Preimplantation Genetic Testing (PGT)

What you need to know

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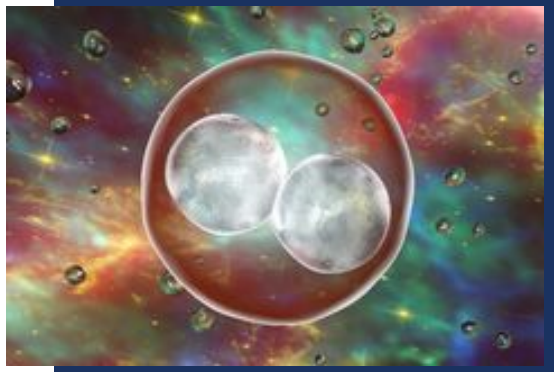
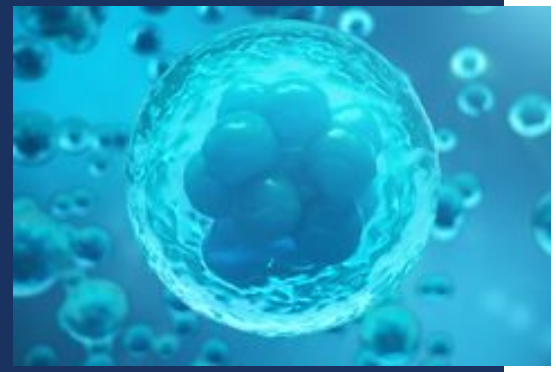


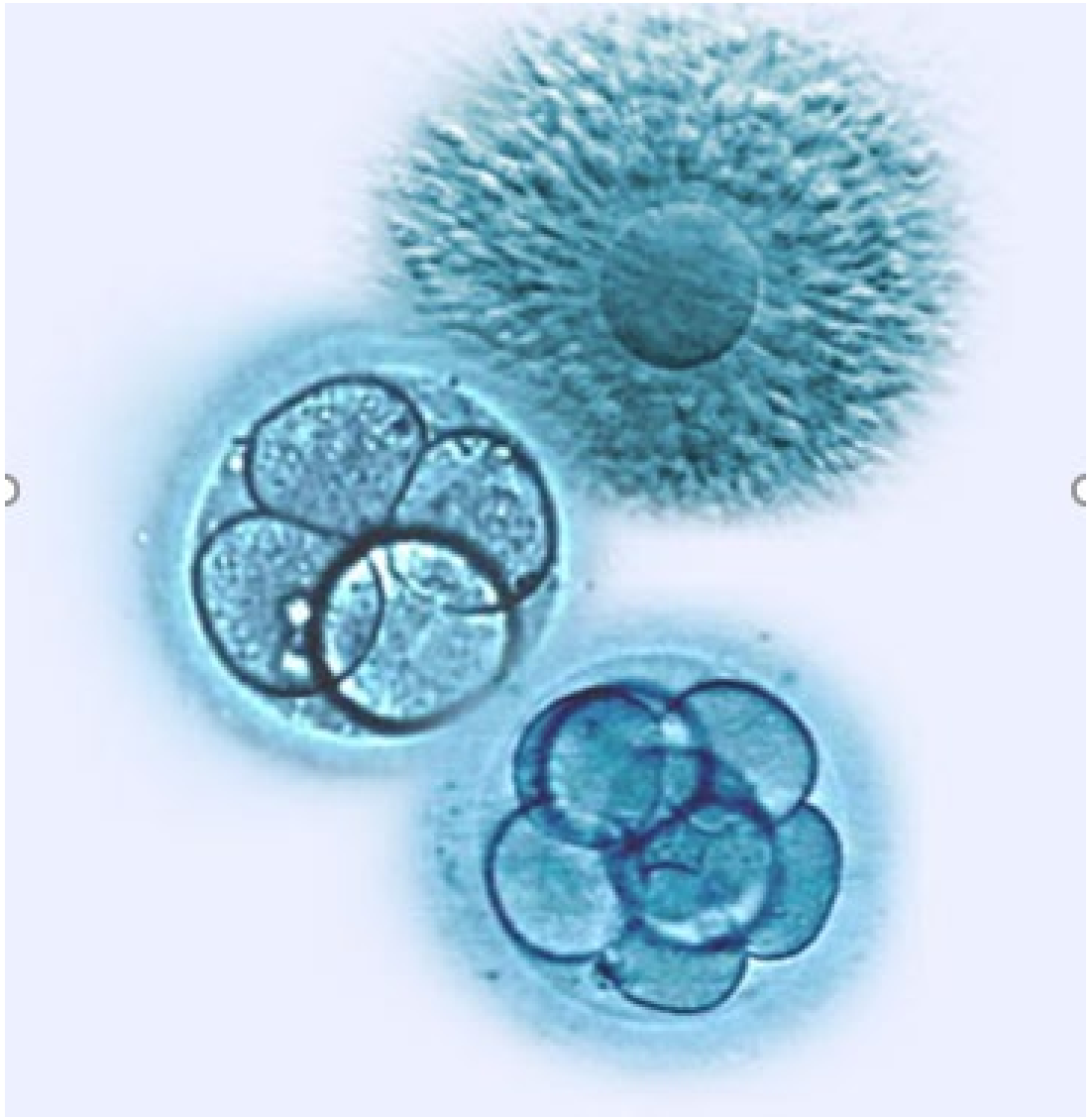


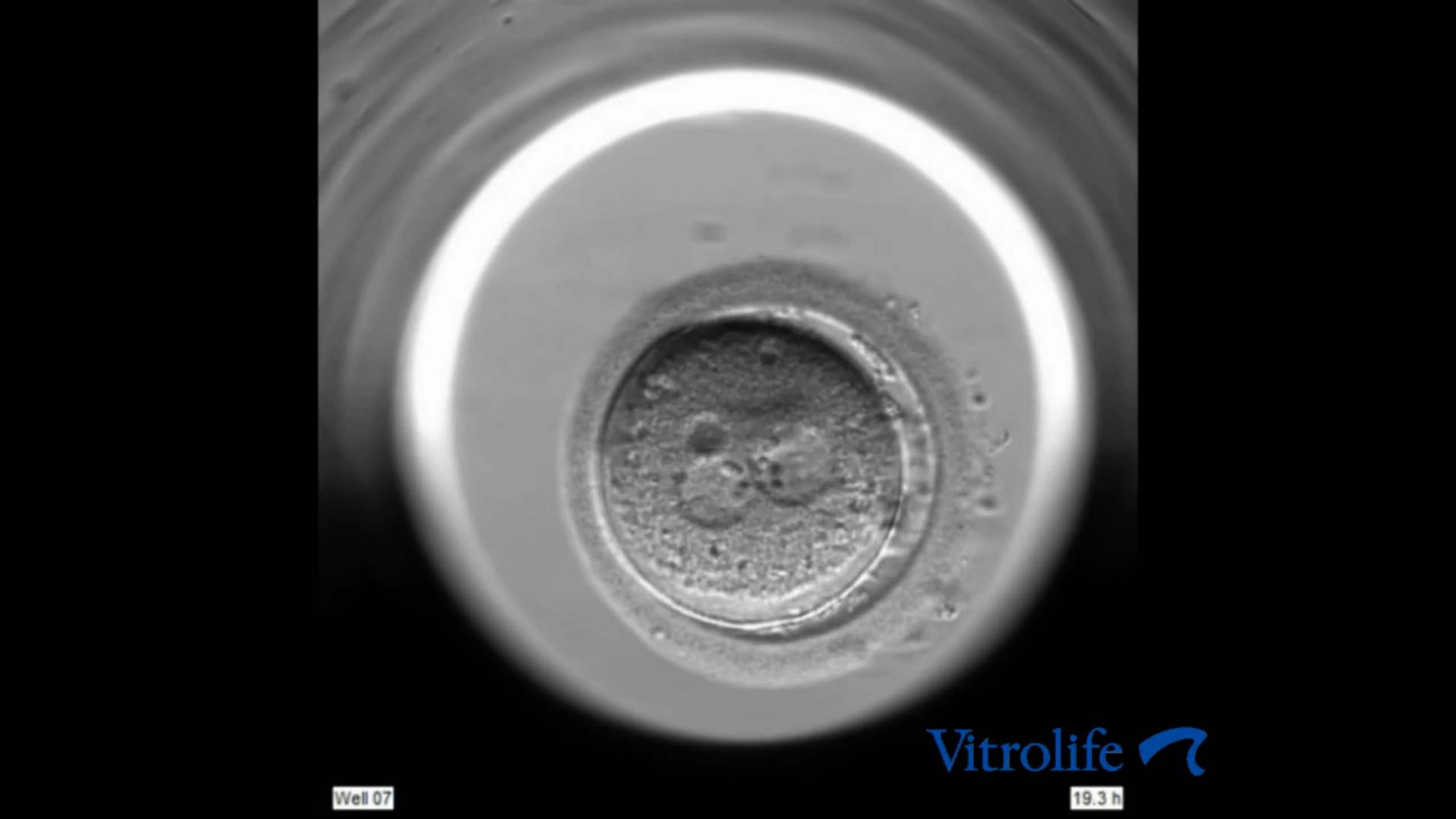
Preimplantation Genetic Testing (PGT)

- An option for women/couples to **reduce** the risk for genetic disorder in a pregnancy by:
  - Using in vitro fertilization (IVF) and genetic testing or screening
  - Implanting only embryos that are not expected to have the genetic abnormality of concern
- Requires
  - IVF
  - Genetic testing of embryos

A VARIETY OF CELLS MAY BE CHOSEN FOR TESTING:

- the first polar body, with or without the corresponding second polar body
- a single blastomere from a cleavage stage embryo
- a group of cells from the trophectoderm at the blastocyst stage

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PGT - Terminology

- A test performed to analyze the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA-typing or for determining genetic abnormalities.

- Types of PGT
  - PGT-A → for aneuploidy
  - PGT-M → for monogenic/single gene conditions
  - PGT-SR → for structural chromosomal rearrangements
  - PGT-HLA → for HLA typing

PGT - Indications

- PGT-M
  - Single gene disorders
- PGT-SR
  - Balanced translocations
  - Inversions
- PGT-A
  - Sex selection for genetic indications
  - Maternal age
  - Recurrent pregnancy loss
  - Repetitive IVF failure
- PGT-HLA
  - When HLA typing is needed/desired

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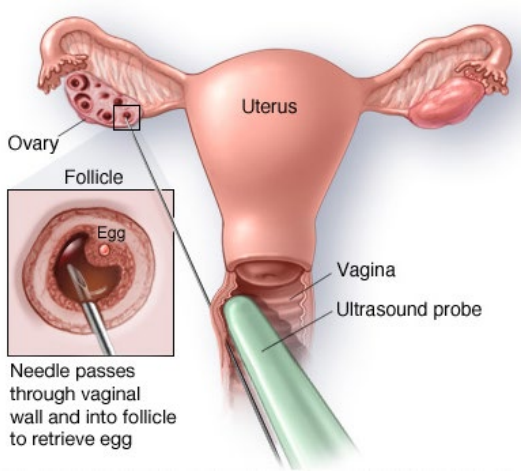
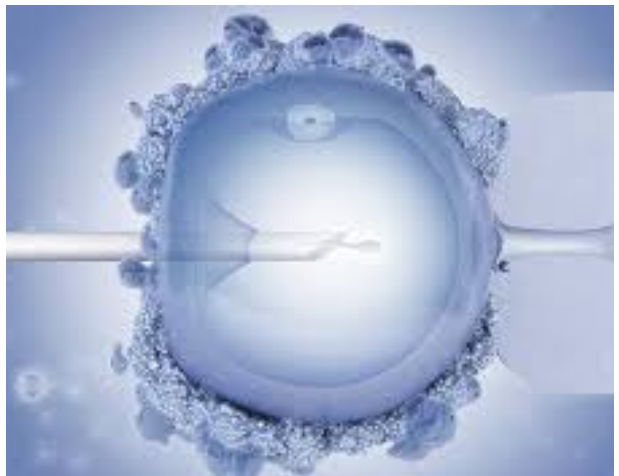
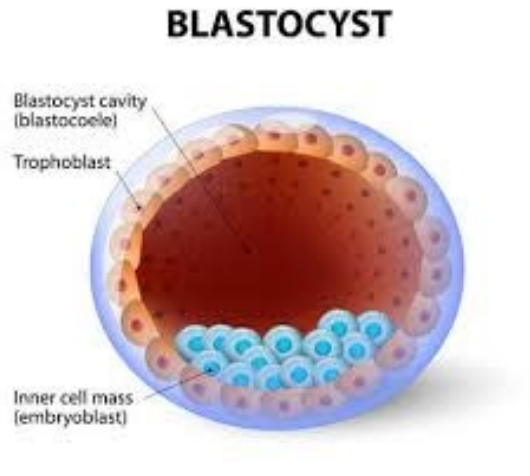
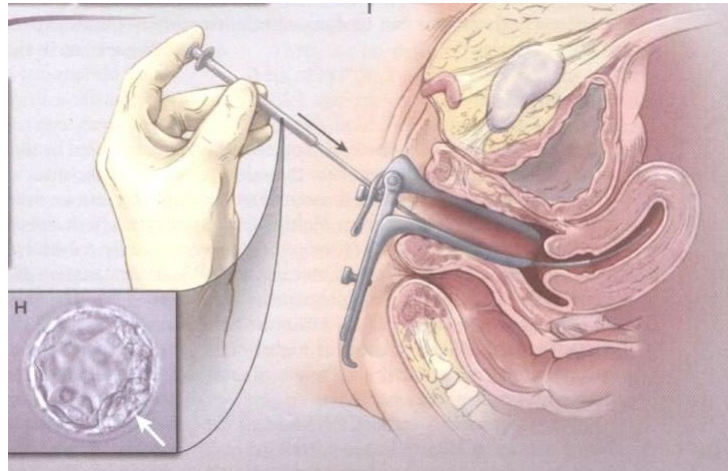
IVF

- The basic steps of an IVF cycle are
  - Ovarian stimulation
  - Egg retrieval
  - Insemination, fertilization, embryo culture
  - Embryo transfer



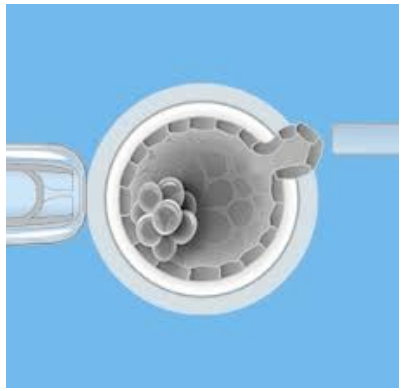


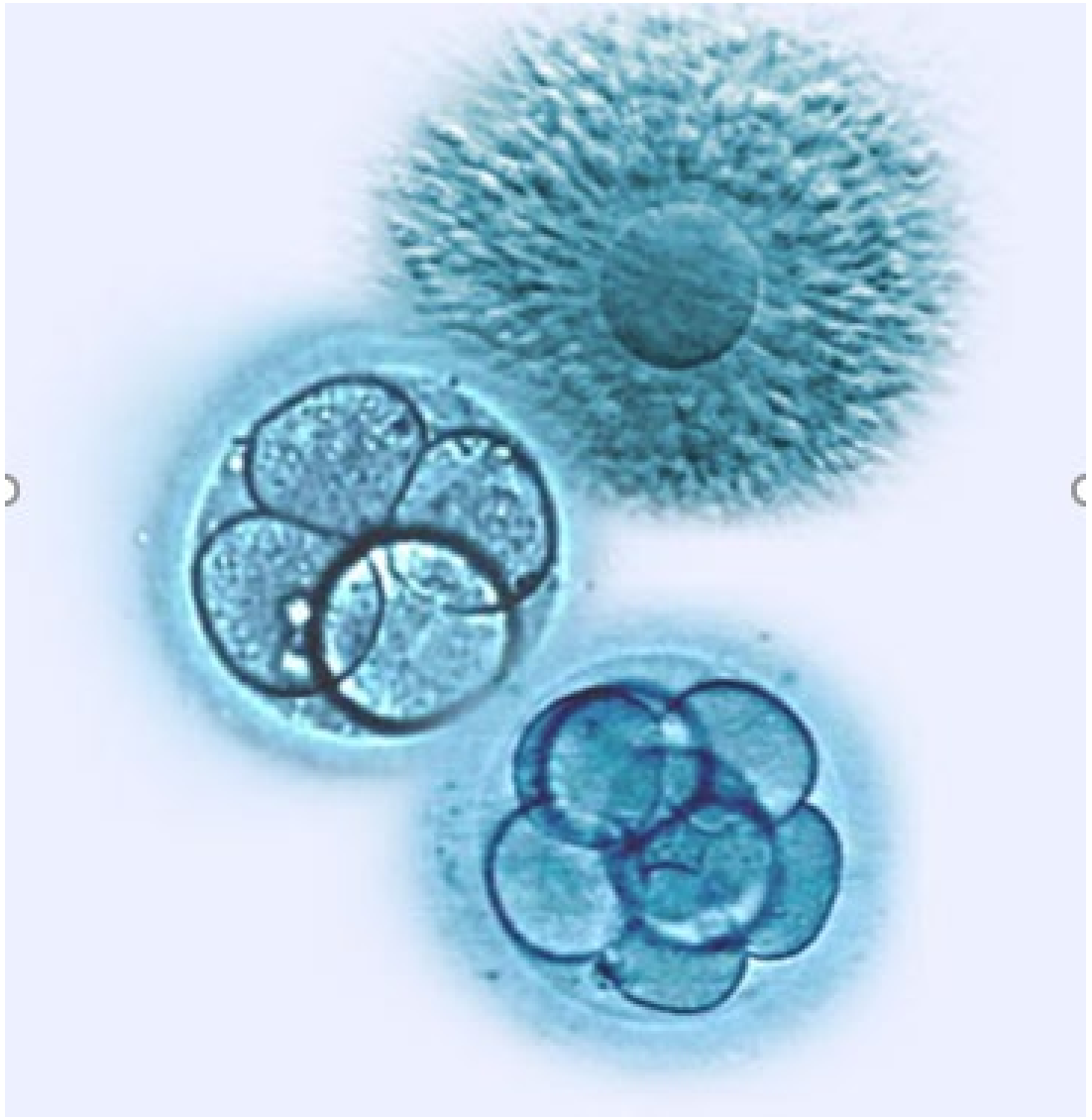




PGT - Requirements

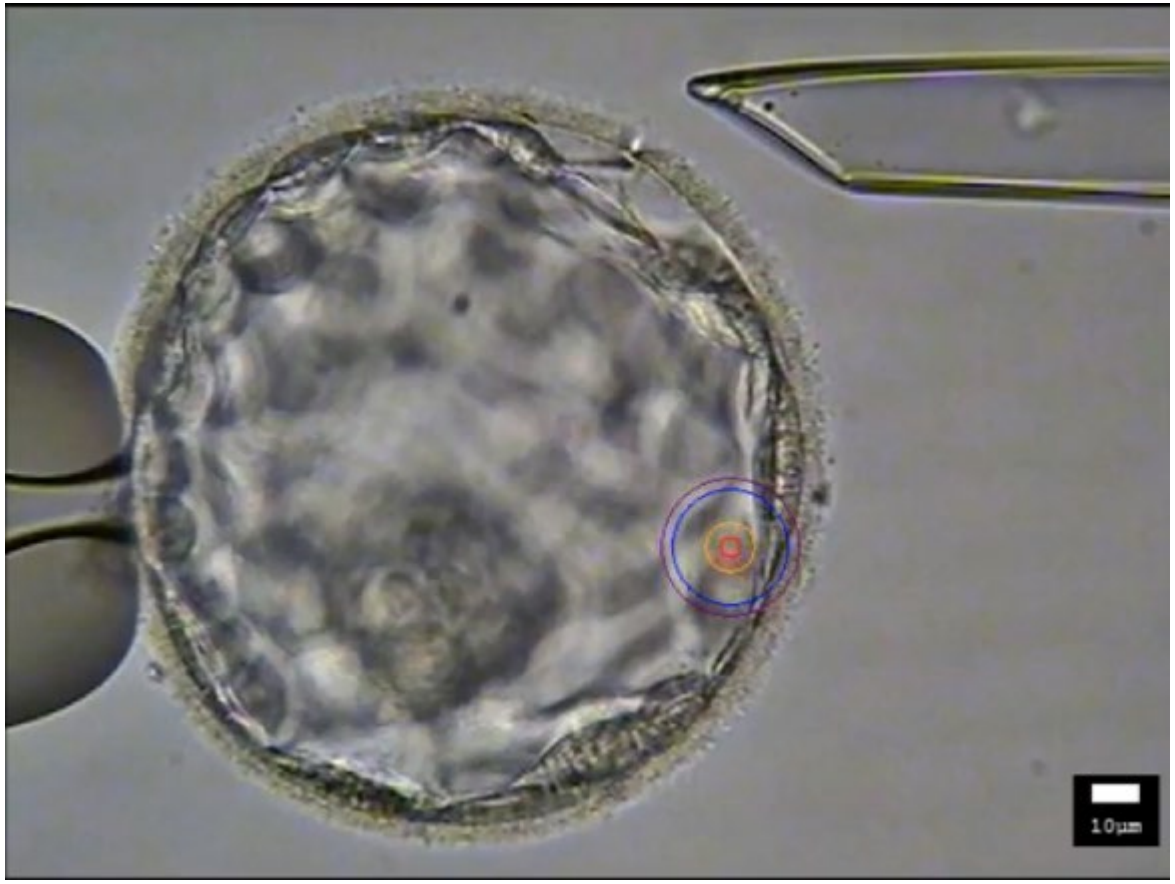
- In addition to IVF, PGT requires:
  - Test set-up
  - Embryo biopsy
  - Genetic testing of biopsied cells (often requiring test set-up)
  - Cryopreservation of embryos





VIDEO OF THE PROCESS OF PGT

https://youtu.be/TZz7GrIId7I



PGT – Test Set-up

- Prior to starting IVF the lab that will be performing the testing will need to design a genetic test specific to the patient/couple's situation
- What do they need?
  - Copies of genetic test results
  - Blood/saliva/DNA samples from the couple and possibly other family members
  - Time
    - Test set typically takes several weeks

PGT-M

- Current standard of practice is to use targeted haplotyping of closely linked or intragenic informative polymorphic markers (single nucleotide polymorphisms: SNPs or short tandem repeats: STRs) with or without direct mutation detection

PGT-M - New Complexities

- Whole exome/genome sequencing (WES/WGS) adds new complexities
  - WES/WGS is often used in cases where other testing has failed to determine an etiology for an individual's condition
    - Variants of uncertain significance
      - Will PGT labs perform testing these variants?
    - New genes
      - Can PGT labs perform testing in genes that are fairly newly described?
      - Do labs have markers for these regions?

Possible Results

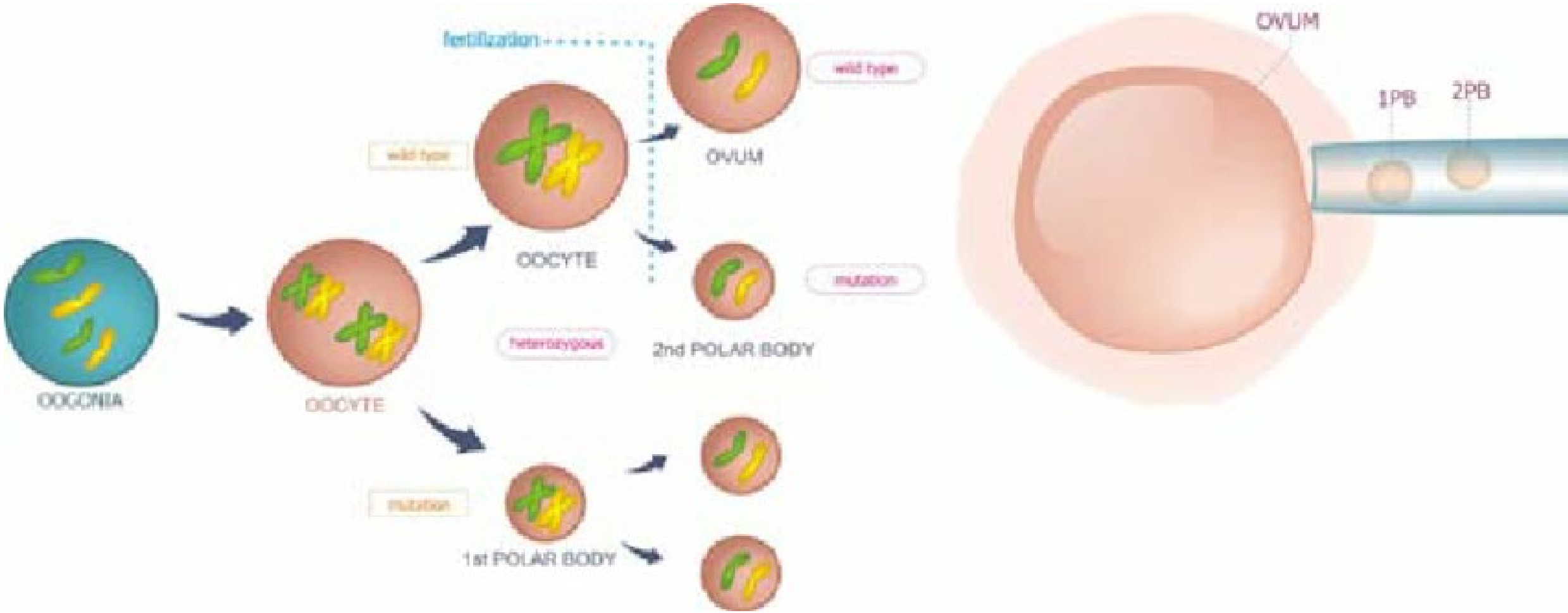
- Normal
  - Limit of detection varies by lab
- Abnormal
- Other results
  - Failed amplification
  - Mosaicism

What do you need to know?

- PGT does not replace prenatal or postnatal testing
- Risk of embryo biopsy
- Cost

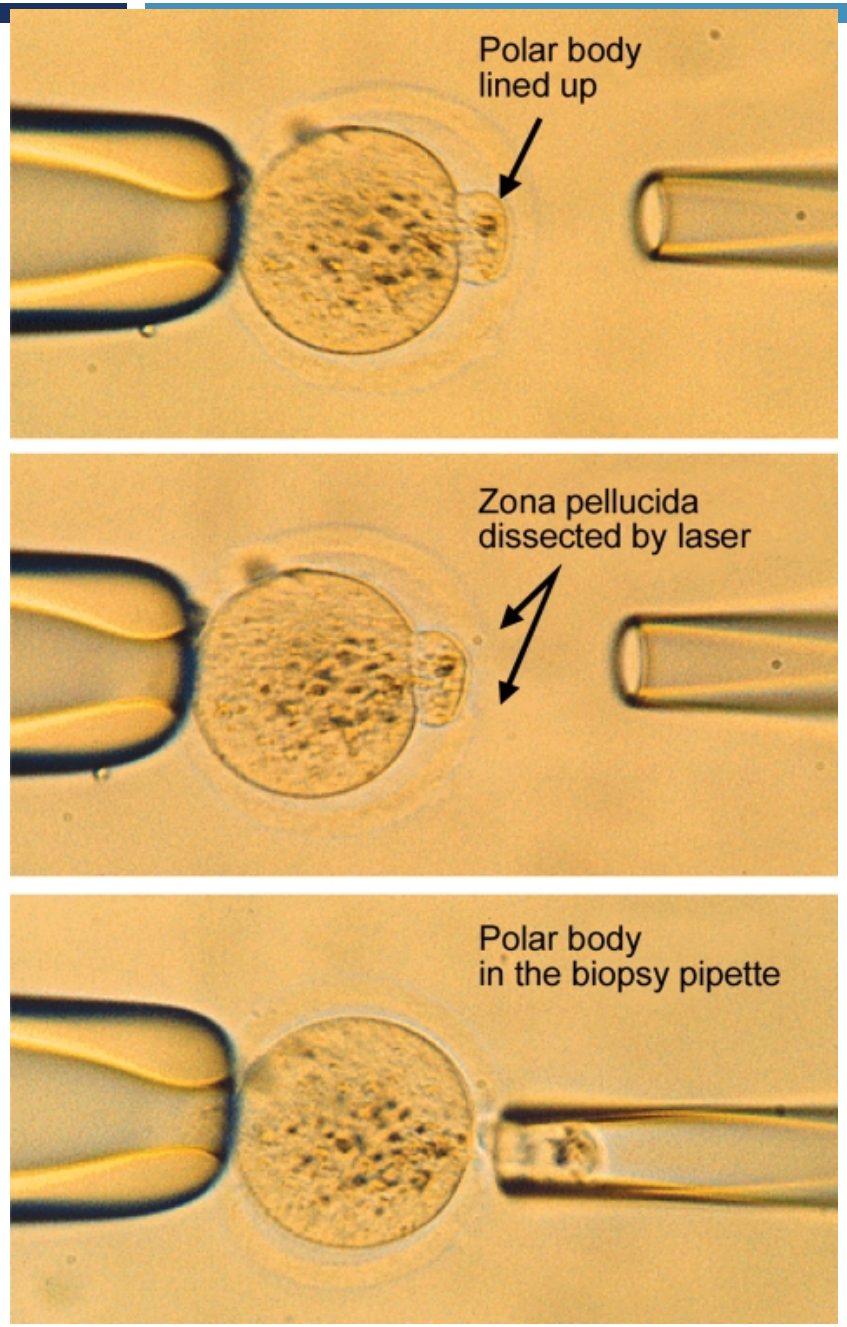
What do patients need to think about?

- IVF with PGT does not guarantee a pregnancy.
- Are they comfortable with the IVF and PGT process and the costs (financial, physical, psychological, etc…)?
- What will they do with affected embryos? Are they comfortable with discarding embryos?
- What will they do if they have no unaffected embryos?
- What will they do with extra "normal" embryos?


fertilization
wild type
OVUM
wild type
OOCYTE
mutation
heterozygous
2nd POLAR BODY
OOGONIA
OOCYTE
mutation
1st POLAR BODY
OVUM
1PB
2PB

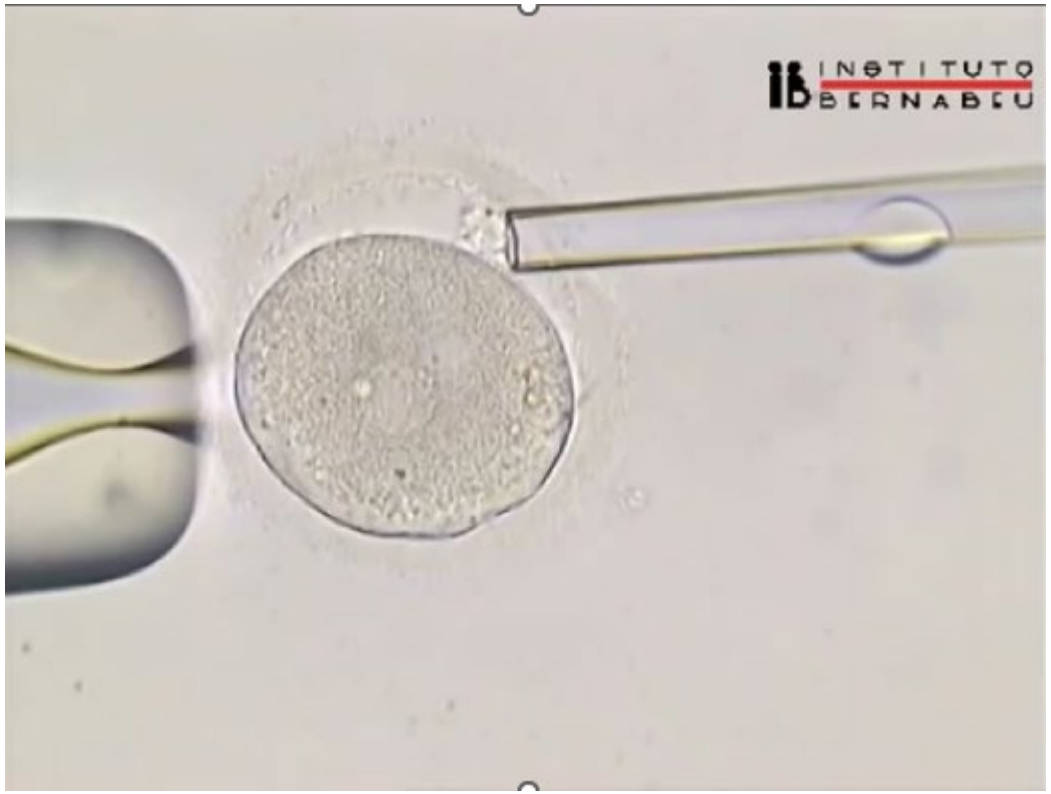
POLAR BODY DIAGNOSIS (PBD)

- PBD is a diagnostic method for the indirect genetic analysis of oocytes.
- Polar bodies are by-products of the meiotic cell cycle, which have no influence on further embryo development.
- PBD is a viable alternative to blastomere biopsy as the embryo's integrity remains unaffected, in contrast to preimplantation genetic diagnosis by blastomere biopsy.


Polar body
lined up
Zona pellucida
dissected by laser
Polar body
in the biopsy pipette

VIDEO

- https://youtu.be/SZFCBtpwtpY



- The major application of PBD is the detection of maternally derived chromosomal aneuploidies and translocations in oocytes.

- The paternal contribution to the genetic constitution of the developing embryo cannot be diagnosed by PBD.

- In AUTOSOMAL RECESSIVE conditions, there is no added advantage to offer PGTM on the embryo, over polar body biopsy on the oocyte, because if the oocyte is not affected by the recessive gene, then the embryo will also not be affected and will not be a carrier either.

WITH REFERENCE TO THE DRAFT PROTOCOL ON PCTM (EPA)

- 8 out of the 9 listed conditions are AUTOSOMAL RECESSIVE.

- Finnish Nephrotic Syndrome; Gangliosidosis; Joubert Syndrome; Maple Syrup Urine Syndrome; Nemaline Myopathy; Spinal Muscular Atrophy; Tay-Sachs Disease and Walker-Warburg Syndrome.

- This means that these conditions can be tested a priori using genetic testing on the parents, followed , if need be, by polar body biopsy of the oocyte.

If the mother is not a carrier, then she does NOT need to be tested and the embryo will not be affected by the condition.

If only the father is the carrier:

- the embryo will have a 50% chance of being completely normal and not a carrier, and
- a 50% chance of being a carrier, like the father, without ever expressing the disease.

- If the mother is indeed a carrier of an autosomal recessive gene, then polar body biopsy will help determine which oocytes do not have the autosomal recessive gene, and these are the ones chosen for fertilisation to create the embryo.
- In this case, the embryo will have a 100% chance of being completely normal and not a carrier.
- In the case that the father is also a carrier, then the embryo has a 50% chance of being a carrier, like the father, without ever expressing the disease.

- Out of the proposed list, Huntington Disease is the only disease which is AUTOSOMAL DOMINANT, meaning that even one copy from either the affected mother or affected father will lead to the disease in the affected parent, and even in the embryo.
- However Huntington's is a particular disease which will show up later in life (typically after the age of 40 years). The parents will be tested to check whether they are carrying that gene, and if one of them does, it is clear that there is a 50% chance that their offspring will develop the disease later in life.
- This means that this condtion is not immediately life threatening, and there is medication which can help to alter the course of the disease (eg. Xenazine, tetrabenazine).