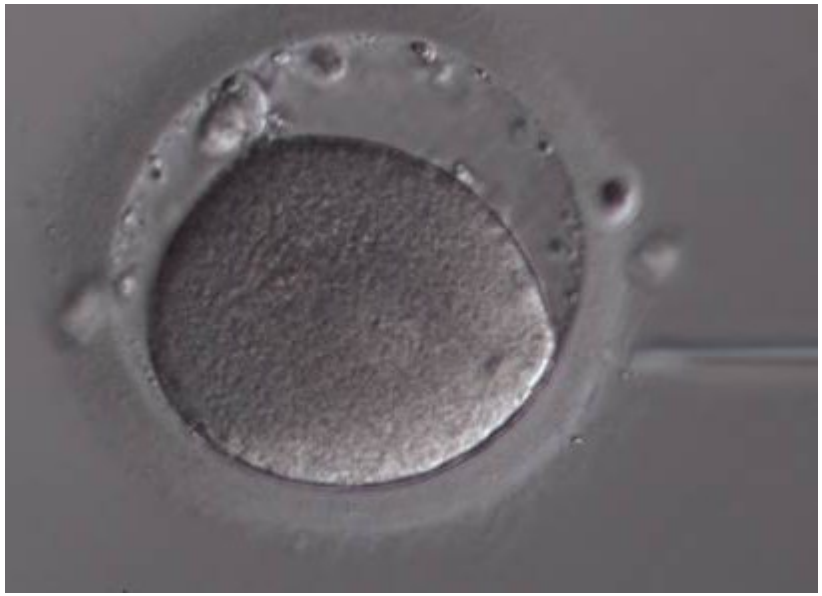


Vacuolated oocyte

PERIVITELLINE SPACE

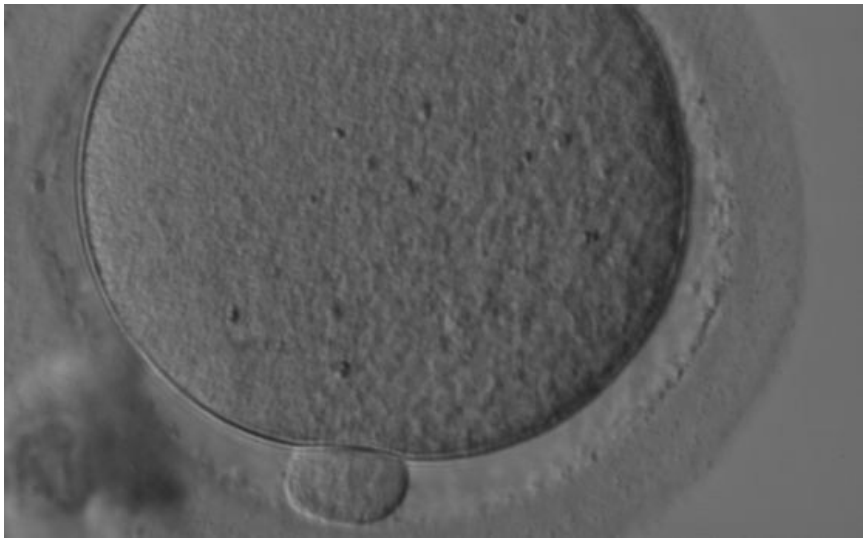


Oocyte with a large PVS and a granular cytoplasm.

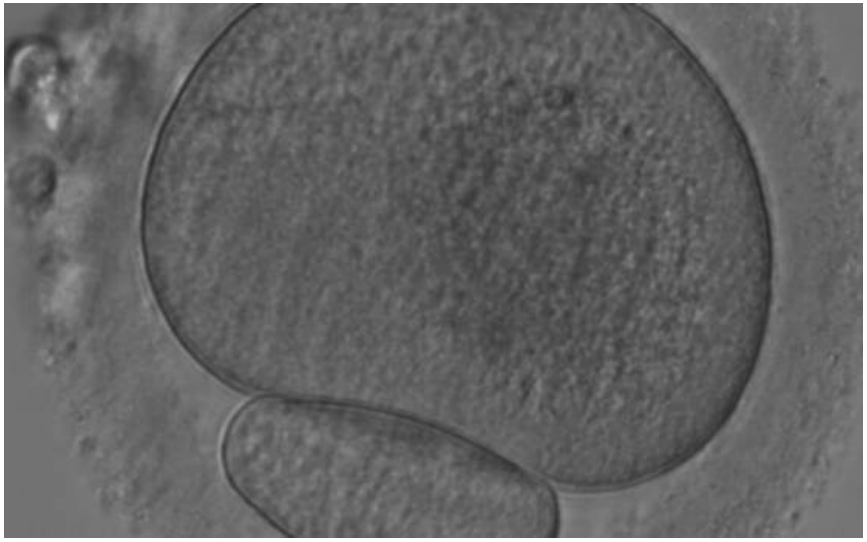


Oocyte with a large PVS.
Several fragments are present in the PVS.

POLAR BODY



A normal-sized PBI.

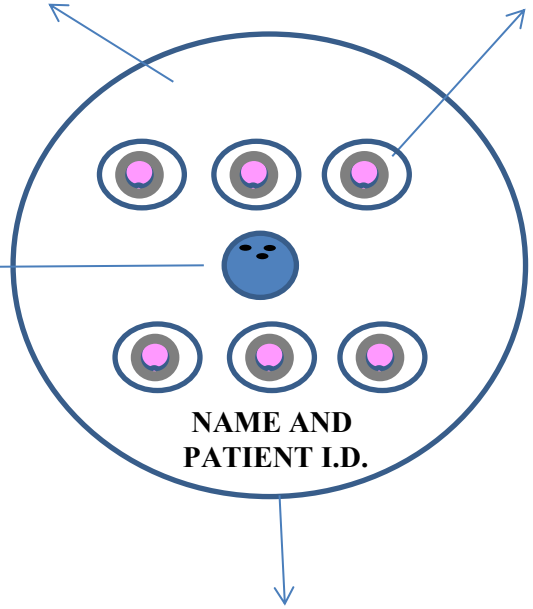


A giant PBI.

THE ICSI DISH

The drops must to be covered with 5 ml of light mineral oil

10 µl HEPES-HTF + oocyte



5 µl PVP +1-2 µl of treated sperms

The lid of a 65 x 10 mm Petri dish has the optimal refringence at the inverted microscope

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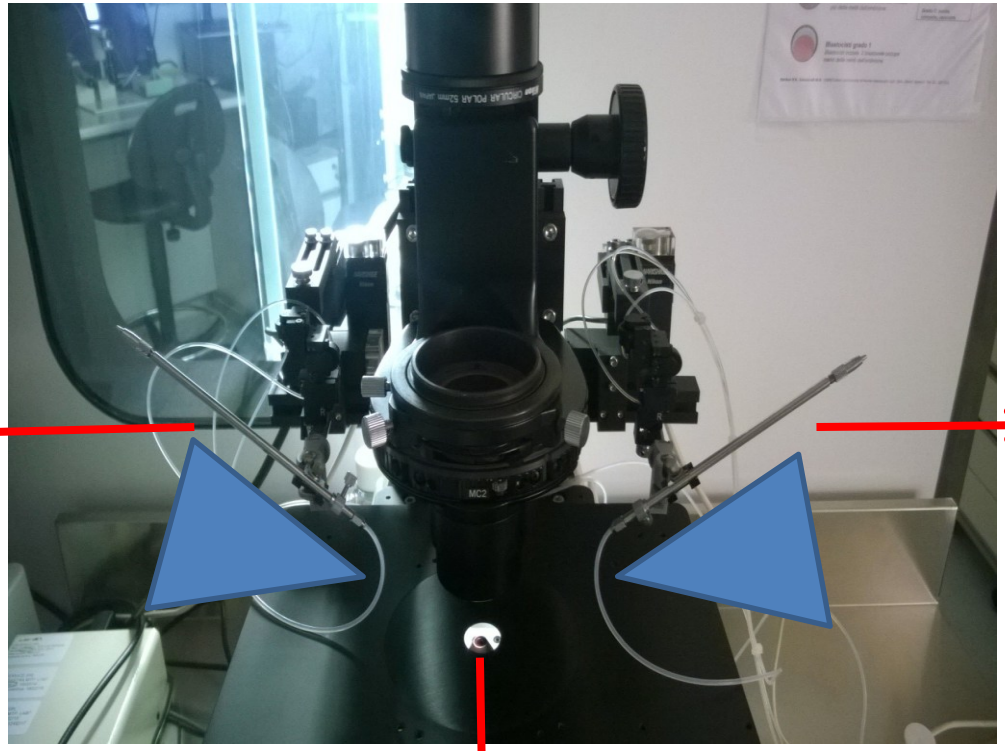
SET UP OF THE MICROMANIPULATION STATION



HOLDING PIPETTE

30-35° BEVEL
PARALLEL WALL
20-25 μM OF ID

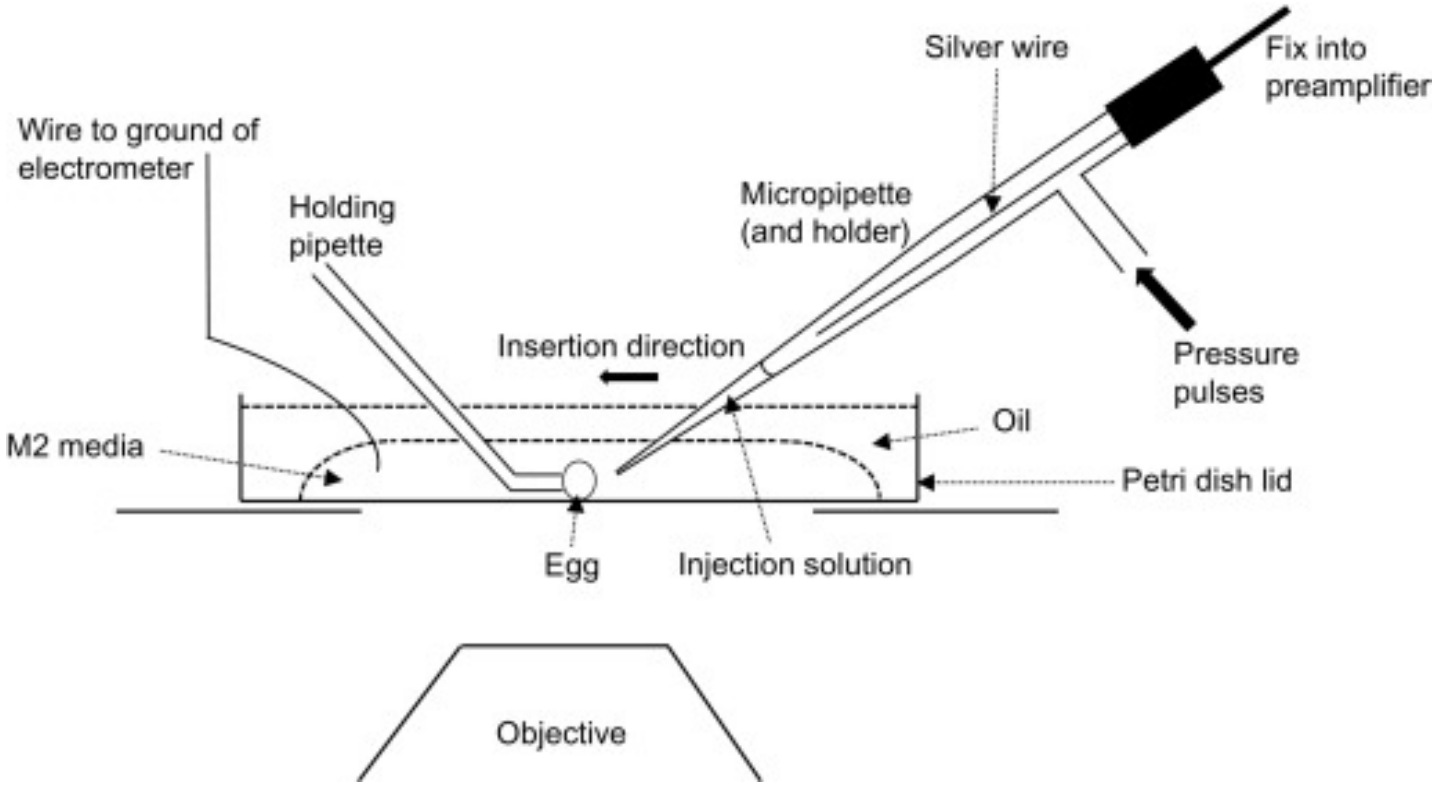
INJECTION PIPETTE

30-35° BEVEL
PARALLEL WALL
4-5 μM OF ID

MAGNIFICATION

40 X FOR BETTER SPERM SELECTION

SET UP OF THE MICROMANIPULATION STATION



https://www.youtube.com/watch?v=bd442apDyus

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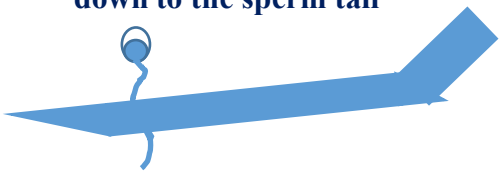
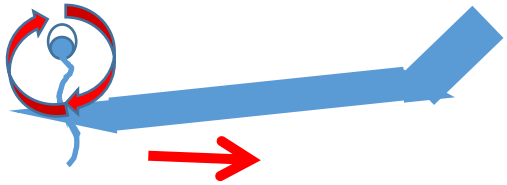
SPERM SELECTION AND IMMOBILIZATION (INSIDE PVP DROP)

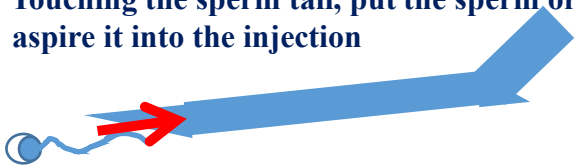
1. **Select the sperm and immobilize it going down to the sperm tail**



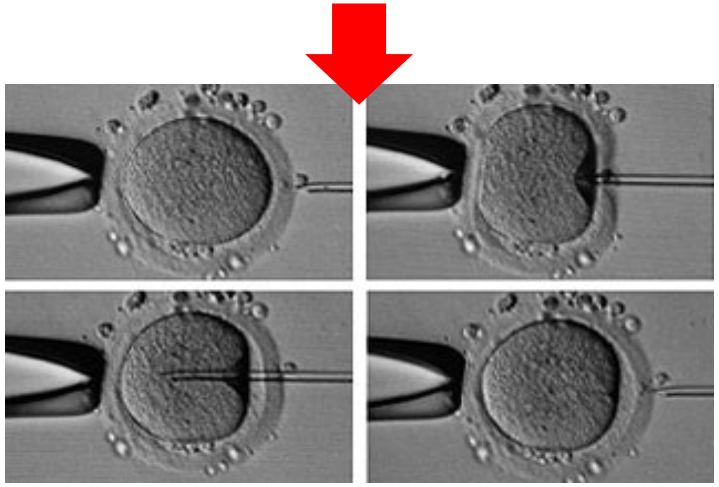
2. **Move the injection to right: the sperm head become to swirl**



3. **Touching the sperm tail, put the sperm orizzontally and aspire it into the injection**

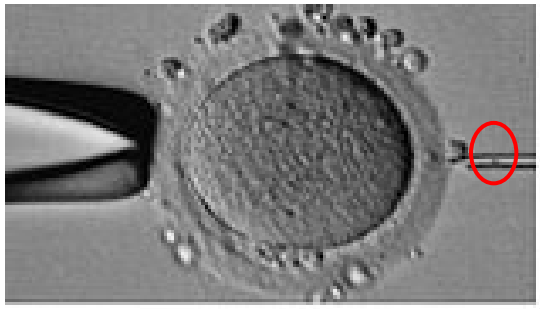


4. **With the sperm inside, move the injection to the drop of the oocyte**

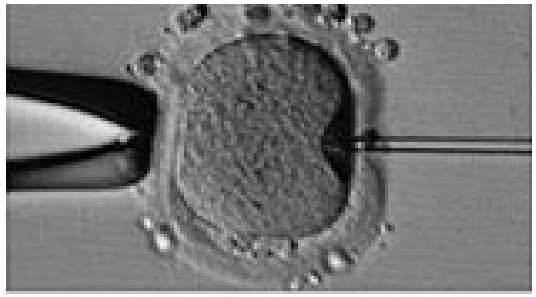


https://www.youtube.com/watch?v=uvmBRTFG7Vo&t=220s

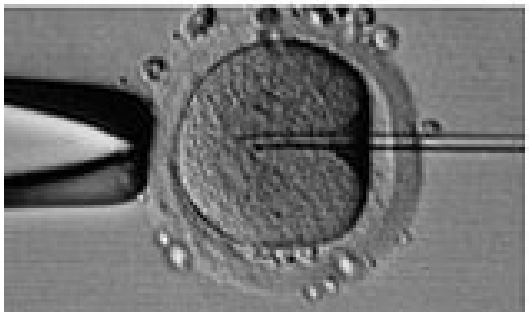
OOCYTE INJECTION (INSIDE OOCYTE DROP)



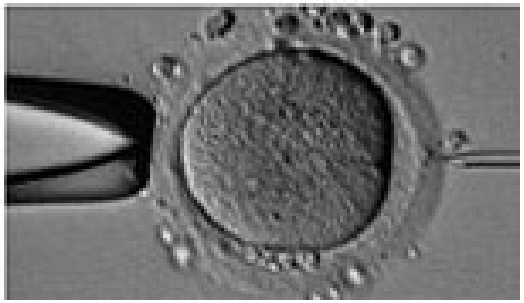
1. Hold the oocyte with a gentle suction putting the pb at 6 or 12 hrs. Focuse on the inner layer of ZP and oolemma. The sperm is at the tip of injection.



2. Move the injection trough the ZP and oolemma. Stop the moviment at ¾ of the oocyte cytoplasm.



3. Perform a vigorous cytoplasm aspiration until the membrane of the oocyte brake down. Immediately, invert the direction in order to release the sperm.



4. Release the sperm inside the cytoplasm and move back the injection pipette. Gently, release the oocyte from the holding suction.

https://www.youtube.com/watch?v=uvmBRTFG7Vo&t=220s

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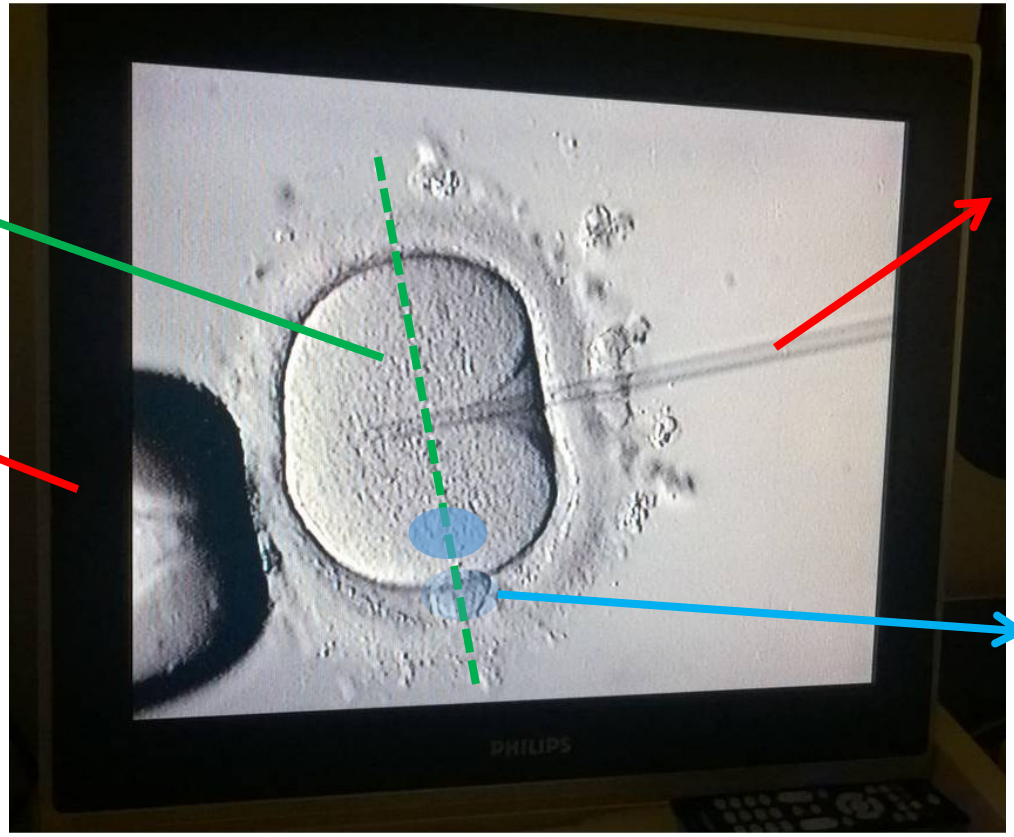
INTRACYTOPLASMATIC SPERM INJECTION (ICSI)

SPERM RELEASE

TIP OF INJECTION
AT ¾ OF THE OOCYTE

HOLDING PIPETTE

30-35° BEVEL
PARALLEL WALL
20-25 µM OF ID



INJECTION PIPETTE

30-35° BEVEL
PARALLEL WALL
4-5 µM OF ID

POLAR BODY

12 OR 6 0'CLOCK
PRESERVE MEIOTIC
SPINDLE

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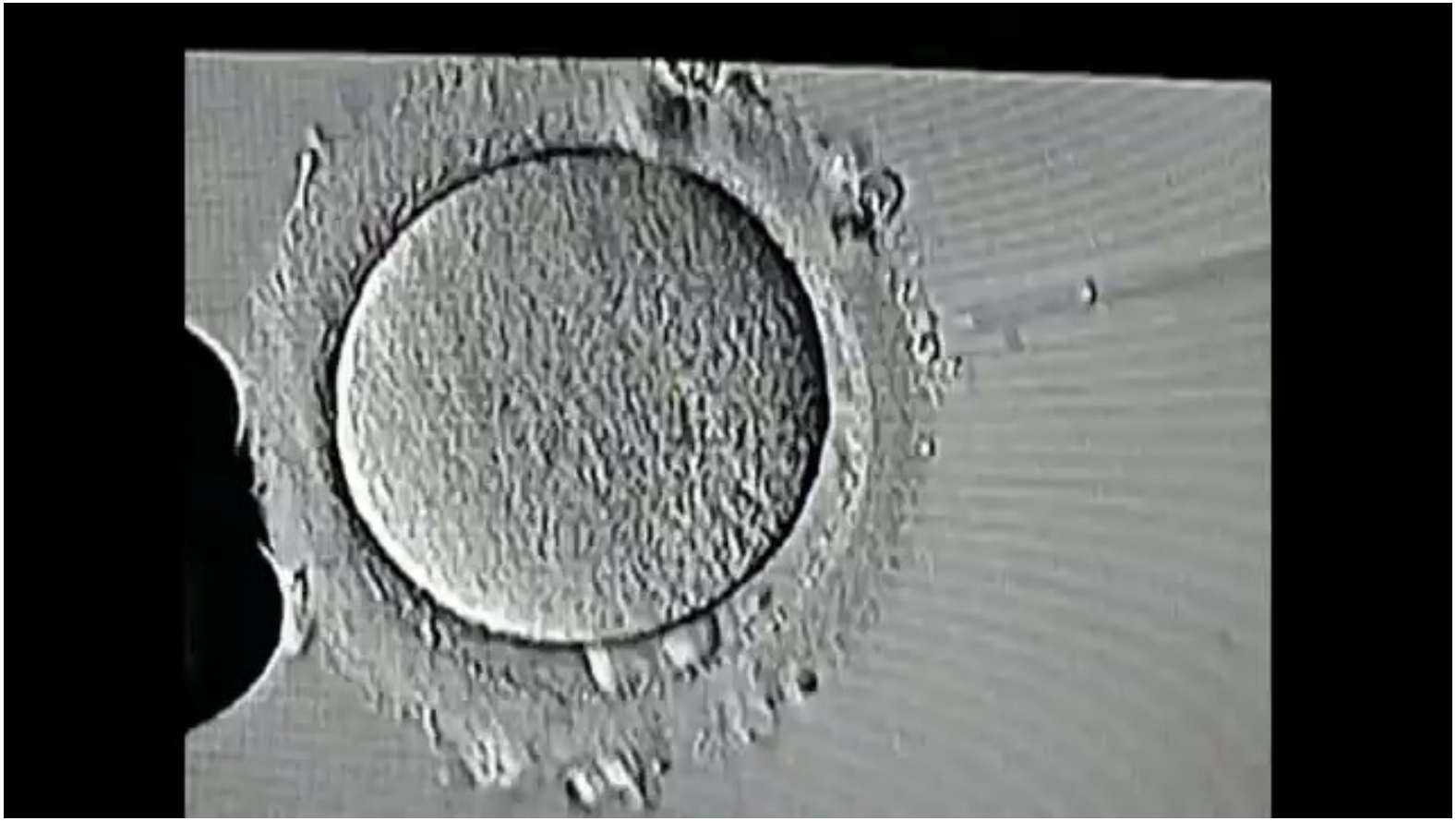
INTRACYTOPLASMATIC SPERM INJECTION (ICSI)



The PIEZO ICSI

The injection process of convetional ICSI may affect fertilization, blastocyst development, and pregnancy rates, as needle penetration of the plasma membrane and the aspiration of cytoplasm by negative pressure can have a negative impact on oocytes, particularly oocytes from older women because their oocytes have fragile plasma membranes.

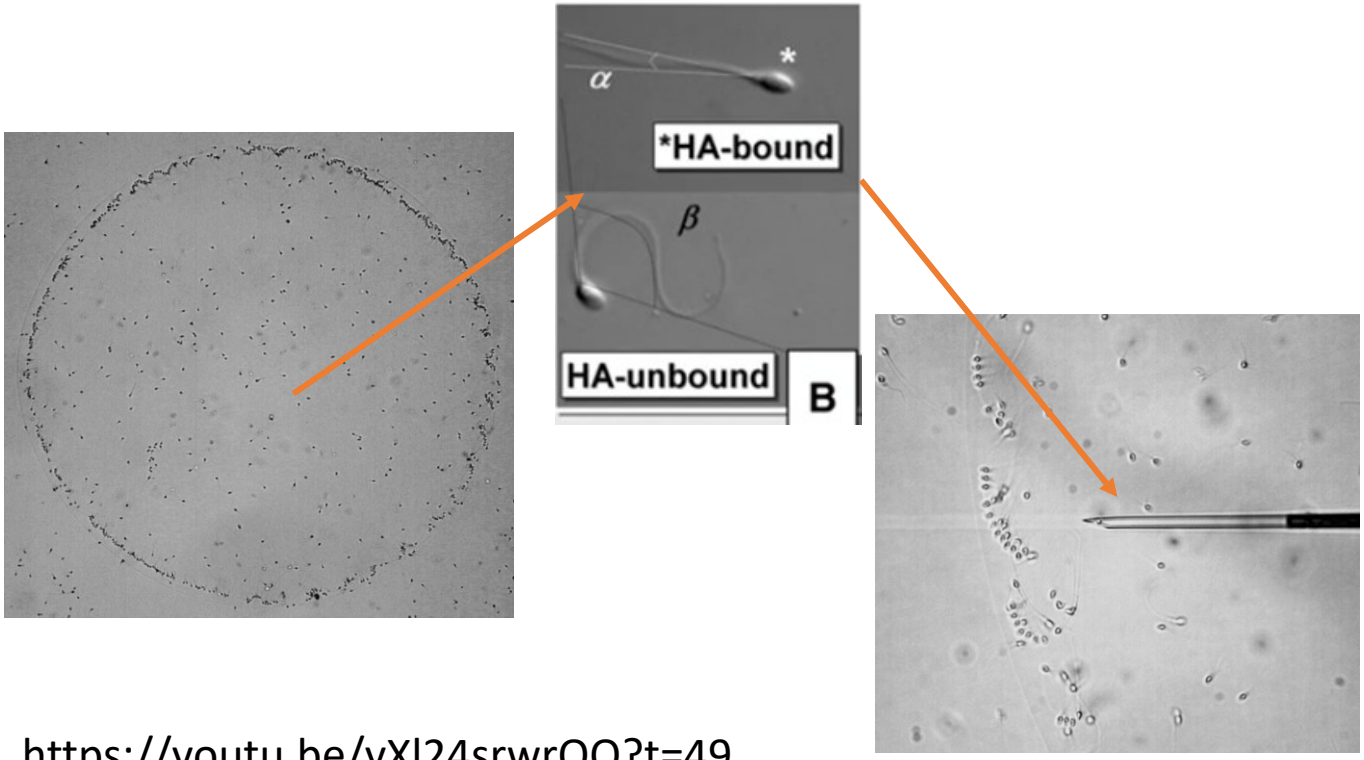
Piezo-assisted ICSI (Piezo-ICSI) markedly decreased physical pressure on the oocyte plasma membra. The Piezo-ICSI could perform the membrane breakage by applying a Piezo pulse. These pulse allow for precise microdrilling of the zona pellucida and smooth penetration of a blunt-ended injection pipette into the egg's cytoplasm.

https://www.youtube.com/watch?v=T_m0fKQrO7k

The PHYSIOLOGICAL ICSI (PICSI)

PICSI is an innovative method for selecting mature sperms based on the use of Hyaluronic Acid (HA). HA is the major component found in the extracellular matrix in OCC. Fully developed spermatozoa has HA binding ability.

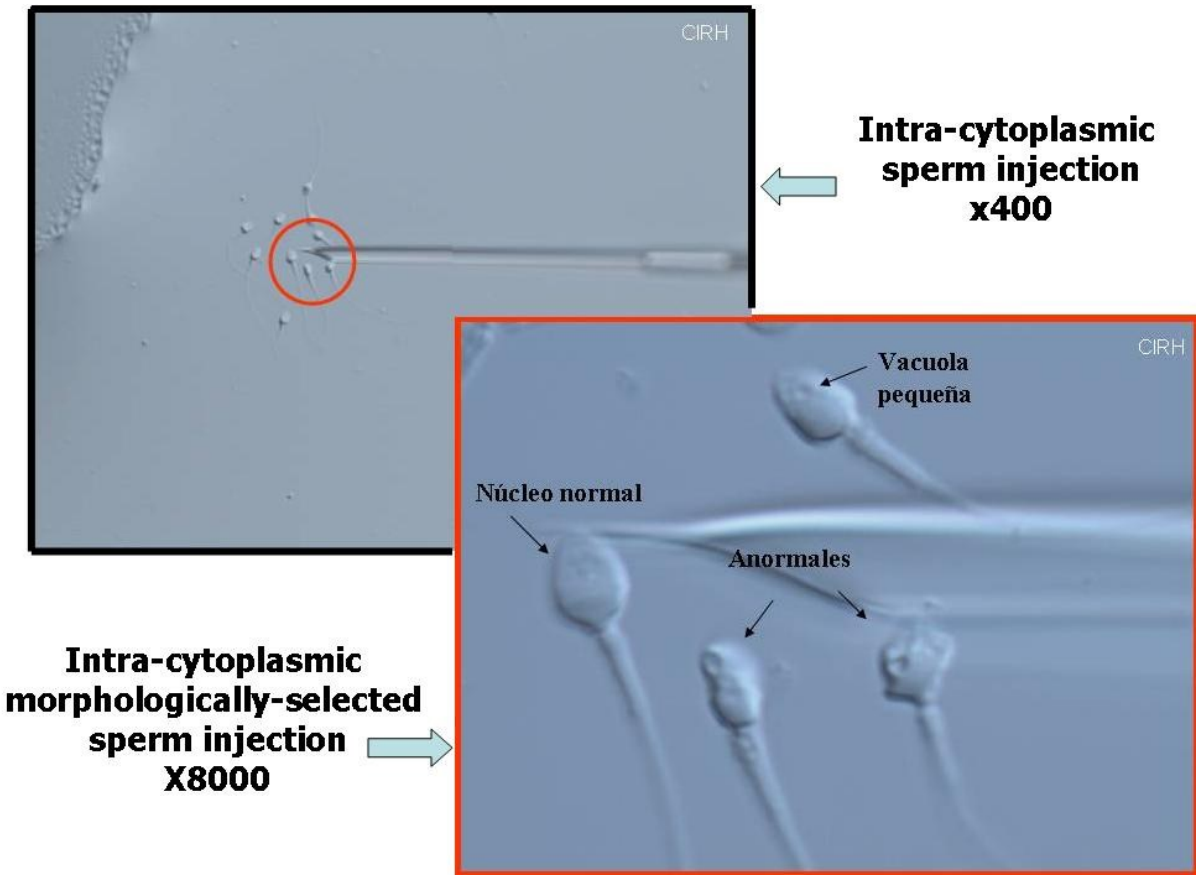
If sperm cells bind to hyaluronan, it will reflect cellular maturity, reduced risk for DNA fragmentation and aneuploidy and increased chromatin integrity.



https://youtu.be/yXl24srwrOQ?t=49

INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION (IMSI)

It is recommended for couples who have experienced repeated failures in conventional in vitro fertilization treatments, such as ICSI, due to fertilization problems or embryonic development arrest. Additionally, it is advised for men with severe teratozoospermia or a high rate of sperm DNA fragmentation.



THE FERTILIZATION CHECK

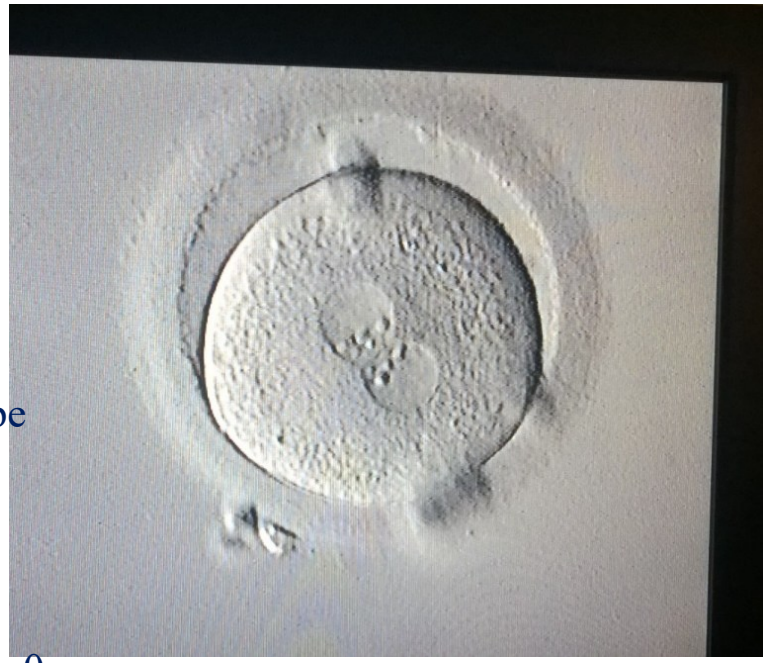
- The occurence of fertilization has to be checked between 16 and 18 hours after insemination

- In case of FIVET insemination, the oocyte must be denuded from the corona cells eventually attached

- In case of ICSI, the oocyte has alredy been denuded, so the check can be done directly at the inverted microscope

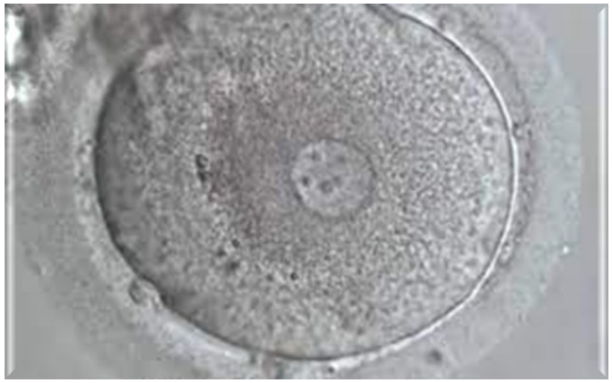
<u>CULTURE ONLY FOR 2PN ZYGOTE !!!</u>

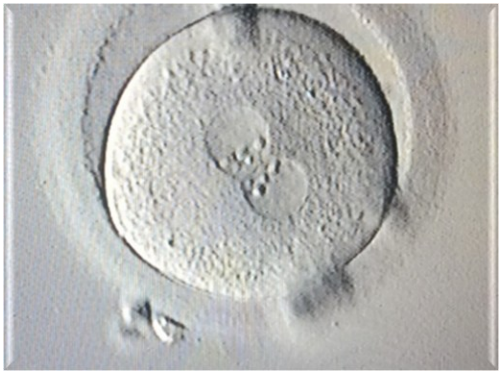
<u>DESCARD: 1 PN ZYGOTE; 3 OR MORE PN ZYGOTE; 0 PN</u>
<u>ZYGOTE WITH ONLY 1 CLEARLY VISIBLE PB</u>

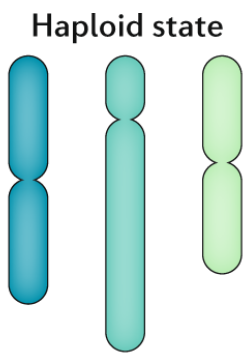


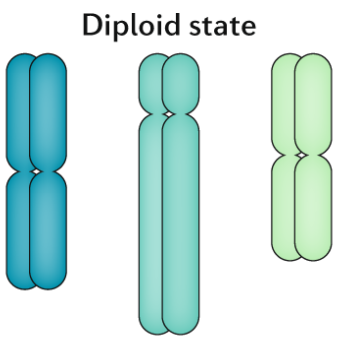
***Grade 1 zygote**: 2 distinct pronucleous with nucleolus aligned and ready to singamy.*

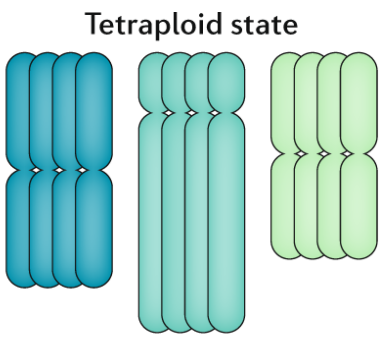
WHAT ABOUT 1PN AND 3PN ZYGOTE?











Haploid refers to the presence of a single set of chromosomes in an organism's cells. Polyploids arise from meiotic nondisjunction, that causes the formation of a diploid gametes that have a complete set of duplicate chromosomes. When a diploid gamete fuses with a haploid gamete, a triploid zygote forms.

CULTURE ONLY FOR 2PN ZYGOTE ?

Article

Clinical use of monopronucleated zygotes following blastocyst culture and preimplantation genetic screening, including verification of biparental chromosome inheritance

Cara K. Bradley, Maria V. Traversa, Natalie Hobson, Alison J. Gee, Steven J. McArthur

Abnormally fertilized oocytes can result in healthy live births: improved genetic technologies for preimplantation genetic testing can be used to rescue viable embryos in in vitro fertilization cycles

Antonio Capalbo, Ph.D.,[a,b] Nathan Treff, Ph.D.,[c] Danilo Cimadomo, M.Sc.,[a,d] Xin Tao, Ph.D.,[c]

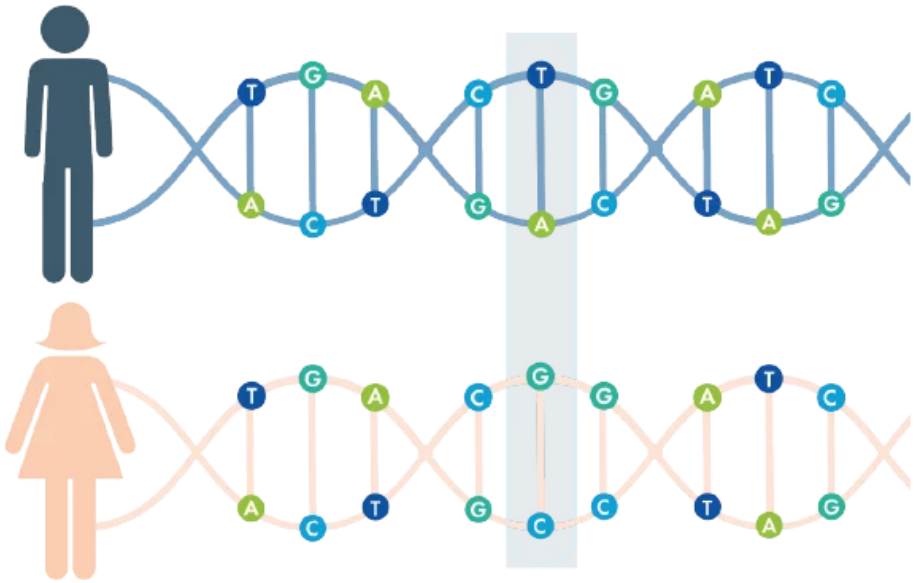
Result(s): Of the 5,026 metaphase II oocytes injected, 5.2% and 0.7% showed 1PN and 2.1PN, respectively. AFOs showed compromised embryo development ($P<.01$). Twenty-seven AFO-derived blastocysts were analyzed for ploidy constitution. The 1PN-derived blastocysts were mostly diploid (n = 9/13; 69.2%), a few were haploid (n = 3/13; 23.1%), and one was triploid (n = 1/13; 7.7%). The 2.1PN-derived blastocysts were also mostly diploid (n = 12/14; 85.7%), and the remainder were triploid. Twenty-six PGT-A cycles resulted in one or more AFO-derived blastocysts (n = 26/719; 3.6%). Overall, eight additional balanced-diploid transferable embryos were obtained from AFOs. In three cycles, the only balanced-diploid blastocyst produced was from an AFO (n = 3/719; 0.4%). Three AFO-derived live births were achieved: one from a 1PN zygote and two from 2.1PN zygotes.

Conclusion(s): Enhanced PGT-A technologies incorporating reliable ploidy assessment provide an effective tool to rescue AFO-derived blastocysts for clinical use. (Fertil Steril® 2017;108:1007–15. ©2017 by American Society for Reproductive Medicine.)

SNPs -PGT-A ANALYSIS

Single nucleotide polymorphisms (SNPs) are unique genetic variations that can be inherited and directly associated with parental DNA.

SNPs can be used for 'DNA fingerprinting' to detect ploidy differences (haploidy -triploidy), or maternal DNA contamination.
This crucial assessment ensures the selection of embryos with the correct chromosomal content, minimizing the risk of genetic abnormalities. SNPs/ PGT-A also increases the number of viable euploid embryos available for transfer by detecting true 2PN (diploid) embryos from among morphologically identified 1 and 2.1/3PN embryos.



EMBRYO PRONUCLEAR MORPHOLOGY & EUPLOIDY

Many factors from the oocyte/sperm or the process of fertilization may affect the zygote formation. The zygote score (Z-score) describes the quality of a human zygote based on its pronuclear morphology, nucleolar precursor bodies, and alignment of polar bodies. **Pronuclear morphology has been proposed as an indicator of embryo development and chromosomal complement.**

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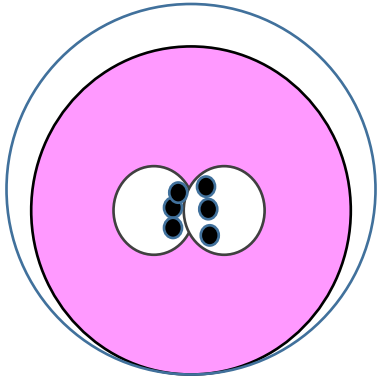
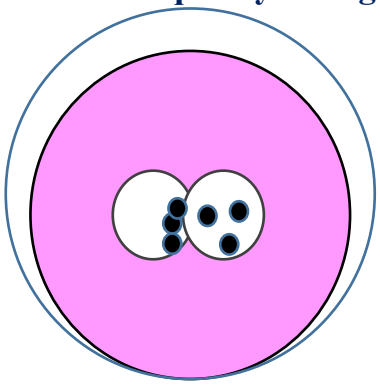
ZYGOTE SCORING

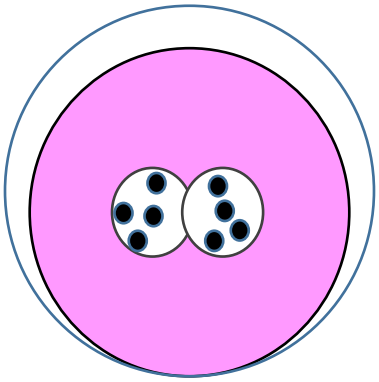
Grade 1: aligned, equality in NPB's



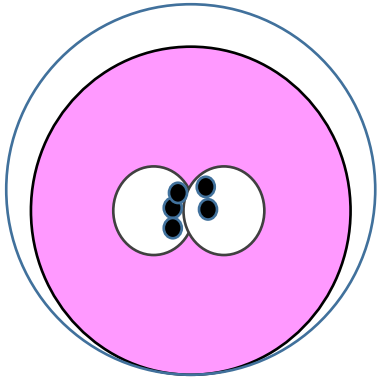
Grade 3: non equality in alignement



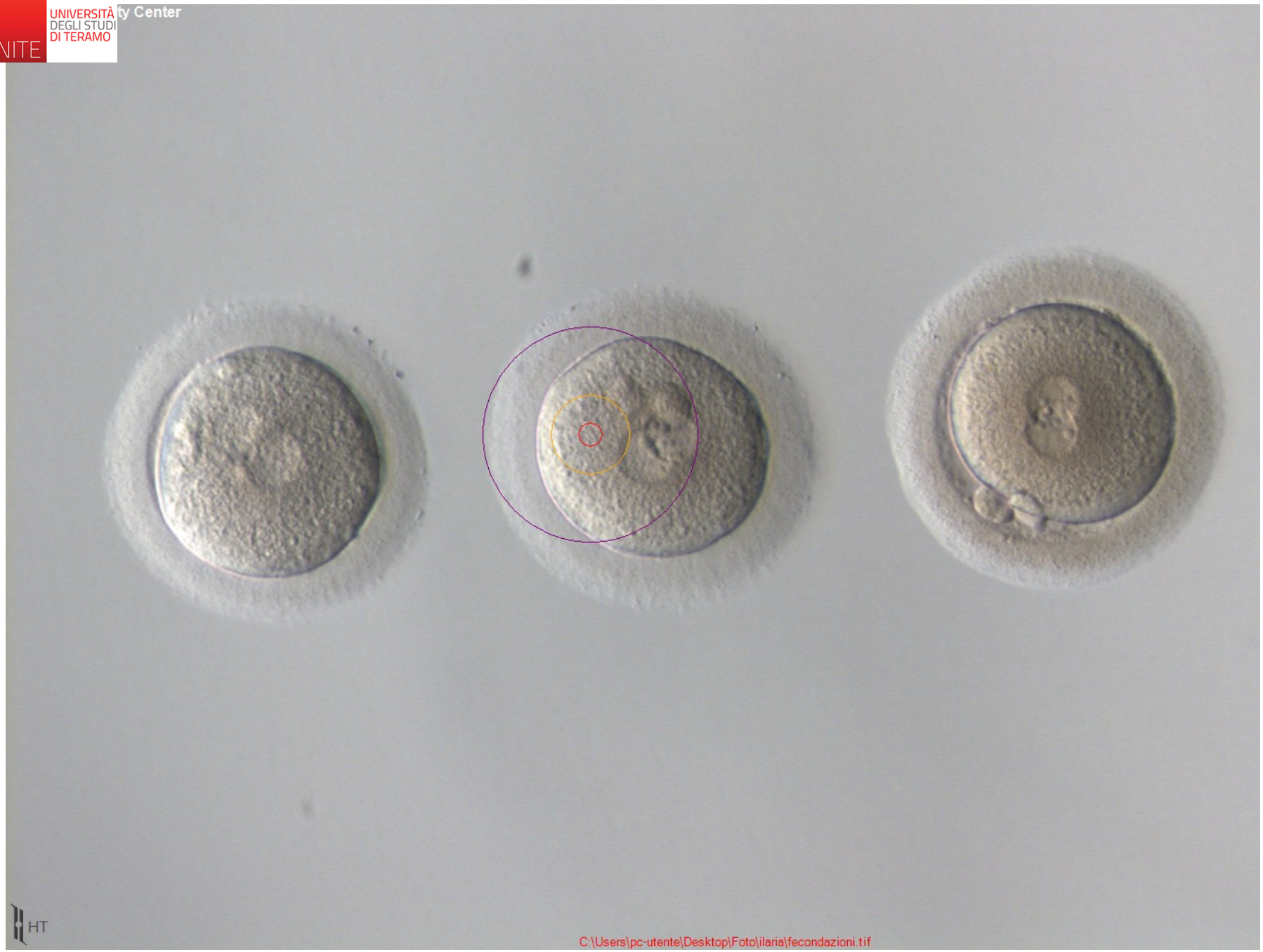
Grade 2: scattered, eqaulity in NPB's



Grade 4: non eqaulity in numbers



Scott, L., Finn, A.,"Morphologic parameters of early cleavage-stage embryos that correlate with fetal development and delivery." 2007. *Human Reproduction,* 22 pp. 230–240.




Bad quality grade 4 zygote
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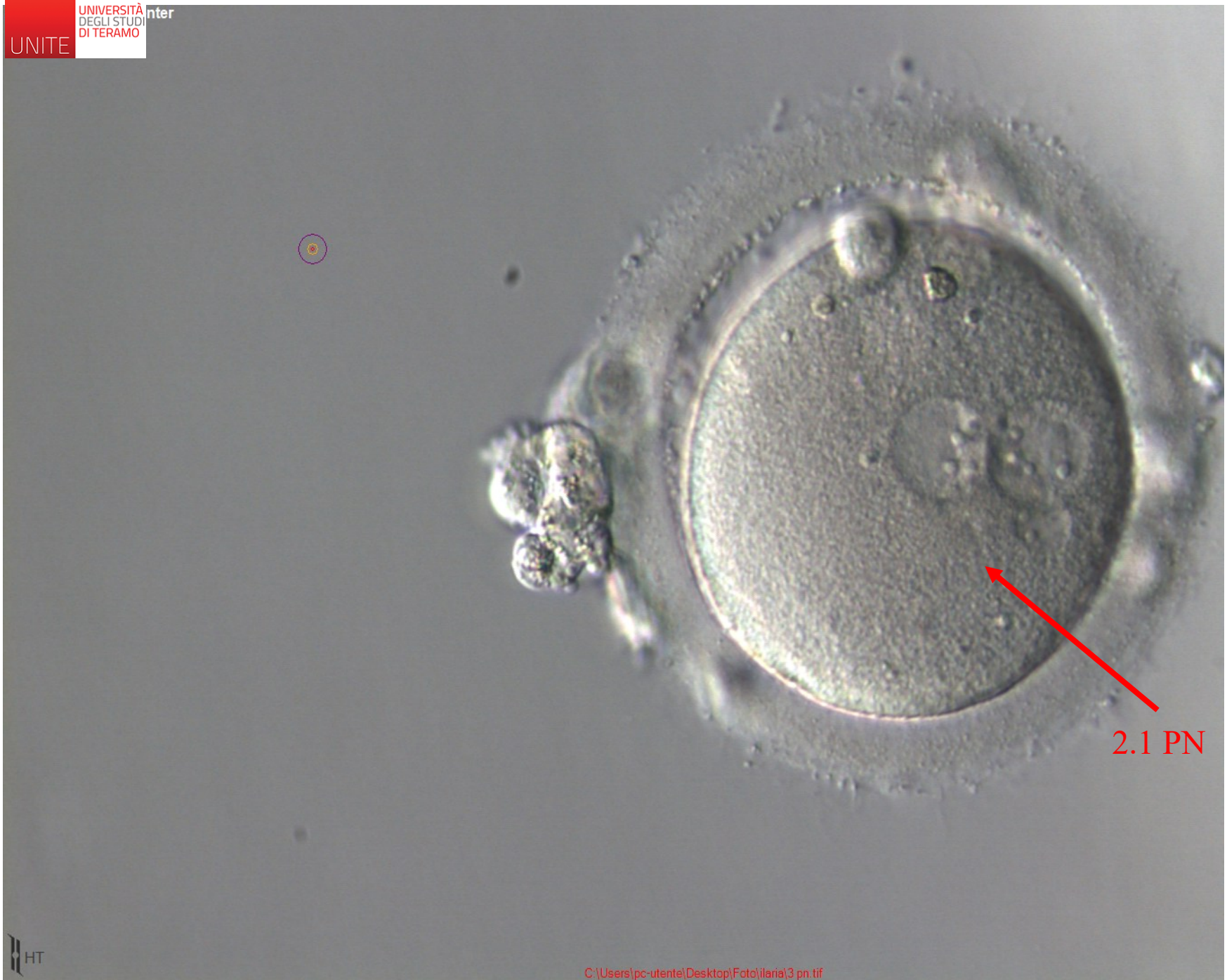
2.1 PN
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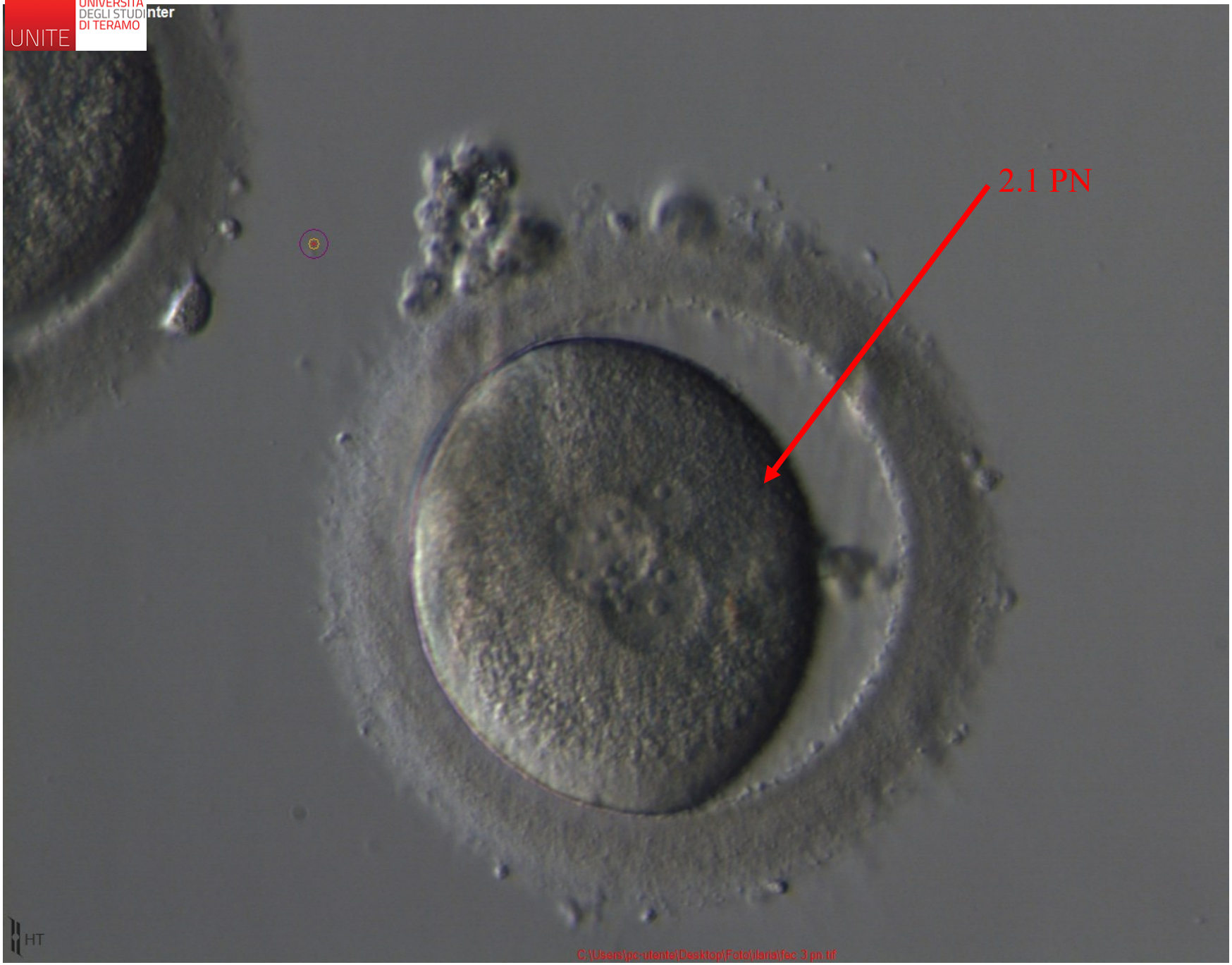
2.1 PN
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1 PN
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Grade 3 Zygote

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