Single-cell DNA sequencing reveals high incidence of numerical and structural abnormalities in human blastocysts

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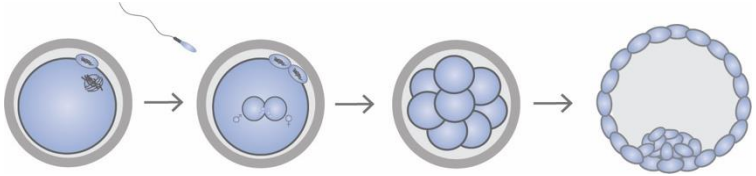
Origin of chromosomal abnormalities

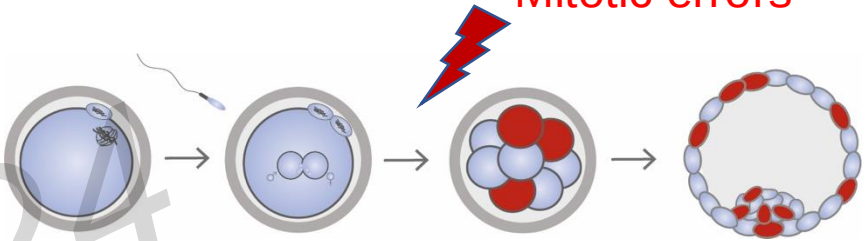
Normal embryo

Normal fertilization



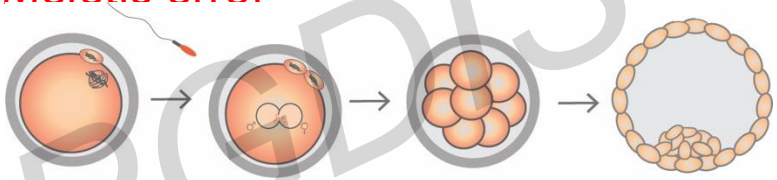
Diploid aneuploid mosaic embryo

Mitotic errors



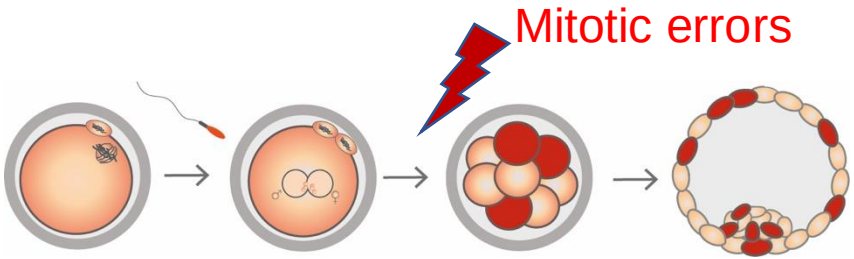
Uniformly abnormal embryo

Meiotic error



Aneuploid mosaic embryo

Mitotic errors



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Chromosomal abnormalities in human embryos

Abnormalities related to

low pregnancy rates

miscarriages

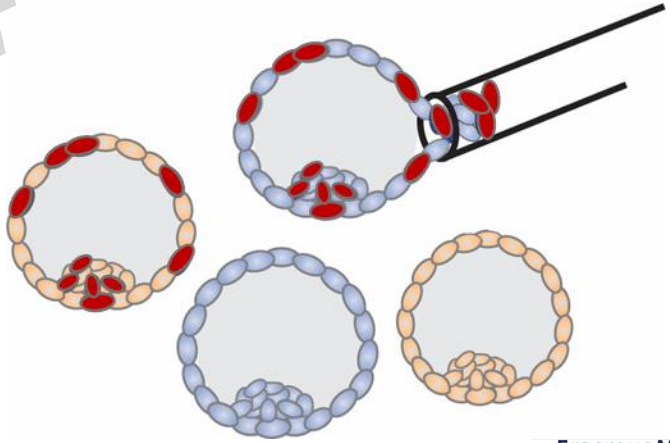
congenital birth defects

Type of abnormality

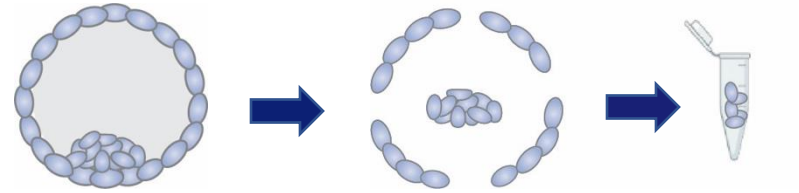
numerical

structural

Embryo selection: PGT-A



Bulk DNA sequencing and technical limitations



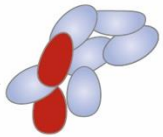
blastocyst → multicellular TE-ICM biopsies → Bulk DNA NGS

Low level mosaicism and products of reciprocal events might go undected

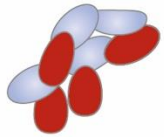
Technical limitations

- Detection limit of 20-30%

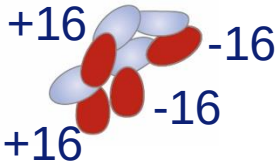
not detected



detected



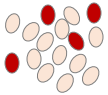
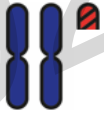
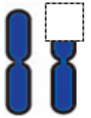
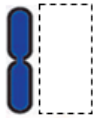
- Average chromosome net gain or loss



→ Normal PGT-A result

Single cell sequencing on human blastocysts

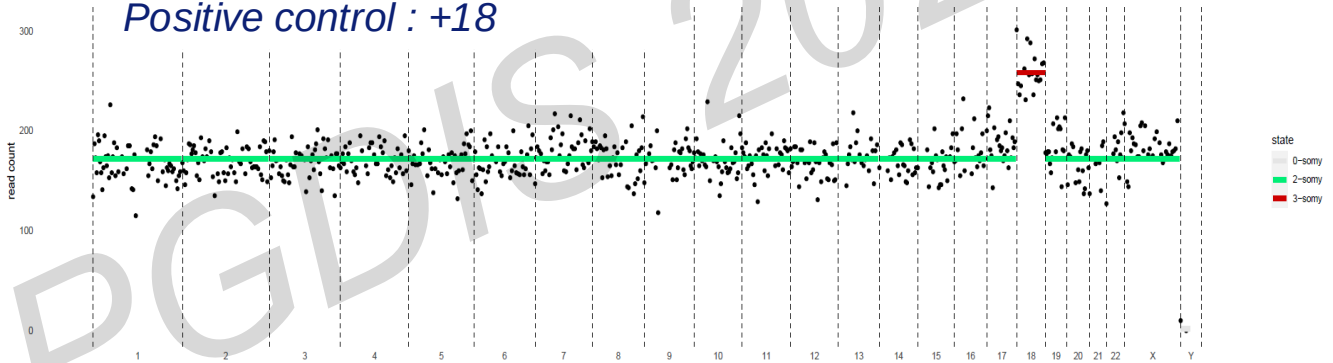
Chromosomal content of the cells of good quality blastocysts

- Mitotic  vs meiotic 
- Type of abnormalities: numerical  vs structural 
- Type of abnormalities: (partial) gain  ,  vs (partial) loss  , 
- Distribution of abnormal cells within ICM & TE and timing of mitotic error
- Mechanisms leading to mosaicism

Validation - technique: Single cell DNA sequencing (scKaryoseq)

- Detects numerical and structural abnormalities in "flow sorted" cells
- Flow cytometry is not feasible for human blastocysts (~50 cells per embryo)
- Validation on ‘’manually plated’’ fetal cells from chorionic villi or amniotic fluid with known abnormalities (numerical + structural)

Positive control : +18



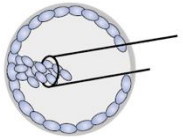
Chavli et al., J Clin Invest. 2024 Jan 4;134(6):e174483

Klaasen et al, Nature 2022:p604

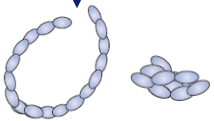
Erasmus MC

Experimental design

Good quality IVF morulas donated for research



ICM biopsy of blastocysts with at least a 3BB morphology score



Dissagregation into single cells (accutase)

Single cell whole genome Sequencing (scKaryo-seq)

55 blastocysts

↓

2322 isolated cells

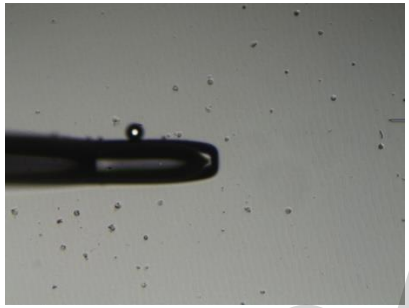
↓

1057 successfully analysed cells

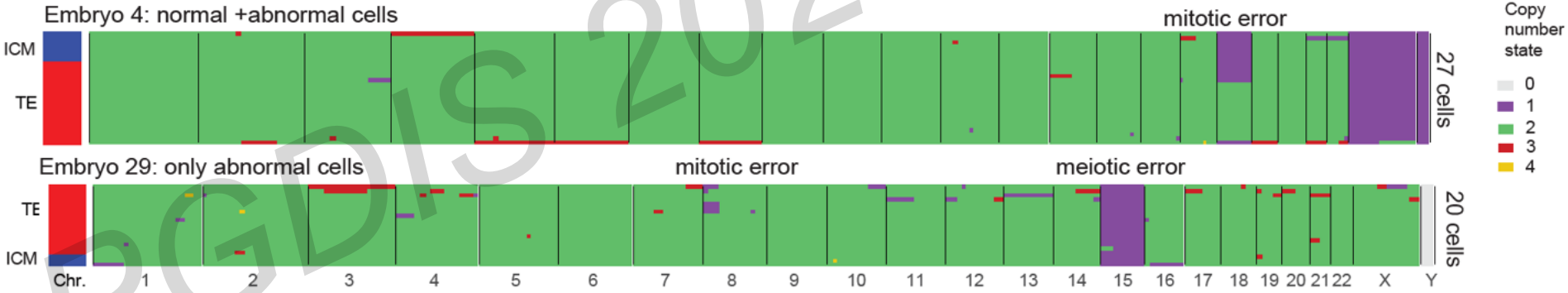
↓

535 abnormal cells
522 normal cells

scKaryo-seq on human blastocysts



2 copies, 1 copy



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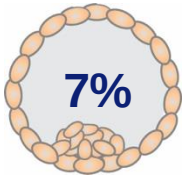
Chromosomal constitution of blastocysts

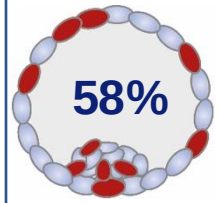
Chromosomal composition

normal

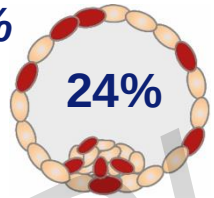


Uniformly abnormal

82%



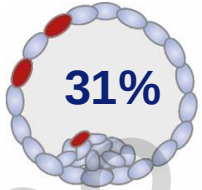
Diploid-aneuploid mosaic



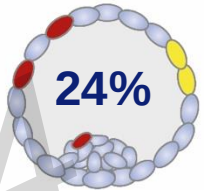
Aneuploid mosaic

In 69% of the embryos >1 mitotic error

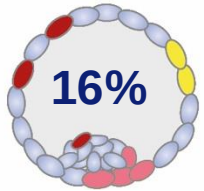
1 error



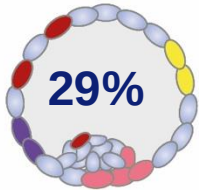
2 errors



3 errors



>3 errors



Abnormal cells

Most blastocyst are mosaic in which several mitotic errors take place

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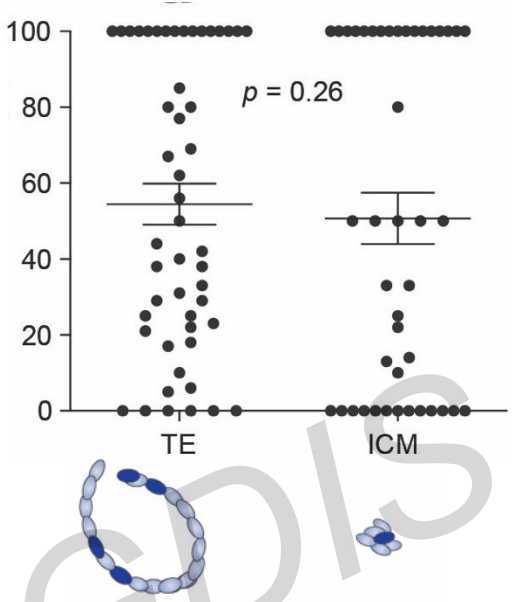
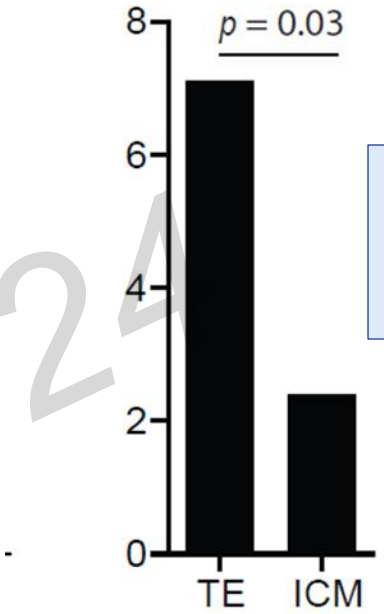
Distribution of abnormal cells in ICM and TE

% abnormal cells/ per embryo



No preferential allocation of abnormal cells towards TE or ICM

% complex abnormal cells



Different ‘‘selective pressure’’ mechanisms in TE and ICM ?

Complex abnormal cells (>4 abnormalities) more common in TE

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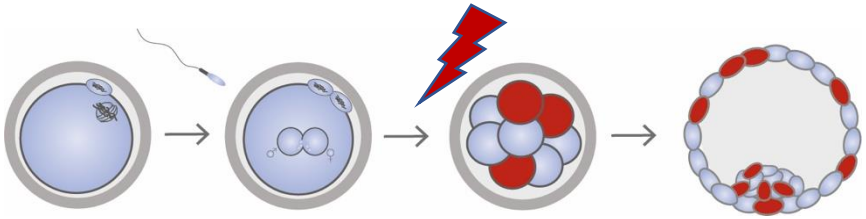
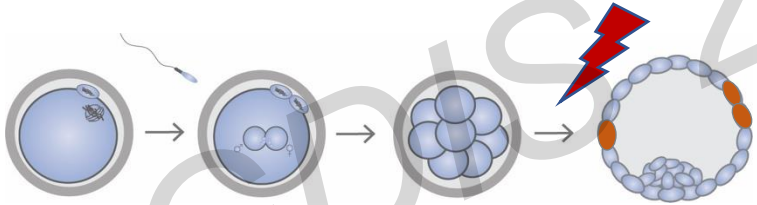
Timing of mitotic error

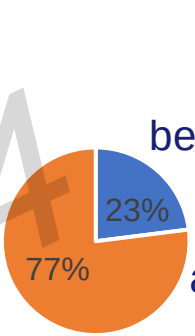
Mitotic error before TE/ICM differentiation



Mitotic error after TE/ICM differentiation



mitotic abnormalities in TE **and** ICM
versus
mitotic abnormalities in TE **or** ICM



Mitotic errors occur possibly also after TE/ICM differentiation

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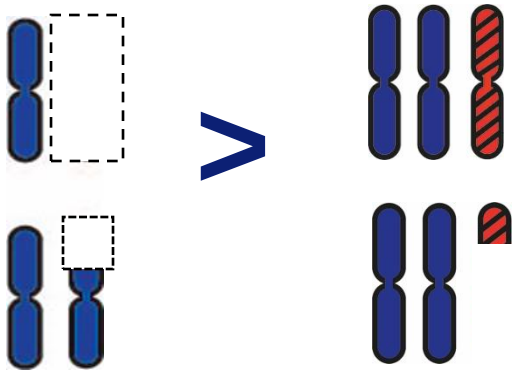
Type of abnormalities in the single cells

numerical structural

loss gain



- High incidence of structural abnormalities
- Structural abnormalities present in 69% of analysed embryos

- In prenatal diagnostics chr. loss is rare
- ‘’Selective pressure’’ for embryos and cells with chr. loss not fully active yet?

Observations: Reciprocal gain + loss

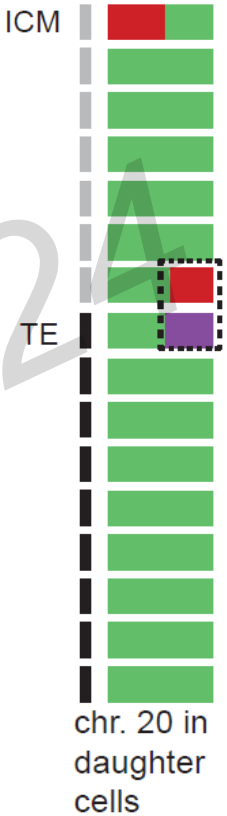
Embryo 1

Reciprocal gain and loss of whole chromosome 21

↓

Missegregation

Embryo 2



Reciprocal gain and loss of a segment of chromosome 20

↓

Incomplete DNA replication
DNA breaks

GAIN LOSS

Palmerola et al, Cell 2022:p2988; Burrell et al, Nature 2013:p492

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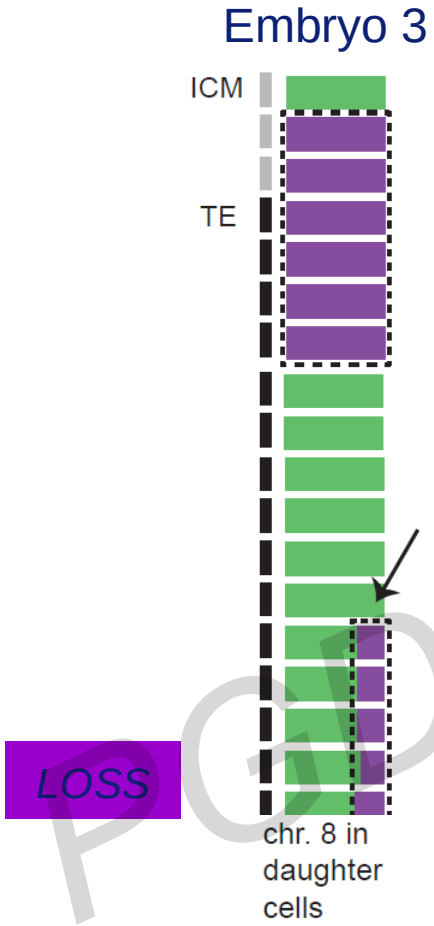
Observation: Numerical & structural loss of the same chr.



DNA break on lagging chromosome – Incomplete DNA replication

↓

Structural loss of chr.

↓ Cell divisions

Complete loss of chr.

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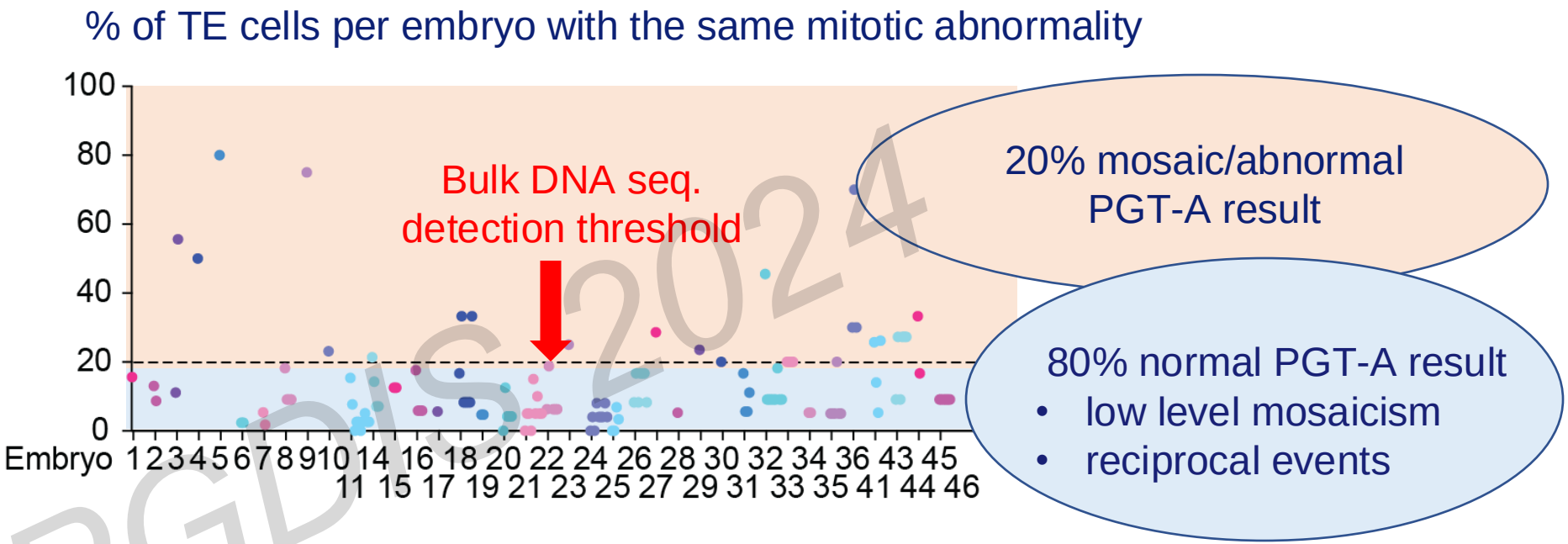
Janssen et al, Science 2011:p1895; Van opstal et al, Prenat Diagn 2019:p1016; Zuffardi et al Eur J Med Genet 2022:p1054

Underestimation of TE mosaicism with bulk DNA seq.

In silico reanalysis of single cell data from embryos with mosaic TE



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Conclusions *in-silico* analysis

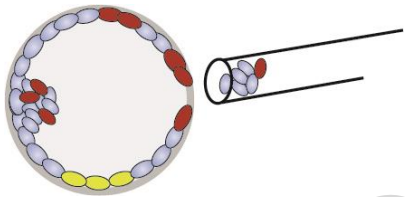
- Most blastocysts show chromosomal mosaicism
- Most mitotic abnormalities are present in less than 20% of the TE-cells/embryo
- These embryos most probably will develop further normally

Possible implications for clinical practise

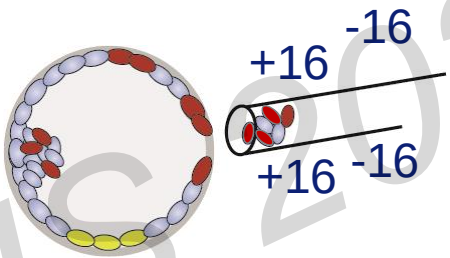
- Clinical outcome of mosaic/ normal embryos uncertain

PGT-A normal result → No Pregnancy

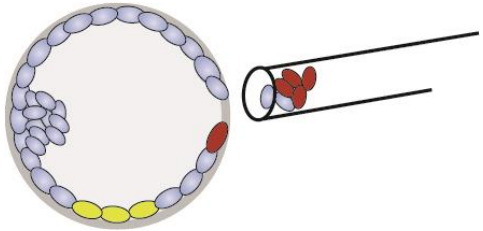
PGT-A mosaic or abnormal result → Healthy baby



mosaic embryo



mosaic embryo
(gains = losses)



mosaic embryo

+ self correction ?

Conclusions

- ✓ No preferential allocation of abnormal cells towards ICM or TE (exception complex abnormal cells)
- ✓ Mitotic errors possibly occur also after TE/ICM differentiation
- ✓ Insights into type of abnormalities and possible mechanisms involved
  - Structural = numerical
  - Chromosome loss > gain
- ✓ Most blastocysts showed chromosomal mosaicism
  - ✓ Possible explanation of unexpected clinical outcomes after the transfer of PGT-A tested embryos

Acknowledgments

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