eurofins | Genoma

Francesca Spinella, PhD
Medical Scientific Liaisons

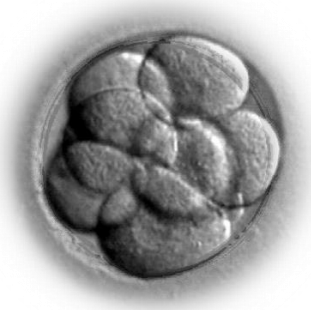
PAST | PRESENT | FUTURE

NEXT GENERATION SEQUNCING

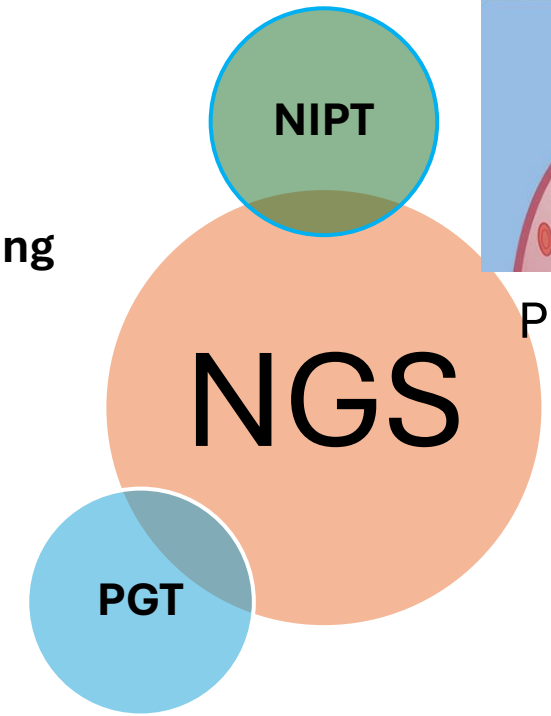
CLINICAL APPLICATION

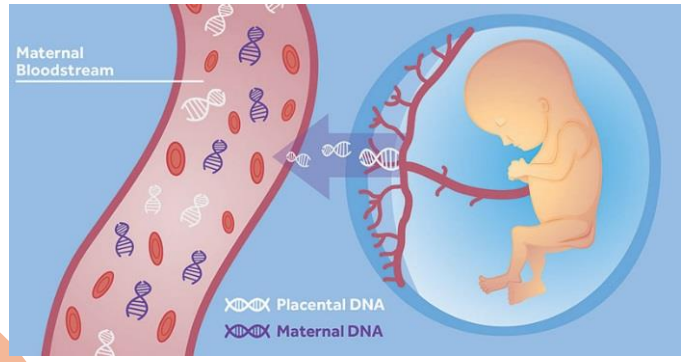
Non Invasive Prenatal Testing

Preimplatation Genetic Testing



Embryonic cells



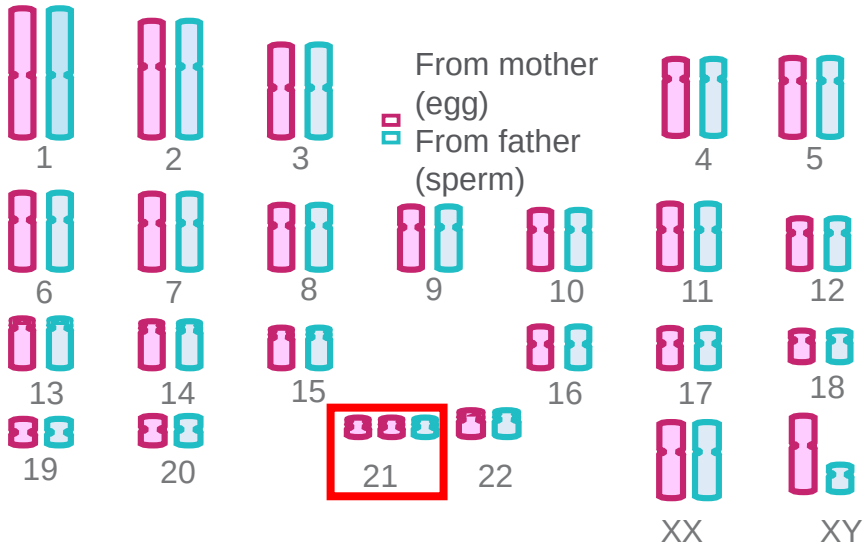


Placenta-derived cfDNA

Chromosome analysis

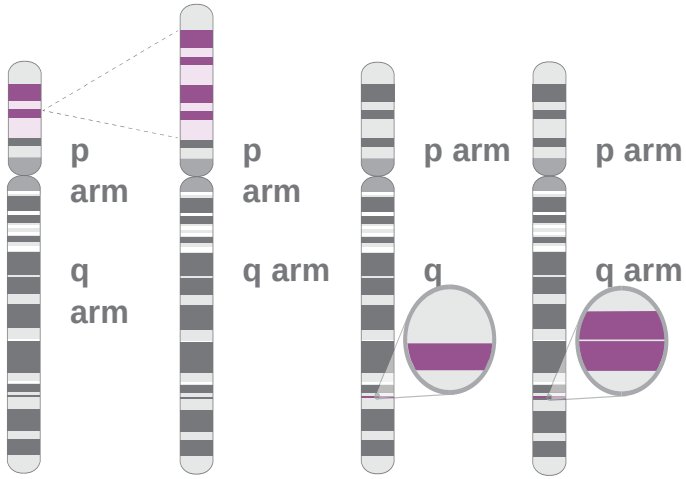
Chromosome analysis is a test to look at the chromosomes in a sample of cells. It can help identify chromosome abnormalities such as aneuploidy and structural abnormalities.

Gain or loss of whole chromosome



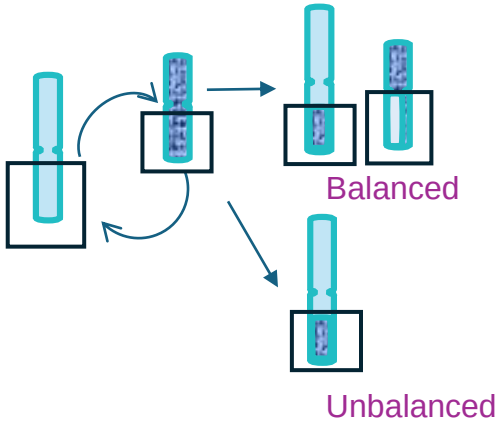
Chromosome 1 = 200 Mb (megabases = millions of bases)
Chromosome 21= 40Mb

Gain or loss of small portion of chromosome (structural abnormalities)

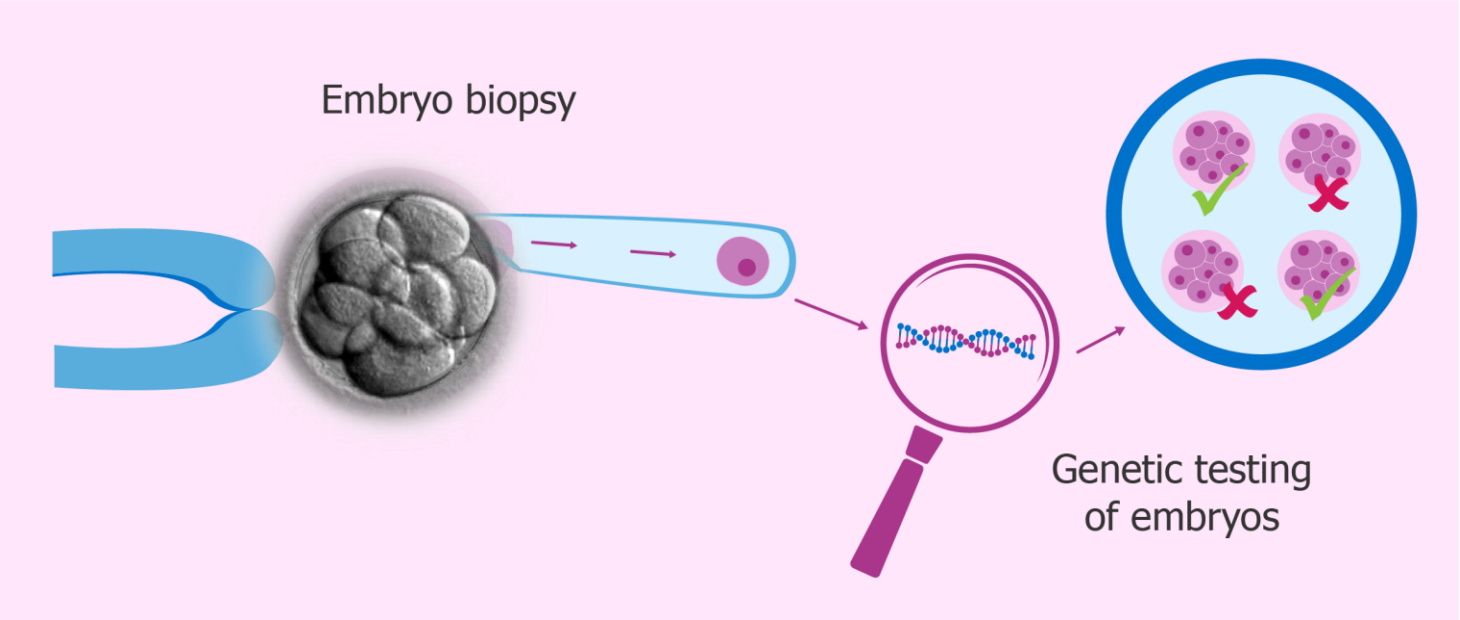


Deletion/duplication >10Mb
Microdeletion/microduplication <10Mb

Traslocation

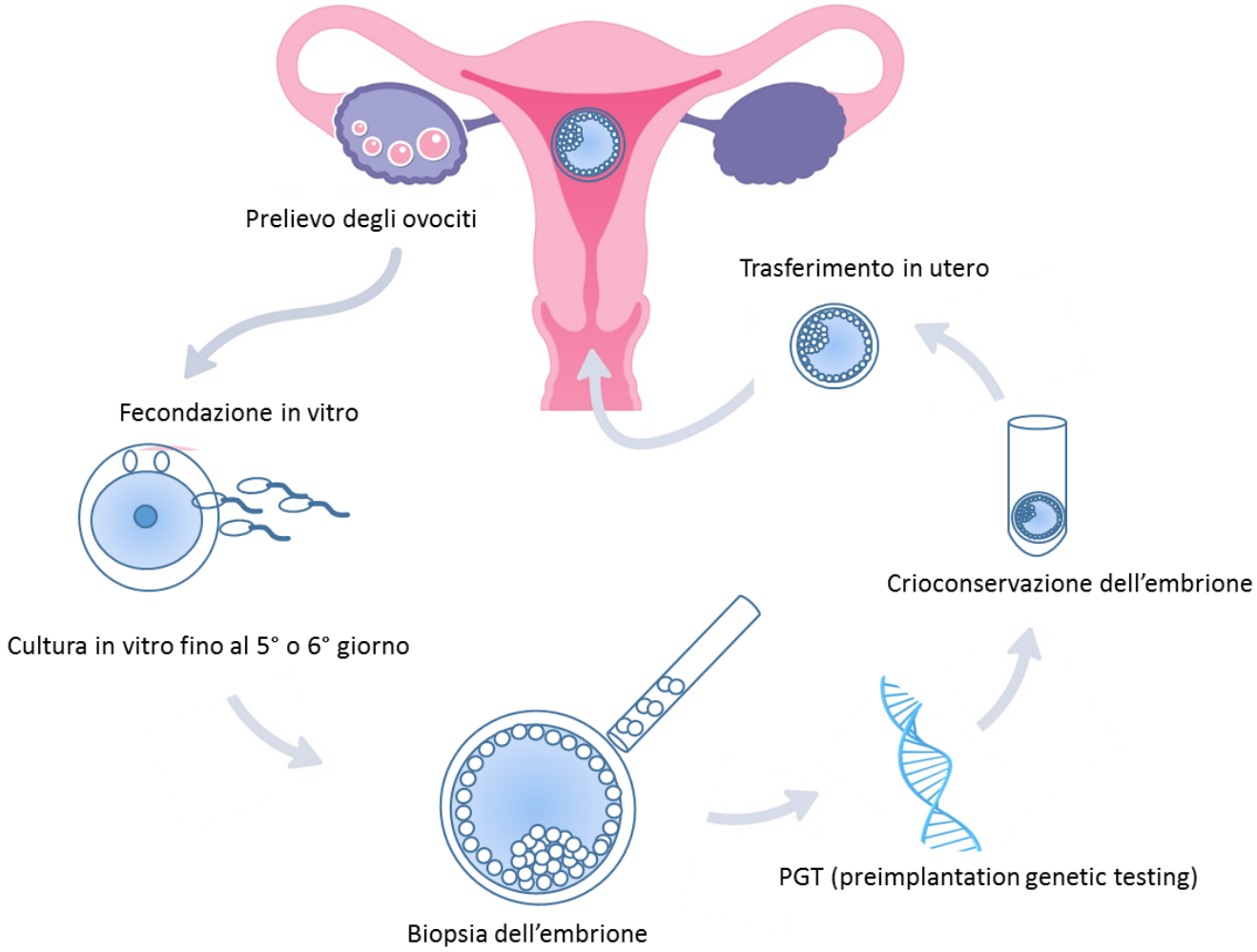


Preimplantation Genetic Testing (PGT)



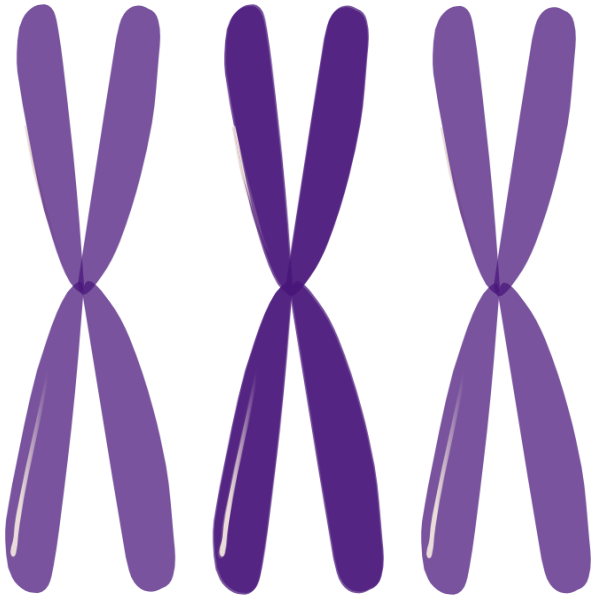
Preimplantation Genetic Testing (PGT) is the study of chromosomal and genetic alterations in the embryo before transfer to the mother's uterus.

Fecondazione in vitro.

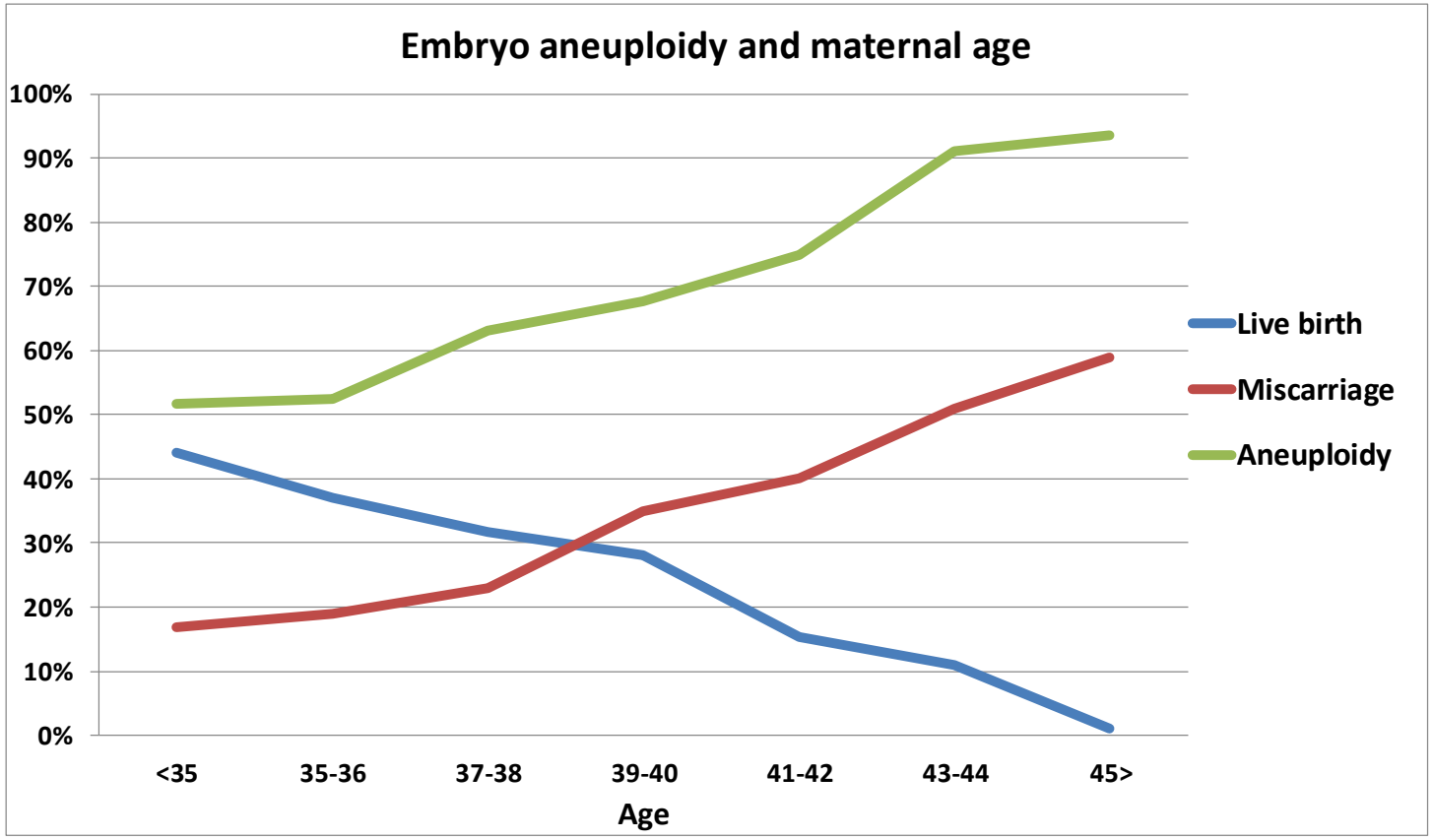


Chromosomal aneuploidy and IVF outcome

Chromosomal aneuploidy is common in preimplantation embryos and provides an explanation for most implantation failures and recurrent miscarriages in in vitro fertilization (IVF) treatments

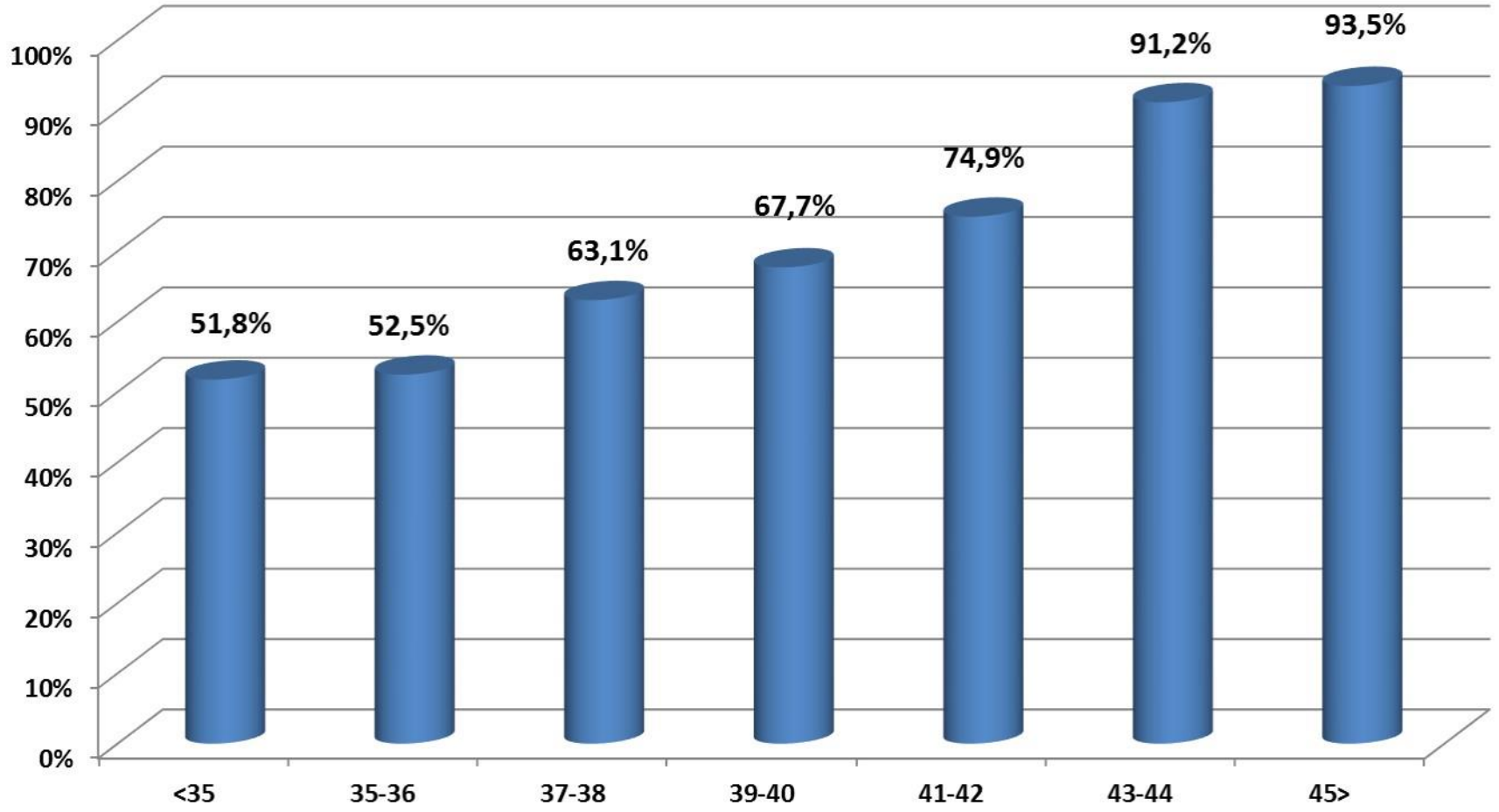


Aneuploidies, maternal age and fertility



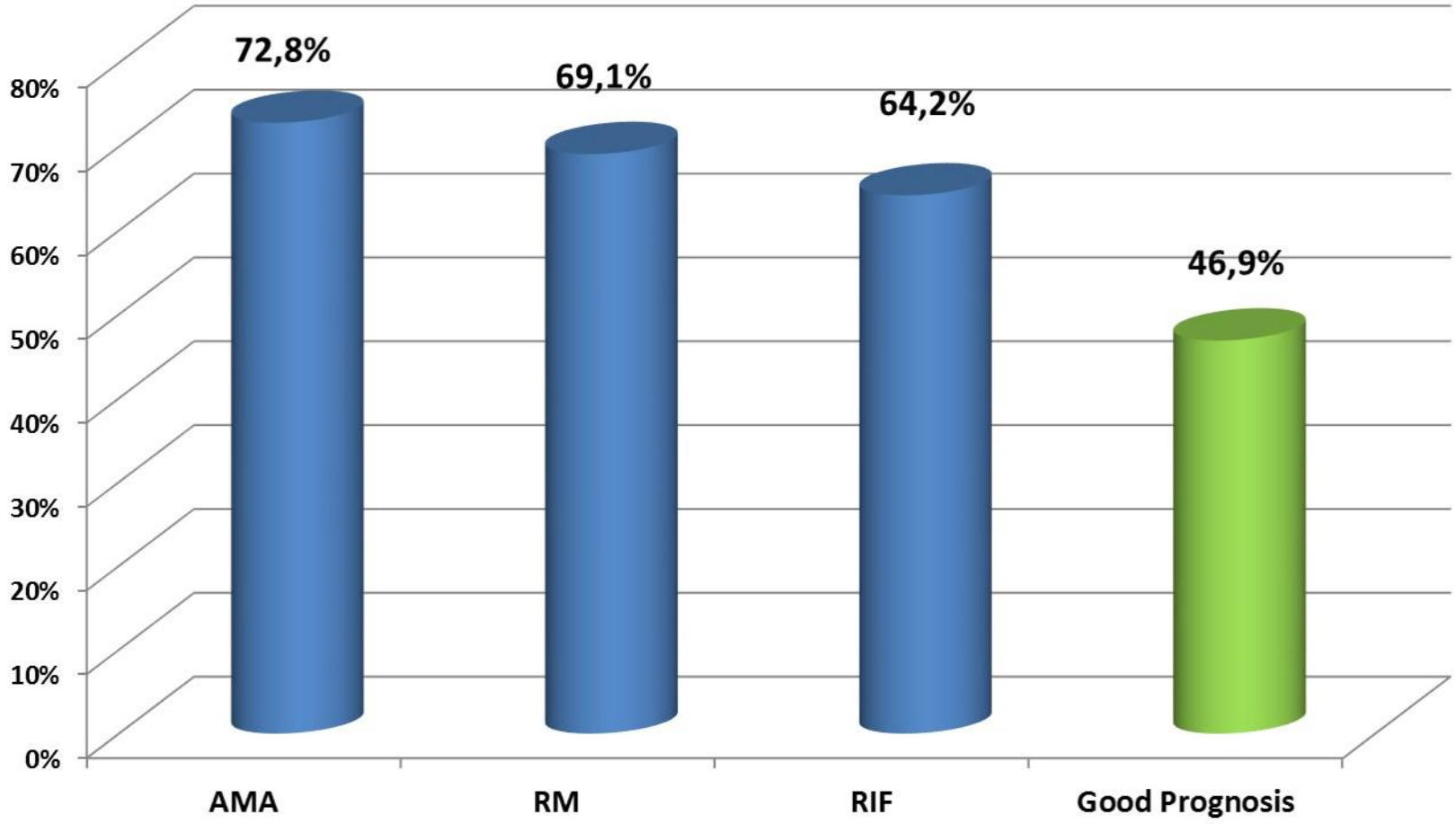
- Aneuploidy increases with advancing maternal age
- Aneuploidy is almost always lethal (failed implantation/miscarriage)
- While aneuploidies increase with age, live birth rate decreases

% aneuploidy in embryos increases with maternal age



Data from 8000 blastocysts tested by NGS

% aneuploidy in embryos according to indication for PGS



Data from 8000 blastocysts tested by MGS

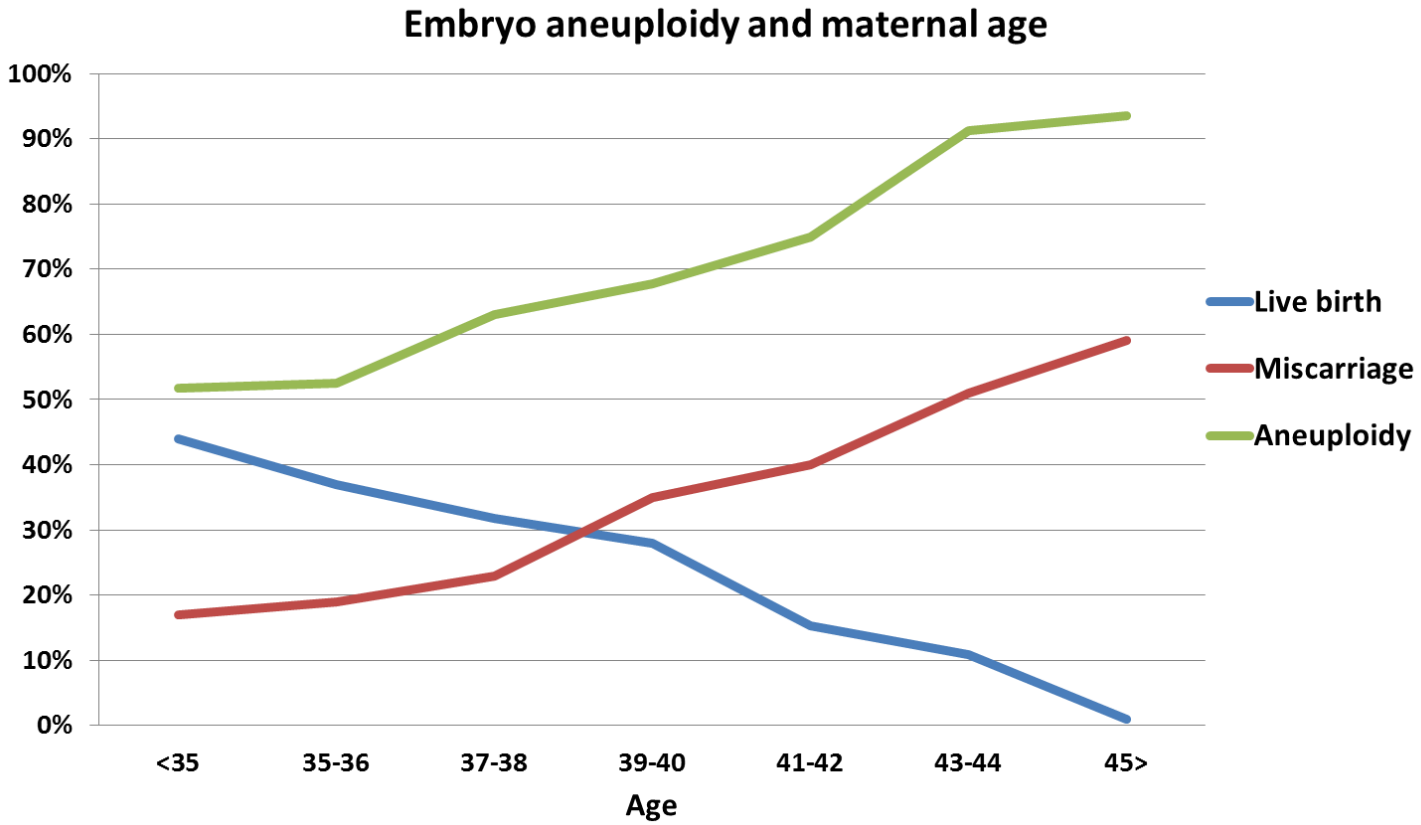
APPLICAZIONE DELLA **PGT**

Genetic testing for aneuploidies (PGT-A), which performs a preimplantation screening for PGT chromosomal abnormalities (increasing IVF success)

- Advanced maternal age
- Repeated implant failure
- Recurrent early miscarriages
- Severe male infertility

- **Genetic testing for PGT-SR structural aneuploidies**
- Translocations (Reciprocal, Robertsonian)
- Deletions
- Duplication
- Inversions

Età materna e autcome clinico



- L‘incidenza delle aneuploidie aumenta con l'avanzare dell'età materna
- L'aneuploidia è quasi sempre letale (impianto fallito / aborto spontaneo)
- Mentre l'aneuploidia aumenta con l'età, il tasso di natalità diminuisce

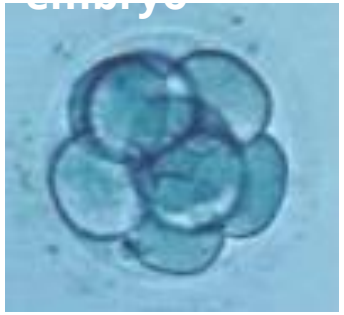
Aims of PGT-A

- Reduce the time it takes to get pregnant
- Selection of the best embryo for single embryo transfer
- Reduce the incidence of miscarriage
- Reduce the risk of having a baby with an aneuploidy condition

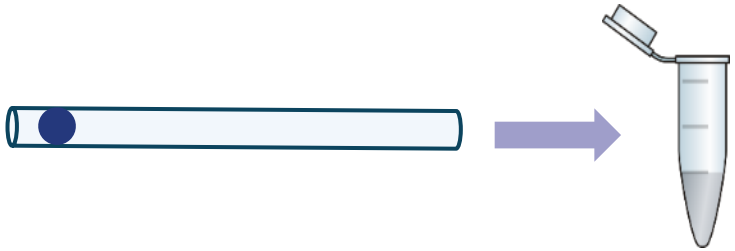
PGT-A

Types of biopsies used

DAY 3



DAY 3 biopsy



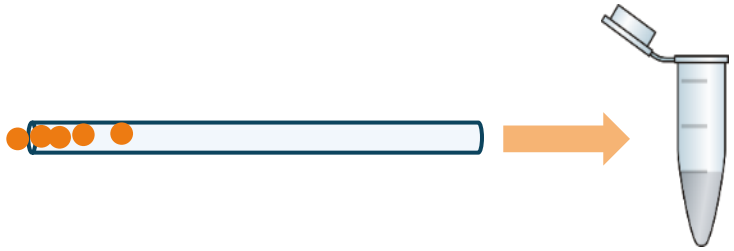
NGS analysis

Embryonic cleavage stage is characterized by chromosomal instability (CIN).

Impossibility to detect mosaicism

DAY 5

Blastocyst or TE biopsy

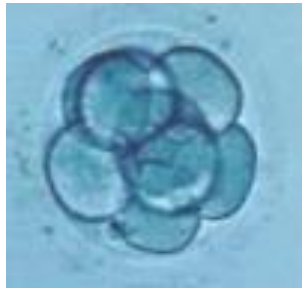


NGS analysis

COME SI ESEGUE UNA PGT-A

IN VITRO FERTILIZATION

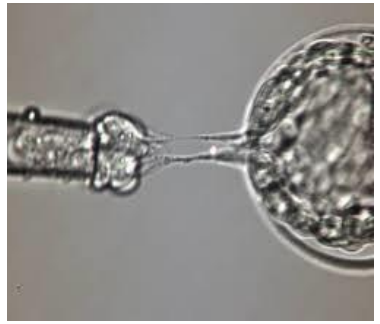
DAY 3 embryo



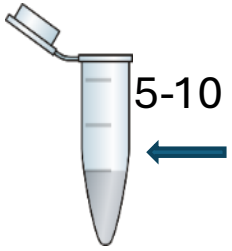
DAY 5 embryo

Embryo biopsy

Trophectoderm cells



5-10 cells

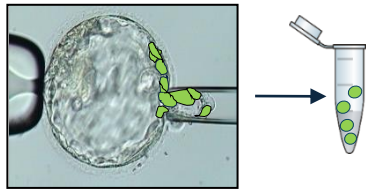


NGS

PGT

Genetic screening workflow

TE biopsy



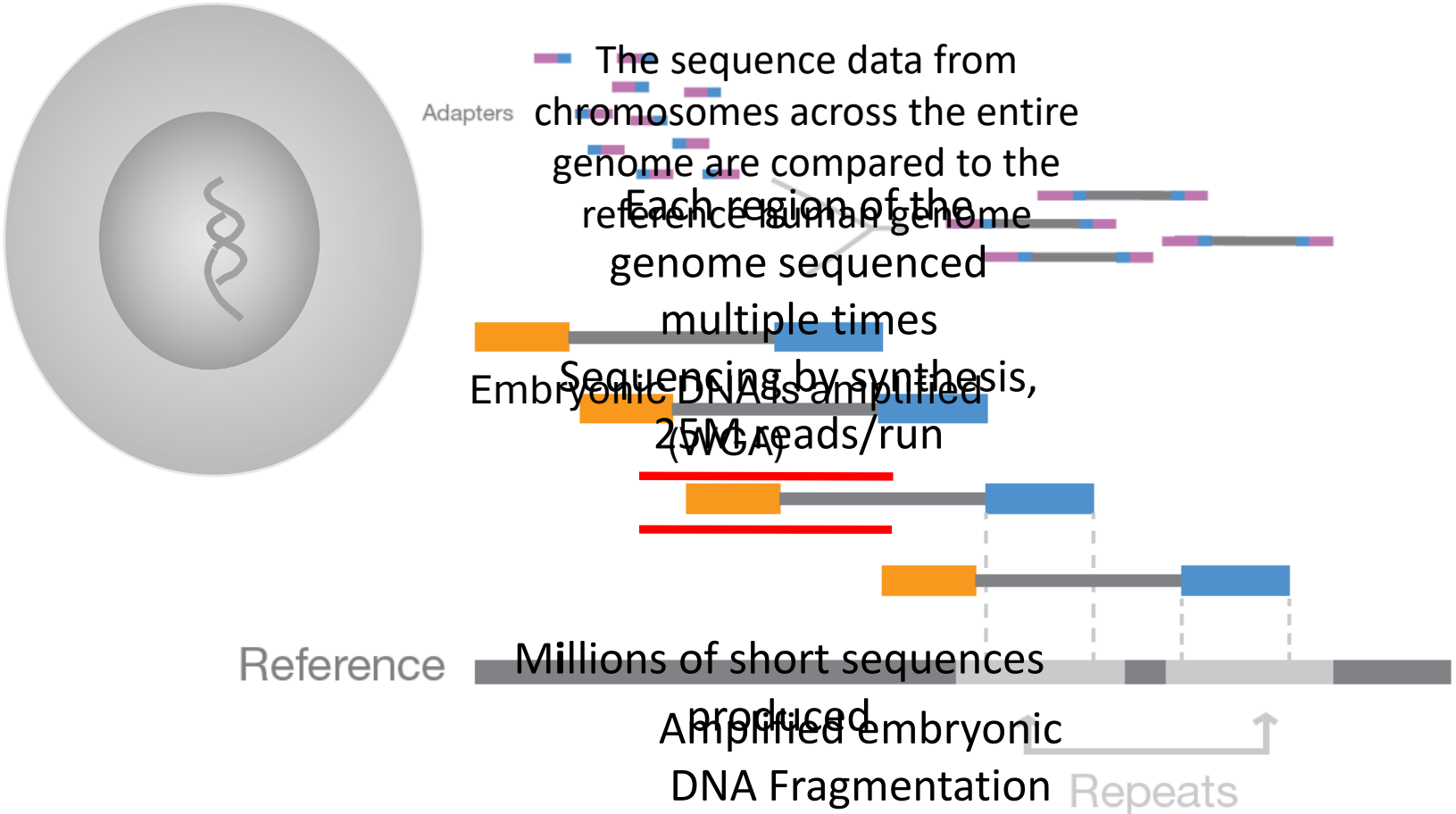
Single cells biopsy

DNA fragmentation and WGA

DNA sequencing

NGS

NGS-based PGS



Embryonic DNA is amplified (WGA)

Amplified embryonic DNA Fragmentation into a Library of smaller fragments (100-200 bp)

Millions of short sequences produced

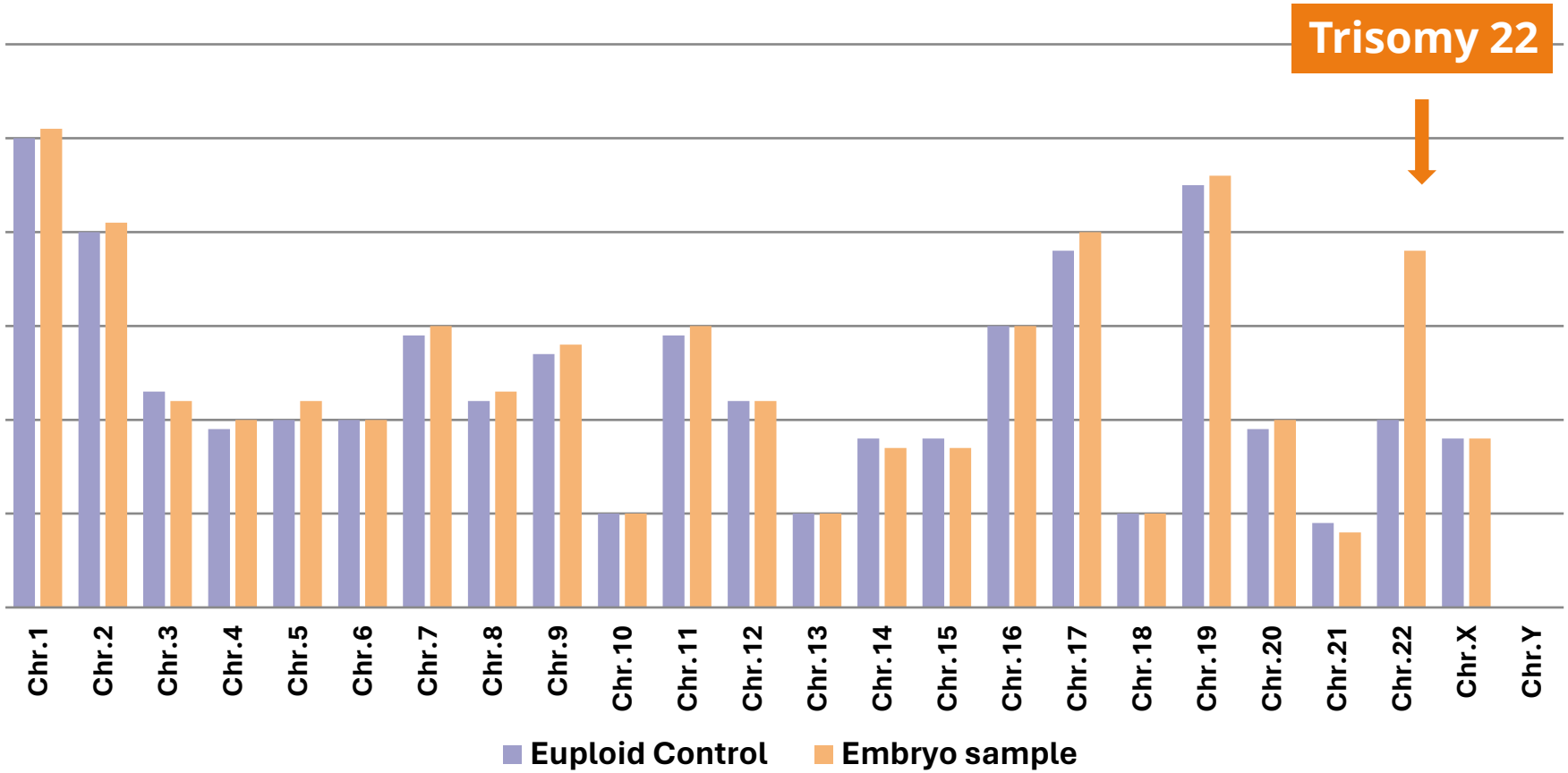
Sequencing by synthesis, 25M reads/run

Each region of the genome sequenced multiple times

The sequence data from chromosomes across the entire genome are compared to the reference human genome

PGT NGS based

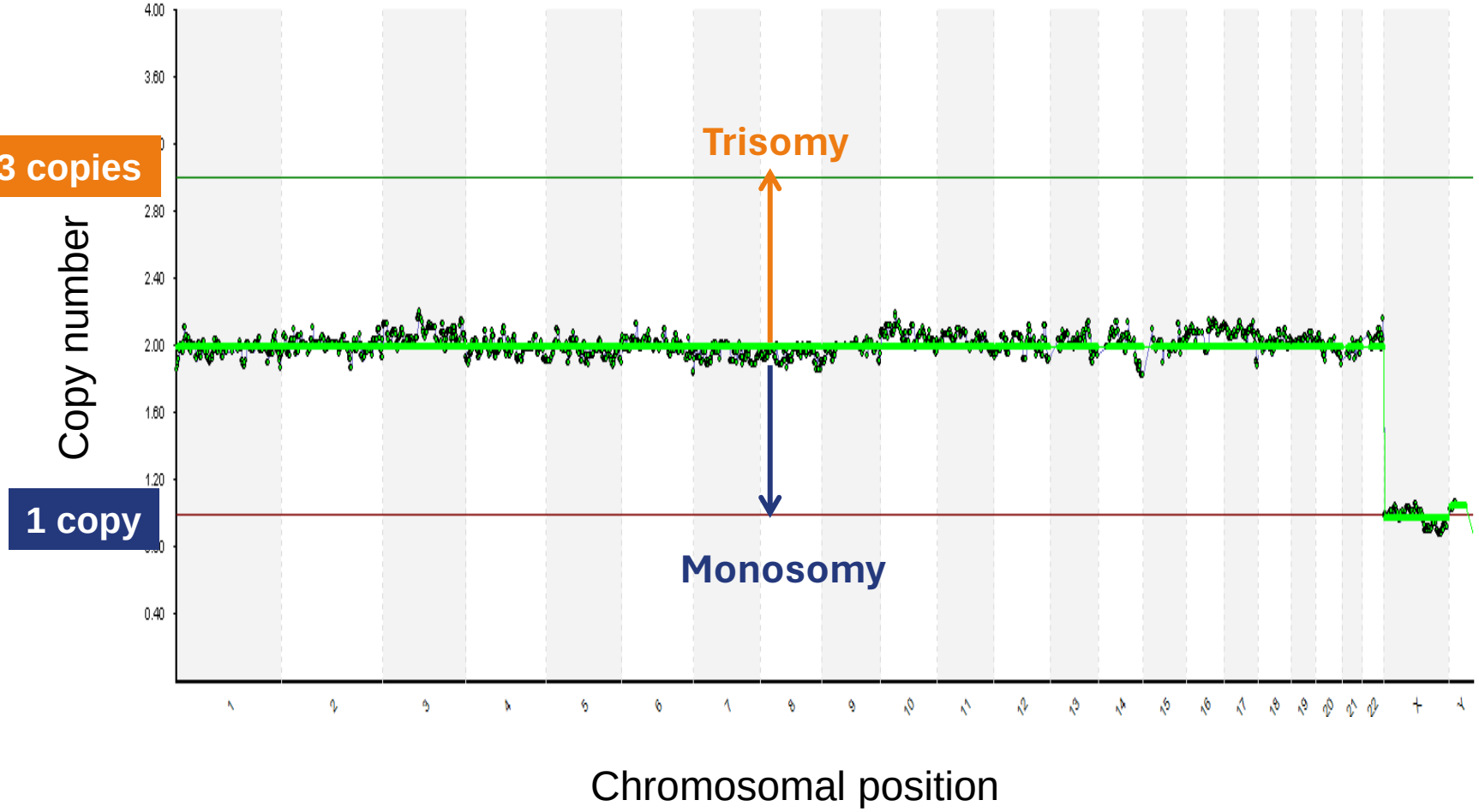
Data analysis



Sequences from each chromosome are counted using a dedicated software and compared with a chromosomically normal reference DNA

PGT results

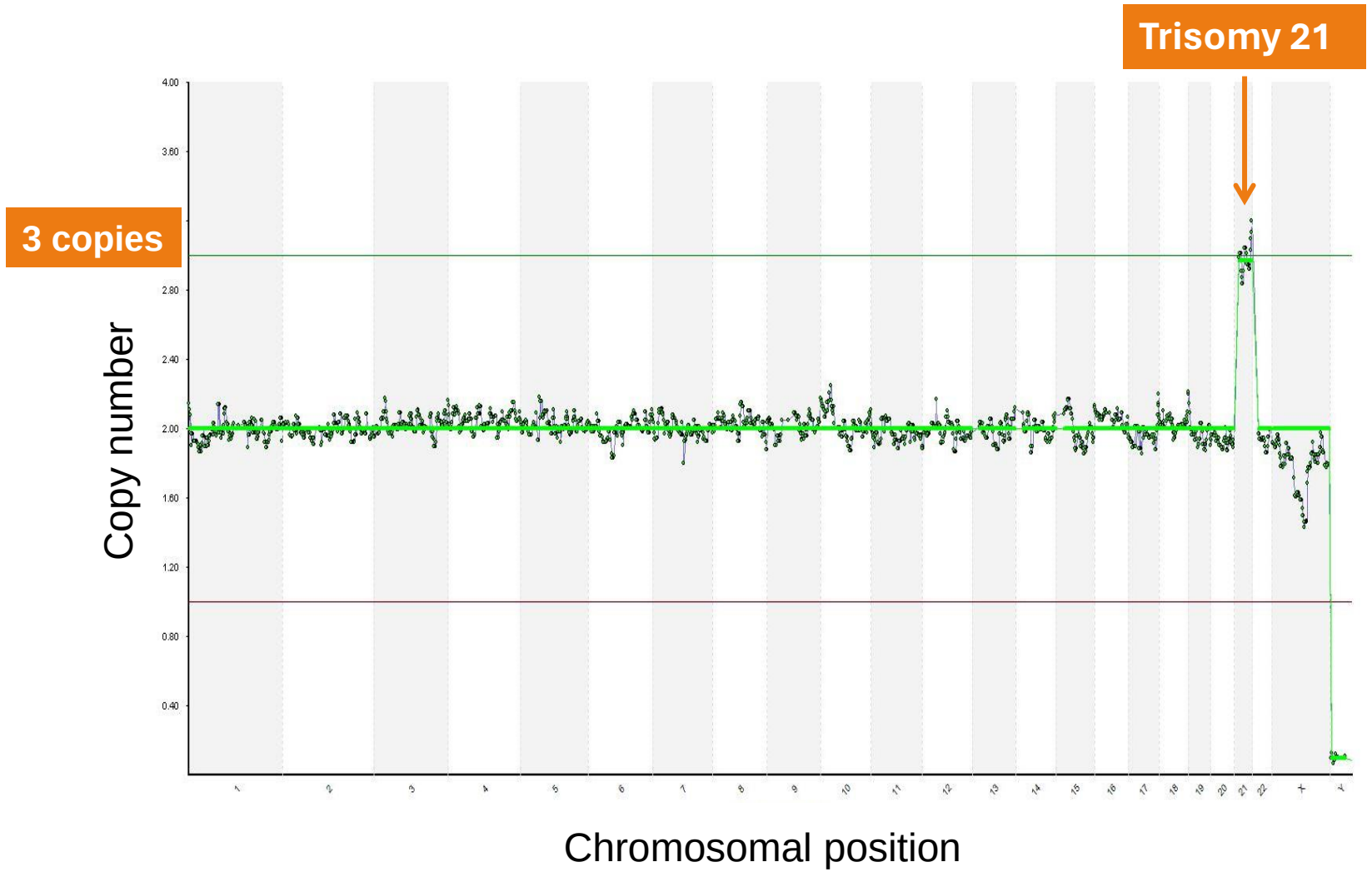
Euploid embryo



NGS enables quantification of chromosome copy number

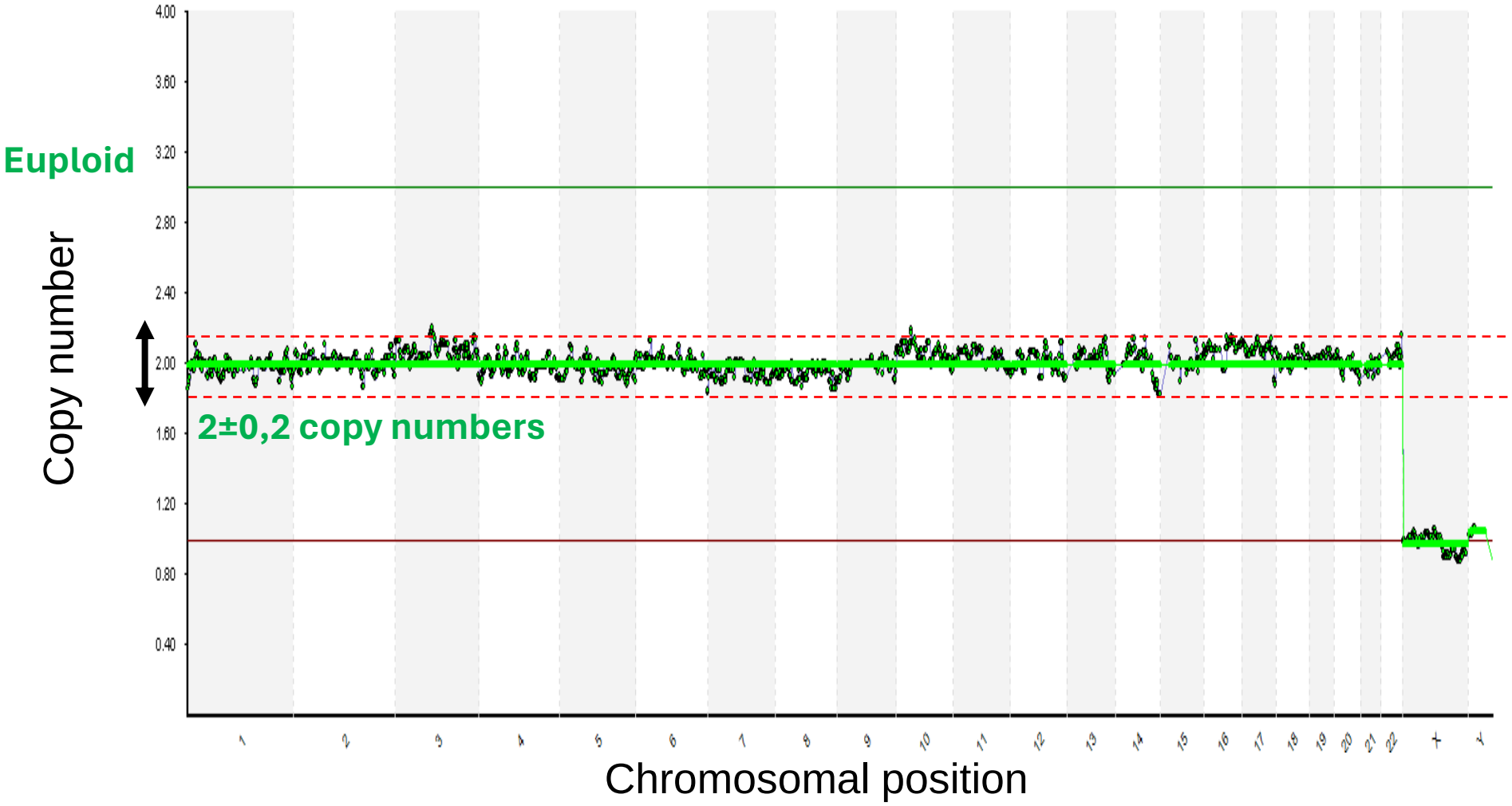
PGT results

Fully aneuploid embryo (trisomy 21)



PGT results

Validation study

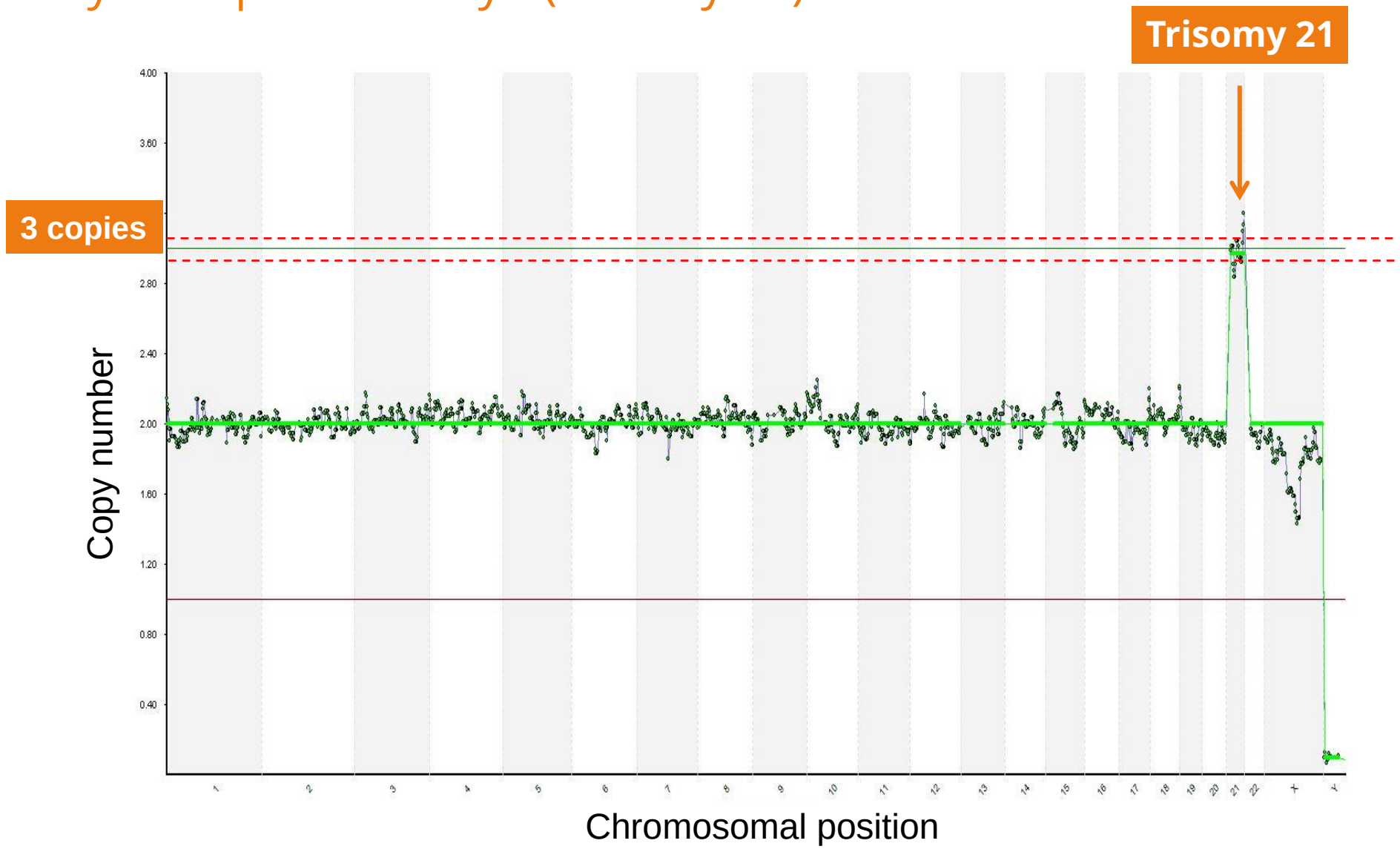


It is recommended to perform validation studies with true aneuploid and euploid cell lines*

*ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. Hum Reprod Open 2020

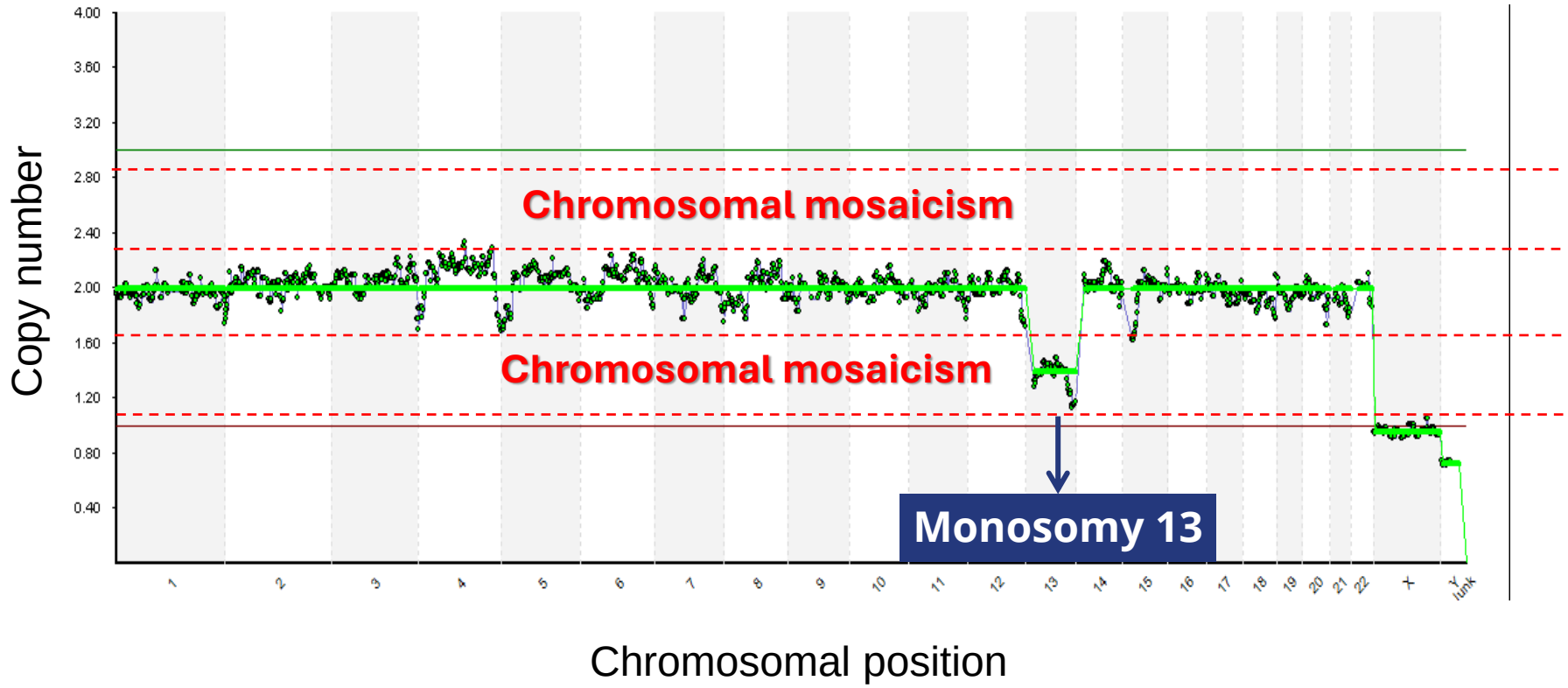
PGT results

Fully aneuploid embryo (trisomy 21)



PGT results

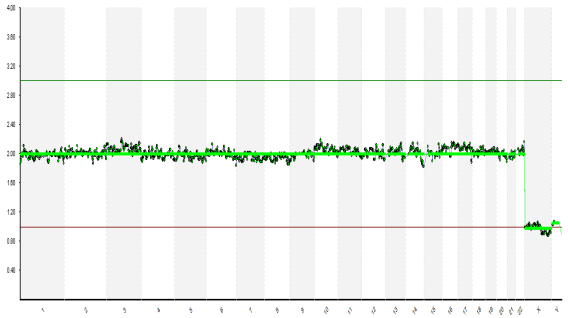
Mosaic embryo (monosomy 13)



PGT results

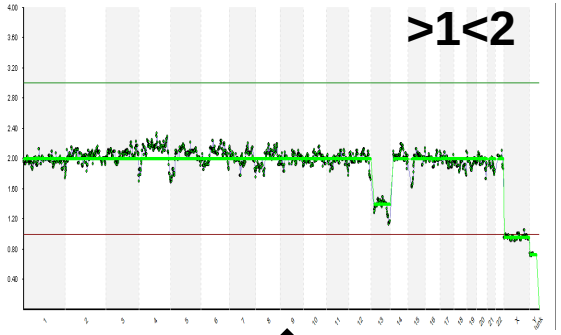
Classification

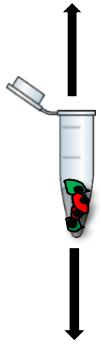
Copy number =2



Euploid

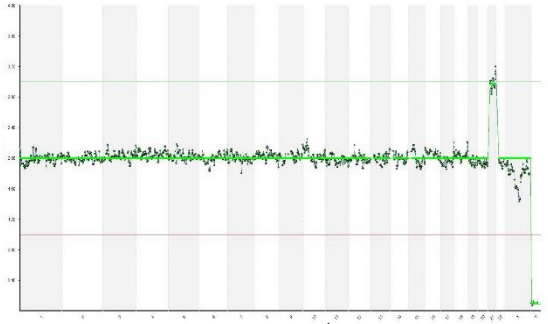
Copy number >2<3 >1<2





Mosaic

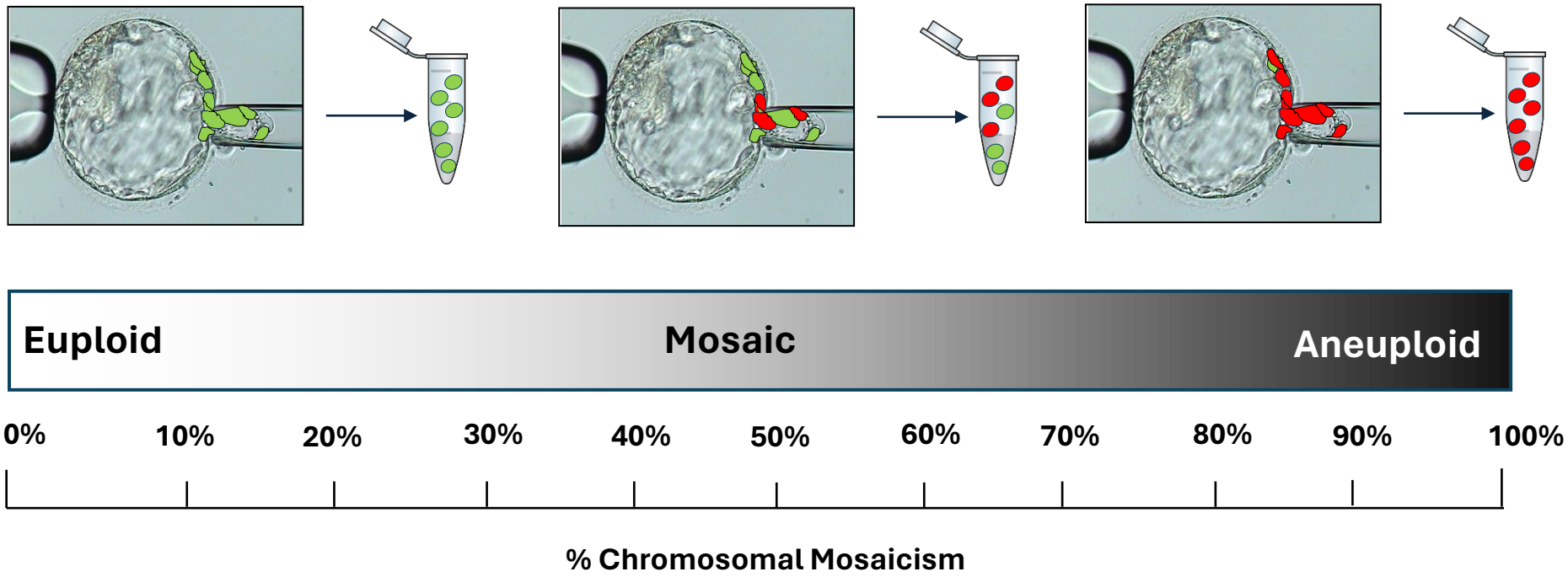
Copy number =3 or 1



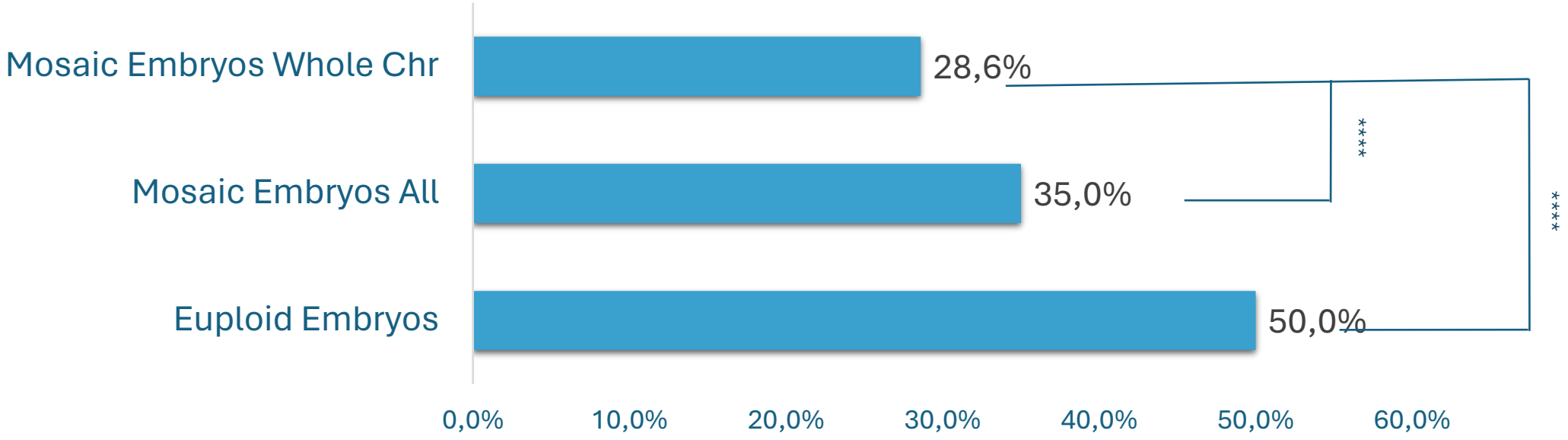
Aneuploid

Clinical outcome based on PGT results

Classification



Clinical Outcomes of Euploid vs. Mosaic Embryos

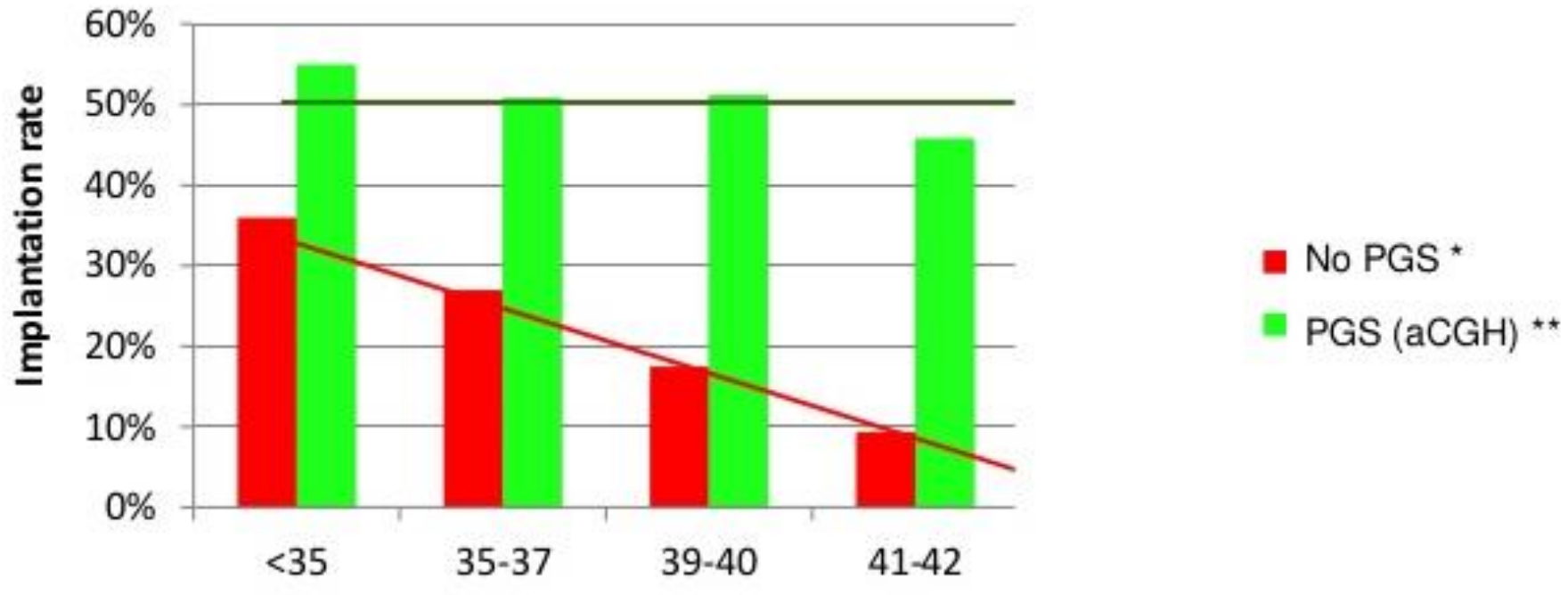


Ongoing Pregnancy/Birth rate

PGT-A

Clinical outcomes

PGT-A allows to avoid the negative effect of maternal age on implantation rate

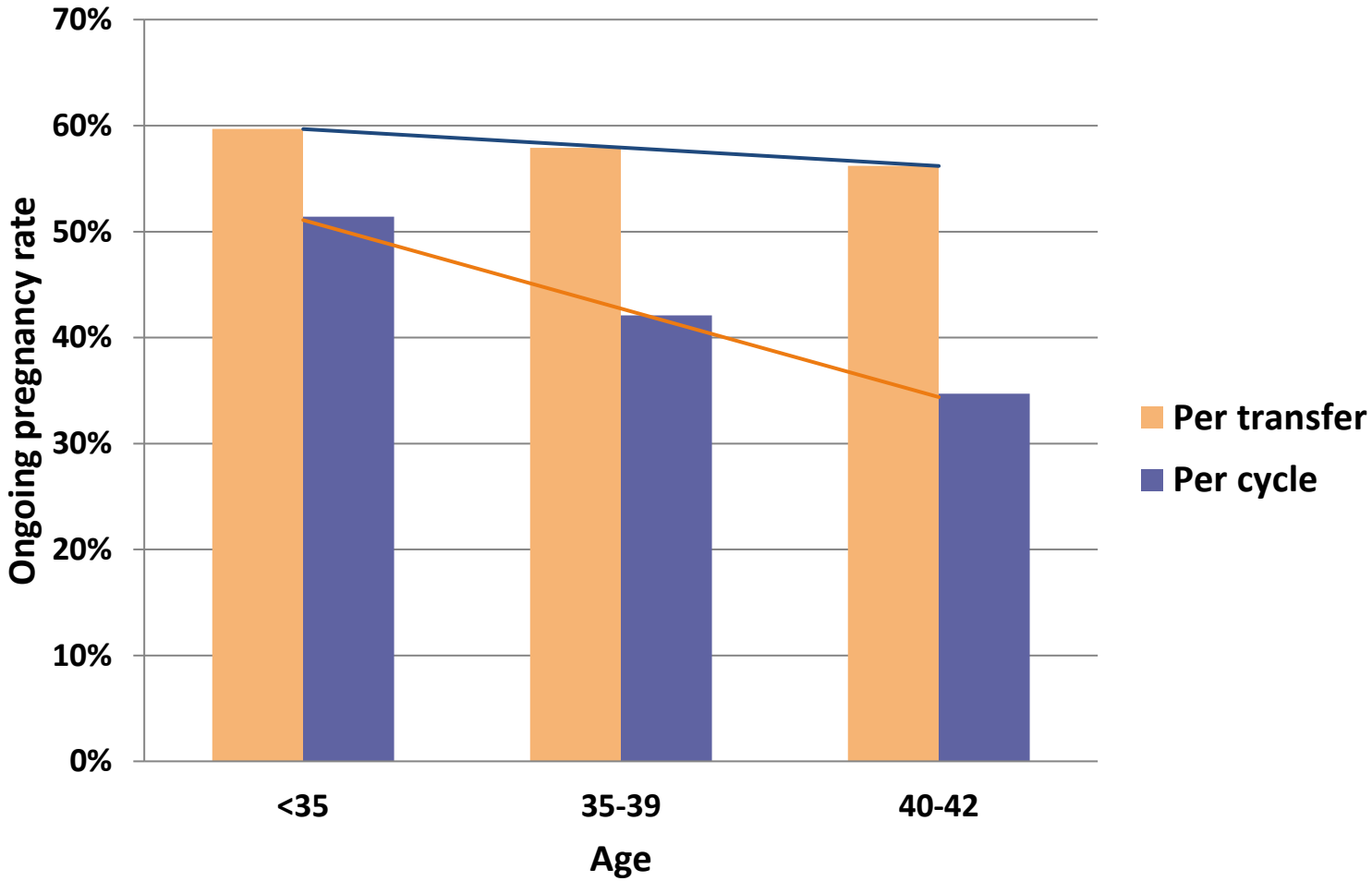


SART National Summary Report: Preliminary CSR for 2014. 2017.

PGT-A

Clinical outcomes

…however the total number of pregnancies will be lower because there will be less transfers per cycle



Data from 1.509 PGT cycles with analysis of 6.150 embryos

PGT

Conclusion

PGT represents a useful tool for embryo assessment before transfer:

- to significantly reduce a couple's risk to have a pregnancy with a genetic disorder or chromosomal abnormality;
- to improve IVF clinical outcome, identifying and selecting for transfer chromosomally normal (euploid) embryos.
- The use of high-depth NGS ensures more accurate results as it also allows intermediate CNVs to be visualized by identifying mosaic embryos

Thank You

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