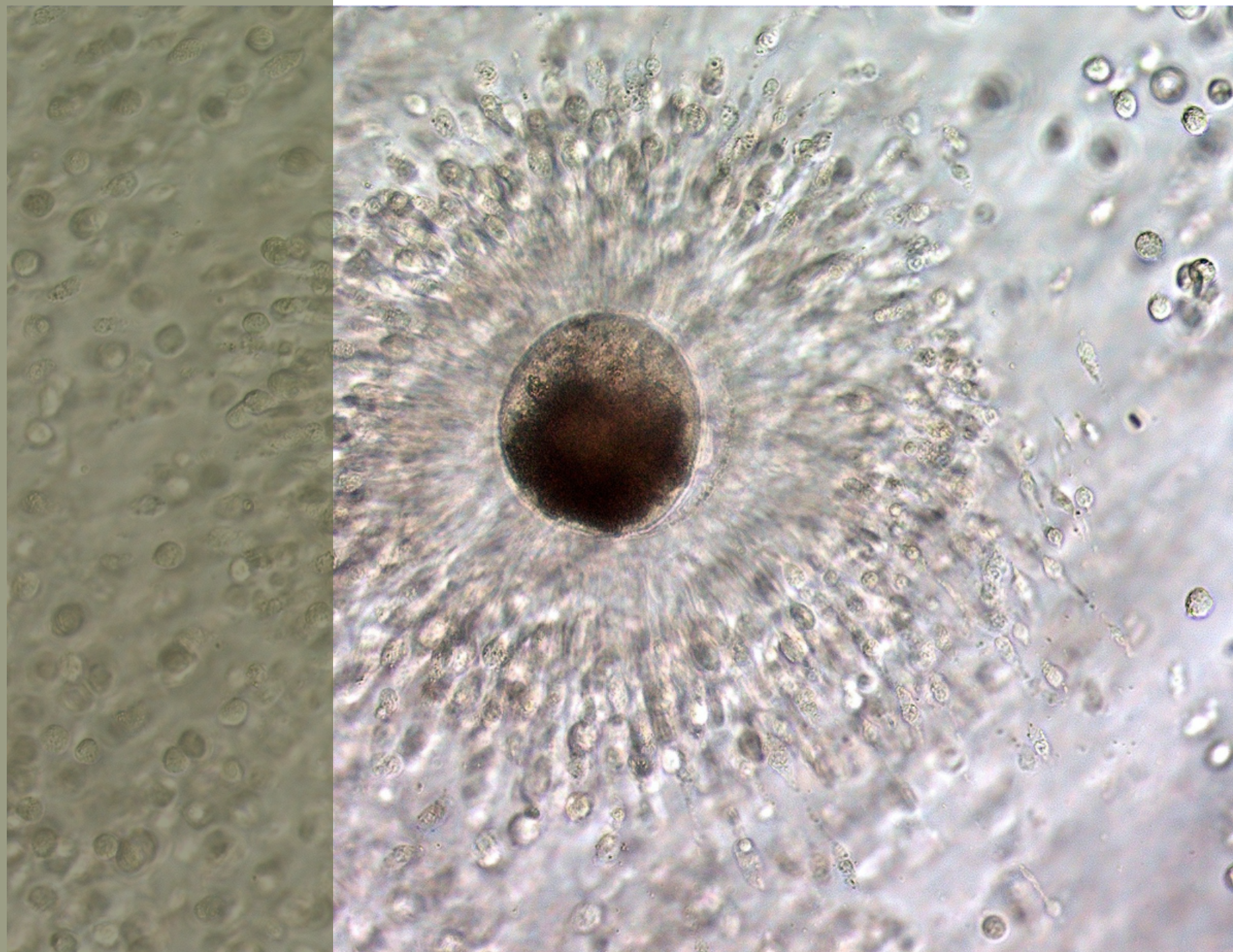




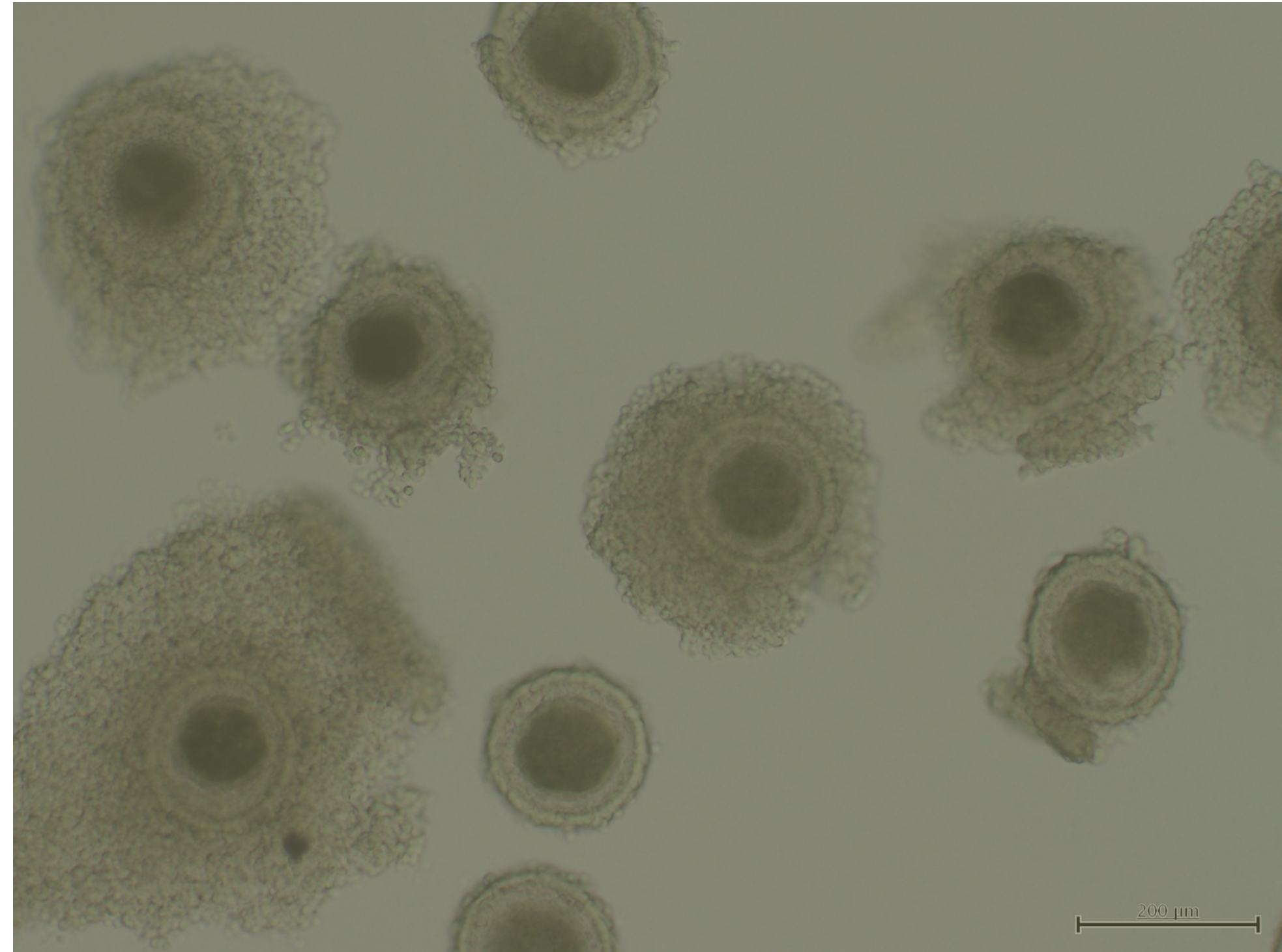
OPU-ICSI FOR PRACTITIONERS

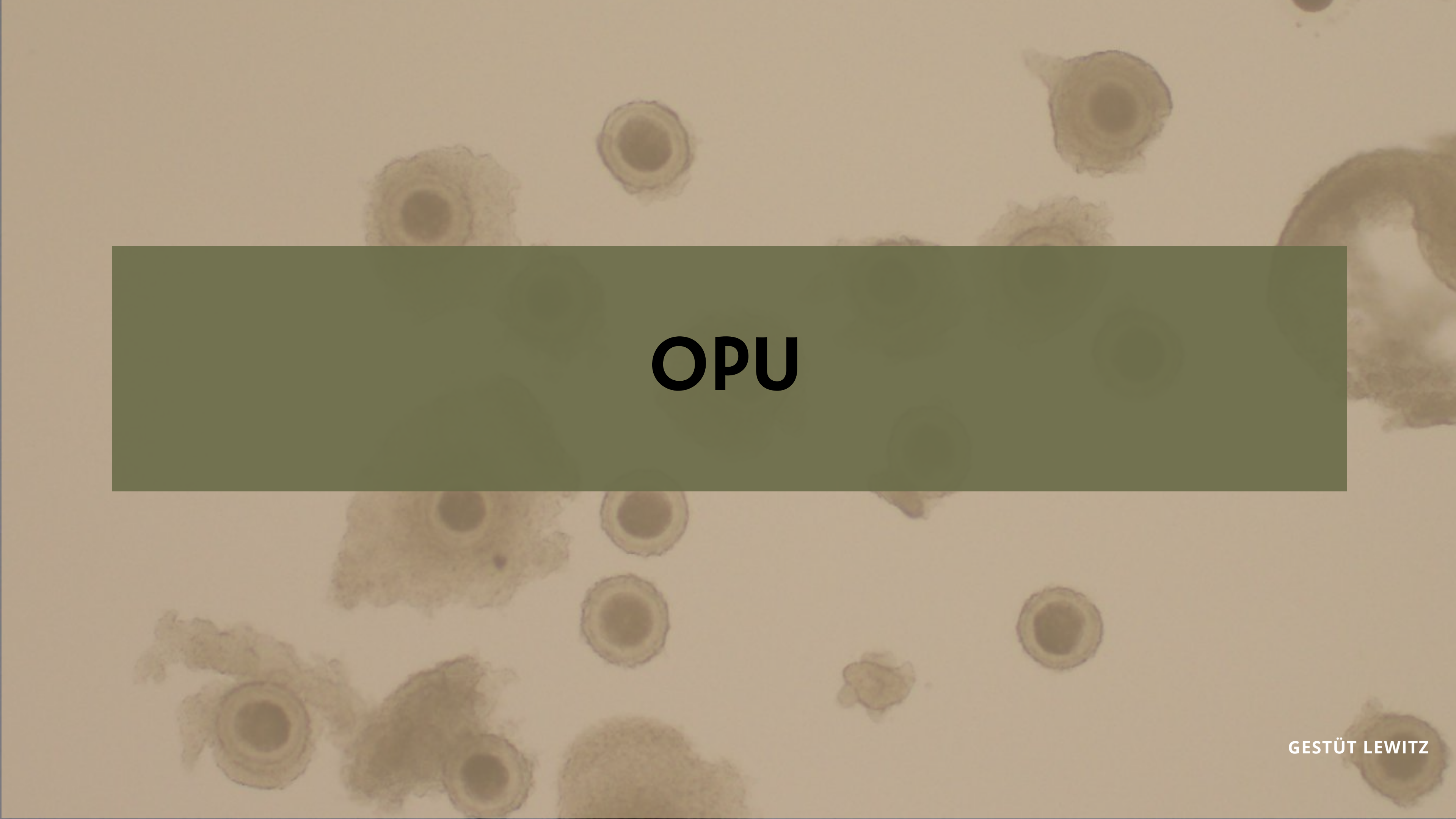
MIGUEL BLANCO

Gestüt Lewitz

May 10th, 2023

- ***O*vum *P*ick-*U*p**
- ***I*ntra*C*ytoplasmatic
*S*perm *I*njection**

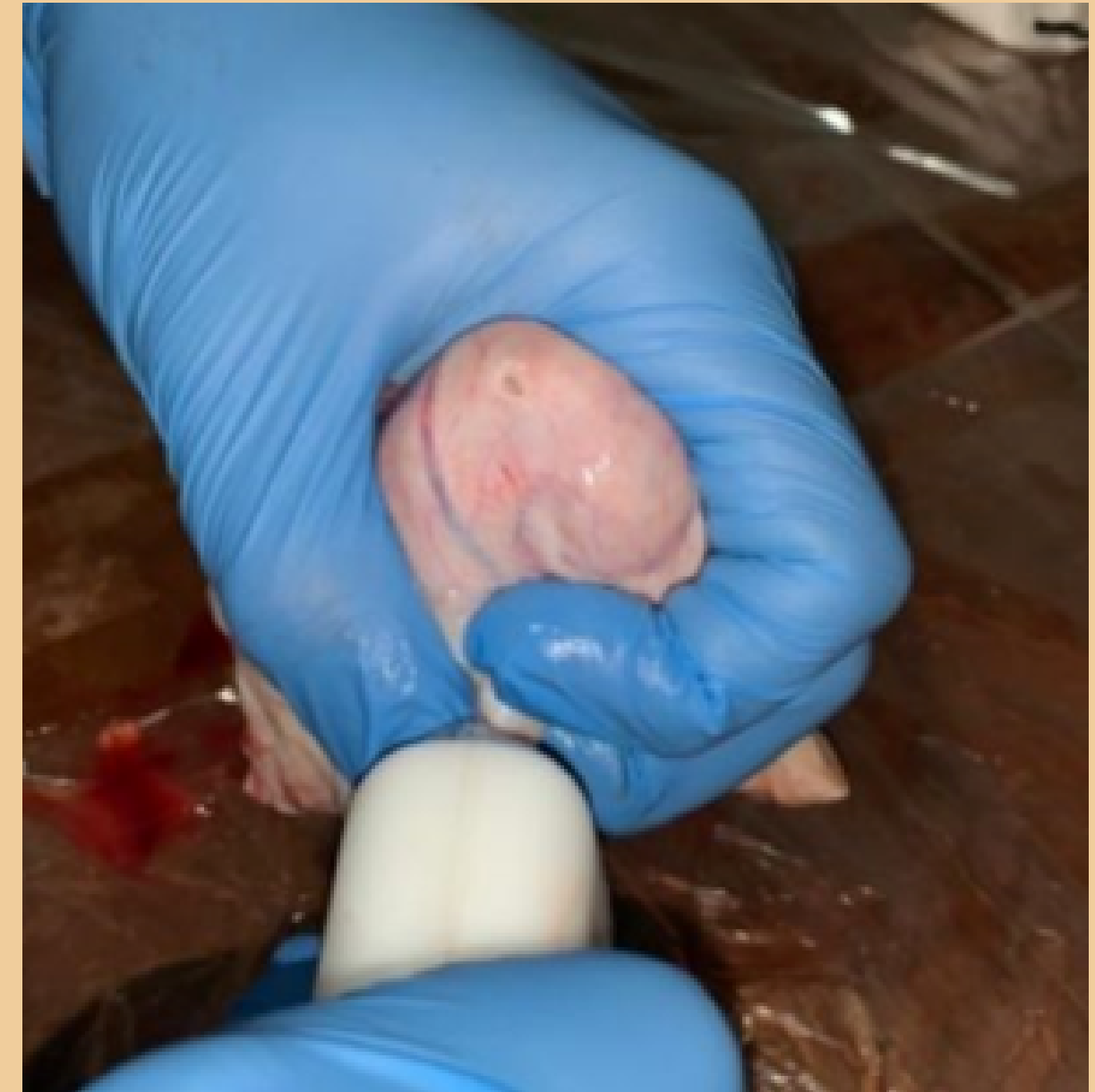


The background of the slide is a microscopic image showing various cells, likely oocytes, with prominent nuclei and surrounding cytoplasm. A solid green rectangle is centered on the slide, containing the text 'OPU'.

OPU

According to the type of aspiration:

- Laparotomy
- Flank
- *Post mortem*
- **Transvaginal**



By follicle type:

N° Follicles

-

+

% Blastocysts

+

-

% Recovery

-

+

M *in vitro*

-

+

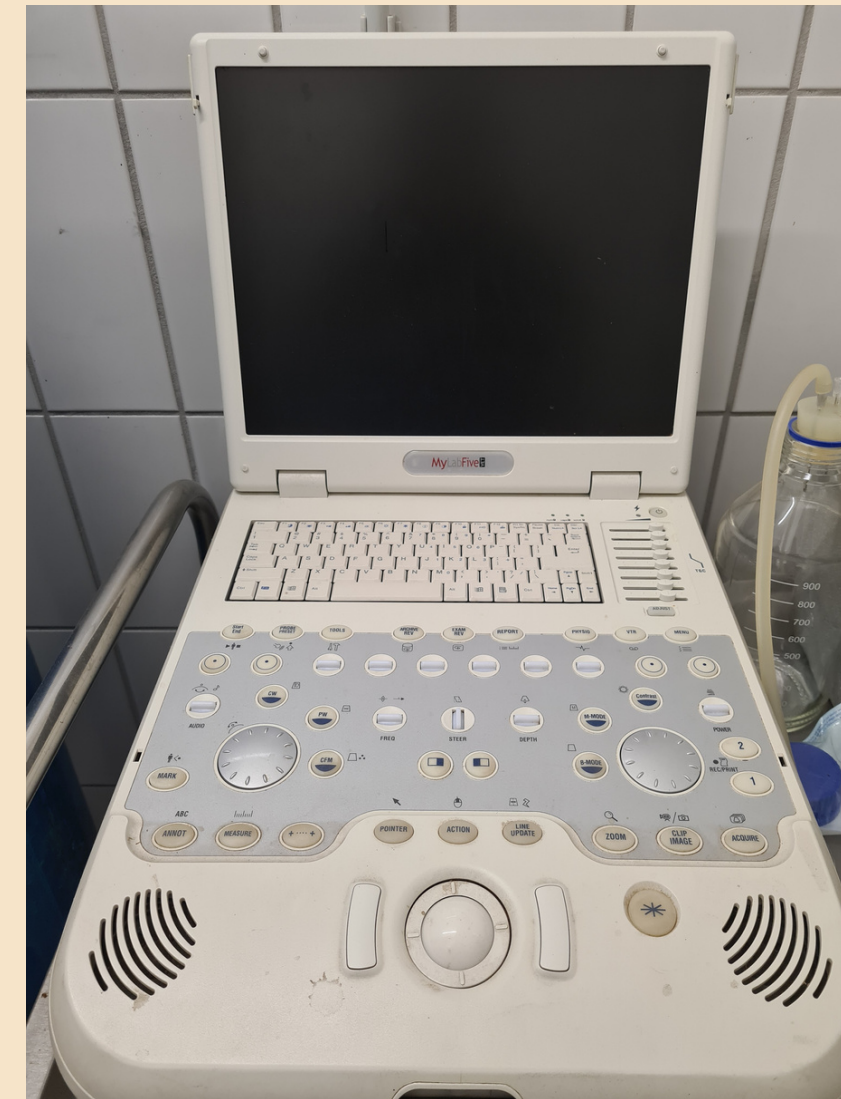
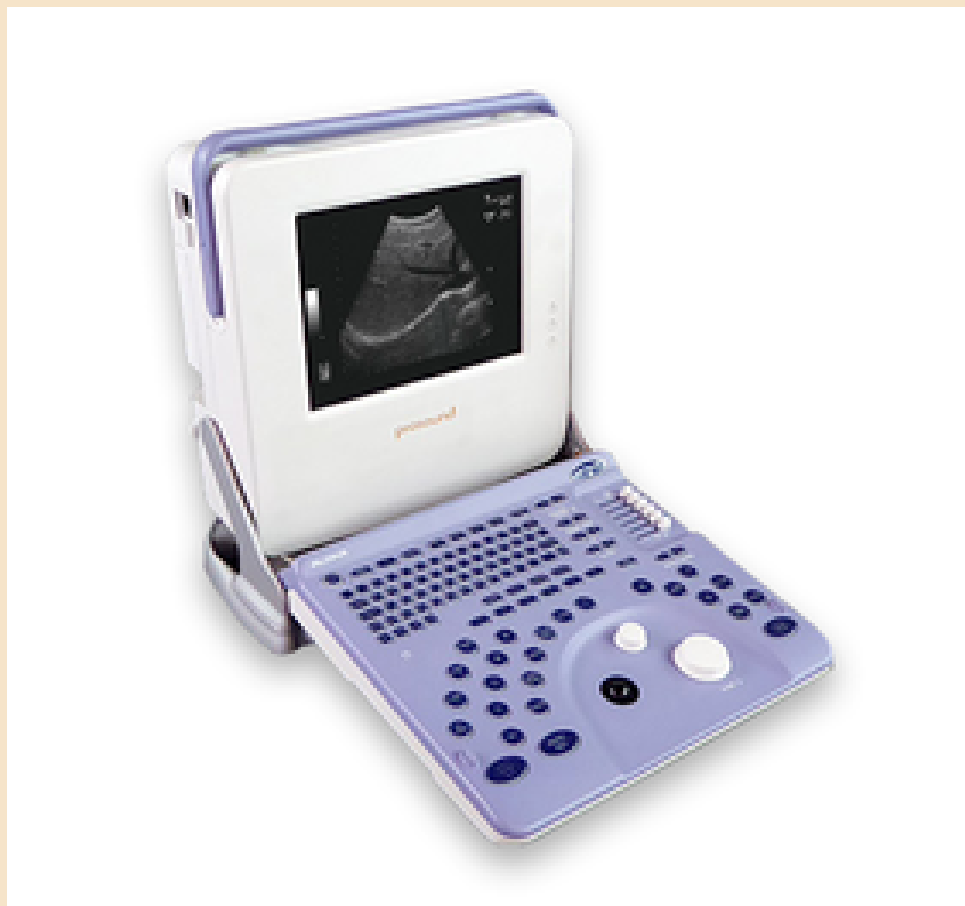
When do we aspirate?

- Dominant follicle: 28-30 hrs after GnRH analogue injection
- Subordinate follicles: follicular control and aspiration
 - >7-8 follicles
 - >5-6mm Ø



What do we need?

Ultrasound



What do we need?

Probe



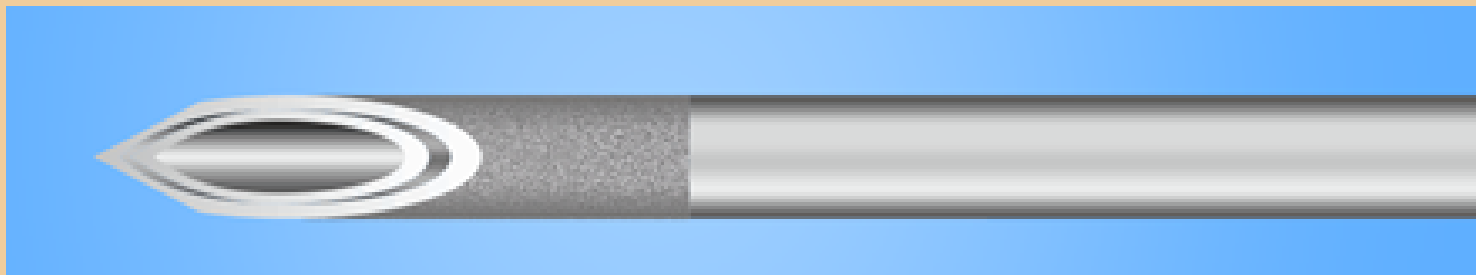
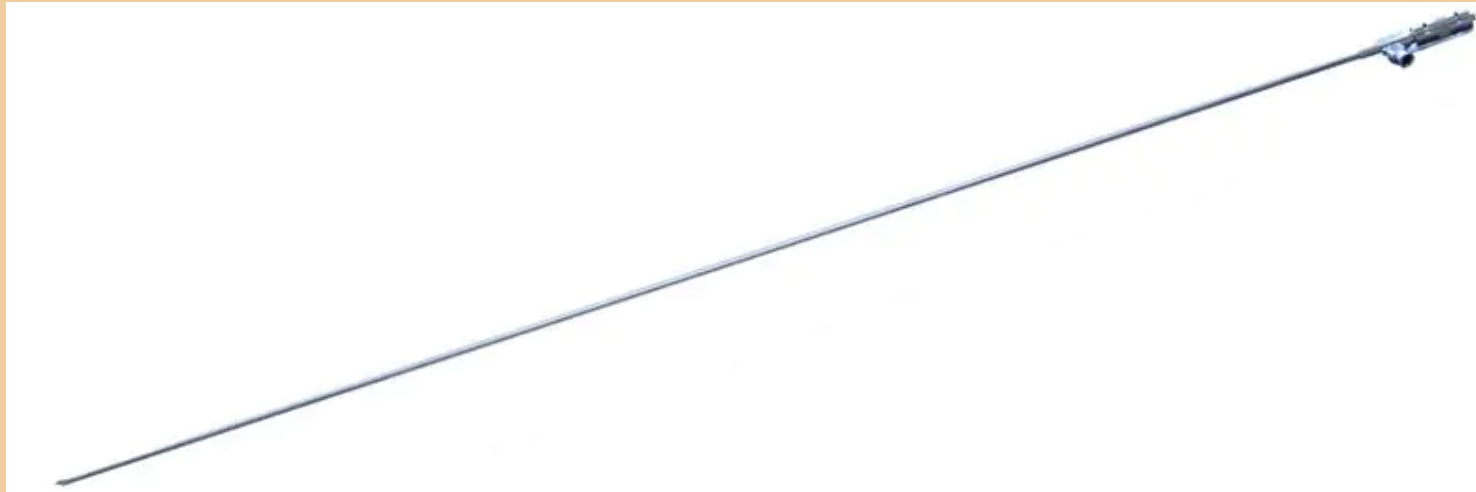
Guide



What do we need?

Needle

- Double lumen
- 12G

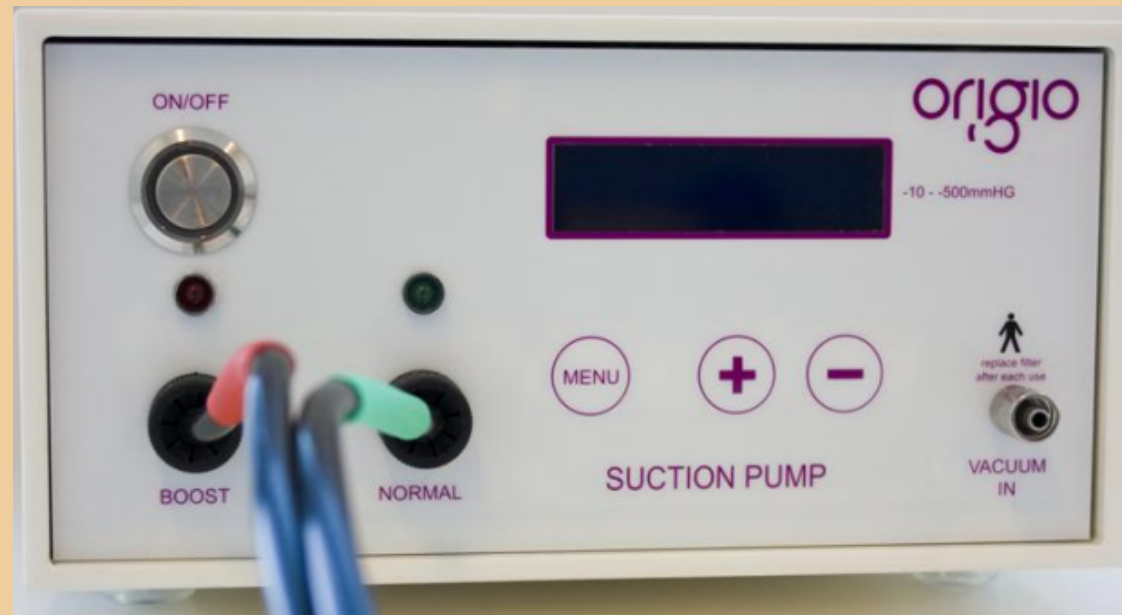


Aspiration media



What do we need?

Vacuum pump



Collection bottle

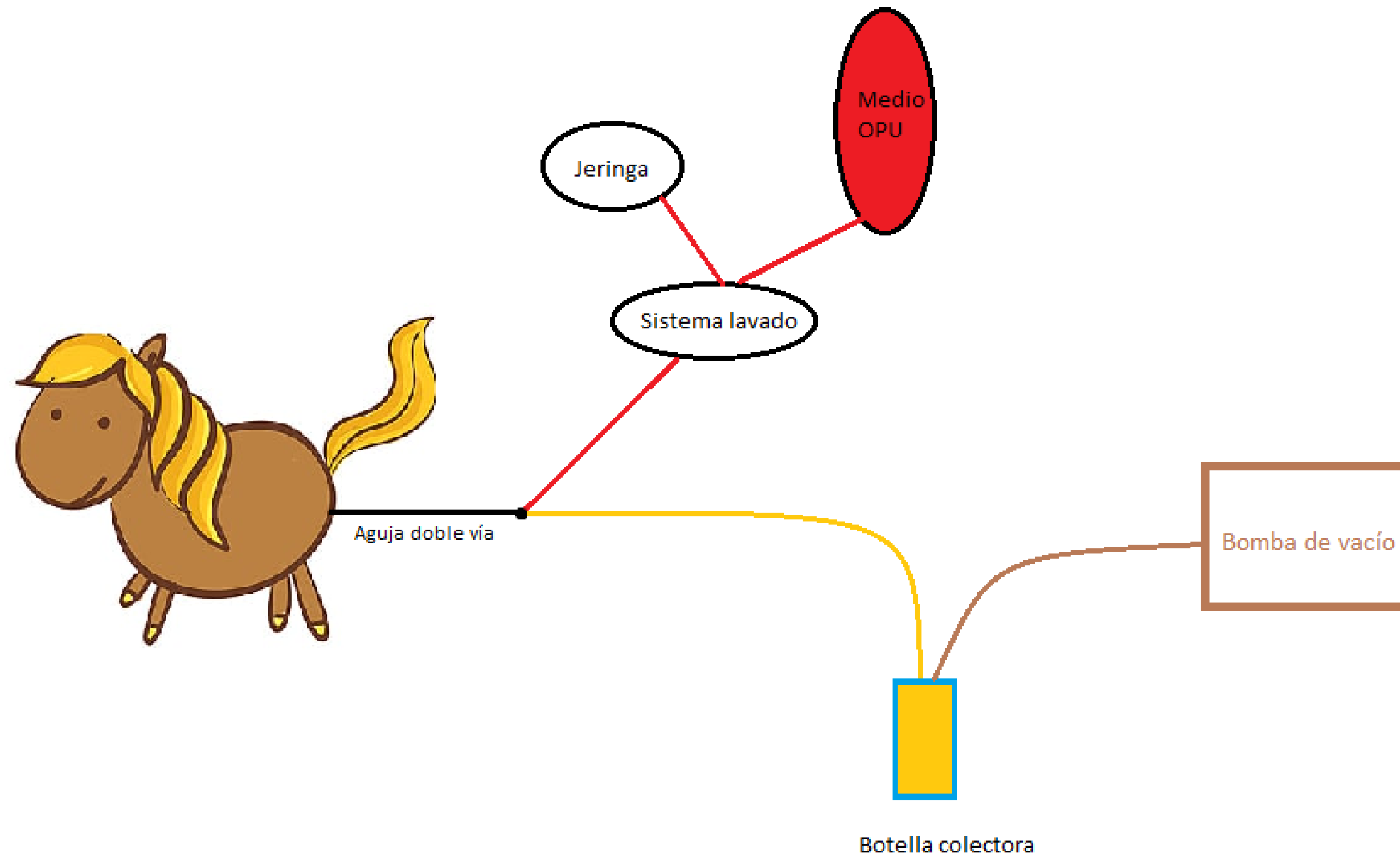


What do we need?

Connect the different elements of the system

- Needle-Collection bottle
 - Needle-Flushing system
- **OPU Media**
- **Syringe**
- Collection bottle-Vacuum pump

The whole system connected



What do we need?

Water bath



Stereo microscope



What do we need?

- Sedation: Detomidine, Butorphanol, Xylazine
- Antispasmodic: Butylscopolamine
- NSAIDs: Flunixin Meglumine
- Antibiotics: Cephalosporin, Peni/Genta...
- Epidural?



MEDICATION



How?

- 3-4 people

Sedation

+

Comfortable work

+

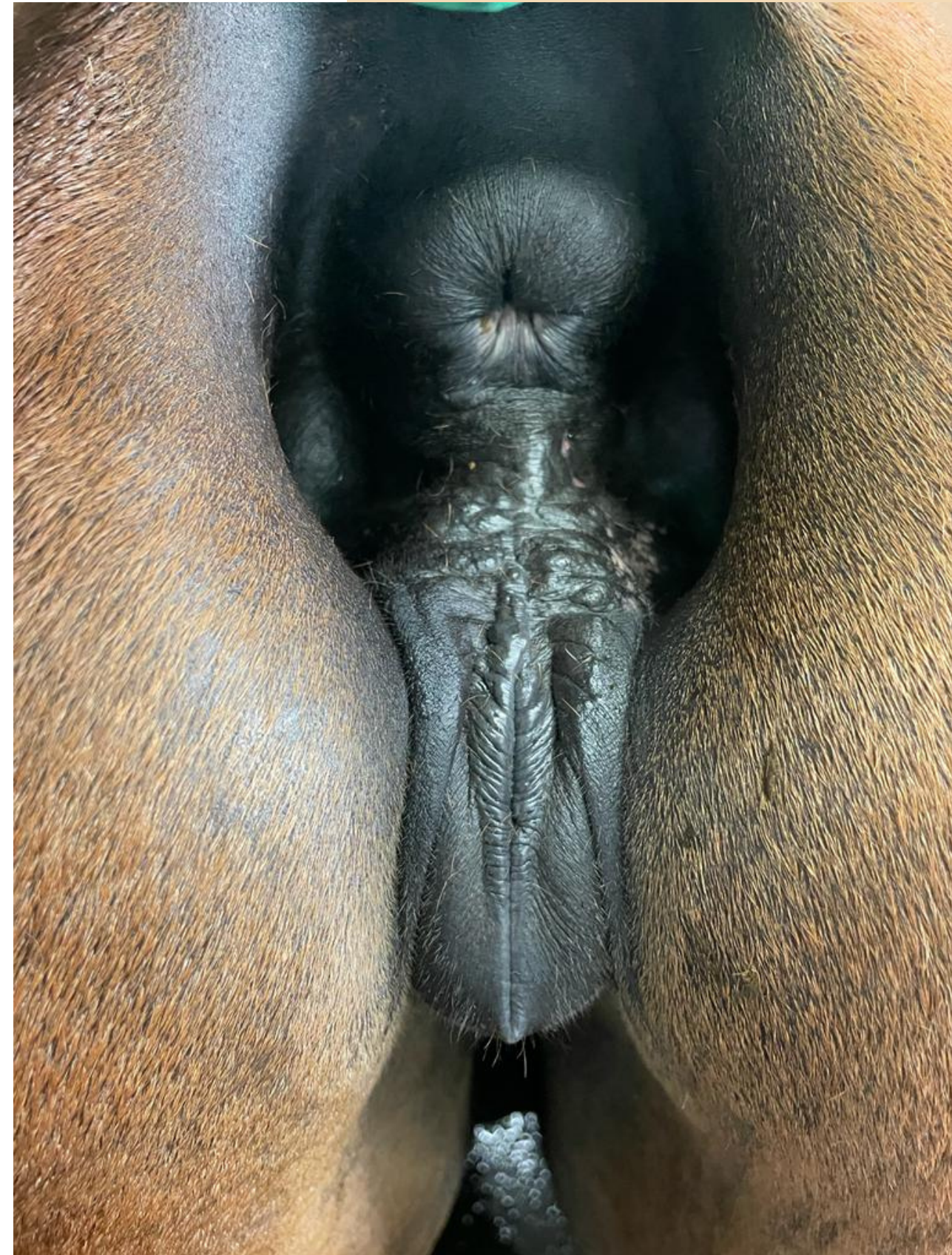
Safe work



GESTÜT LEWITZ

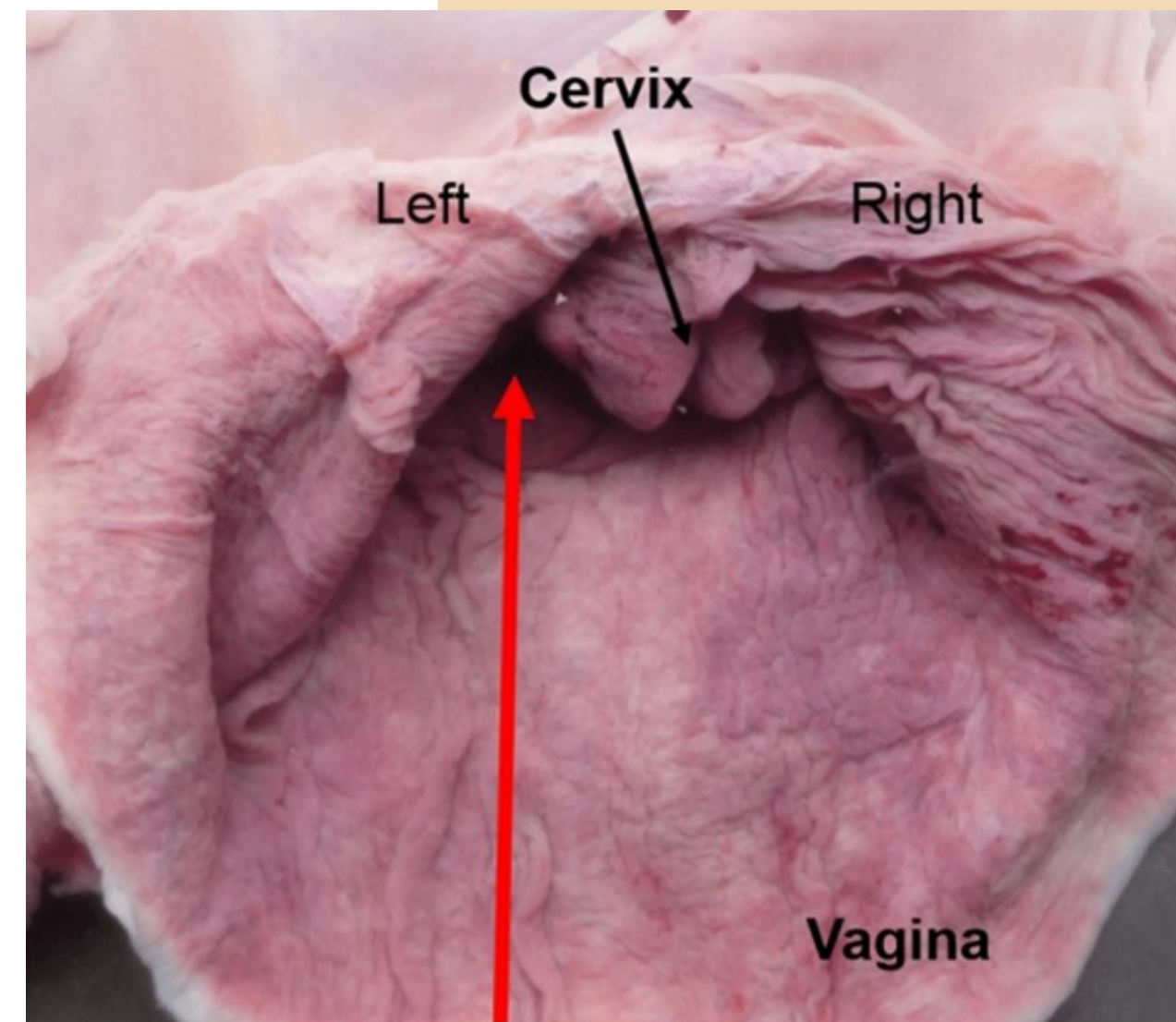
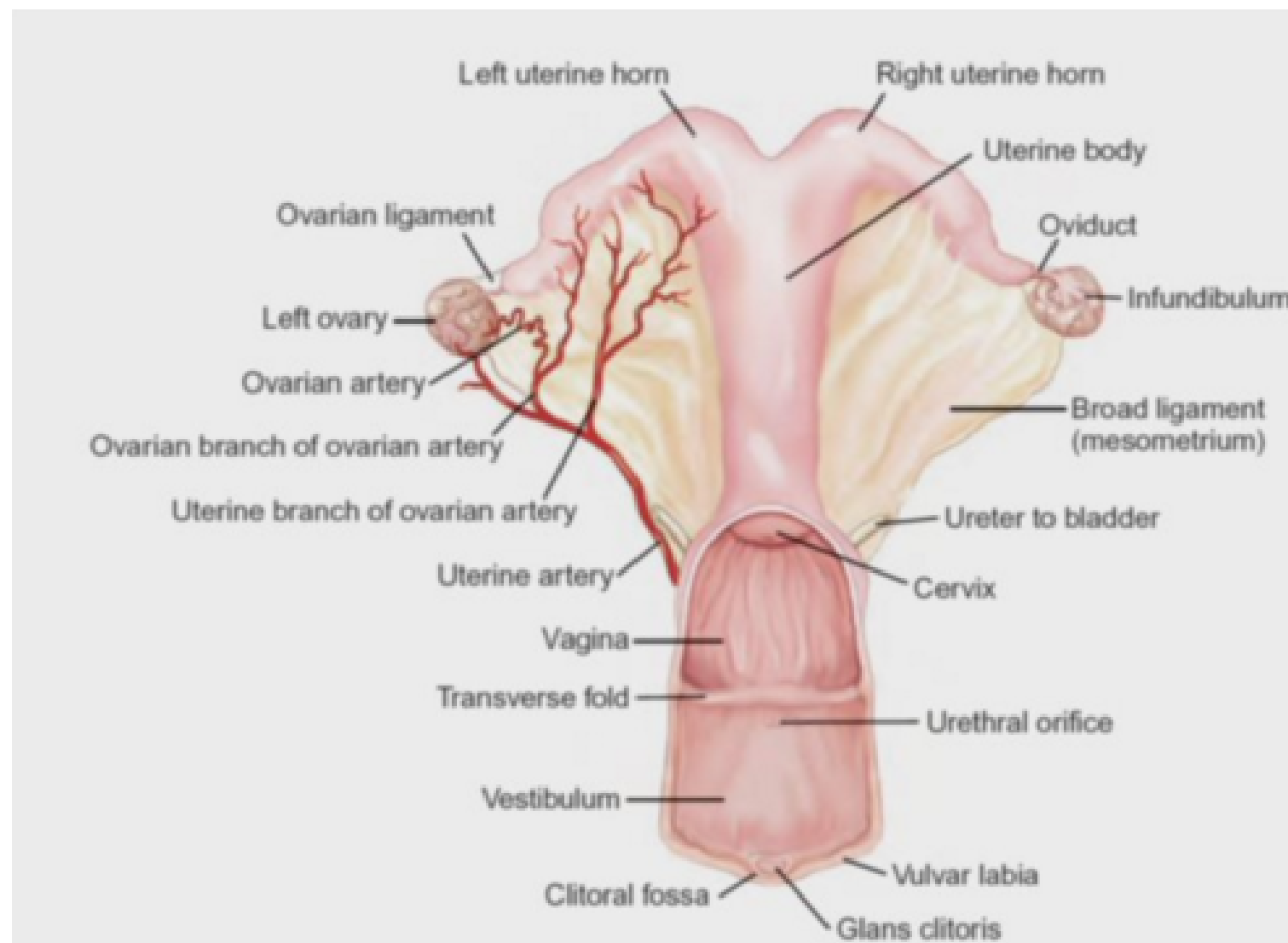
How?

- Perineal area as aseptic as possible



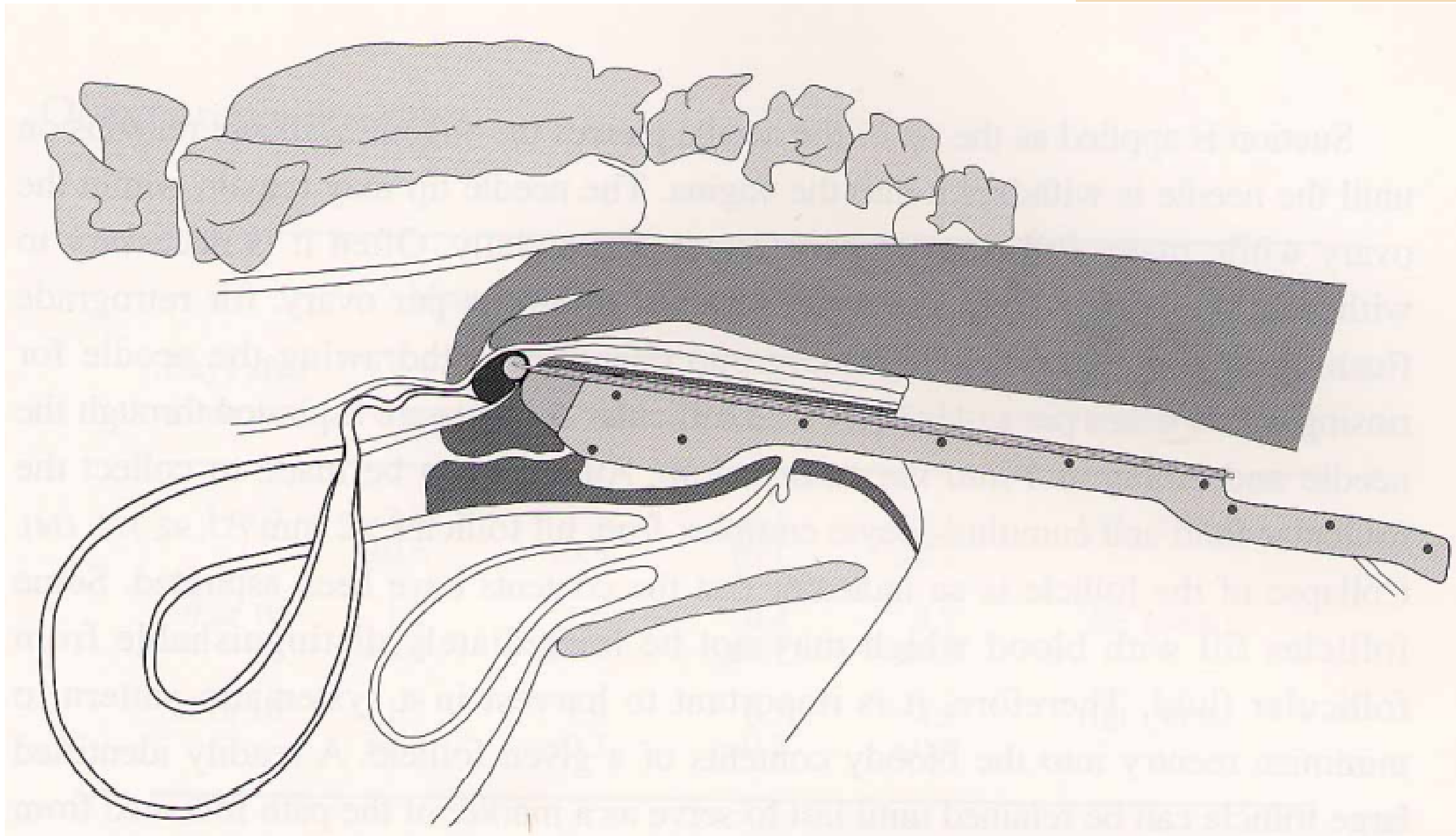
How?

- Disinfected probe-guide combination
- Sanitary sheath around the set
- We pass through the vulva, vestibule and vagina



How?

- Transrectally, we place the ovary in the cranial part of the vagina



How?

- We face the ovary with the probe
- When we see the follicle with no other opposition than the wall of the vagina, we introduce the needle

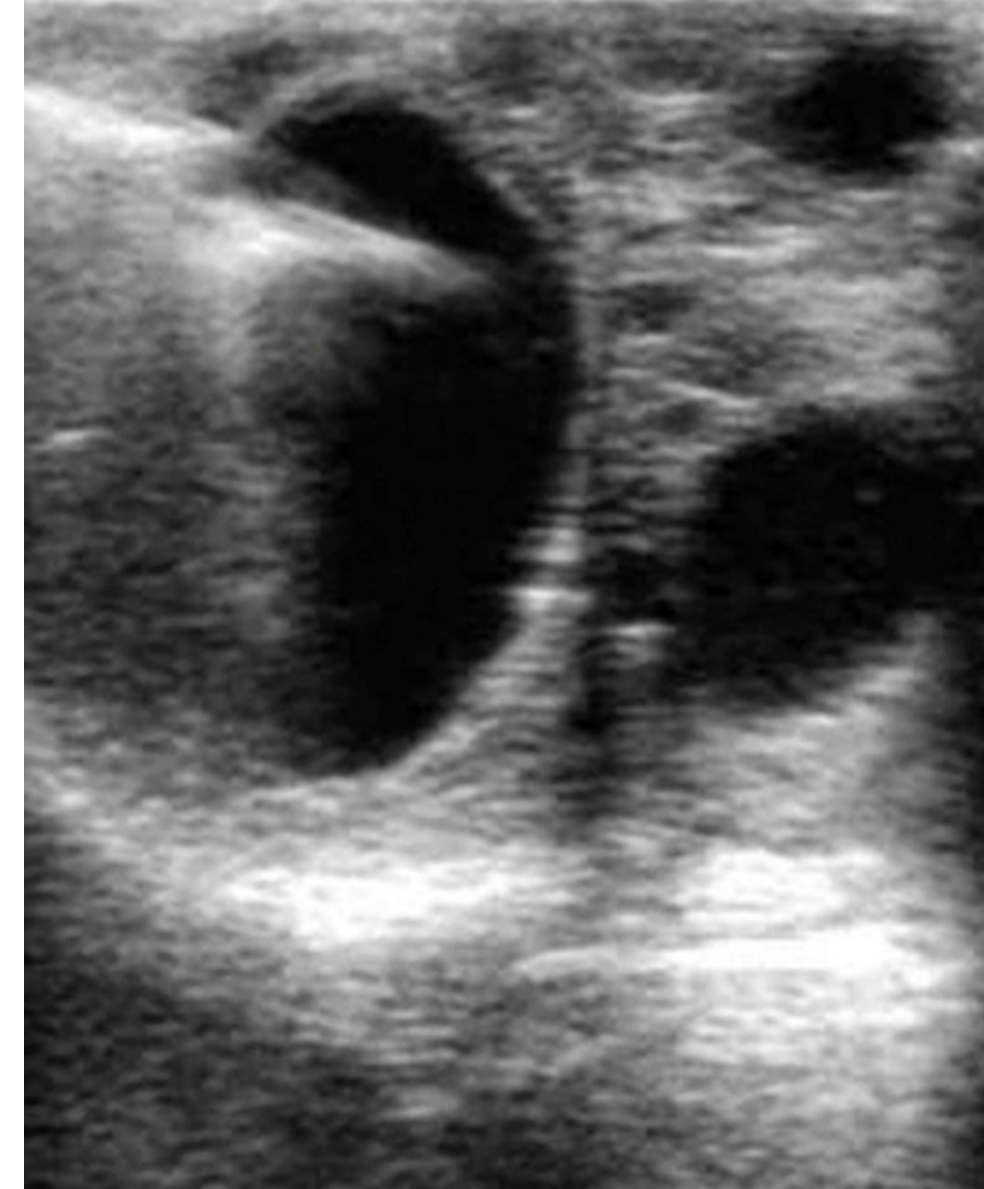


How?

With the needle inside the follicle:

- We hold the ovary firmly and rotate it around the needle
- We rotate the needle over the wall of the follicle
- We wash the follicle several times with aspiration medium

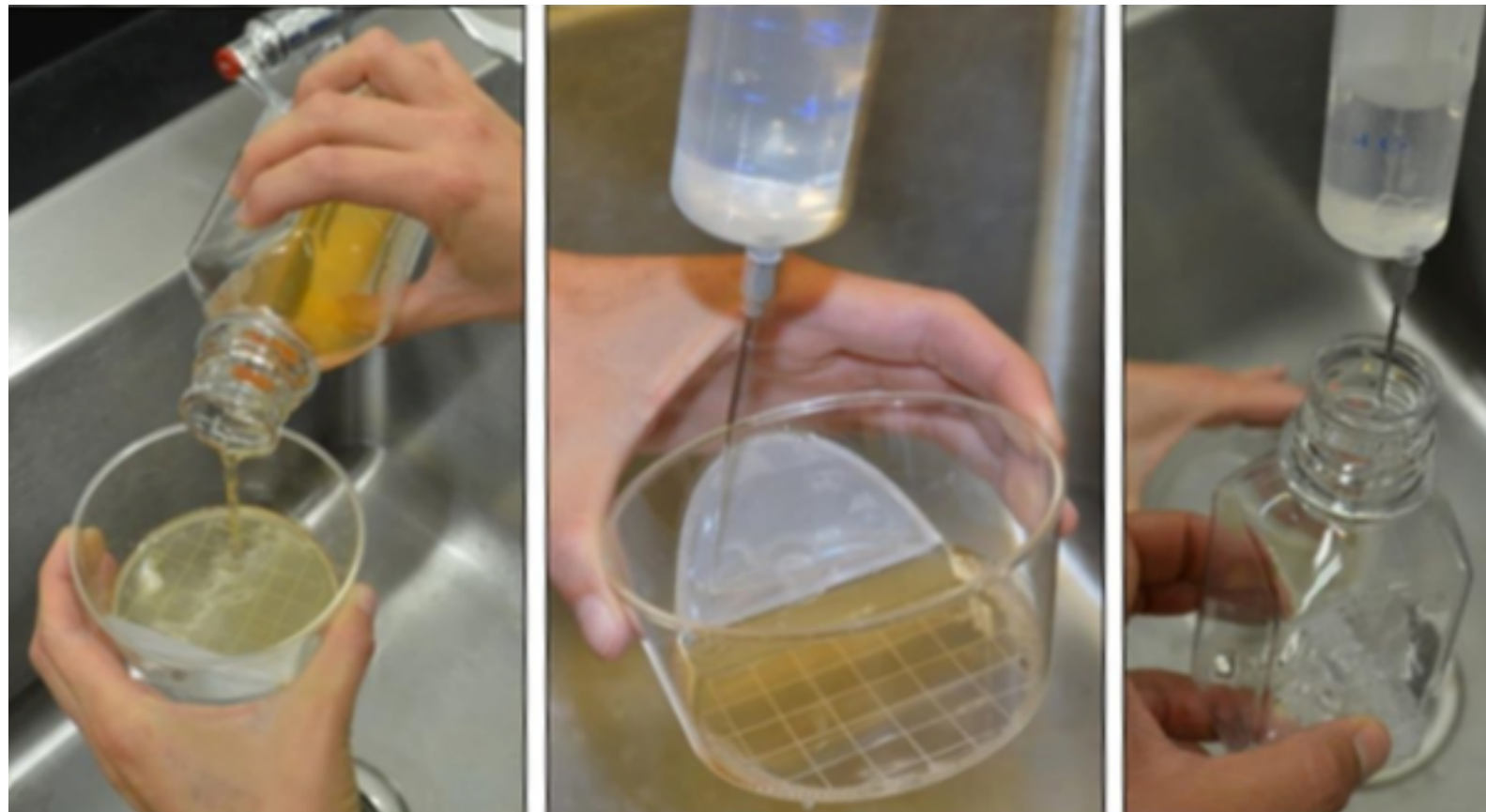
***REPEAT SAME PROCEDURE FOR
EACH FOLLICLE >5MM Ø***



How?

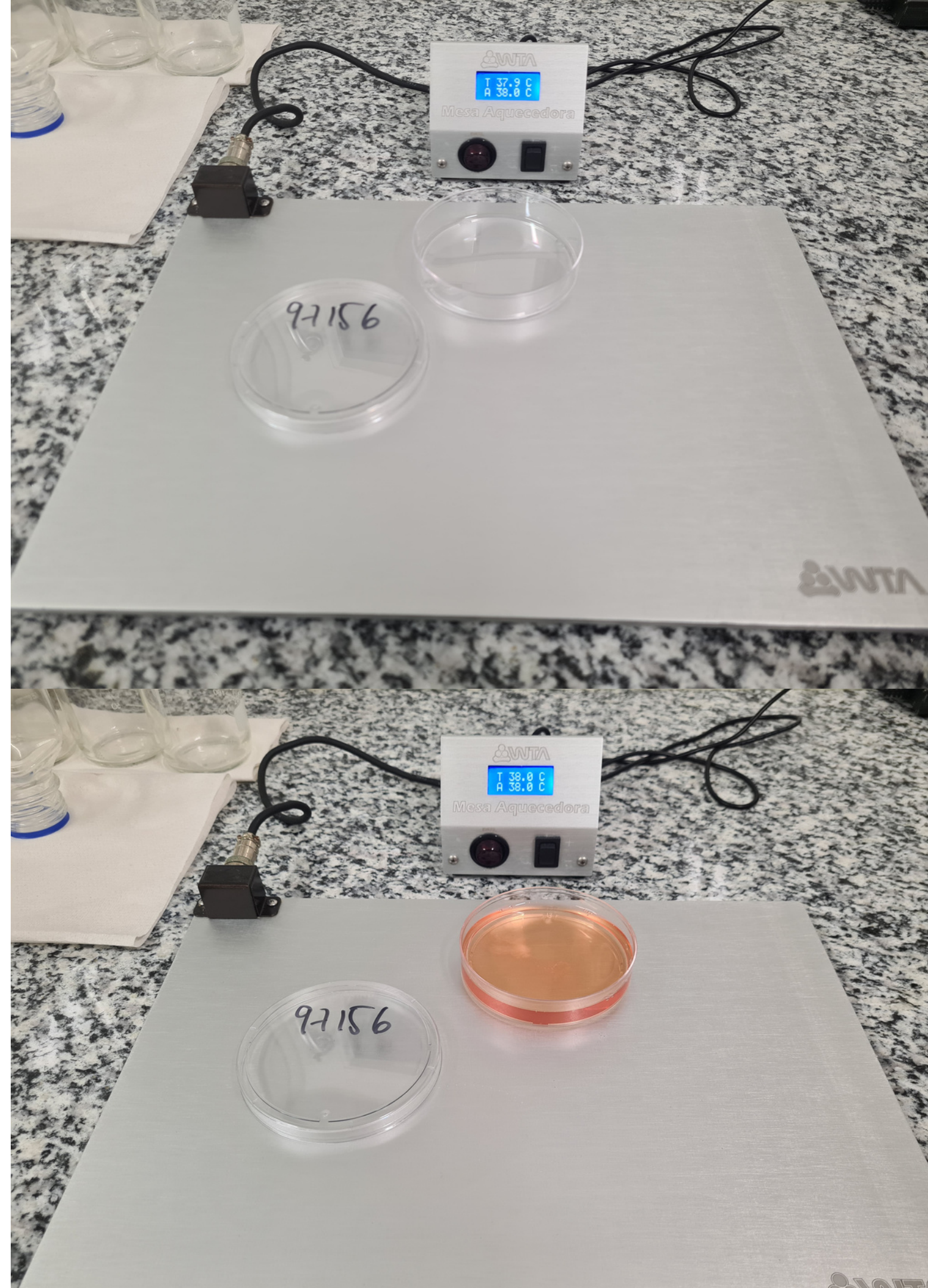
At the end of the OPU of both ovaries, and trying to keep the temperature, we filter the contents of the collection bottle.

Dominant follicle



How?

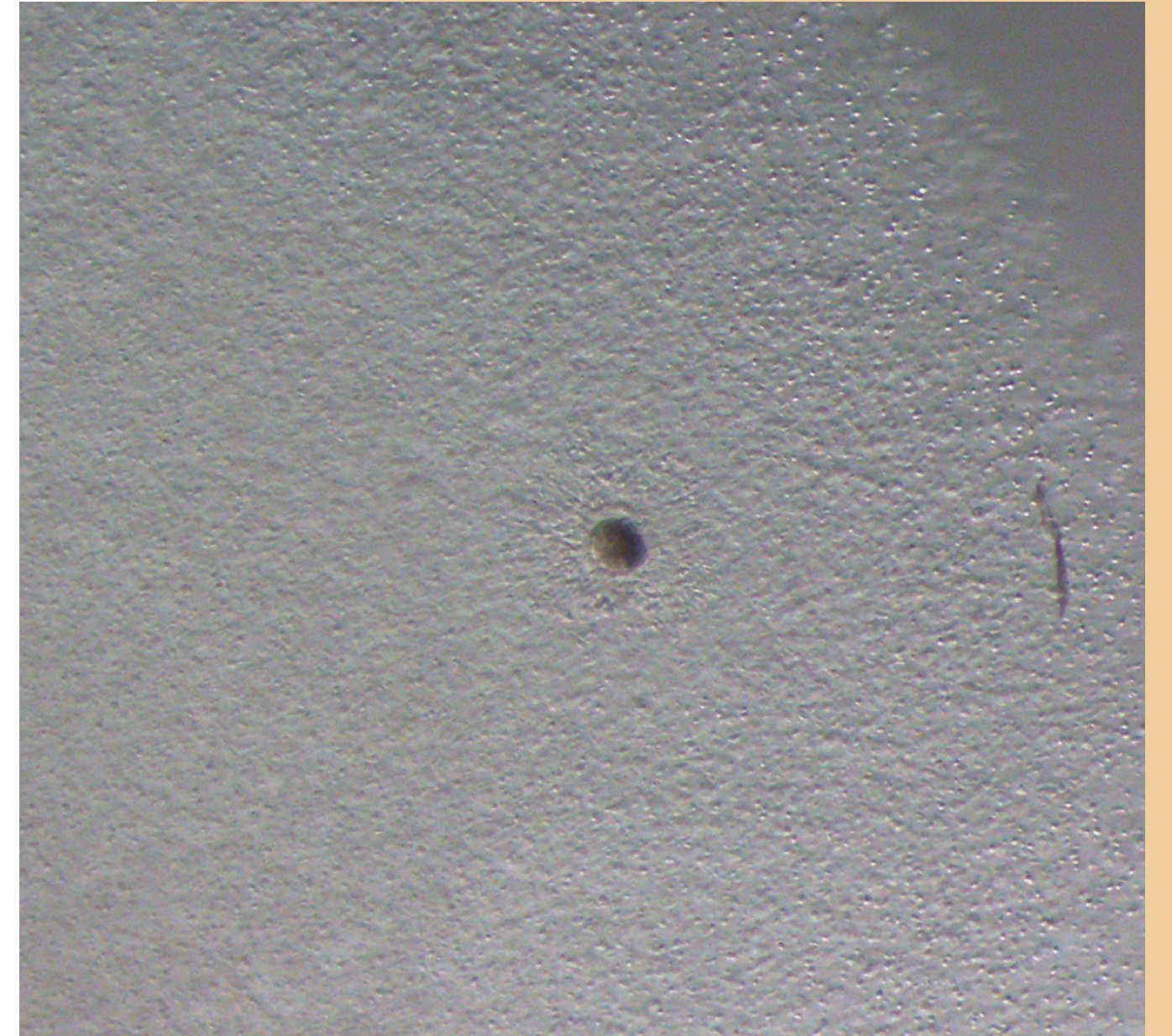
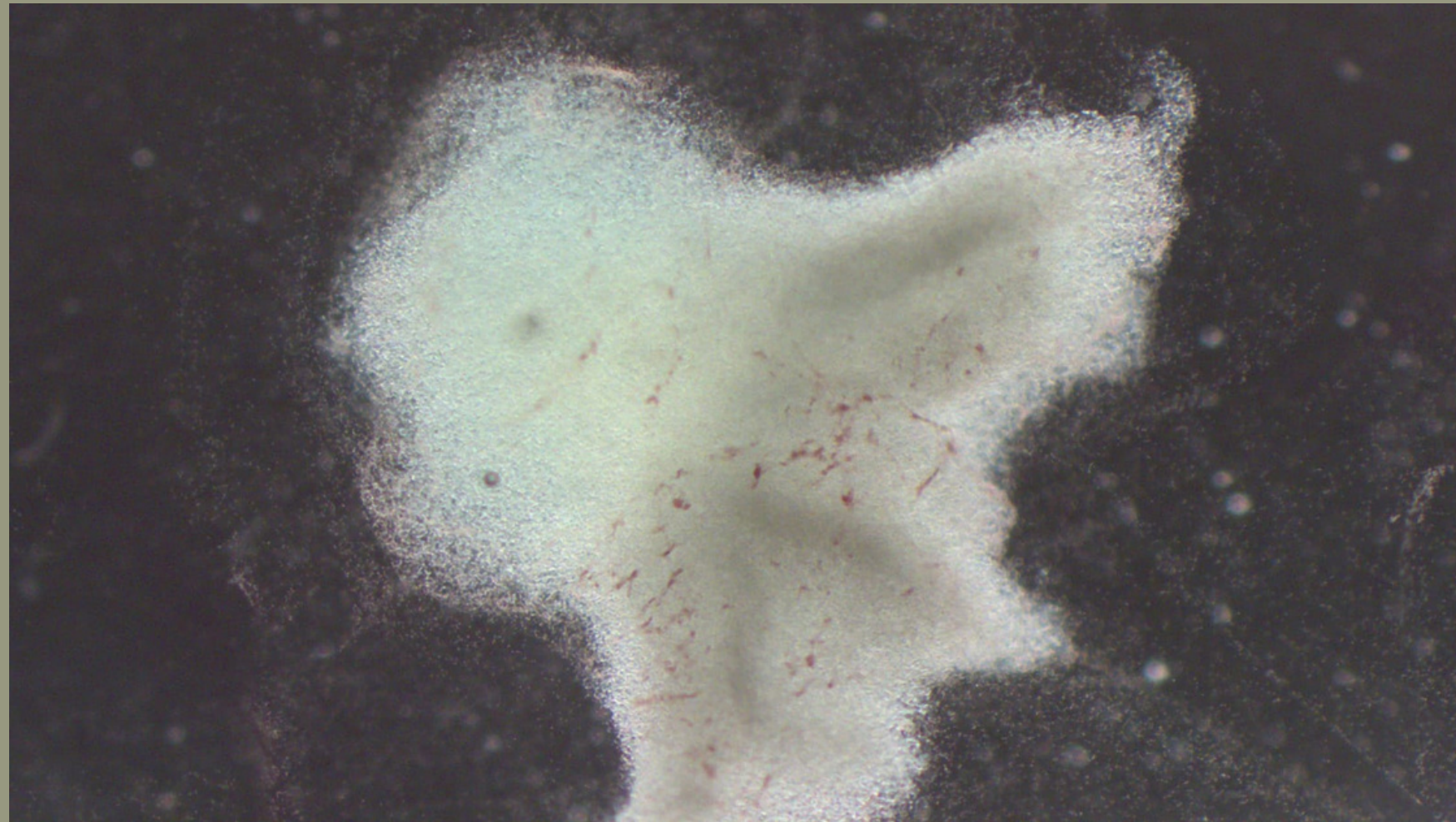
**Pour the contents
of the filter into a
Petri dish**



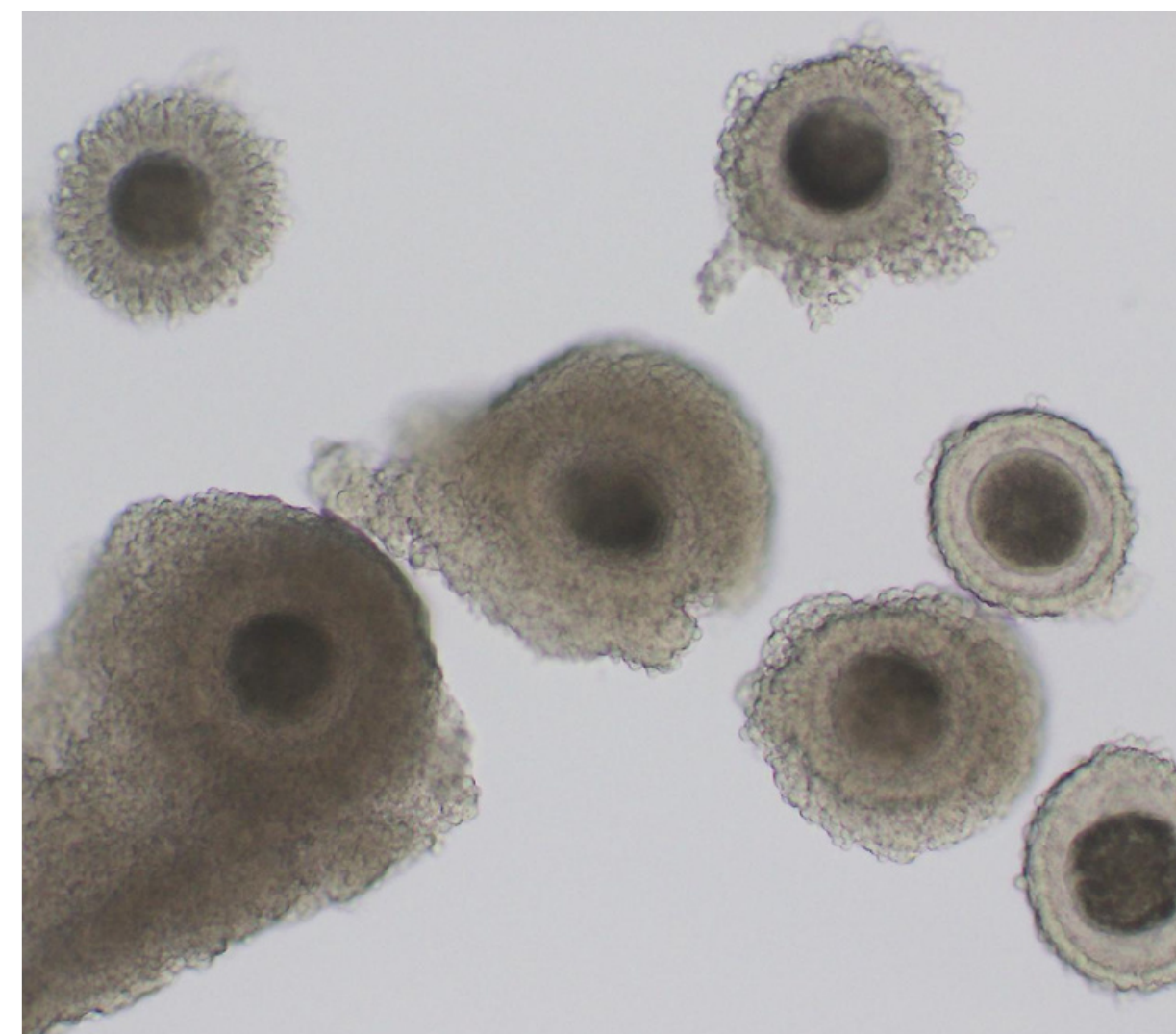
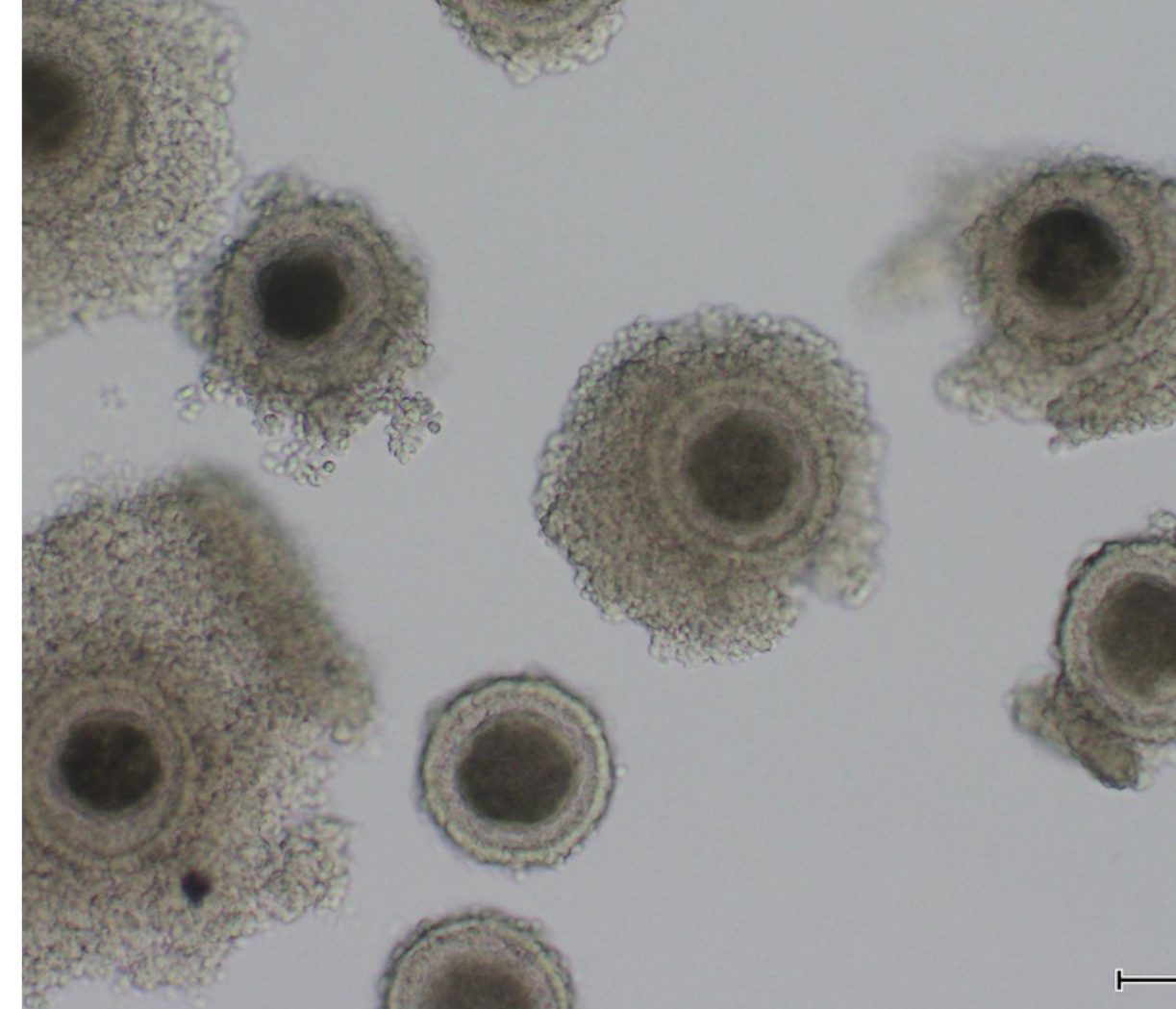
How?

**Under the stereo microscope, we look for and isolate the cumulus-
ocyte complexes (CCO) on
another plate with
aspiration fluid.**





GESTÜT LEWITZ





Once we have all the oocytes, we place them in **holding media**:

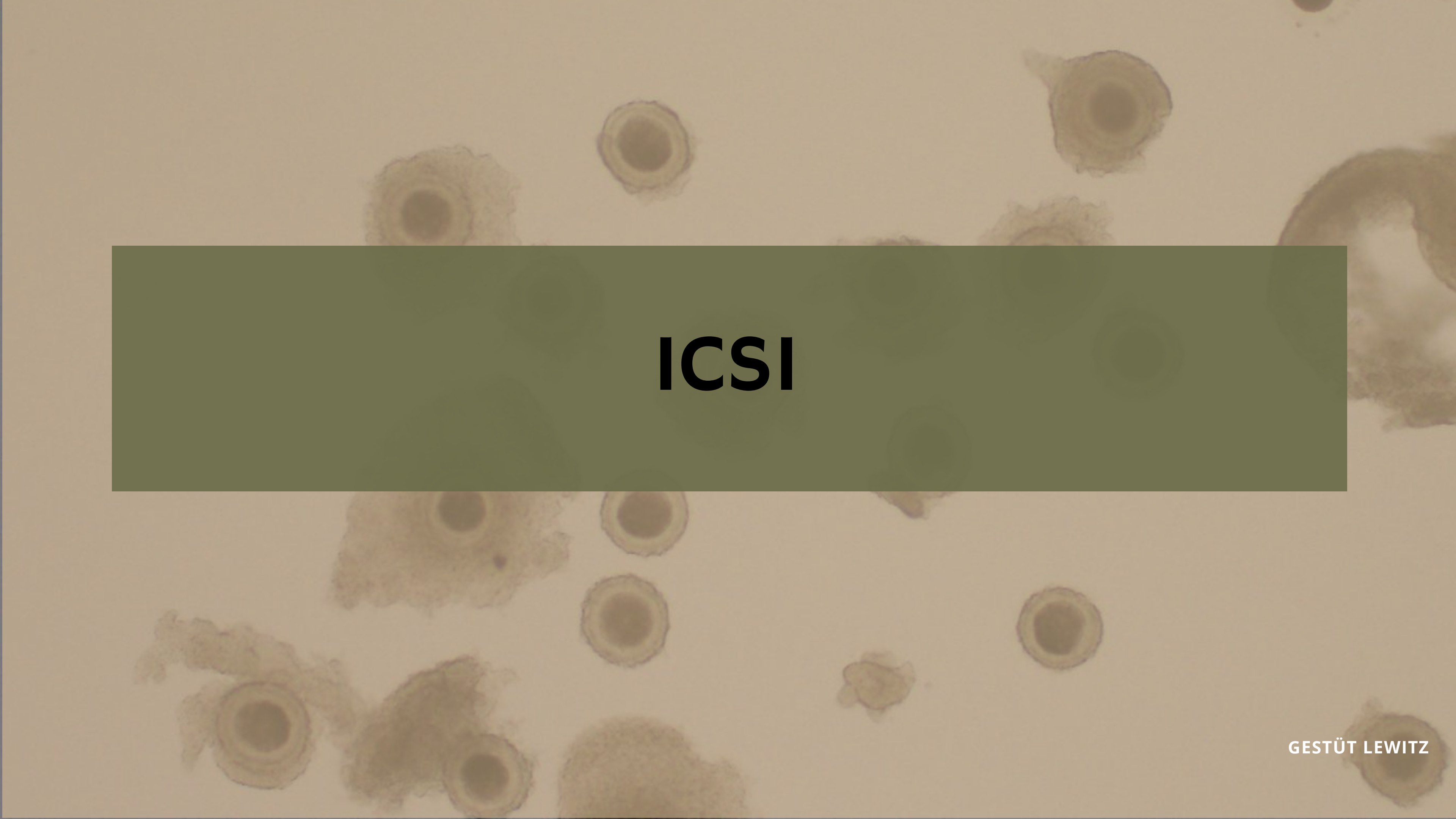
- Modified culture media, buffer, serum, AB...
- Embryo holding

To:

- Send to an external laboratory
- Choose a suitable maturation time

- 20-25°C
- 24hrs



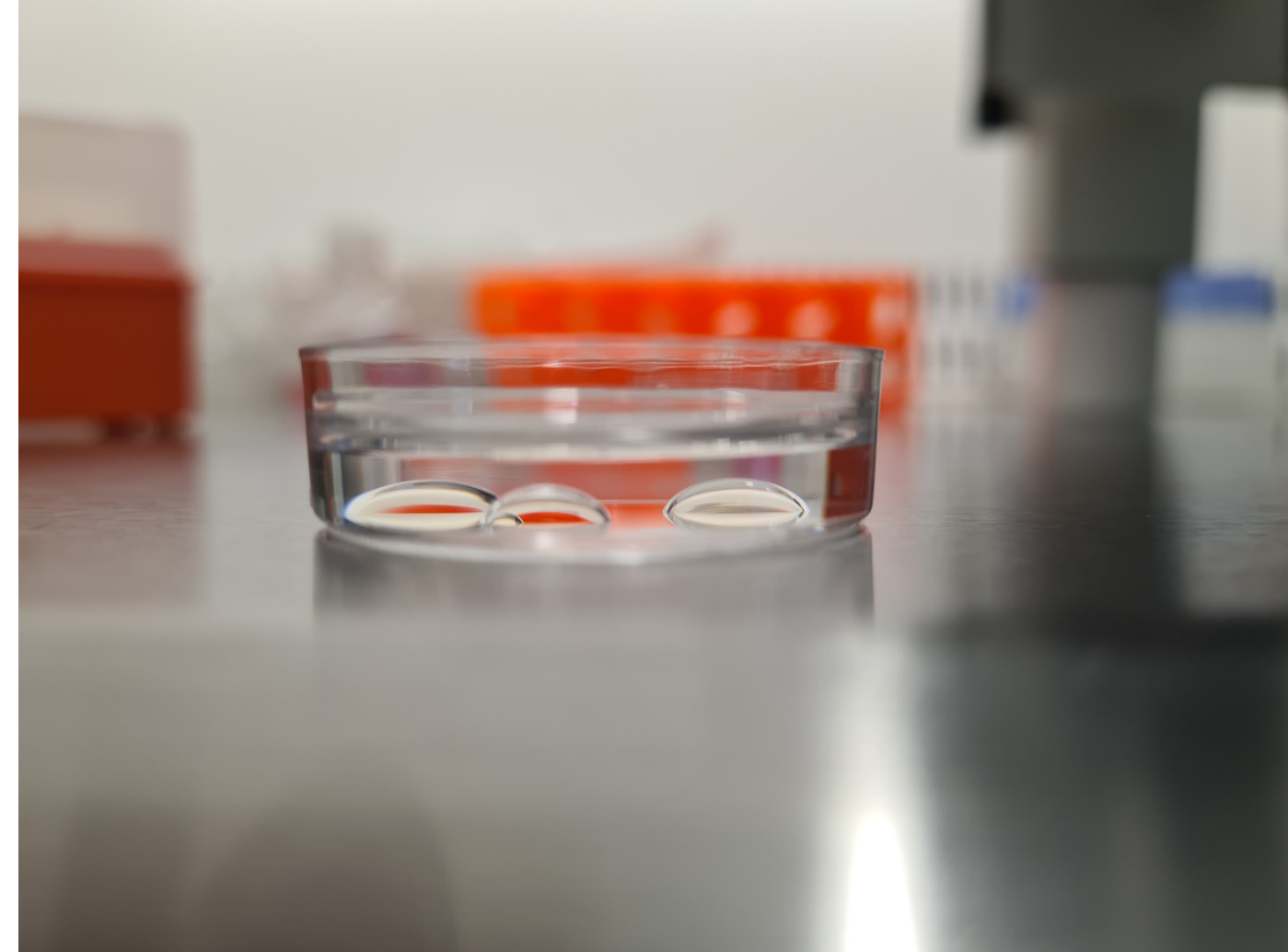
The background of the slide is a microscopic image of several oocytes. These cells are roughly spherical with a prominent, dark, circular nucleus in the center. The cytoplasm is lighter and has a granular texture. Some oocytes are surrounded by a thin, darker outer layer, likely the zona pellucida. The overall color palette is muted, with various shades of beige, tan, and light brown.

ICSI

MATURATION

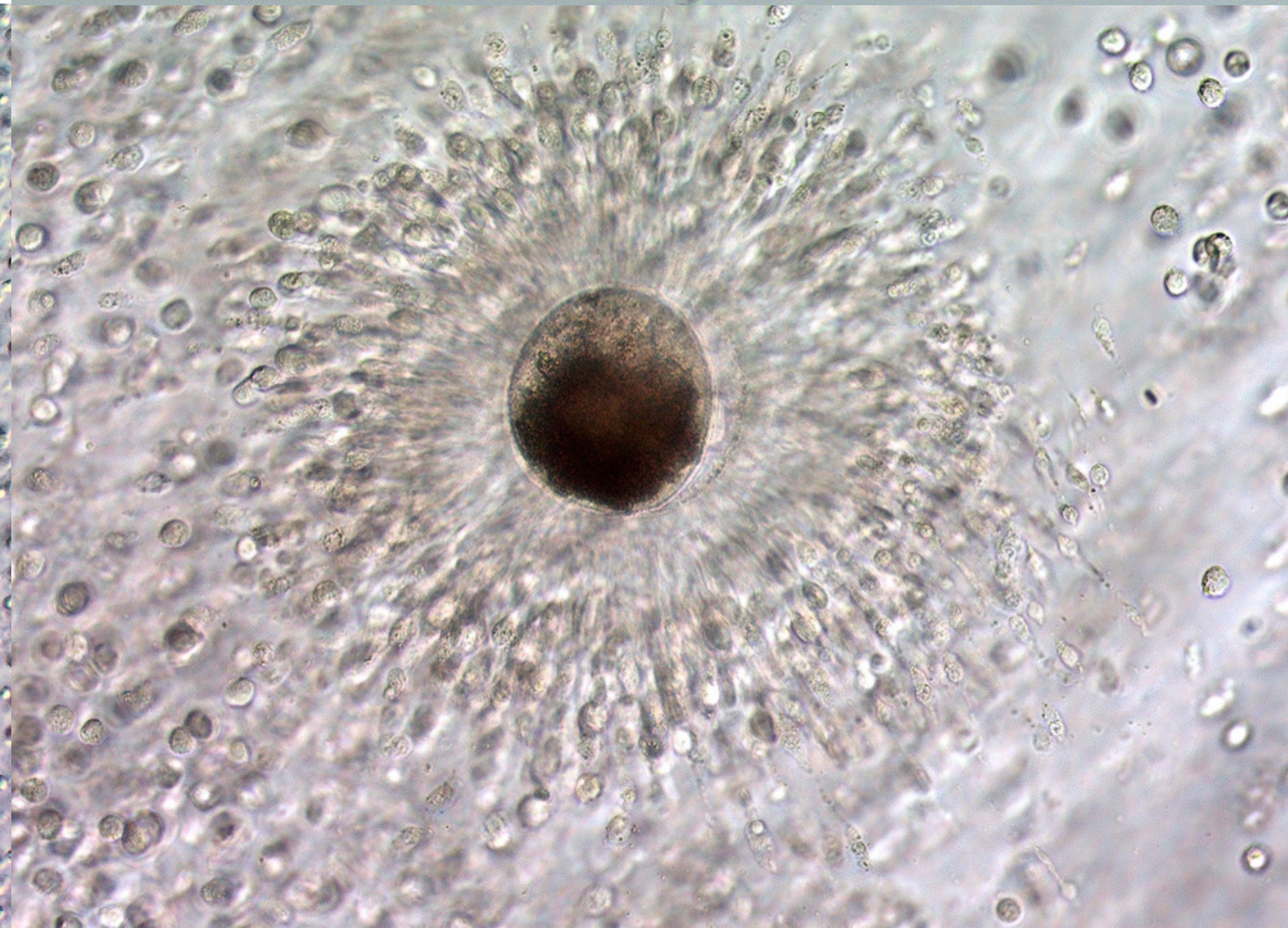
$T^a/CO_2/O_2$
controlled

Micro drops of 30 μ L
until 8 oocytes



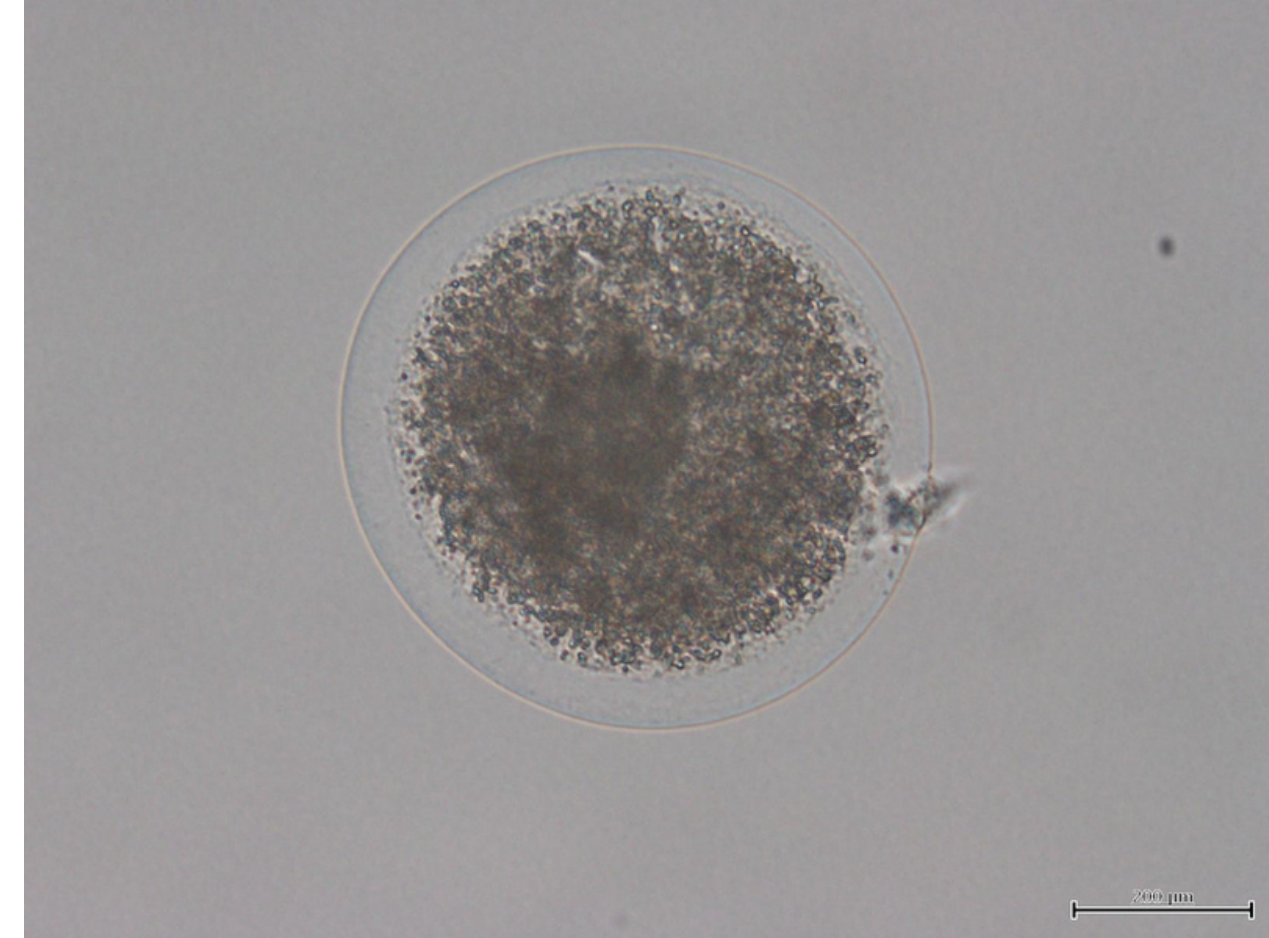
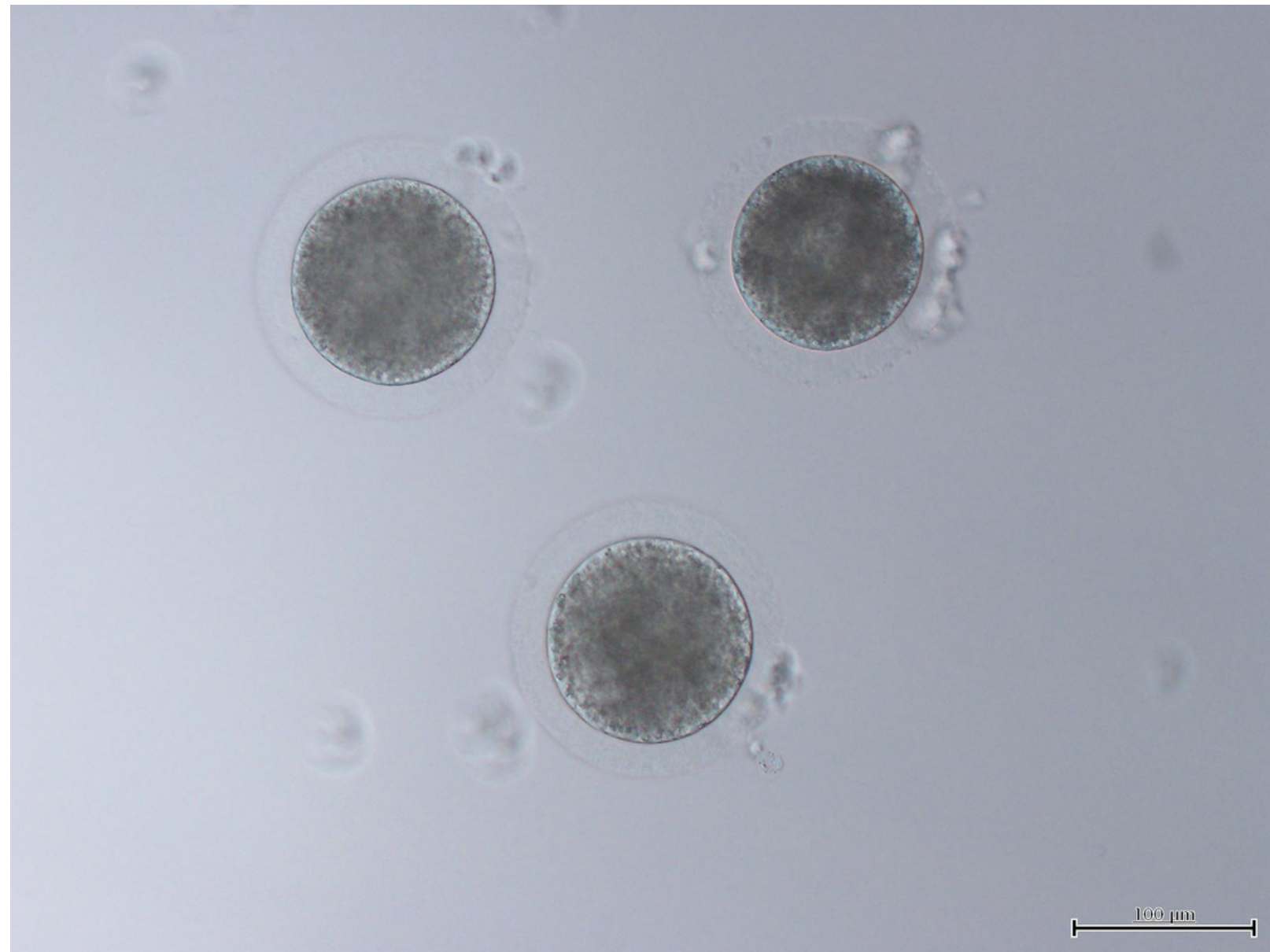
MATURATION

After 28-30 hrs, we evaluate the maturation through polar body extrusion (nuclear) and cumulus expansion



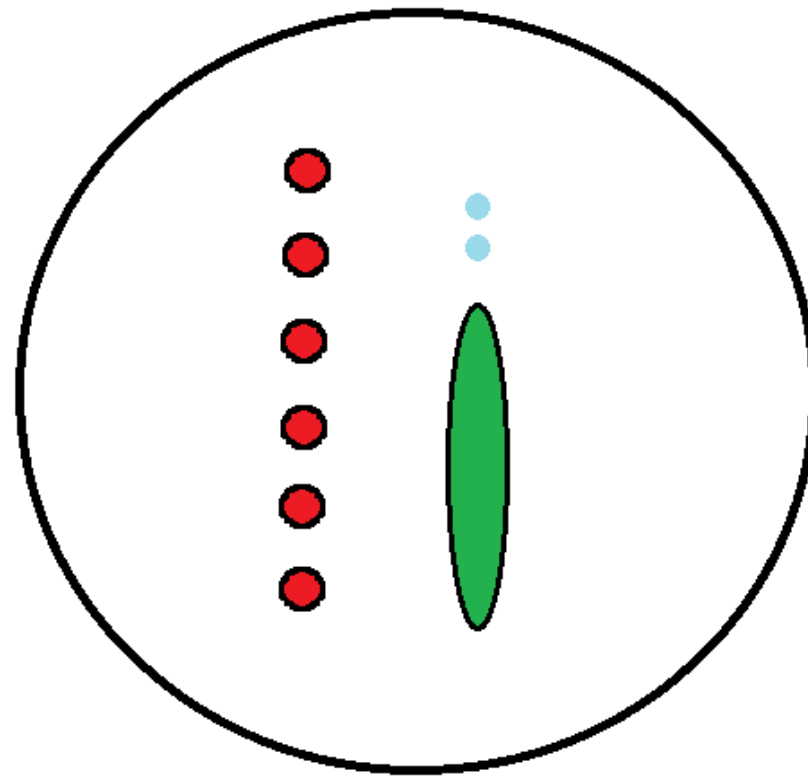
DENUDATION

We remove cumulus cells
using hyaluronidase



Microinjection (¿FIV?)

Every oocyte with PB extrusion (M II)

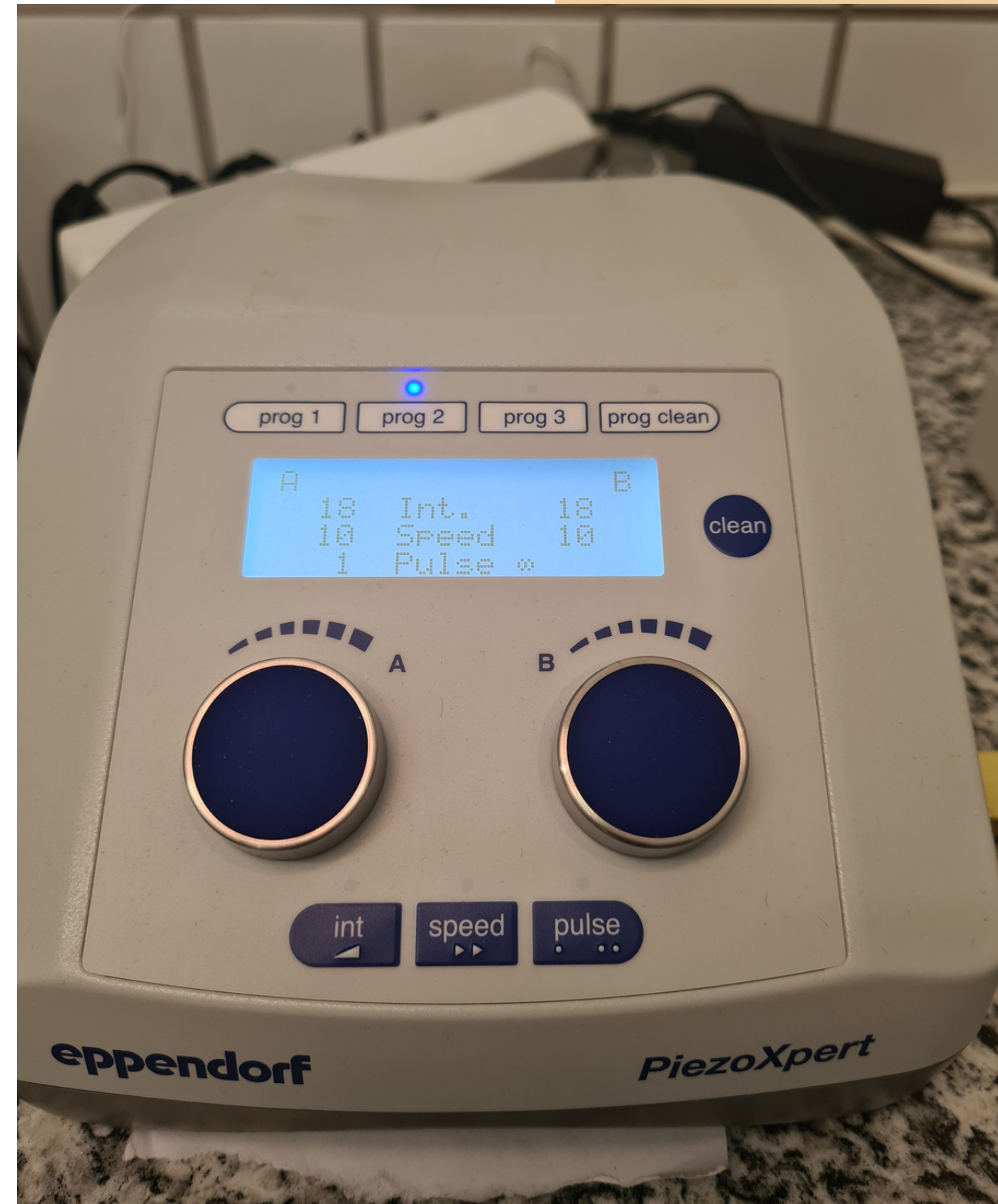


Microinjectionn

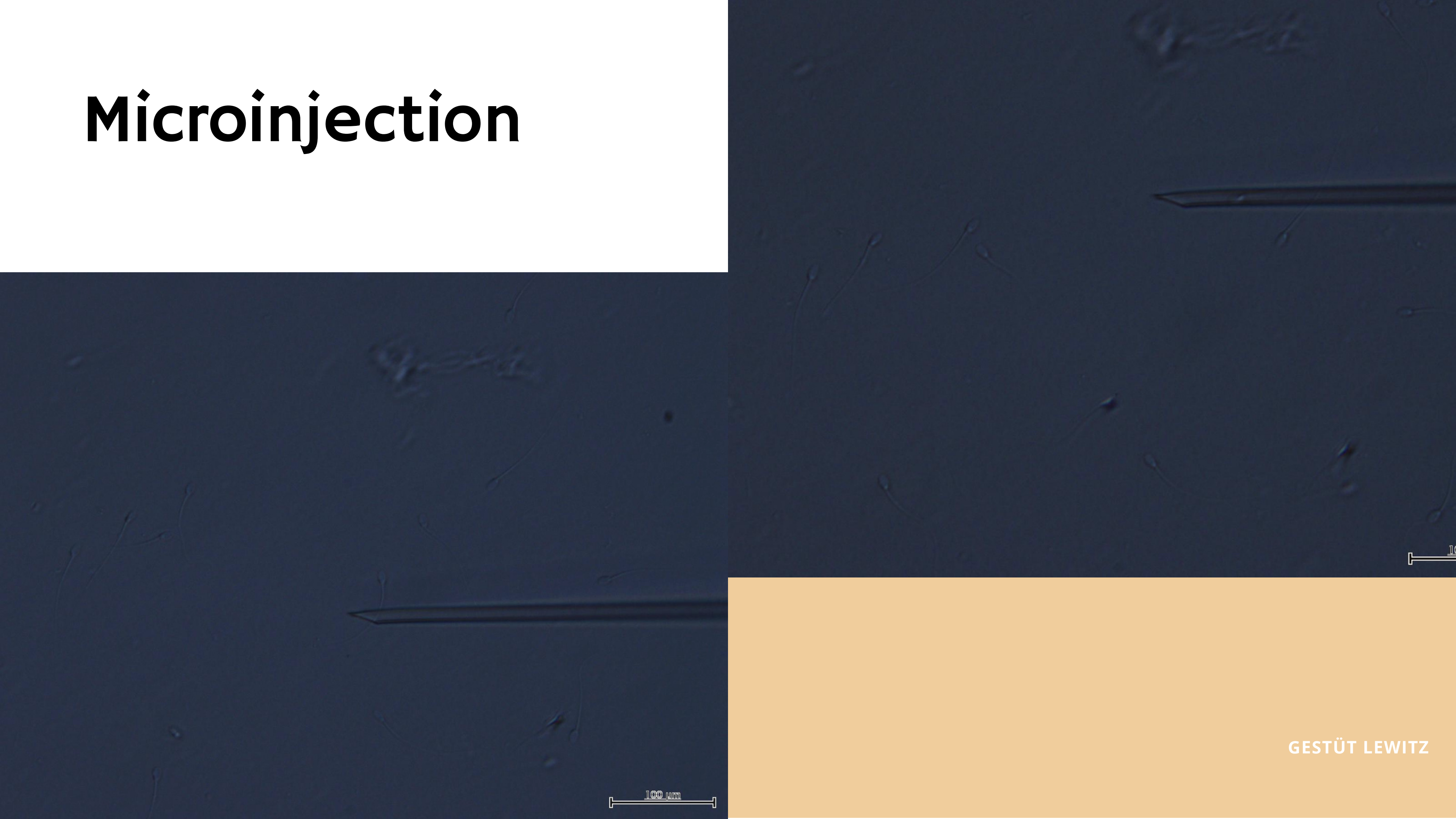
Immobilisation and aspiration
of the spermatozoa

We place the oocyte in the
correct plane and orientation.

Piezo-assisted sperm injection
into the centre of the
cytoplasm



Microinjection



100 μm

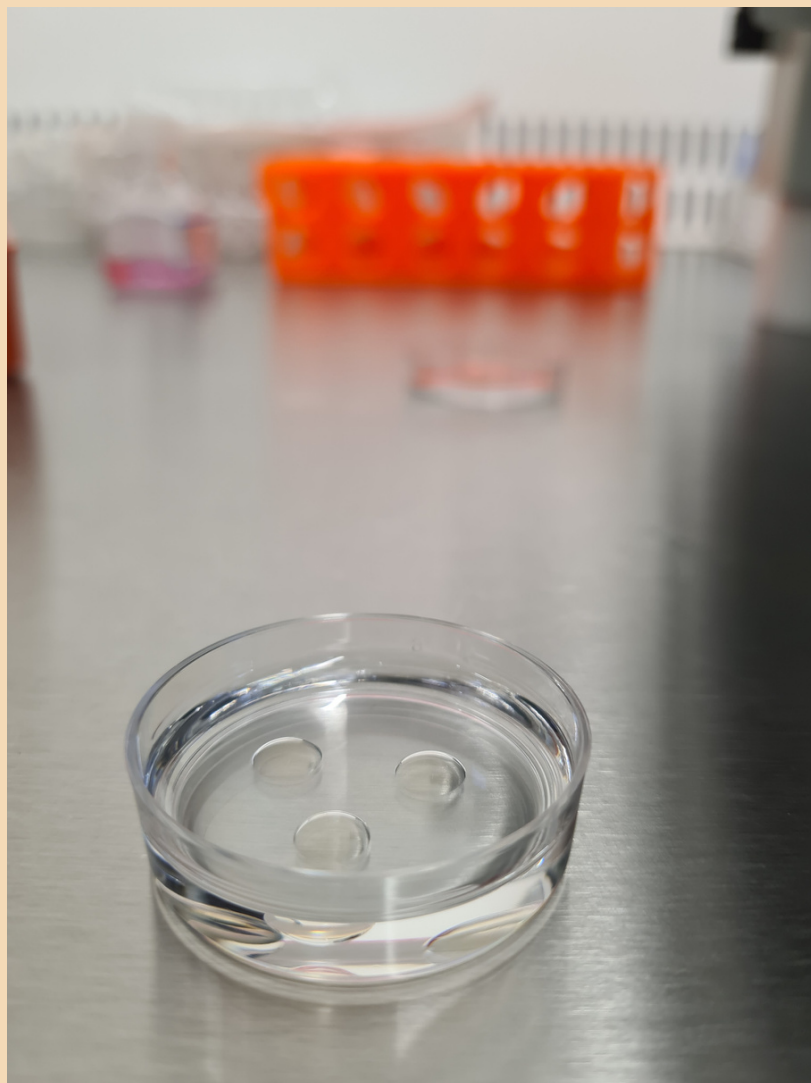
GESTÜT LEWITZ

Microinjection



CULTURE

After injecting the oocytes (MII), we place them in culture media (C) and put them in the incubator



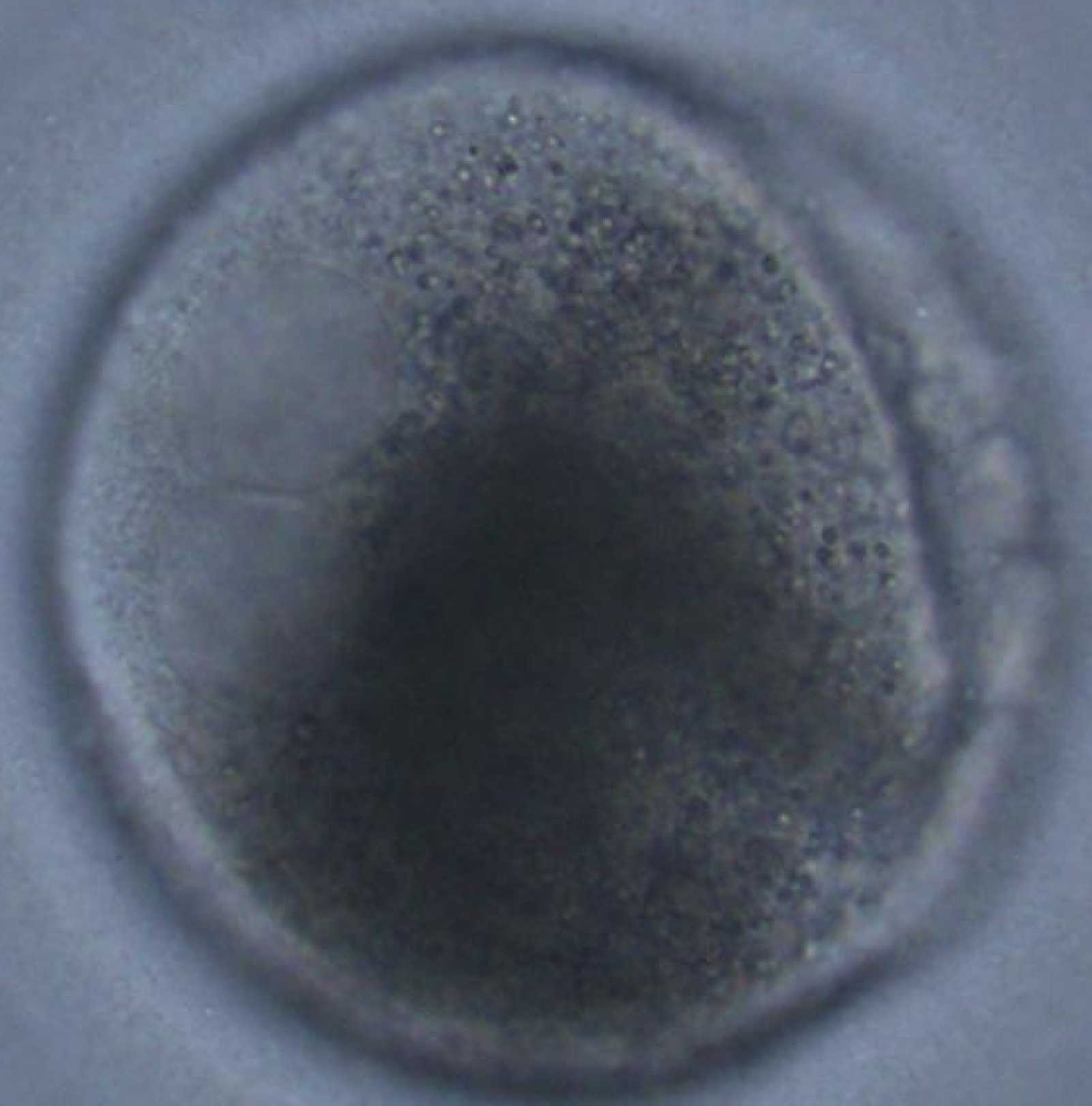
CULTURE

6 hours



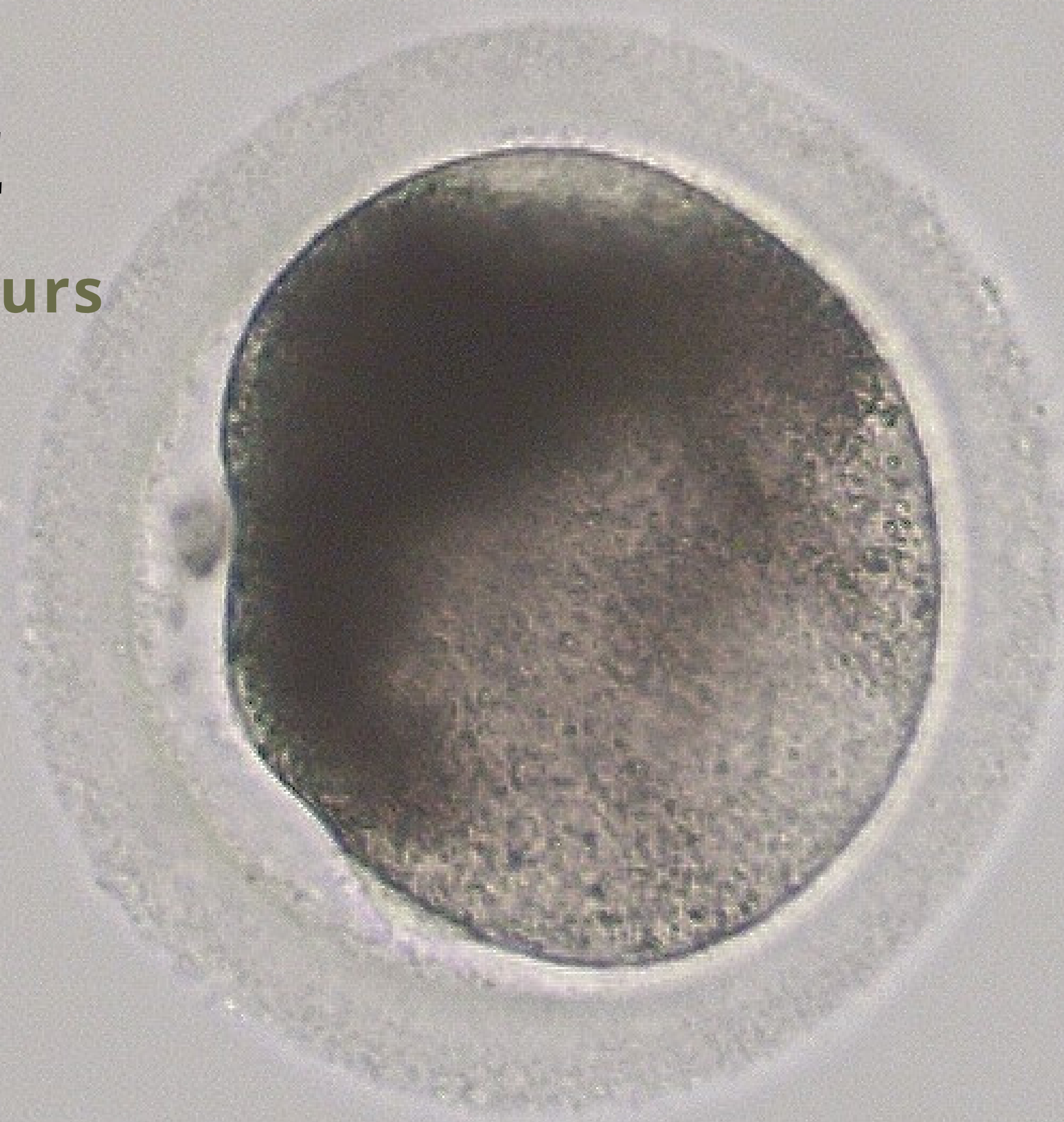
CULTURE

18-24 hours



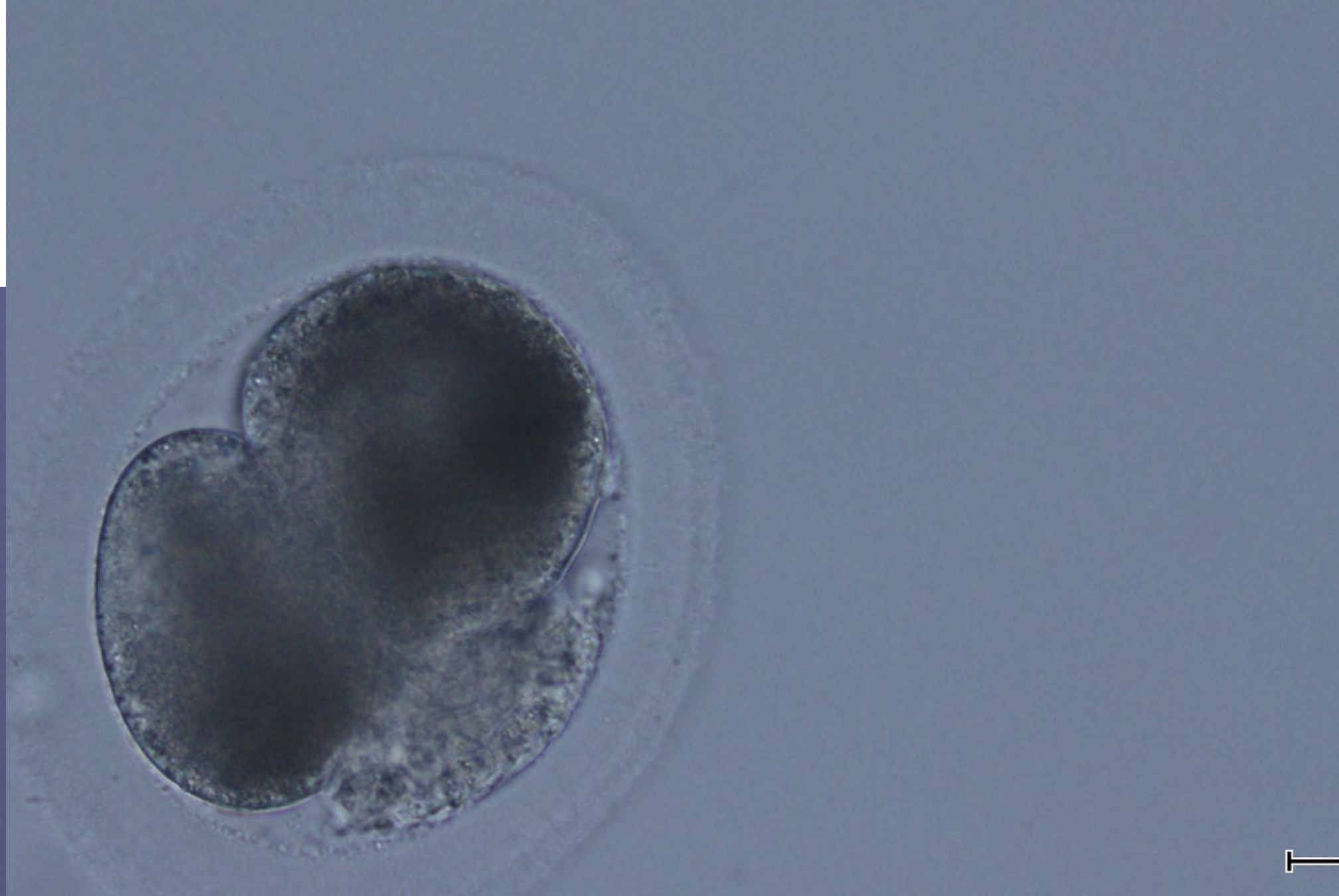
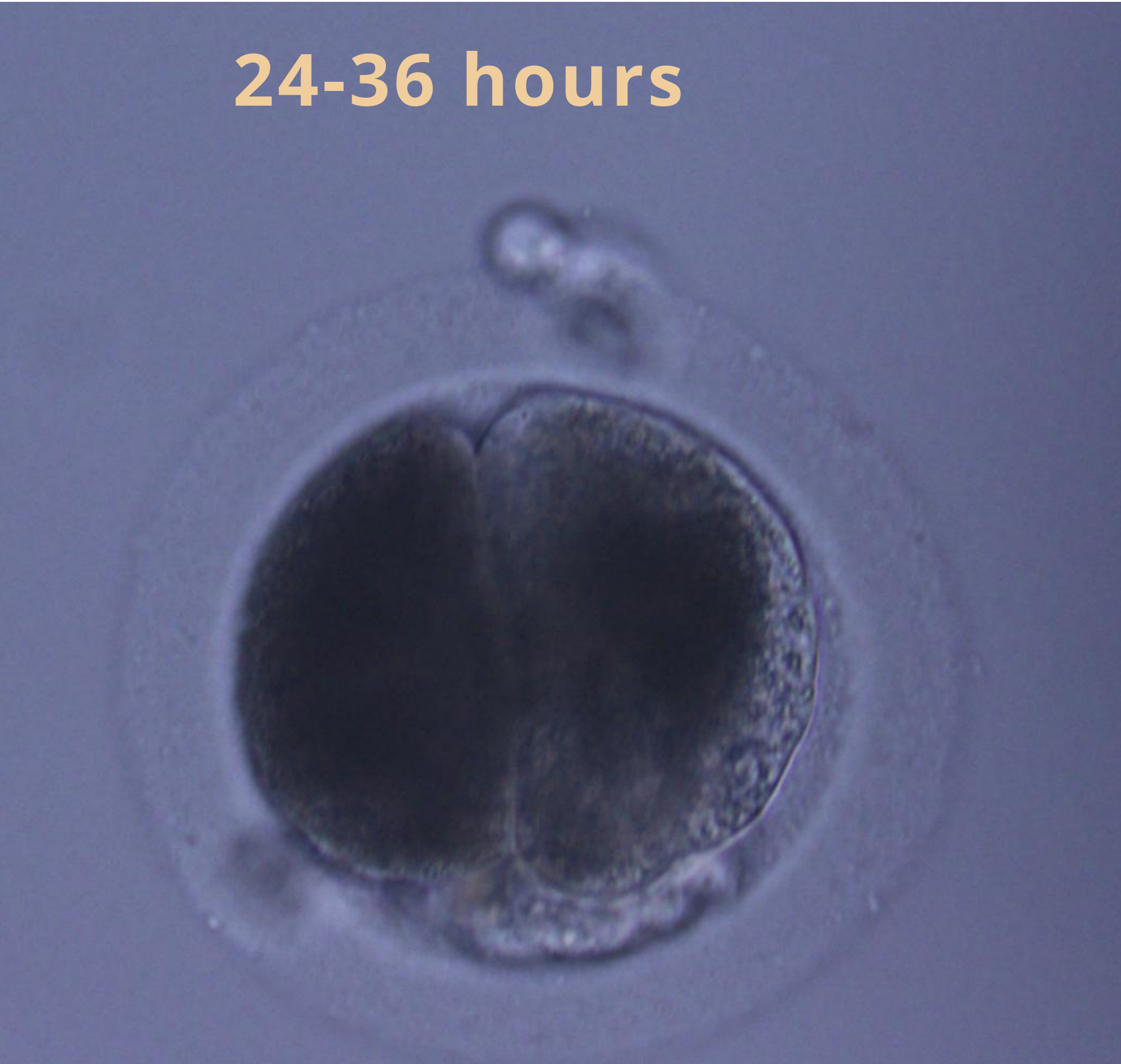
CULTURE

18-24 hours



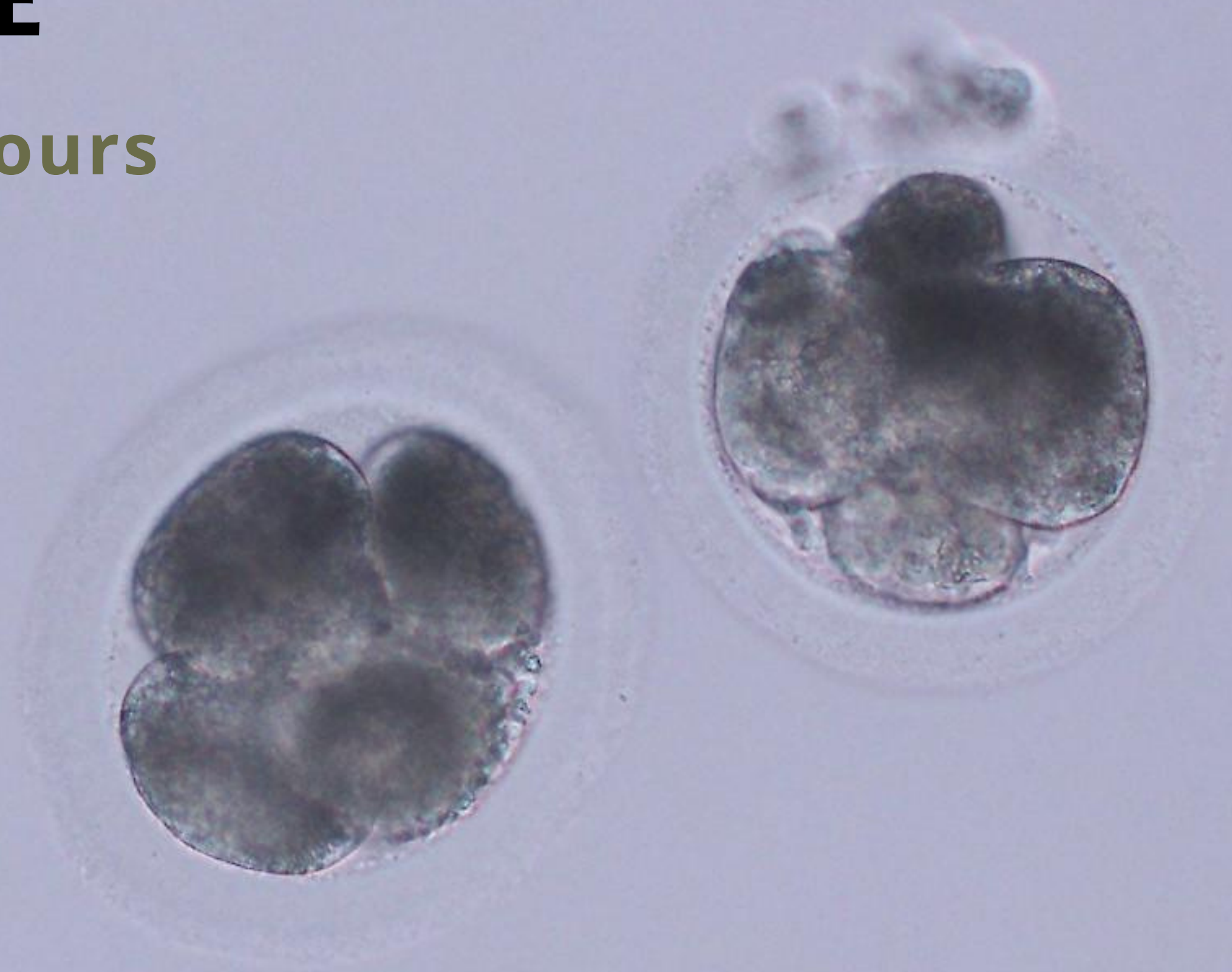
CULTURE

24-36 hours



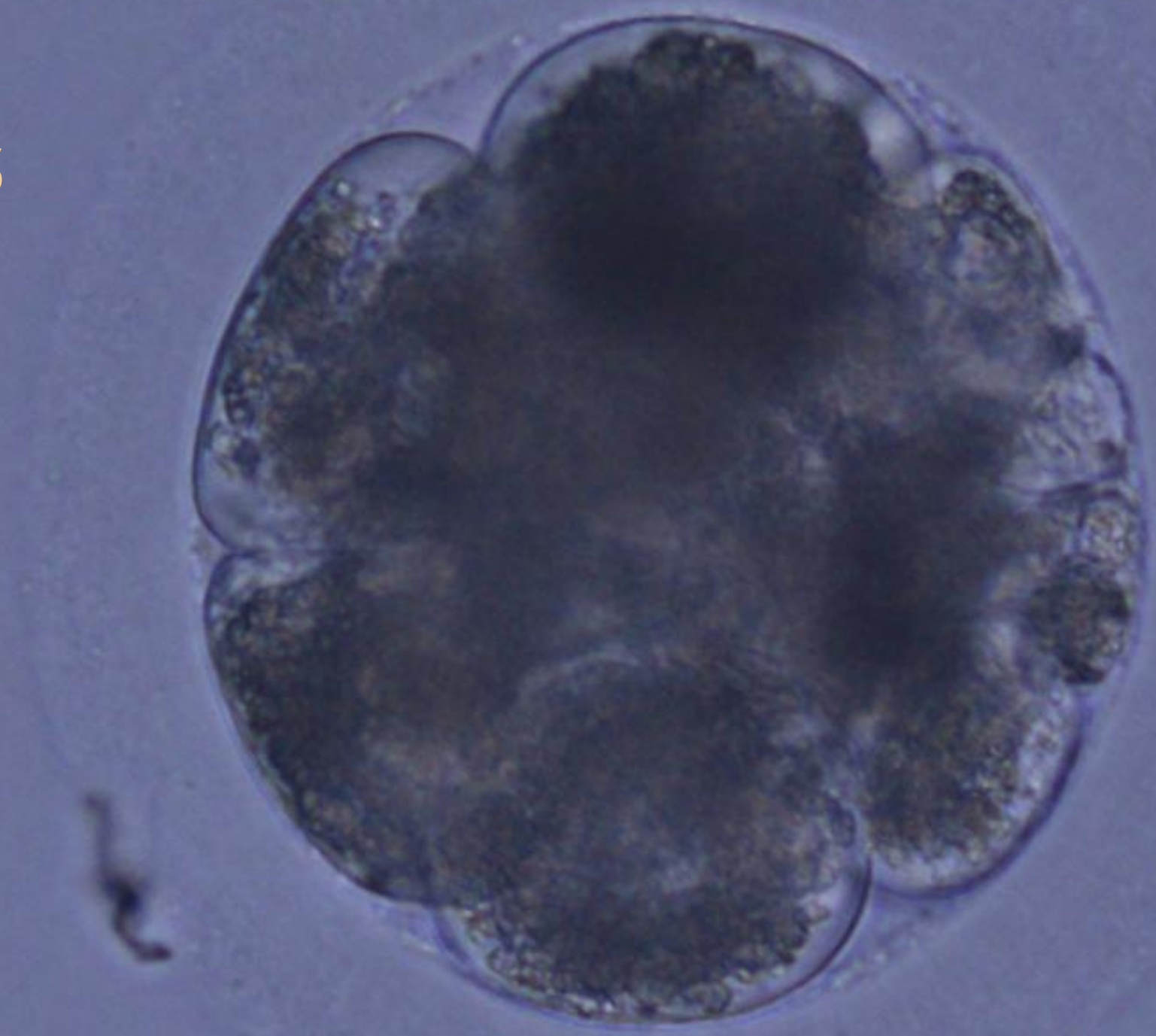
CULTURE

36-48 hours



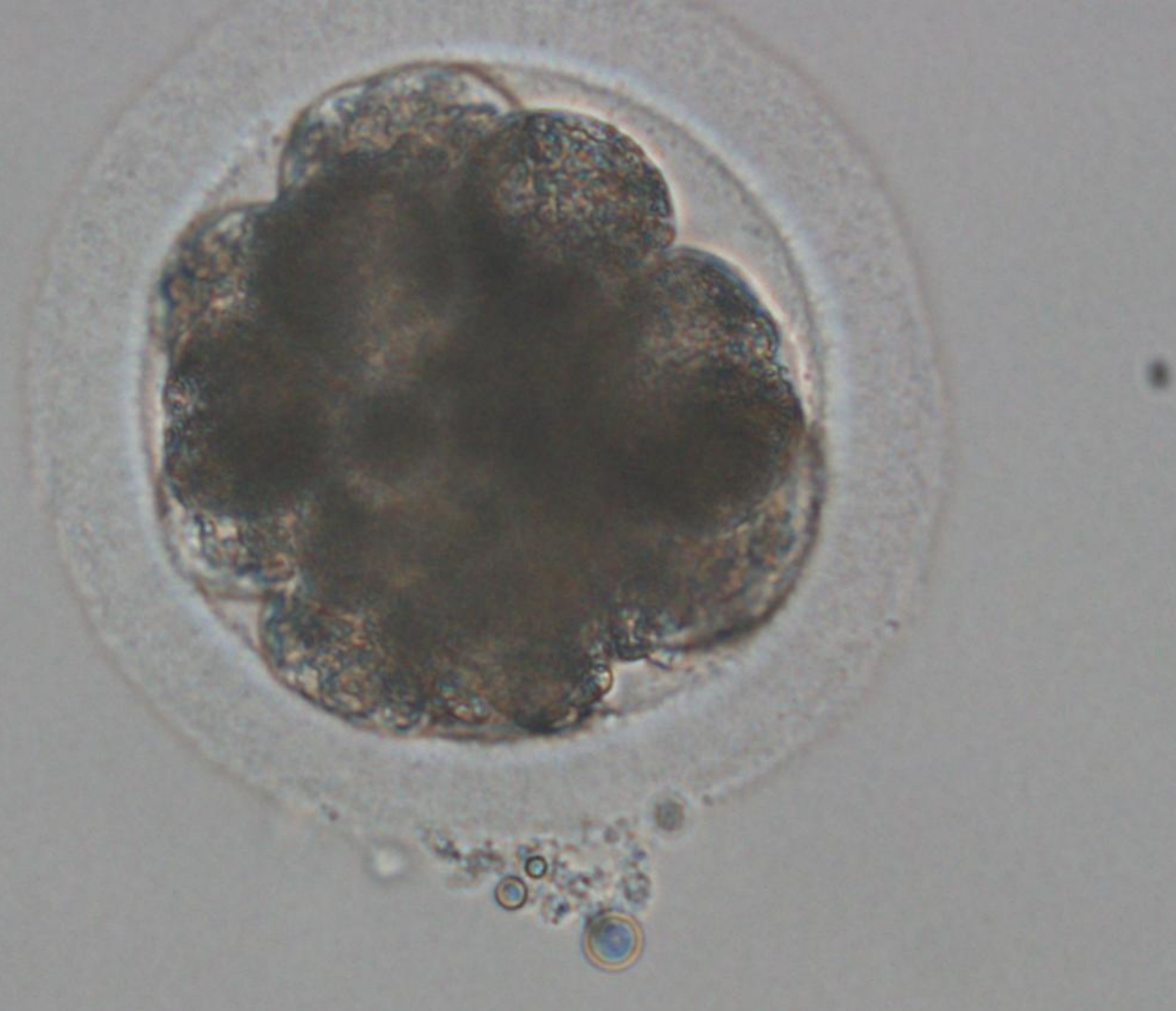
CULTURE

48-72 hours



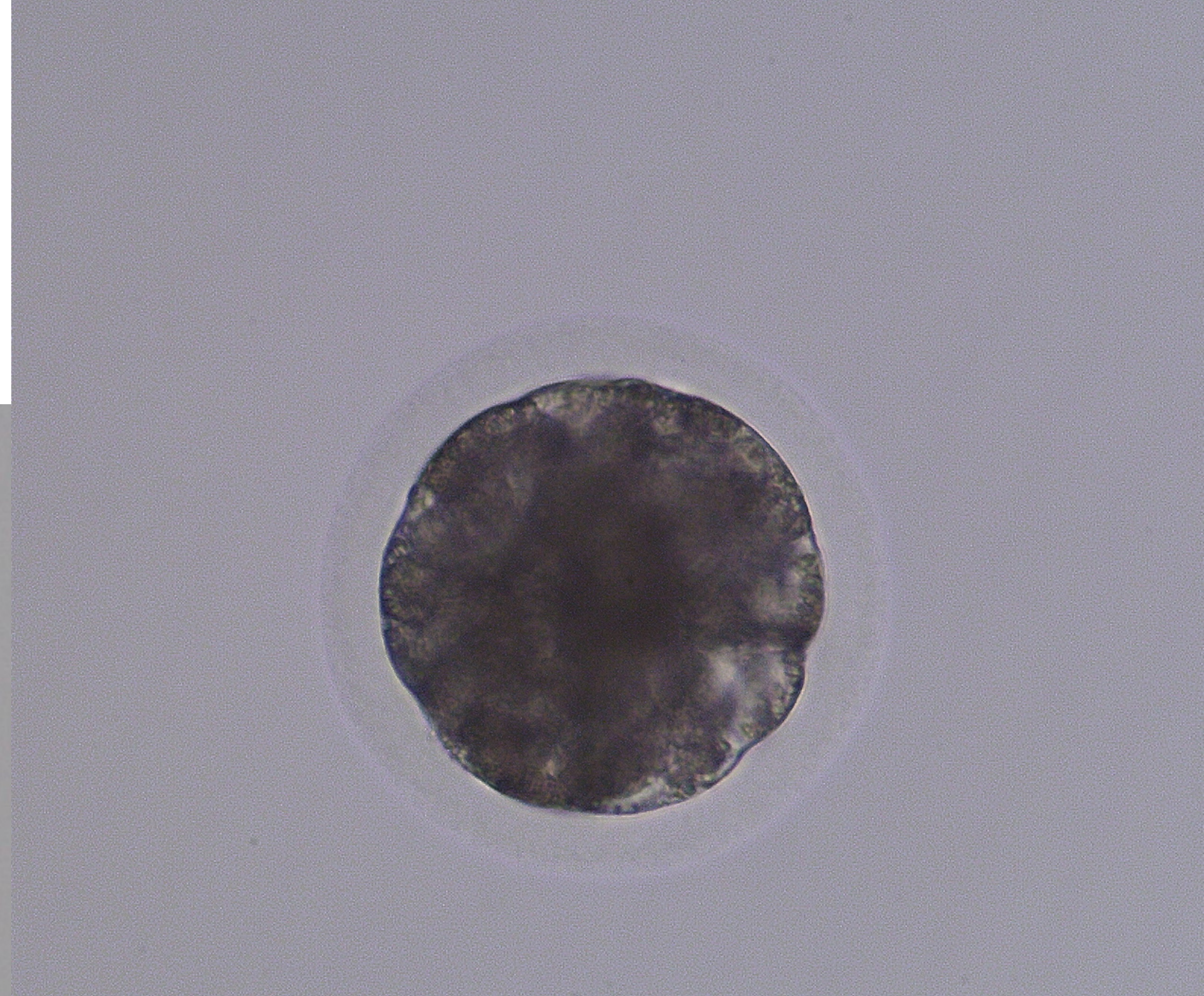
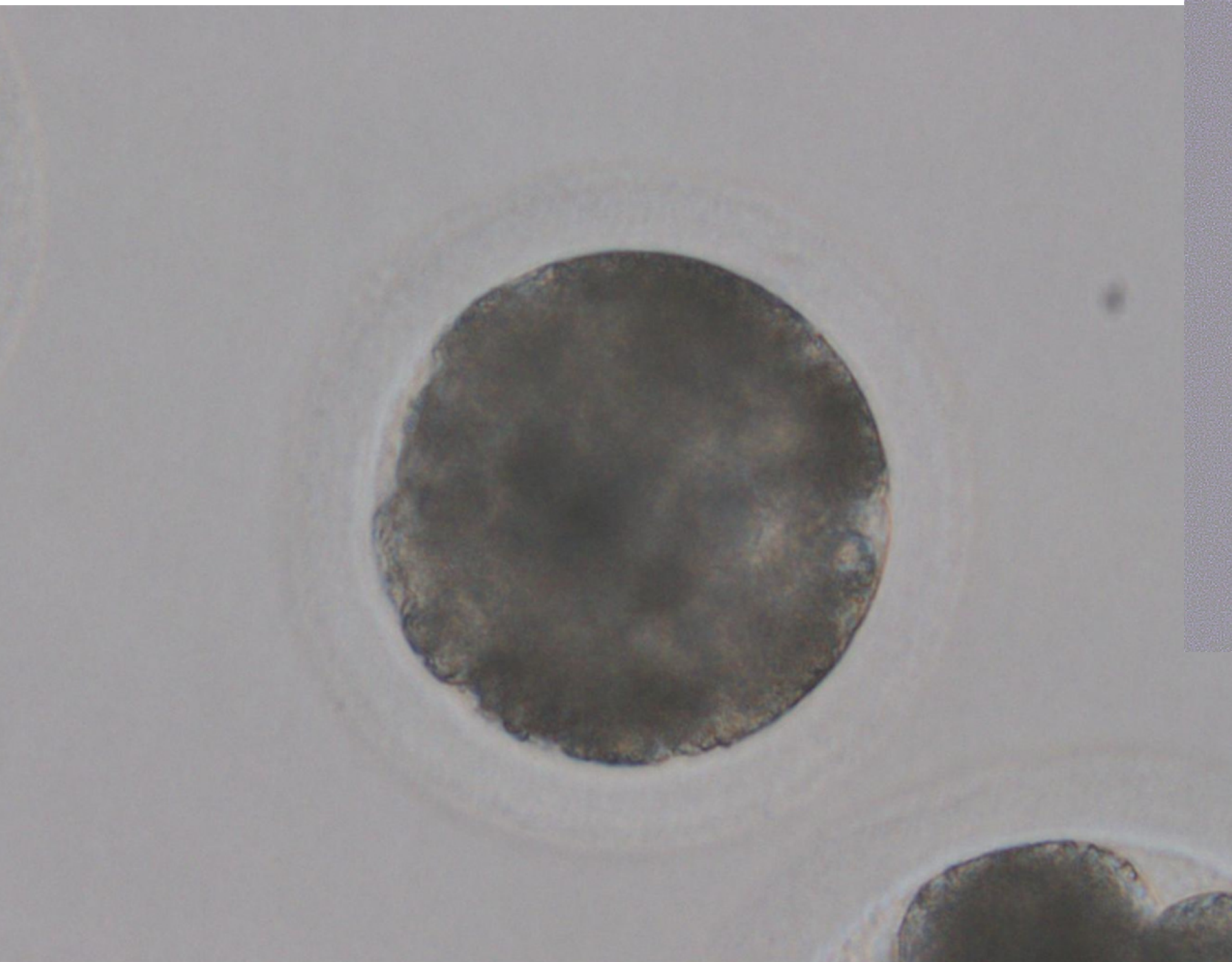
CULTURE

3-4 days



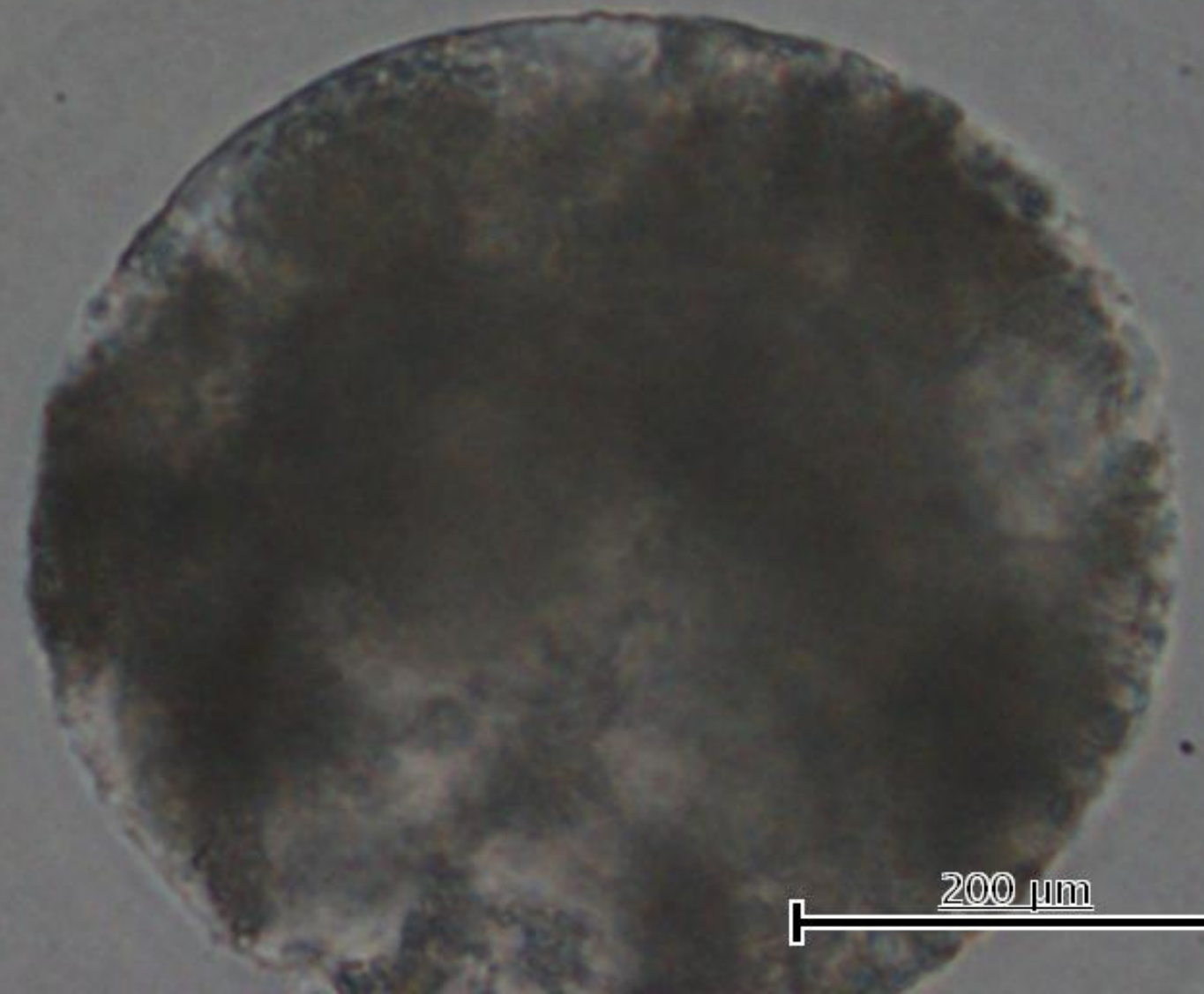
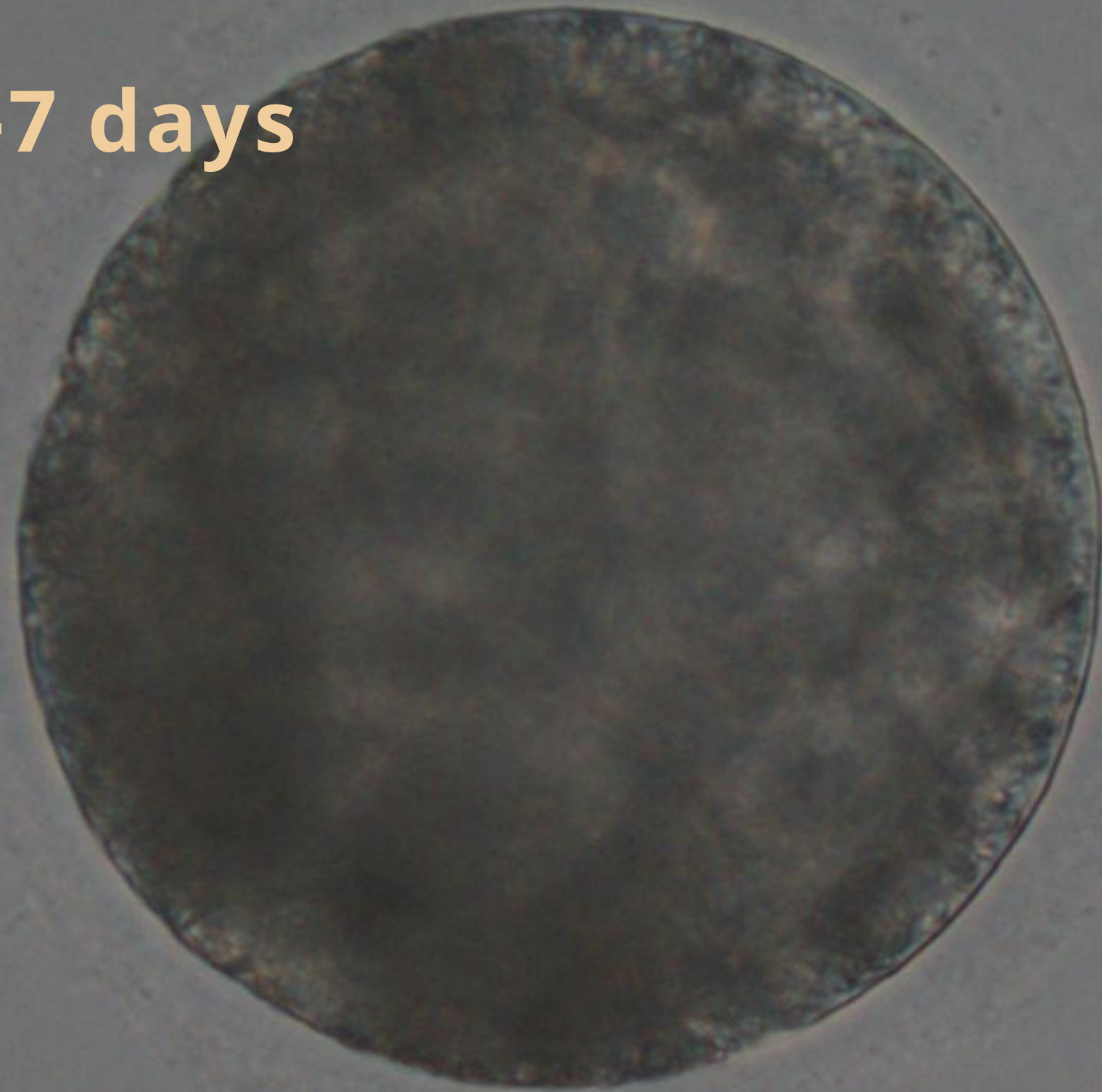
CULTURE

5-6 days



CULTURE

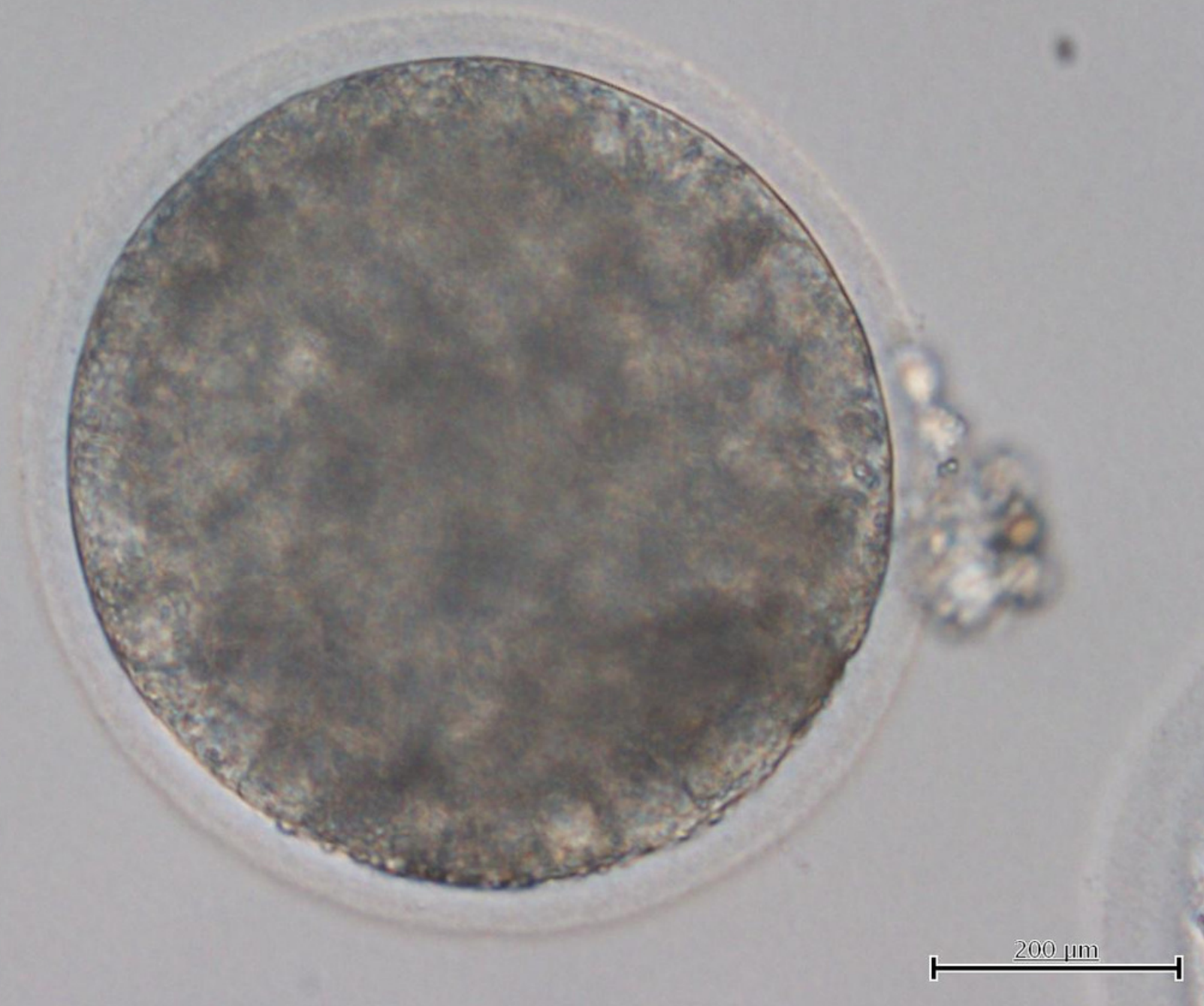
6-7 days



200 μm

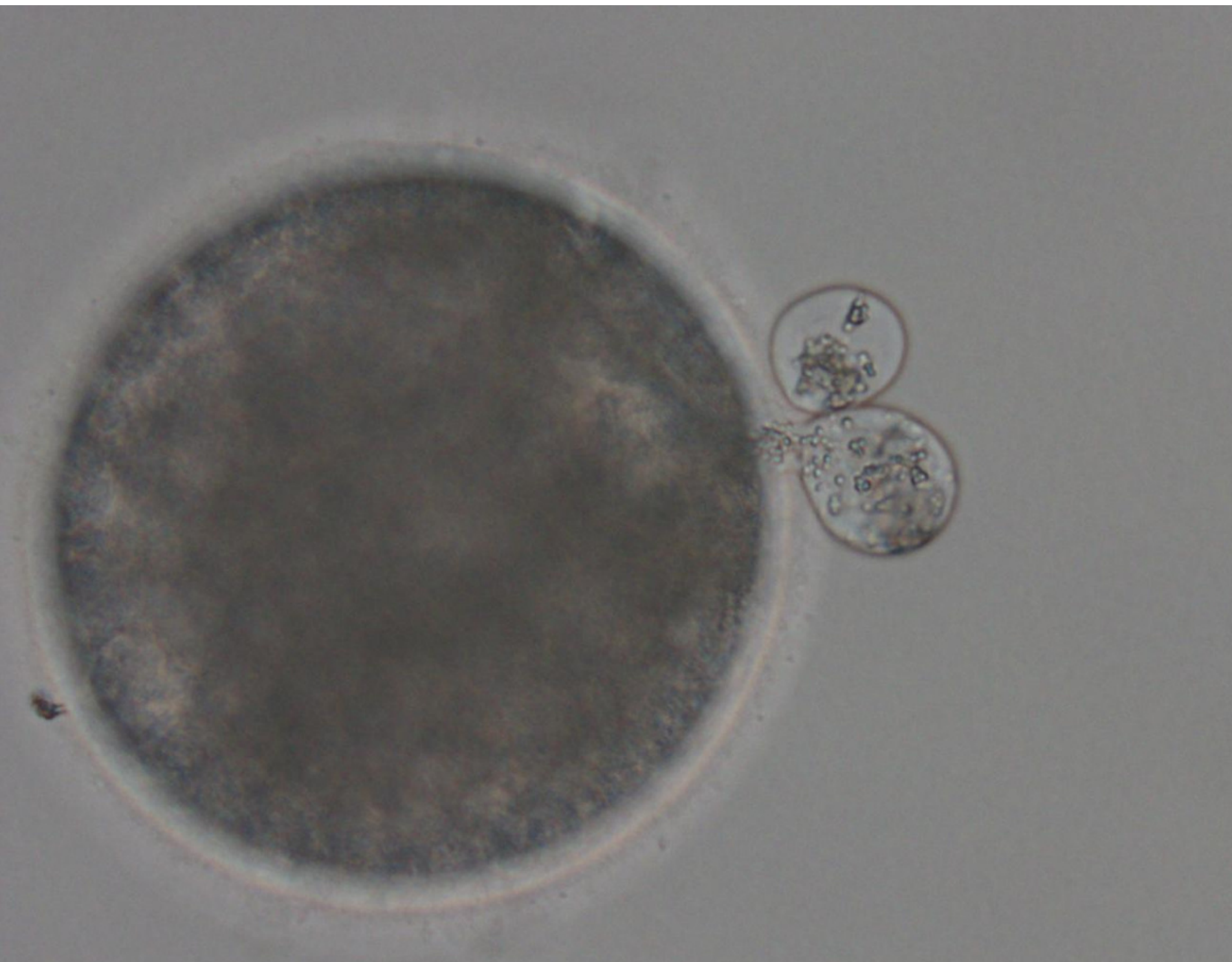
CULTURE

7-8 days



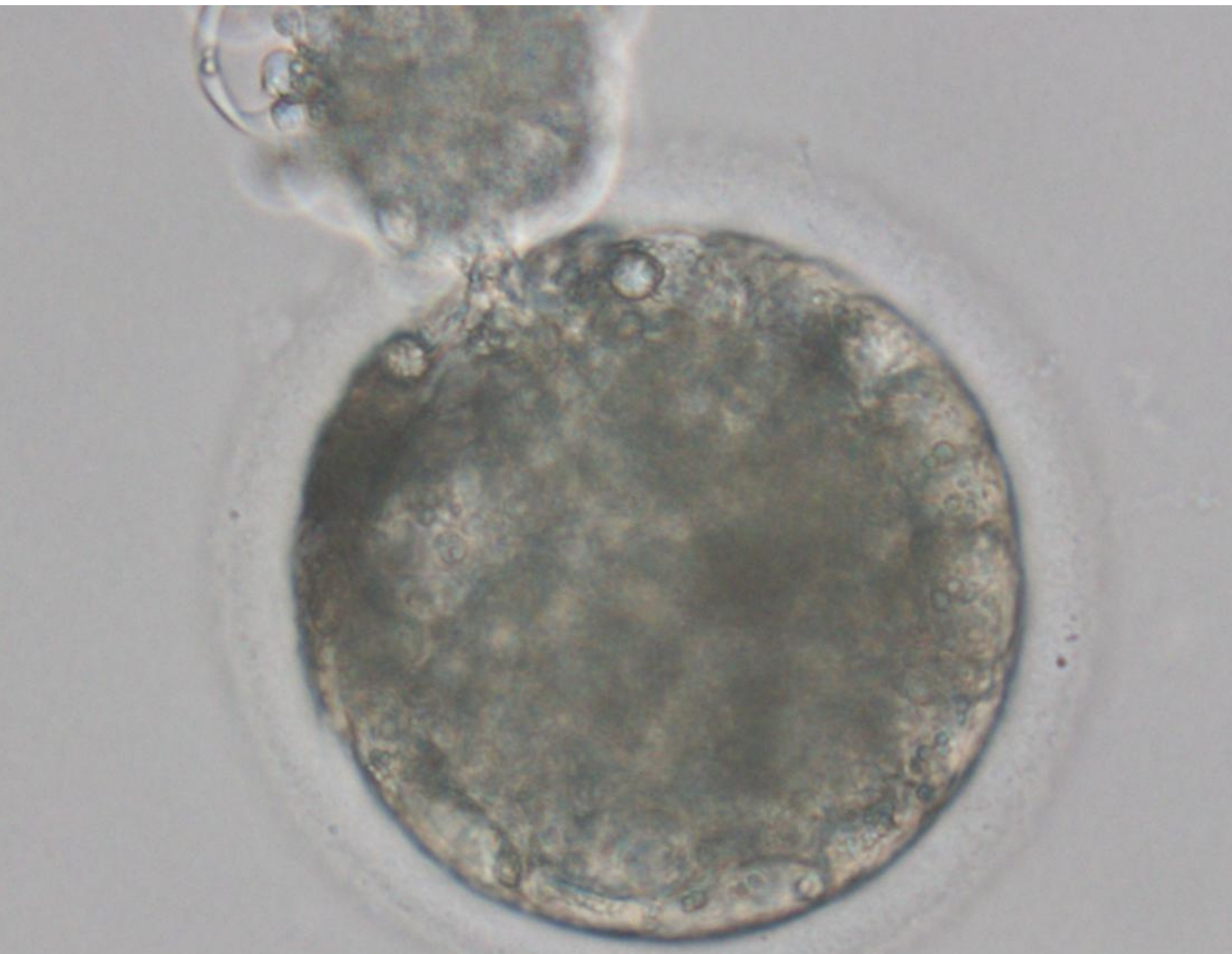
CULTURE

+8 days



CULTURE

+8 days



Factors affecting the success of the technique

- Adequate equipment
- Expertise
 - Aspiration technique
 - Asepsis/oocytes handling (T^a, light...)
- Quality control asp media/holding
- Mare individual factors
 - Oocyte quality (age, treatments, chronic diseases)
 - Character/temperament
- Individual sire factors (activation)



Benefits

- **Use of out-of-season cycles**
- **Subfertile mares (ovulation problems, oviductal obstruction, endometriosis)**
- **Sub-fertile stallions**
- **Limited or high cost semen**
- **Mares in training/competition**
- **Optimal time to cryopreserve embryos**



Disadvantages

- **Cost of equipment**
- **Requires training/experience**
- **Risk**
- **Difficult/young mares**
- **Individual factor**
- **Monozygotic twins**



**More efficient
than
traditional ET?**





OPU-ICSI vs ET

OPU-ICSI

ET

Embryo/technique

0,8-2,2

0,5-0,8

% Pregnancy

70

80

% Resorption

15

6



OPU-ICSI vs ET

OPU-ICSI

0,41-1,23 pregnancies 60 days/OPU

ET

0,38-0,58 pregnancies 60 days/flush

**Thank you
for your
time!**