Complete genome sequencing of embryos

Santiago Munné

Affiliations and Disclosures

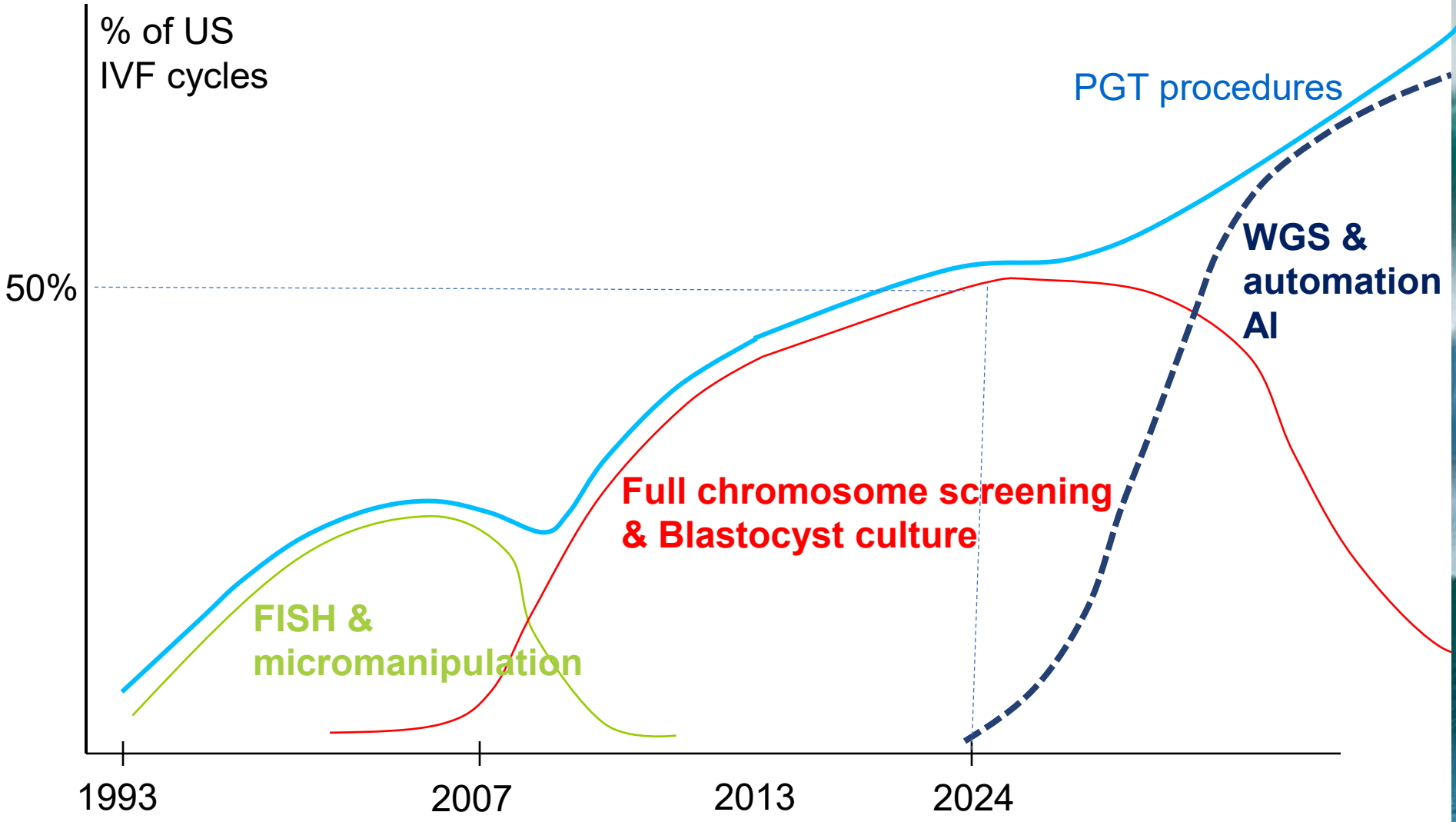
Scientific Director @ **Progenesis**

President, founder @ **HoMu Health Ventures**

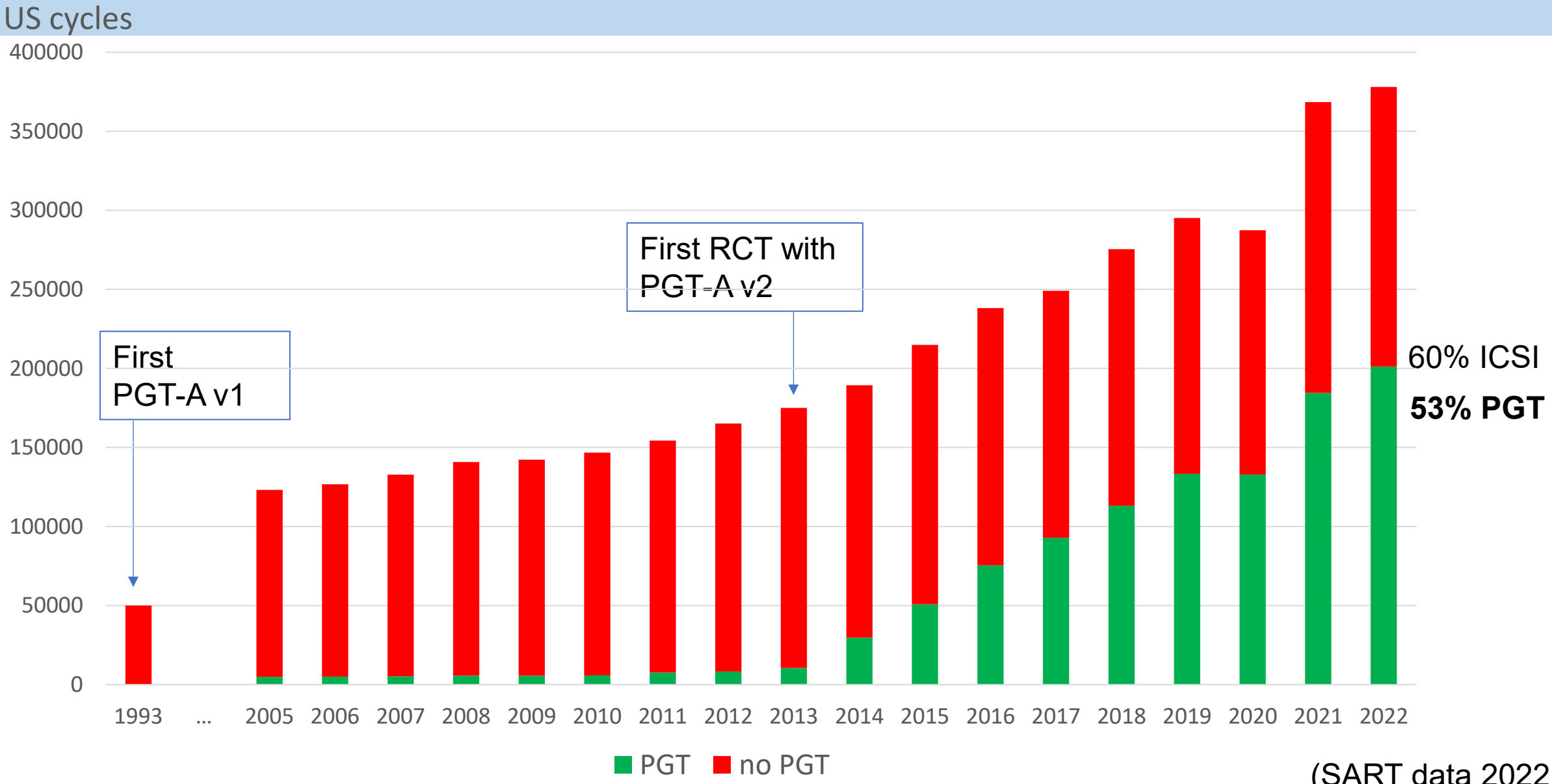
Chief Innovation Officer, founder @ **Overture Life**

BOD @ **GenEmbryomics**, **Butterfly Bio**, **Sama, Ovum Health**

Waves of technology



PGT usage: in the US is the standard of care



(SART data 2022)

My journey through PGT

Genetics before PGT:
Not actionable

1993 - 2012
PGT-A v1:
Actionable
Needed skills
More depth

2013- 2020
PGT-A v2:
Genetics automated
IVF and Biopsy was not
PGT-A is boring

2020-2025
PGT-WGS, PGT-P
Fun again
Not enough eggs

TBC

2025
Lab automation / non invasives:
IVF lab automated
Non-invasives still need work

Comprehensive PGT methods

SNP ARRAYS GENOTYPING

- **Karyomapping** (1)
- **Haplarithmisis** (2)
- **High throughput SNP array** (13)
- HaploSeq
- APCAD (4)
- Genome prediction PGT-PS (3)
- Haplotype-Aware (5)
- Whole genome prediction (6)

GENOTYPE BY SEQUENCING

- **PGT Complete** (12)
- **Targeted NGS**
- One PGT (7)
- Genotyping by sequencing (8)
- Chen et al. (9)
- HaploPGT (10)
- S-HaploSeek (11)

SEQUENCING + ADDING SNPs

- **PGT-Seq**
- OneGene PGT (14)

(1) Handyside et al. J. Med. Genet. 47, 651–658 (2010); (2) Zamani Esteki, et al. , Am. J. Hum. Genet. 96, 894–912 (2015). (3): Treff et al. (2019) Fronteers Endocrinol (4) Verdyck et al. 2022, (5) Ariad et al. (2021) . (6) Kumar (2022) Nature Medicine 28:513–516, (7) Masset et al. Hum. Reprod. 34, 1608–1619 (2019); (8) Masset et al. (2022) Nucleic Acid Res, (9) Chen et al. (2020) Human Reprod; (10) Xie et al. (2022) Human Reprod 37:2546–59.(11) Backenroth et al. (2023) Nature Scientific Reprots 13:18036 (12) Buldo-Licciardi et al. 2020. ASRM; (13) Treff et al. Eur J Med Genet. 2019;62: 103647. (14) Hornak et al(2024) JARG

Genotyping:
High throughput SNP arrays

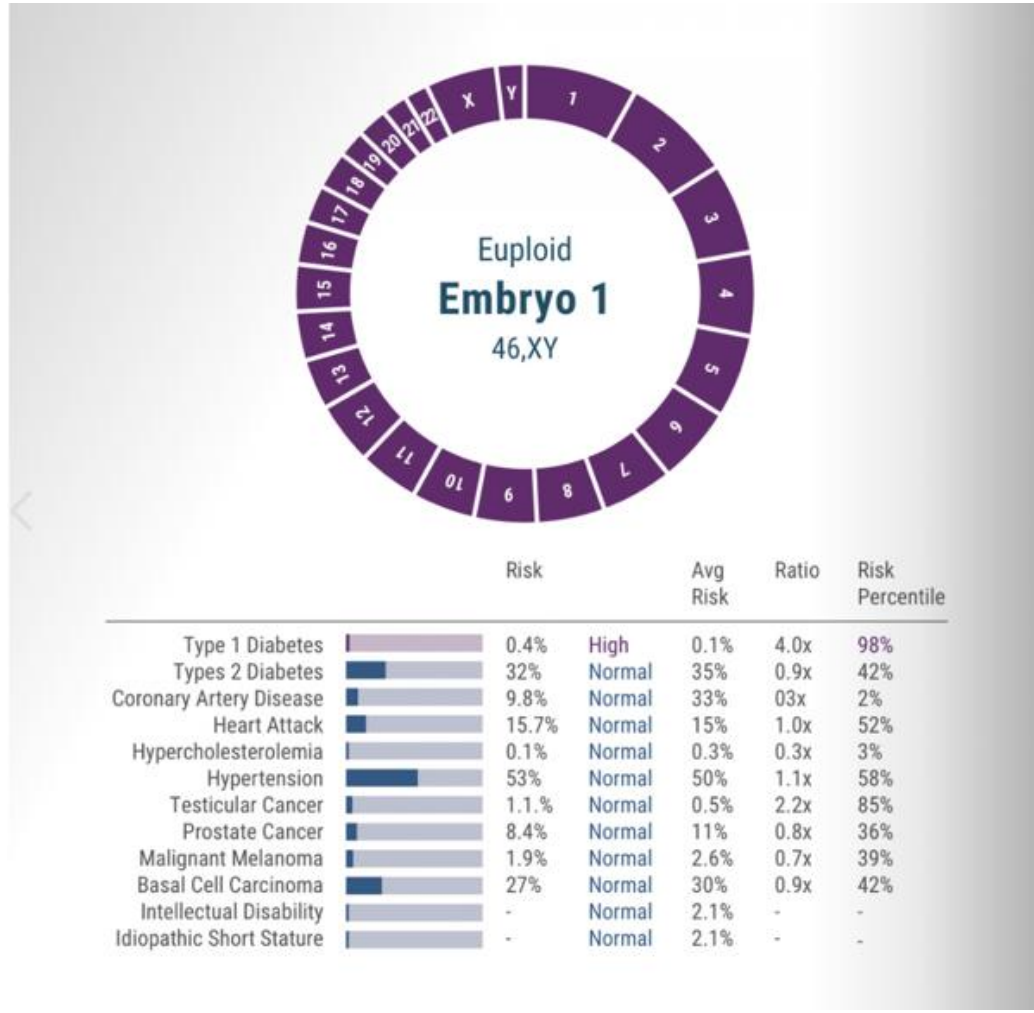
High throughput SNP microarray (800K SNPs)

PGT-A: quantitative copy number analysis and **qualitative** by genotyping: with **99.3% accuracy** (1) and detects **all polyploidies** and UPD (2)

PGT-M: using **parental DNA** it does **linkage analysis** and detects 20-500 SNPs within 2M window of the gene defect

PGT-SR: differentiates **Normal from Balanced**, with a 10Mb resolution (1)

PGT-P: for several diseases, with AUC of 0.65-0.75



	Risk		Avg Risk	Ratio	Risk Percentile
Type 1 Diabetes	0.4%	High	0.1%	4.0x	98%
Types 2 Diabetes	32%	Normal	35%	0.9x	42%
Coronary Artery Disease	9.8%	Normal	33%	03x	2%
Heart Attack	15.7%	Normal	15%	1.0x	52%
Hypercholesterolemia	0.1%	Normal	0.3%	0.3x	3%
Hypertension	53%	Normal	50%	1.1x	58%
Testicular Cancer	1.1.%	Normal	0.5%	2.2x	85%
Prostate Cancer	8.4%	Normal	11%	0.8x	36%
Malignant Melanoma	1.9%	Normal	2.6%	0.7x	39%
Basal Cell Carcinoma	27%	Normal	30%	0.9x	42%
Intellectual Disability	-	Normal	2.1%	-	-
Idiopathic Short Stature	-	Normal	2.1%	-	-

(1) Treff et al. (2019) Fronteers Endocrinol.; Leahy et al.. JARG 41: 121-126; (2): *(2) Kratka et al. (2023) F&S science,* ***Diego Marin in da room***

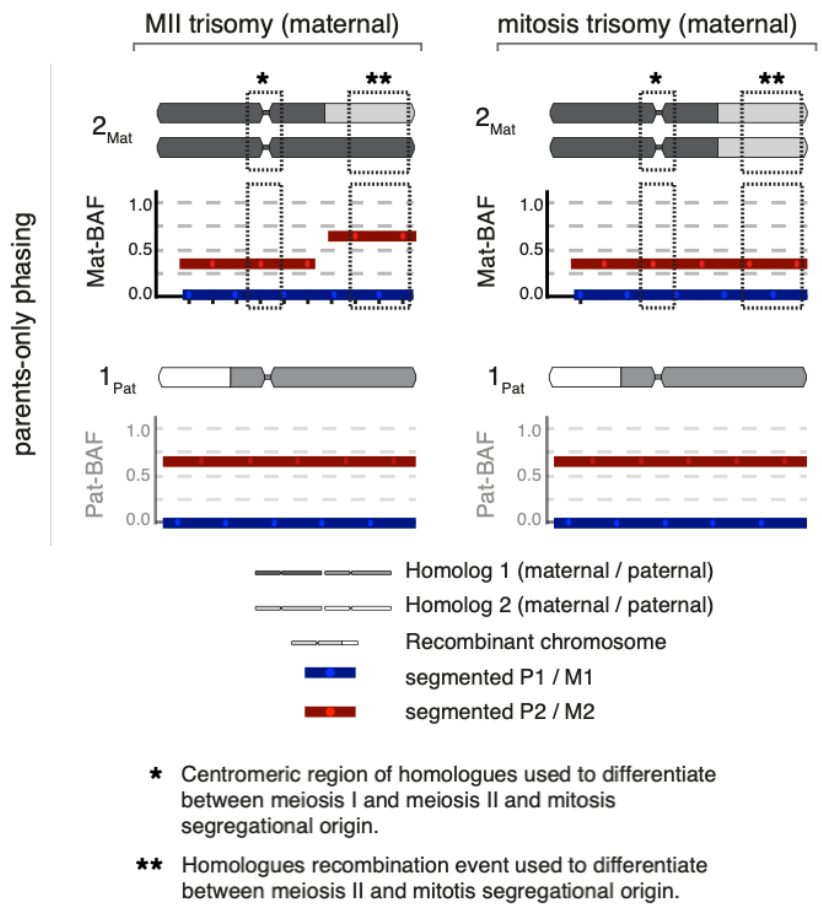
Genotyping by sequencing: Haplorithmisis

METHOD:

- MDA amplification
- Parental and embryo DNA is **sequenced at x10 depth**
- haplotyping analysis using **HAPLORITHMISIS**

RESULTS:

- 80% genome coverage
- **PGT-M**: **direct mutation for 82%** embryos and rest by haplotyping
- **PGT-A**: Detects **mitotic origin** of mosaicism, all **triploids**
- **PGT-SR**: differentiates **balanced from normal**

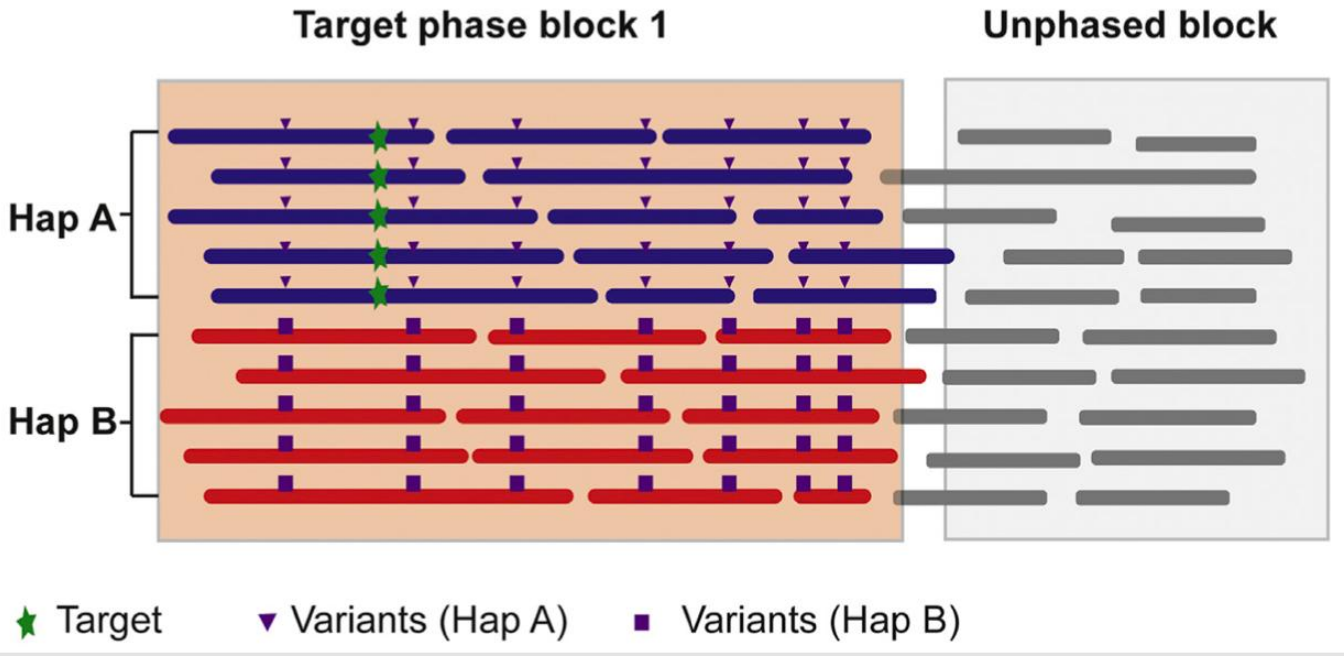


Jansen et al. (2023) MedRxiv

HaploPhasing by Long Read sequencing

- **Long read sequencing** of the **parents** provides parental variant maps
- **Tiling of variants** allows SNP phasing around the target sequence.
- **PGT-A**: standard method
- **PGT-M and PGT-SR**: through SNP PCR for mutation or or breakpoint. No proband needed

HaploPhasing:



NextSeq, 8M reads.

Cheng et al. (2021) Fertil. Steril. 116:774-783

Why do WGS?

***De novo* mutations** are not detectable in the parents.

Rare Diseases and de novo mutations

- There are **20,000 genes** coding for proteins
- **10,000 genetic diseases** have been identified, of which **>7000 are rare**[1]
- The basis for **these rare diseases** can be identified by **whole exome sequencing**[2]:

  32% in non-consanguineous patients

  50% in consanguineous patients

- **300 million people** worldwide have a **rare disease**[3,4]
- Over **80% of severe developmental disorders** are caused by ***de novo* mutations**

(1) Boycott et al. 2013; (2) Quaio et al. (2020), (3): Frederiksen et al. (2022); (4) Nguengang Wakap et al (2019) EJHG 28:165-173

40% more congenital abnormalities in ART

PROGENESIS

IVF babies have **40% more congenital abnormalities** than naturally conceived babies.

Reasons:

- **higher parental age** of IVF patients
- **underlying mutations** causing infertility.

ginal Sound for Musicians: Off ● Recording

The Fetal Medicine Foundation

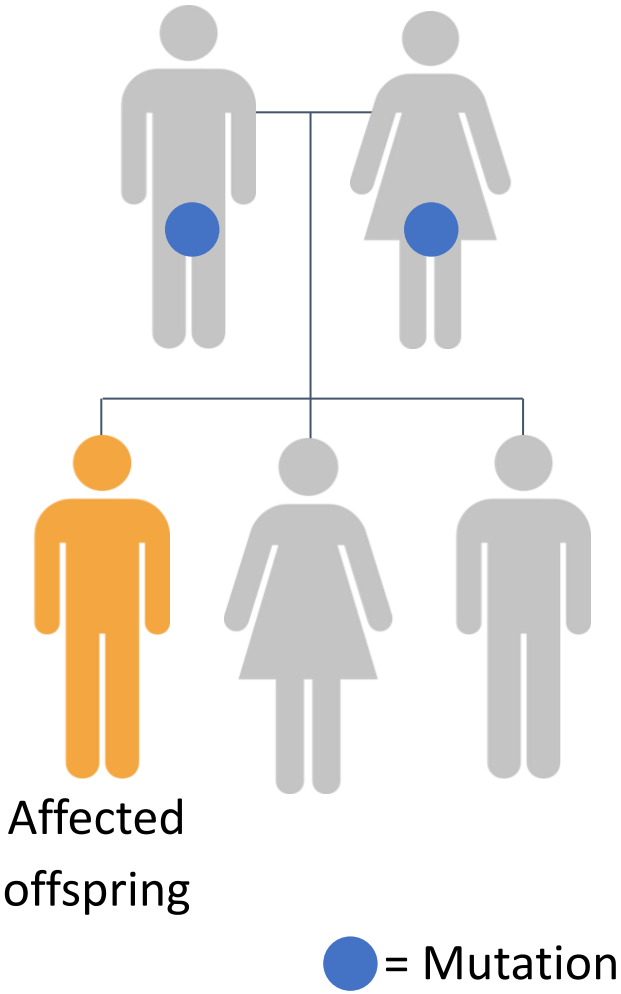
Congenital anomalies ART vs natural

Meta-analyses	Articles	ART infants	Pooled estimate (95% CI)
Wen *et al* (2012)	46	124,468	1.37 (1.26, 1.48)
Hansen *et al* (2013)	45	92,671	1.32 (1.24, 1.42)
Zhao *et al* (2018)	46		1.40 (1.31, 1.49)
Hoosnan *et al* (2017)	30		1.99 (1.87, 2.11)
- Cardiac defects			1.43 (1.27, 1.62)
- CNS defects			1.36 (1.10, 1.70)
- Urogenital defects			1.58 (1.28, 1.94)
- Musculoskeletal			1.35 (1.12, 1.64)
- Chromosomal			1.14 (0.90, 1.44)
Giorgione *et al* (2018)	8	25,856 CHD	1.45 (1.20, 1.76)
Zhao *et al* (2019)	46	112,913	1.43 (1.31, 3.52)

ARTs increase risk of birth defects by about 40%

De novo mutations (DNV)

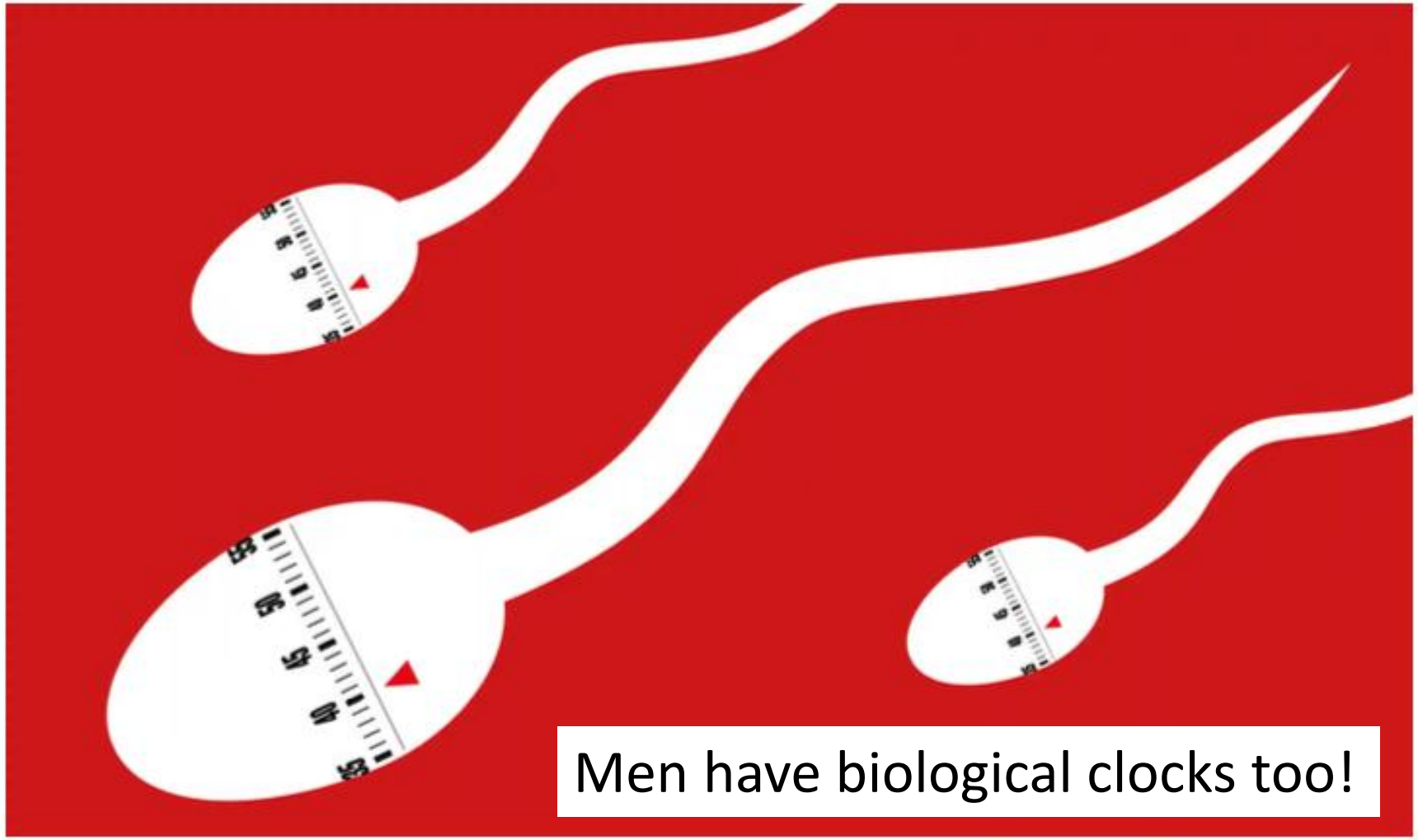
- DNV mutations accumulate in the parents' gametes and are **not detectable by carrier screening.**
- There are **74 DNV mutations per embryo: 1-2 pathogenic** (1-3)
- DNV mutations are **more detrimental** than inherited mutations not exposed to natural selection (11)
- DNV mutations **increase with paternal age** (4). Fathers 45 years old have x3.5 risk of autististic (5,6) and x27 risk of bipolar offspring than 25 years old (7).
- **1/488 children are born with a DNV mutation causing developmental malformation** (8,9), Autism (10), ….



(1) Acuna-Hidalgo et al. (2015) Am J Human Genet 97, 67–74, (2) Kondrashov (2003) Human Mutation 21, 12–27, (3) Acuna-Hidalgo et al. (2016) Genome Biology 17, 241. (4) Kong et al. 2012, Nature, (5): D'Onofrio et al. 2014, JAMA (4) Sanders Nature 2012;485:237–41.(7) Sandin S et al. (2016). Mol Psychiatry. 21:693-700 (8) Lord Lancet 2019;393:747–57, (9) de Ligt N Engl J Med 2012;367:1921–9. (10) Kong et al. 2012, Nature; (11): Veltman Nat Rev Genet 2012;13:565–75.

The perils of putting off fatherhood: why it poses risks to children's physical and mental health

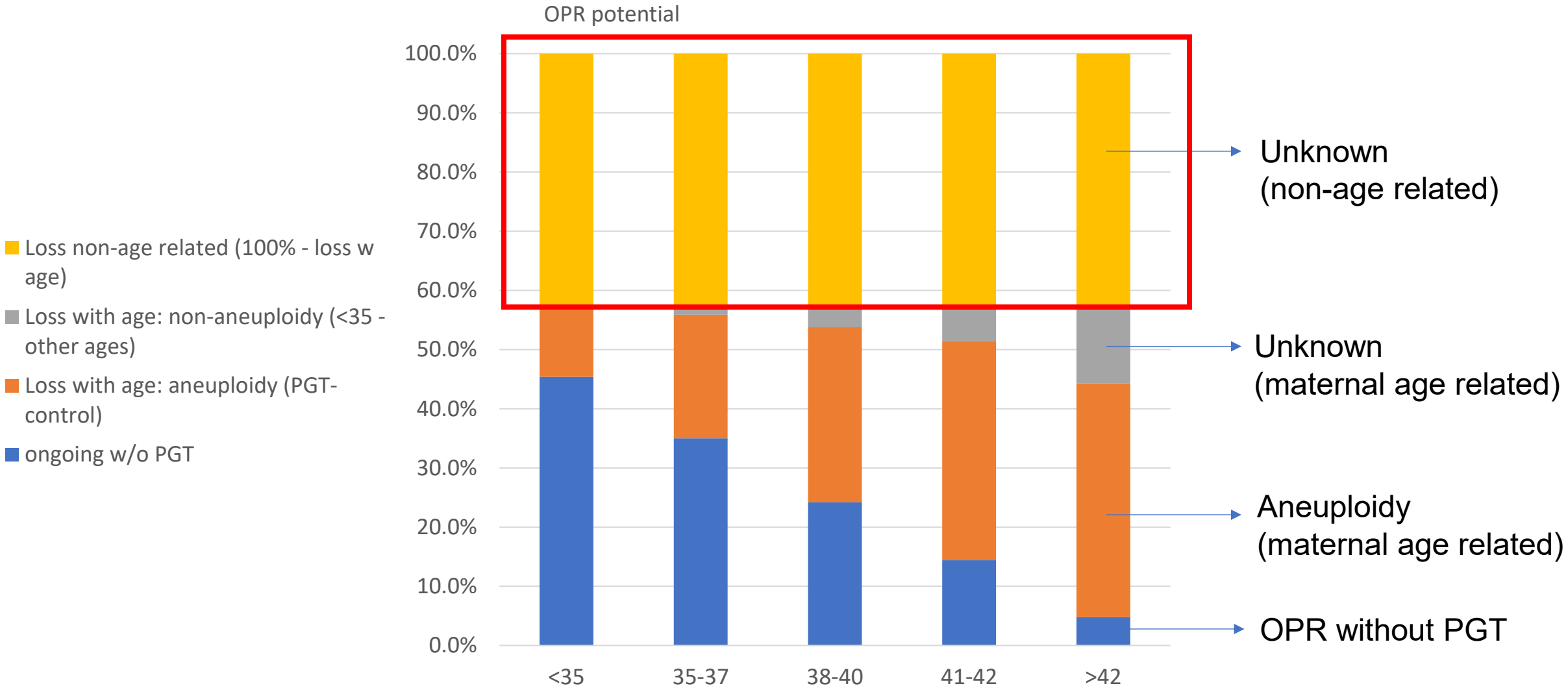
The Guardian



Older men are not generally discouraged from using fertility services, unlike women. Illustration: Philip Lay/Observer Design

Men have biological clocks too. Fertility drops with age, and the likelihood of offspring having conditions such as autism, schizophrenia and leukaemia rises

De novo mutations could produce embryo lethality



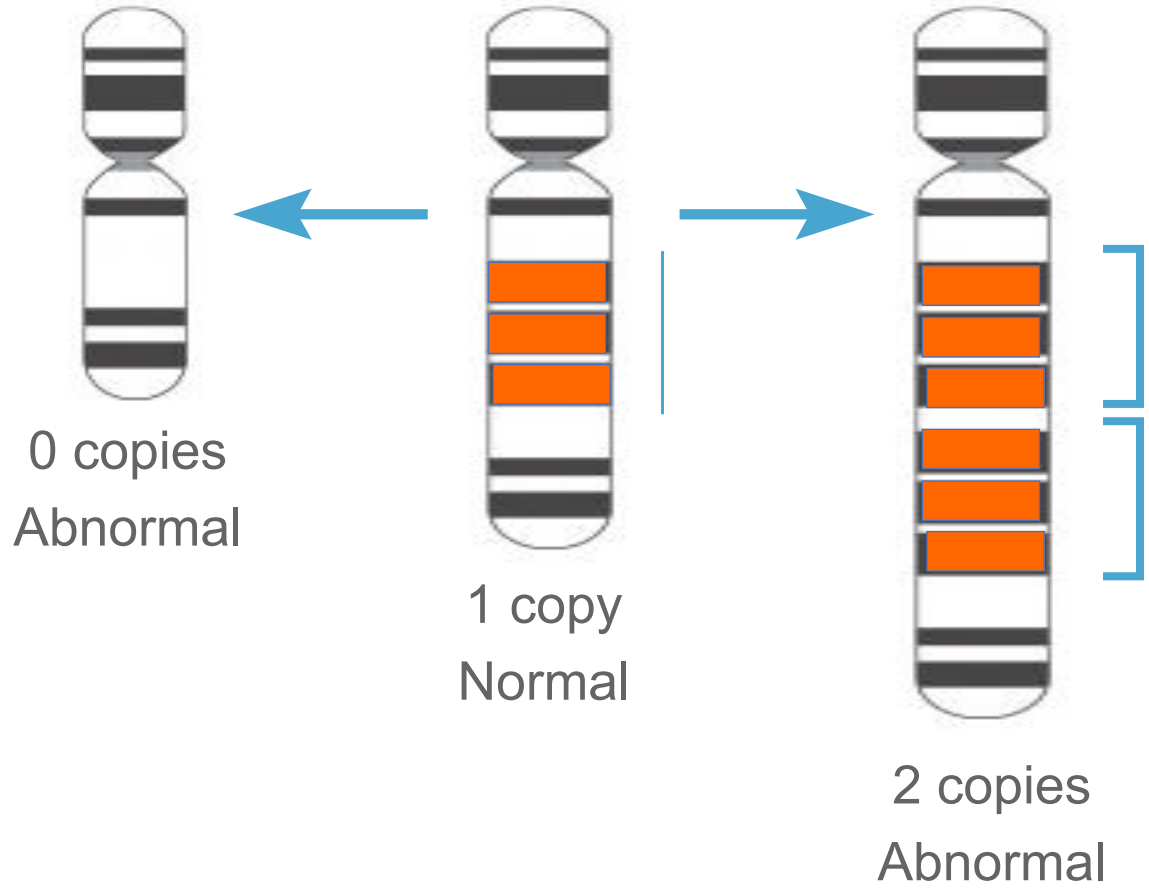
SART 2020 (29,546 PGT transfers vs. 35,199 non PGT)

De novo copy number variants (CNV)

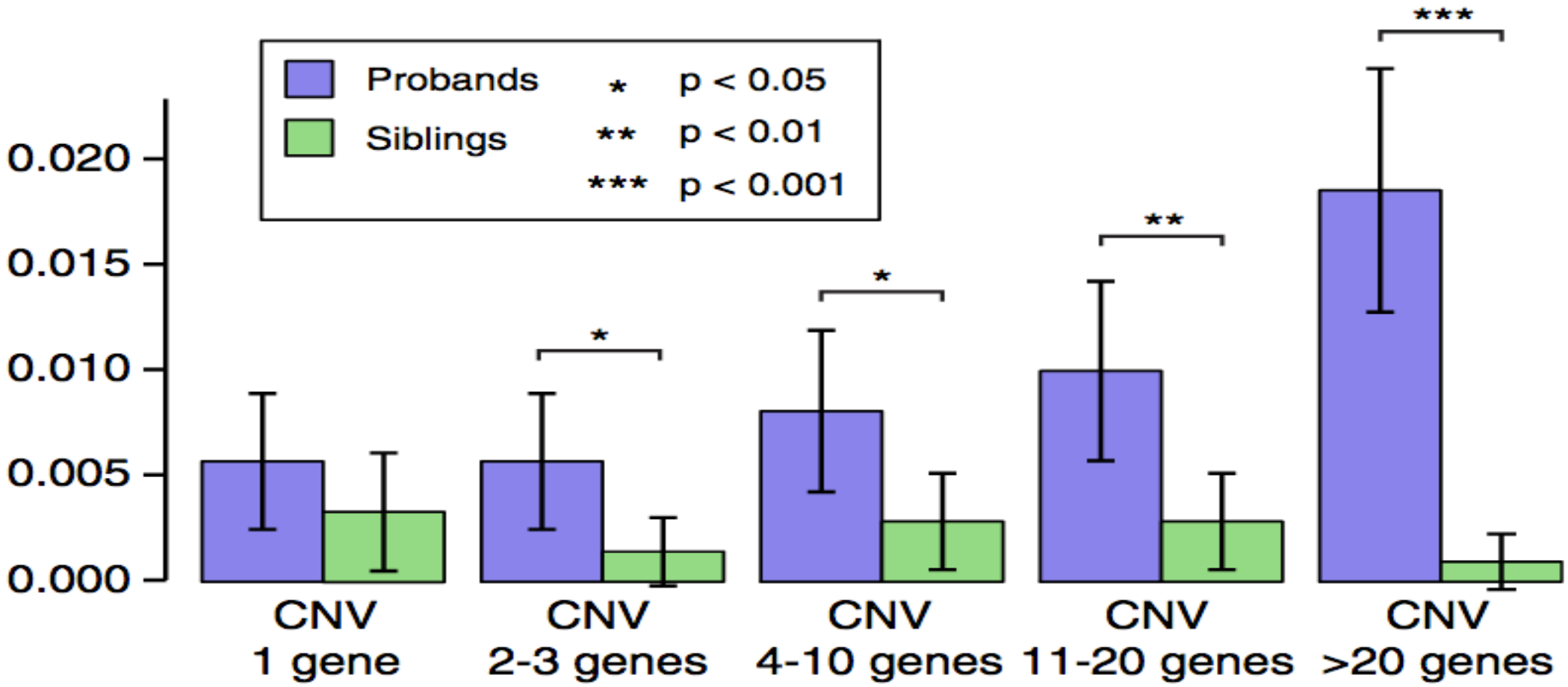
- **Copy number variants (CNV)** are the result of segmental duplications and /or deletions >500bp

- Unlike point mutations, **CNVs are extremely unlikely to be amplification artifacts**

- **1-2% of conceptions** carry CNVs that are 100kb to 10Mb, not detectable by low-coverage PGT-A



High number of CNVs is very predictive of autism



Sanders et al. (2015)

PGT methods for Whole Genome sequencing

1. **Pieters, Munné et al (2015):** Individually sequence 384 DNA fragments to allow haplotyping to detect amplification artifacts.

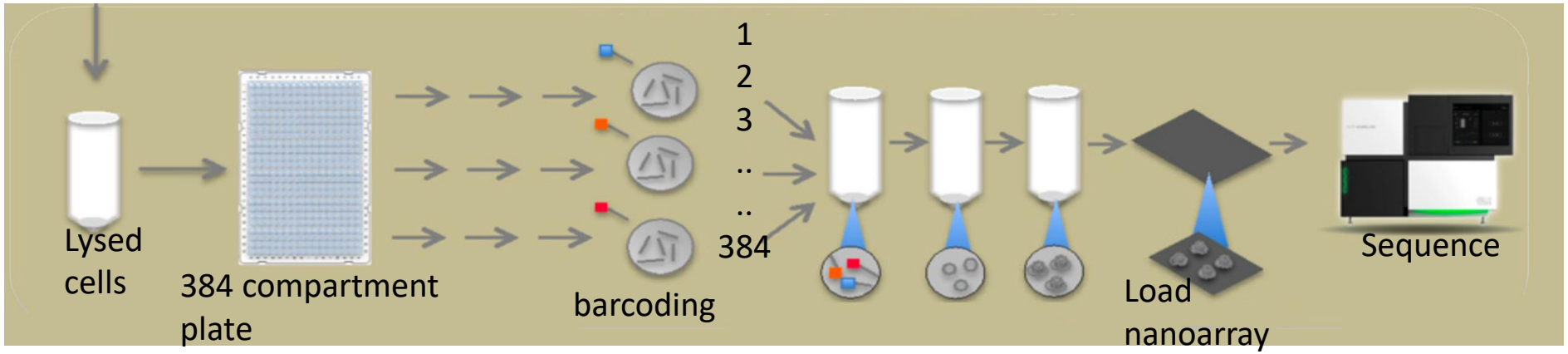
2. **Xia et al. (2021):** Uses PTA to amplify the DNA biopsy

3. **Murphy et al. (2020):** standard amplification with bioinformatics filtering of false positives

1. WGS using Large Fragment Reads (LFRs) (Pieters et. al 2015)

METHOD:

- DNA was dispersed across **384 compartments,** each with 5% haploid genome
- Amplification, fragmentation, barcoding, and **s**equencing at x23 depth, **were performed individually for every compartment**



RESULTS:

- **97%** of the biopsy genome was sequenced
- **3000 de novo variants** per embryo, mostly **VUS**, but **30 pathogenic**

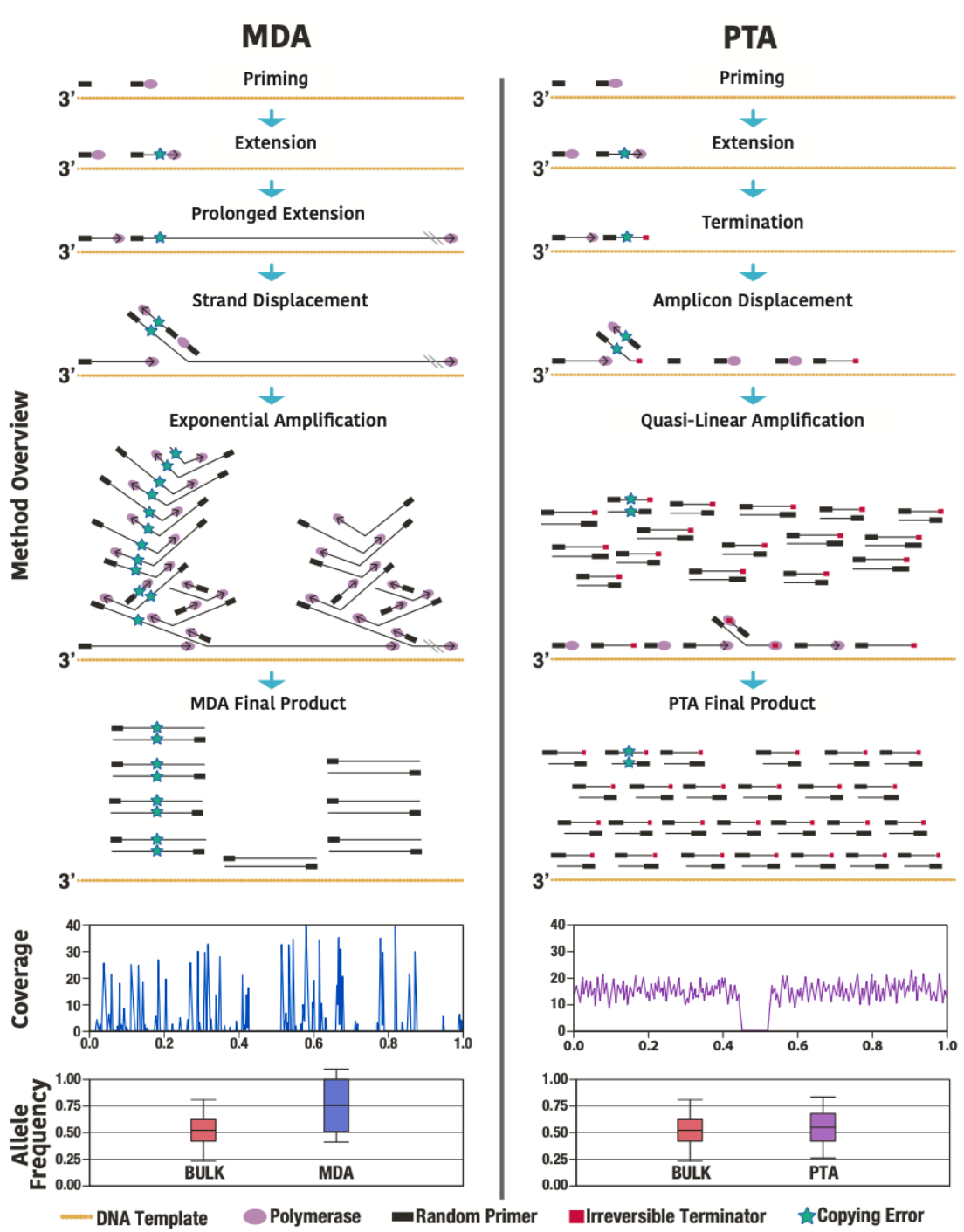
***Peters**, **Munné**, et. al (2015) Genomics Research, 25: 426-434, Gulbahce et al. (2017) Cancer Res. 77: 4530-41*

2. Primary template-directed amplification (PTA)

PTA method:

- Uses MDA polymerase but **exonuclease-resistant terminators** that create smaller amplicons.
- The amplicons undergo **limited subsequent amplification** with more of the amplification occurring from the primary template
- **Any errors** in daughter amplicons have **limited propagation** during the following amplification

Gonzalez et al. (2020), Xia et al. (2021)



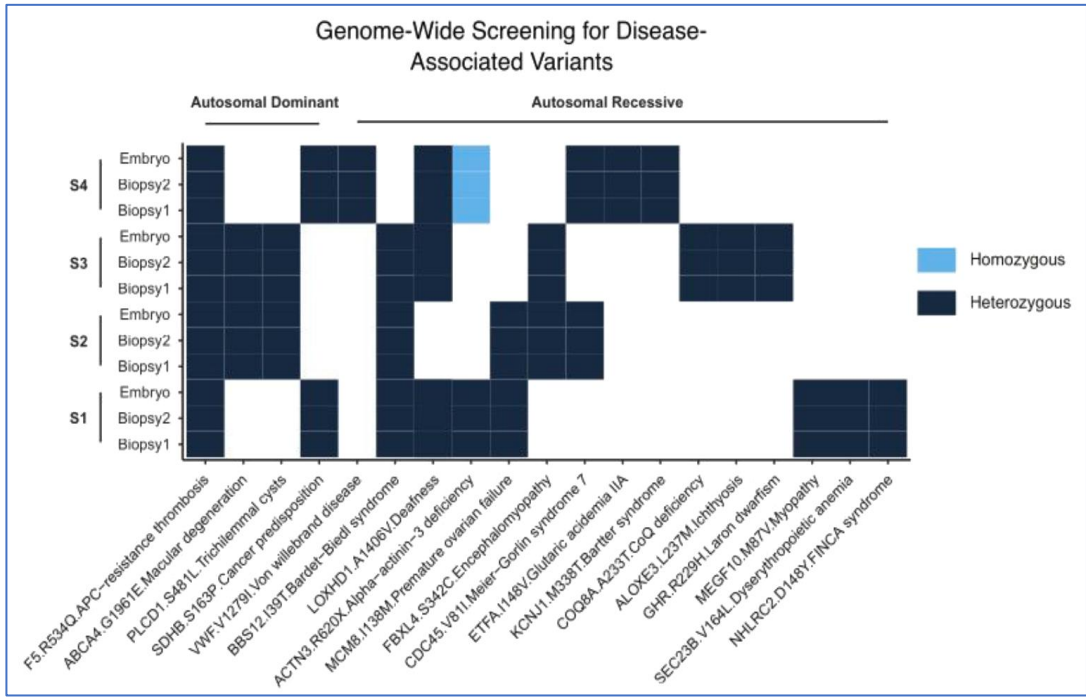
2. PTA: Results

ORCHID

PGT-A: **99% concordance** for chromosome abnormalities in 110 reanalized embryos[(2)].

PGT-SR: CNVs greater than **400Kb** were detectable.

PGT-M: no need of **parental DNA** at **x30** depth



7-9 pathogenic variants per embryo[(1)]:

- 11 inherited (appearing in >1 embryo).
- 7 De novo (appearing only in one embryo)

Detected 3.27M SNVs, 96% concordant with the whole embryo (70,000 SNVs different)[(1)]

(1) Xia et al. (2021) bioRxiv, (2) Xia et al. (**2022**). bioRxiv

ORCHID

- Uses **PTA** amplification which provides **better coverage** of the genome
- Reporting on **1200 genes**
- **Offered clinically** with over 250 performed procedures ($2500 per embryo)
- They **do not test the parents**:
  - Cannot **confirm the mutation** using haplotype phasing from parental DNA
  - **Cannot differentiate de novo mutations from artifacts**

3. GenEmbryomics: method

Method:

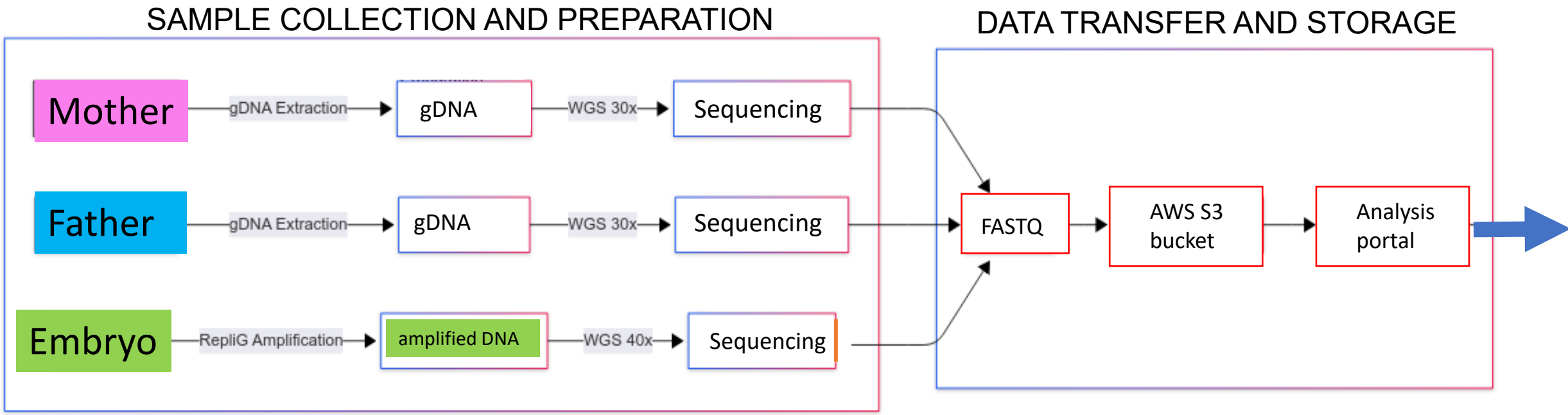
- **WGA** by MDA
- **Whole genome sequence** of **parents** at **x30** and embryos at **x40 depth**
- **Extensive Variant filtration** to eliminate false-positives
- **Variants annotation is** performed from >50 annotation sources, pathogenicity prediction algorithms, and ACMG guidelines.

Murphy et al. (2020) Scientific Reports 10:3795

Outcomes:

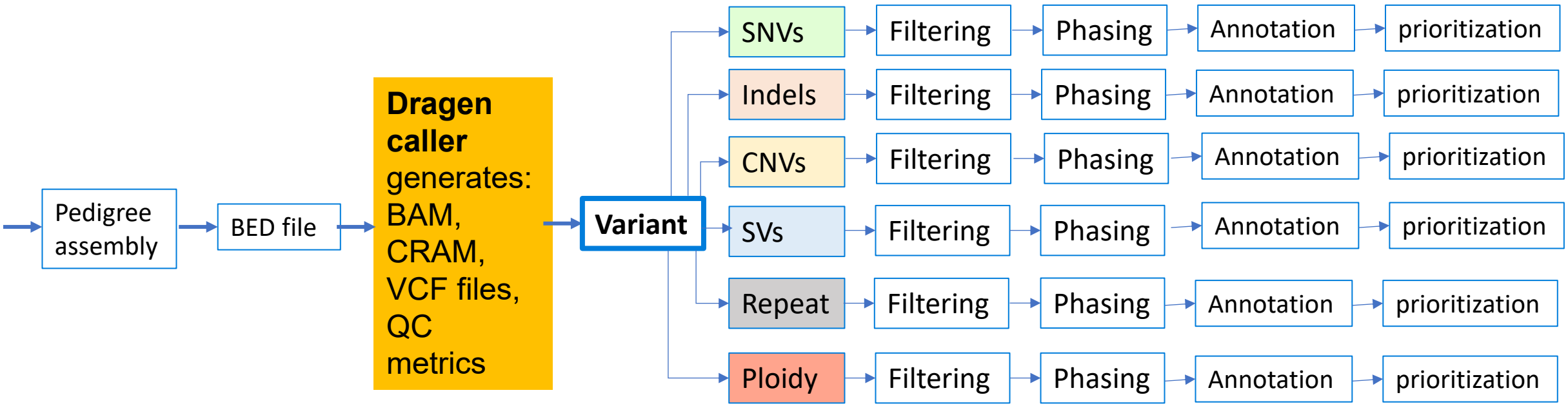
- **Inherited** mutations
- ***de novo*** mutations
- Aneuploidies, translocations, triploidy, mosaicism, Copy number variants >10**K**b
- Carrier **trinucleotide repeats**
- **Polygenic** gene disorders and traits

Pipeline Part 1: Sample Collection & Data Transfer



- **Mother and father** samples undergo gDNA extraction followed by WGS **sequencing at x30**
- The **embryo biopsy** is **amplified** by RepliG followed by WGS **sequencing at x40**
- Sequencing data is **converted to FASTQ format** for analysis

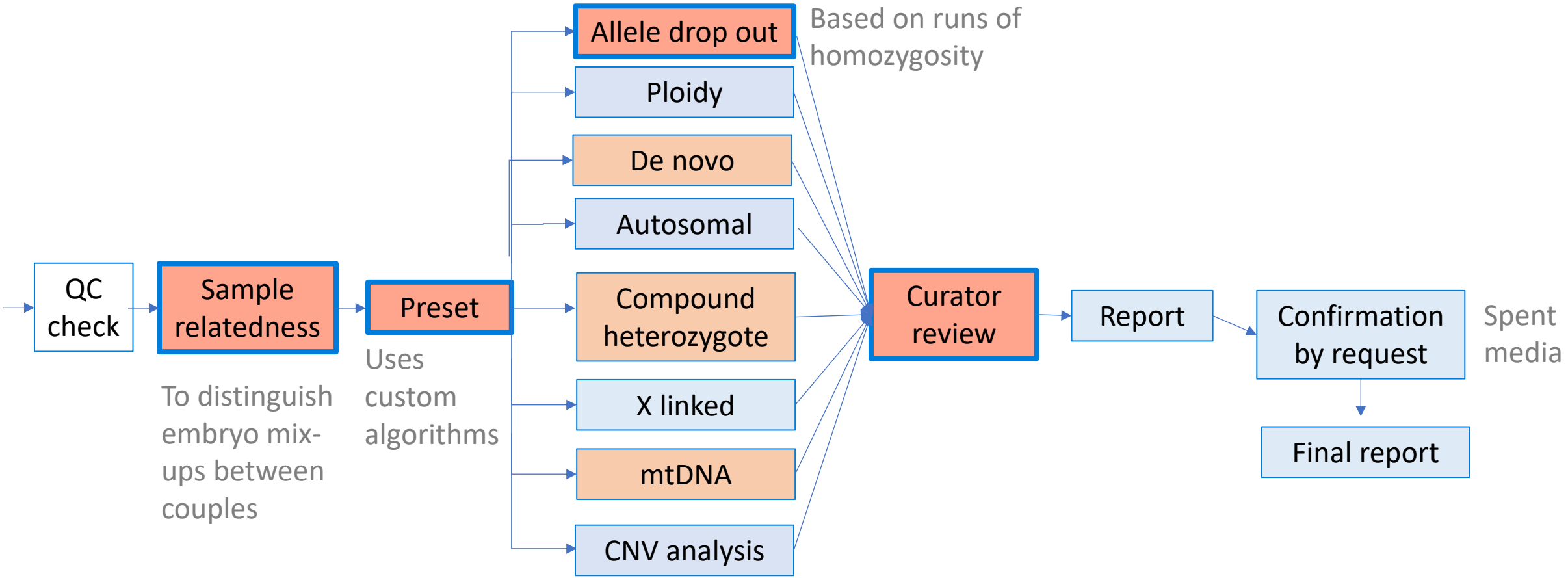
Pipeline Part 2: Analysis Pipeline



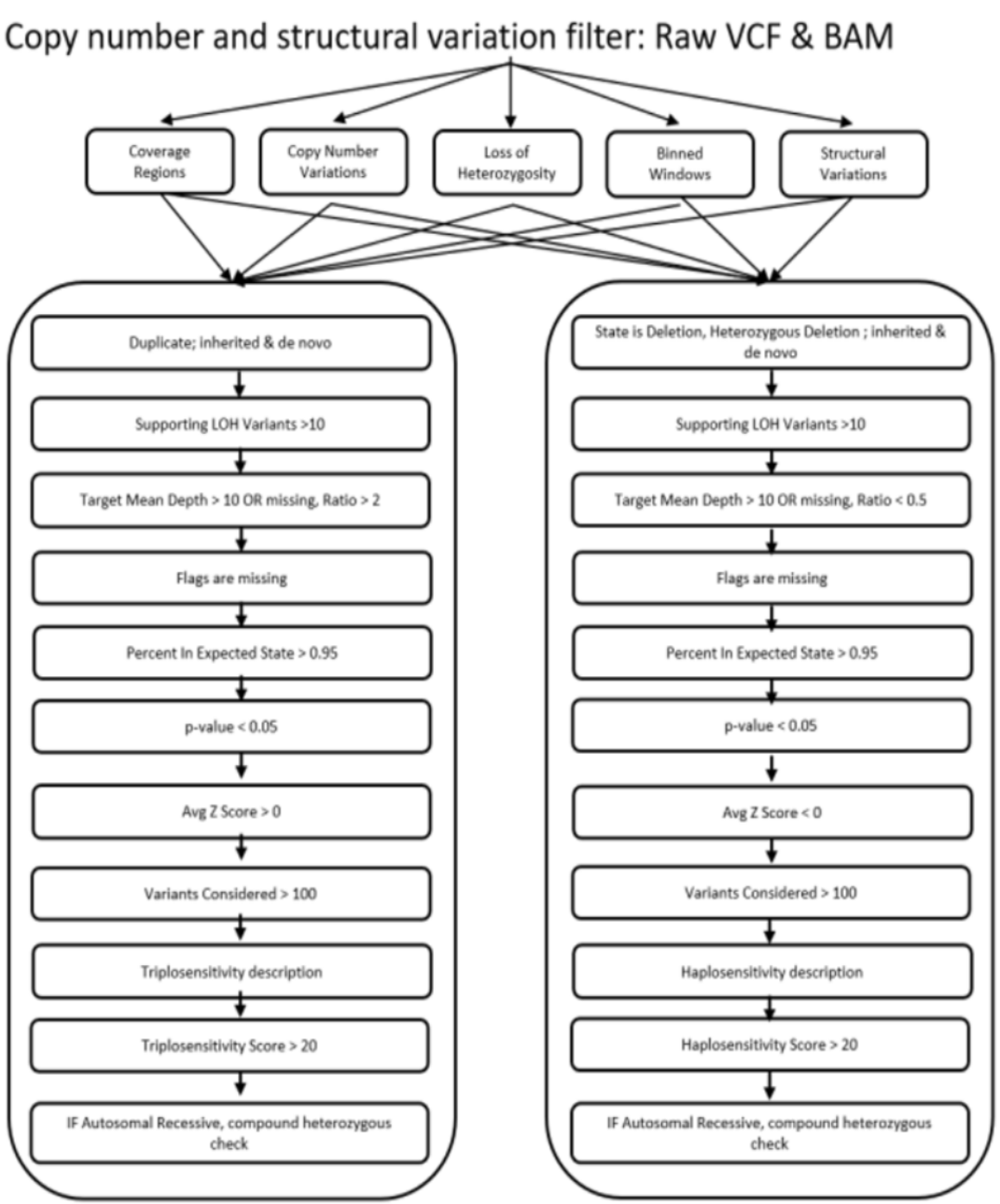
- **Pedigree** is assembled and converted to a **BED file.**
- The **Dragen** calling suite **generates** VCF, CRAM, BAM files and QC metrics
- **Variant calling branches into** SNVs, Indels, CNVs, SVs, Repeat analysis, and Ploidy calling.
- All variants are then **filtered, phased, annotated, and prioritized**

Pipeline Part 3: Curation & Reporting

After QC checks and sample relatedness verification, the analysis branches into multiple preset categories for curation.



Variant filtration



Variants pathogenicity annotation

Variants annotation performed from >50 annotation databases, pathogenicity prediction algorithms, and ACMG guidelines.

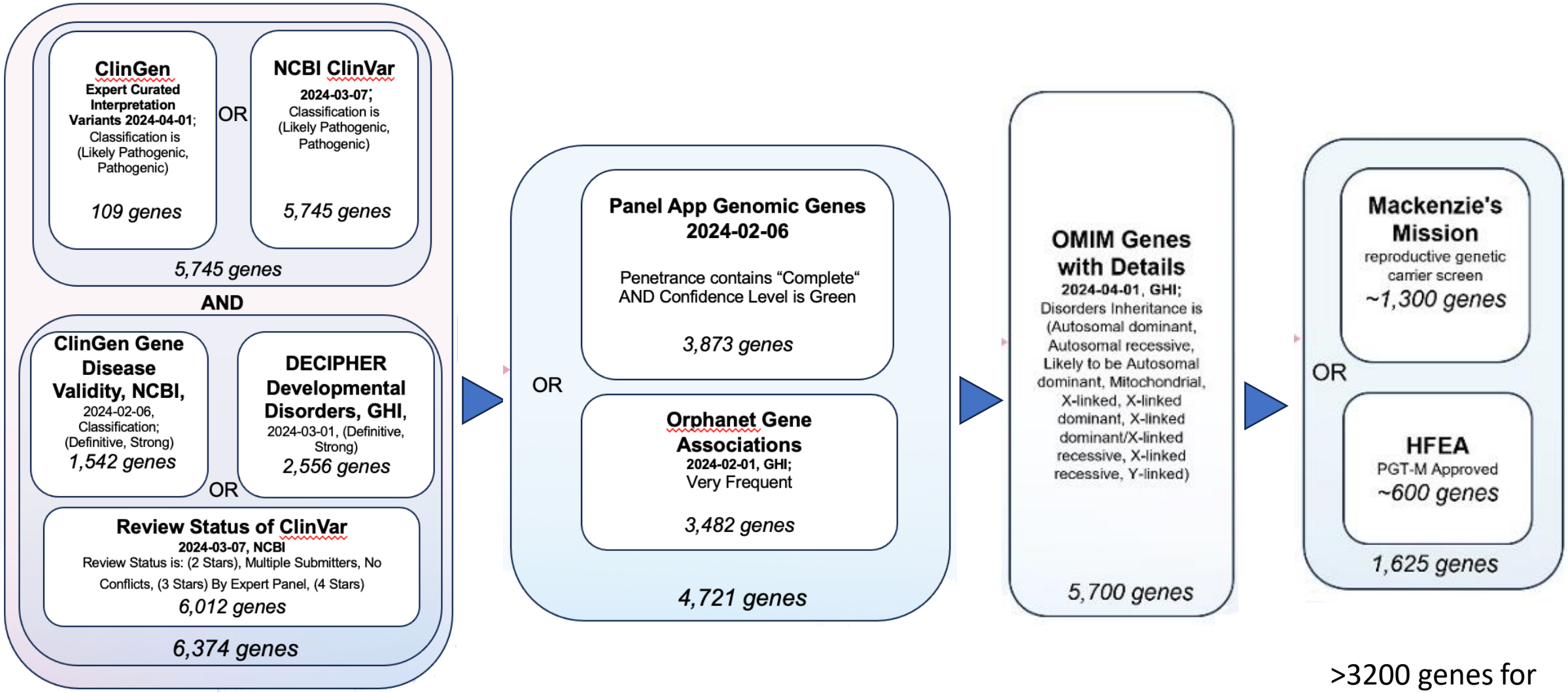
1kG Phase3 - Variant Frequencies 5a with Genotype Counts, GHI 1kG Phase3 CNVs and Large Variants 5b V2, GHI , CADD Scores InDels v1.5, UW, ClinGen Gene Disease Validity 2021-01-14, NCBI, ClinGen Gene Dosage Sensitivity 2022-02-03, NCBI , ClinGen Region Dosage Sensitivity 2022-01-07, NCBI , Clinical Genomic Database 2022-02-23, GHI, ClinVar 2022-01-06, NCBI , ClinVar Assessments 2022-01-06, NCBI ClinVar CNVs and Large Variants 2022-01-06, NCBI , ClinVar Transcript Counts 2022-01-06, NCBI, ClinVarCNVsandLargeVariant Assessments 2022-01-06, NCBI , Conservation Scores Exonic, GHI , dbSNP 155, NCBI , DECIPHER Developmental Disorders 2021-12-02, GHI, DECIPHER Population CNV v9.2DGV CNVs - Gold Standard Variants 2016-05-15 v3, DGV , Gene Identifiers and Descriptions 2021-11-30, GHI , Genetics Home Reference 2022-05-12, GHI, Genomic Super Dups 2014-10-19, UCSC gnomAD - Gene Constraint 2.1.1 v2, BROAD, gnomAD Exomes Variant Frequencies 2.0.1, BROAD , GnomAD High Frequency CNV Regions 2019-11-25, GHI , gnomAD Structural Variants 2.1, BROAD, Haploinsufficiency Predictions Version 3, DECIPHER, Human Phenotype Ontology 2022-02-21 , InterPro Regions 2019-09-18, GHI , Low Complexity Regions and Universal Mask-GHI, Missense Badness and MPC, BROAD, MONDO 2021-05-13, GHI, Mondo Gene Disease Association 2020-07-25, MI , Multiple Sequence Alignments of 100 Vertebrates, UCSC, OMIM Genes 2022-02-01, GHI, Orphanet Gene Associations 2022-02-01, GHI, Reference Sequence GRCH37 g1k, 1000Genomes, Reference Sequence GRCh38, NCBI, RefSeq Genes 109.20211119, NCBI, Repeating Elements by RepeatMasker, UCSC , SIFT and PolyPHen2 Missense Predictions 2021-04-21, GHI

Murphy et al. (2020) Scientific Reports 10:3795

Gene selection criteria

- **Pathogenic** or Likely Pathogenic
- >90% certainty of being **disease-causing**
- **several submissions** with no conflicts between submissions
- High to **complete penetrance** (>80% of individuals develop the disease)
- Simple (**monogenic**) mode of inheritance:
  - Initially only Autosomal dominant or recessive, X-linked, Mitochondrial
- **No cure** available
- **Severe** phenotype

3200 genes selected



3586 genes → 3366 genes → 3042 genes

>3200 genes for variant curation

Gene Selection

- **3,200 genes** included as the standard test
- **Can include any other on demand**

validation method using GIAB

validation method

- Flow sorted cells from several Genome-in-a-bottle (GIAB vs 3.3.2): NA12878, NA12877, ..
- Compared genomic DNA to amplified DNA (using RepliG)
- CNV & ploidy were assessed via transmission & read binning for Copy Number
- Tandem repeat disorders were reported via carrier repeat number
- De Novo mutation accuracy was calculated after the filtering but BEFORE annotation.
- Annotation significantly improved the accuracy.
- *De novo* mutations after annotation were reconfirmed by Sanger.

validation results using GIAB

Mutation performance metrics prior to annotation (SNVs)	
Specificity	99.998%
Accuracy	99.997%
Sensitivity	92.2%
Precision	98.0%
Negative Predictive Value	99.992%
False Positive Rate	0.0023%
False Negative Rate	2.93%

Validation based on samples of 5 cells of Genome-in-a-bottle NA12878

Rate of Pathogenic *de novo* mutations

Pre-annotation detection of the novo mutations**:**
3,632 DNVs in NA12878 5-cells model **biopsies**

Post-annotation detection of the novo mutations**:**
Average of **1.17 pathogenic DNVs** per sample

Further confirmation pre-reporting:
-**Sanger** re-testing for DNV prior to reporting
- Re-confirmation using **Spent culture media**

>3000 DNVs
Pre-annotation

1.2 DNVs
After annotation

validation method using rebiopsy vs whole embryo

Clinical validation study (NCT05739890):

- **Patients**: - 100 carrier couples of monogenic diseases undergoing PGT-M

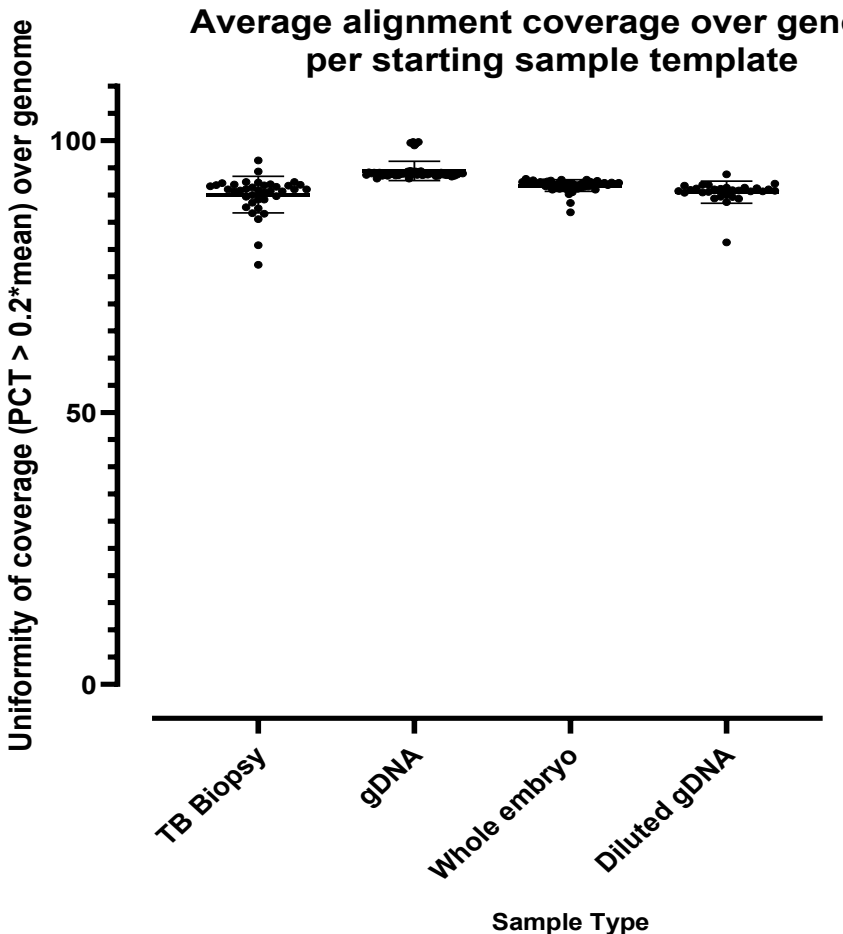
- **Procedure**: - Embryo biopsy and MDA amplification
  - PGT-WGS at x40 for embryos and parents at x30
  - Affected embryos re-biopsied

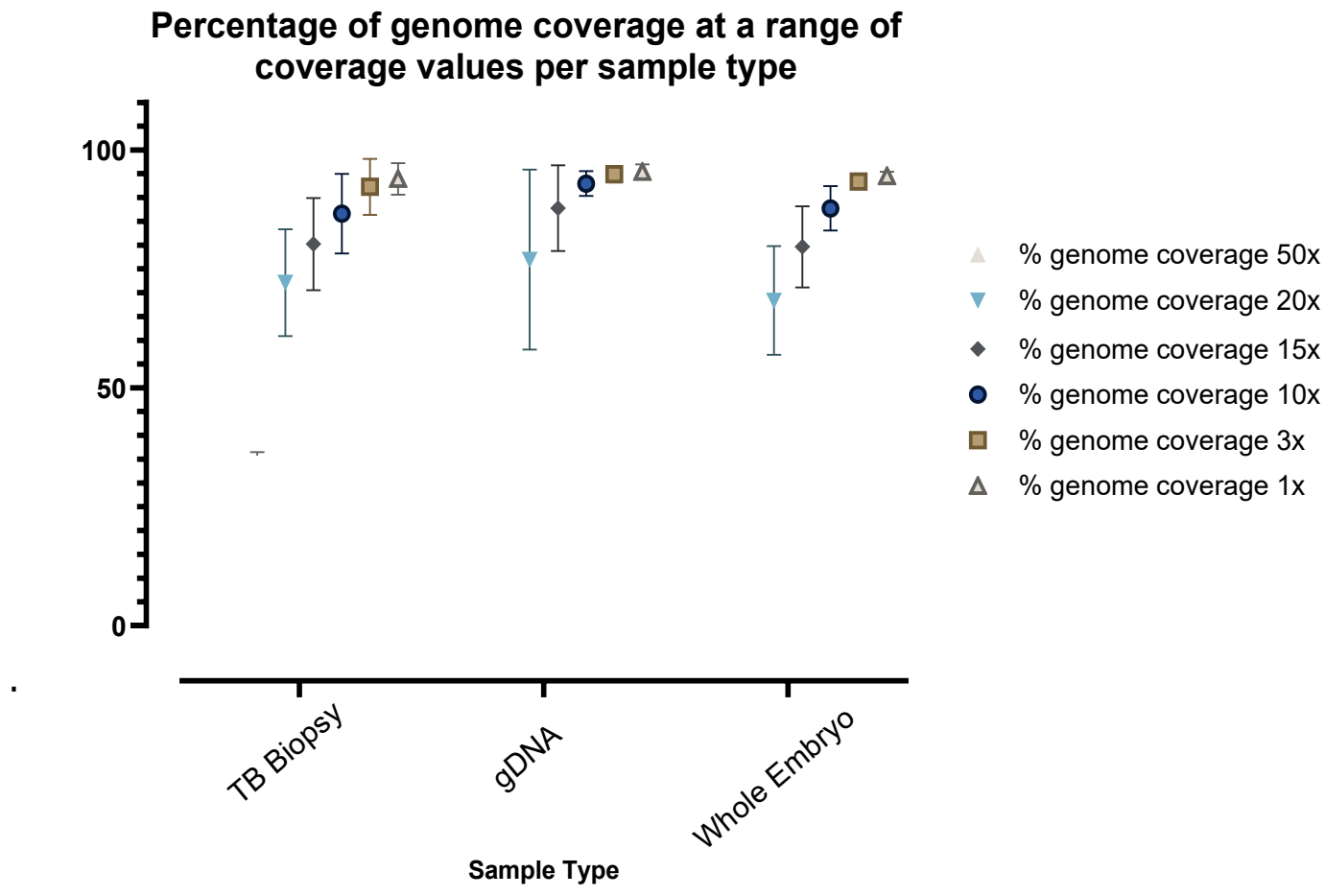
- **Aim:** - Compare PGT-WGS of TE biopsy (query) vs. whole embryo (Truth).
  - Determine at what paternal age is WGS more indicated

- **Metrix**: - genome in a bottle benchmarking standards (**recall, precission, F1**)

Clinical Validation: uniform coverage

Uniform coverage was very similar between TB, whole embryo and gDNA





Clinical validation results:

Whole embryo	biopsy WGS	Biopsy PGT-M	whole Embryo WGS	biopsy PGT-WGS aneuploidy	Biopsy PGT-A	#
affected	affected	affected	euploid	euploid	euploid	16
affected	affected	affected	euploid	euploid	NR	4
affected	affected	affected	euploid	euploid	chaotic	1
affected	affected	affected	Aneuploid	Aneuploid	Aneuploid	8
affected	affected	affected	47XX,+3	47XX,+3	47XX,+3, mos -1	1
affected	affected	affected	45XX,-19	45XX,-19, -5q	46XX,-19, +21	1
affected	affected	affected	Complex	complex	complex	3
affected	affected	affected	69,XXY	69,XXY	complex	1
affected	affected	affected	45XX,-18	44XX,-18, -9	NR	1
TOTAL	**100%**			**95%**		**36**

Kahraman, Cetinkaya, Munné, Murphy (submitted)

Clinical Validation Results: Pathogenic *de novo* Variants

195 candidate mutations detected in 22 embryos

Filter applied: Allele freq thresholding, depth >3 reads, Map Quality >20, pop. freq <10%, high severity, de novo, disease associated genes; SNVs & indels known variants only.

4 De novo mutations selected for confirmation

DMD	rs104894797	Duchenne muscular dystrophy
TRMU	rs779022860	Liver Failure, Infantile
LOX	rs1754538399	Aortic aneurysm, familial thoracic
EYA4	rs1554275988	Deafness, autosomal dominant

2 De novo mutations confirmed in the whole embryo

9% embryos have confirmed *de novo* mutations (2/22)

Genetic counseling

Advantage over other PGT-M platforms

- Embryos classified as heterozygous for a recessive disease **may still carry a de novo** mutation and be affected.

- PGT-WGS can detect other **inherited** or ***de novo*** mutation in other genes not tested for the PGT-M indication.

- **Initial indications:**
  - PGT-M
  - Advanced paternal age
  - Consanguinity

Genetic counseling and reporting

Two approaches:

- **Report only pathogenic:**
  - **Only pathogenic** or likely pathogenic variants are reported following the prenatal diagnosis approach to WGS
  - This is **more conductive** to current PGT reporting in IVF centers which have limited time and capabilities for genetic counseling.

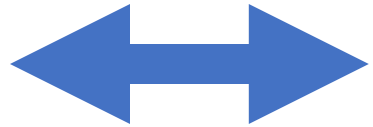
- **Report other variants and traits** (i.e. PGT-P for IQ):
  - Some demanding parents may want **extensive counseling** on each embryo, each gene and each trait requiring specialized services beyond regular IVF set ups

Conclusions

- It took **30 years** (1993 – 2023) to go from the first PGT-A (XY,13,18,21) to PGT-WGS of embryos

- **30% of euploid embryos still do not implant**. **Metabolomics** and **WGS** may identify others, while 10% loss may be due to the embryo transfer process itself.

- Although there is a variety of platforms that can perform **comprehensive PGT for** –A –M –SR –P, only **WGS** can detect **de novo mutations PLUS** everything else.



GenEmbryomics PGT-WGS test is distributed by Progenesis

Credits to:

GenEmbryomics:

- Nick Murphy, PhD
- Kyle Day, PhD
- Shannon Wieloch, PhD
- Kim Skellington, PhD
- Vanessa Cortes, PhD

Memorial Sisli Hospital:

- Prof. Semra Kahraman, M.D
- Murat Cetinkaya, M.D., PhD

Questions?

Santiago.munne@gmail.com
Nick.Murphy@genembryomics.com