In Vitro Fertilisation (IVF) & Embryology

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IVF and the Reproductive Technology Revolution

- There are ~5 million IVF offspring worldwide
- ~800,000 couples seek infertility treatment each year worldwide
- 1 in 6 Australian couples seek infertility treatment in Australia
- In 2010, 3.3% of babies born in Australia were from IVF
- IVF hormones cost the Pharmaceutical Benefits Scheme ~$100 million/year

Indications for IVF

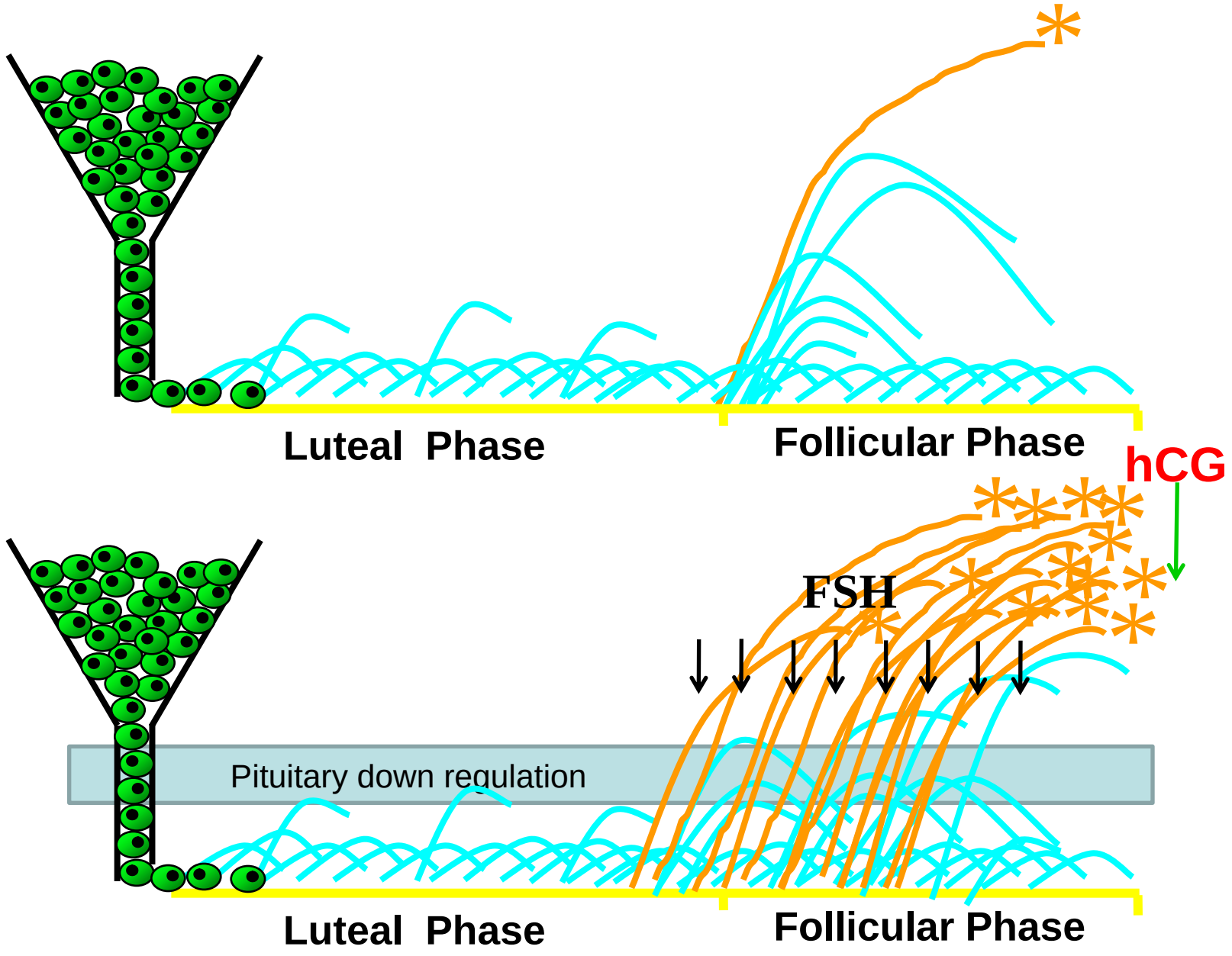
- Tubal infertility
  - Past tubal ligation, salpingectomy
  - Adhesions due to infection, endometriosis
- Male factor infertility (ICSI)
- Anovulation (failed 1st and second line treatments)
- Unexplained infertility
- Advanced maternal age…??
- Genetic disorders or recurrent miscarriage (PGD)

Step 1: Ovarian stimulation

Normal ovary before ovulation

Stimulated ovary for IVF

Natural cycle vs ovarian hyperstimulation



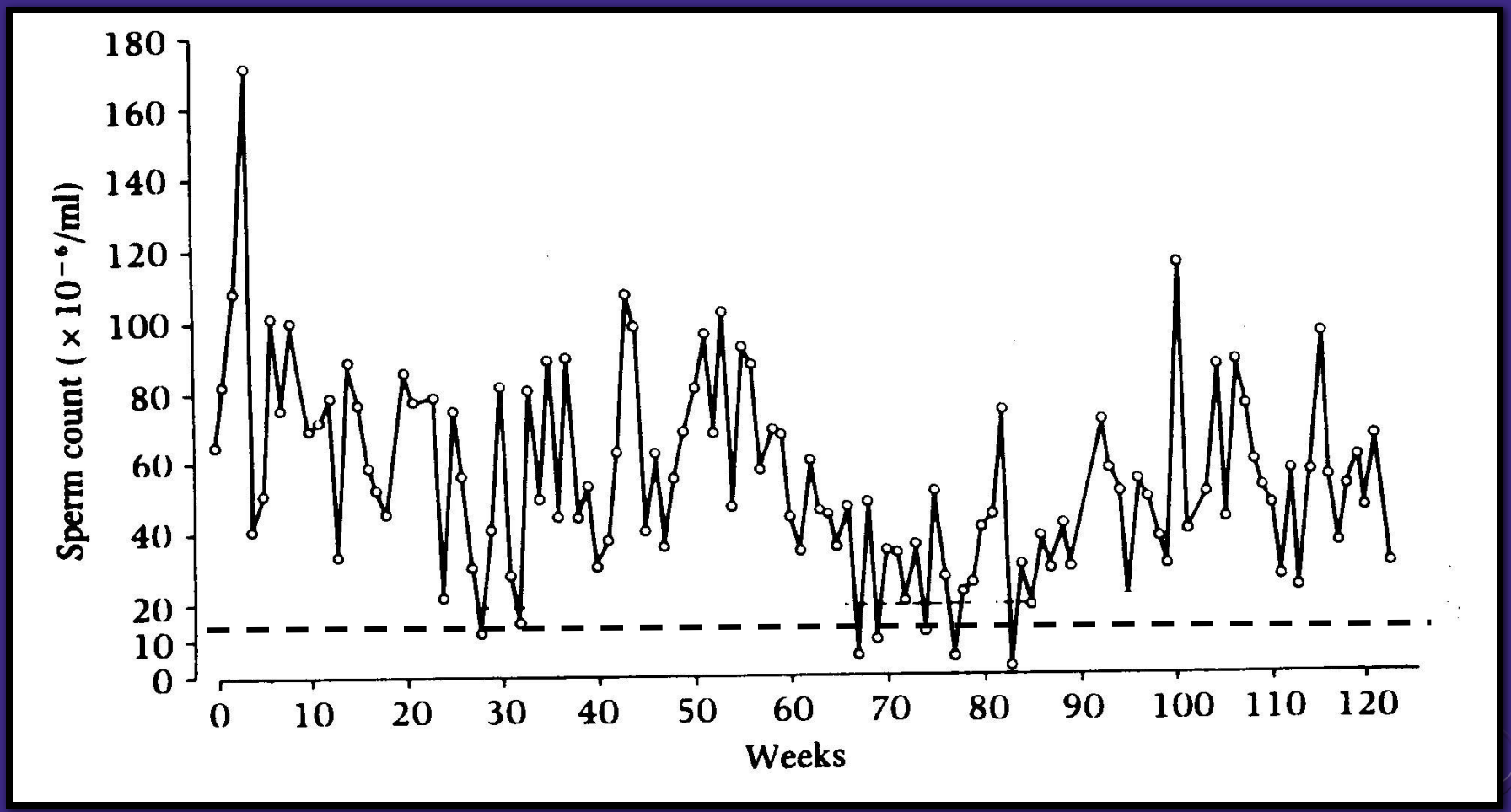
Step 2: sperm retrieval

- Options for sperm collection
  - Home (needs to arrive at lab within 1 hour)
  - On site collection
  - Surgical sperm collection (when no sperm in ejaculate)

2. Andrology

Sperm Concentration

W.H.O 5th >15 M/mL



2. Andrology

Motility: How many moving?

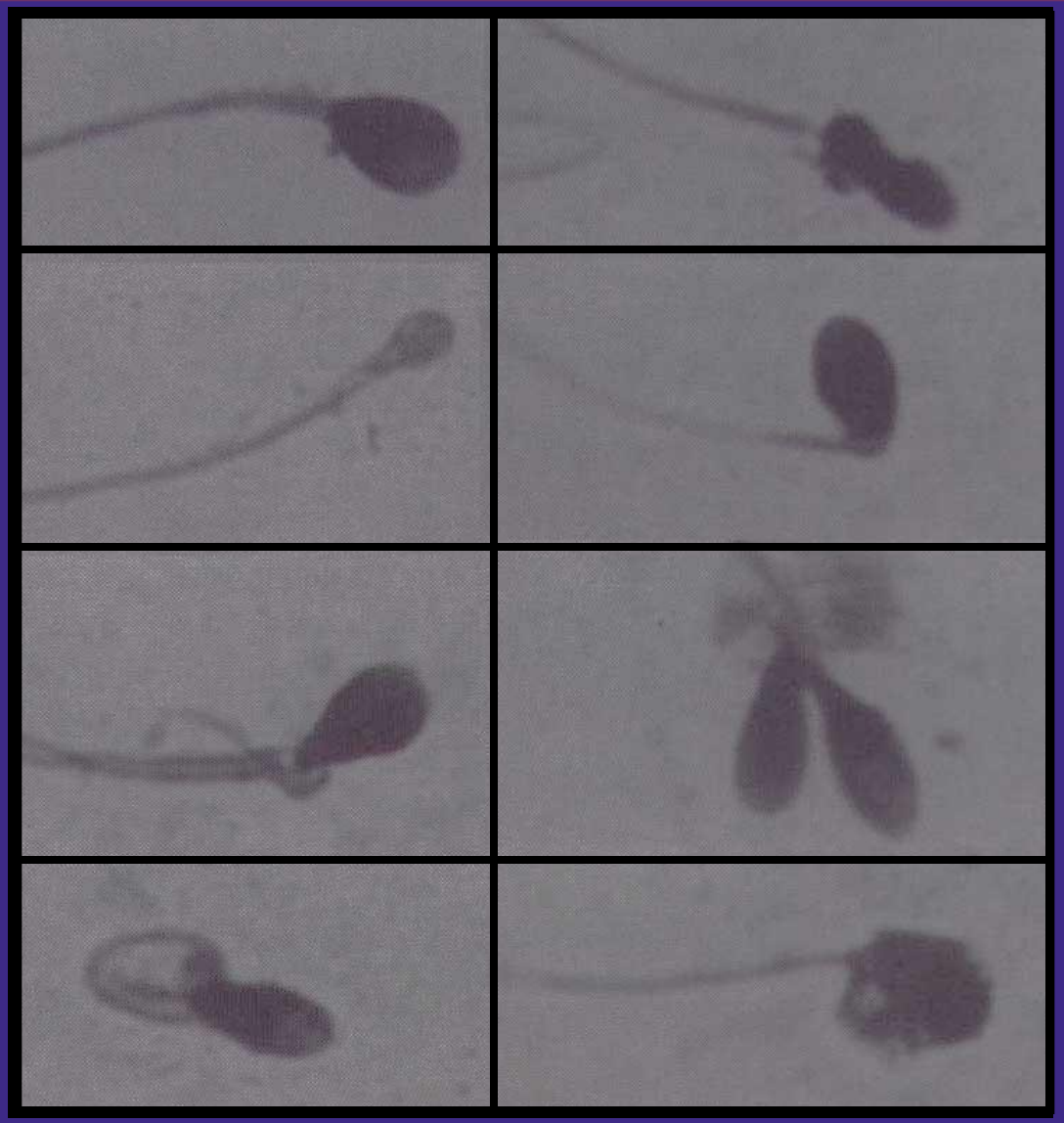
- We do not assess the speed of motility progression, as humans eyes are not accurate.

- only whether it is moving (regardless of speed).

W.H.O 5th
>32 %
progressive

2. Andrology

Morphology

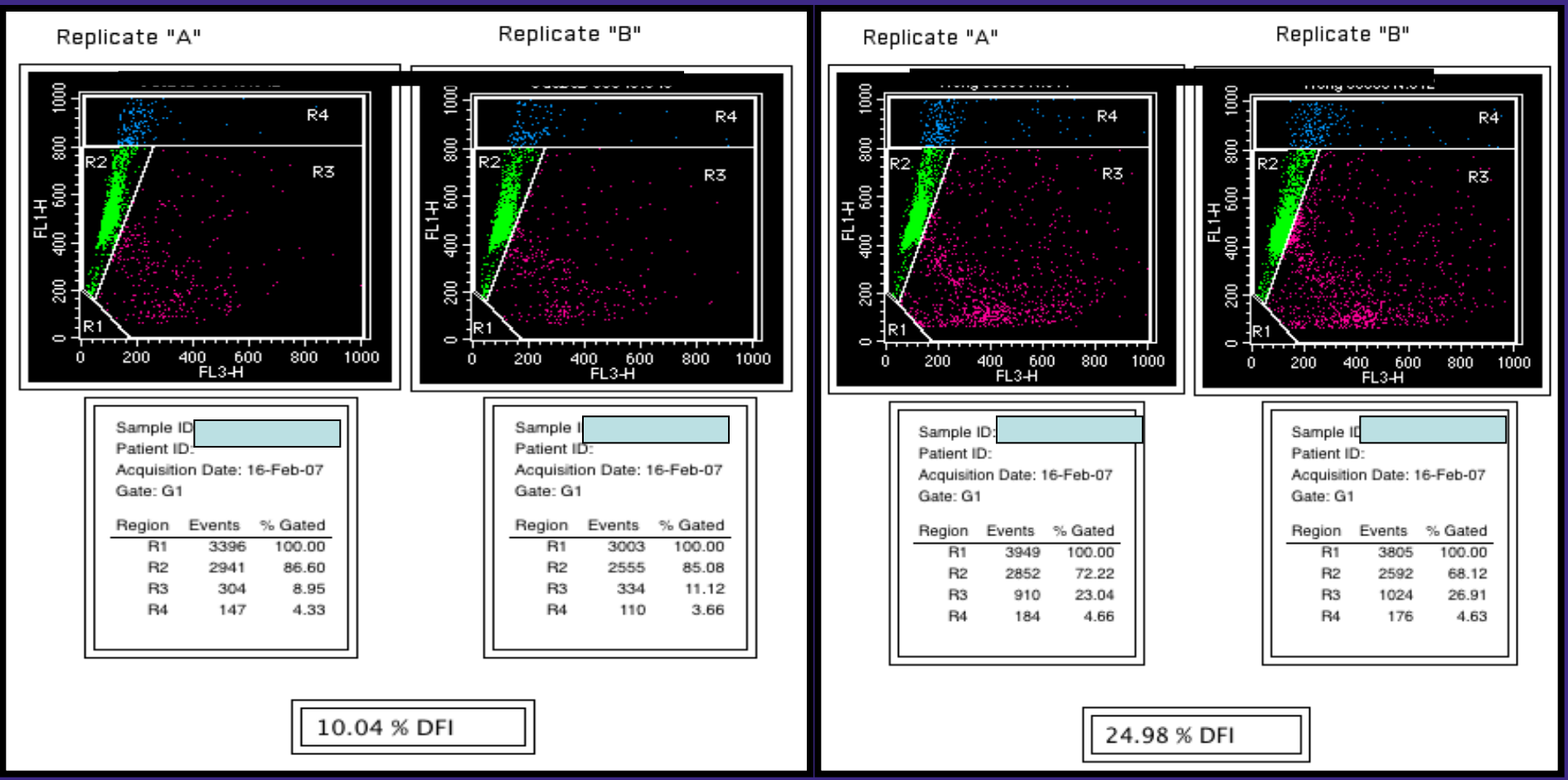


W.H.O 5th >4 %

2. Andrology - DNA Fragmentation (SCSA)

Excellent DNA Integrity

Borderline Poor DNA Integrity



2. Andrology - DNA Fragmentation (SCSA)

- DFI >30% (~7+% of patients)
  - Longer to get pregnant
    - Metaanalysis [Evenson and Wixon, 2006]
  - Increased miscarriages
  - Is not always apparent from embryo morphology.

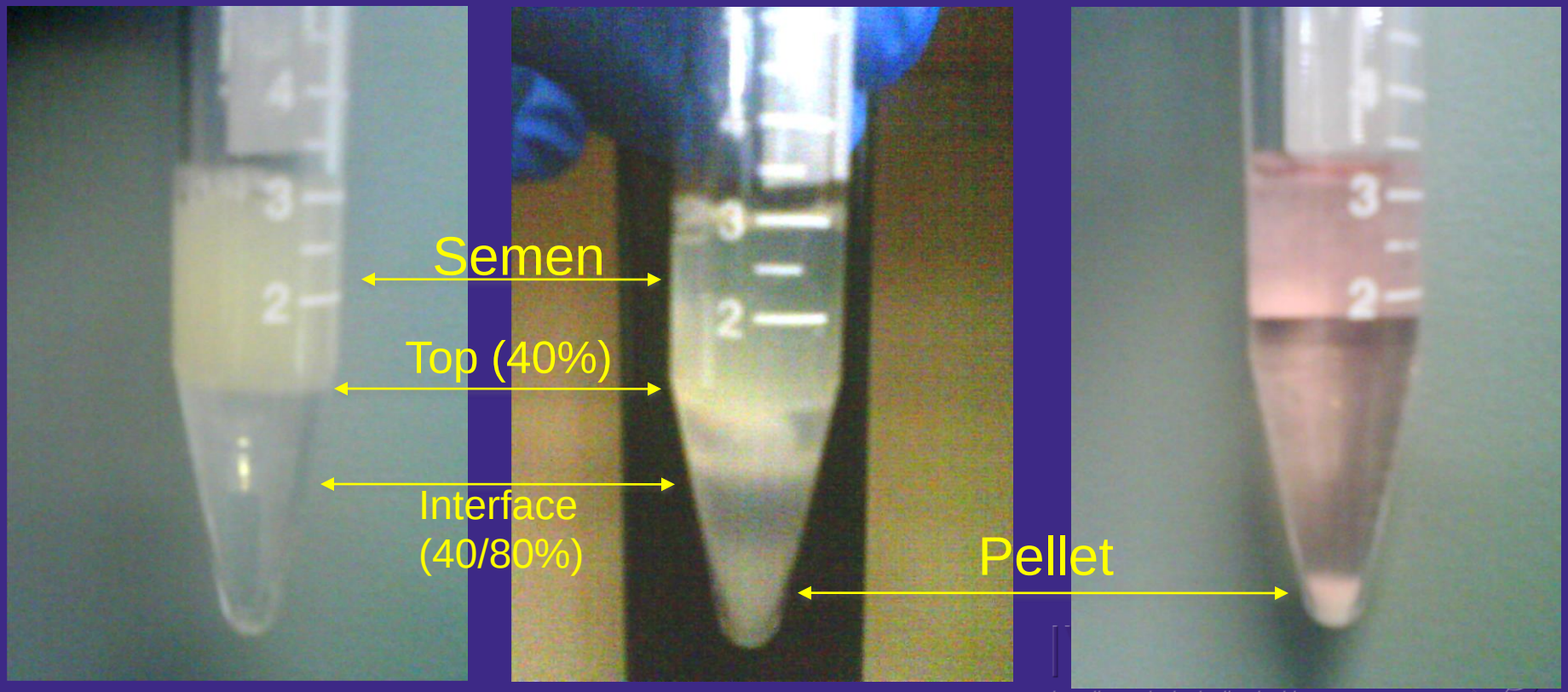
2. Preparing ejaculated sperm:

Discontinuous Density Gradient Separation

Semen on Gradient

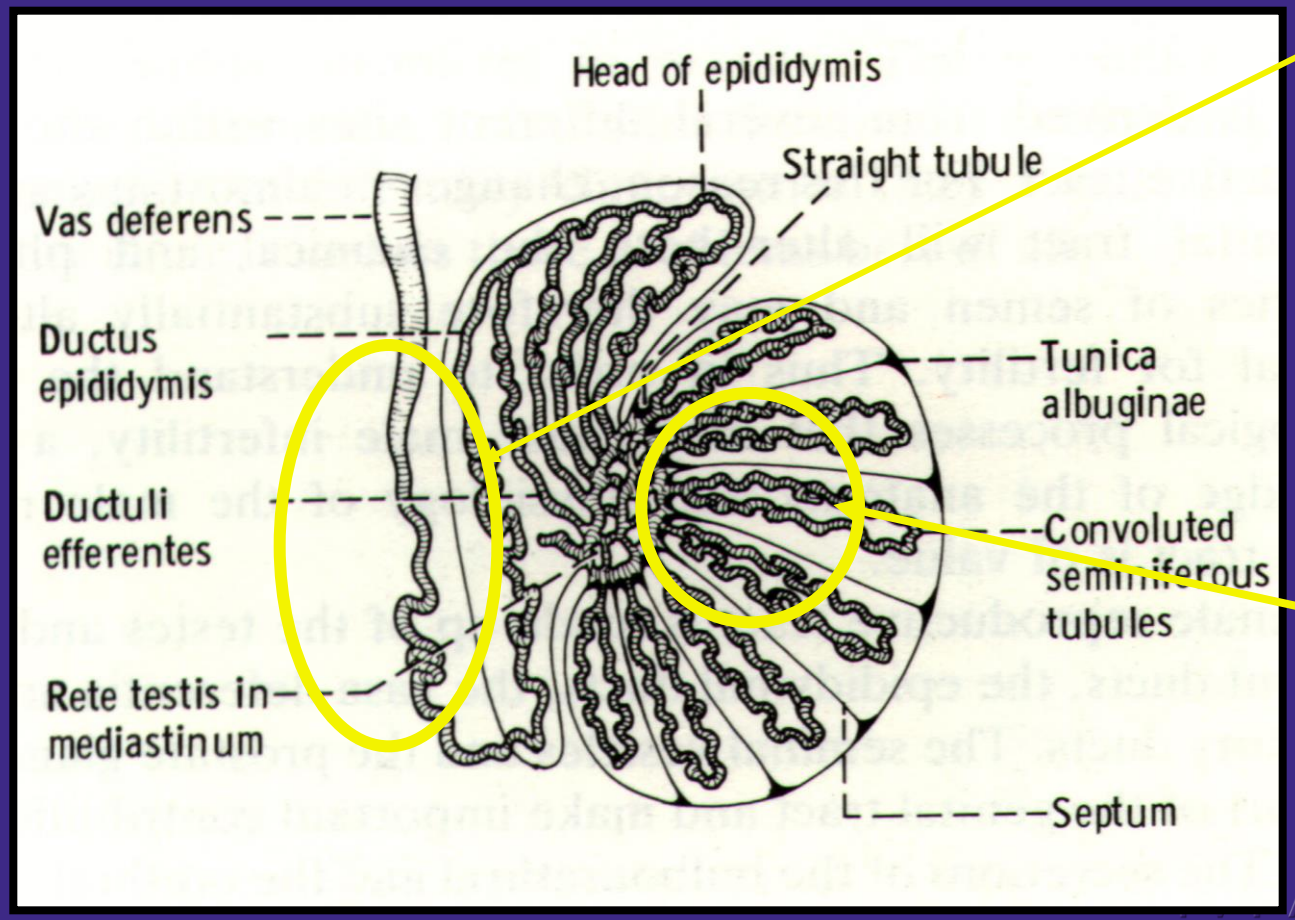
Semen post Spin

Sperm pellet post wash



2. What if there is no ejaculated sperm?

Surgical Sperm Collections



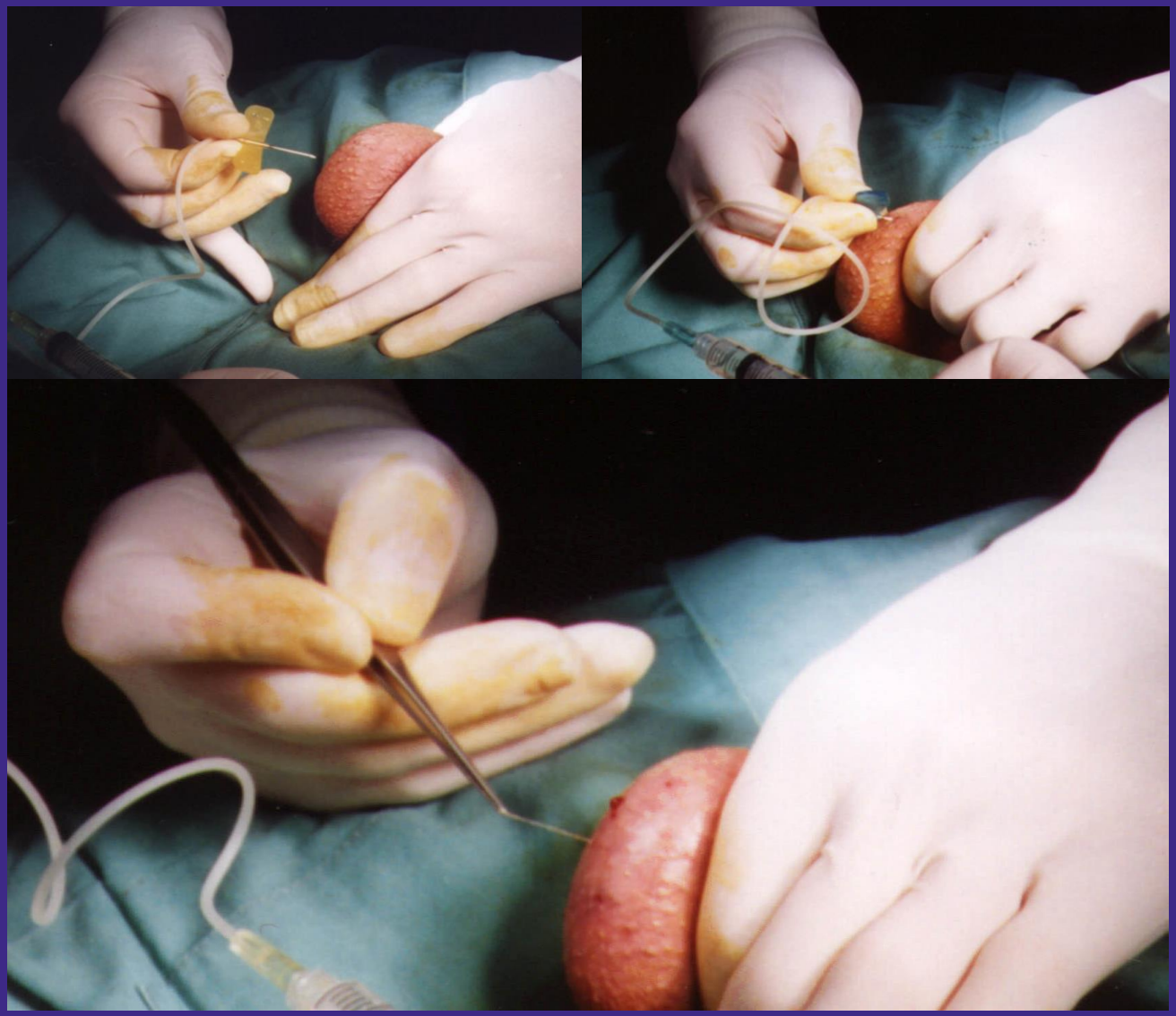
PESA

Percutaneous

TESA

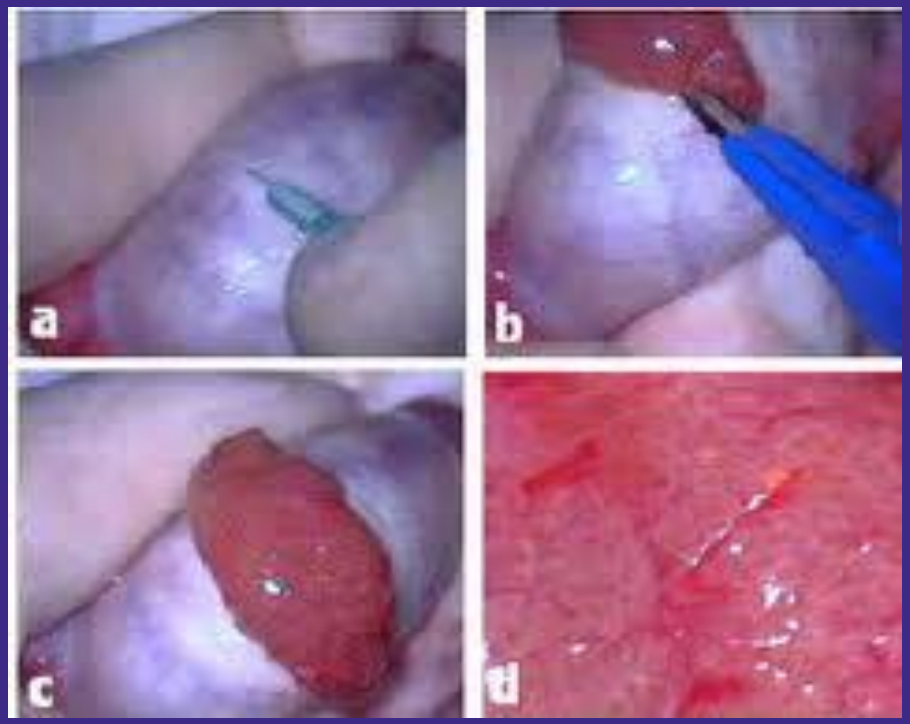
Testicular

2. What if no ejaculated sperm?



2. What if no ejaculated sperm?

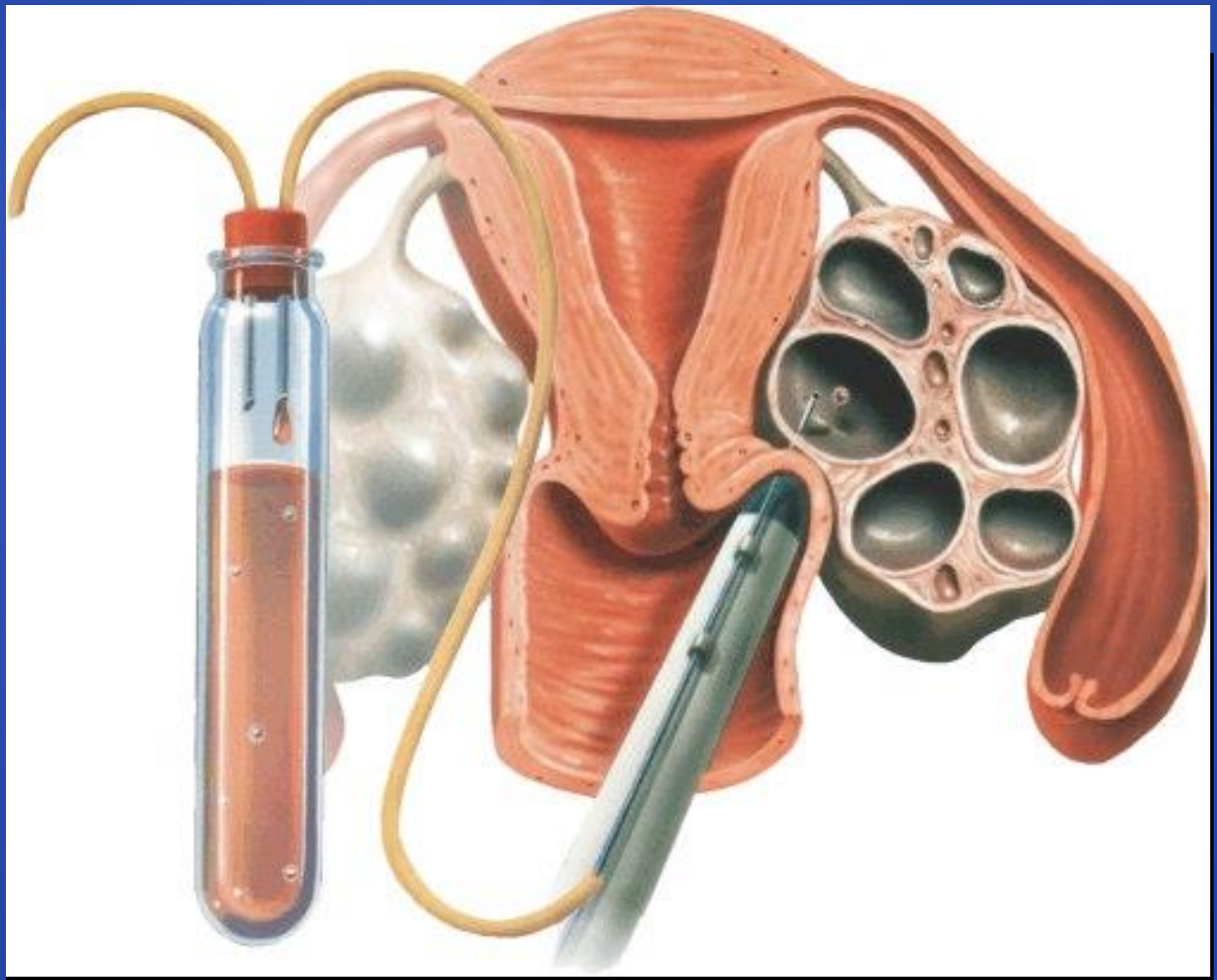
Open Testicular Biopsy



Step 3: oocyte retrieval

- Day surgery / hospital approx 4 hours
- Procedure takes about 30 mins
- Anaesthetic options
  - Light general anaesthetic
  - Local anaesthetic

3. Egg pick-up (OPU) procedure

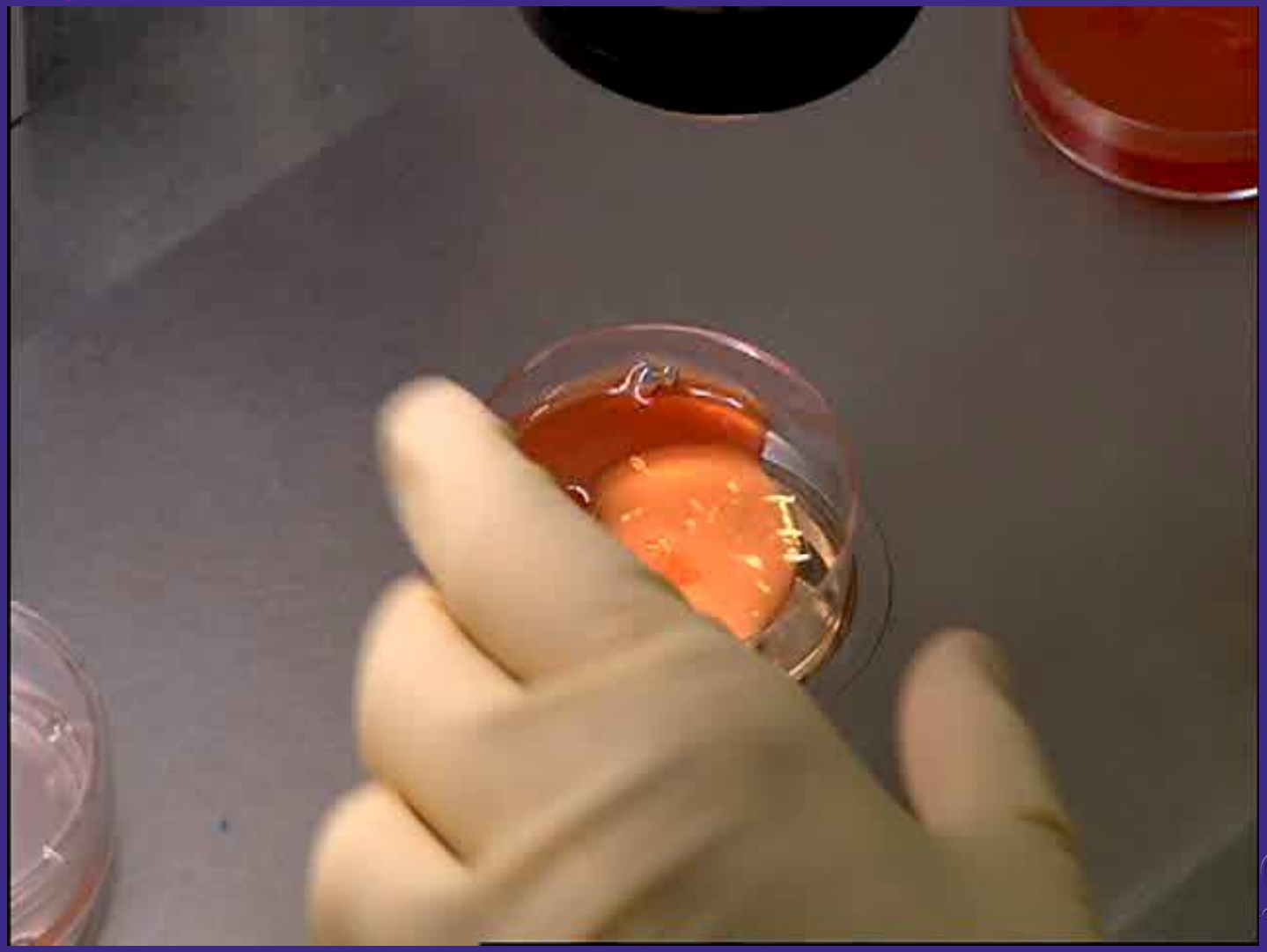


3. IVF Lab: OPU Process

Oocyte Collection

3. IVF Lab: Eggs Located

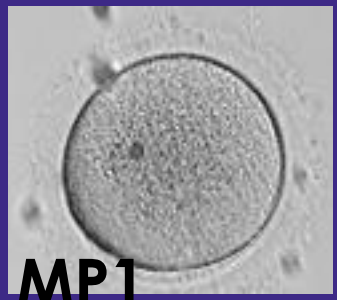
Oocytes collected



3. IVF Lab: Maturity Stages


GV (Prophase1)

GV (Germinal Vesicle) at prophase 1 then undergoes **GVBD** (Germinal Vesicle Break Down) to form a **MPI** (Metaphase I) stage oocyte.


MP1

This oocyte completes meiosis 1, and starts

Meiosis 2, and arrests at the **MPII** (Metaphase II) stage.
It has **1PB** (one Polar Body). It stays at this stage until after sperm enters. It then completes Meiosis 2, and forms a,


MP2

2PN (Two Pro Nuclei), a normally fertilised oocyte with **2PB's** (Two Polar Bodies).


2PN

3. IVF Lab: Usable oocyte

Step 4. Insemination & embryo culture

Insemination Day 0

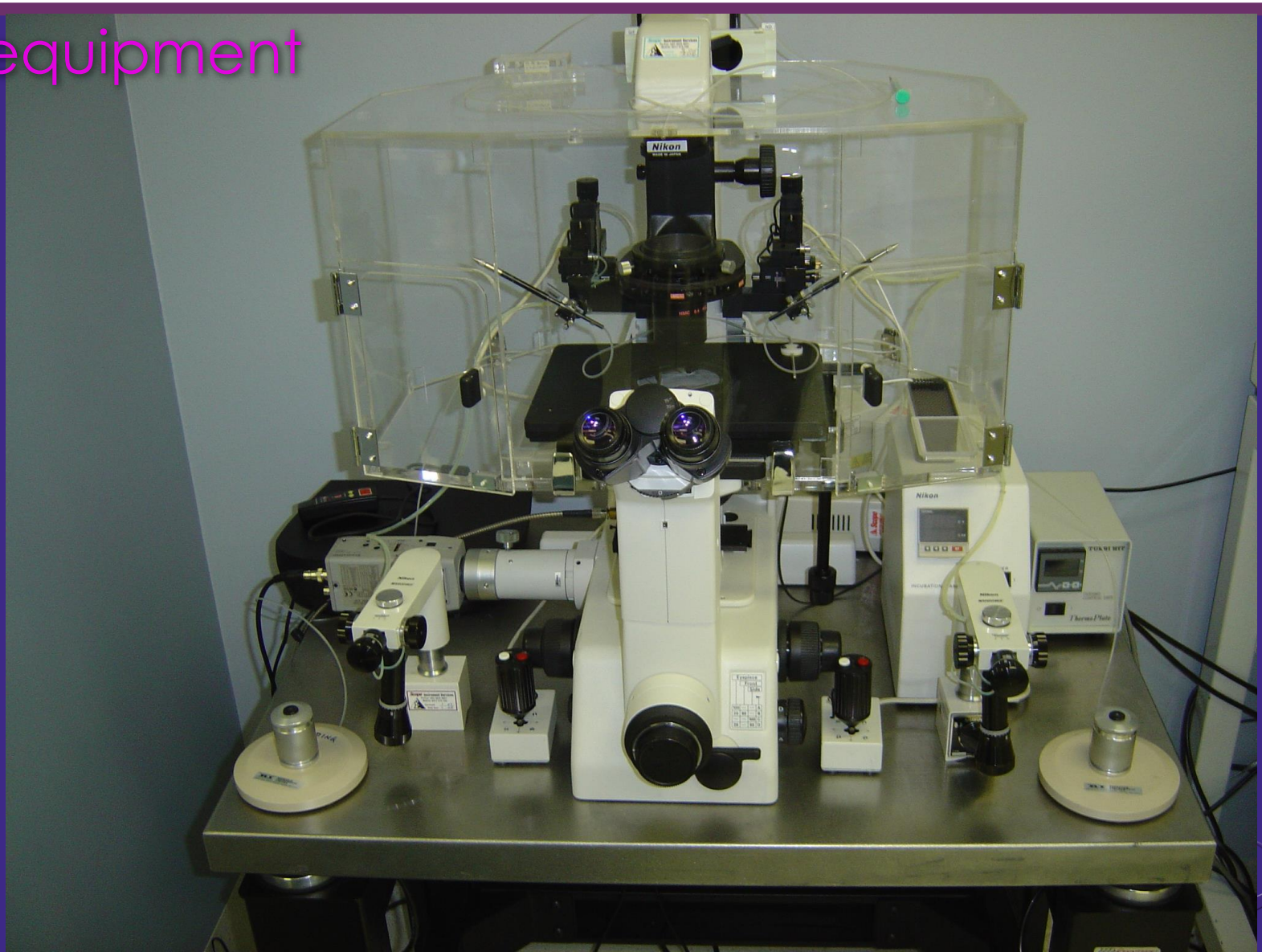
Add lots sperm (IVF)

4. IVF Lab: ICSI Denuding

ICSI denuding

4. IVF Lab: Microinjector

ICSI equipment



4. IVF Lab: ICSI Process

Insemination Day 0

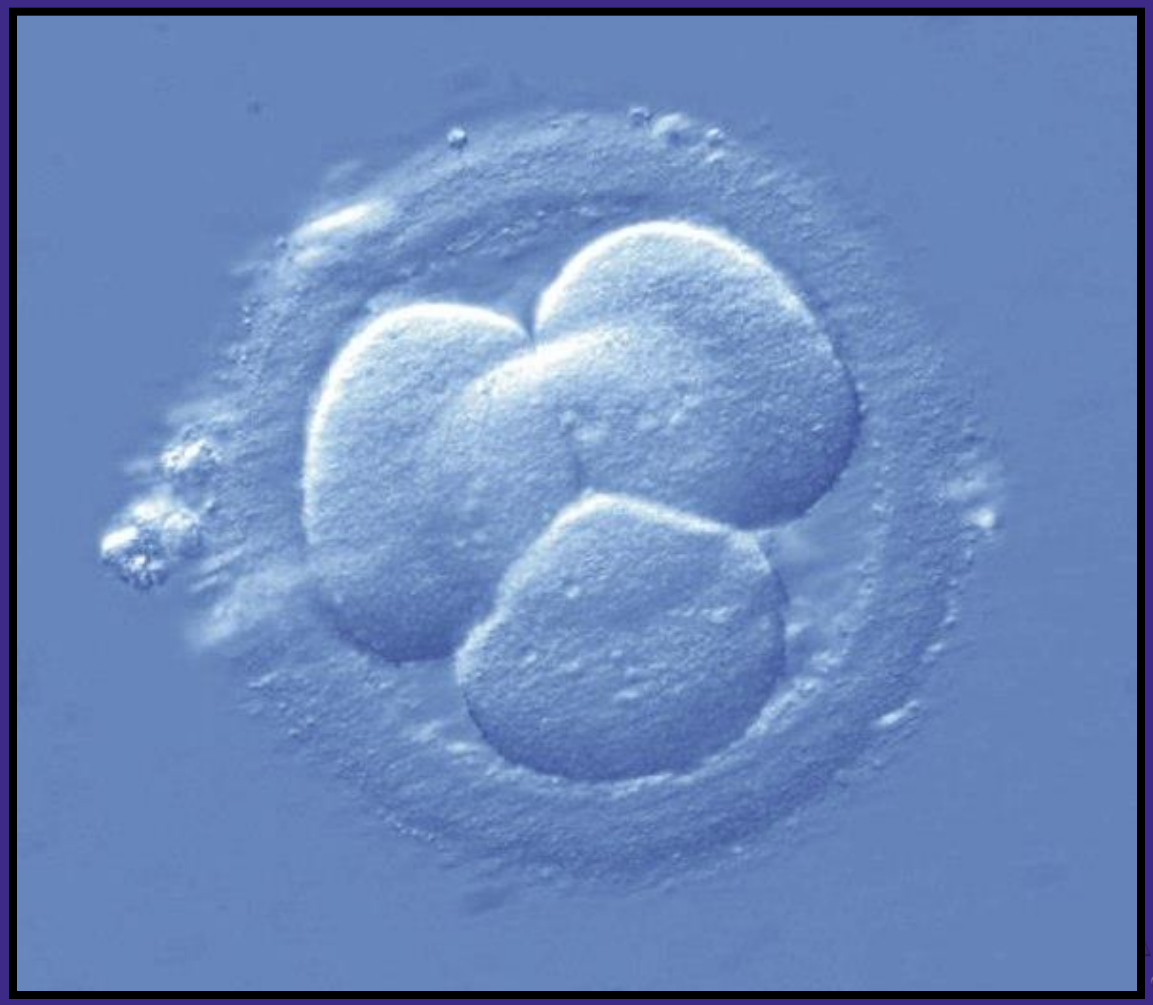
Inject single sperm (ICSI)

4. IVF Lab: Fertilisation

Fertilisation - Day 1

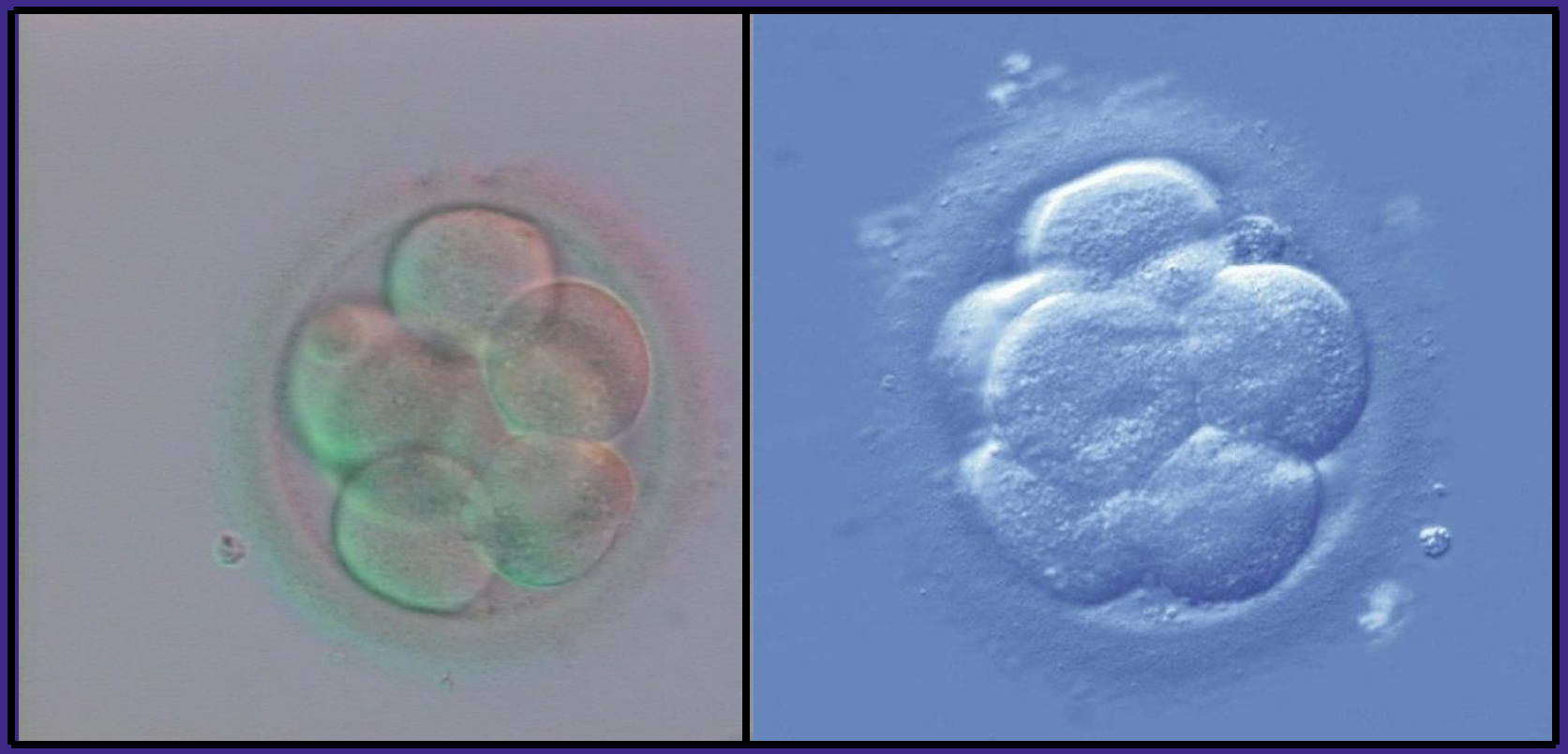
4. IVF Lab: Cleavage Culture

Early Cleavage - Day 2



4. IVF Lab: Cleavage Culture

Early Cleavage - Day 3



4. IVF Lab: Morula

Extended Culture - Day 4

4. IVF Lab: Blastocysts

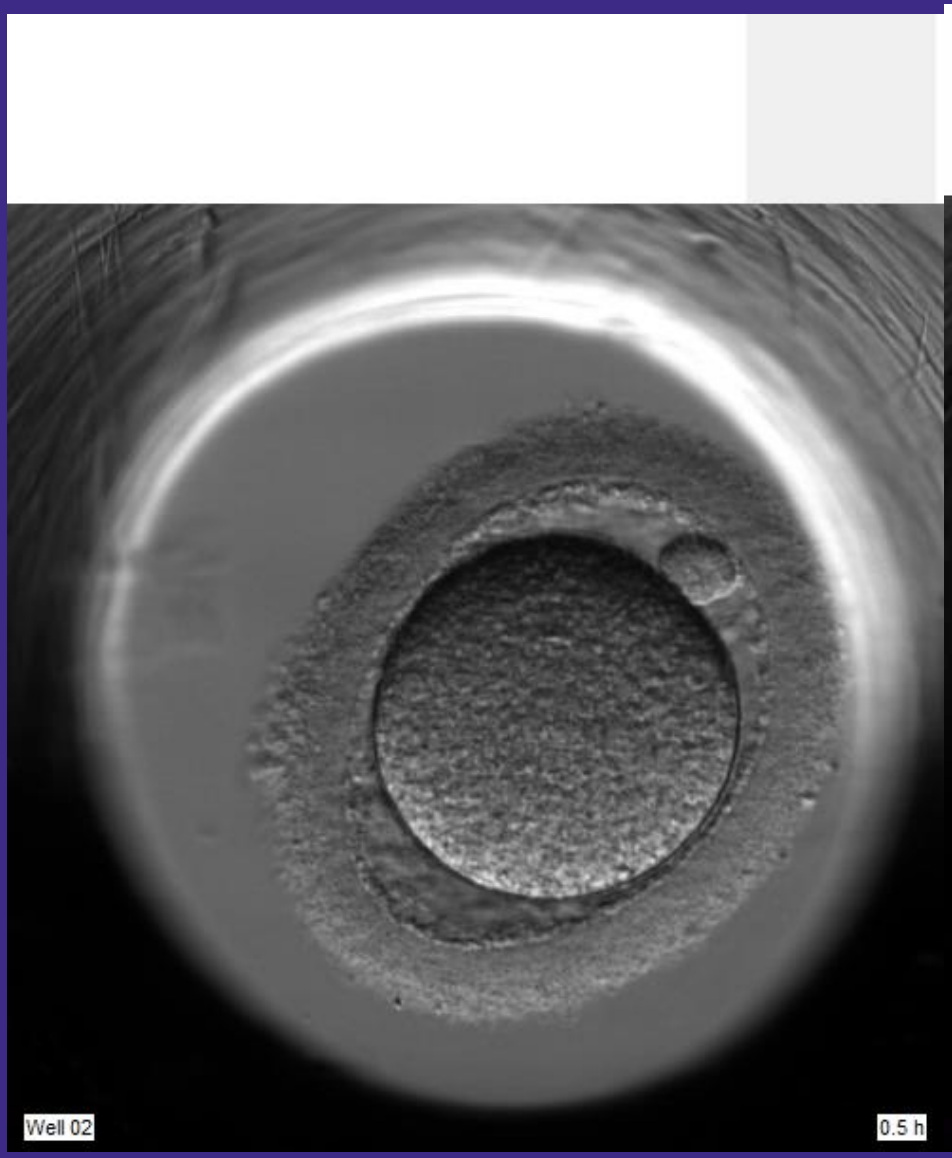
Blastocyst - Day 5

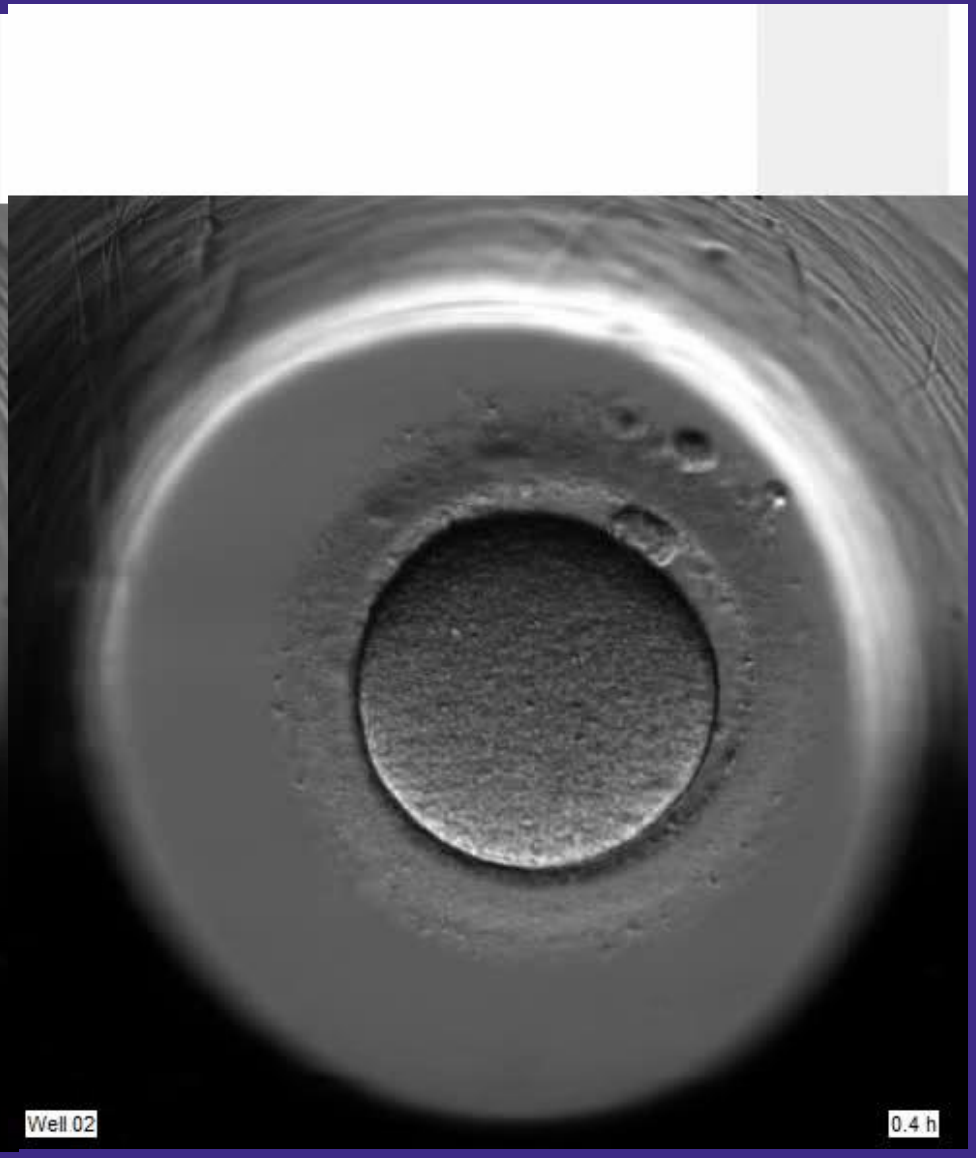
4. IVF Lab: Blastocyst tissue types

Trophectoderm
(forms Placenta)

Inner Cell Mass
(forms Fetus)

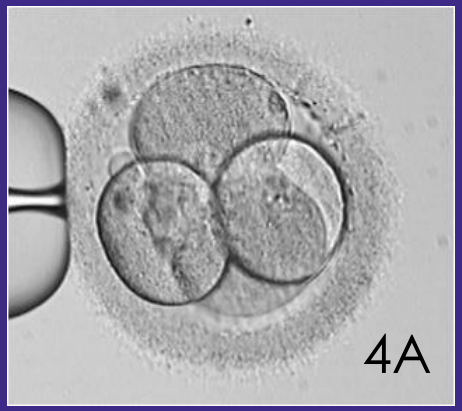
4. IVF Lab: All growth days together

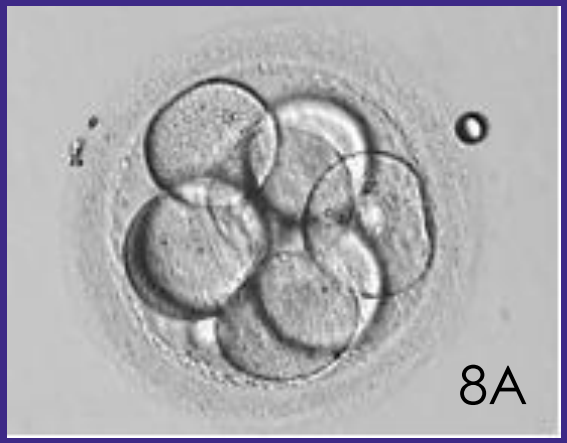




4. IVF Lab: Not all embryos are usable

High Preg rate

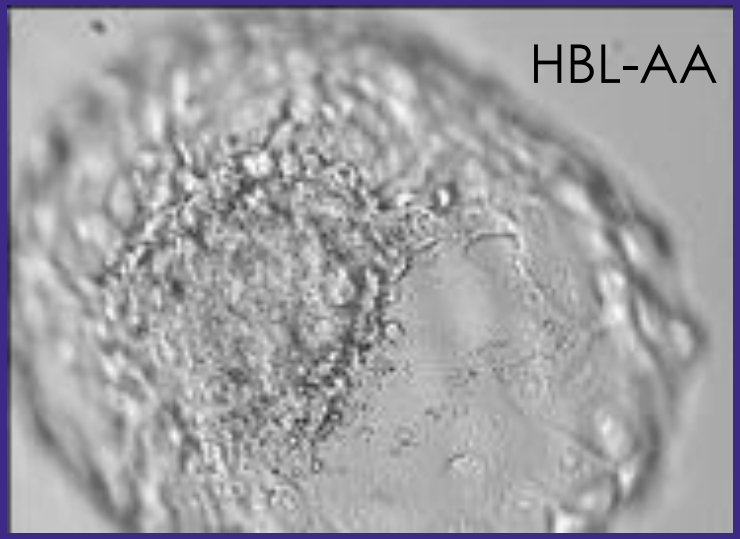


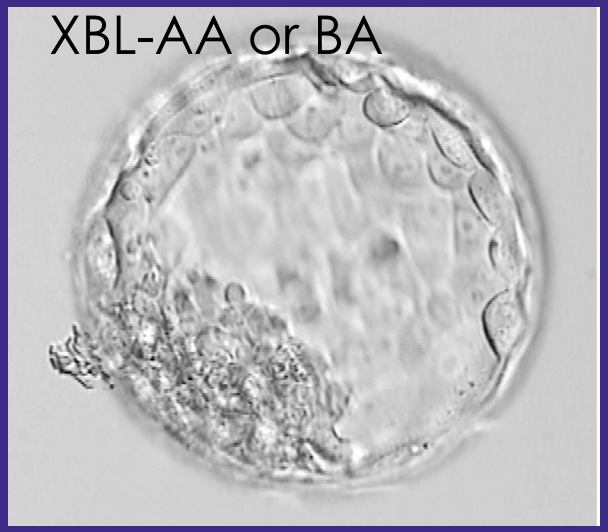


Low Preg rate.

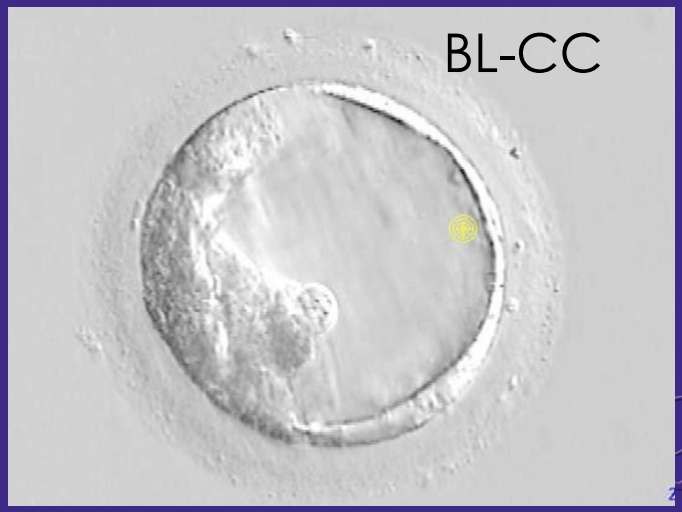
4. IVF Lab: Not all Blasts are usable

High Preg rate





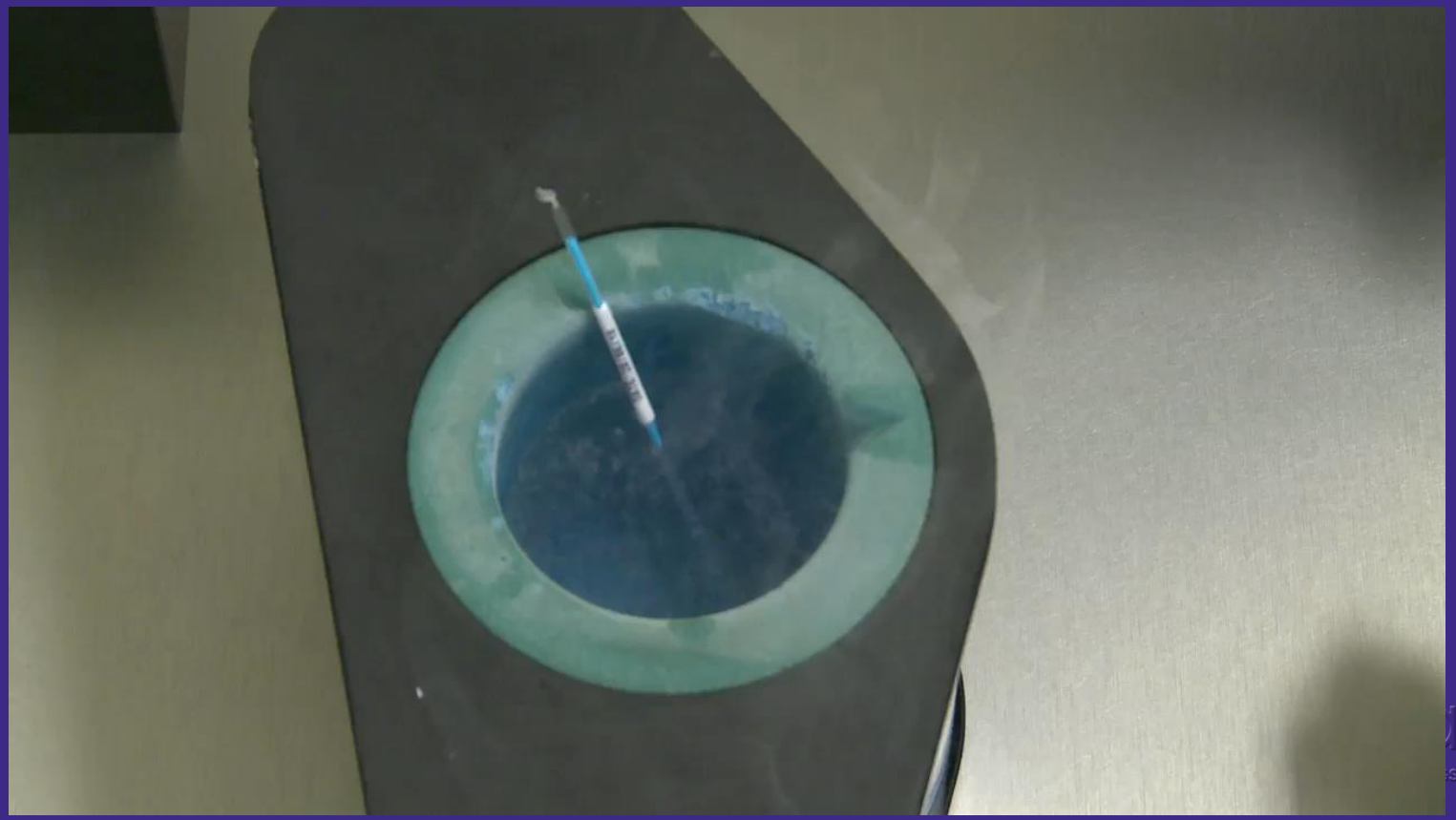
Low (or zero) Preg rate.



4. IVF Lab: Freezing of embryos

Cryopreservation

- 40-50% of all pregnancies come from Frozen Transfers.
  - Lose 5-8% of embryos using vitrification, or,
  - 15-20% using slow freeze



4. Preimplantation genetic diagnosis (PGD)

PG Diagnosis – Known disease or disorders

- Single gene defects …ie. CF, DMD, etc, or Translocations.
- Normally by DNA amplification techniques

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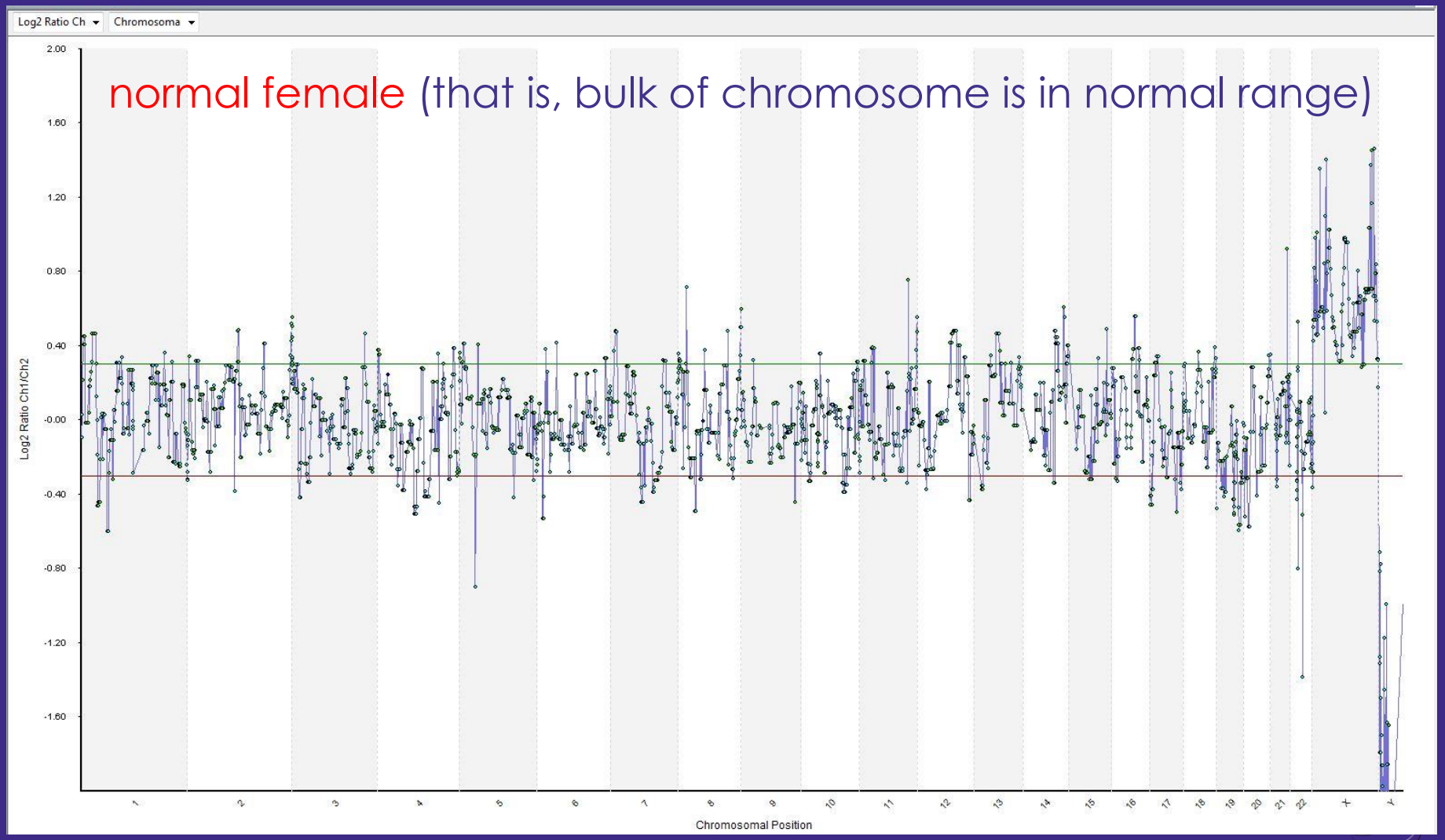
PG Screening – Looking for whole chromosome aneuploidy

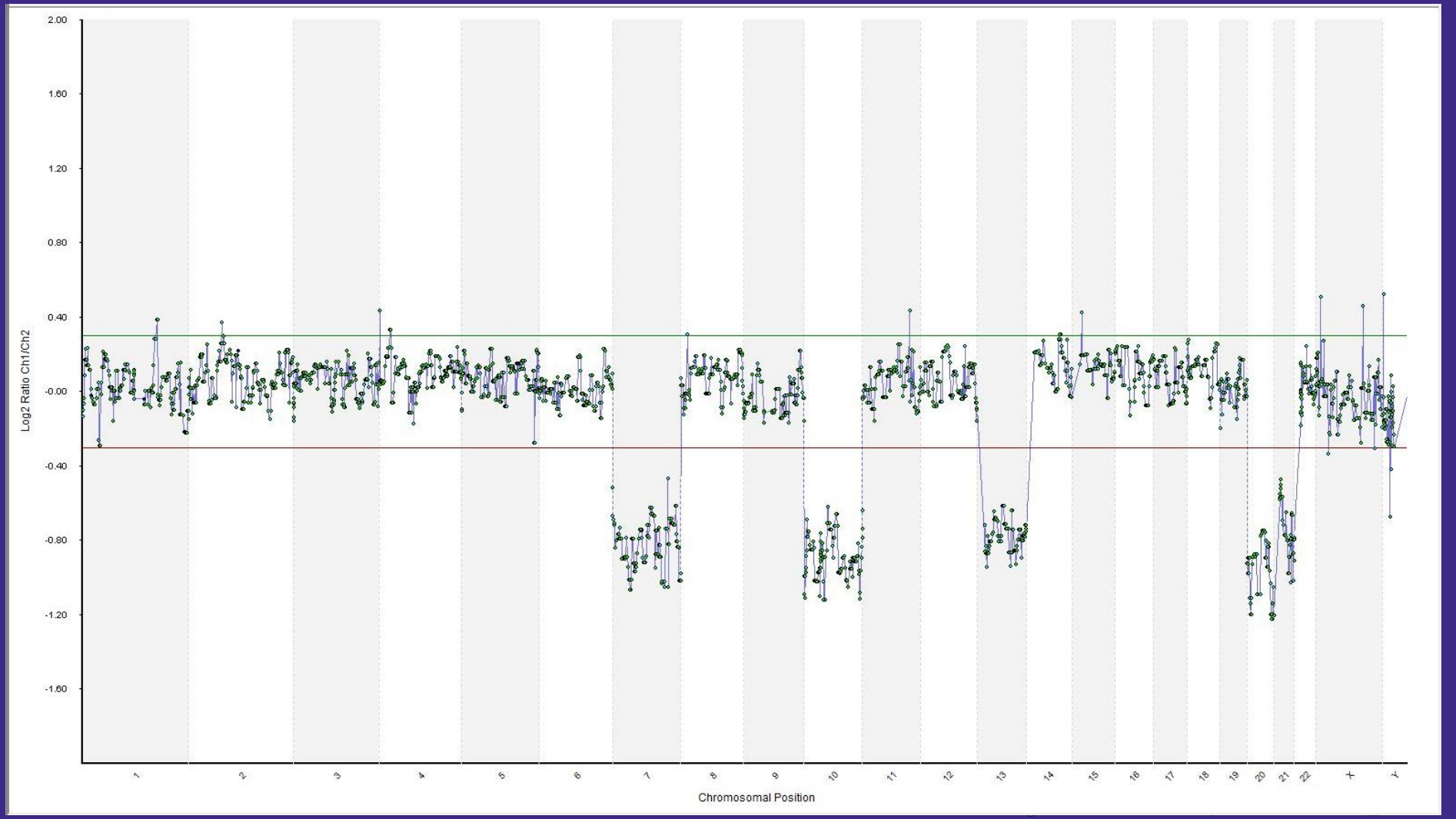
- Repeat miscarriage (> 2 or 3 M/C)
- RIF = repeat implantation failure (>5 embryos or > 4-5 transfers)
- AMA = Advanced maternal age (RIF but now >38 yrs of age)

4. PGD processes (Day 5 Biopsy)

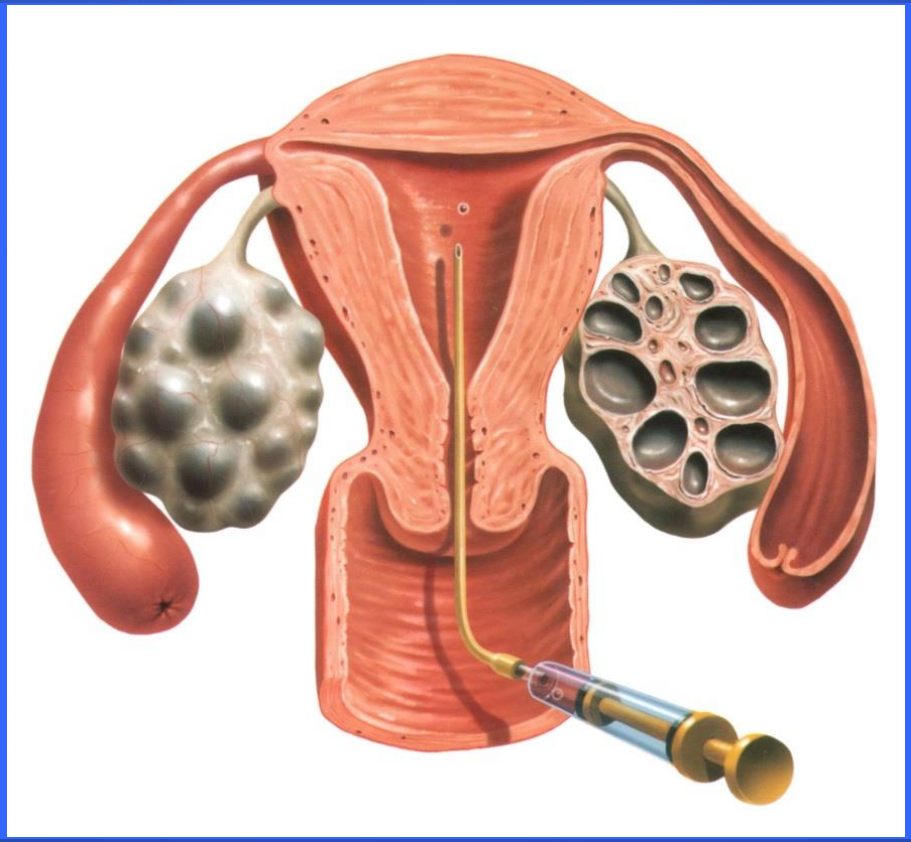
4. PGD processes: array PGD: (results for all chromosomes in <8hrs)



4. PGD processes: array PGD: 41,XY-7-10-13-20-21



Step 5: Embyro transfer

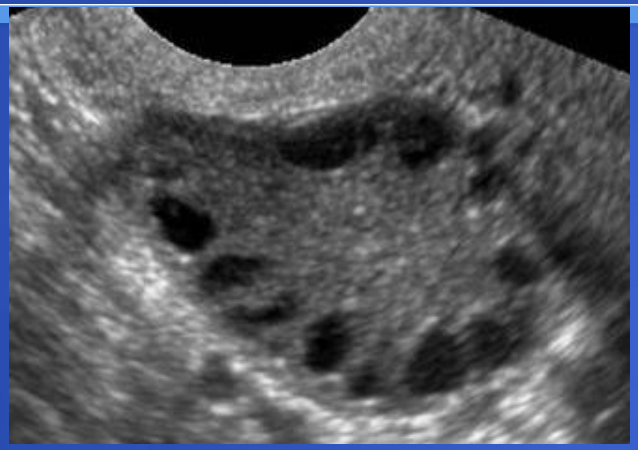


Complications of IVF

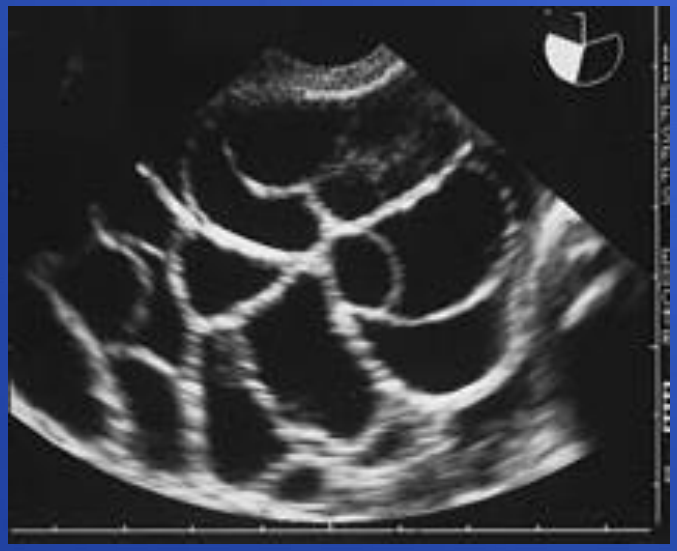
- Side effects of medications
  - Local reactions
    - Injection site
    - Nasal/sinus irritation
  - Breast tenderness
  - Abdominal bloating/ fluid retention
  - Nausea
  - Fatigue
  - Mood swings
  - Hot flushes

- Failure of stimulation

Ovarian hyperstimulation syndrome



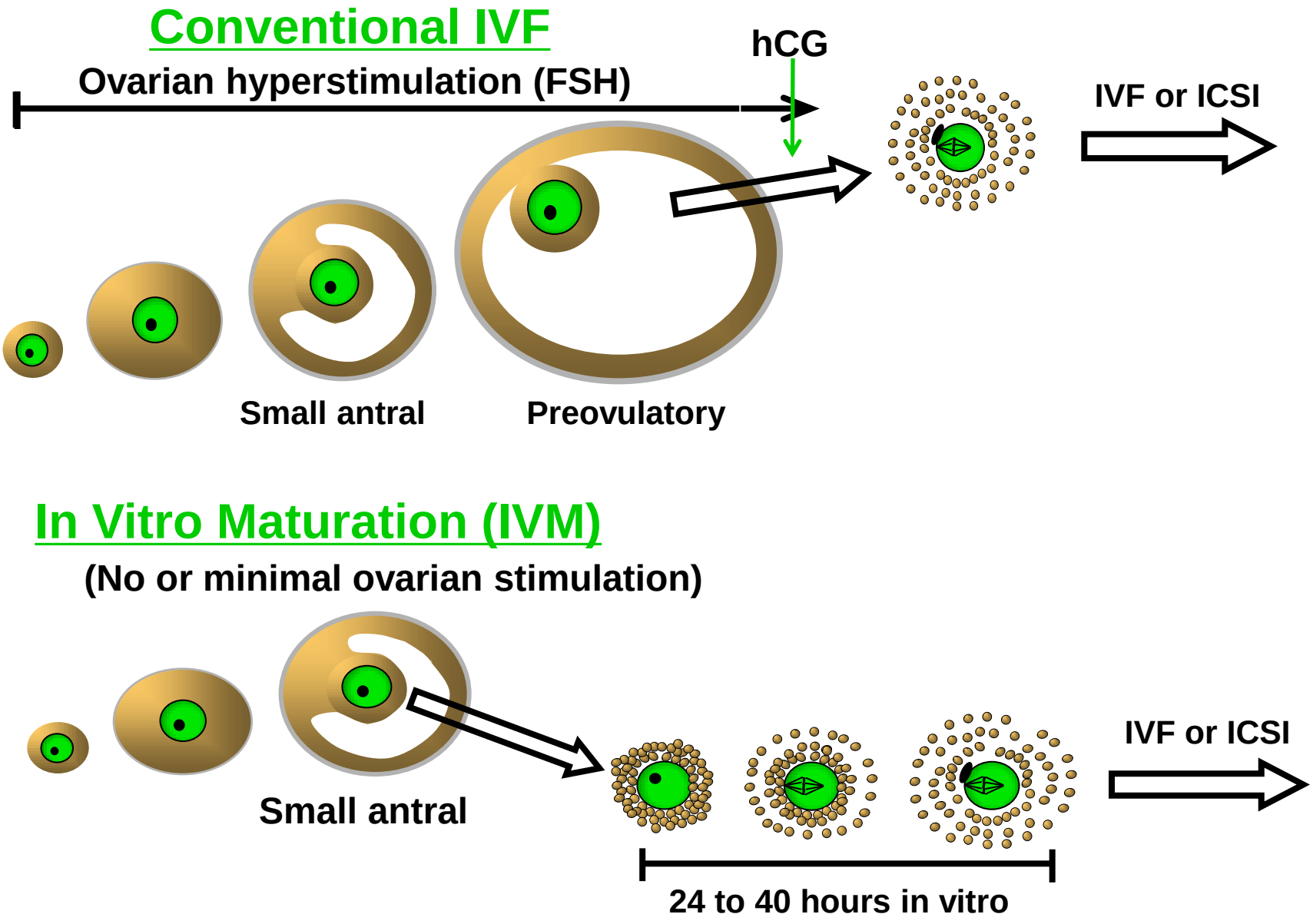
Polycystic ovary



Ovarian
hyperstimulation (OHSS)

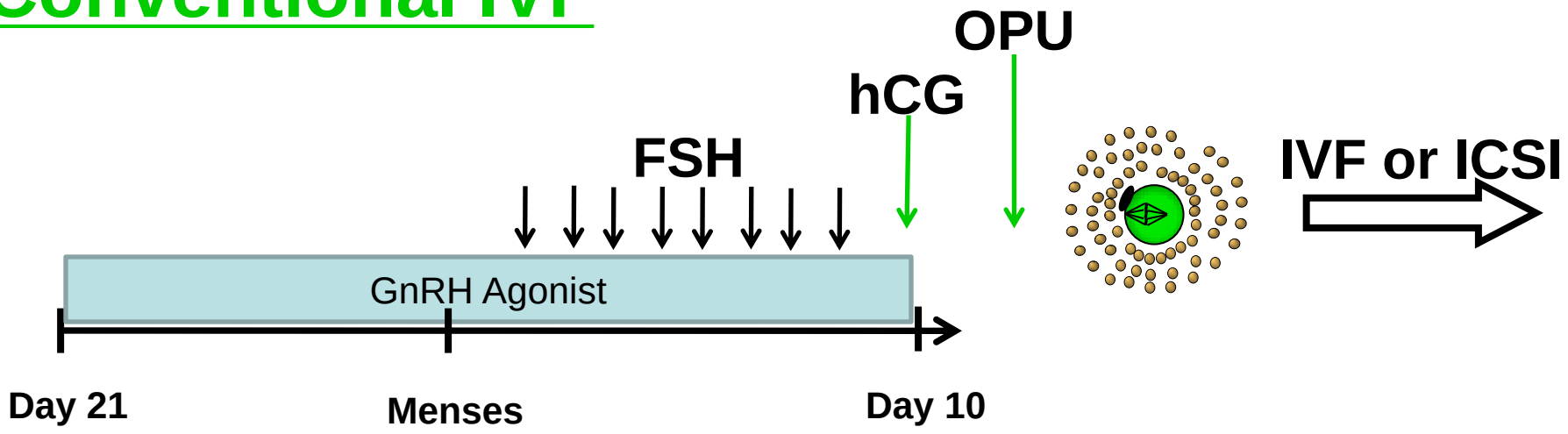
OHSS

- Symptoms
  - Abdominal bloating and pain
  - Decreasing urine output
  - Severe nausea and vomiting
  - Diarrhoea
  - Shortness of breath
  - Increasing thirst
- Less than 1% require hospitalisation treatment



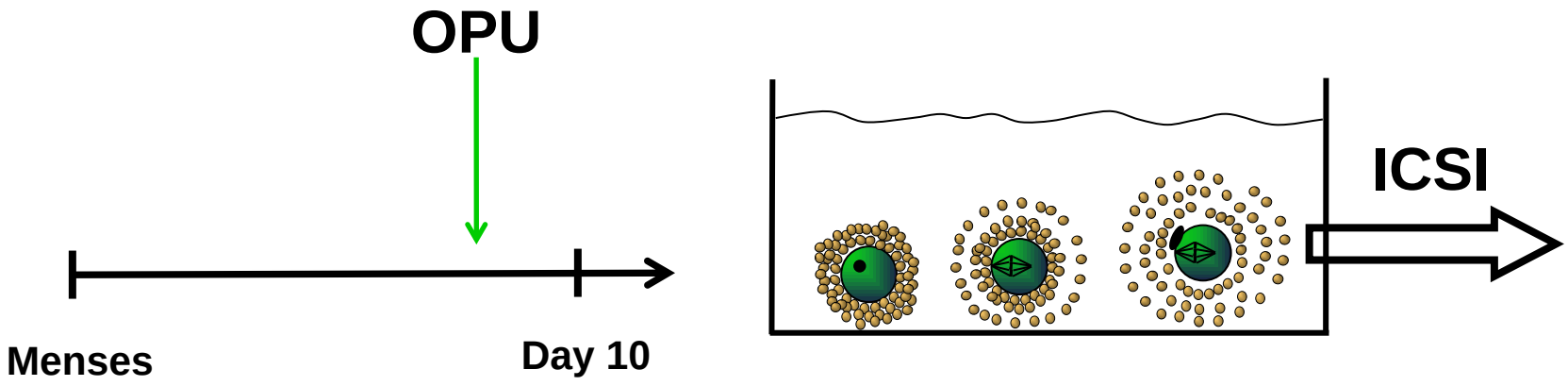
Thompson JG & Gilchrist RB (2013) In: Biology and Pathology of the Oocyte (Cambridge University Press)

Conventional IVF



In Vitro Maturation (IVM)

See YouTube clip:
- search “Enhanced IVM”



Pregnancy complications

- Ectopic preganancy, miscarriage : same as natural conception
- Multiple pregnancy
  - National figures - up to 16% of IVF cycles (but falling)
  - Mostly caused by transferring two or more embryos
  - Embryos can split after implantation
- Multiple pregnancy risks include:
  - Premature birth
  - Threefold increase in risk of baby dying during or soon after birth
  - Fourfold increase in the chance of cerebral palsy
- Most Australian clinics aim to transfer Single Embryos

Success rates

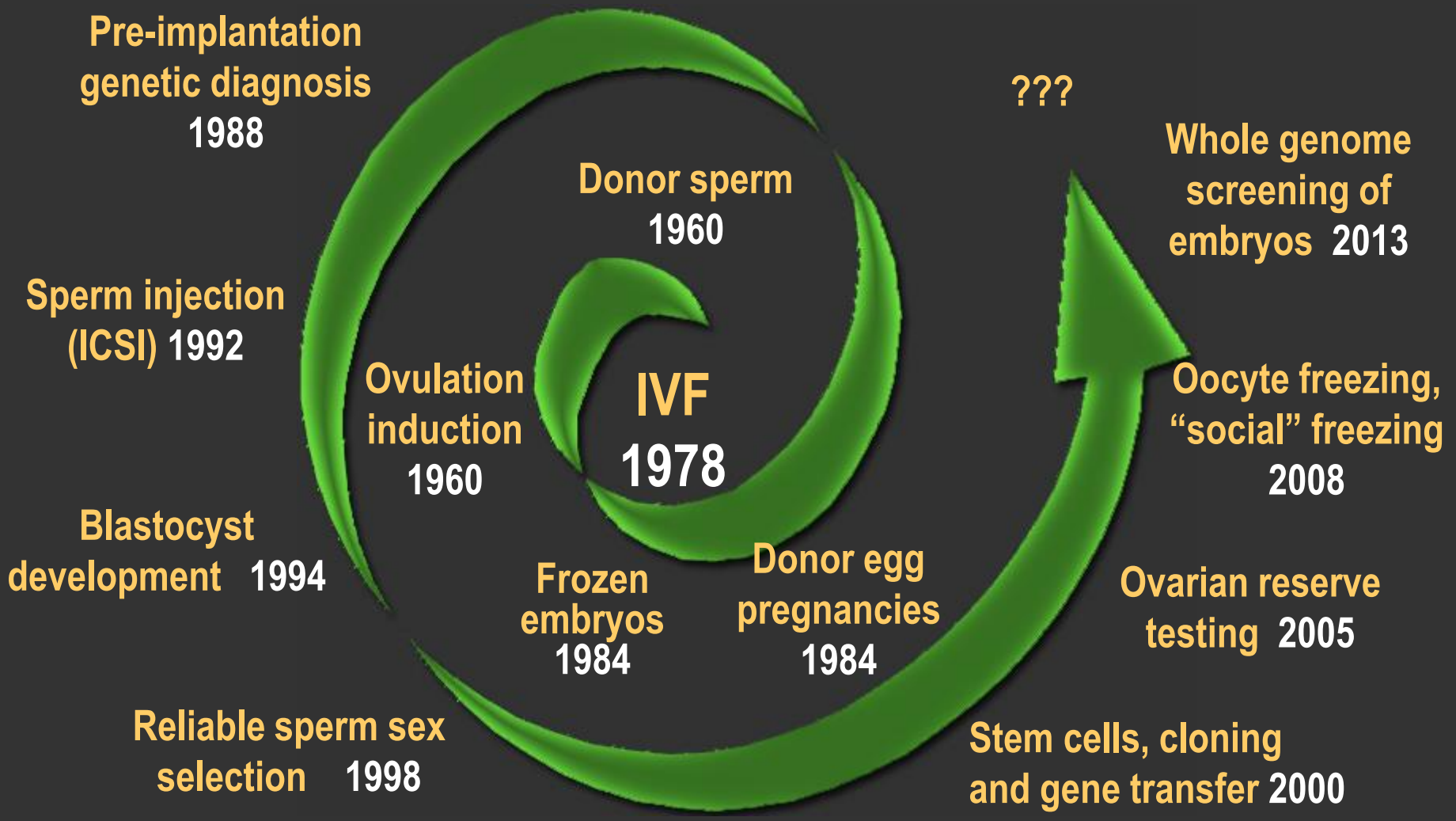
- Depend on
  - FEMALE AGE
  - Duration and cause of infertility
  - Quality and number of embryos created
  - (Health of woman's uterus)

2013 Fresh ET: CPR% and IR% Australia

TABLE 1: SUMMARY (2013 NPESU data)

FRESH CYCLES	<30 years	30-34 years	35-39 years	40+ years	All ages Overall Percentile	
	number	number	number	number		
Number of cycle with OPU	4,447	10,632	14,340	11,109		
OPU with FSH administration (ov_stim=y)	4,418	10,546	14,187	10,932	25th percentile	75th percentile
total oocytes collected	52,995	112,774	125,264	71,646		
average oocytes collected per OPU	11.9	10.6	8.7	6.4	10.8	12.3
OPU with embryo transfer	3,131	8,029	10,654	7,803		
total embryos transferred	3,446	9,178	13,438	11,429	25th percentile	75th percentile
average embryos transferred per transfer	**1.10**	**1.14**	**1.26**	**1.46**	1.2	1.4
Implantation rate	**41.3%**	**33.4%**	**23.2%**	**8.6%**	25th percentile	75th percentile
clinical pregnancy per OPU (%)	32.4 *	30.2 *	22.9 *	10.6 *	19.0	25.3
clinical pregnancy per ET (%)	**46.1**	**40.0**	**30.8**	**15.1**	**25.4**	**32.9**
% FH pregnancies with >1 fetal heart	5.1	6.2	8.2	6.3	4.0	9.4

The Artificial Reproductive Technology (ART) Revolution



IVF: conclusions

- IVF is a safe and very effective treatment for many causes of infertility
- Single embryo transfer minimizes risks
- IVF is not a solution for age related infertility as the success falls with increasing age