INTERNATIONAL FEDERATION OF FERTILITY SOCIETIES

Preimplantation Genetic Diagnosis
Clinical and Laboratory Aspects

IFFS Embryology Workshop
TARTU, ESTONIA
21-23 September 2018

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MERCK

Preimplantation Genetic Tests

Preimplantation Genetic **Diagnosis** (PGD)

- Test for a specific mutation or chromosomal abnormality, where there is a known risk due to **parental abnormality**
- Includes tests for **known** translocations

Preimplantation Genetic **Screening** (PGS) or **Testing** (PGT)

- Test for aneuploidies, where prospective parents do not have a **known** genetic abnormality but may be considered at risk, eg
    - due to age
    - previous aneuploid pregnancy

Preimplantation Genetic Diagnosis

Is for those who **know** they have a significant chance of a pregnancy that has inherited a serious genetic disorder, and who wish to avoid that risk

Key points for patients considering PGD

- Couples **must** be seen by **clinical geneticist** in order to:
  - Confirm genetic diagnosis
  - Understand inheritance risk
  - Calculate risk within family
  - **Review reproductive options including PGD**
- For single gene disorders, PGD is only possible if the gene mutation has been identified
  - Linkage studies may be used
  - May not be possible eg for some rare metabolic disorders

Preimplantation Genetic Screening

PGS is for those who **hope** they may have an increased chance of achieving a pregnancy with ART if their embryos are screened for any **sporadic** chromosomal abnormalities they may have, and only those free of abnormalities are transferred

While used widely internationally, PGS remains a **theoretically** beneficial treatment

Key points when considering PGS

- In PGS, no specific diagnosis is tested for
- The parents are presumed to be normal and embryos are tested for abnormalities in chromosome numbers
- Most numerical chromosome abnormalities are **sporadic**
  - Usually result in miscarriage
  - **This is used as argument in support of PGS**
  - Efficacy still a matter for debate

Main indications cited for PGS

- **Advanced maternal age** (usually defined as maternal age over 37 or 38 years)
- **Repeated implantation failure** (usually defined as ≥3 failed embryo transfer procedures with good quality embryos)
- **Recurrent miscarriage** in patients with normal karyotypes (usually at least three previous miscarriages)
- **Severe male factor** infertility

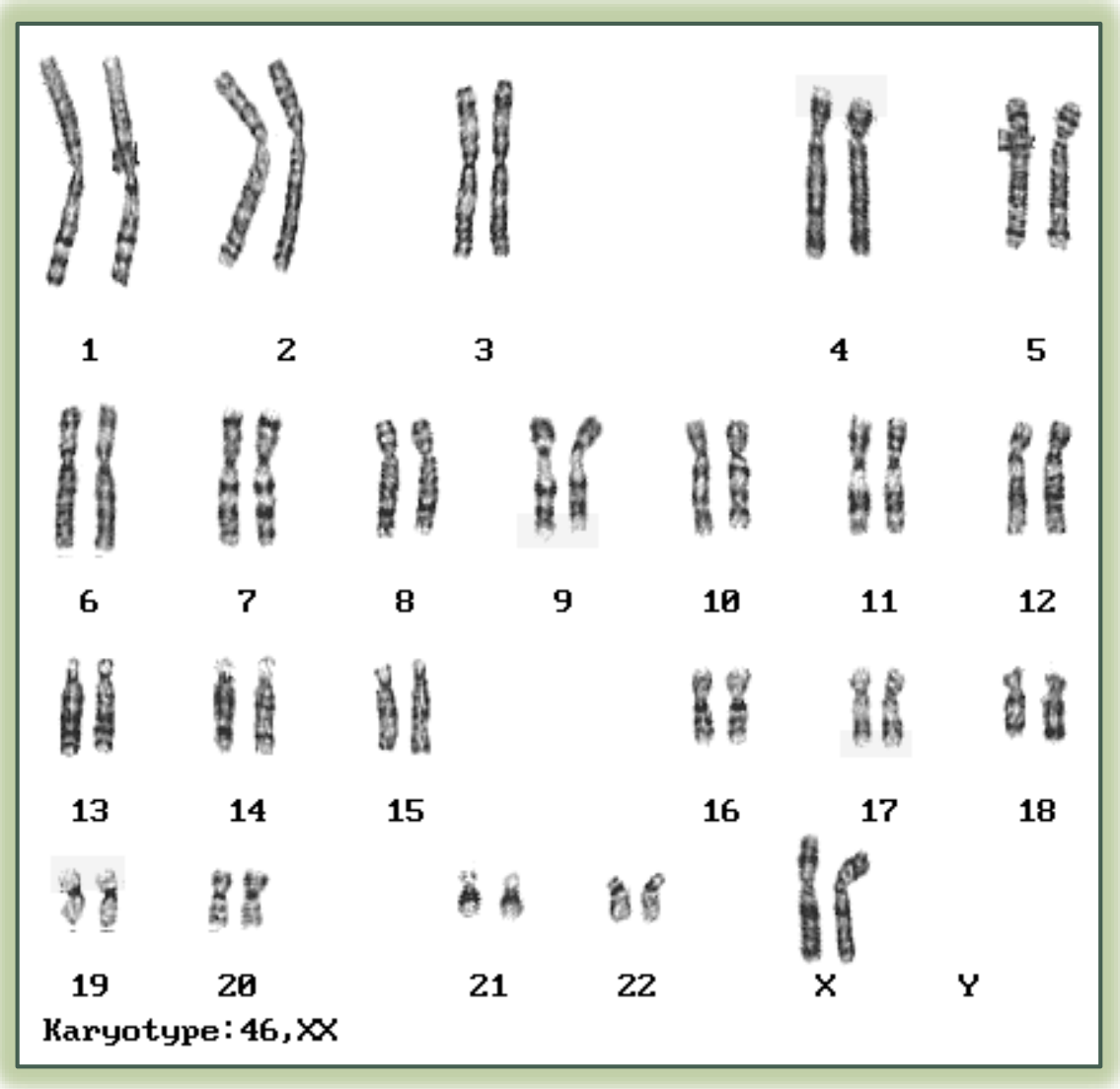
NHS Clinical Commissioning Policy (2014)

"In the absence of evidence of its clinical and cost effectiveness, there is no intention to support the introduction of PGS into NHS clinical practice"

Basic Genetics

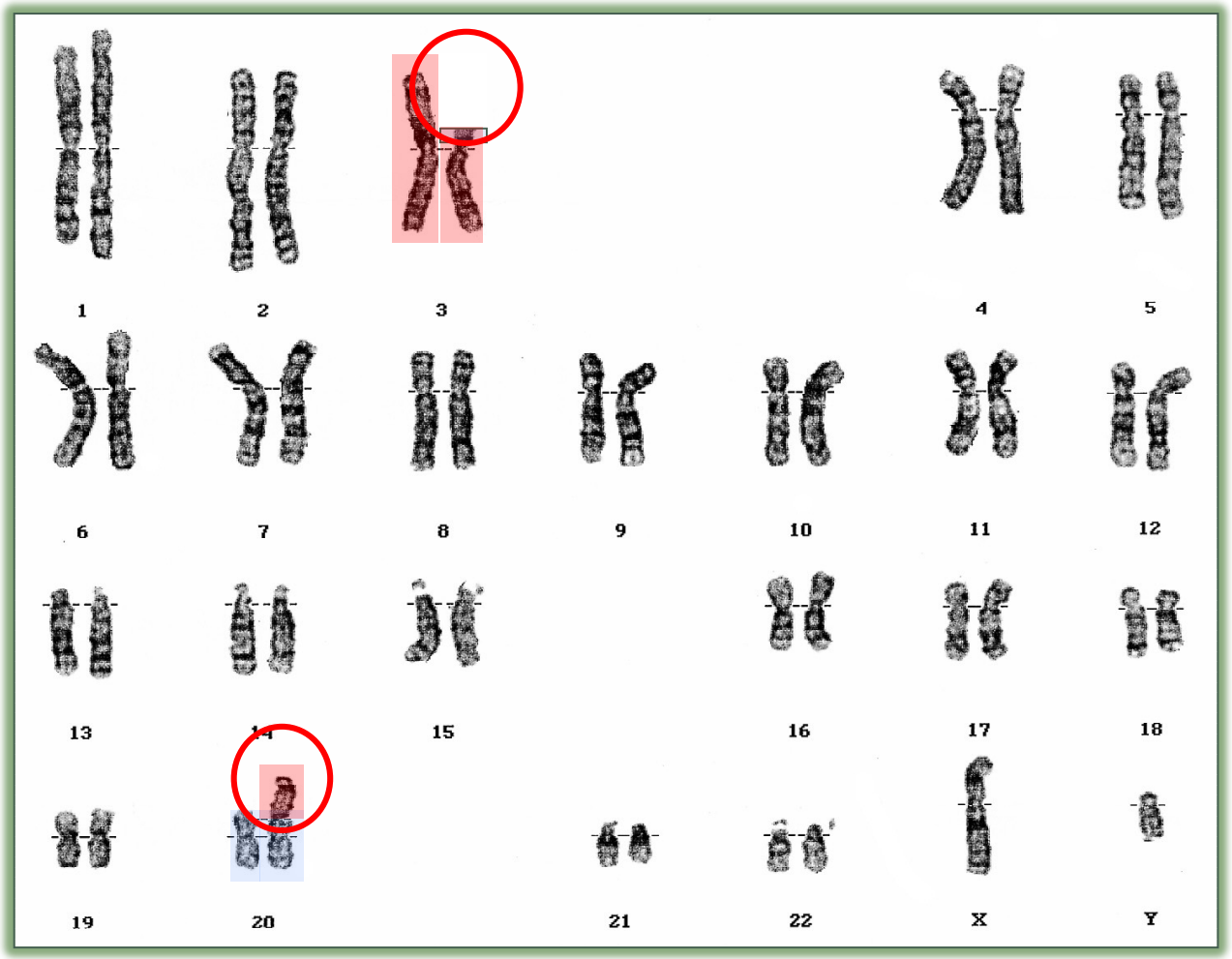
1. **Chromosome** Abnormalities

- Abnormal copy number
  - Polyploidies eg triploidy
  - Monosomies
  - Trisomies (eg 21, 13, 18)
- Sex chromosome abnormalities
- Chromosome rearrangements
  - Deletions
  - Translocations
    - Reciprocal
    - Robertsonian



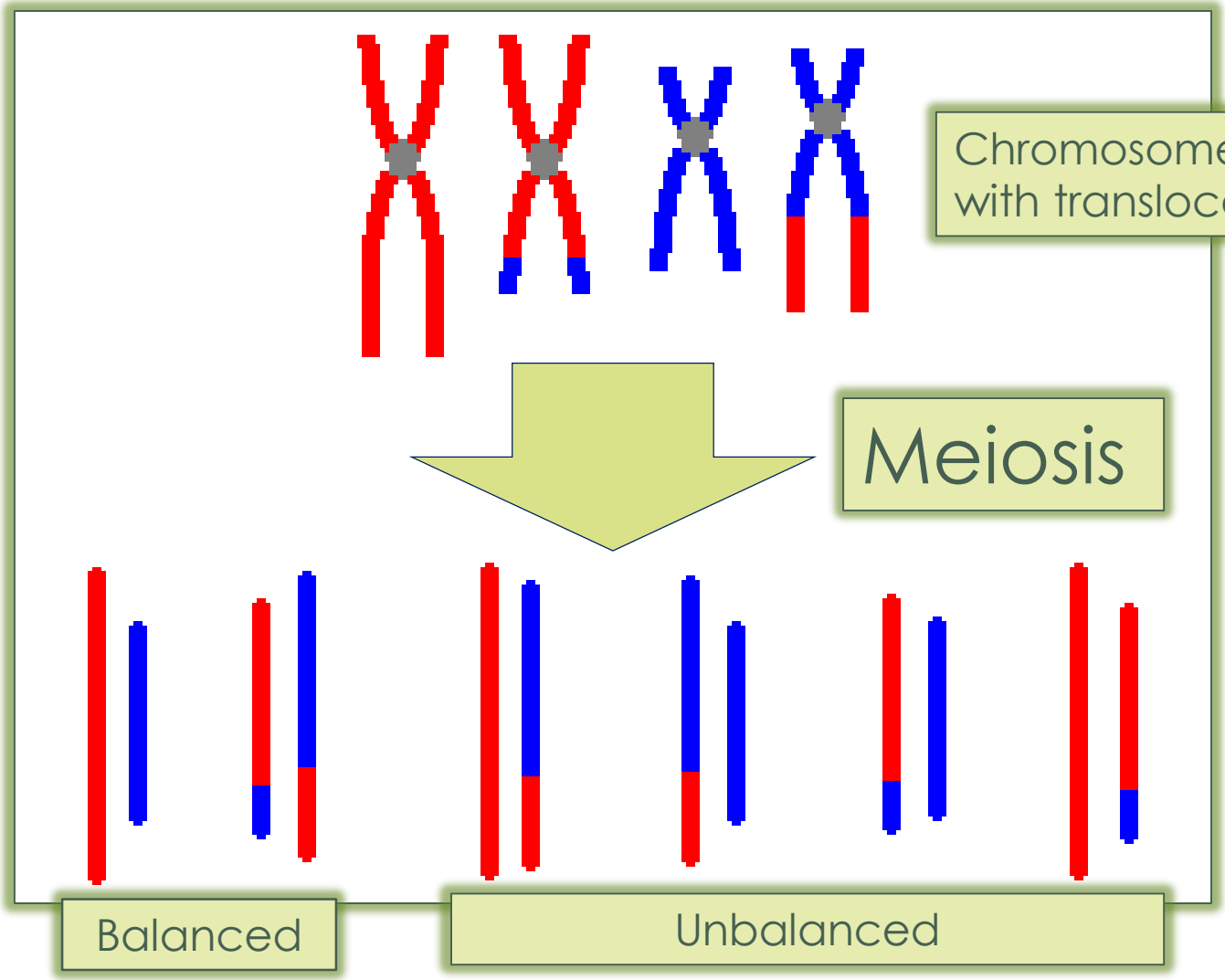
Chromosomal abnormalities:

Translocation



Chromosomal abnormalities:

Segregation of translocations at meiosis



Chromosomal Abnormalities

Segregation of translocations at meiosis

Theoretically, chromosomal constitutions in secondary oocytes could be:

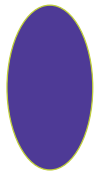
- 16 different (reciprocal) translocations
- 6 different (Robertsonian) translocations
- **Only 2 of them are balanced**
- Which means 14 out of 16 (87%) are **unbalanced**

Basic Genetics

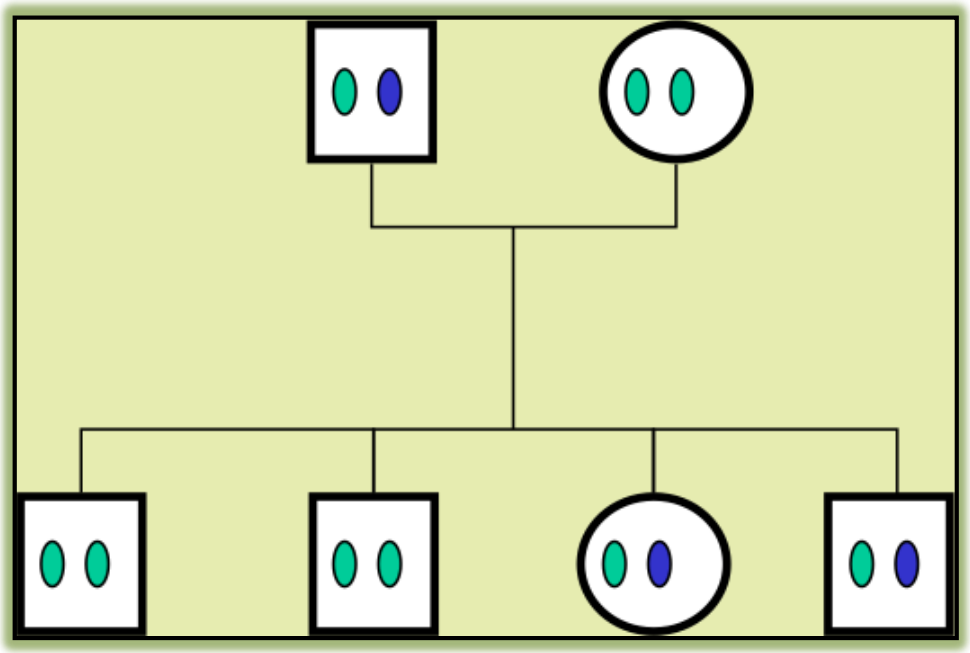
2. **Genetic** mutations

- Autosomal dominant conditions
  - Affect 1:200 people
  - Single mutation in 1 copy of gene pair
  - Examples: Huntington's; myotonic dystrophy
  - **1:2 risk for children**
- Autosomal recessive conditions
  - Much rarer
  - Risk only if both parents are carriers
  - Examples: cystic fibrosis; spinal muscular atrophy
  - **1:4 risk for children**
- X-linked inheritance
  - Females carriers; 50% chance of passing to son
  - **Males affected**

Monogenic Autosomal Dominant Disorders Classic Mendelian Inheritance



Disease-Causing Allele
50% chance of inheritance

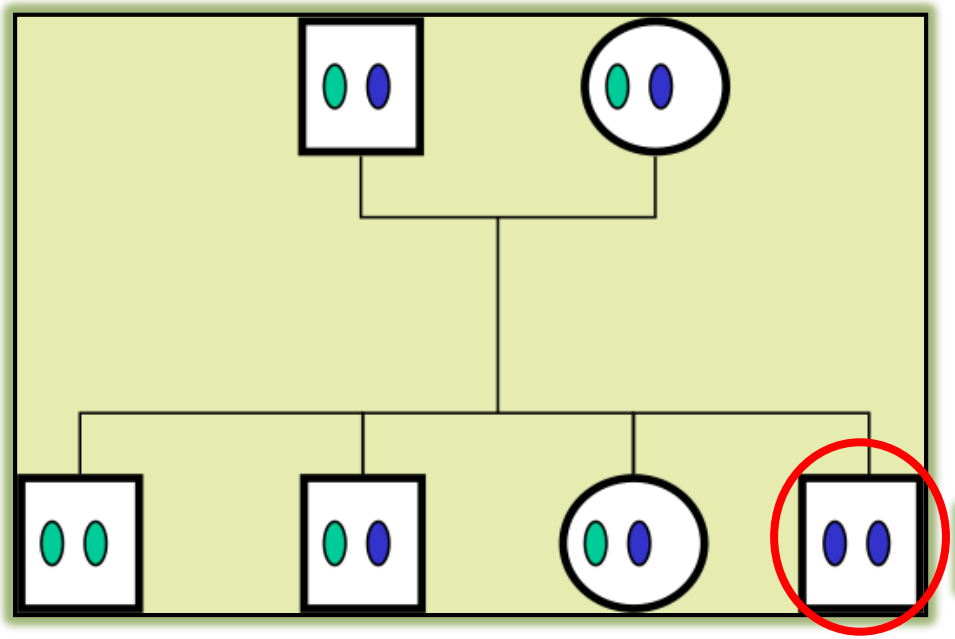


One parent carrier may have mild/minor/no symptoms, or late onset

Offspring may have more severe symptoms depending on penetrance and expressivity

Monogenic Autosomal Recessive Disorders
Classic Mendelian Inheritance

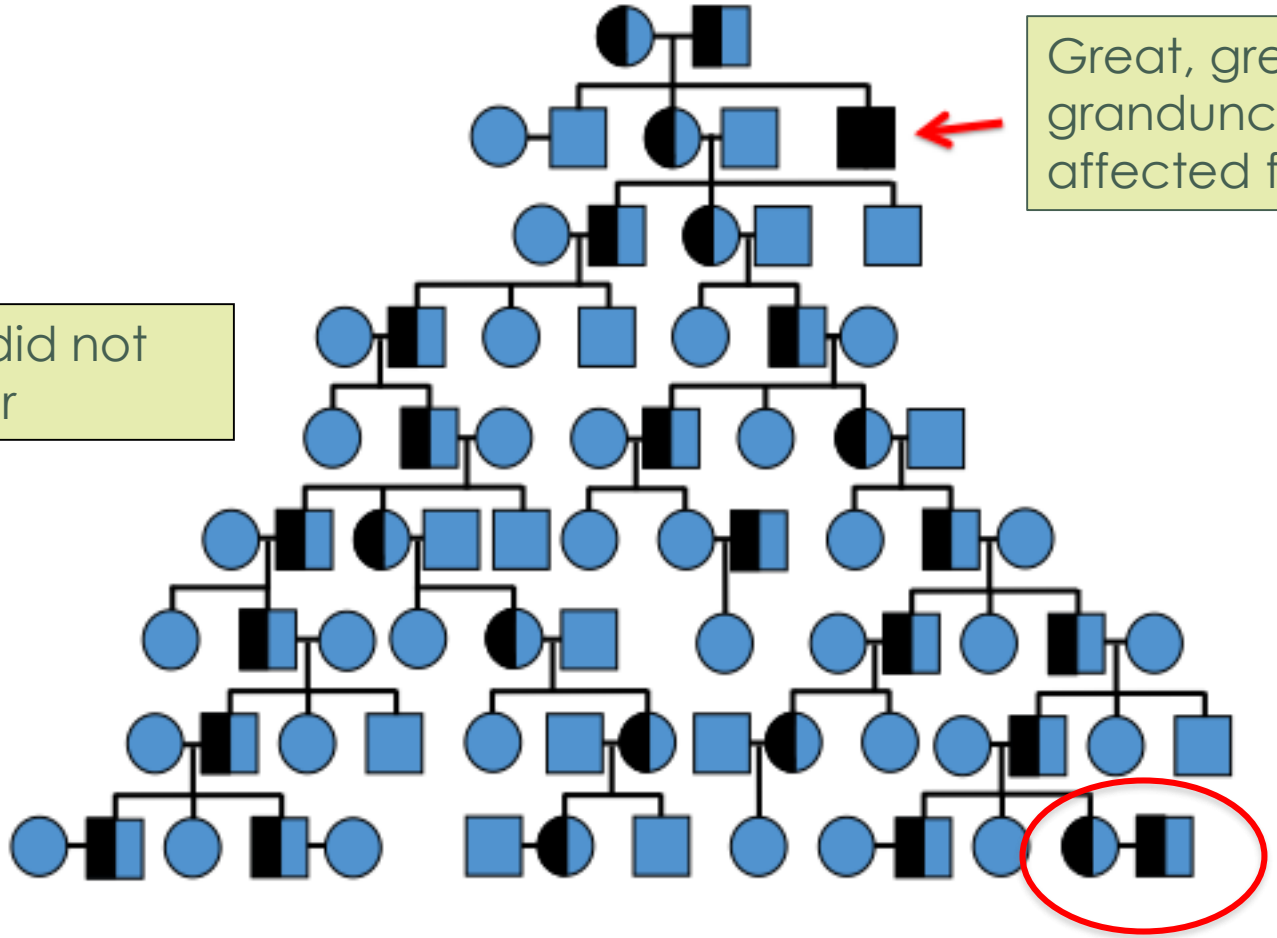
Disease-Causing Allele
25% chance of inheritance



Both parents **carriers**
Usually no symptoms

1:4 pregnancies severe symptoms/disorder

Family History: Autosomal recessive disorders



Great, great, great, great granduncle was the previous affected family member

24 family members did not meet another carrier

Courtsey J.A.Horcajadas - Recombine

Genetic Counselling and its role in PGD

Specialist counselling, separate from Fertility Counselling

- Family history and reason for requesting PGD
- Understand genetic risk
- Explore alternative reproductive options
- Understand PGD process, physical and emotional impact, and chance of successful pregnancy
- Understand limitations of genetic testing
- Consider implications of genetic testing in case of late onset disorders (eg Huntington's; BRCA1 & BRCA2)
- Receive written summary, along with relevant information leaflets

Preimplantation Genetic Testing

Laboratory Tests for Chromosomal Abnormalities

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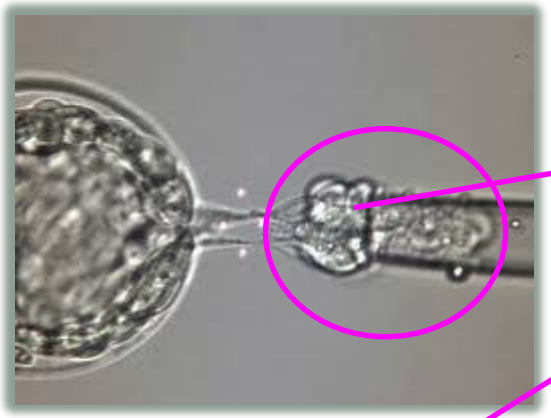
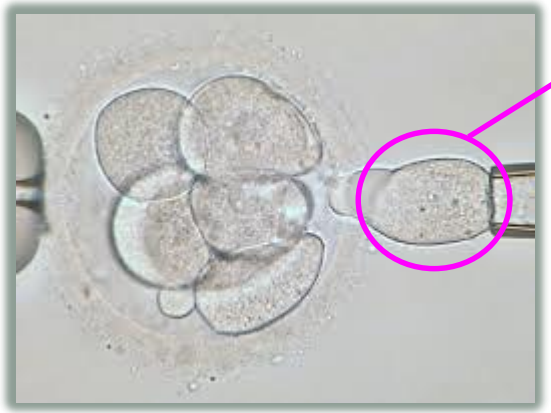
First amplify the DNA
Whole Genome Amplification (WGA)

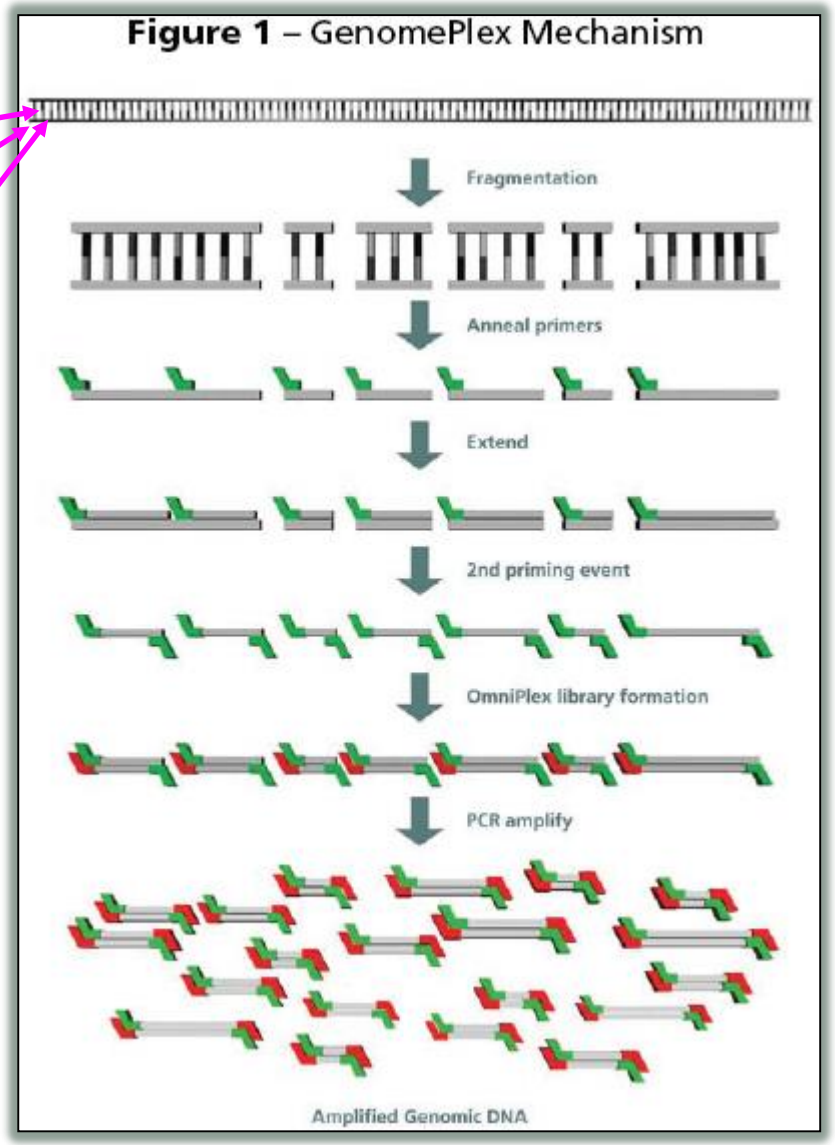


Trophectoderm biopsy

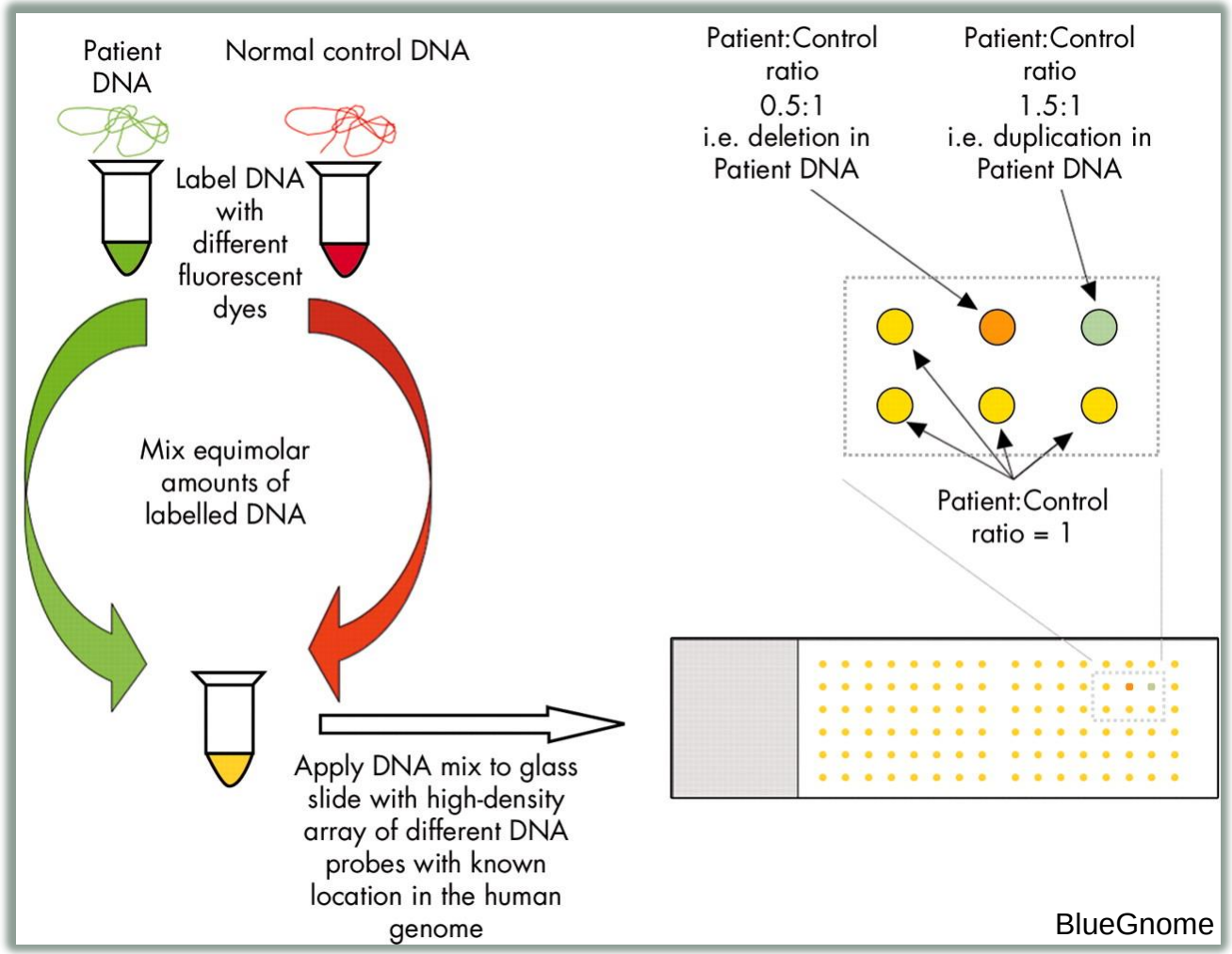


Blastomere biopsy

Polar body biopsy

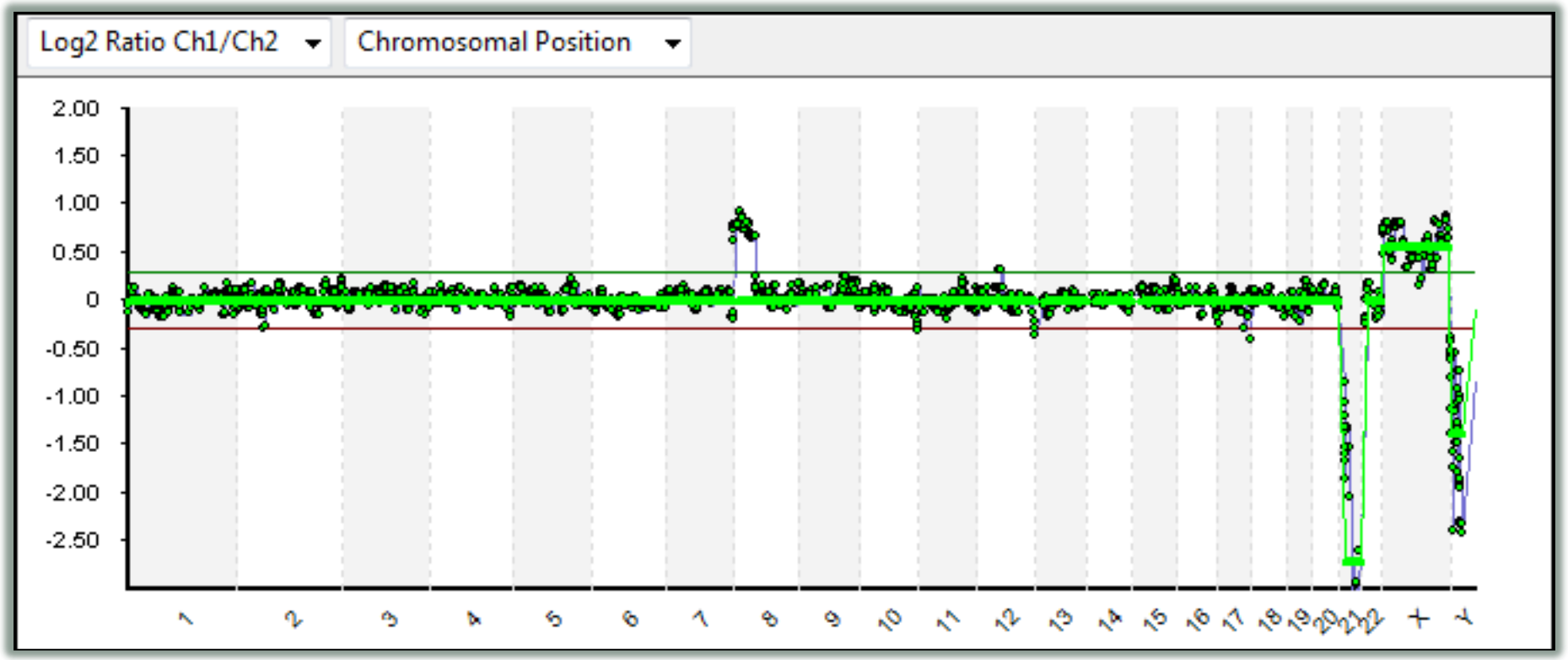


Array CGH (Comparative Genome Hybridisation)

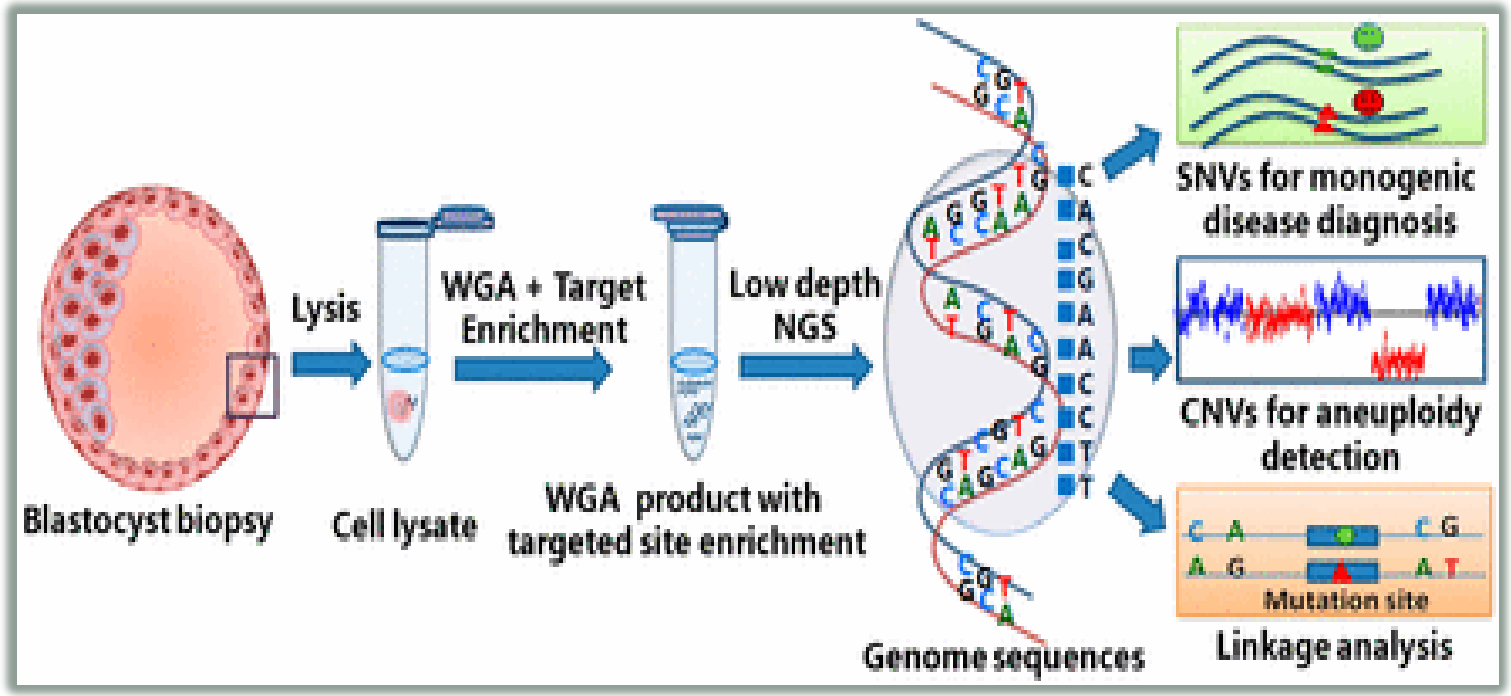


- Compares DNA of the test cells with known normal karyotype
- Hybridise with oligo nucleotides on slide
- Limited resolution

Translocation Analysis



Next Generation Sequencing (NGS)



pnas.org

- Identification of chromosomal abnormalities *and* monogenic disorders
- Only labour and cost effective with high throughput
- Major implications with parallel sequencing options

Arrays vs Next Generation Sequencing

	FISH	Micro Array	NGS
Sensitivity	Low	Medium	High
Coverage	1	3000 probes	150,000 reads
False positive rate	Moderate	Low	Very low
No. chromosomes tested	≤12	24	24
Detect single mutation?	No	No	Yes
Detection method	Indirect	Indirect	Direct

Preimplantation Genetic Testing

Strategies for embryo biopsy

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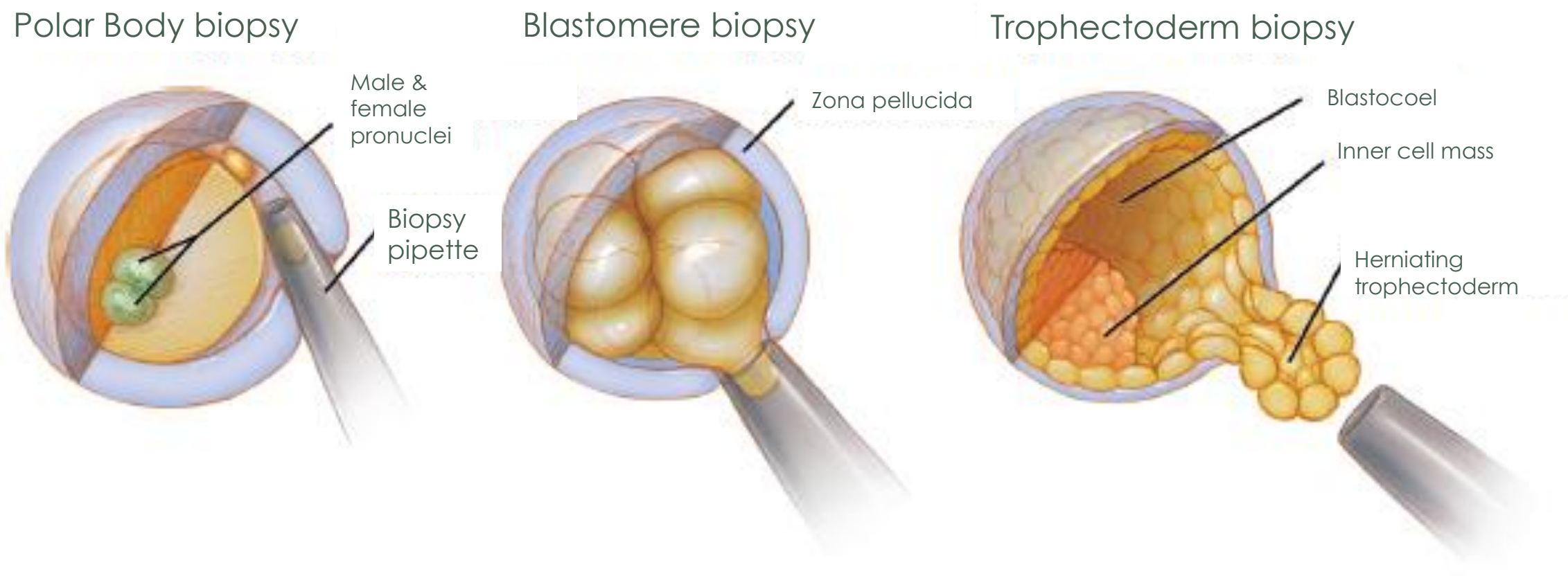
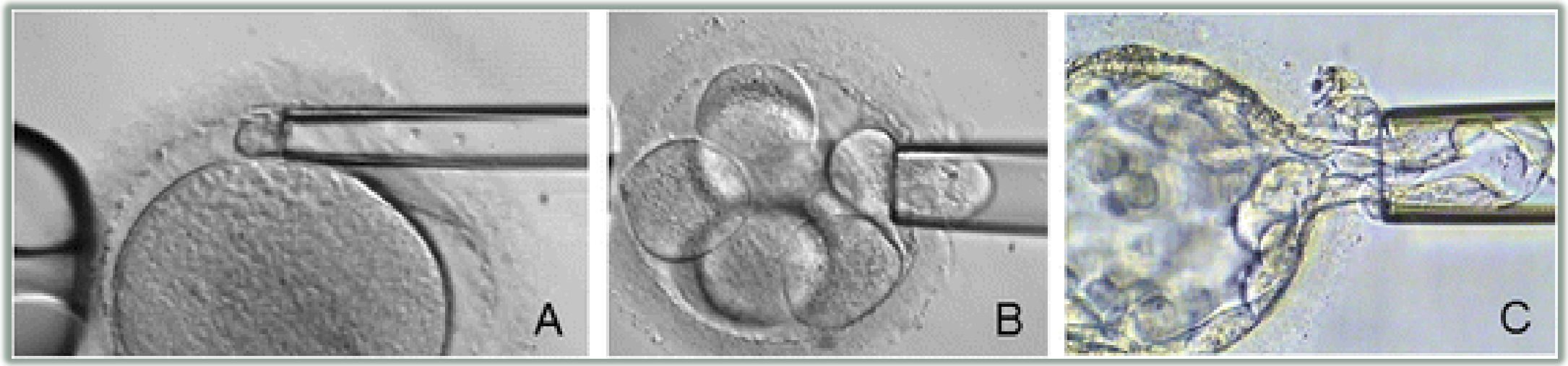
Strategies for embryo biopsy



Hyde & Schust, Cold Spring Harbour Press 2015

Strategies for embryo biopsy



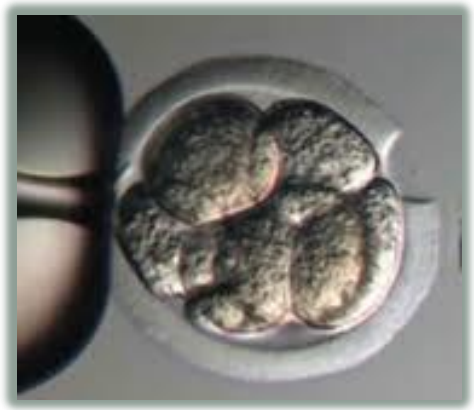
Polar body biopsy

Blastomere biopsy

Trophectoderm biopsy

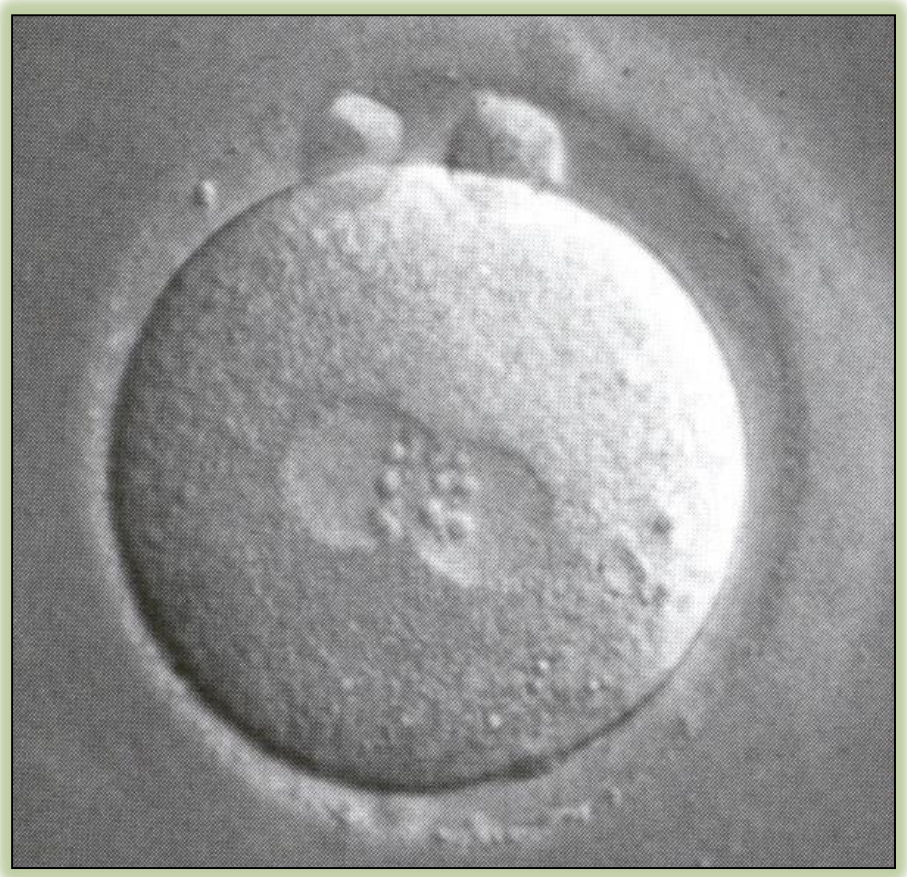
Breaching the zona pellucida

- Chemical – acid Tyrode's
  - Relatively cheap
  - Inter-procedure variables
  - Risk of exposure to chemical
- Mechanical – laser
  - Expensive
  - Programmed
  - Risk of heat damage



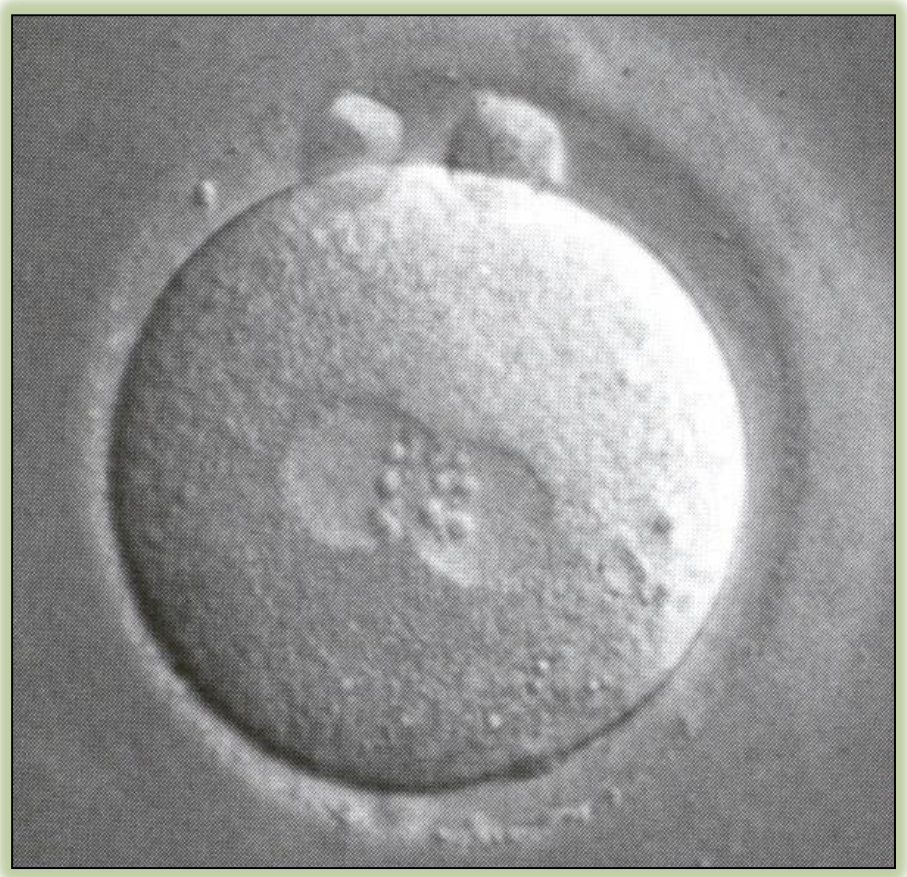
Polar body analysis

- Polar bodies are the result of two meiotic divisions
- Polar bodies carry the complementary chromosomal content to the oocyte after each division
- Diagnosis only of oocyte
- **Only for disorders of maternal origin**



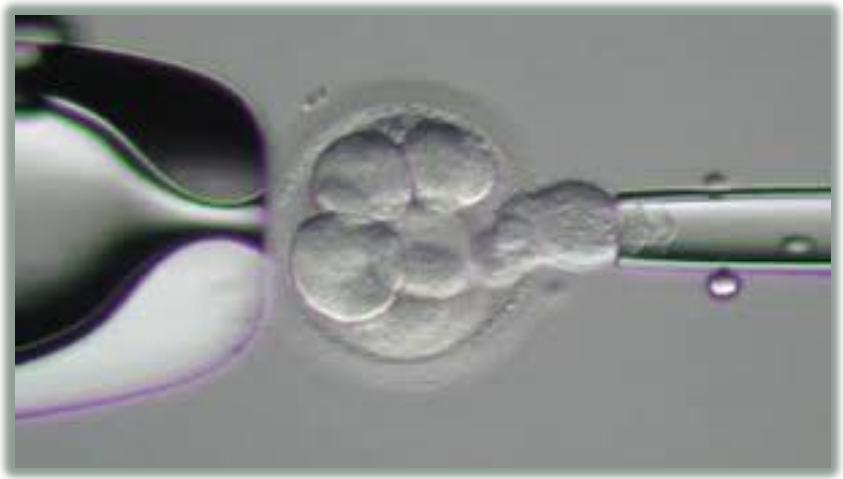
Polar body analysis

- Removal of extra-embryonic DNA
- Allowed in countries where embryo testing is prohibited
- DNA from maximum 2 cells
- Technically challenging
- Polar bodies may fragment/degenerate

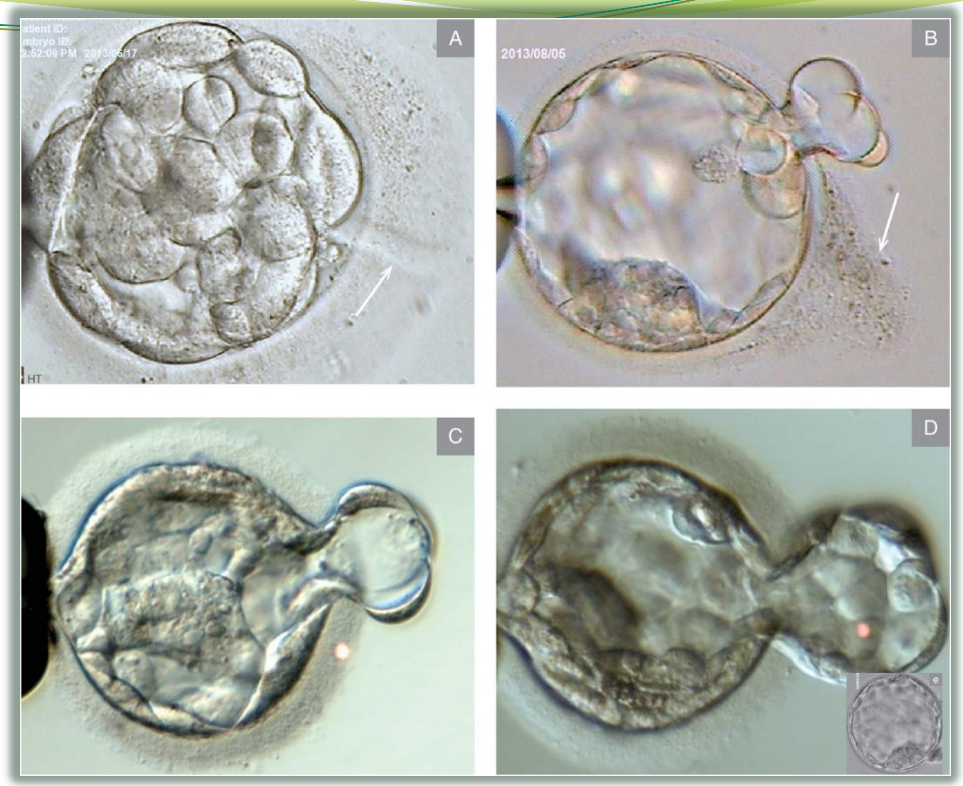


Cleavage stage biopsy

- Day 3 biopsy; 6- to 8-cell stage
- Embryos may have compacted
- Pre-biopsy incubation in calcium-free medium
- Leave embryos in culture waiting for genetic analysis
- Biopsy embryos that may not develop
- Maximum 2 blastomeres biopsied
- Fresh embryo transfer on Day 5



Blastocyst biopsy



- Current method of choice
- Breach zona on Day 3/4
- Allow trophectoderm to herniate
- Excise herniated TE cells
- Excise mechanically or using laser
- Biopsy 6-10 cells
- Cryopreserve biopsied blastocysts waiting for genetic analysis
- Only biopsy embryos that have developed into blastocysts
- Can "batch" genetic test runs – economy of scale
- Successful cryopreservation service essential

Use of the Embryoscope

Ablated zona, awaiting herniation

Herniated cells, Ready for biopsy

Some words of caution regarding PGS

- Is the biopsied material representative of the embryo?
- Might the embryo develop normally, even if some cells are chromosomally abnormal?
- Might PGS lead to some embryos being discarded when they might have corrected errors and developed normally?

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