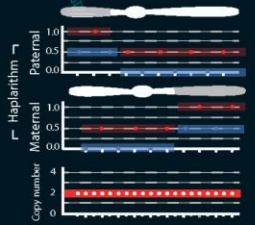
All-in-one PGT



Masoud Zamani Esteki

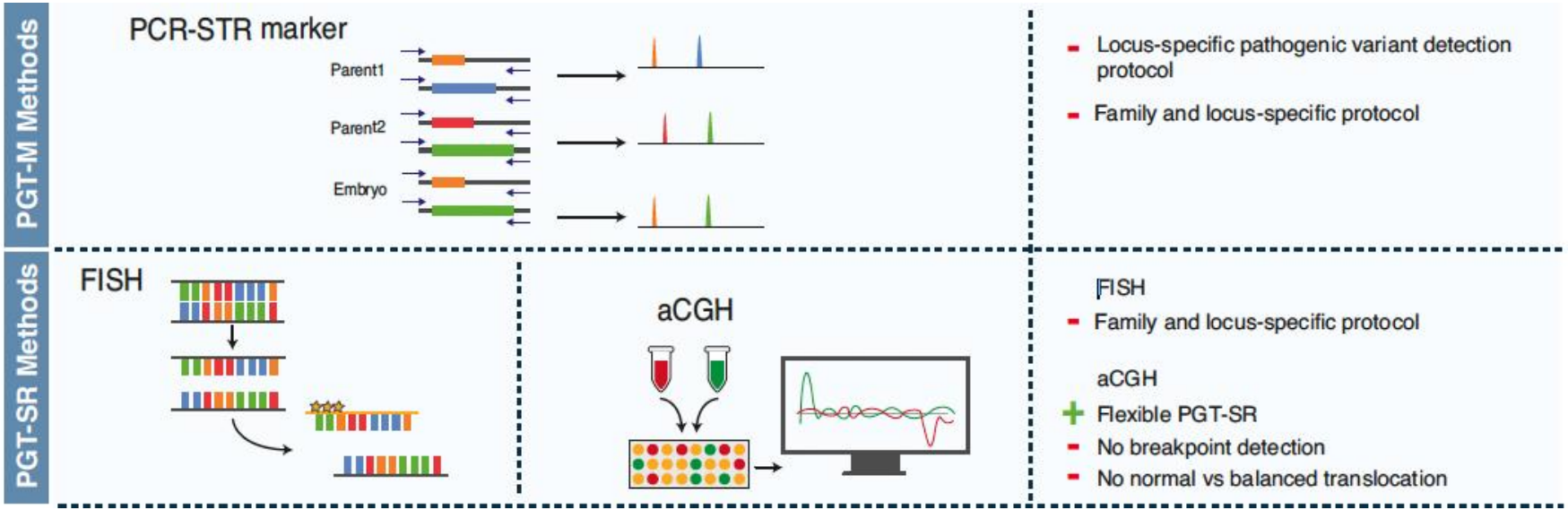
Cellular Genomic Medicine
DEPARTMENT OF CLINICAL GENETICS
MAASTRICHT UMC+

www.zamanilab.org
masoud.zamaniesteki@mumc.nl

Outline

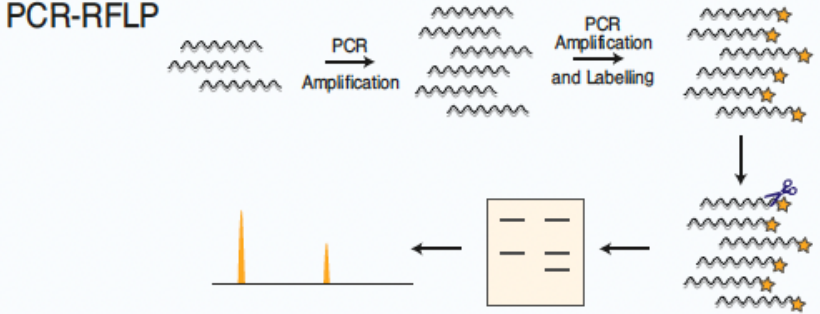
- Traditional PGT
- Haplotyping-based methods **development** & **translation**
  - Generic PGT via haplarithmisis, prallel haplotyping & copy-number typing
  - Mechanistic origin of aberrations, i.e. Meiotic vs. Mitotic
  - Lineage origin of aberrations, i.e. ICM vs. TE
- Prevalance of aberrations in **successful pregnancy**
- Prevalance of aberrations in **pregnancy loss**
- **Clinical follow up** of embryos following PGT-M

Traditional PGT – nuclear DNA

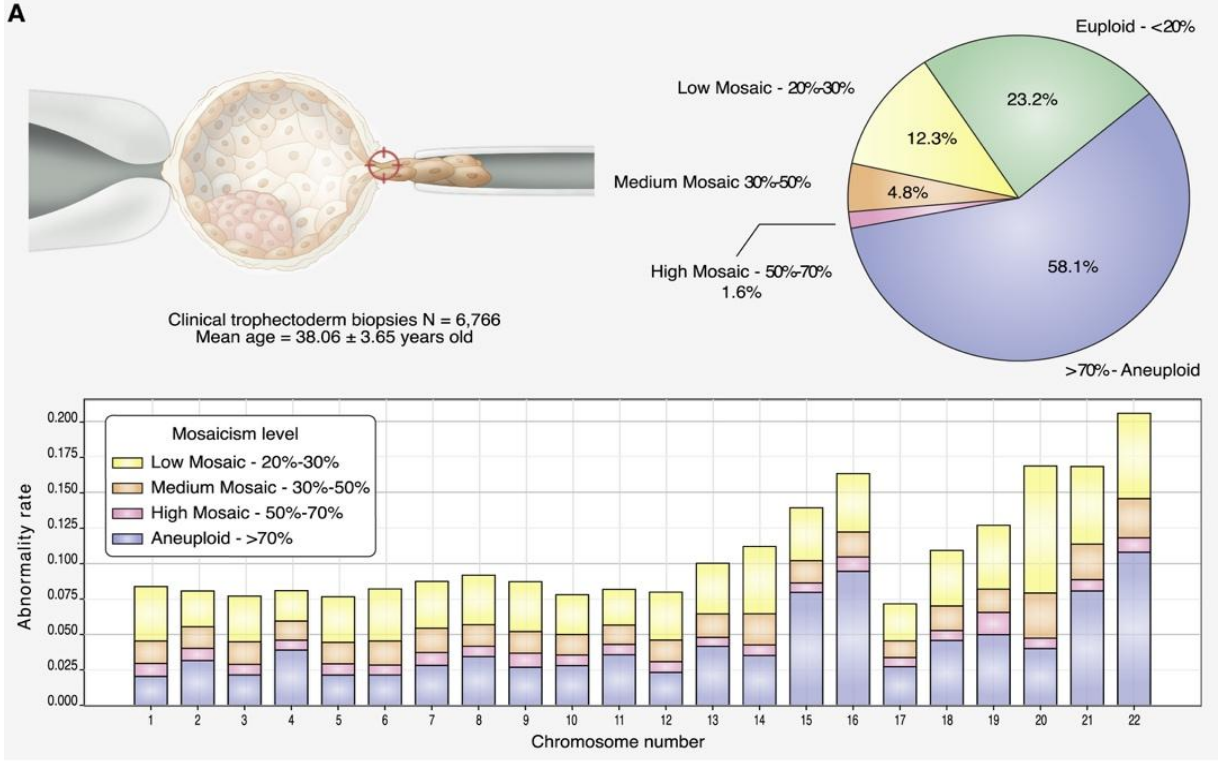


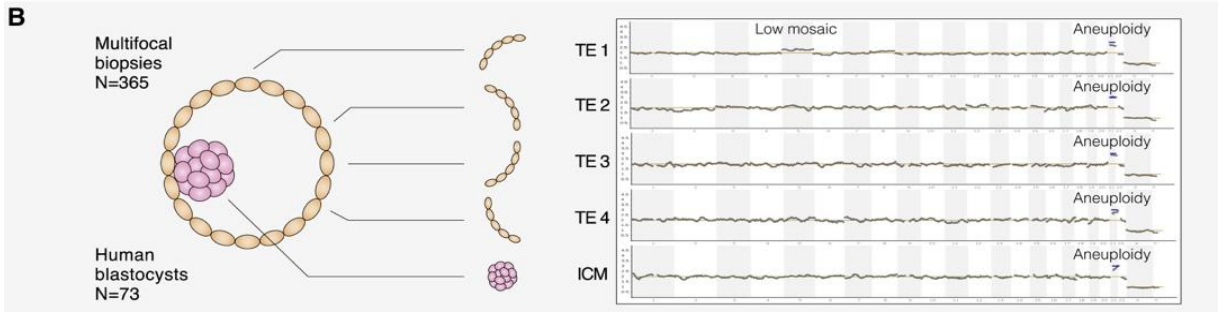
Traditional PGT – mitochondrial DNA

PGT-MT Methods



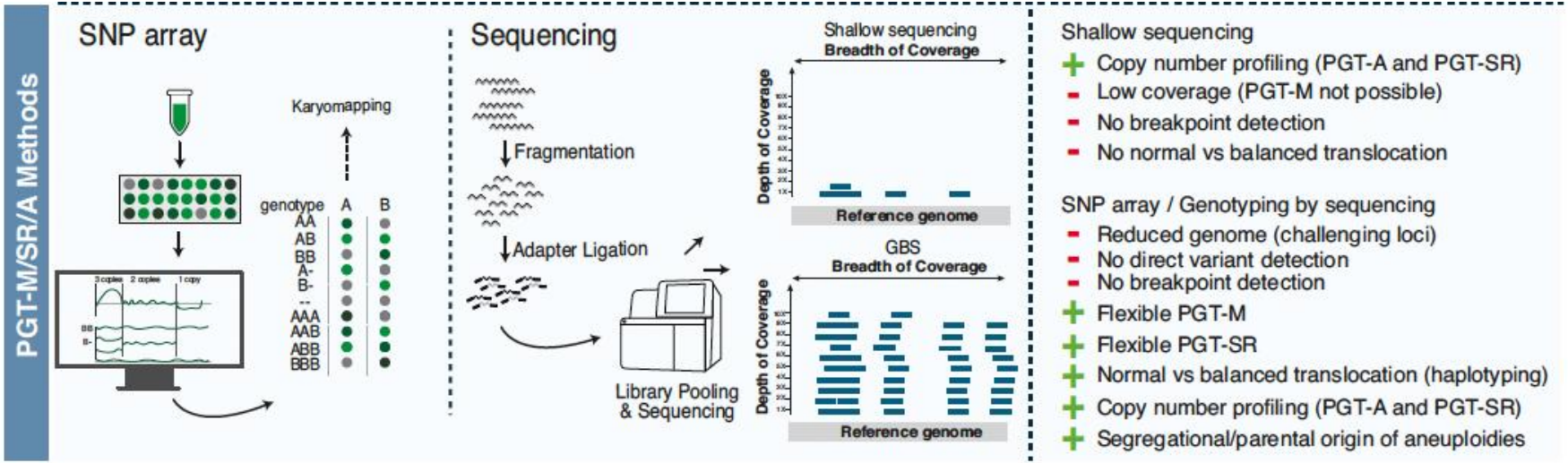
- Family and locus-specific protocol
- Protocol duration of 2 days
- Day-3 biopsy
- Calibration curve needed for signal quantification (indirect method)





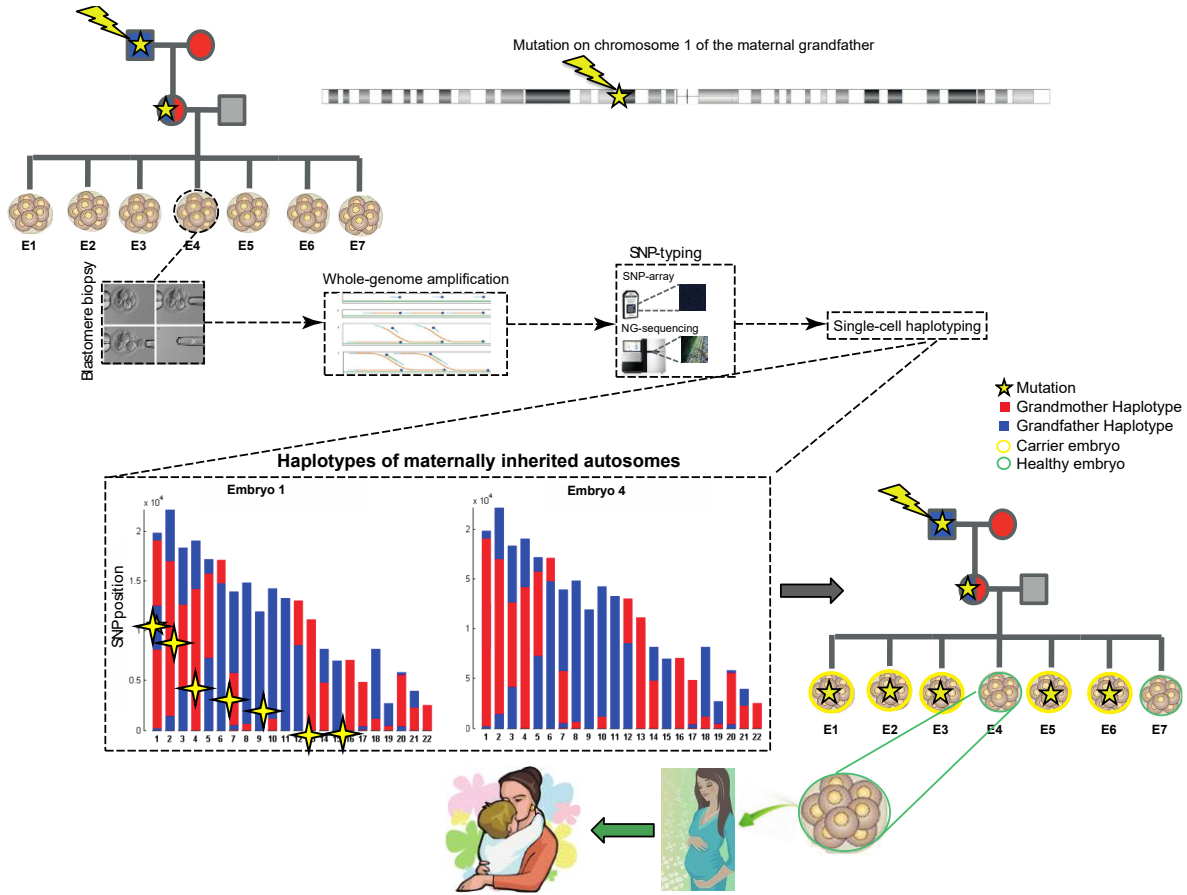
Capalbo et al. 2021 *AJHG*

SNP + copy-number profiling



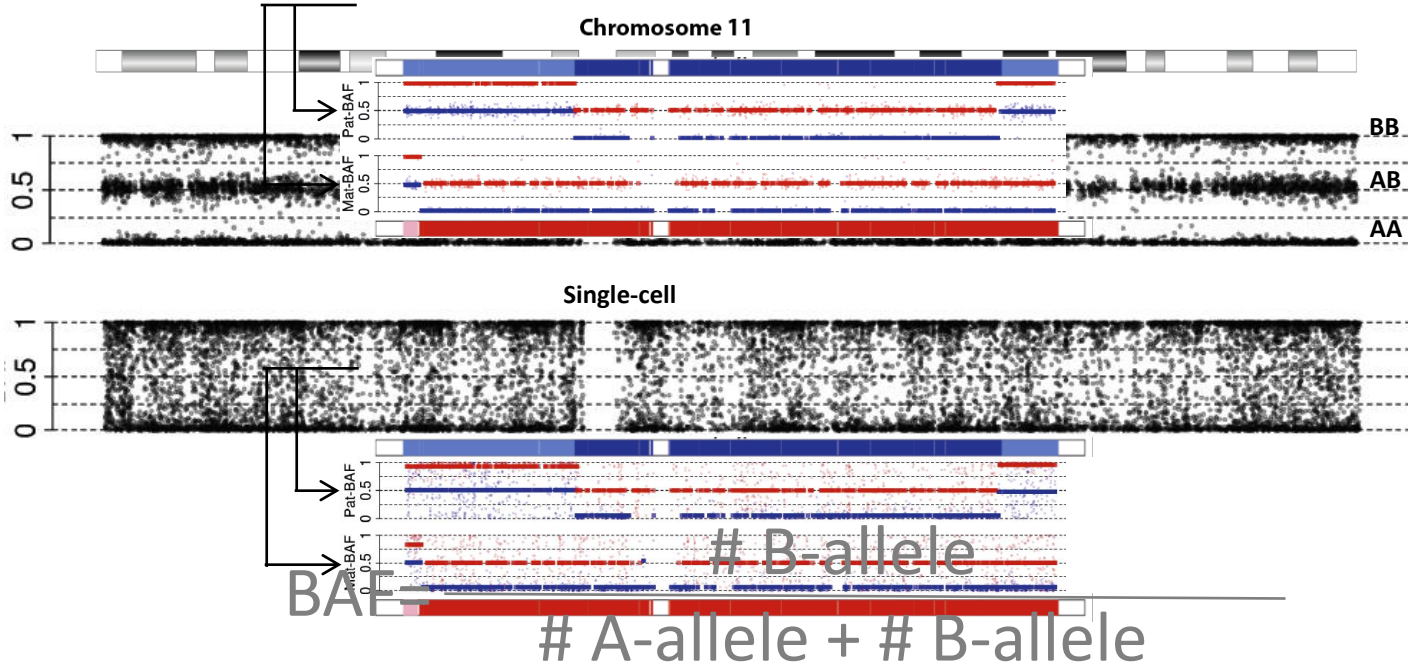
Karyomapping (Handyside et al. 2009) **Haplotyping**
Haplarithmisis (Zamani Esteki et al. 2015) **Haplotyping + copy-number typing**

Towards all-in-one PGT



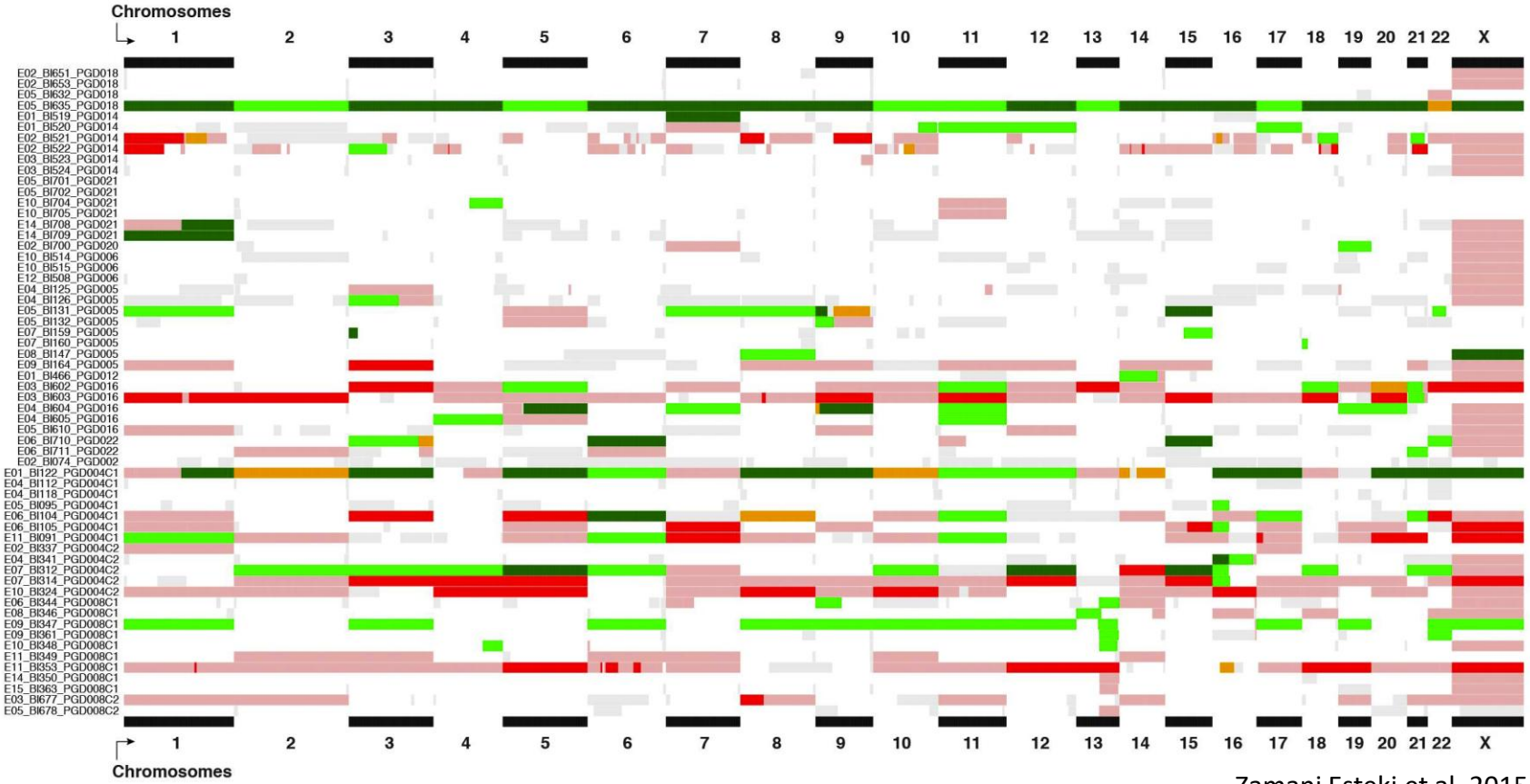
Single-cell SNP B-allele fractions

(Haplotype numbering)



Zamani Esteki et al. 2015, *AJHG*

Chromosome anomalies in >80% of the IVF embryos



Zamani Esteki et al. 2015, AJHG

All forms of PGT via massively parallel sequencing

Human Reproduction, Vol.34, No.8, pp. 1608–1619, 2019
Advance Access Publication on July 26, 2019 doi:10.1093/humrep/dez106

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Multi-centre evaluation of a comprehensive preimplantation genetic test through haplotyping-by-sequencing

Heleen Masset[1,†], Masoud Zamani Esteki[1,2,3,†], Eftychia Dimitriadou[4], Jos Dreesen[2,3], Sophie Debrock[5], Josien Derhaag[3,6], Kasper Derks[2], Aspasia Destouni[1,7,8], Marion Drüsedau[2], Jeroen Meekels[2], Cindy Melotte[4], Karen Peeraer[5], Olga Tšuiko[1], Chris van Uum[2], Joke Allemeersch[9], Benoit Devogelaere[9], Katrien Omer François[9], Scott Happe[10], Dennis Lorson[9], Rebecca Louise Richards[9,11], Jessie Theuns[11], Han Brunner[2,3], Christine de Die-Smulders[2,3], Thierry Voet[12], Aimée Paulussen[2,3], Edith Coonen[2,3], and Joris Robert Vermeesch[1,4,*]

Published online 25 February 2022

Nucleic Acids Research, 2022, Vol. 50, No. 11 ***e63***
https://doi.org/10.1093/nar/gkac134

Single-cell genome-wide concurrent haplotyping and copy-number profiling through genotyping-by-sequencing

Heleen Masset[1], Jia Ding[2], Eftychia Dimitriadou[2], Amin Ardeshirdavani[8], Sophie Debrock[3], Olga Tšuiko[1,2], Katrien Smits[4], Karen Peeraer[3], Yves Moreau[8], Thierry Voet[5], Masoud Zamani Esteki[6,7] and Joris R. Vermeesch[1,2,*]

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

GENType: all-in-one preimplantation genetic testing by pedigree haplotyping and copy number profiling suitable for third-party reproduction

L. De Witte [1], L. Raman[1], M. Baetens[2], A. De Koker[3], N. Callewaert[3], S. Symoens[2], K. Tilleman [4], F. Vanden Meerschaut [4], A. Dheedene[2], and B. Menten [1,*]

[1]Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium [2]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium [3]VIB-UGent Center for Medical Biotechnology, Department of Biochemistry and Microbiology, Ghent University, Ghent-Zwijnaarde, Belgium [4]Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium

*Correspondence address. Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University, Corneel Heymanslaan 10, Ghent 9000, Belgium. Tel: +32-93325284; E-mail: bjorn.menten@ugent.be https://orcid.org/0000-0001-8182-659X

Submitted on November 22, 2021; resubmitted on March 30, 2022; editorial decision on April 12, 2022

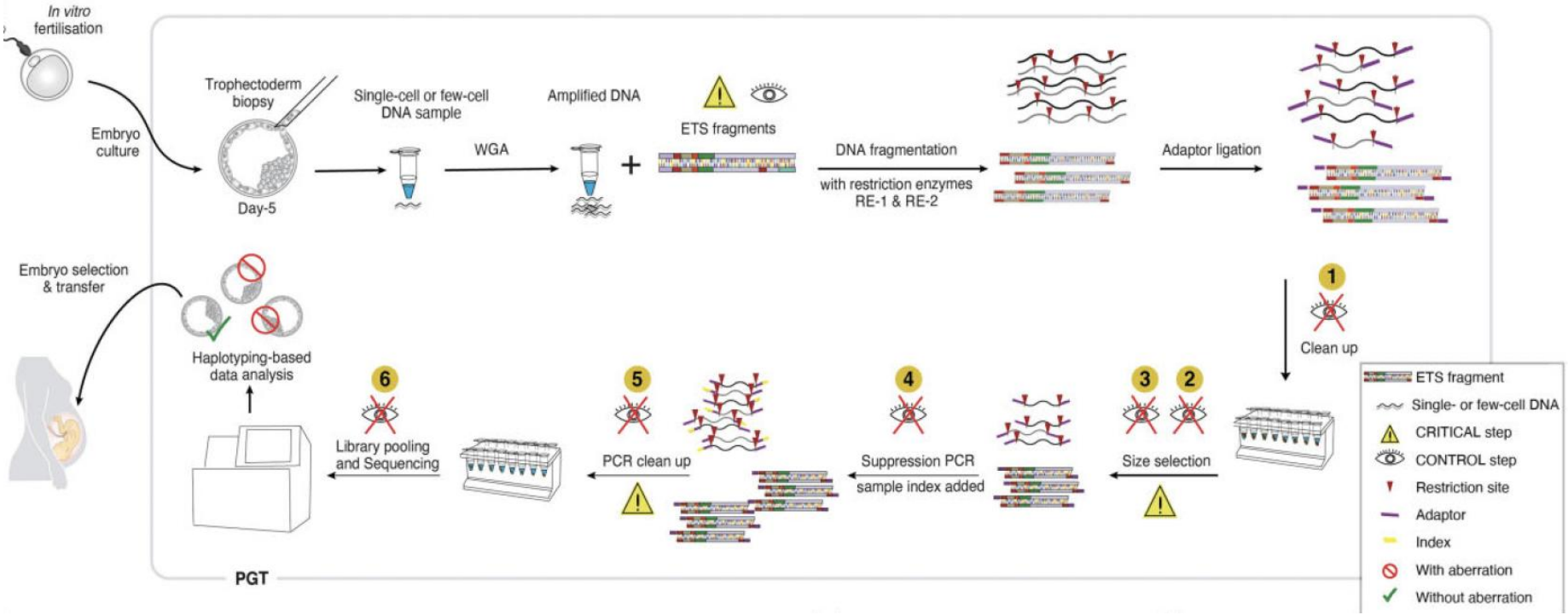
Human Reproduction, pp. 1–9, 2022
https://doi.org/10.1093/humrep/deac208

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Embryo tracking system for high-throughput sequencing-based preimplantation genetic testing

Wanwisa van Dijk[1,†], Kasper Derks[1,†], Marion Drüsedau[1,†], Jeroen Meekels[1], Rebekka Koeck [1,2], Rick Essers [1,2], Joseph Dreesen[1,2], Edith Coonen[1,3], Christine de Die-Smulders[1,2], Servi J.C. Stevens [1,2], Han G. Brunner[1,2,4], Arthur van den Wijngaard[1,2], Aimée D.C. Paulussen [1,2], and Masoud Zamani Esteki [1,2,*]

Towards automation of genotyping-by-sequencing



nature communications

Article

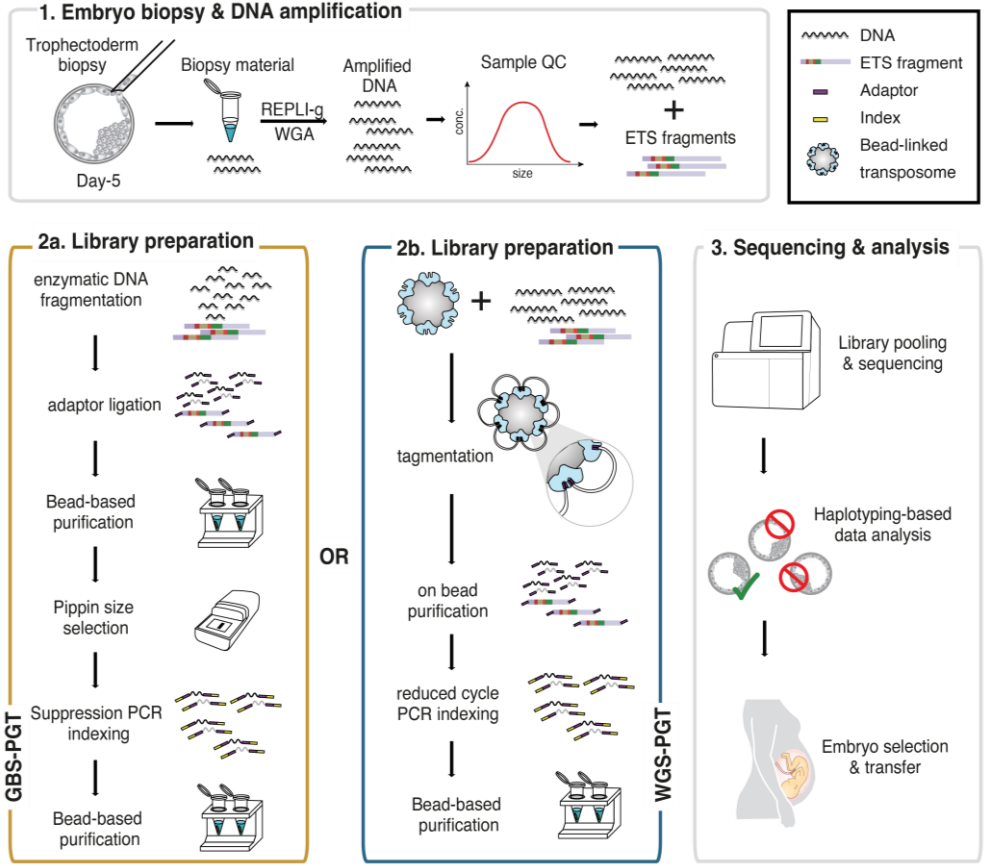
https://doi.org/10.1038/s41467-024-51508-1

Clinical-grade whole genome sequencing-based haplarithmisis enables all forms of preimplantation genetic testing

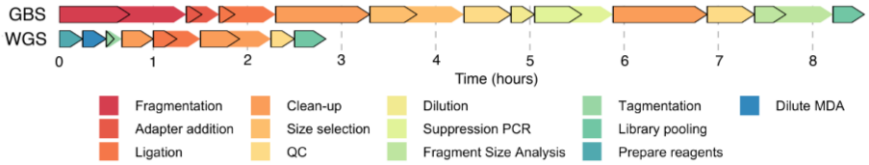
Anouk E. J. Janssen[1,2,9], Rebekka M. Koeck[1,2,9], Rick Essers[1,2,9], Ping Cao[1,2], Wanwisa van Dijk[1], Marion Drüsedau[1], Jeroen Meekels[1], Burcu Yaldiz[1], Maartje van de Vorst[1], Bart de Koning[1], Debby M. E. I. Hellebrekers[1], Servi J. C. Stevens[1], Su Ming Sun[1], Malou Heijligers[1], Sonja A. de Munnik[1], Chris M. J. van Uum[1], Jelle Achten[1], Lars Hamers[1], Marjan Naghdi[1,2,3], Lisenka E. L. M. Vissers[4], Ron J. T. van Golde[5], Guido de Wert[6,7], Jos C. F. M. Dreesen[1], Christine de Die-Smulders[1,2], Edith Coonen[1,5], Han G. Brunner[1,2,4], Arthur van den Wijngaard[1], Aimee D. C. Paulussen[1,2] & Masoud Zamani Esteki[1,2,8]

[1]Department of Clinical Genetics, Maastricht University Medical Centre (MUMC+), Maastricht, The Netherlands. [2]Department of Genetics and Cell Biology, GROW Research Institute Oncology and Reproduction, Maastricht University, Maastricht, The Netherlands. [3]Faculty of Psychology and Neuroscience, Section Applied Social Psychology, Maastricht University, Maastricht, The Netherlands. [4]Department of Human Genetics, Research Institute for Medical Innovation, Radboud University Medical Center, Nijmegen, The Netherlands. [5]Department of Obstetrics and Gynaecology, GROW Research Institute for Oncology and Reproduction, Maastricht University, Maastricht, The Netherlands. [6]Department of Health, Ethics and Society, GROW Research Institute for Oncology and Reproduction, Maastricht University, Maastricht, The Netherlands. [7]CAPHRI Research Institute for Public Health and Primary Care, Maastricht University,

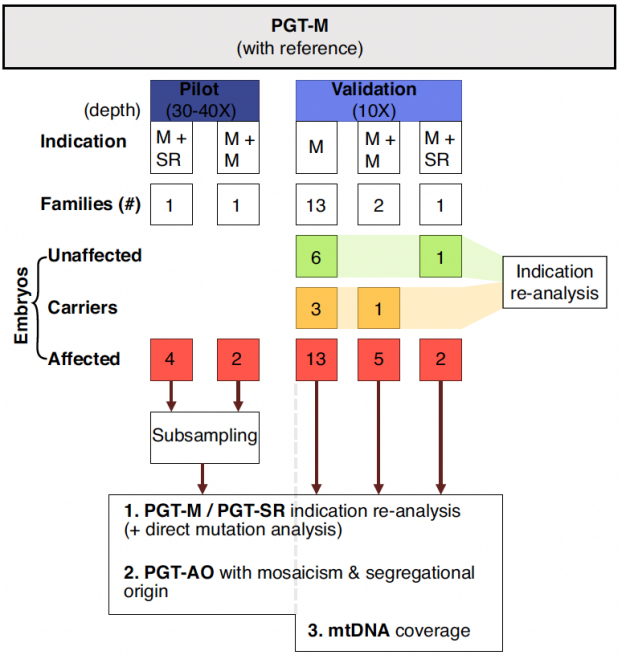
Clinical-grade whole-genome sequencing-based haplarithmisis

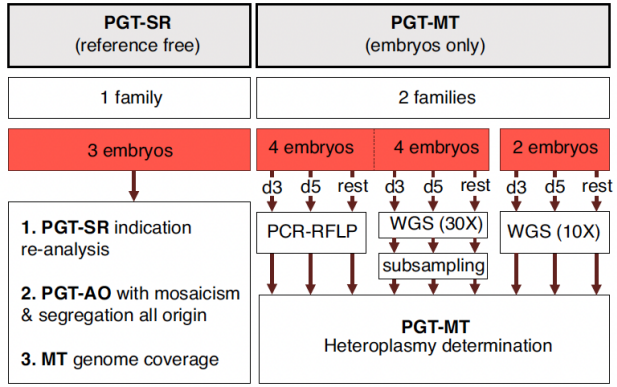


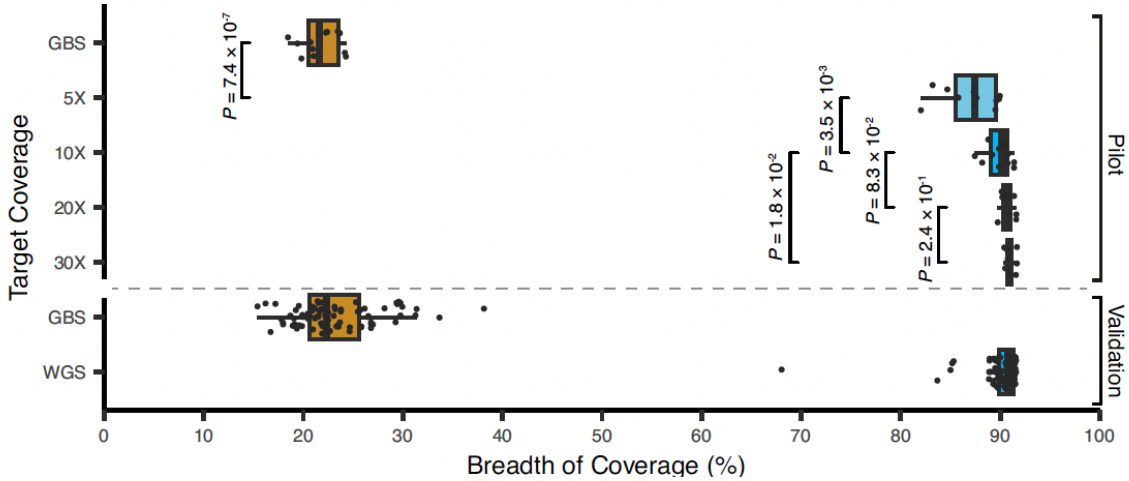
Anouk Janssen
PhD candidate

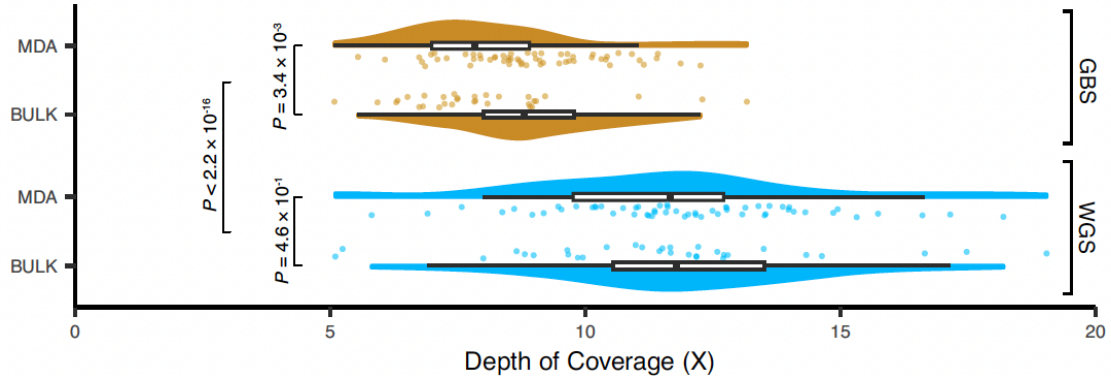


Janssen et al. 2024, *Nature Communications*



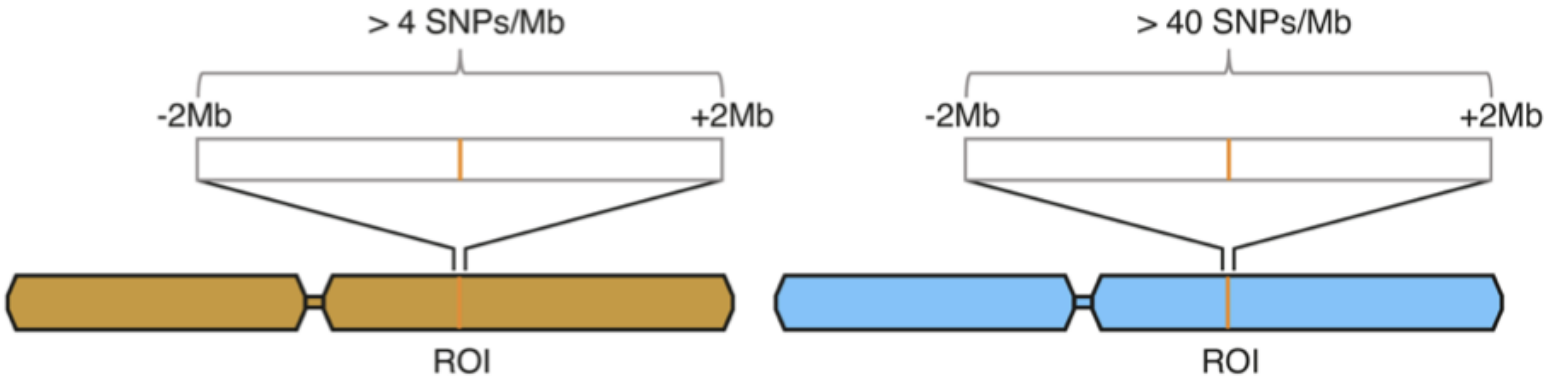






Janssen et al. 2024, nature communications

PGT-M with extreme accuracy

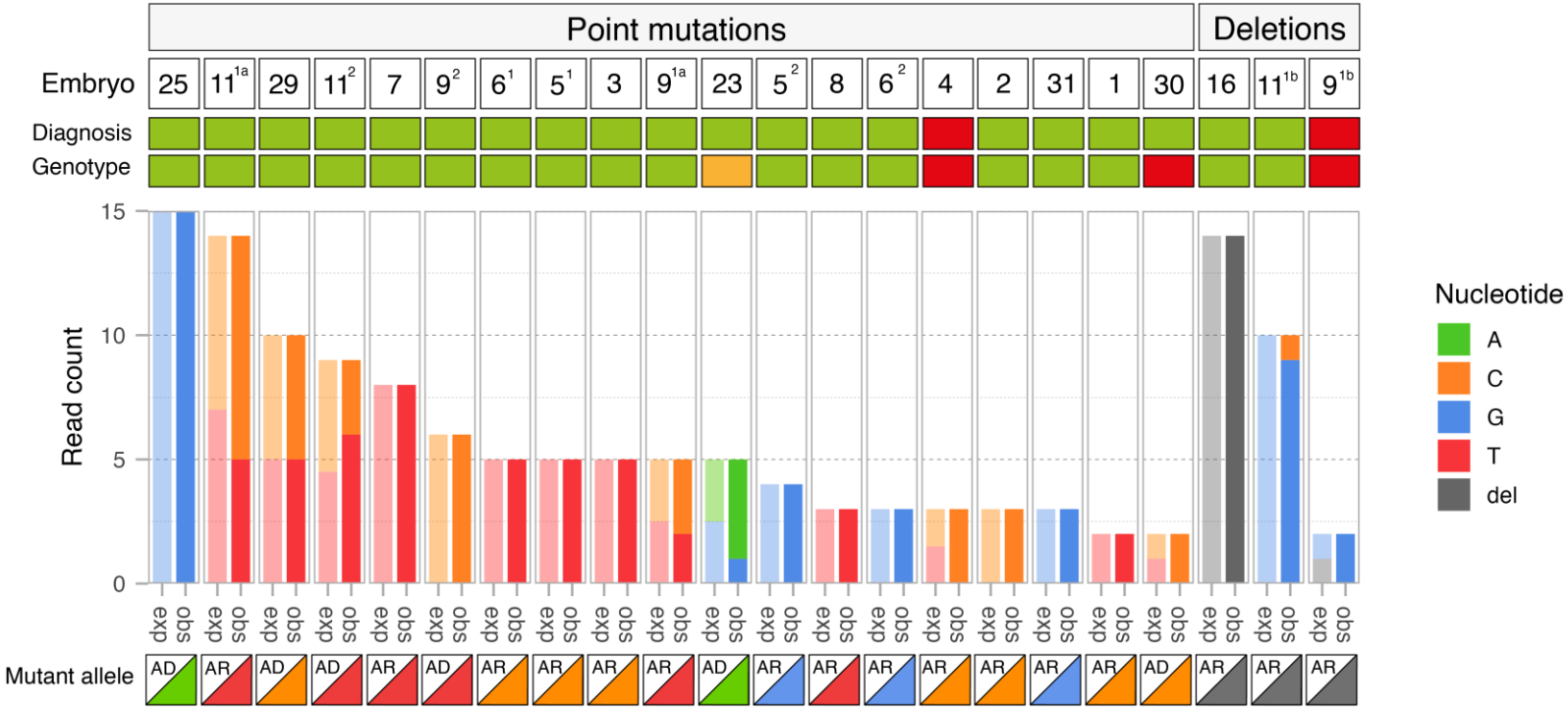


$$P(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

$$P = 4.77 \times 10^{-74}$$

Janssen et al. 2024, *Nature Communications*

Direct detection of point mutations

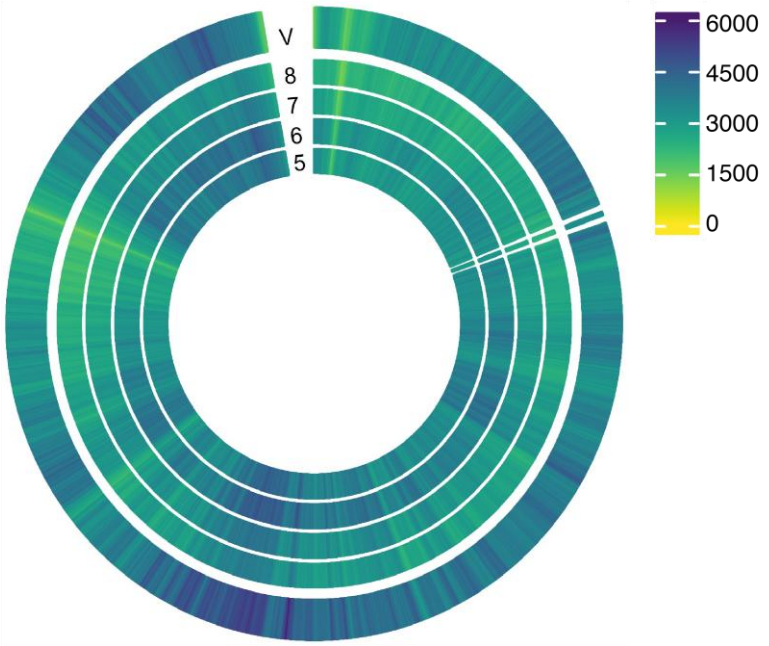


Janssen et al. 2024, *Nature Communications*

Clinical whole-genome sequencing for PGT-MT

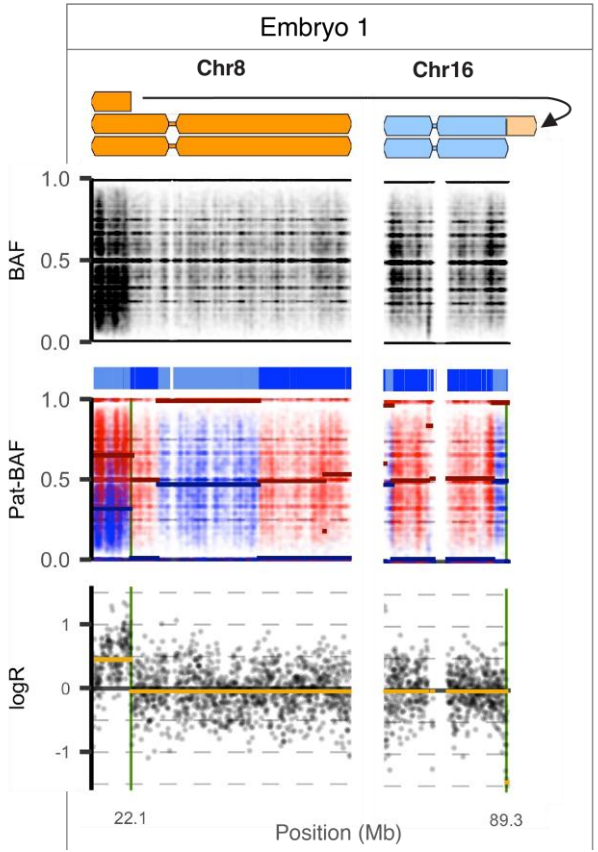
PGT-MT heteroplasmy

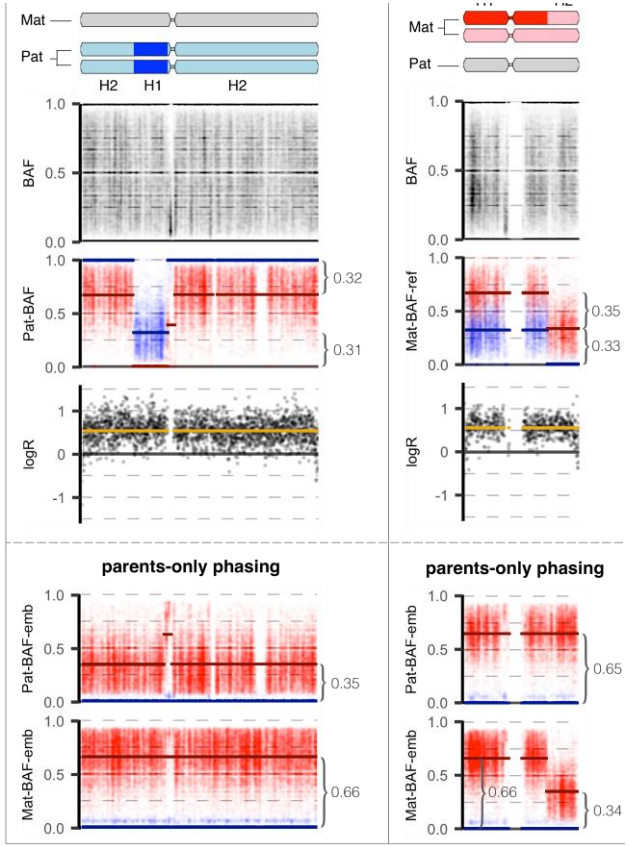
Embryo	Day-3 (PCR)	Day-5 Method	Day-5 (TE biopsy)	Day-5 (Rest embryo)
41	38%	PCR	35%	38%
42	58%		56%	57%
43	71%		70%	67%
44	78%		74%	77%
45	48%	WGS	49.6%	49.9%
46	64%		67.3%	67.6%
47	77%		75.5%	77.0%
48	-		41.0%	39.4%



Janssen et al. 2024, *Nature Communications*

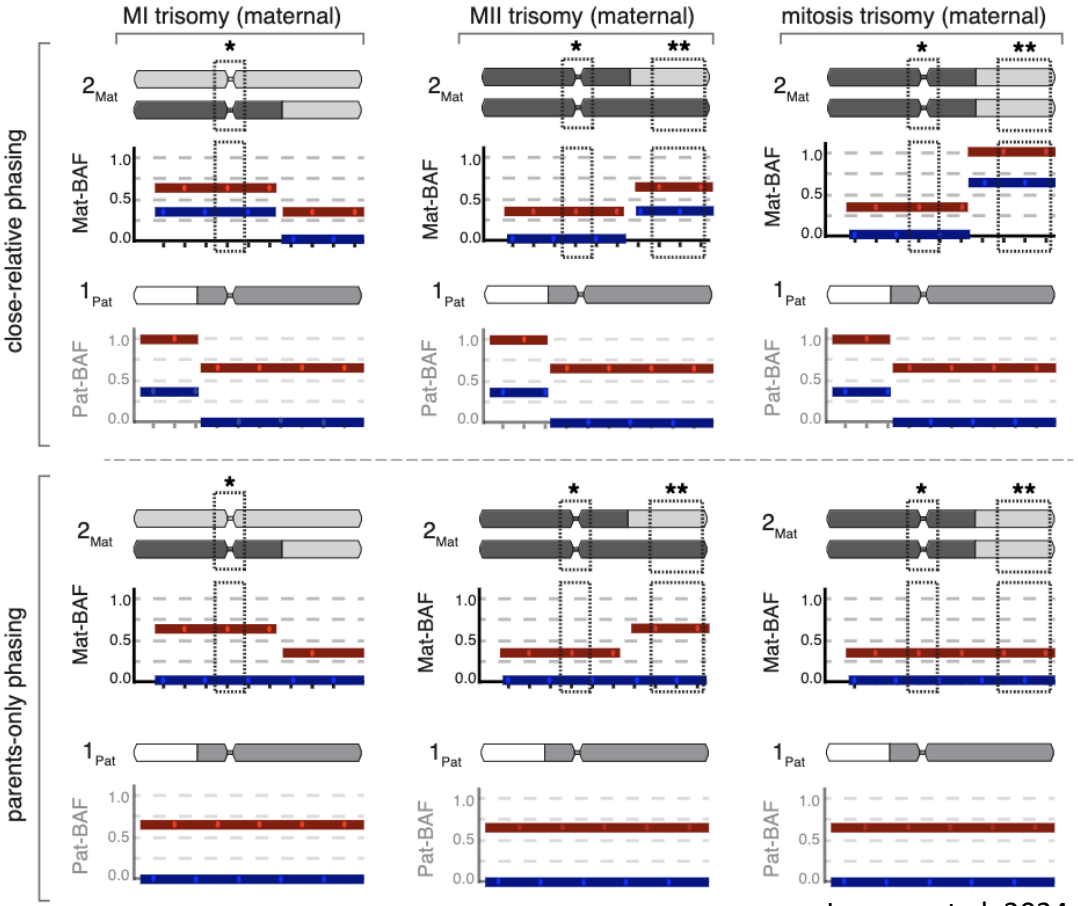
Clinical whole-genome sequencing for PGT-SR





Janssen et al. 2024, *Nature Communications*

Parents-only PGT for aneuploidy origin (PGT-AO)

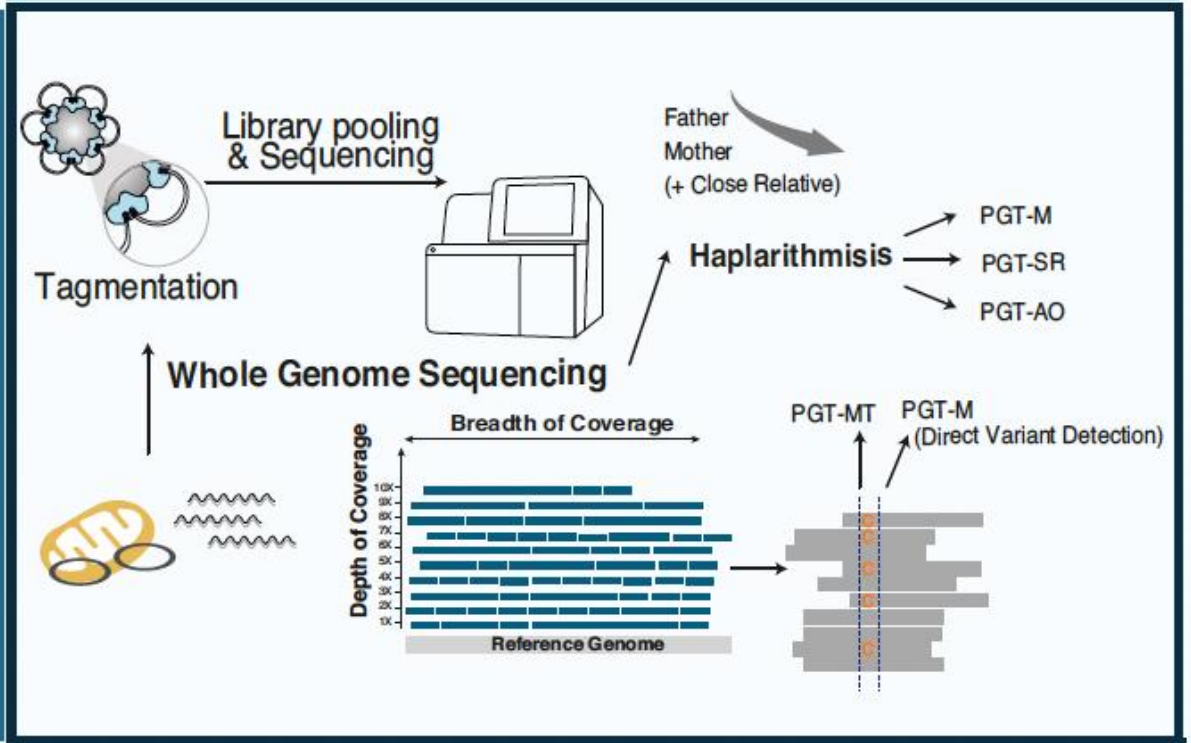


Janssen et al. 2024, *Nature Communications*

All-in-one PGT

All-in-one Method

Method



characteristics

- Flexible PGT-M
- **Generic pathogenic variant detection at base resolution**
- Flexible PGT-SR
- Normal vs balanced translocation (haplotypin
- **Breakpoint detection at base resolution**
- Copy number profiling (PGT-A)
- Segregational/parental origin of aneuploidies
- Level of mosaicism of aneuploidies
- **Mitochondrial DNA (heteroplasmy level)**

Outline

- Traditional PGT
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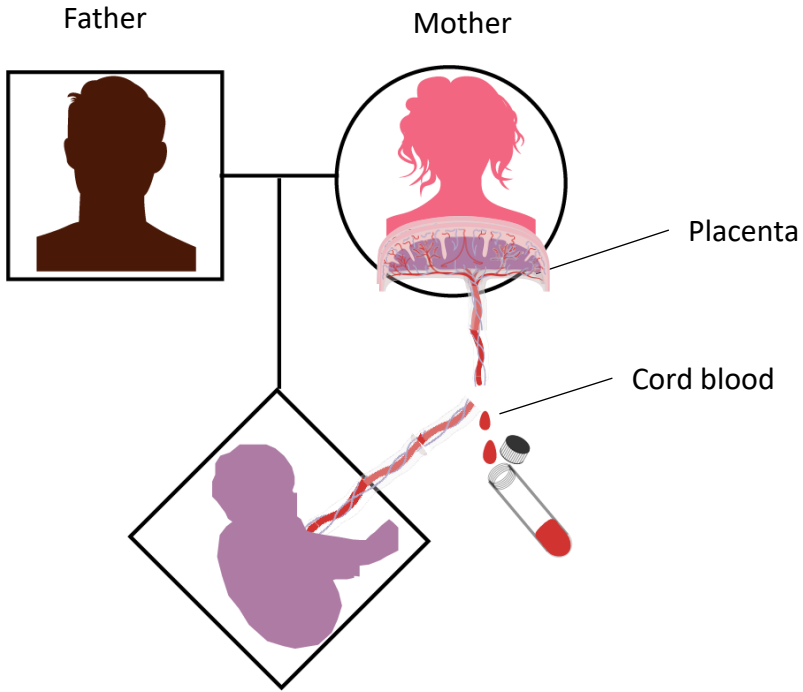
- Prevalance of aberrations in **pregnancy loss**
- **Clinical follow up** of embryos following PGT-M

Zamani Esteki et al. 2019
Nature Medicine

Quartets to Study Nature and Extend of CIN

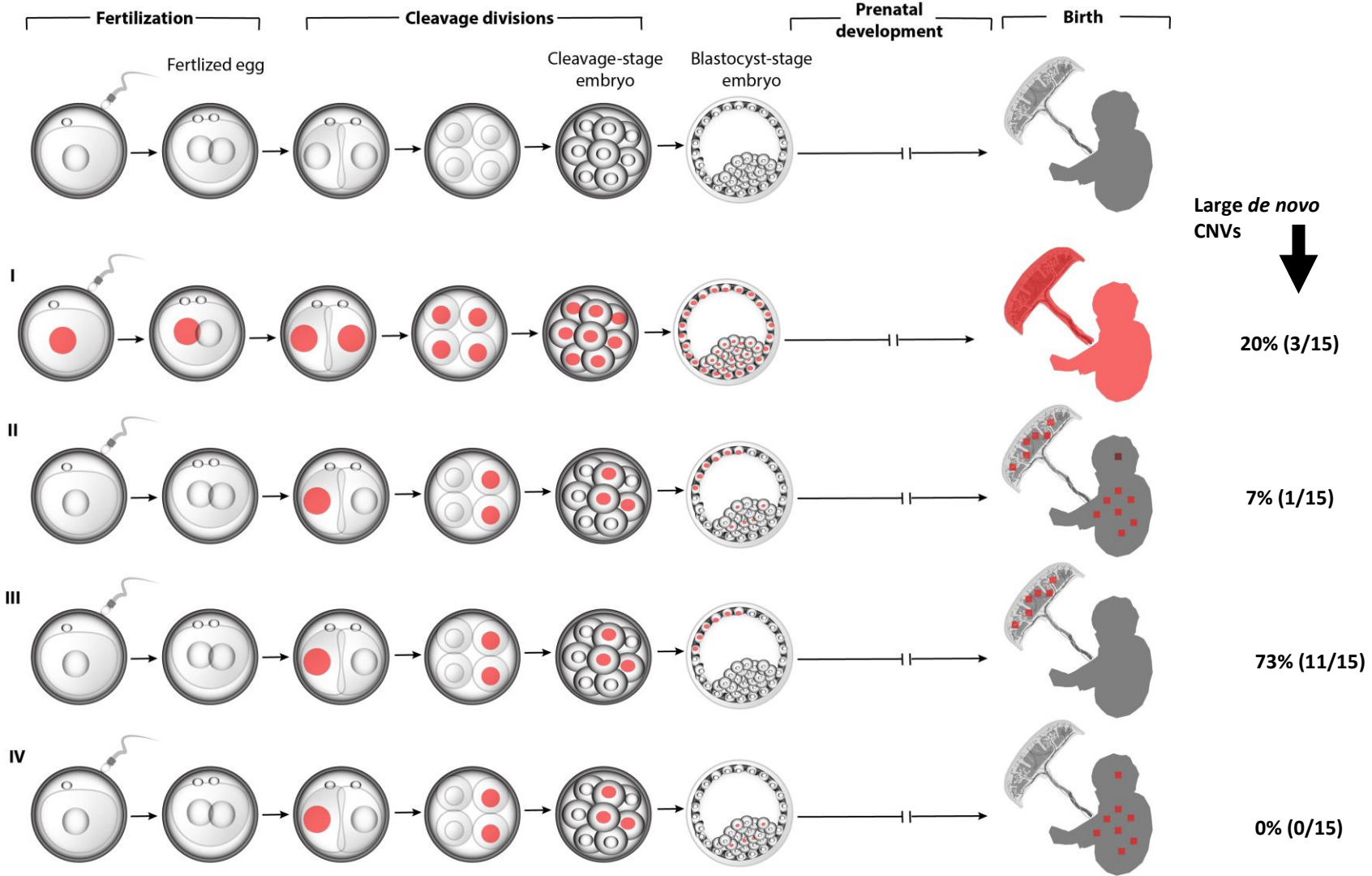


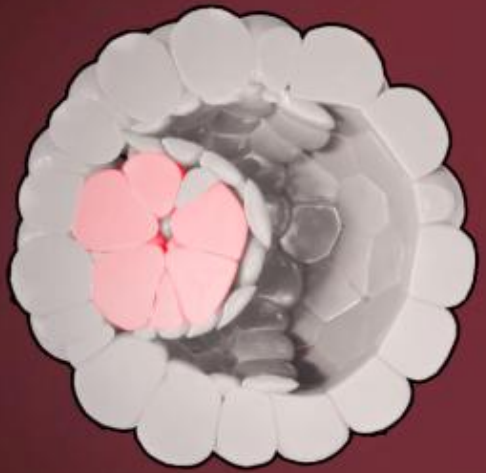
Maternal

Fetal

48 IVF vs **50 naturally-conceived** AGA neonates

16 naturally-conceived SGA neonates


Fertilization
Cleavage divisions
Prenatal development
Birth
Fertlized egg
Cleavage-stage embryo
Blastocyst-stage embryo
I
II
III
IV
Large *de novo* CNVs
20% (3/15)
7% (1/15)
73% (11/15)
0% (0/15)



?

Fetal Development

Vanneste et al., 2009
Nature Medicine

Zamani Esteki et al., 2015
American Journal of Human Genetics

Zamani Esteki et al., 2019
Nature Medicine

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- Prevalance of aberrations in **pregnancy loss**
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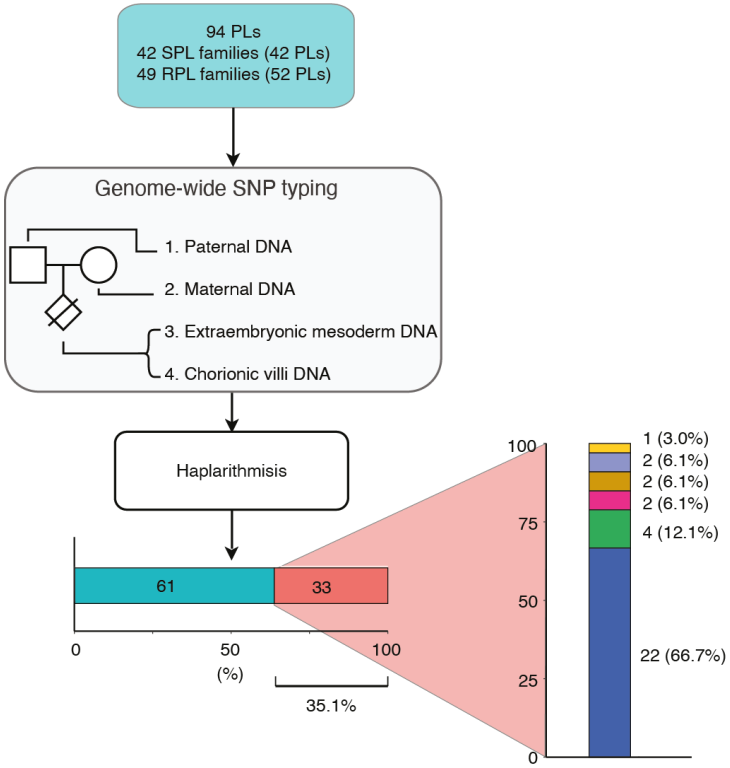
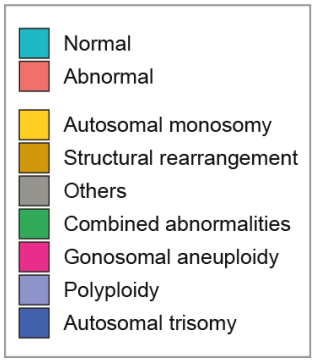
True aberration rate

Rick Essers
PhD candidate

True aberration rate:

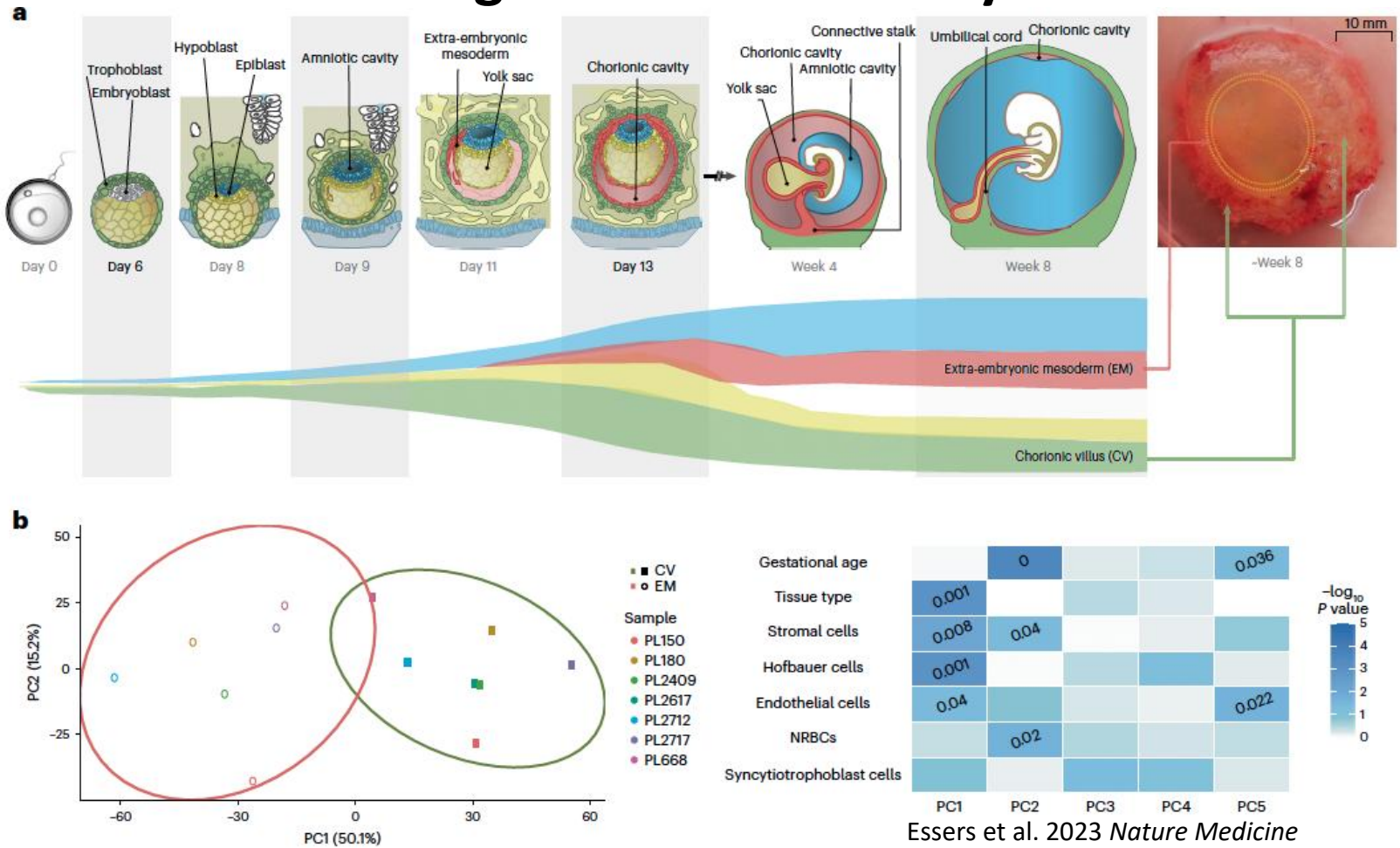
50.4% → **67.8%**





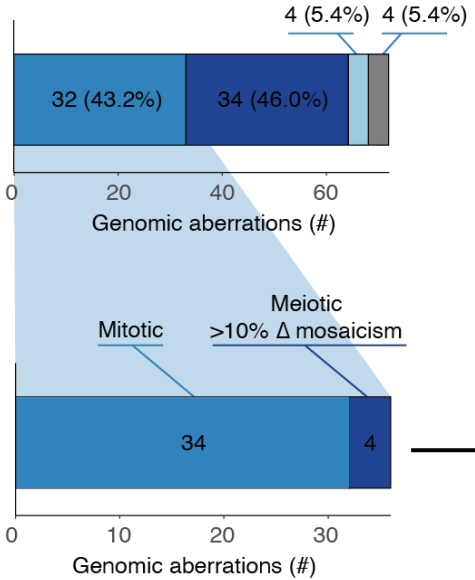
Essers et al. 2023 *Nature Medicine*

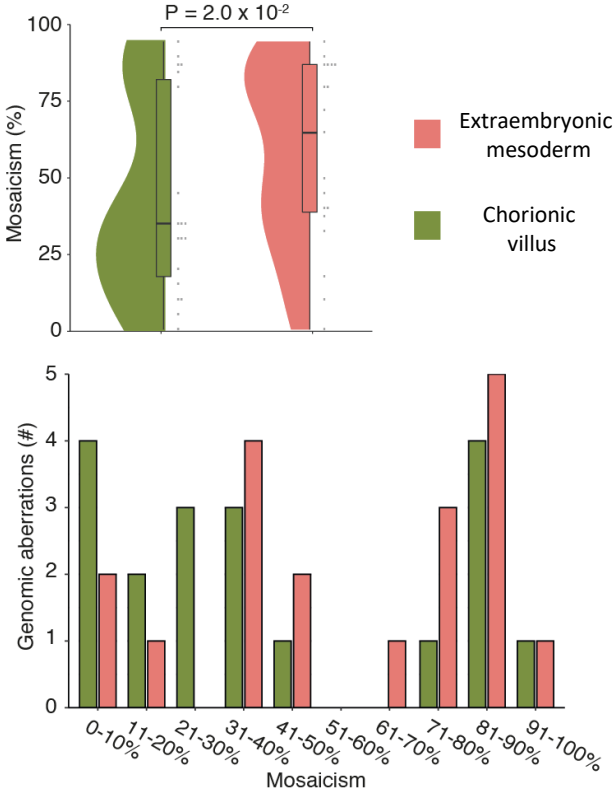
Cell lineages in human embryos



Essers et al. 2023 *Nature Medicine*

Genetic abnormalities likely less tolerated in fetal lineage





Essers et al. 2023, Nature Medicine

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- Prevalance of aberrations in **pregnancy loss**
- **Non-slection study: Clinical follow up** of embryos following PGT-M

Ongoing PGT-A debate in the field

Not effective

Cochrane Library
Trusted evidence.
Informed decisions.
Better health.

Cochrane Database of Systematic Reviews

[Intervention Review]

Preimplantation genetic testing for aneuploidies (abnormal number of chromosomes) in in vitro fertilisation

Simone Cornelisse[1], Miriam Zagers[2], Elena Kostova[2], Kathrin Fleischer[1,3], Madelon van Wely[2], Sebastiaan Mastenbroek[2]

ORIGINAL ARTICLE

Live Birth with or without Preimplantation Genetic Testing for Aneuploidy

J. Yan, Y. Qin, H. Zhao, Y. Sun, F. Gong, R. Li, X. Sun, X. Ling, H. Li, C. Hao, J. Tan, J. Yang, Y. Zhu, F. Liu, D. Chen, D. Wei, J. Lu, T. Ni, W. Zhou, K. Wu, Y. Gao, Y. Shi, Y. Lu, T. Zhang, W. Wu, X. Ma, H. Ma, J. Fu, J. Zhang, Q. Meng, H. Zhang, R.S. Legro, and Z.-J. Chen

The Imperative of Responsible Innovation in Reproductive Medicine

Sebastiaan Mastenbroek, Ph.D., Guido de Wert, Ph.D., and Eli Y. Adashi, M.

Effective

In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study

Carmen Rubio, Ph.D.,[a] José Bellver, M.D.,[b,c] Lorena Rodrigo, Ph.D.,[a] Gema Castillón, M.D.,[d] Alfredo Guillén, M.D.,[e] Carmina Vidal, M.D.,[b] Juan Giles, M.D.,[b] Marcos Ferrando, M.D.,[f] Sergio Cabanillas, M.D.,[b] José Remohí, M.D., Ph.D.,[b,c] Antonio Pellicer, M.D., Ph.D.,[b,c,g] and Carlos Simón, M.D., Ph.D.[a,b,c]

ASSISTED REPRODUCTION TECHNOLOGIES

Check for updates

Preimplantation Genetic Testing for Aneuploidy Improves Clinical, Gestational, and Neonatal Outcomes in Advanced Maternal Age Patients Without Compromising Cumulative Live-Birth Rate.

Laura Sacchi[1] · Elena Albani[1] · Amalia Cesana[1] · Antonella Smeraldi[1] · Valentina Parini[1] · Marco Fabiani[2] · Maurizio Poli[2] · Antonio Capalbo[2] · Paolo Emanuele Levi-Setti[1]

International Journal of *Molecular Sciences*

MDPI

Review

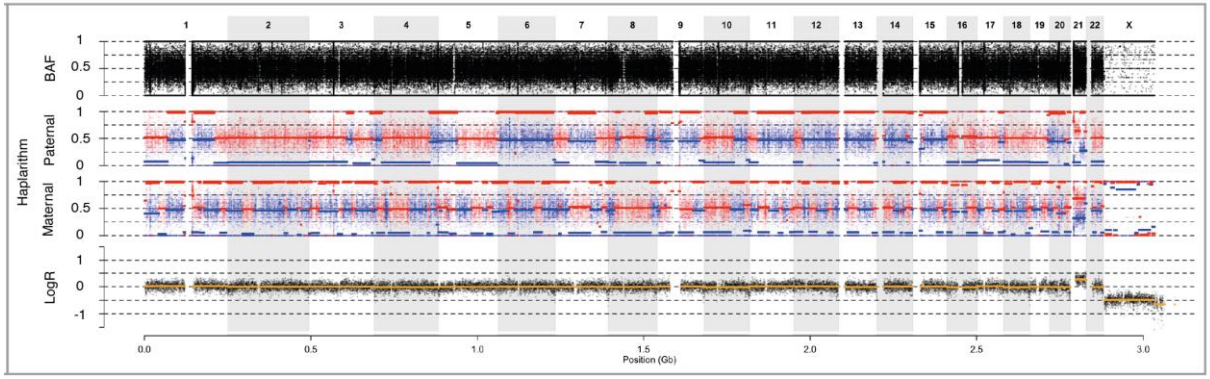
Preimplantation Genetic Testing: Where We Are Today

Ermanno Greco [1,2], Katarzyna Litwicka [1,*], Maria Giulia Minasi [1], Elisabetta Cursio [1], Pier Francesco Greco [1] and Paolo Barillari [1]

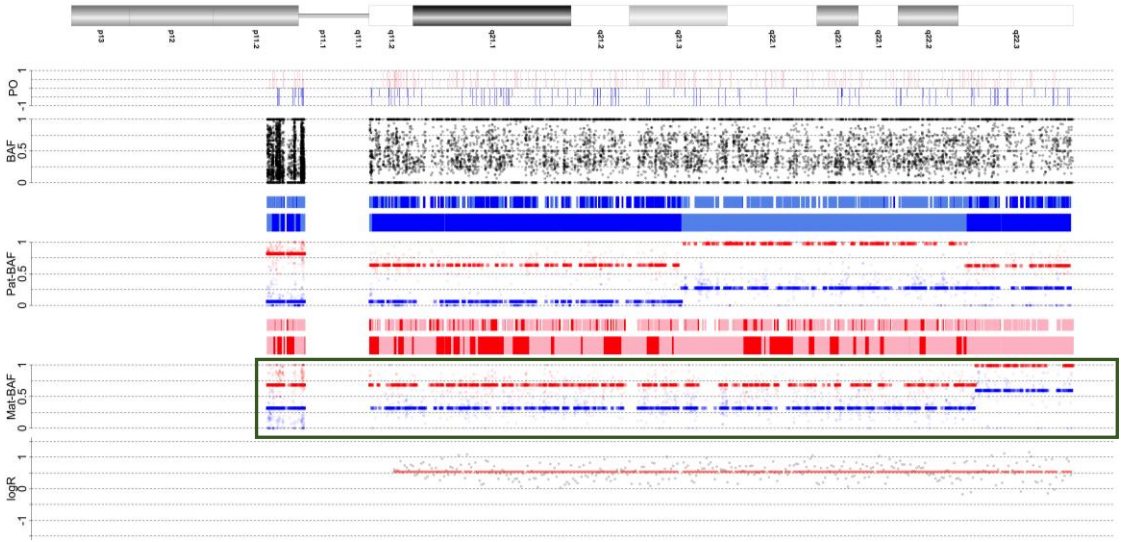
Termination of pregnancy after PGT

5 termination of pregnancy cases

- 1 T18
- 4 T21
  - 2 meiotic I
  - 2 meiotic II



Chromosome 21



Meiotic I origin

Maternal origin

Trisomy

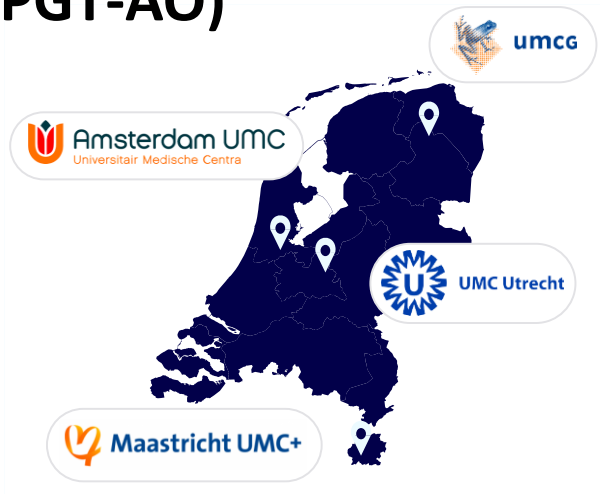
PGT Aneuploidy Origin (PGT-AO)

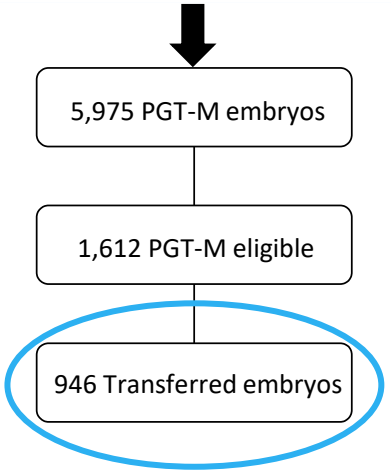
- Retrospective study of PGT-M embryos
  - PGT-A not performed in The Netherlands

- Genome wide evaluation by haplarithmisis on PGT-M embryos

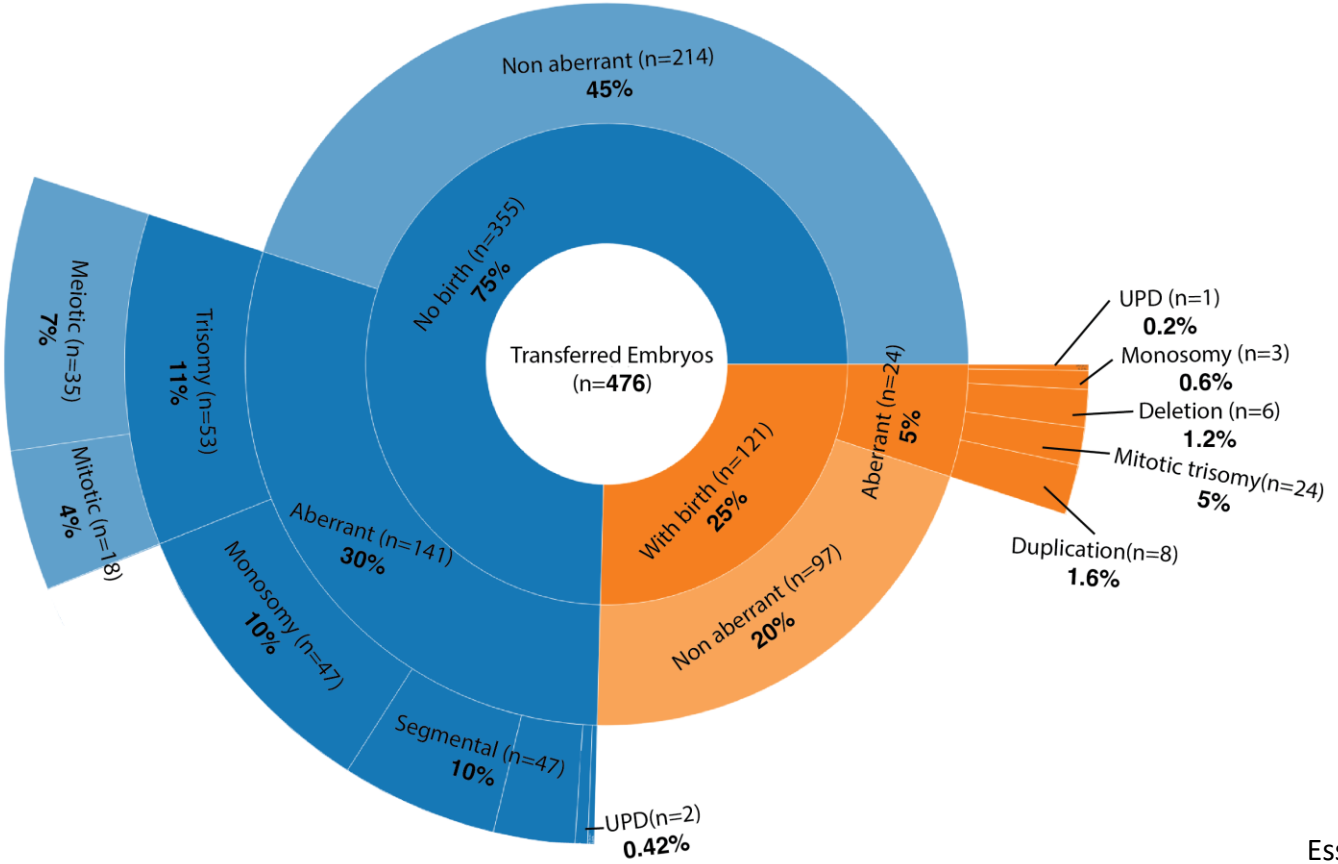
- Link with transfer/pregnancy outcome
- Comprehensive exploration of aberrations

Rick Essers
PhD candidate



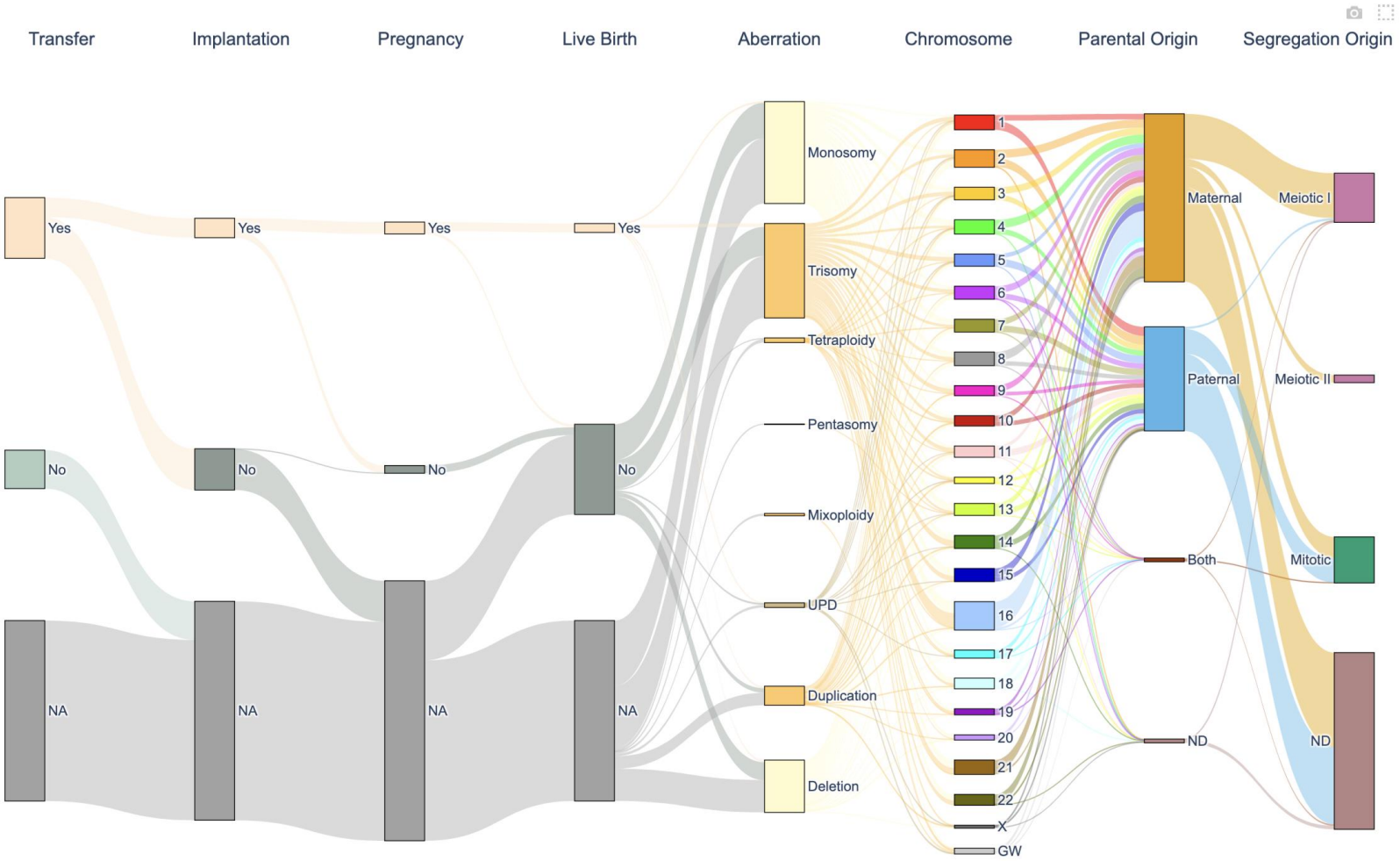


PGT for aneuploidy origin (PGT-AO)
6000 embryos



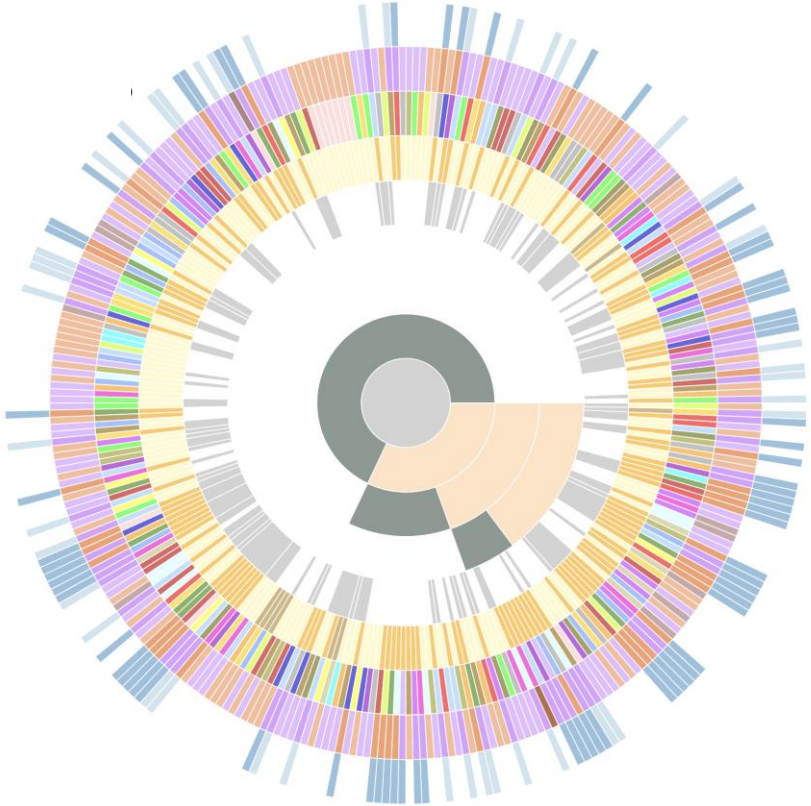
Essers et al. unpublished

Genomic alterations & early life bottleneck

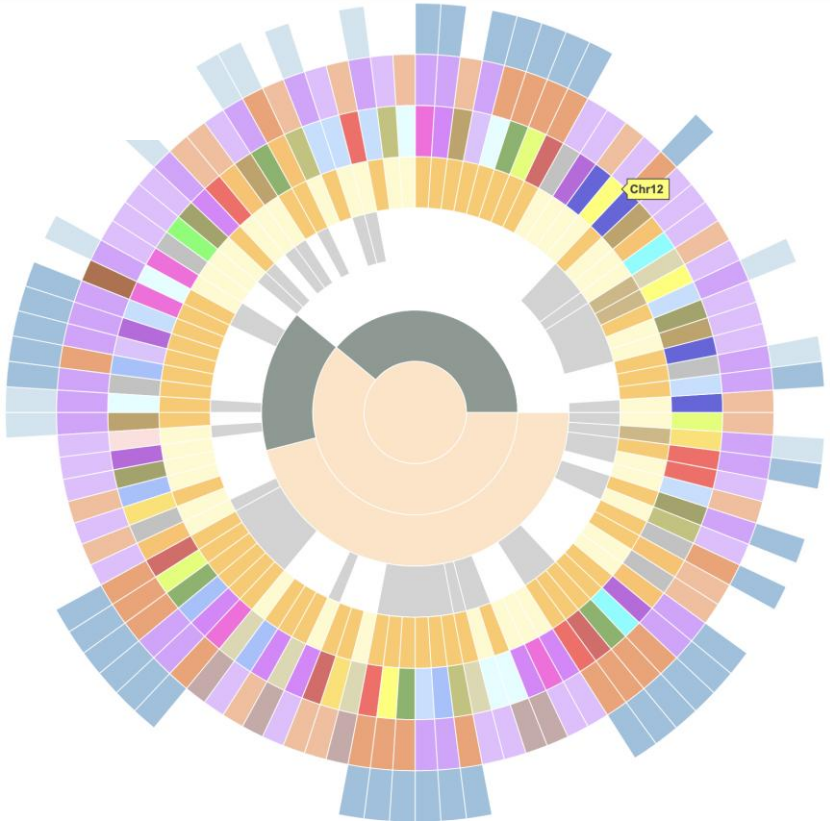


et al. unpublished

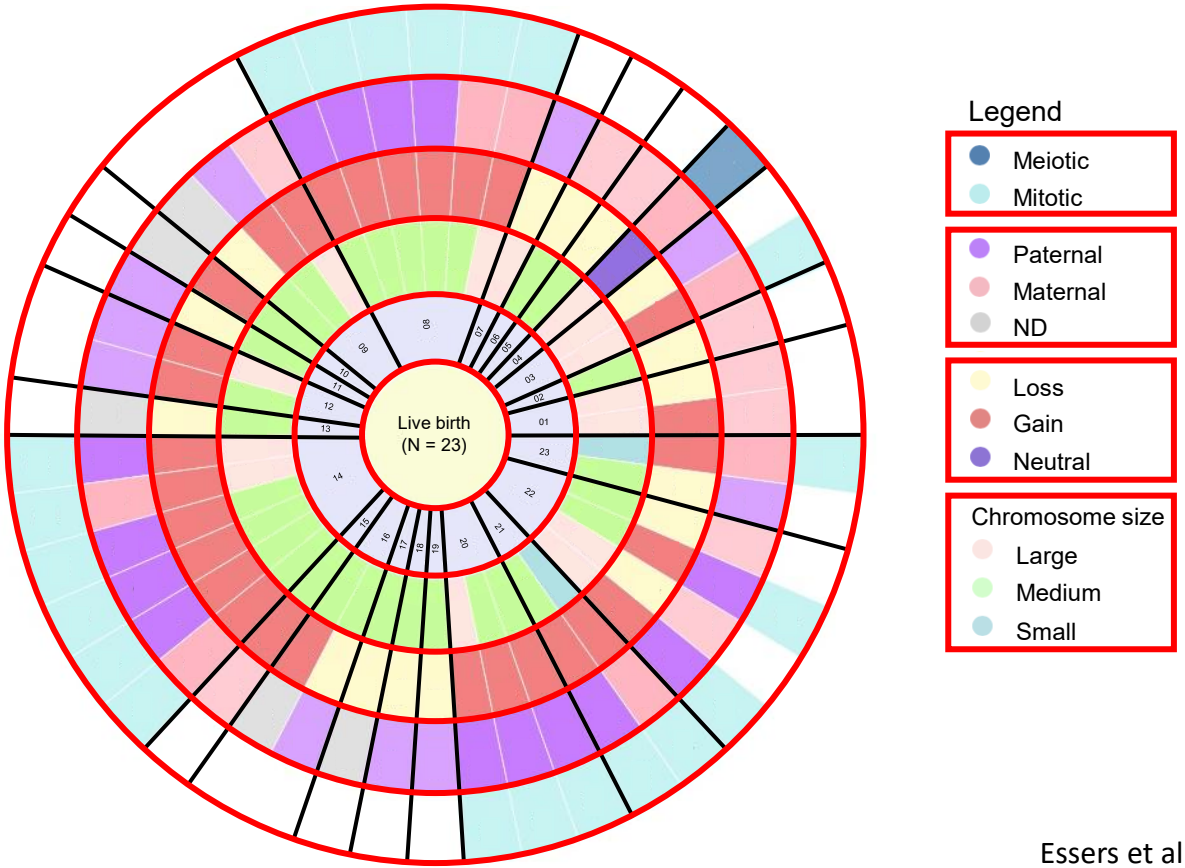
Transfered



Positive HCG

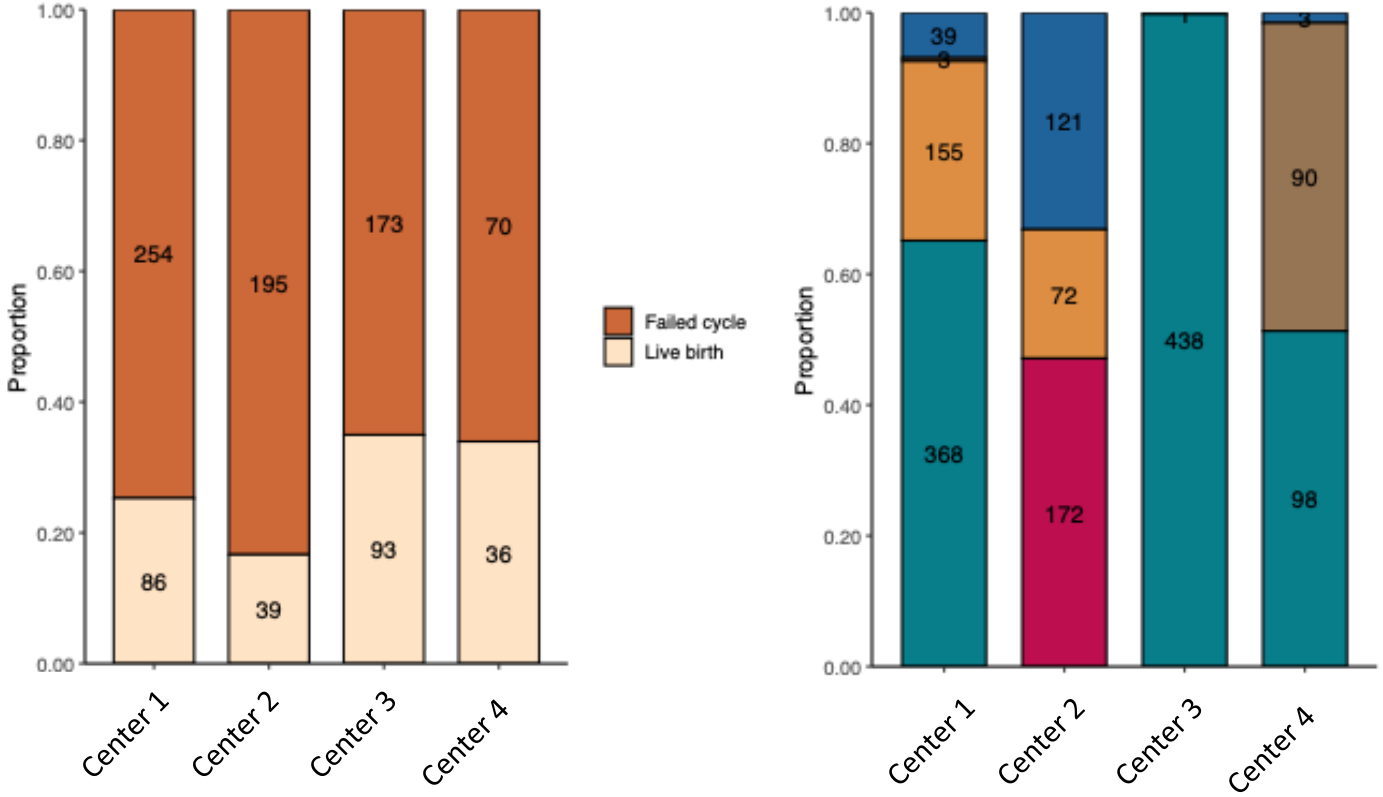


Live births with Chromosomal Aberrations



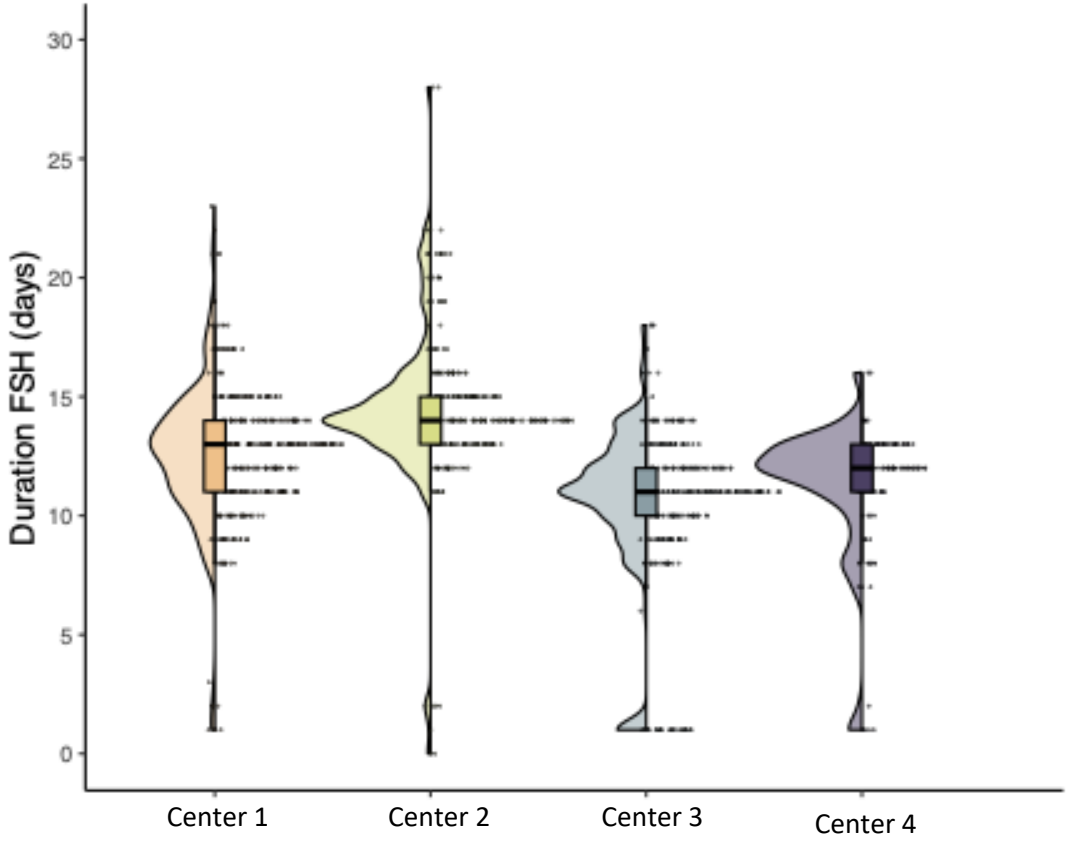
Essers et al. unpublished

Birth status vs. centers & medication



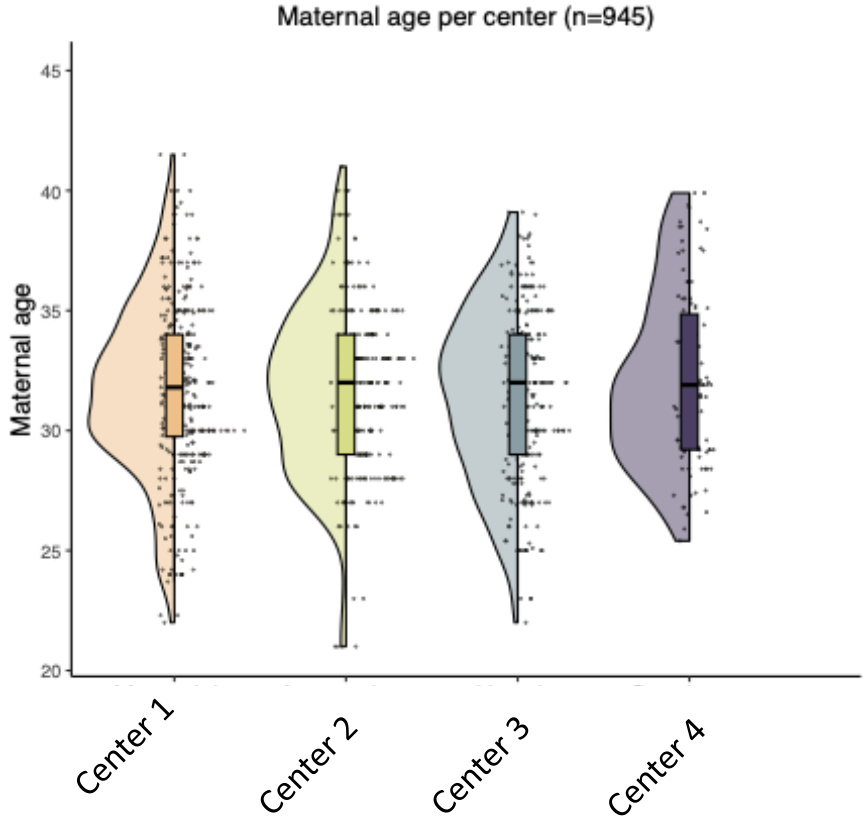
Essers et al. unpublished

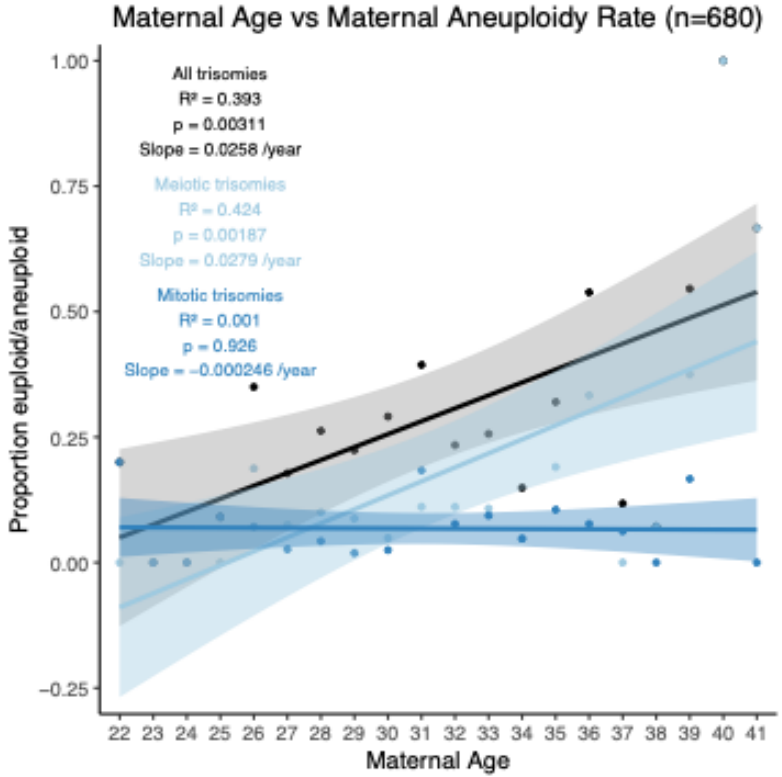
Duration FSH administration



Essers et al. unpublished

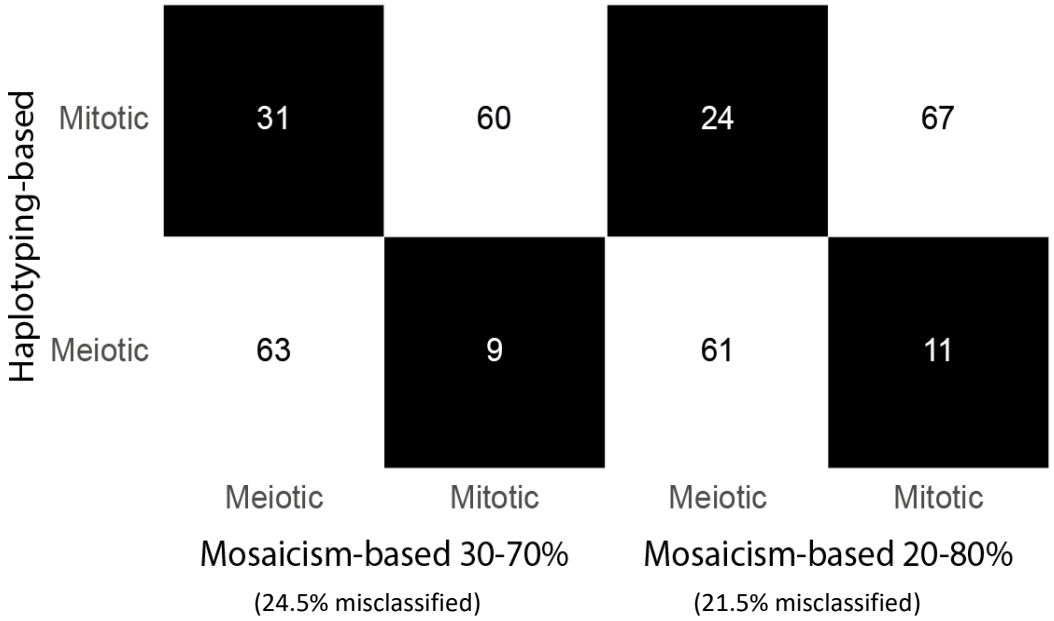
Maternal age vs centers



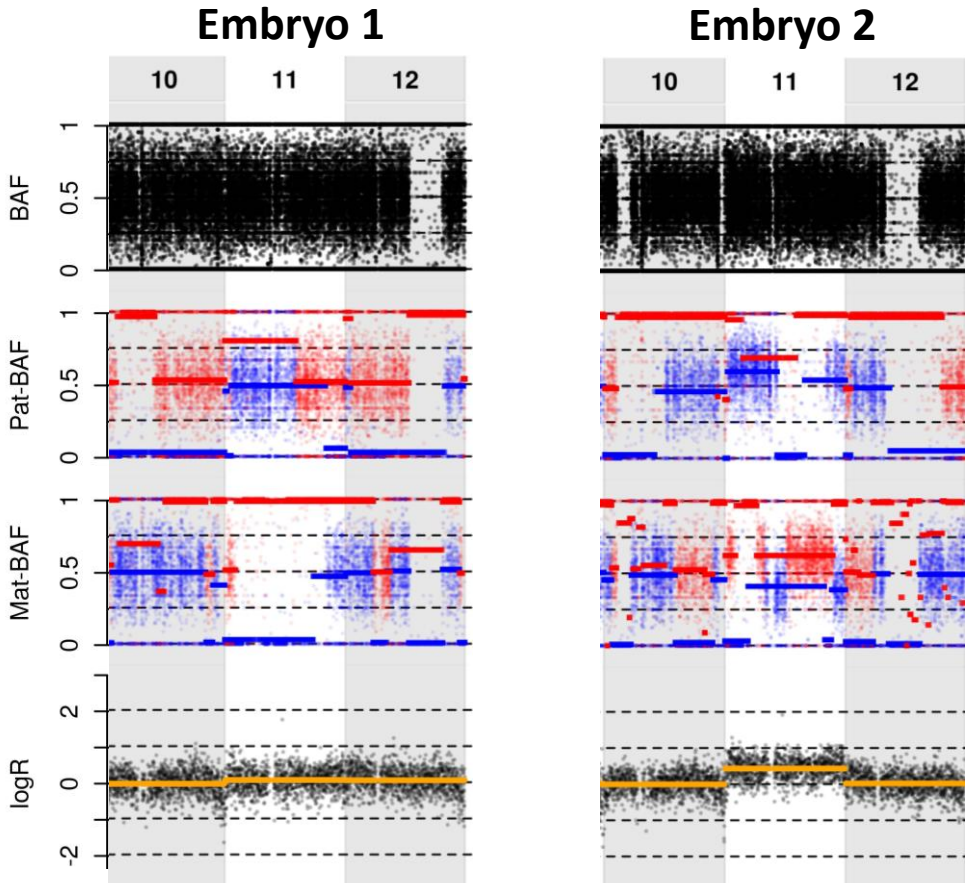


Essers et al. unpublished

Discordance in segregational origin

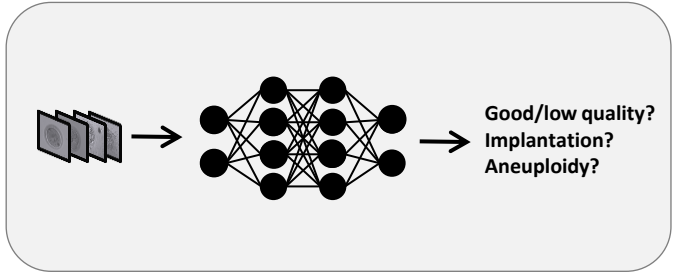


Double transfer: embryo with **meiotic trisomy could not** make it

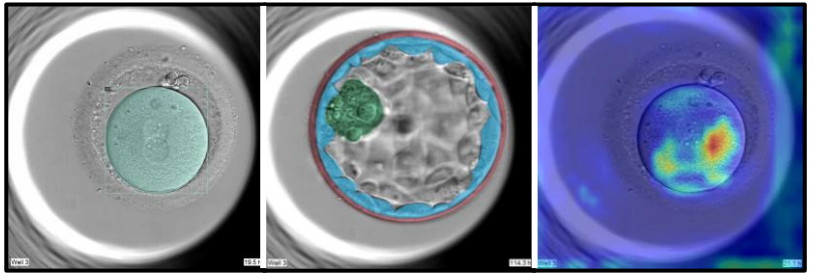


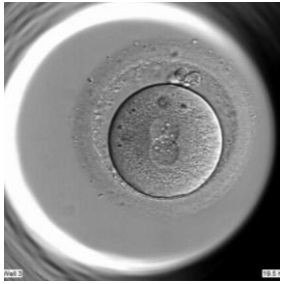
Essers et al. unpublished

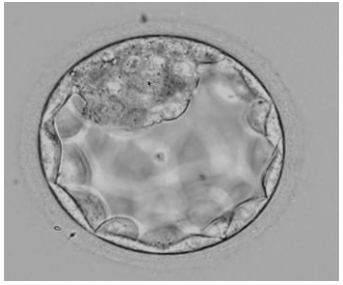
Multimodal deep learning for embryo selection in assisted reproduction



Prediction model

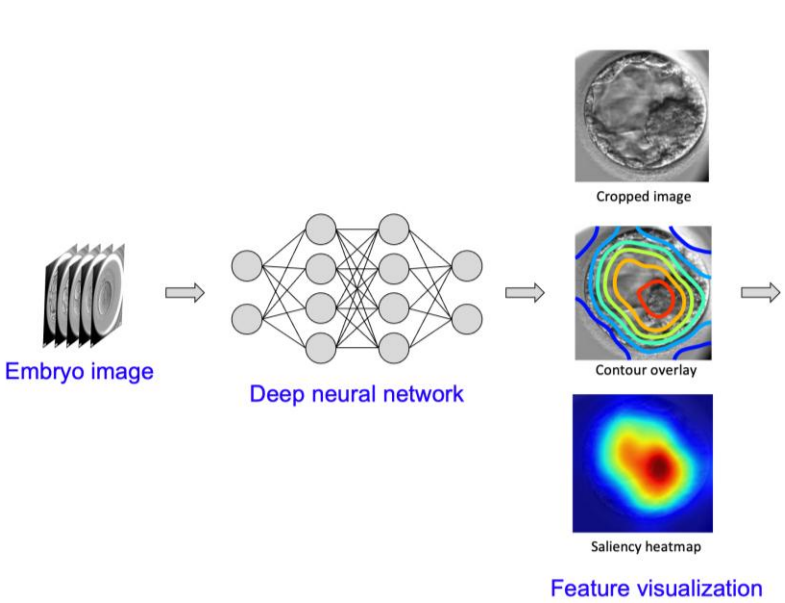


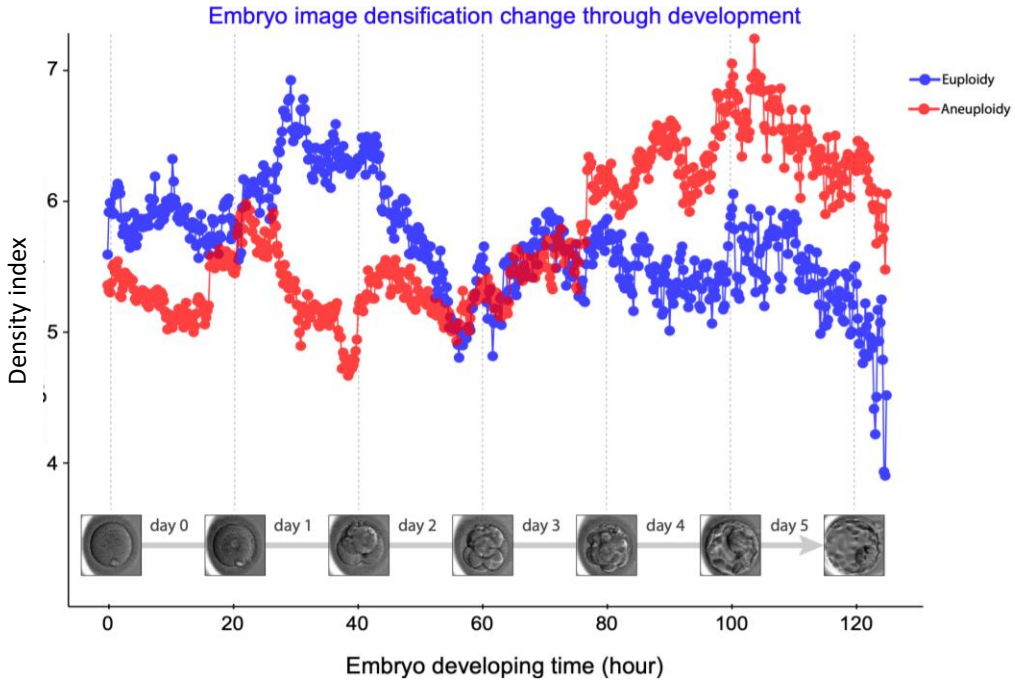




Ping Cao
PhD candidate

Embryo selection model: genome integrity + morphology

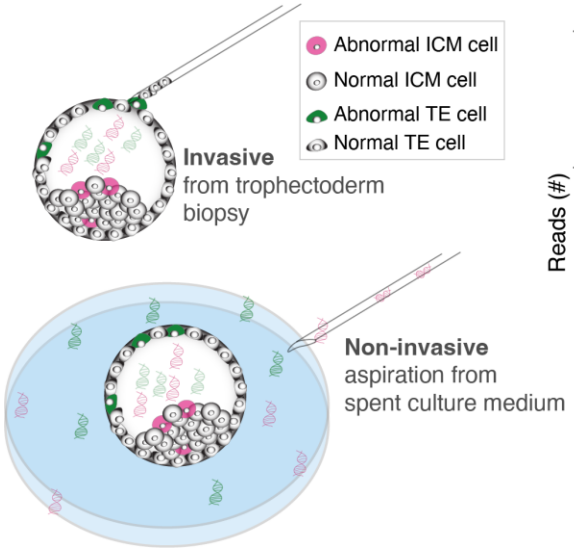


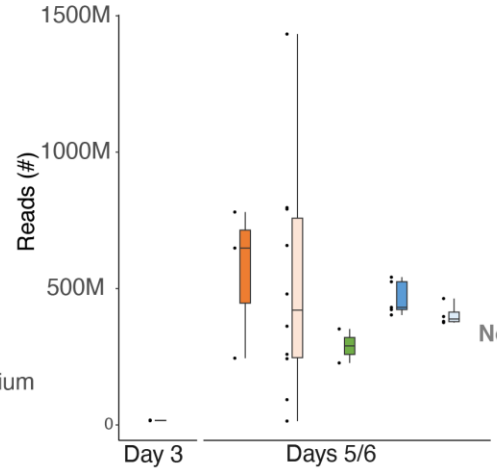


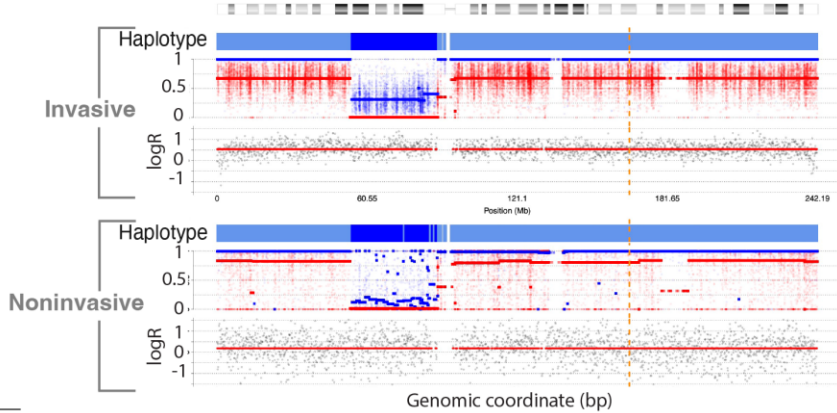
Cao et al. unpublished

Non-invasive PGT

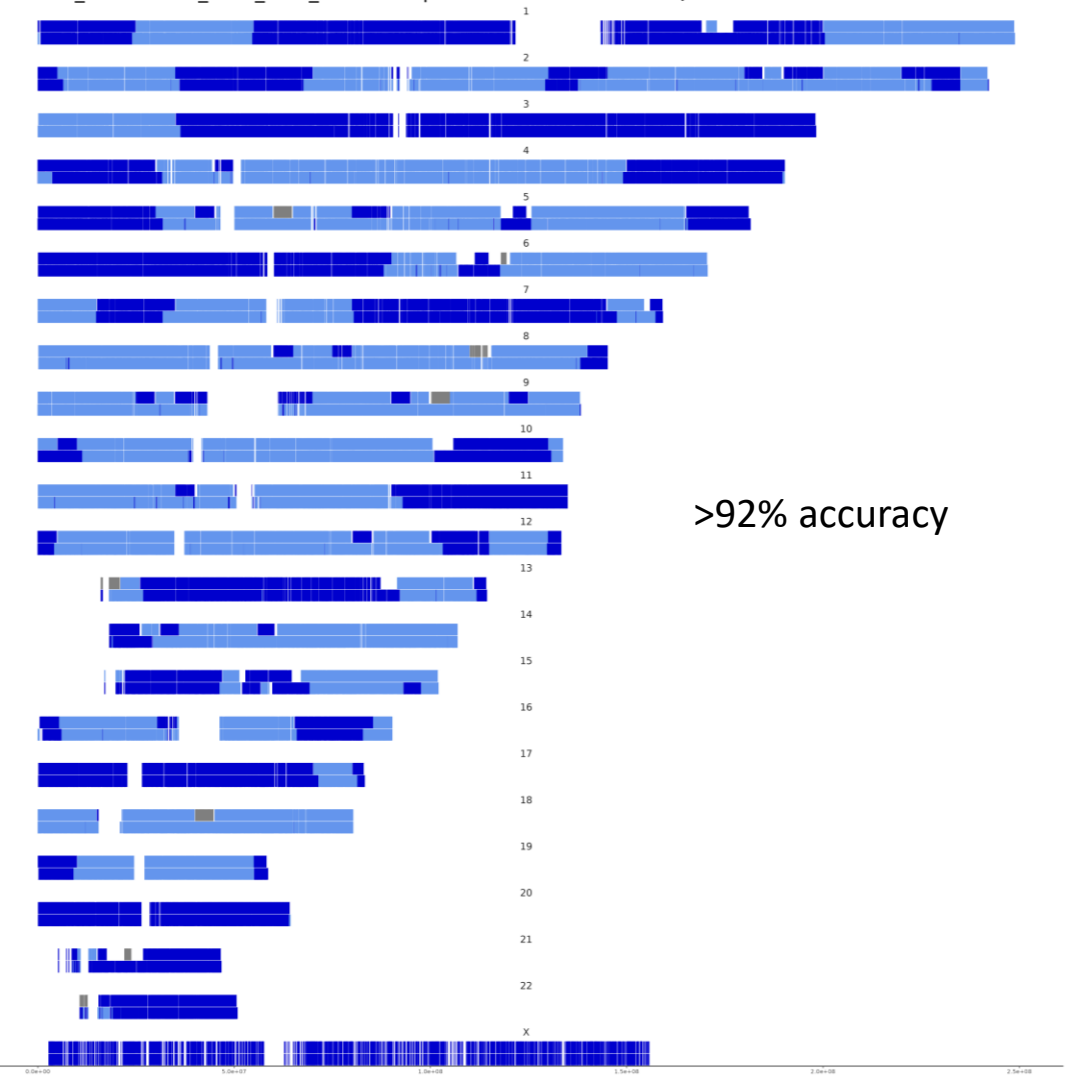
Anouk Janssen
PhD candidate



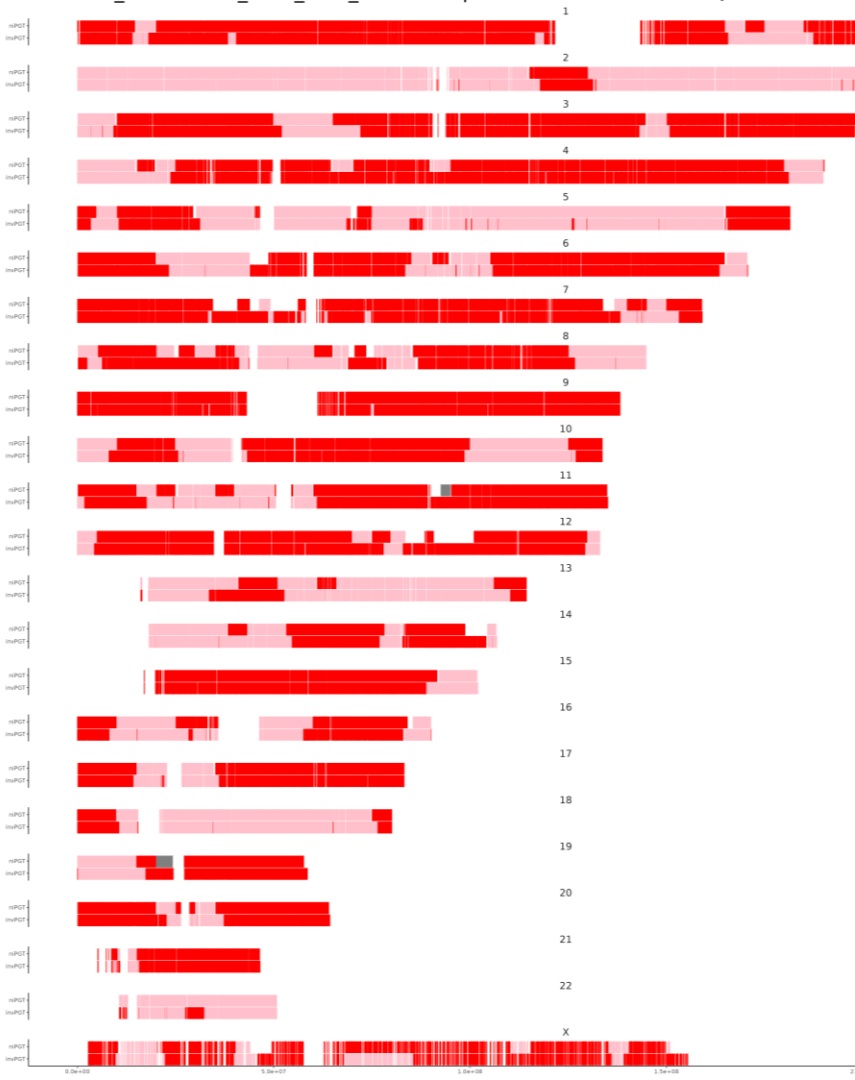




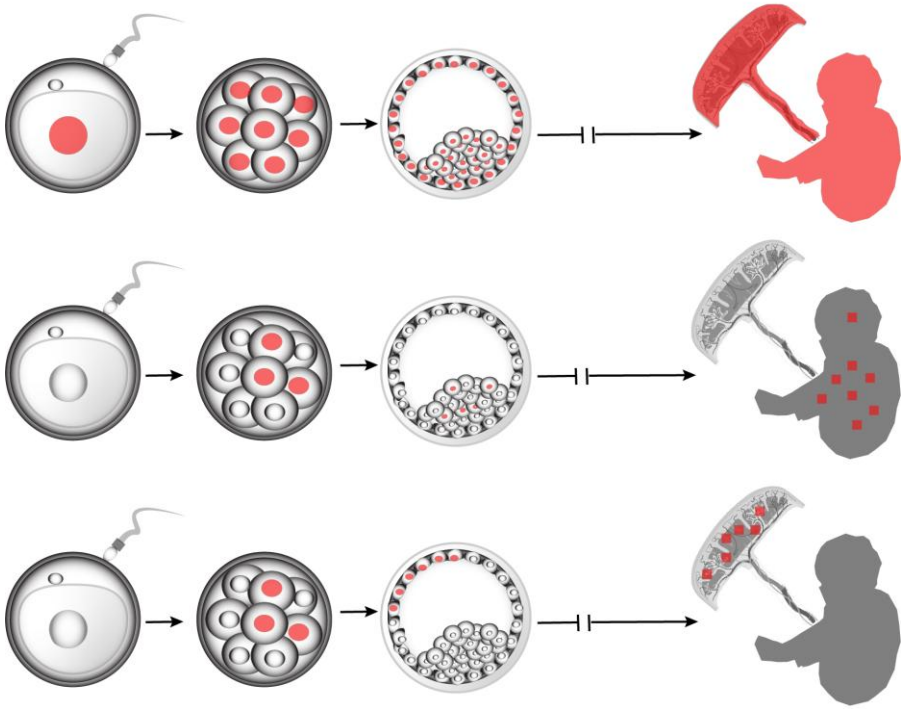
Janssen et al. unpublished



>92% accuracy



Conclusions (impact of PGT-AO)



Before fertilization, everywhere ➔ Failed Implantation Miscarriage

Janssen, […], Zamani Esteki, Nat. Com. 2024
Essers, […], Zamani Esteki, unpublished

After fertilization, fetus only ➔ Miscarriage

Essers, […], Zamani Esteki, Nat. Med. 2023

After fertilization, placenta only ➔ Healthy newborns

Zamani Esteki, et al, Nat. Med. 2019

Summary & Conclusions

All-in-one haplarithmisis-based PGT

Simplified workflow with automation

1. **PGT-M: Enhanced sequencing by even coverage**
   - Better testing in proximity of complex genomic regions
   - Direct mutation detection at base resolution
2. **PGT-AO: Parent's only PGT for aneuploidy origin**
   - Segregational origin: Identification origin of trisomies and UPDs
3. **PGT-SR:** Differentiation between balanced and chromosomally normal embryos at base resolution
4. **PGT-MT:** Mitochondrial heteroplasmy detection after WGA

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PGT NEDE
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KATHOLIEKE UNIVERSITEIT LEUVEN

Department of Human Genetics
Joris Vermeesch
Thierry Voet
Aspasia Destouni
Olga Tsuiko
Eftychia Dimitriadu
Sophie de Broek

University of Helsinki

Nina Kaminen-Ahola
Aila Tiitinen
Heidi Marjonen
Hanna Kahila
Viveca Söderström-Anttila
Anne-Maria Suikkari

Masoud Zamani Esteki
Principal investigator

See Masoud's vision here

Wanwisa van Dijk
Project manager & senior technician

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Internship student

NIPGT (fragmentome)

Sanne Romans
Internship student

NIPT-M

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BSc student

PGT-MT

Dimitra Christopikou
Visiting Scientist | PGT Specialist

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