# Assessing the Embryo Genome: Promise, Practice, and Peril

2026 PCRS ANNUAL MEETING
REPRODUCTIVE FRONTIERS:
BRIDGING BIOLOGY, PRACTICE, AND POSSIBILITY
MARCH 18-22 | RANCHO MIRAGE, CA

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# Disclosures

- Employee: Fairfax EggBank (full-time)
- Consulting Fee (e.g., Advisory Board): Mavin Clinic, LabCorp

# Learning Objectives

- Assess the clinical utility and validity of emerging technologies in human embryo testing
- Consider clinical, social, and ethical aspects of embryo whole genome sequencing
- Recognize the implications of embryo test results for stakeholders in third party reproduction including gamete donors and donor-conceived persons

# Agenda

- Introduction to PGT and WGS in embryos
- Clinical Utility of WGS in embryos
- Ethical and Clinical Considerations

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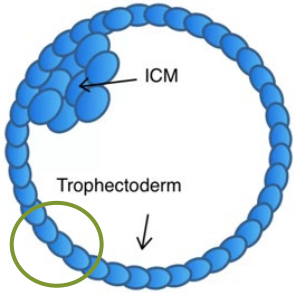
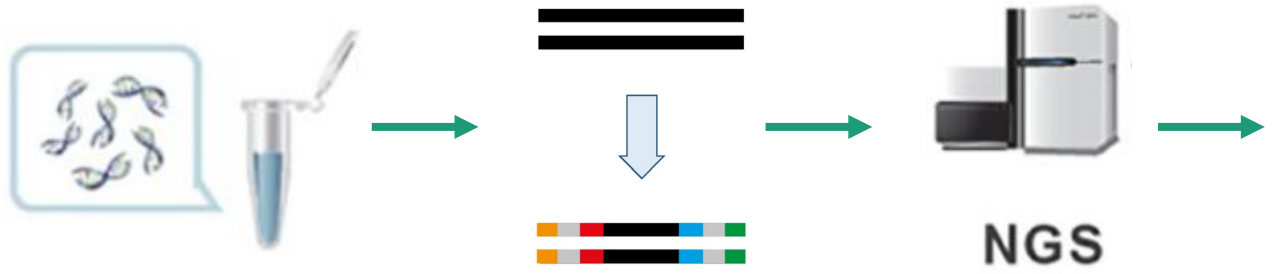
# Traditional PGT Technology

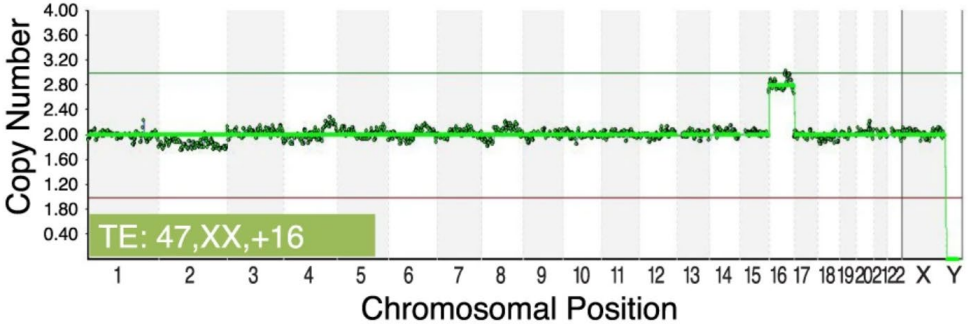
| Test | Indication | Method | Typically Detects |
|---|---|---|---|
| **PGT-A** | General aneuploidy screening | Low coverage sequencing | Whole chromosome imbalance.<br>May detect:<br>*Partial imbalance ≥10 Mb<br>*Possible mosaicism |
| **PGT-SR** | Gamete source with rearrangement (e.g. translocation) | Low coverage sequencing | Chromosome copy number variation ≥5 Mb |
| **PGT-M** | Known single gene condition/variant | Linkage analysis with or without direct mutation analysis | Presence/absence of variant(s) of interest |

# NGS-Based PGT-A




Biopsy




DNA isolation and whole genome amplification (e.g. SurePlex)

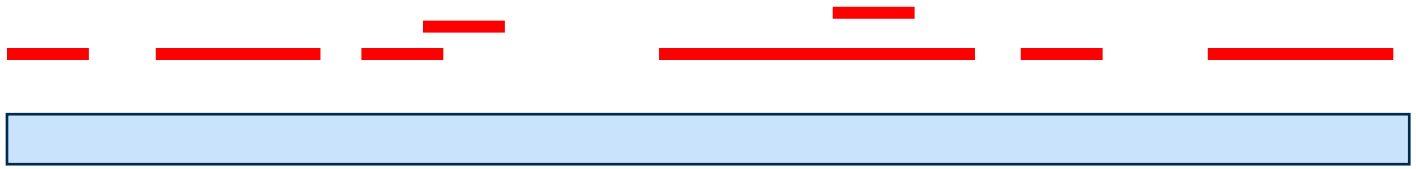
Library preparation

Low coverage sequencing

Bioinformatic Analysis

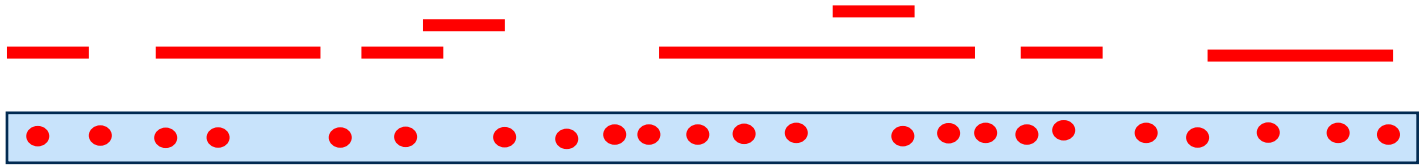
# PGT-A with the addition of SNP Analysis

PGT-A via NGS (no SNP)



Chromosome

PGT-A via genome-wide SNP analysis



Chromosome

NGS based PGT-A detects:

- Whole copy number changes
- Segmental aneuploidy
- Potential mosaicism

SNP based PGT-A additionally detects:

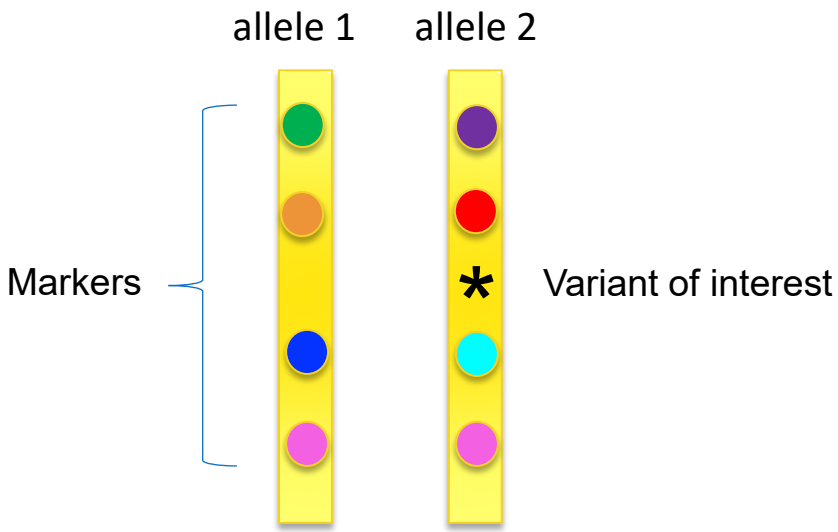
- Polyploidy
- Uniparental disomy (UPD)
- Cumulus cell contamination
- Cohort QC (embryo sibship)
- PN number

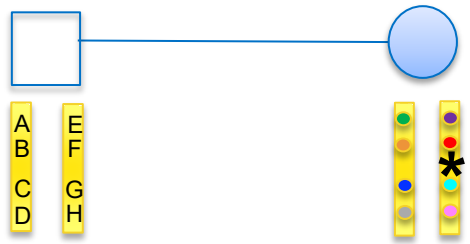
With parental samples:

- Parent of origin
- Source of aneuploidy

# PGT-M traditionally requires test development

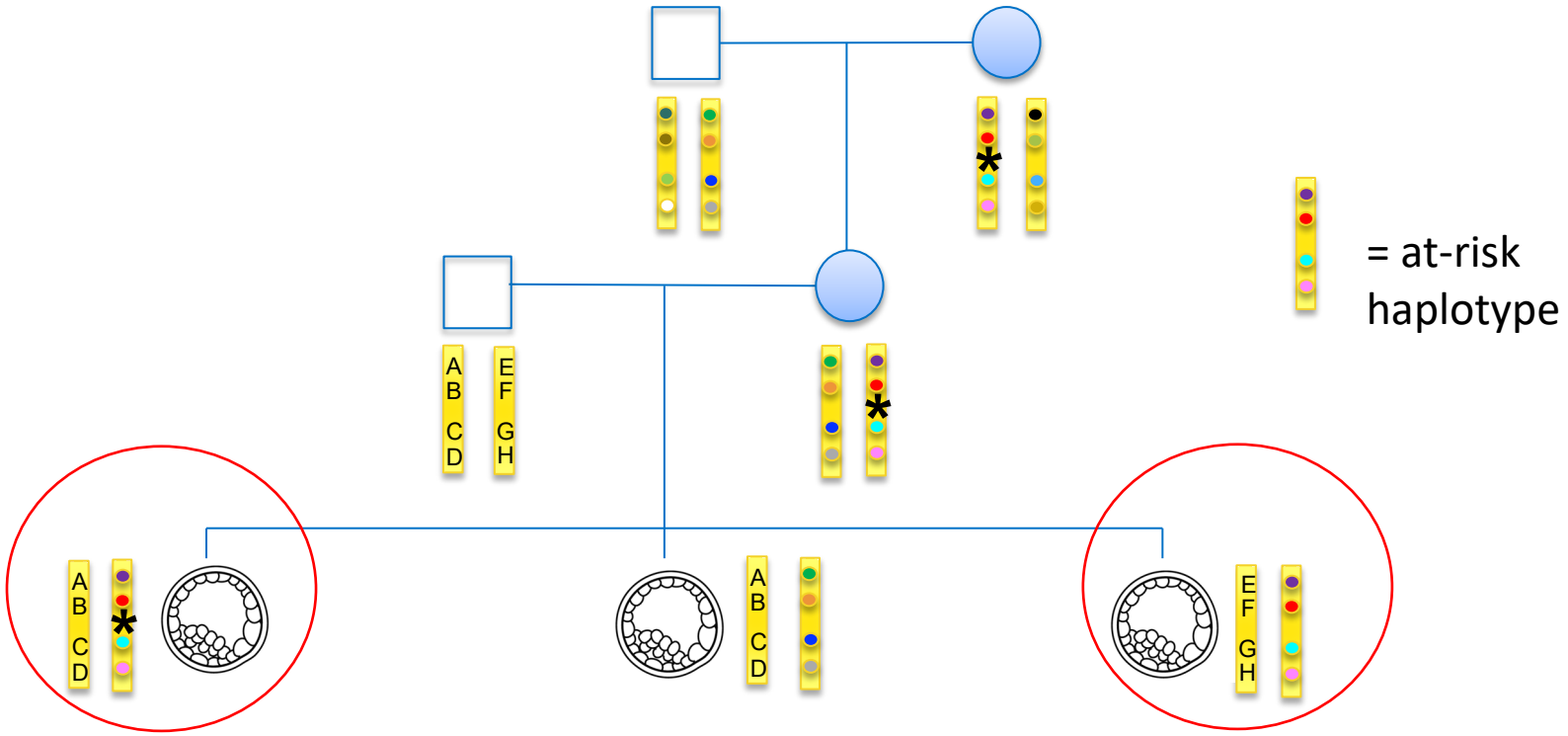
Set of custom-made primers for direct detection of variant(s) and linked markers (at-risk haplotype). Unique for each couple/family. Typically takes weeks to complete.



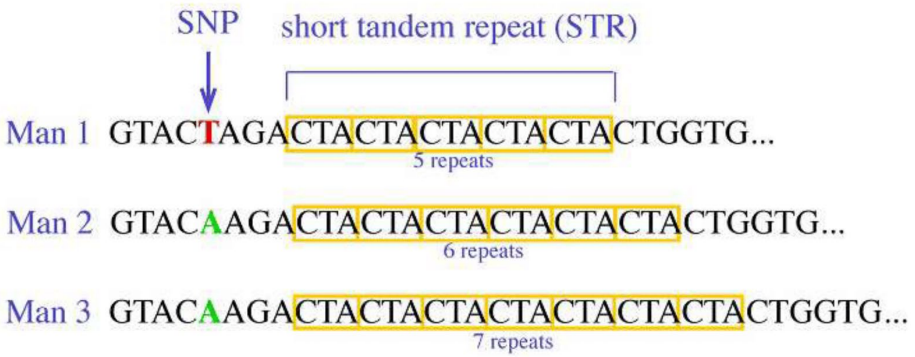

Markers must be informative for PGT-M to work

# PGT-M with linkage analysis



# PGT-M moved to using SNPs for markers

**Markers**: either short tandem repeats (**STR**) or single nucleotide polymorphisms (**SNP**)



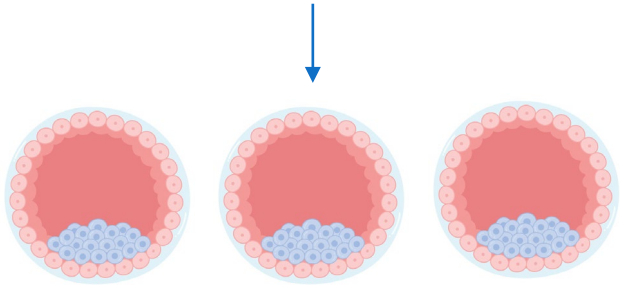

| STRs | SNPs |
|---|---|
| • Present in less frequently.<br>• Require custom set up for each family.<br>• May not always be informative.<br>• Higher risk of allele drop out. | • Present in higher frequency across genome.<br>• No custom set up required.<br>• Allows simultaneous PGT-A and PGT-M. |

# PGT for Polygenic Conditions (PGT-P)

- Assessment of thousands of SNPs by statistical models.
- Based on GWAS in adults comparing SNPs in affected and control samples.
- Combined into polygenic risk scores (PRS) for common, multifactorial conditions.
- Embryos ranked based on probability of risk.

Family history of multifactorial conditions:

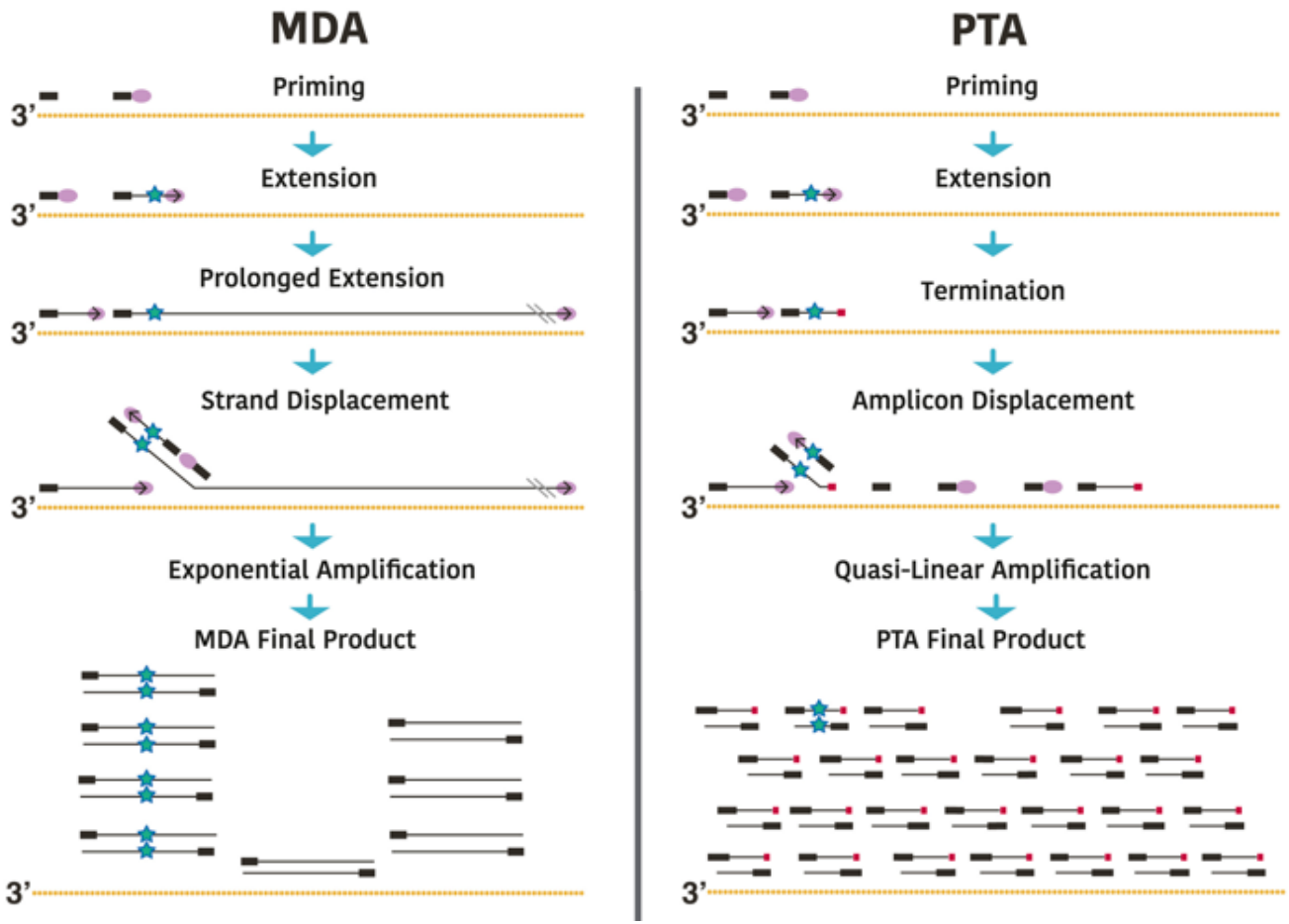
- Diabetes
- Alzheimer's disease
- Schizophrenia



All embryos essentially at risk
PRS theoretically offers modest reduction in risk

Treff, N. R., *et. al.* (2019). Utility and First Clinical Application of Screening Embryos for Polygenic Disease Risk Reduction. *Frontiers in endocrinology*, *10*, 845.

# Primary Template-Directed Amplification (PTA)




Significantly improved genome sequencing coverage and variant detection from a single cell!

V. Gonzalez-Pena *et. al.*, Accurate genomic variant detection in single cells with primary template-directed amplification, Proc. Natl. Acad. Sci. U.S.A. 118 (2021).

# Whole Genome Sequencing (WGS) on Embryos

Advanced technology that enables:

- More comprehensive coverage of genomic regions
- Lower allele drop out and miscall rates
- Higher number of SNPs and increased sensitivity
- Ability to perform “universal PGT”
- **Ability to detect de novo variants**

# WGS on Embryos

- **PGT-A/SR** with ability to include:
  - ✓ Segmental aneuploidy (> 2Mb)
  - ✓ Microdeletions/Microduplications (>400 kb)
  - ✓ Triploidy
  - ✓ Uniparental disomy (UPD)

- **PGT-M**
  - ✓ Known and de novo genetic variants
  - ✓ Use direct detection - no probe design. Reduces TAT and potentially eliminates use of relatives' samples

- **PGT-P**
  - ✓ Predisposition screening for multifactorial conditions

# WGS on Embryos may include:

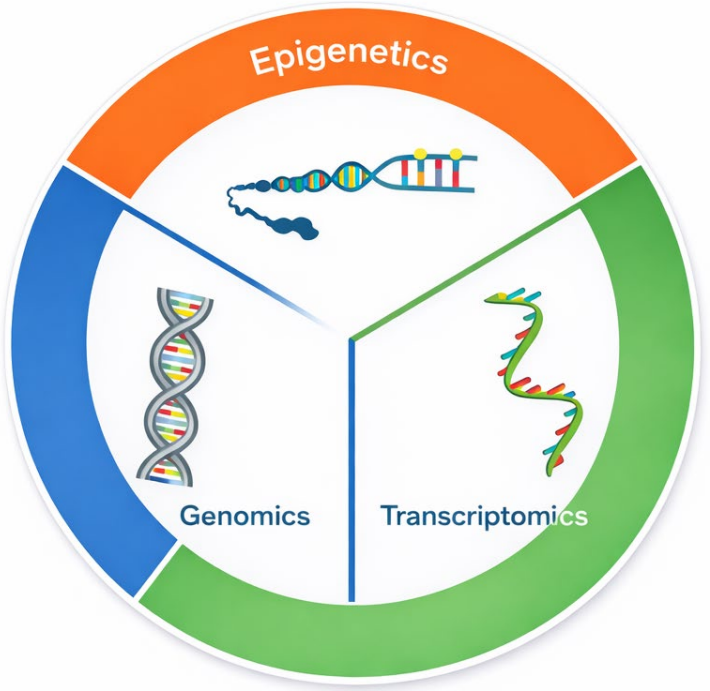
- **Preset panels**, examples:
  - ✓ Hereditary cancers, cardiovascular diseases, neurodevelopmental disorders, skeletal dysplasias
  - ✓ Not limited to recessive and X-linked
  - ✓ May include mitochondrial DNA

- **Assessment of reduced viability variants**
  - ✓ Use whole genome trio and transcriptome (RNA) sequencing
  - ✓ Prioritize embryo transfer based on the highest chance for viabilty

- **Assessment of methylome**
  - ✓ Provides risk stratification based on overall methylation signal

# Universal PGT?

- Now becoming possible with advanced DNA amplification, WGS, and trio testing
- PGT-WGS vs. PGT-G*
- Future direction is likely multi-omics embryo profiling: simultaneously analyzing DNA (genome), RNA (transcriptome), and epigenetic markers (methylome)




Multi-Omics Embryo Profiling

*Grushcow, Jeremy *et al.* (2025). Whole genome and transcriptome sequencing of embryo biopsies may better facilitate transfer decisions by identifying more euploid embryos and fewer false-positive segmental and/or mosaic embryos. *Fertility and Sterility*, Volume 124, Issue 6, e66 - e67

# Possible indications for WGS on Embryos

- Technically challenging PGT-M and PGT-SR indications
- Repeat failed euploid transfers
- Increased paternal age: de novo variant assessment
- Family history of multifactorial conditions
- Desire for improved PGT technology
- **Information seeking – just want to know more!**

# Technical Limitations of WGS on Embryos

No, or limited detection for:

- Trinucleotide repeats  (e.g. FMR1)
- Intragenic inversions (e.g. common inversion in F8)
- Pseudogenes (e.g. CYP21A2)
- Homopolymer regions
- Low coverage genes
- Methylation analysis*
- Balanced rearrangements*

*Currently pending validation

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- Clinical Utility of WGS in embryos
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# What is the Clinical Utility of WGS in Embryos?

For a genetic test to have clinical utility, it must demonstrate:

- ✓ **Analytical validity:** accurately determine the genotype of interest
- ✓ **Clinical validity:** accurately predict a phenotype

Raf Winand, Kristien Hens, Wybo Dondorp, Guido de Wert, Yves Moreau, Joris Robert Vermeesch, Inge Liebaers, Jan Aerts, *In vitro* screening of embryos by whole-genome sequencing: now, in the future or never?, *Human Reproduction*, Volume 29, Issue 4, April 2014, Pages 842–851.

# Several Validation Studies

Article | Open access | Published: 21 March 2022

**Whole-genome risk prediction of common diseases in human preimplantation embryos**

Akash Kumar, Kate Im, Milena Banjevic, Pauline C. Ng, Tate Tunstall, Geronimo Garcia, Luisa Galhardo, Jiayi Sun, Oren N. Schaedel, Brynn Levy, Donna Hongo, Dusan Kijacic, Michelle Kiehl, Nam D. Tran, Peter C. Klatsky & Matthew Rabinowitz

*Nature Medicine* **28**, 513–516 (2022) | Cite this article

Eur J Med Genet. 2019 Aug;62(8):103647. doi: 10.1016/j.ejmg.2019.04.004. Epub 2019 Apr 23.

**Validation of concurrent preimplantation genetic testing for polygenic and monogenic disorders, structural rearrangements, and whole and segmental chromosome aneuploidy with a single universal platform**

Nathan R Treff [1], Raymond Zimmerman [2], Elan Bechor [2], Jeff Hsu [2], Bhavini Rana [2], Jens Jensen [2], Jeremy Li [2], Artem Samoilenko [2], William Mowrey [2], James Van Alstine [2], Mark Leondires [3], Kathy Miller [3], Erica Paganetti [3], Louis Lello [4], Steven Avery [4], Stephen Hsu [4], Laurent C A Melchior Tellier [2]

Article | Open access | Published: 02 March 2020

**Genome sequencing of human *in vitro* fertilisation embryos for pathogenic variation screening**

Nicholas M. Murphy, Tanya S. Samarasekera, Lisa Macaskill, Jayne Mullen & Luk J. F. Rombauts

*Scientific Reports* **10**, Article number: 3795 (2020) | Cite this article

**15k** Accesses | **26** Citations | **38** Altmetric | Metrics

Nat Commun. 2024 Sep 2;15:7164. doi: 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,#], Rebekka M Koeck [1,2,#], Rick Essers [1,2,#], 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,✉]

**ABSTRACT ONLY** · Volume 124, Issue 6, Supplement , E321-E322, December 2025 Fertility and Sterility, Volume 124, Issue 6, e321 - e322

VALIDATION OF A PREIMPLANTATION GENETICS TEST USING WHOLE GENOME SEQUENCING (PGT-WGS) FOR THE PURPOSE OF DETECTING INHERITED AND DE NOVO PATHOGENIC VARIANTS

Santiago Munne, PhD [1] · Nick M. Murphy, Ph.D [2]

**ORIGINAL ARTICLE** · Volume 5, Issue 1, P63-71, March 2024 · *Open Access* Download Full Issue

The first clinical validation of whole-genome screening on standard trophectoderm biopsies of preimplantation embryos

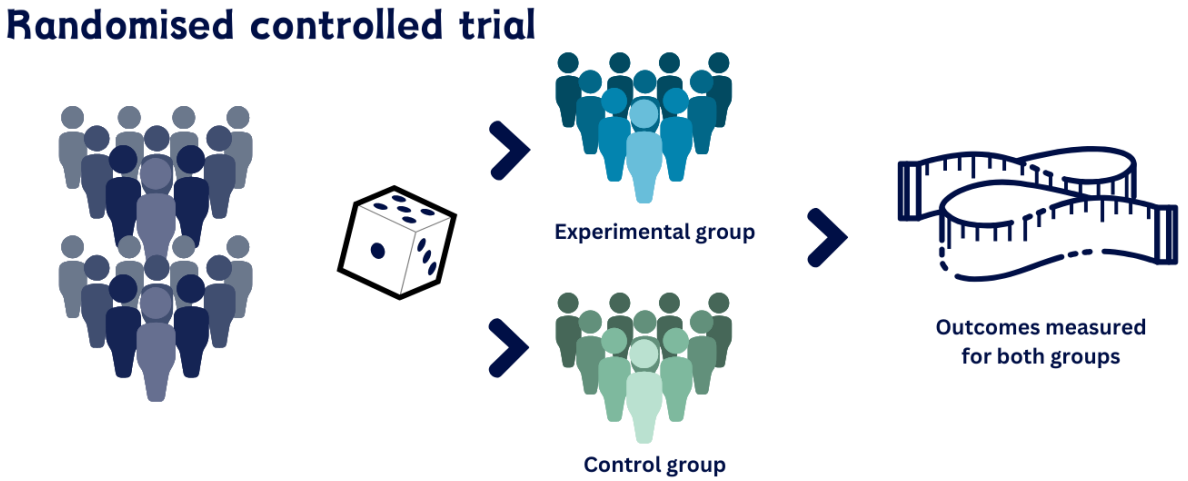
Yuntao Xia, Ph.D. [a] ✉ · Maria Katz, M.Sc. [a] · Dhruva Chandramohan, Ph.D. [a] · ... · Barry Behr, Ph.D. [c] · Jacques Cohen, Ph.D. [d] · Noor Siddiqui, M.Sc. [a] ... Show more

# Analytical Validation

- Typically involves comparing results of WGS to reference genome, samples with known genetic status, or the whole embryo.
- Studies include validation for chromosomal CNV as well as single gene variants.
- Overall averages vary by platform:
    - ✓ Sensitivity 92->99%
    - ✓ Specificity ~99.9%
    - ✓ Positive predictive value ~97-98%

# Clinical Validation

- More challenging since most “abnormal PGT” embryos are not transferred
- May require randomized controlled trials and/or blinded transfers

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# Clinical Validation Studies

- Ultimate goal is to confirm that:
  - ✓ Variant(s) of interest is present in resulting pregnancy/child
  - ✓ Variant(s) of interest is associated with a predetermined risk (e.g. risk of disease or risk of pregnancy loss) relative to control

# De Novo Variants

- An estimated 74 de novo SNP mutations are introduced at embryogenesis
- In one embryo, ~0-2 may be pathogenic
- Trio sequencing is crucial in assessment

Questions:

1. What is the chance that a de novo variant is a true positive?
2. Is the genotype-phenotype relationship of the de novo variant with disease status clear?


1. Acuna-Hidalgo, R. *et al*. Post-zygotic Point Mutations Are an Underrecognized Source of De Novo Genomic Variation. *The American Journal of Human Genetics* **97**, 67–74, (2015).
2. Kondrashov, A. S. Direct estimates of human per nucleotide mutation rates at 20 loci causing mendelian diseases. *Human Mutation* **21**, 12–27, (2003).
3. Acuna-Hidalgo, R., Veltman, J. A. & Hoischen, A. New insights into the generation and role of de novo mutations in health and disease. *Genome Biology* **17**, 241, (2016).

# De Novo Variants

- Examples:
  - Pathogenic variant in FBN1, associated with Marfan Syndrome, versus
  - Likely pathogenic variant in TNFRSF13B associated with Common Variable Immunodeficiency 2. Variant is associated with AR and AD forms and has documented reduced penetrance.

# Reduced Viability Variants

- ~50-70% of euploid transfers result in an ongoing pregnancy and livebirth
- Identification of reduced viability variants (RVV) may improve outcomes by offering means of prioritization
- RVV are based on variants associated with fetal lethality, spontaneous pregnancy loss, intrauterine fetal demise, stillbirth, and neonatal mortality.

Questions:

1. What confirmation exists in association with reduced viability of embryos?
2. How does that compare to euploid embryos without RVV detected?

Ready et. al. (2025). Whole genome and transcriptome sequencing identifies variants associated with reduced embryo viability, providing an opportunity to improve embryo selection and clinical outcomes. *Fertility and Sterility*, Volume 124, Issue 6, e67

# Polygenic Risk Scores

- Clinical validation may require longitudinal studies following health of IVF-born children
- Validation is needed to demonstrate utility of PRS among “sibling embryos”
- Most GWAS were based on subjects with European ethnicity
- Antagonistic pliotropy
- ACMG does not currently recommend using PRS

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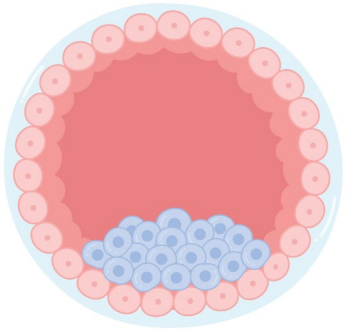
# WGS on Donor-Conceived Embryos

Egg donor



Recipient's embryo

WGS Results

Multiple recipients

- Indirectly assess genetic information on anonymous donors
- Results relevant to donors and donor-conceived persons

# Gene Panels included in WGS

Depending on lab, can be a predetermined panel or whole exome/genome analysis

Panels are curated based on:

- Published guidelines (e.g. ACMG secondary findings)
- ClinGen classification in terms of association with disease
- Available panels on markets, like carrier screening or diagnostic panels
- Other

# Gene Panels included in WGS

- Guidelines genes don't always have clear genotype-phenotype relationships

| Gene Name | Associated Diseases | ClinGen Classification |
|---|---|---|
| MCCC2 | 3-methylcrotonyl-CoA carboxylase deficiency | Definitive |

  - Definitive relationship with disease ≠ pathogenicity
  - Most compound heterozygotes or homozygotes aren't clinically affected
  - Of note, MCCC2 is also part of ACMG Tier 3 carrier screening panel

- ACMG secondary findings genes may have low penetrance
  - HFE – hemochromatosis, most common variants are mild
  - MYH7 → hypertrophic cardiomyopathy, variable expressivity

# Gene Panels included in WGS

- Recessive genes with potential “manifestation of symptoms”
  - Examples: GBA and Parkinson’s
  - Will decisions on transfer be based on this information?

- Rare variants with no published case studies implicating pathogenicity
  - These can still be classified as “likely pathogenic”

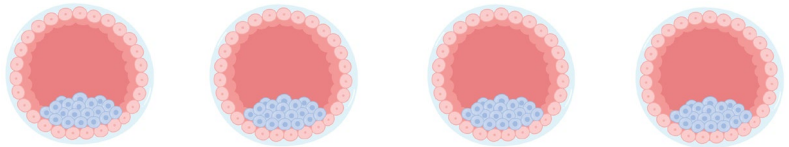
# WGS on Embryos – Polygenic Risk Scores

- “Procreative beneficence’’: an ethical obligation to select the embryo(s) with the best chance of the best life.
- What constitutes the ‘‘best life?”
- Parental expectations and quality of parent-child relationship
- Potential devaluation of individuals with disability

Houtz C, Largent E. Pursuing the best life in the age of preimplantation whole genome sequencing: ethical considerations for preimplantation genetic testing. Fertil Steril. 2025 Nov 1;124(5 Pt 1):902-903.

# WGS on Embryos – May not be for all patients

- Patients have long reproductive journeys. Emotionally, physically, & financially exhausting.
- More testing means less embryos available to transfer.
- Complicated results = Agonizing decisions on embryo transfer.
- Feelings of guilt about passing down disease.
- We must manage expectations regarding a perfectly healthy baby.



| 46,XX | 47,XX,+5 | 47,XX,+7 (LM) | 46,XY |
|---|---|---|---|
| HFE carrier | Not carrier | Not carrier | Not carrier |
| Low PRS | Low PRS | Low PRS | High PRS |

Raf Winand, et.al. (2024). , *In vitro* screening of embryos by whole-genome sequencing: now, in the future or never?, *Human Reproduction*, Volume 29, Issue 4, April 2014, Pages 842–851.

# WGS – Informed Consent and Transfer Decisions

- Pre- and post-test counseling.
  Which counseling service to use?
  - ✓ Lab vs clinical GC: different scopes of practice

- Are the clinic's transfer policies communicated upfront?

# WGS on Embryos - Barriers for use

**Expensive!**

- $2,500 - $10,000 per embryo
- Not currently covered by insurance

**Limited accessibility**

- Select labs
- Select clinics

# Conclusions

- WGS on embryos offers an exciting, new alternative to traditional PGT methods of analysis.
- As the technology evolves, it will continue to present complex scenarios for results interpretation and risk management.
- At this time, more validation is needed to justify clinical utility.
- Third party reproduction has additional challenges such as the need for disclosure of new genetic information to other recipients/donor-conceived persons.
- In all scenarios, focus should be on availability of services to facilitate patient-centered care.

# Thank you!

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