On (mosaic) aneuploidy through haplotyping perspective

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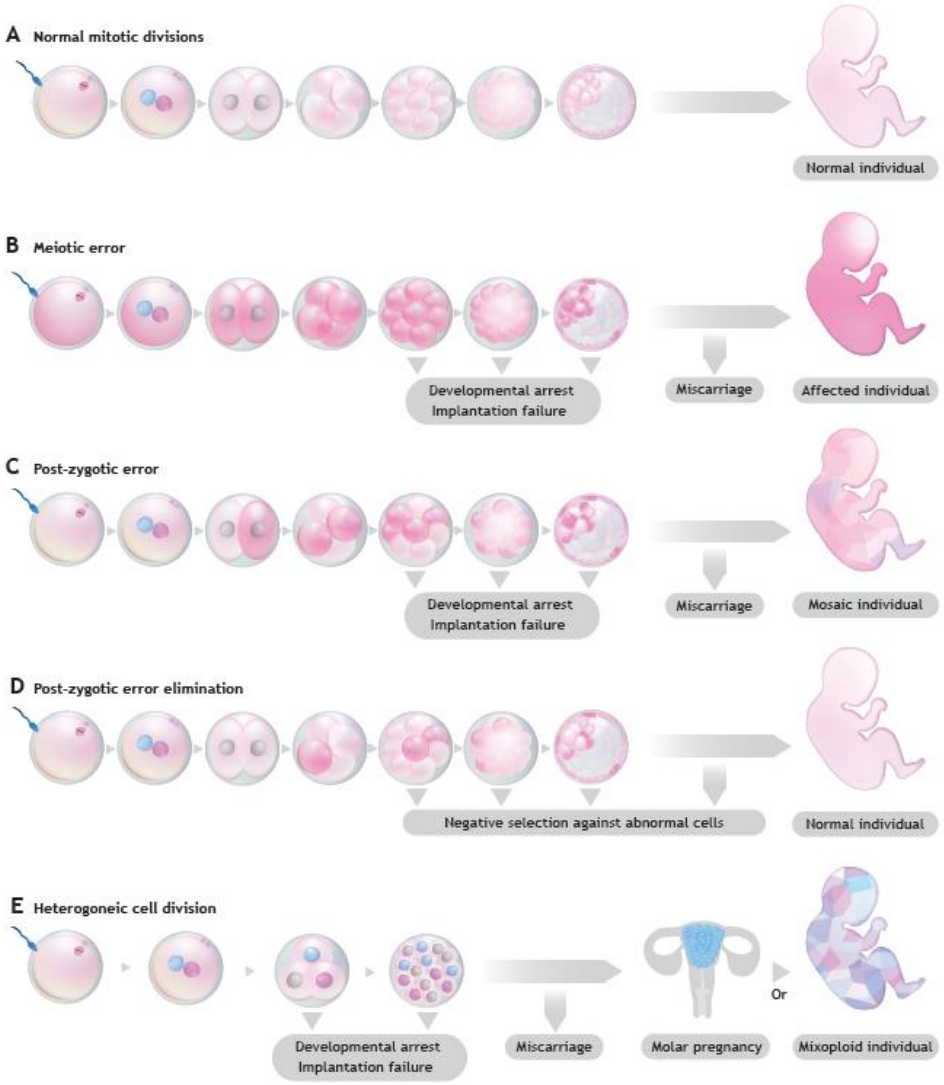
The impact of aneuploidy on embryo development



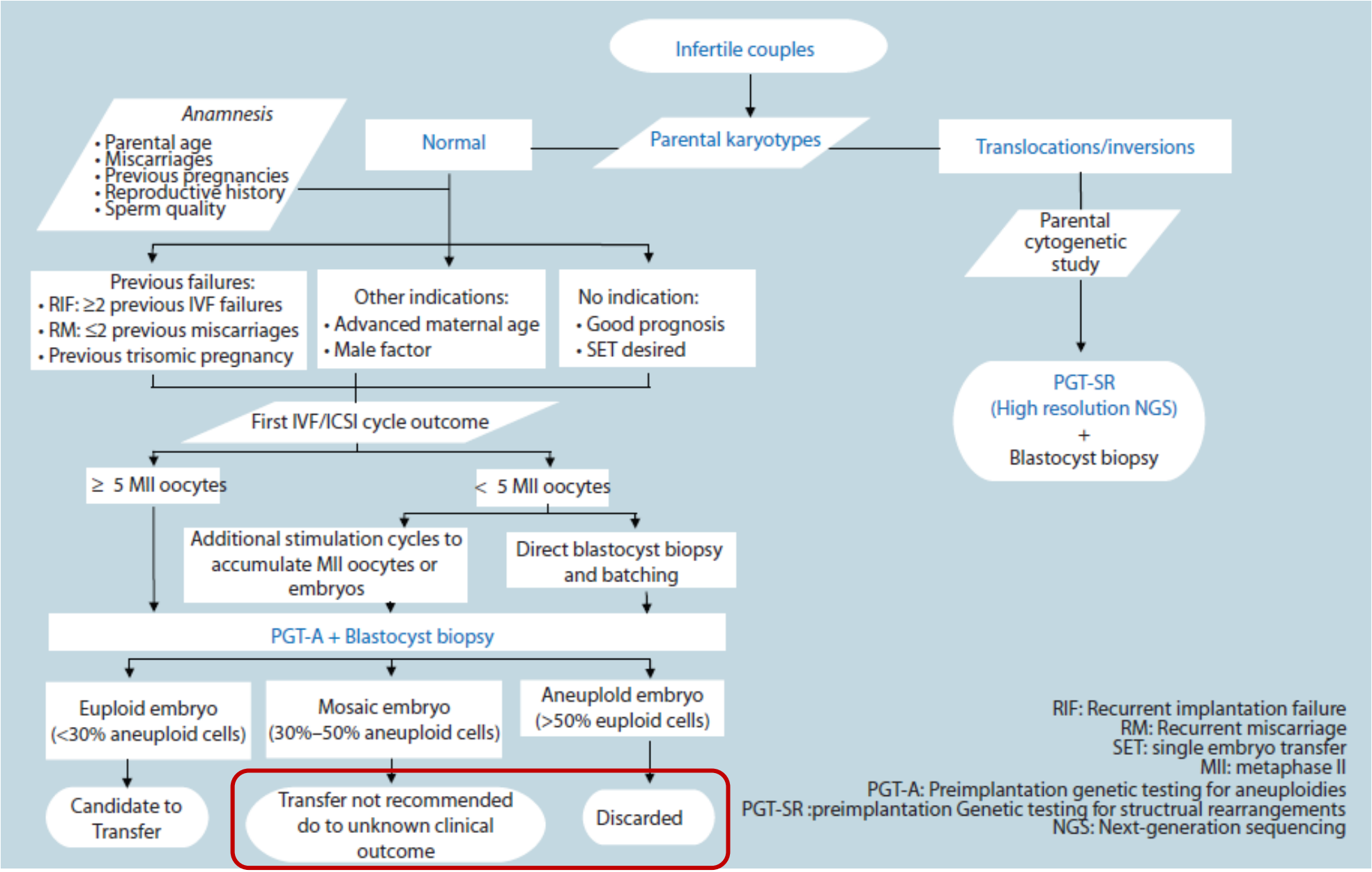
Meiotic aneuploidy – inherited from oocyte (~95%) or sperm (~5%) and all cells carry aneuploidy (increases with maternal age)
Viable aneuploidy: T13, T18, T21; XO, XXY, XXX, XYY

Mitotic (postzygotic) aneuploidy – errors occur during early embryo development, leading to cells with different karyotypes

- **Belgium:** transfer of mosaic embryos (except 13, 16, 18, 21 and mosaic XO)

Rare **genome-wide aberrations (haploidy, triploidy):** miscarriage, formation of molar pregnancy or mixoploidy in humans

Tsuiko, Fernandez-Gallardo et al 2020 (PMID: 33065545)

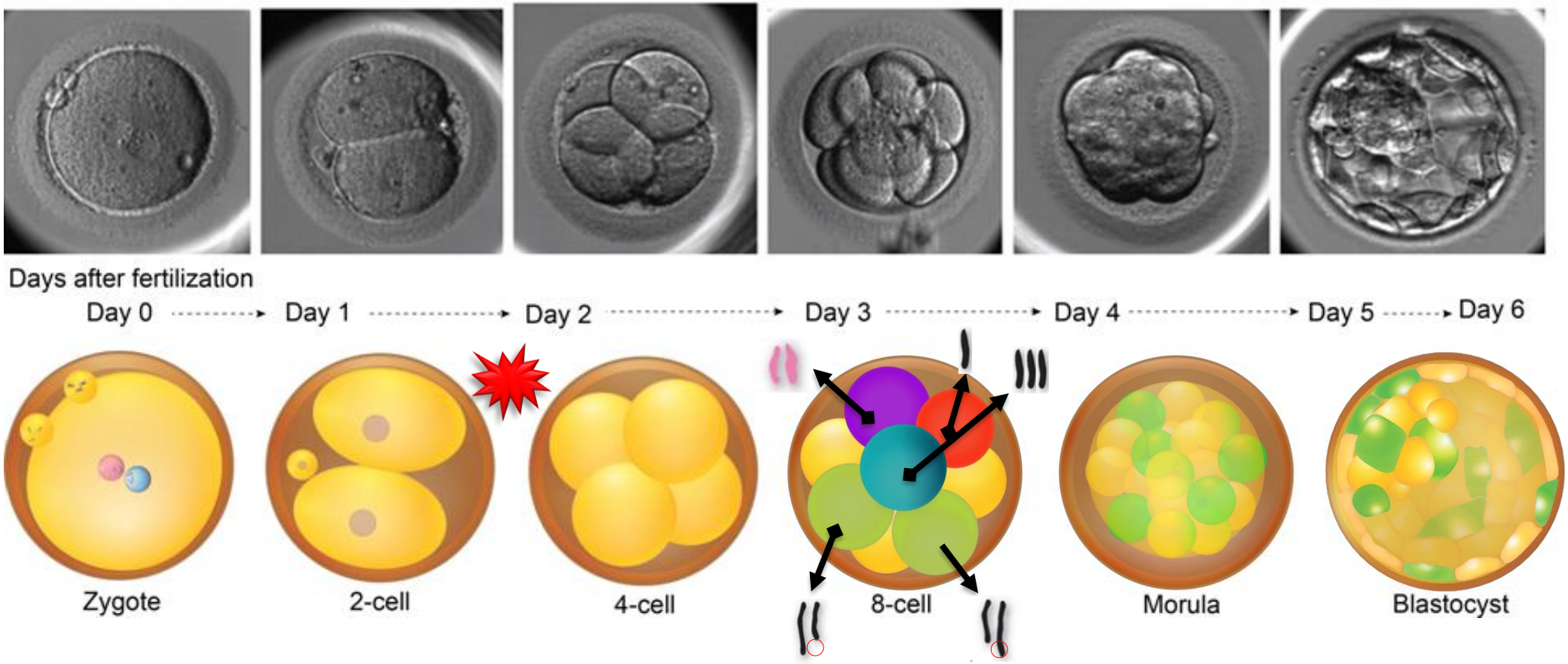


Rubio, Simón - Handbook of New Genetic Diagnostic Technologies in Reproductive Medicine

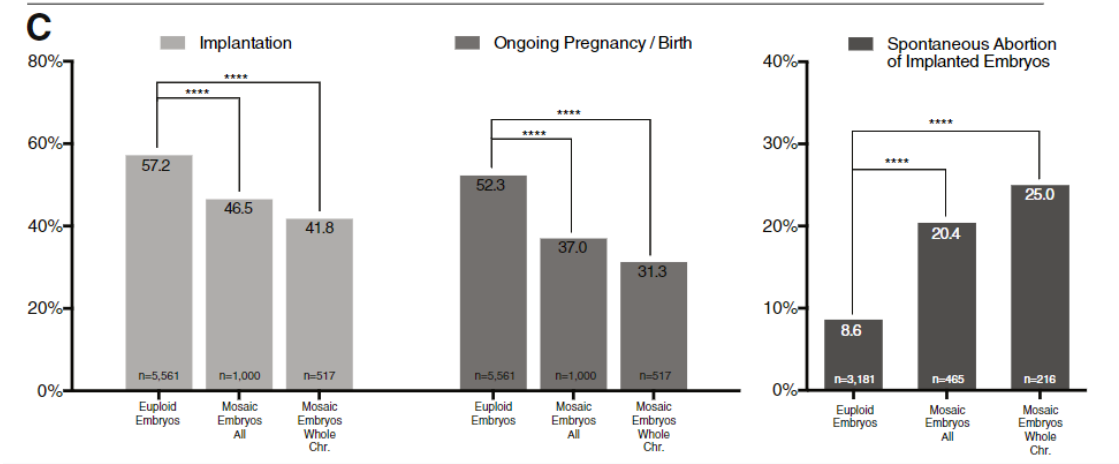
Understanding embryo biology guides clinical decision-making

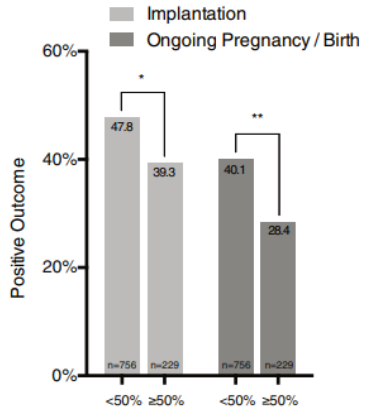
Single cells studies:

- embryos that contain at least one cell with aneuploidy - 80% for cleavage-stage;
- up to 80% for blastocysts, often with less than 20% of cells affected per embryo! (Chavli et al, 2024, PMID: 38175717)



Large-scale follow up studies of mosaic embryo transfer





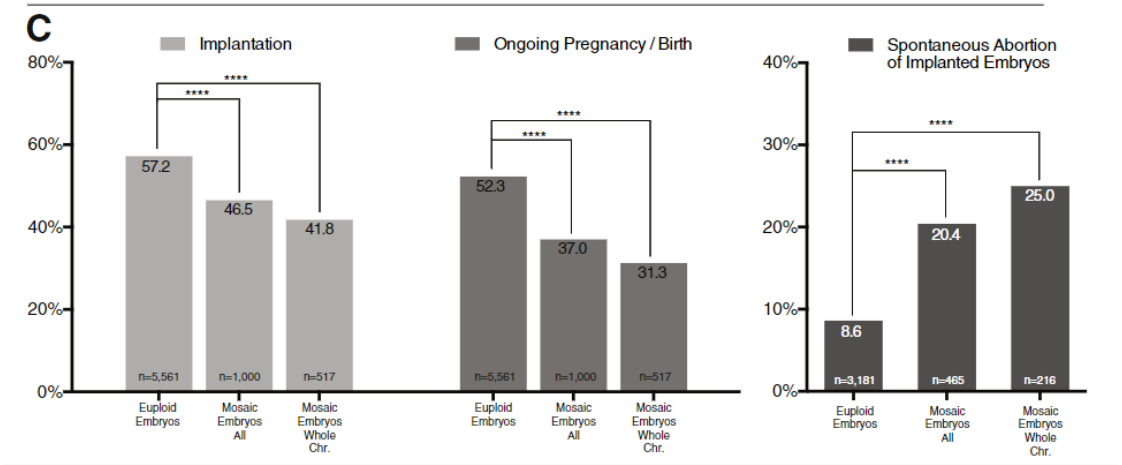
(IRMET)

- Can lead to live birth
- ↓ implantation rate
- ↑ miscarriage rate
- Low-grade mosaicism (<50%) superior to high-grade (>50%)

Viotti et al 2021 (PMID: 33685629)

Updated study 2023: 1.2% (3/250) of prenatal test results reflect mosaicism detected at the embryonic stage

Large-scale follow up studies of mosaic embryo transfer



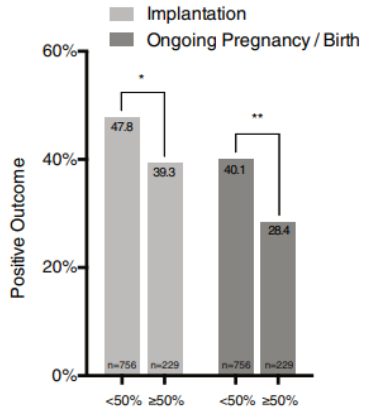


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Table 1. Reproductive outcomes of euploid and mosaic embryos

	Group A: Euploid	Group B: Low-grade mosaic (20–30% variation)	Group C: Medium-grade mosaic (30–50% variation)	Adjusted OR (95% CI; p value)
Test sets, n	484	282	131	-
Positive pregnancy test, % (n)	55.8% (270/484)	55.0% (155/282)	55.7% (73/131)	0.98 (0.75–1.27; 0.86)
Biochemical pregnancy loss, % (n)	10.7% (29/270)	12.3% (19/155)	13.7% (10/73)	1.18 (0.69–2.02; 0.53)
Miscarriage, % (n)	12.0% (29/241)	11.0% (15/136)	12.7% (8/63)	0.89 (0.50–1.55; 0.69)
Live birth, % (n)	43.4% (210/484)	42.9% (121/282)	42.0% (55/131)	0.97 (0.74–1.26; 0.82)
Monochorial twins delivery, n	1	1	1	-
Gestational age, mean (95% CI)	38.4 (38.0–38.7)	38.2 (37.9–38.6)	38.1 (38.0–38.5)	-
Birth weight, mean (95% CI)	3,286 (3,200–3,371)	3,174 (3,080–3,267)	3,130 (2,950–3,310)	-

Capalbo et al 2021 (PMID: 34798051)

(IRMET)

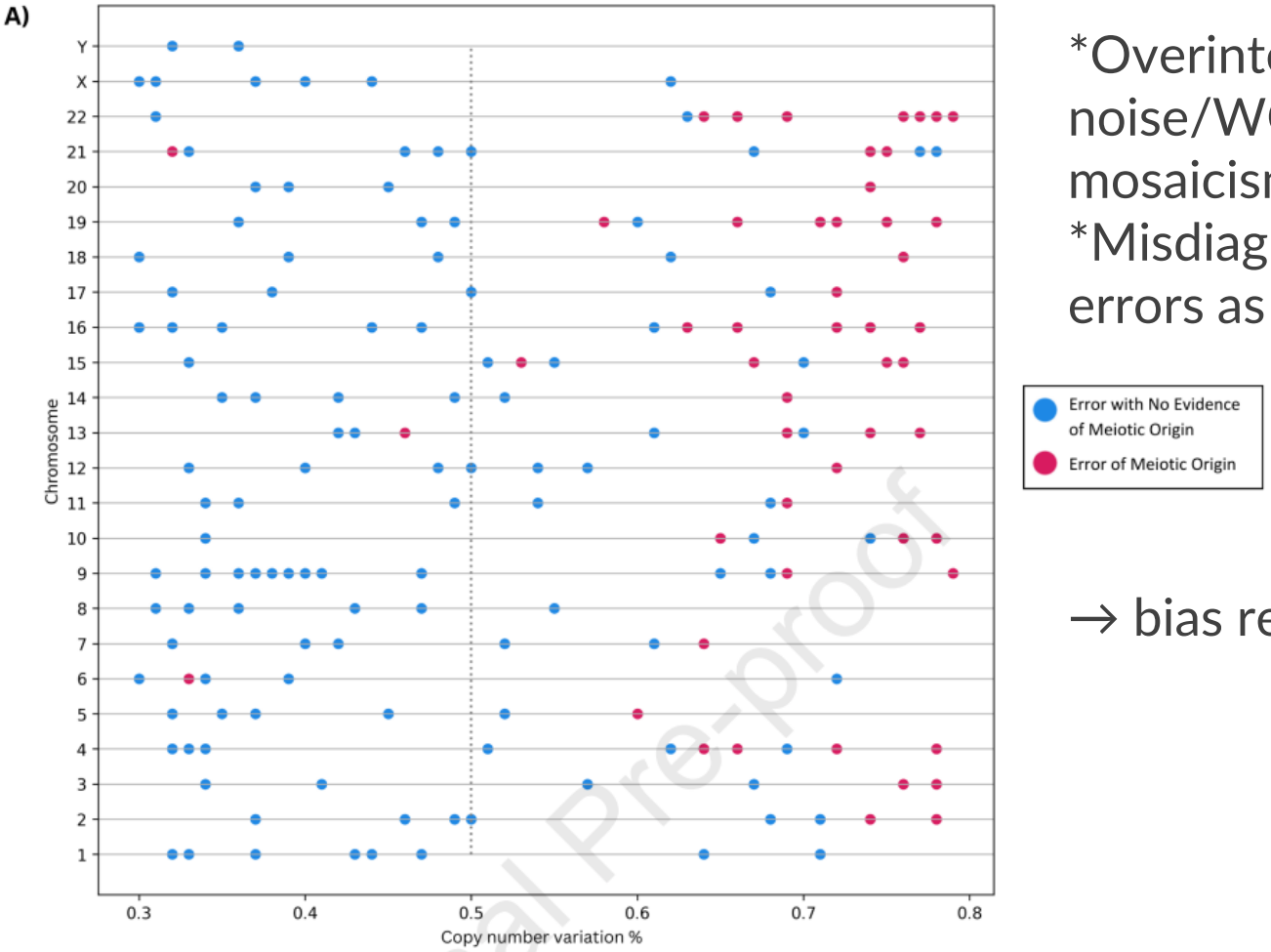
- Can lead to live birth
- ↓ implantation rate
- ↑ miscarriage rate
- Low-grade mosaicism (<50%) superior to high-grade (>50%)

- <50% mosaics have the same ET outcome as euploid ones

The issue of embryo misclassification

Current quantitative methodologies for pre-implantation genetic testing frequently misclassify meiotic aneuploidies as mosaic

Teodora Popa, PhD, Colin Davis, MBBS, Leoni Xanthopoulou, PhD, Evangelia Bakosi, MSc, Chloe He, MSc, Helen O'Neill, PhD, Christian Ottolini, PhD

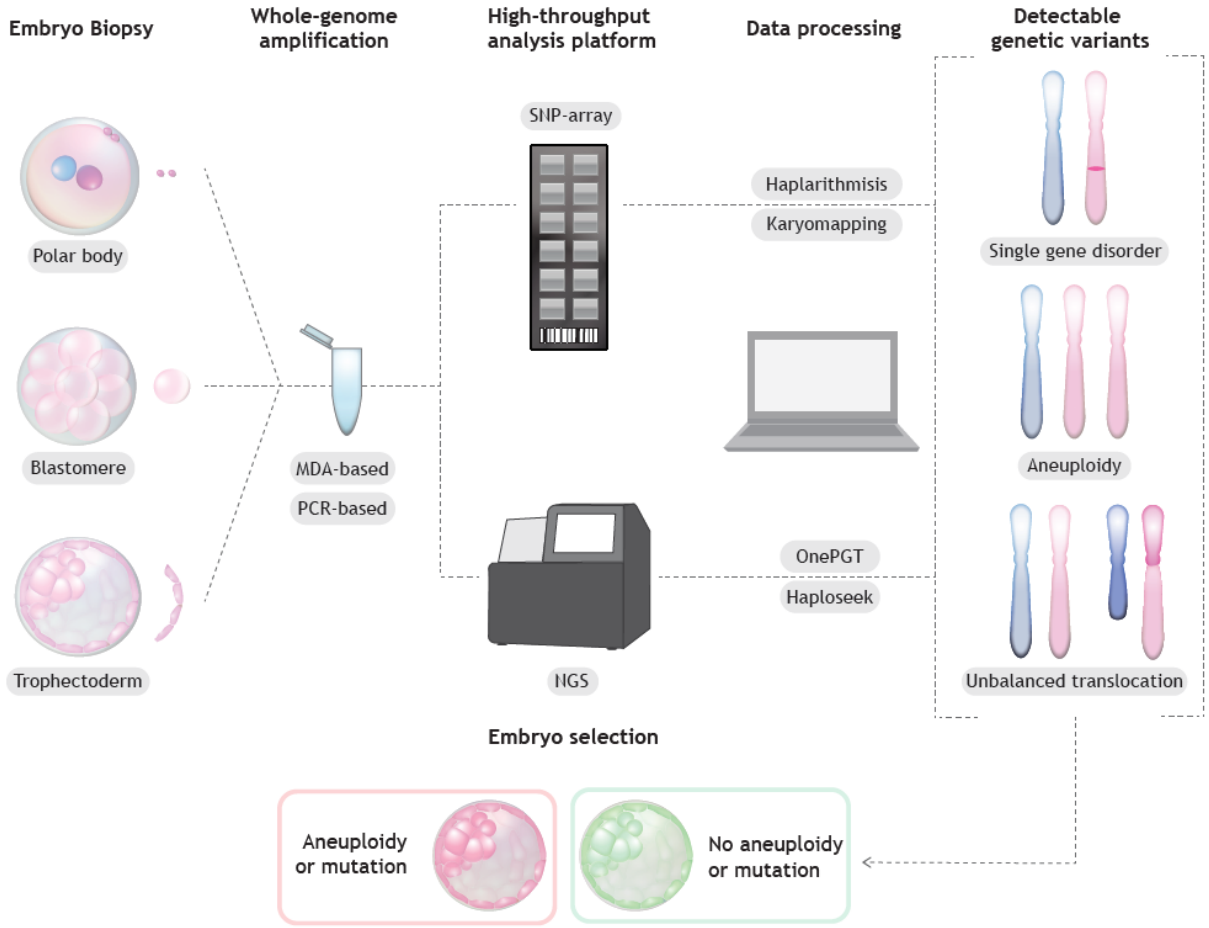


Copy number-based solutions may lead to embryo misclassification:

*Overinterpretation of technical noise/WGA artefacts as low-grade mosaicism
*Misdiagnosis of embryos with meiotic errors as 'high-grade' mosaics

→ bias retrospective MET studies(?)

Next phase: PGT-A with origins of aneuploidy



PGT-M

- Generic: any Mendelian disorder
- Multiple indications / multiple loci

PGT-A

- Parental origin (maternal vs paternal)
- Mechanistic origin (meiosis vs mitosis)

PGT-SR

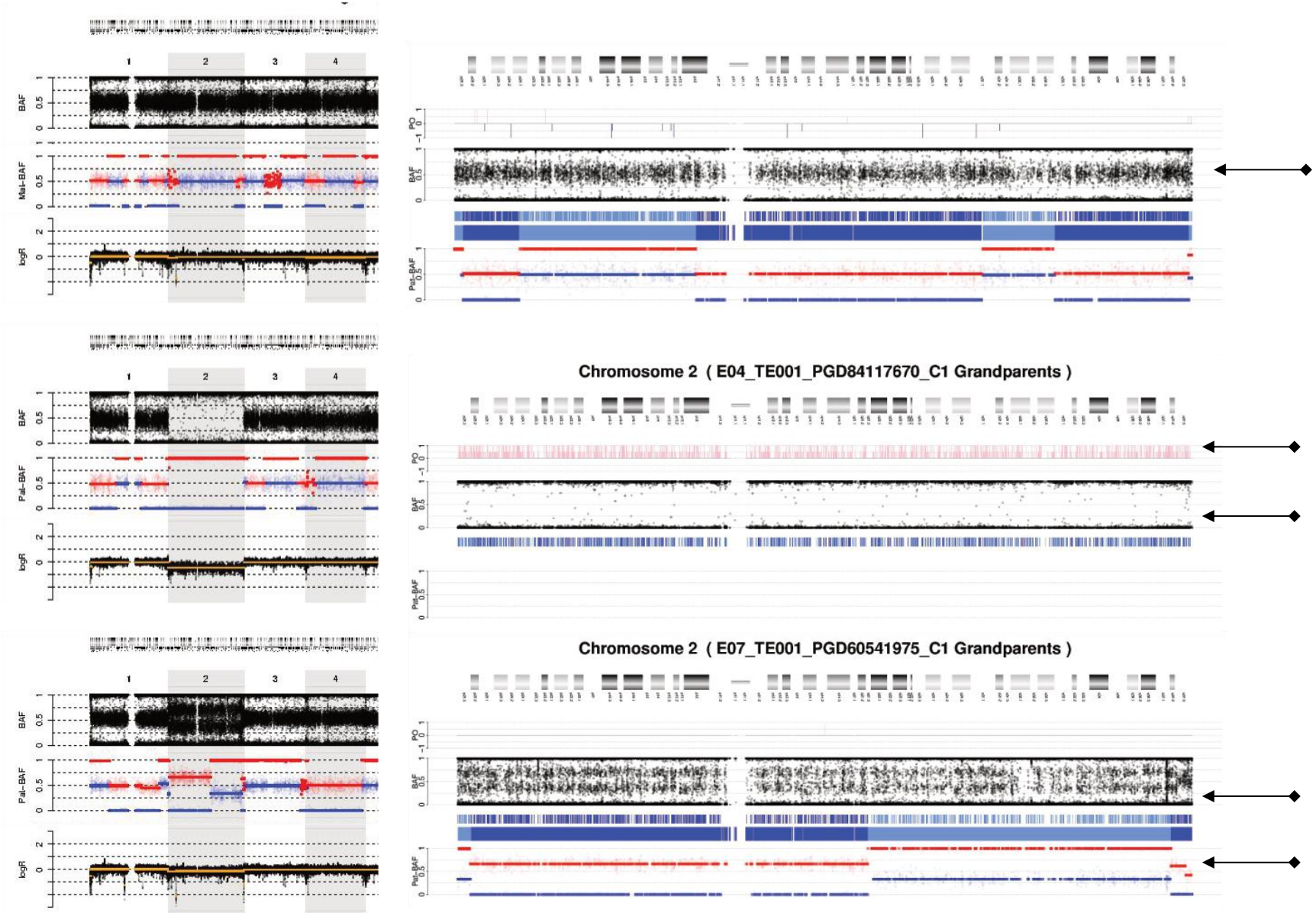
- identify balanced translocation carriers

Tsuiko, Fernandez-Gallardo et al 2020. Reprod (PMID: 33065545)

Handyside et al. 2010 J Med Gen; Zamani Esteki et al. 2015 Am J Hum Gen; Masset et al. 2019 Hum Reprod; Backenroth et al. 2019 Genet Med; Backenroth et al. 2023 Sci Rep; De Witte et al. 2022 Hum Reprod; Masset et al. 2023 Nucleic Acids Res; Janssen et al 2024 Nat Commun; Verdyck et al 2025 Genes; ….etc.

What are the advantages of using genome-wide haplotyping for PGT(-OA)?

Haplotyping-based monosomy detection

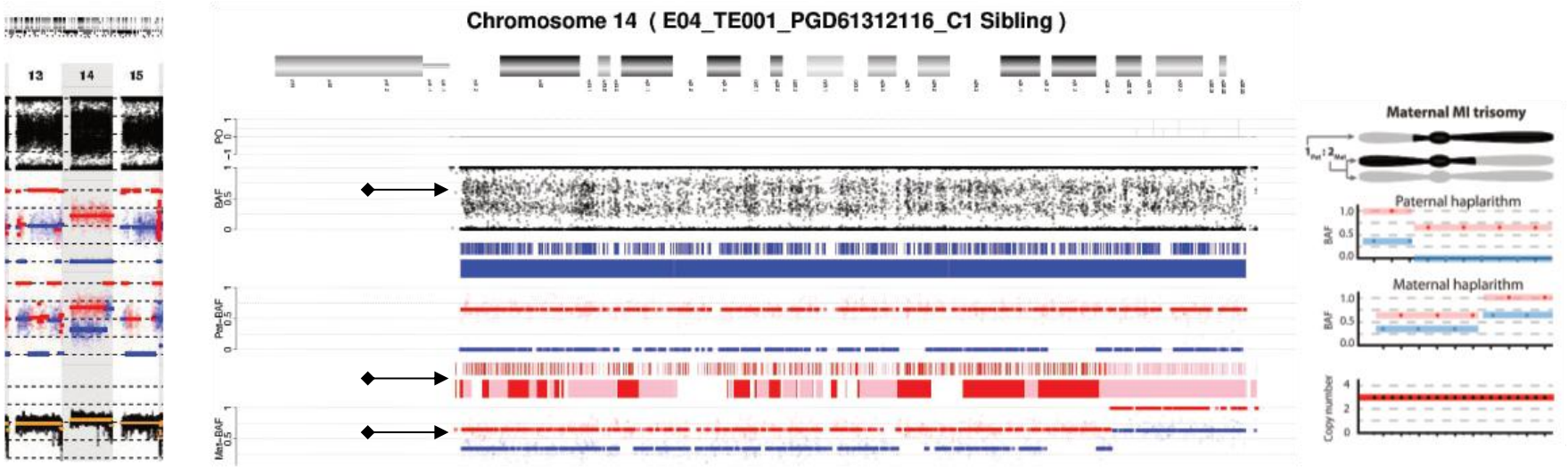


Normal euploid

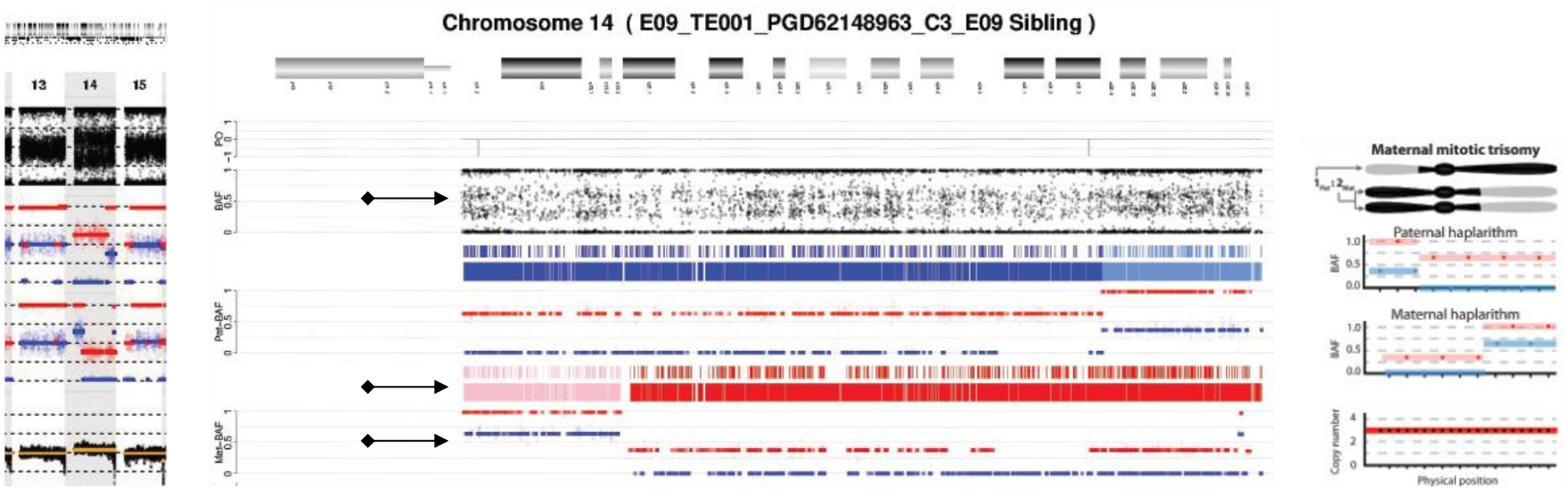
Monosomy 2

Mos monosomy 2 (ca. 50%)

Haplotyping-based trisomy detection

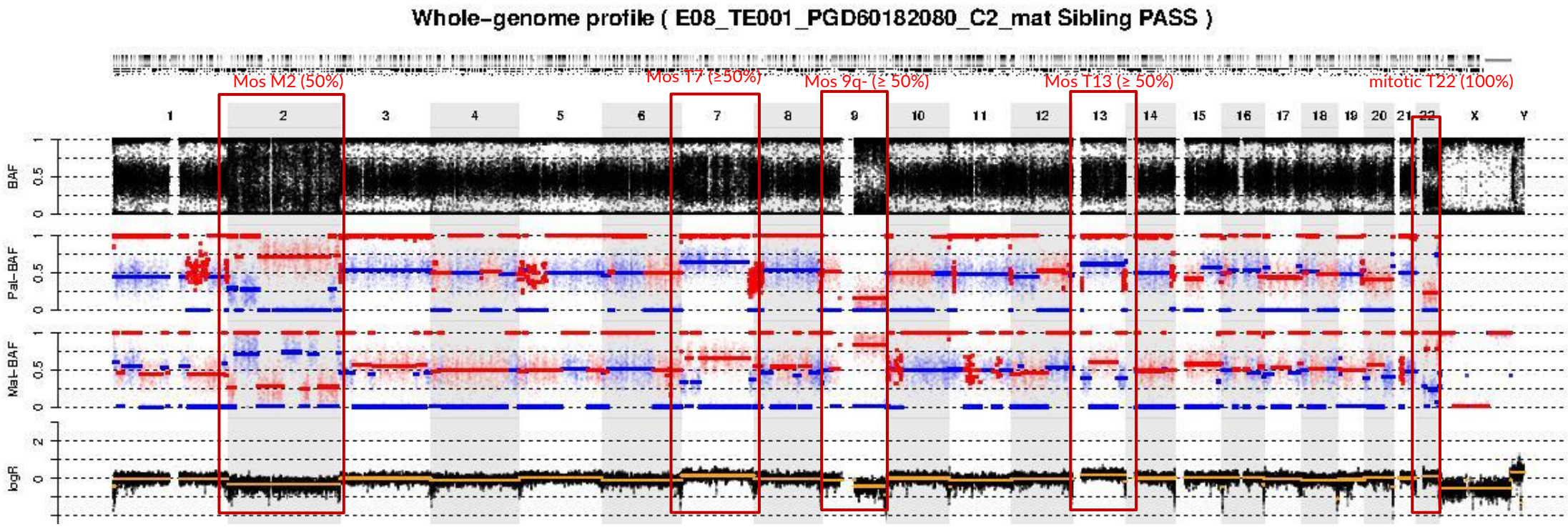


Meiotic trisomy



Mitotic trisomy

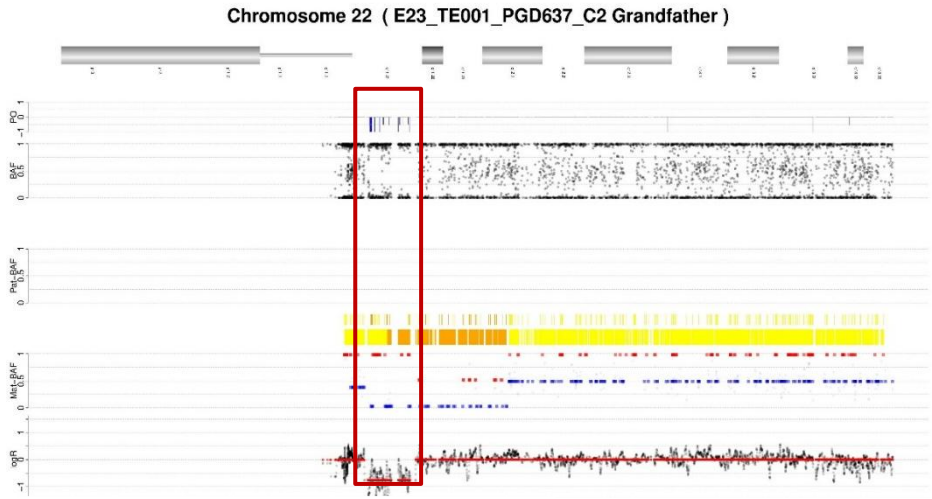
Genome-wide mosaicism detection



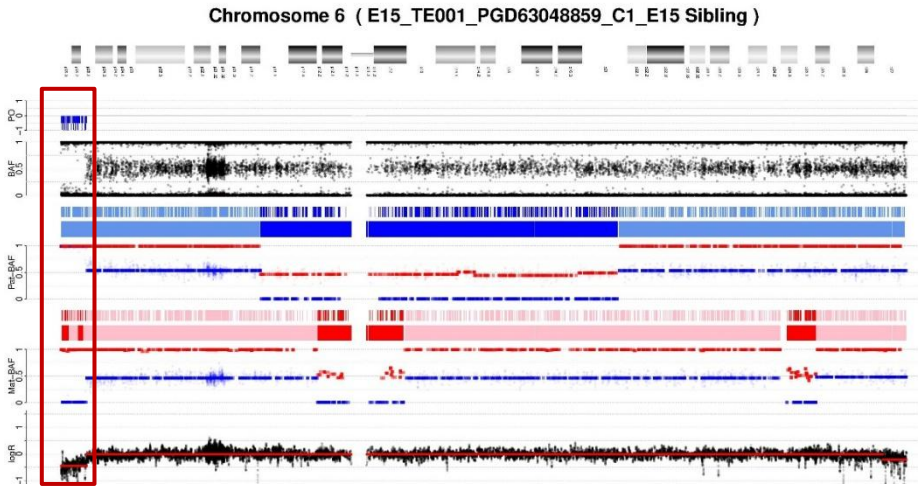
- BAF, haplotype and logR are DNA quality dependent → mosaicism calling threshold >30%
  - Technical – avoid overinterpretation
  - Biological – based on studies low-grade mosaicism can be neglectable
  - Patient-perspective – couples are still uncomfortable with the word 'mosaic'

- The better the quality – the more confident the call, detection limit can go below 30%
- The % is not important: meiosis vs mitosis is!

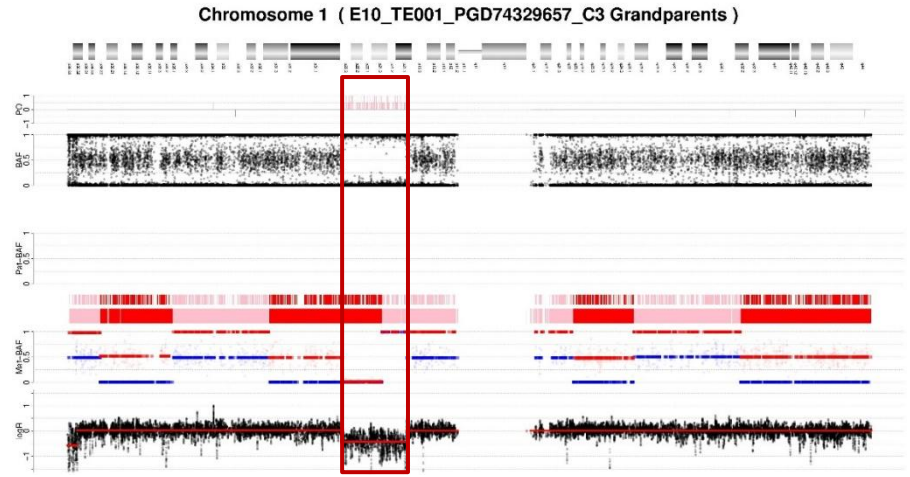
Improved detection limit for segmental aneuploidy (down to 1Mb)



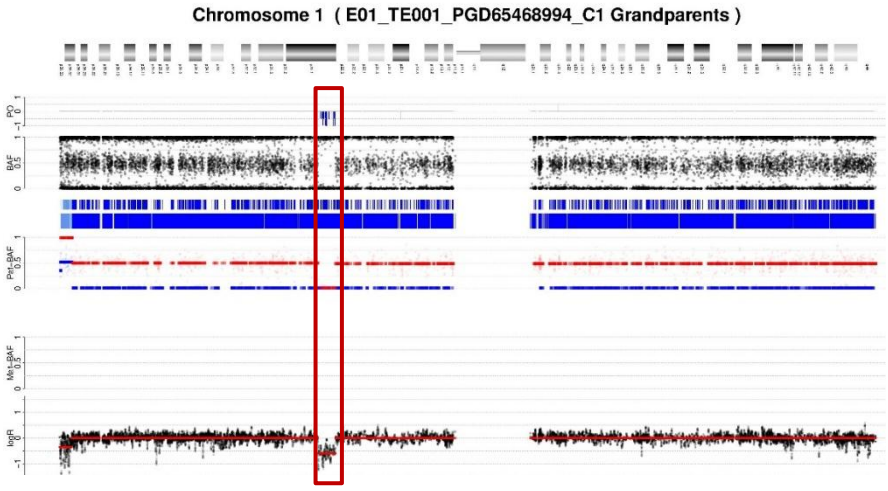
22q11.2 deletion syndrome



Axenfeld-Rieger syndrome



Critical overlap: 1p21.3 microdeletion syndrome

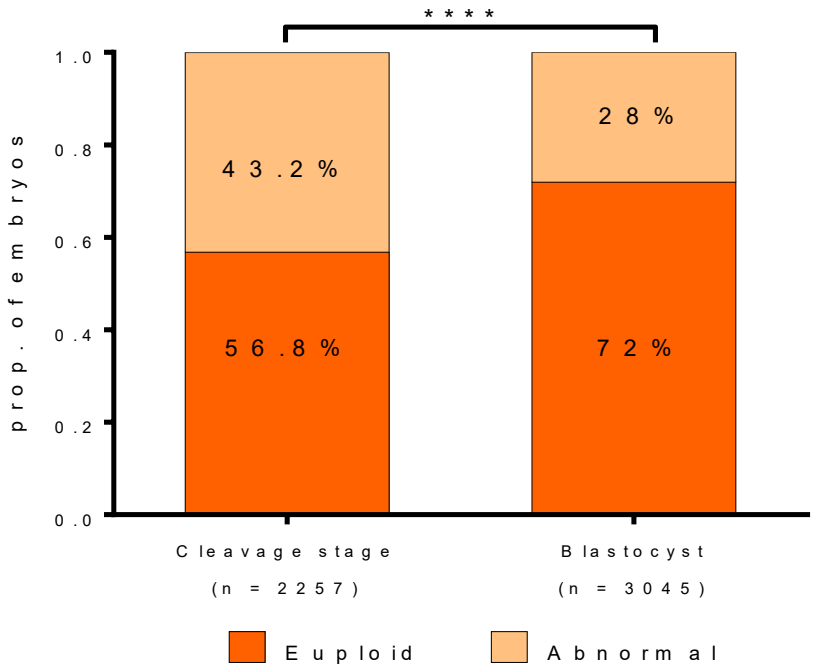


1p31.1 region: clinical significance?

Can we learn more about embryos using 'PGT-OA'?

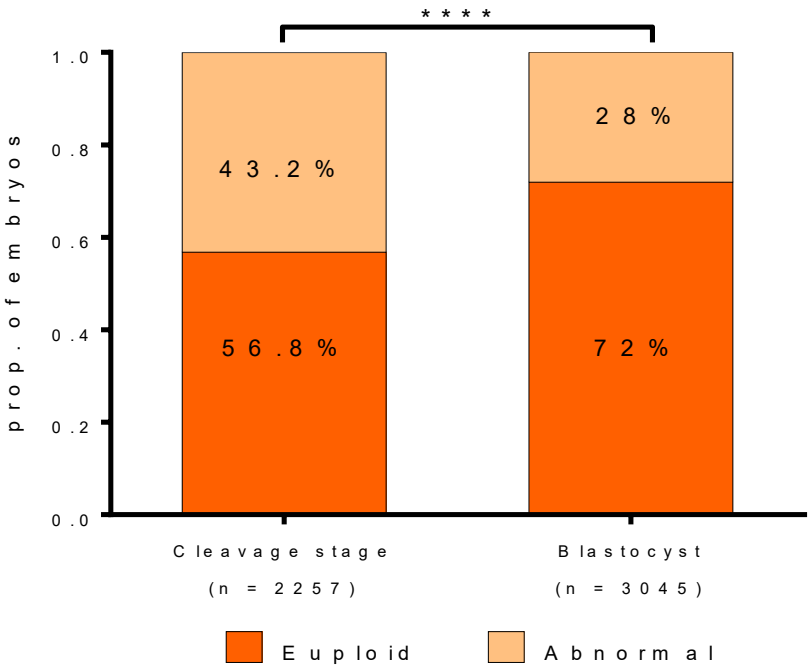
- siCHILD/haplarithmisis (Zamani Esteki et al. 2015 Am J Hum Gen)
  - Illumina HumanCyto-12 SNP array (~300,000 SNPs)
  - Since October 2024: Illumina Global Screening Array (~600,000 SNPs)
- 2021-2024: 485 PGT-M couples, 788 PGT cycles
  - PGT-SR cycles were excluded
  - Average maternal age: ±31.24 (min: 21, max: 43)
  - Average # of biopsies per cycle: ±3.9 (min: 1, max: 20)
- 3045 TE biopsies
  - already excl. embryos with 'no result' or low quality for mosaicism calling

Incidence of abnormalities per biopsy type

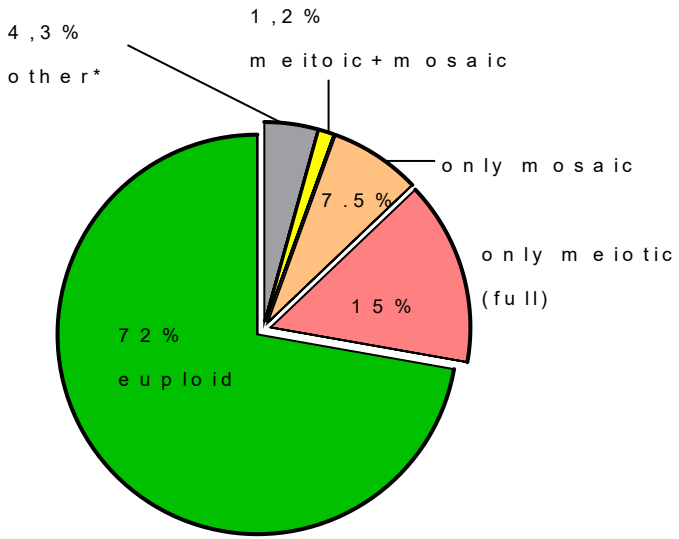


- (Complex) aneuploidy rate is reduced by blastocyst stage: selective pressure

Incidence of abnormalities per biopsy type



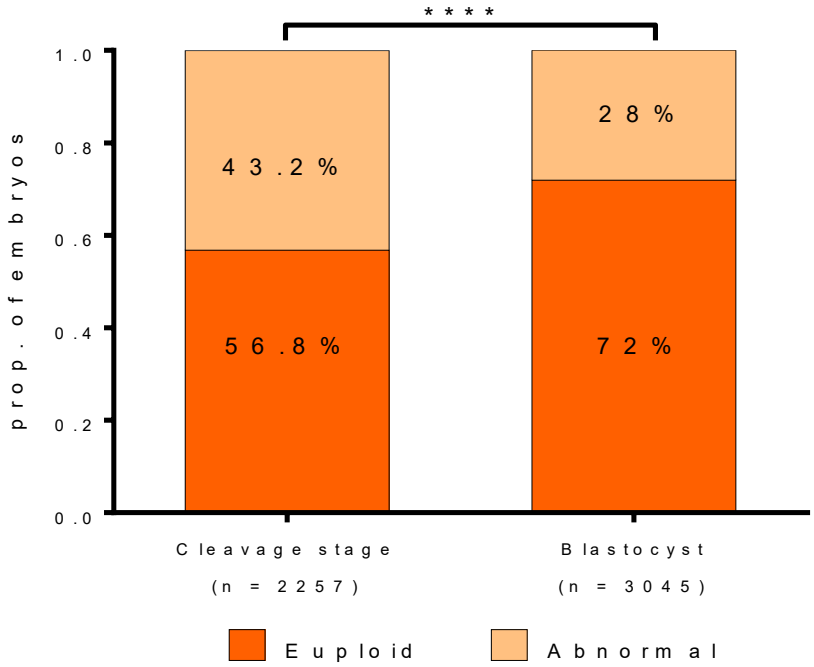
Embryo configuration based on TE biopsy

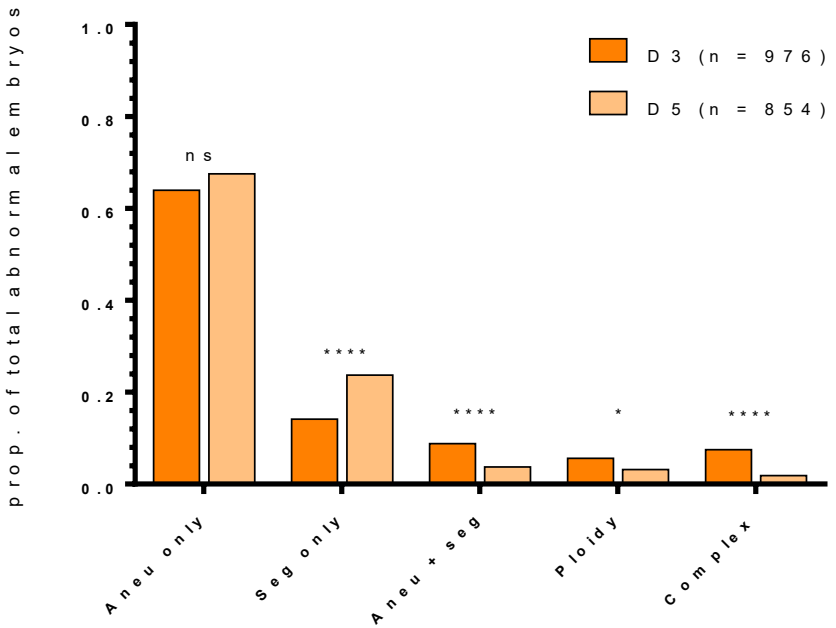


- (Complex) aneuploidy rate is reduced by blastocyst stage: selective pressure
- Mosaicism (>30% cutoff) detection rate in TE biopsy: ~9%

- *Other: genome-wide aberrations and segmental aneuploidy with undetermined origin

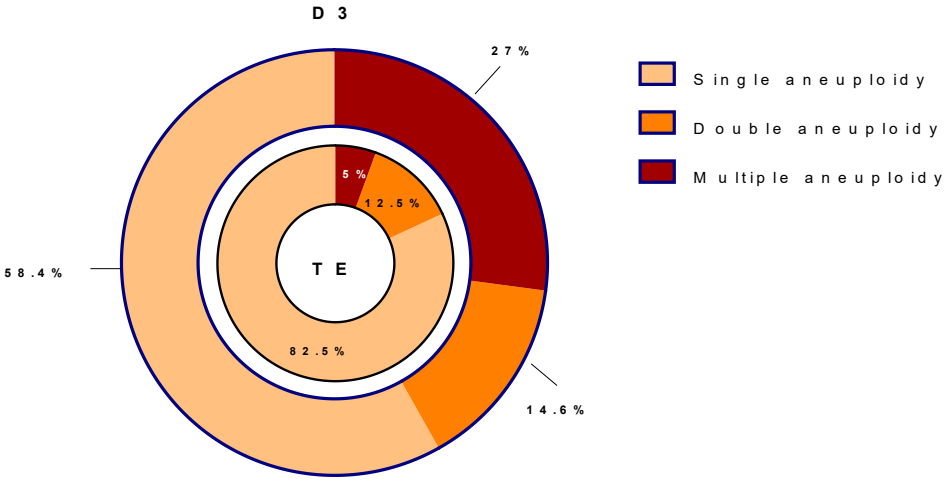
Incidence of abnormalities per biopsy type



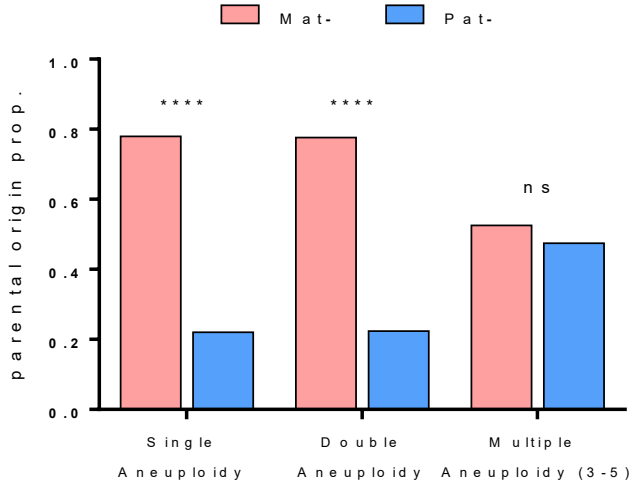


- (Complex) aneuploidy rate is reduced by blastocyst stage: selective pressure
- Mosaicism (>30% cutoff) detection rate in TE biopsy: ~9%
- Ploidy aberrations: 2.4% of all D3 biopsies and 0.8% of all TE biopsies
- Complex profiles look very different in D3 and TE biopsies

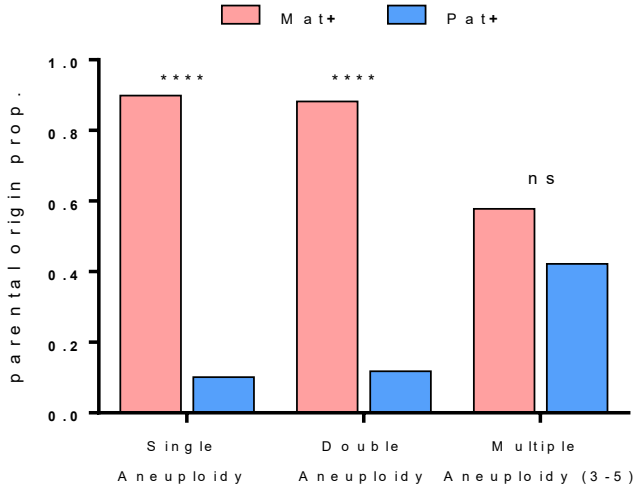
Aneuploidy in blastocysts is mainly due to maternal meiotic errors



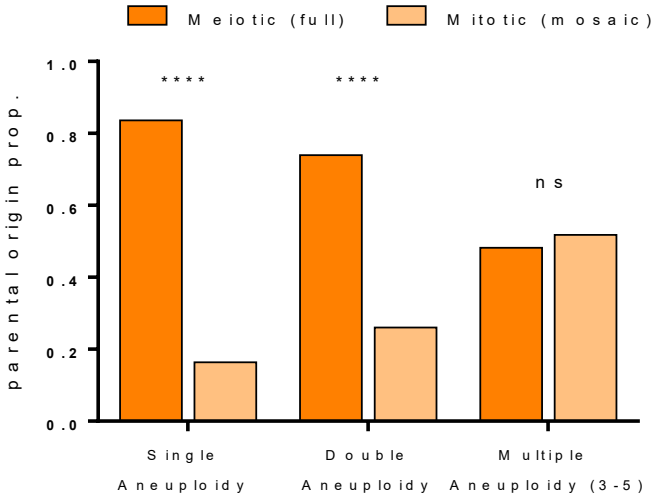
- The bias towards maternal chromosomes in single aneuploidy is due to meiotic errors in the oocyte
- Post-zygotic aneuploidy does not discriminate between maternal and paternal chromosomes
- This association is less strong than in D3 biopsies



Whole chr loss

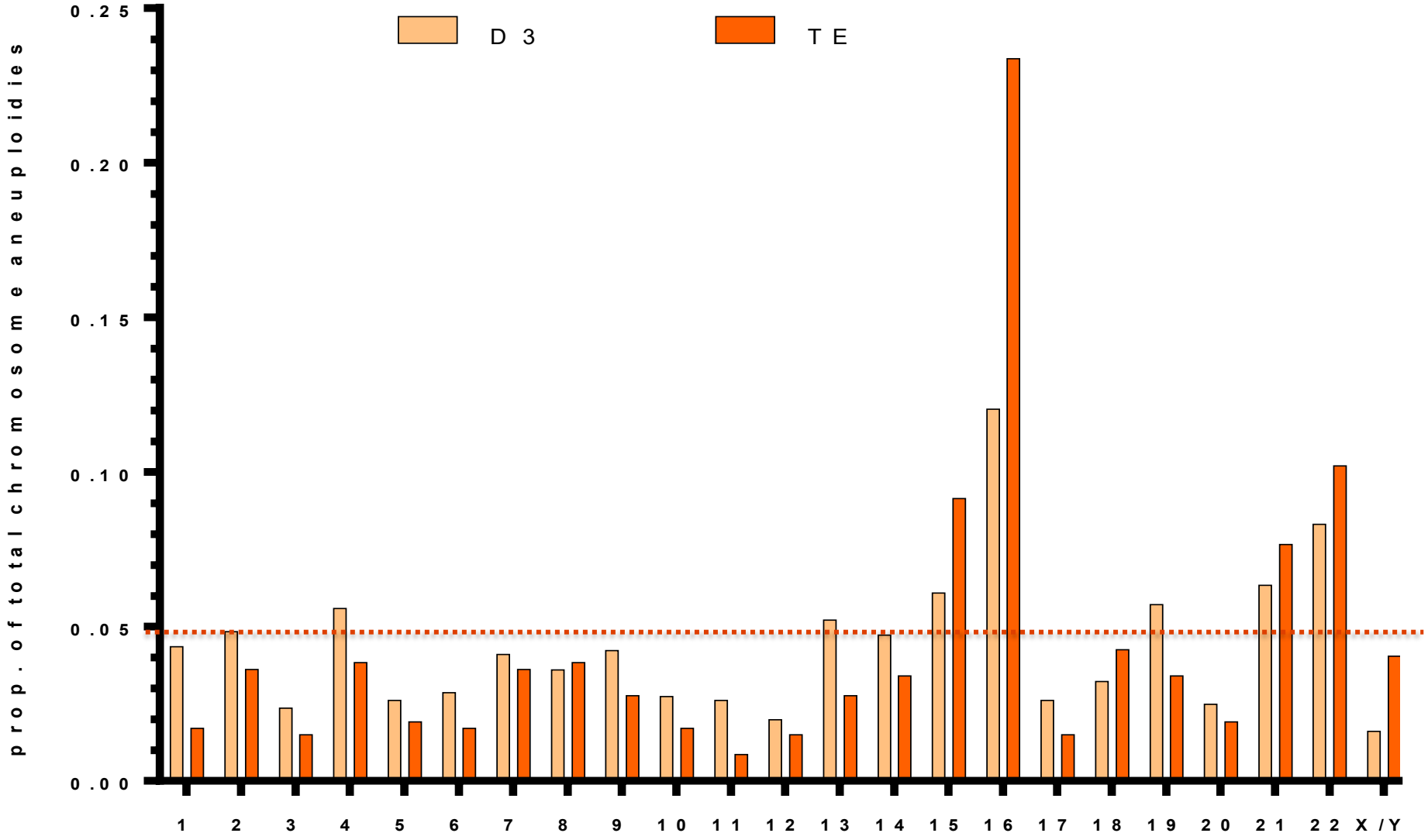


Whole chr gain

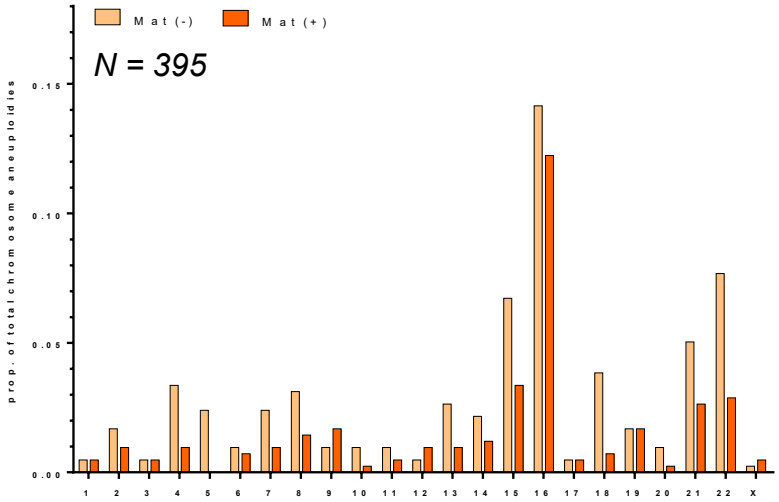


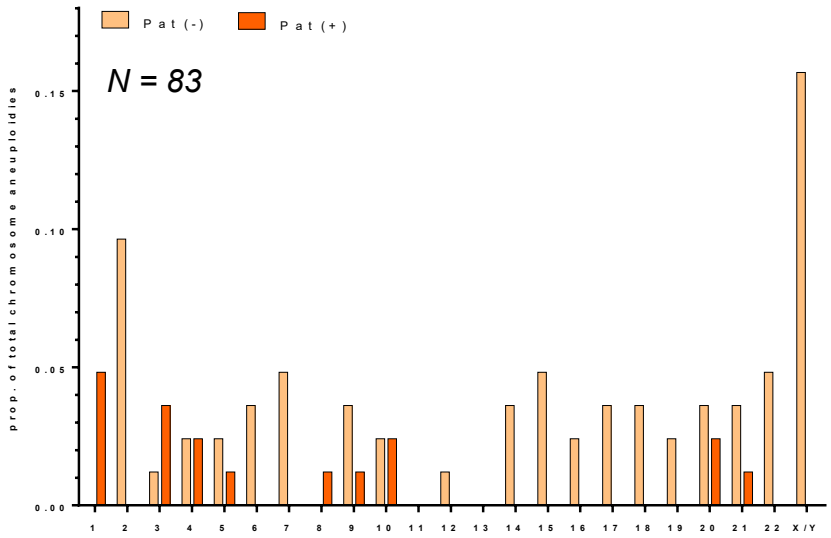
Mechanism of aneuploidy

Frequency of single aneuploidy per chromosome and biopsy type



Parental and mechanistic origin of single aneuploidy





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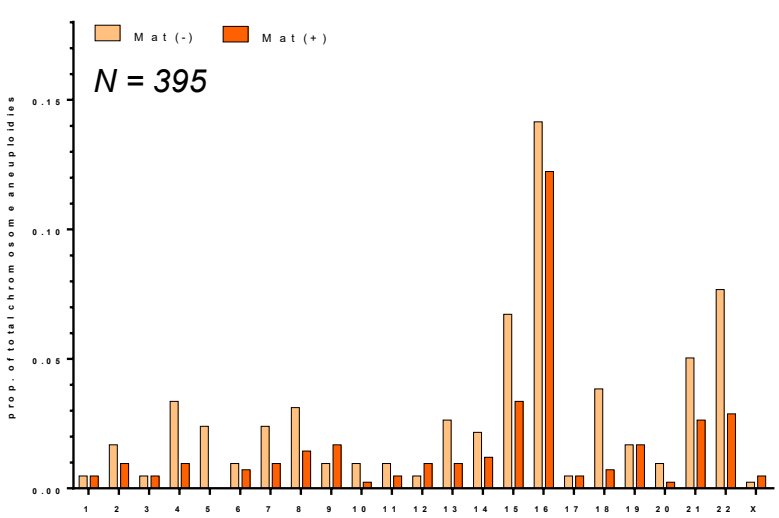
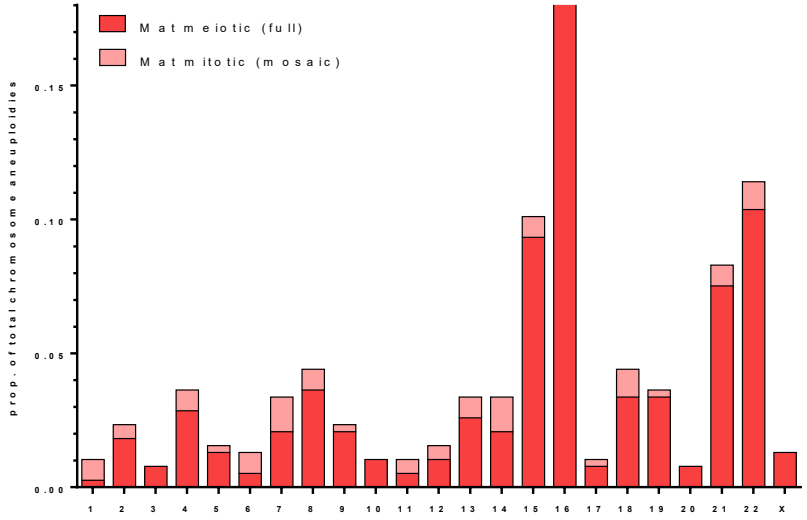
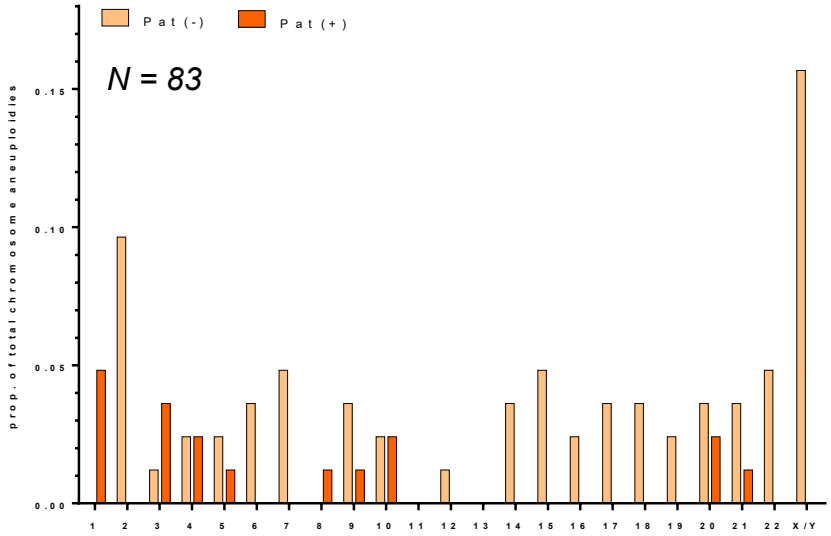
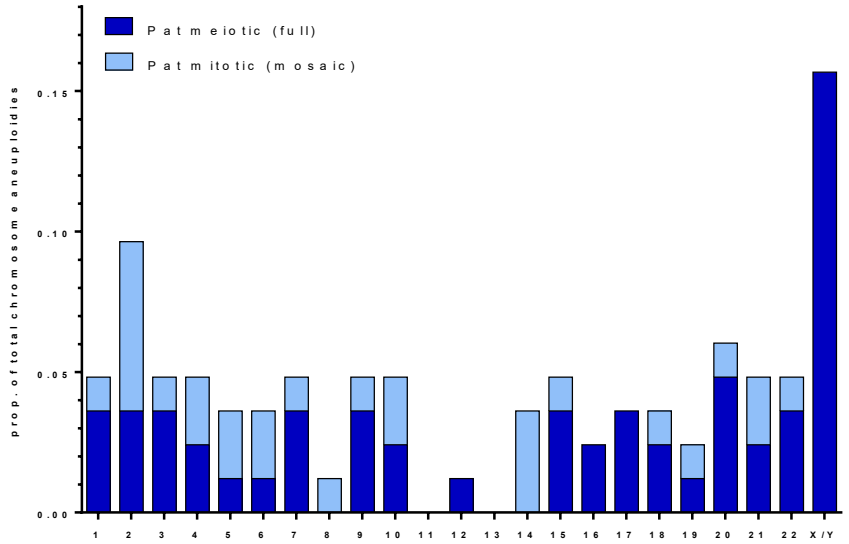
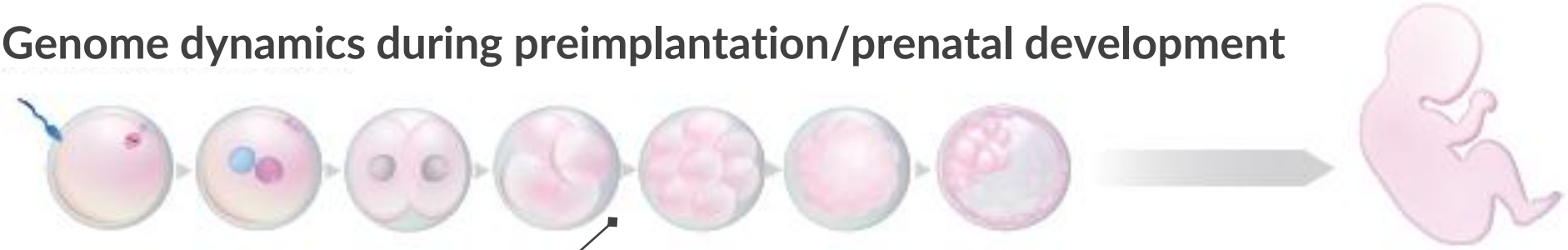




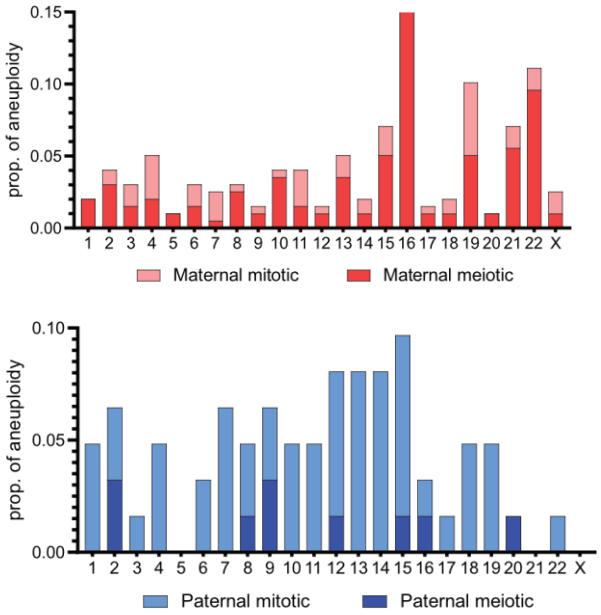




Genome dynamics during preimplantation/prenatal development



Cleavage-stage embryo



Tsuiko et al. 2021 (PMID: 34620870)

Genome dynamics during preimplantation/prenatal development

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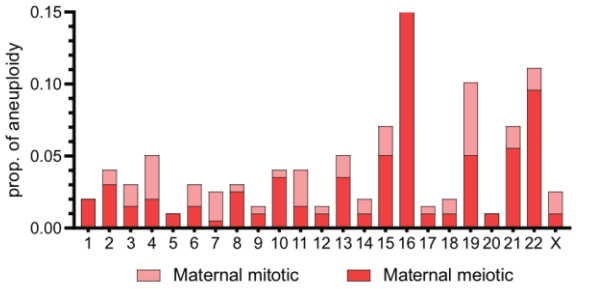
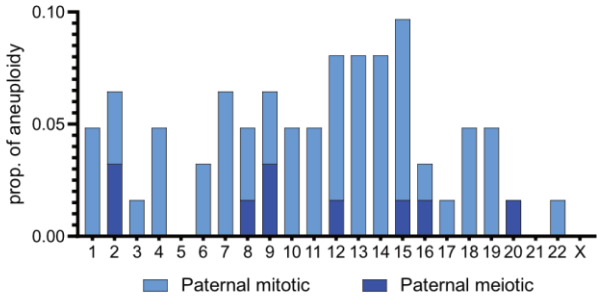
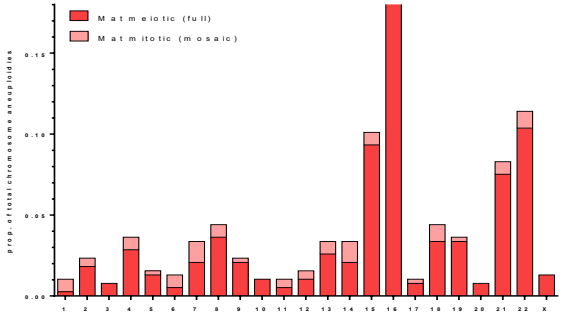
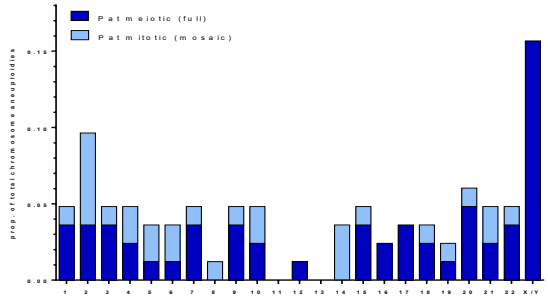




Blastocyst





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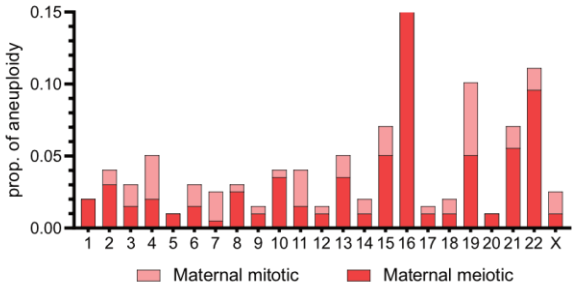

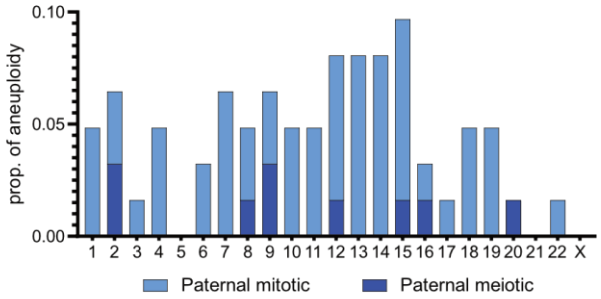

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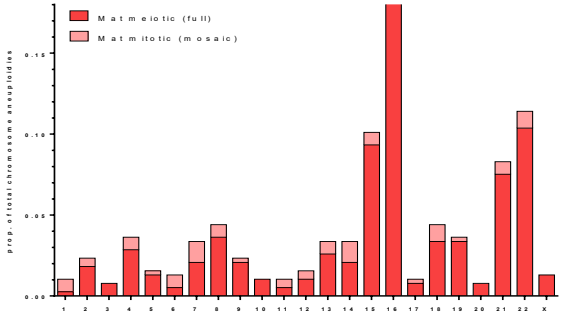
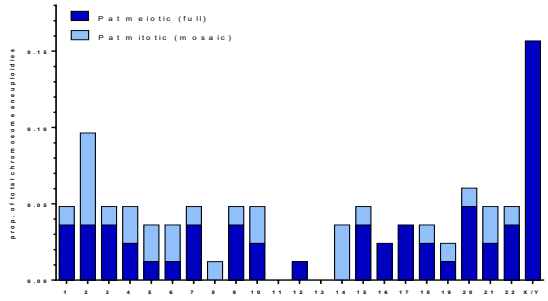
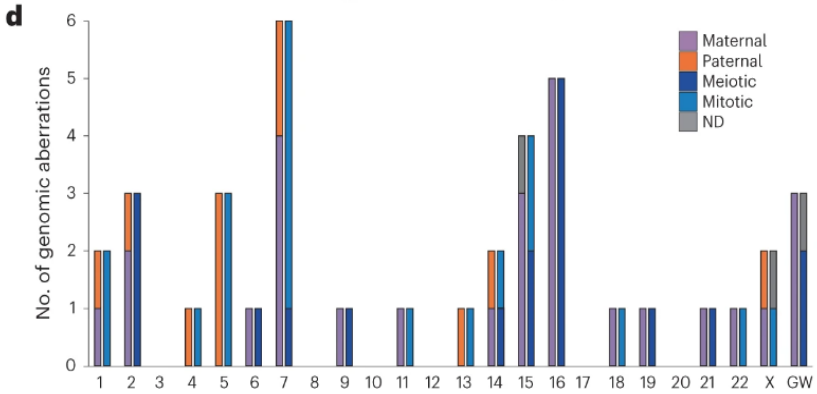




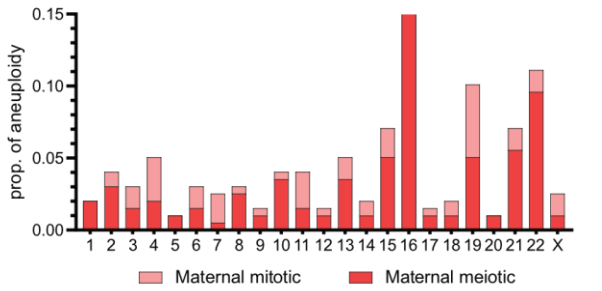
Early miscarriage

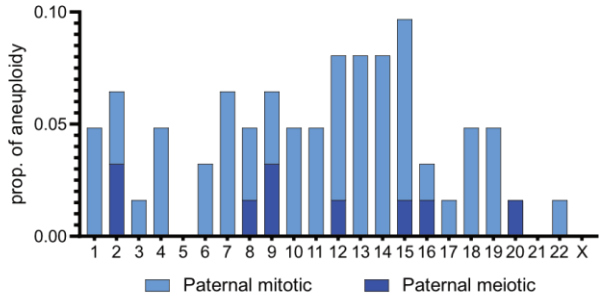


Essers et al. 2023 (PMID: 37996709)

Genome dynamics during preimplantation/prenatal development

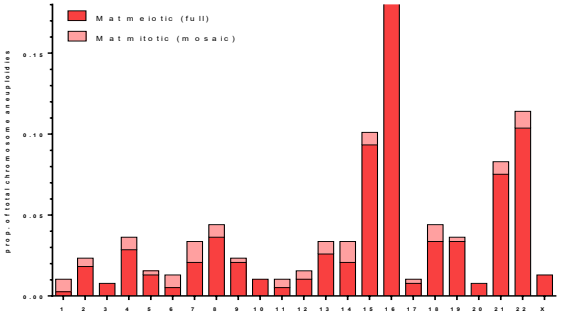
Cleavage-stage embryo

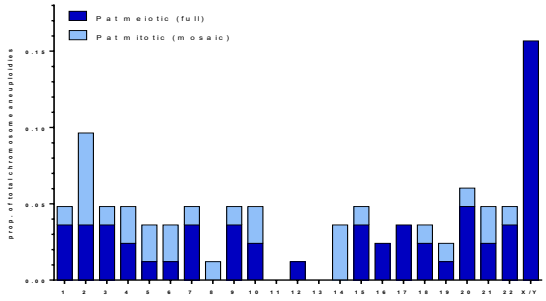




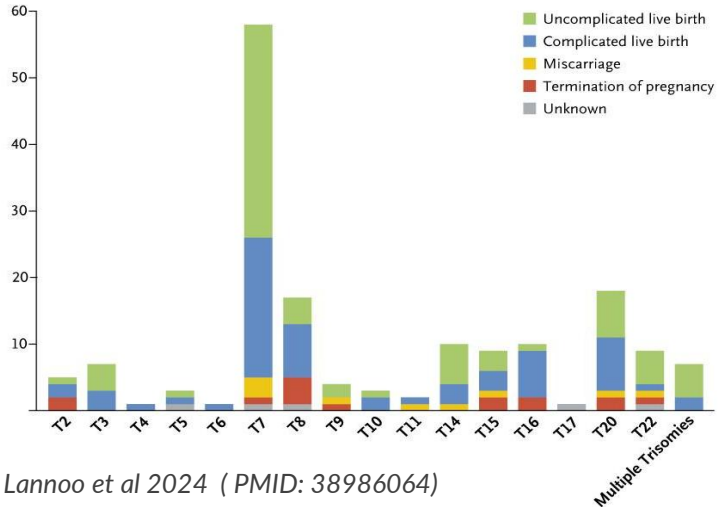
Tsuiko et al. 2021 (PMID: 34620870)

Blastocyst



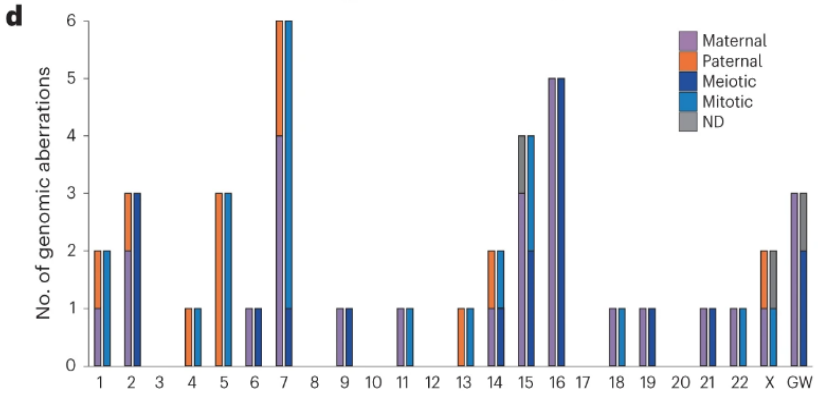


NIPT (RAAs)



Lannoo et al 2024 (PMID: 38986064)

Early miscarriage



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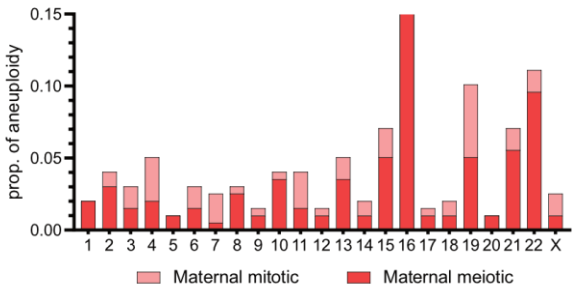

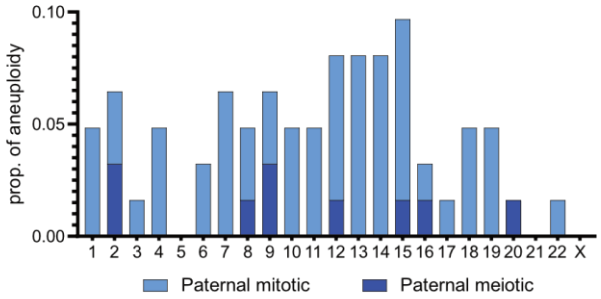

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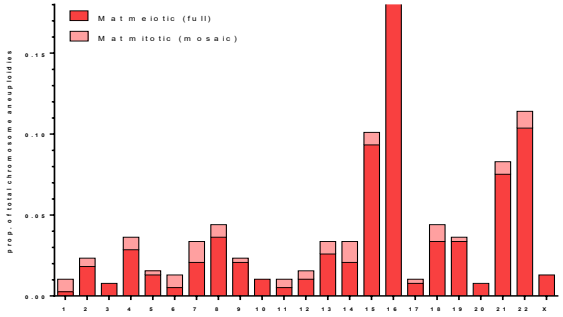

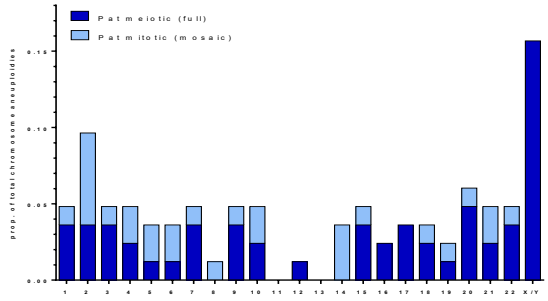

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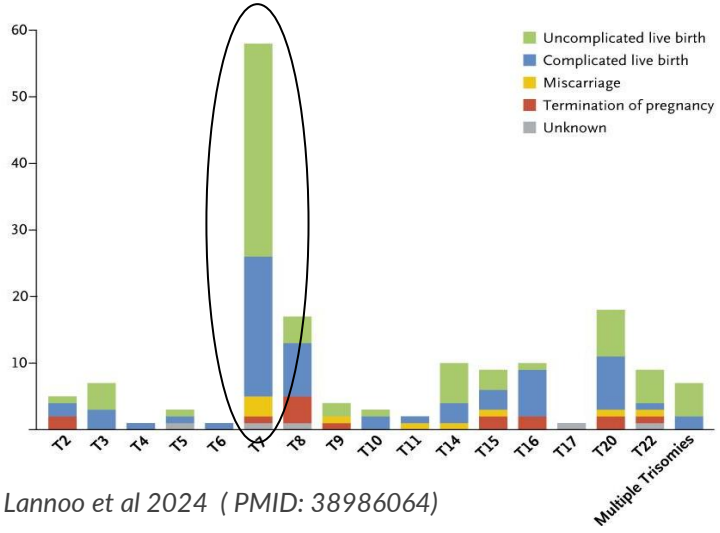

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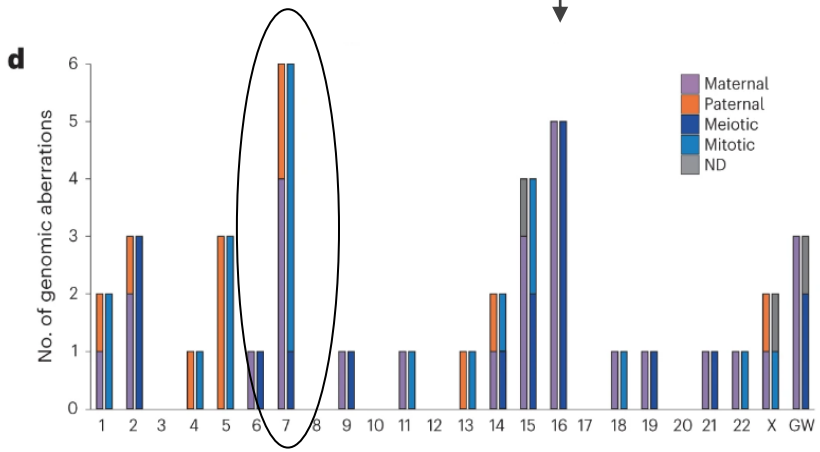
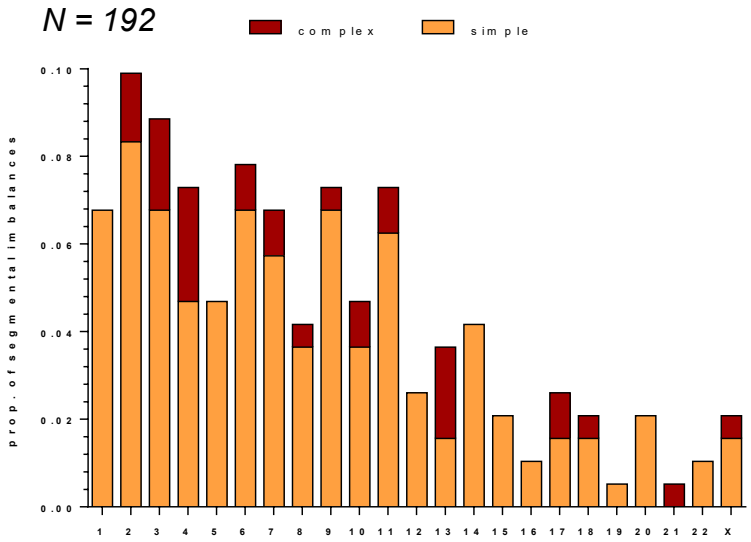


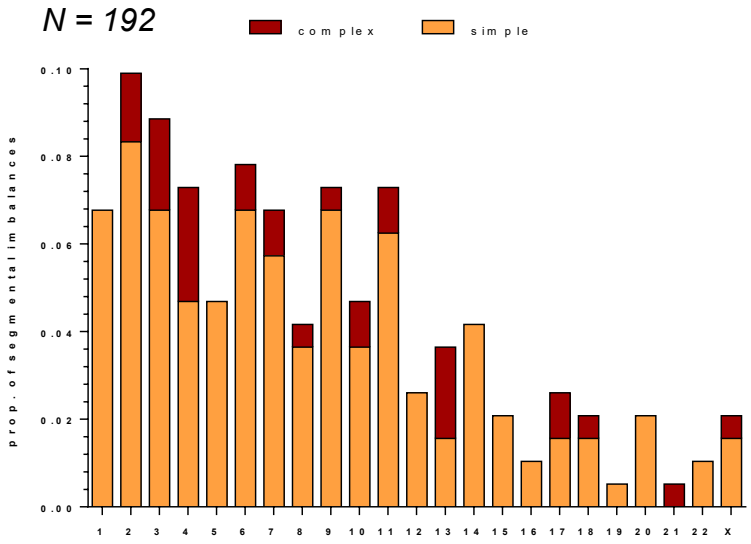
Essers et al. 2023 (PMID: 37996709)

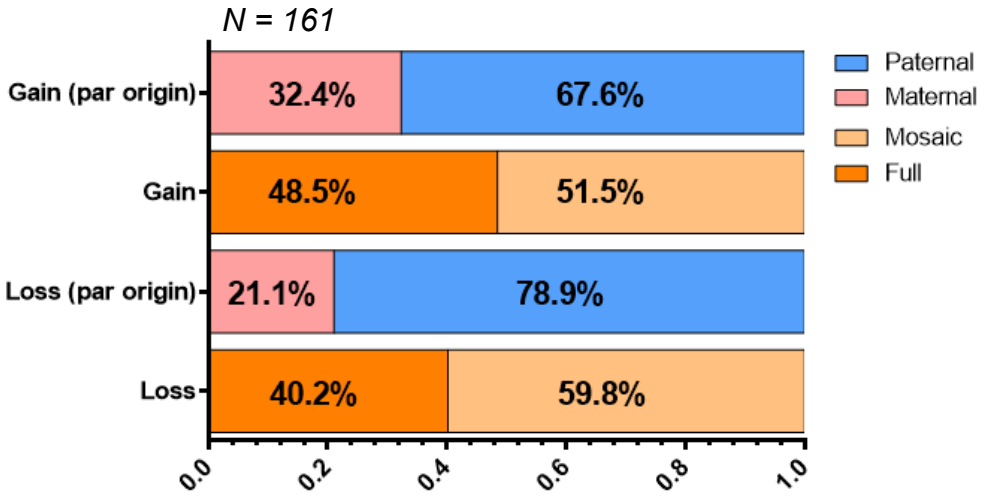
Single segmental imbalances in trophectoderm



- Larger chromosomes have a higher rate of segmental imbalances
- Complex segmental aneuploidy is detected in >1% of all TE biopsies

Single segmental imbalances in trophectoderm





- Larger chromosomes have a higher rate of segmental imbalances
- Complex segmental aneuploidy is detected in >1% of all TE biopsies

- Unlike wh chr aneuploidy, segmental imbalances are mainly of paternal origin
- Trophectoderm biopsy data is echoing findings in cleavage-stage biopsies
  - *Kubicek et al, 2019 (RBMO) – TE biopsies*
  - *Handyside et al, 2024 (biorxiv) – TE biopsies*
- The rate of meiotic segmental imbalances in the sperm is 0.4% *(Bell et al. 2020 Nature)*
- The vast majority of segmental aneuploidy is mitotic in origin? → challenge to the clinic?

Take home messages

- Haplotyping-based PGT can uncover a wide spectrum of genomic aberrations
  - PGT-OA leads to improved embryo selection → deselection of unviable embryos
  - Single aneuploidy mainly arises due to (chromosome-specific) meiotic errors in the oocyte
  - Segmental imbalances are more likely to affect paternal chromosomes
  - (post-zygotic) aneuploidy diminishes over time

- Uncertainty in mosaicism management will continue to pose challenges
  - There is a “black box” between preimplantation and prenatal development
  - Internationally no consensus on chromosomal mosaicism management (i.e. detection limits, reporting, transfer policy)
  - 1.2% of mosaic findings detected during PGT can be traced back in prenatal results → risk evaluation should be done per chromosome
  - What about pregnancy complications?

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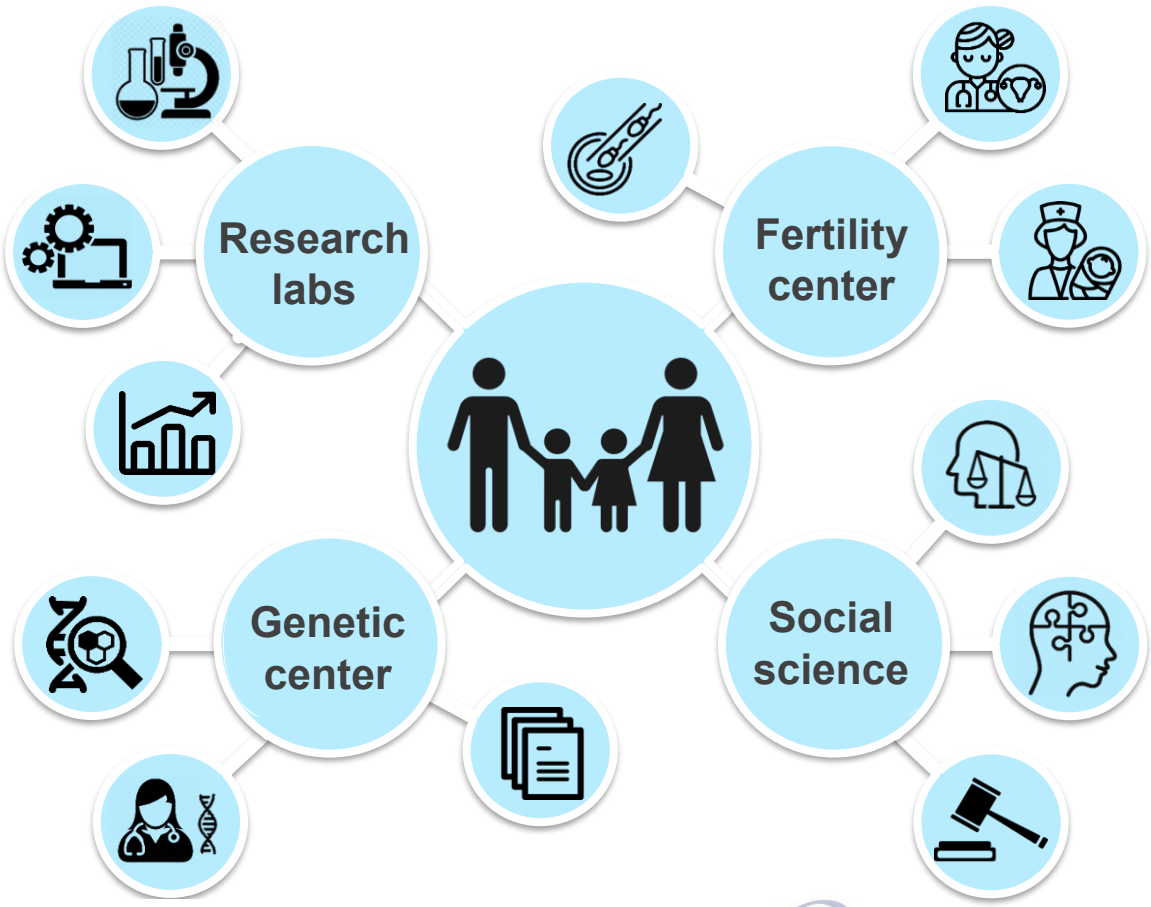

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