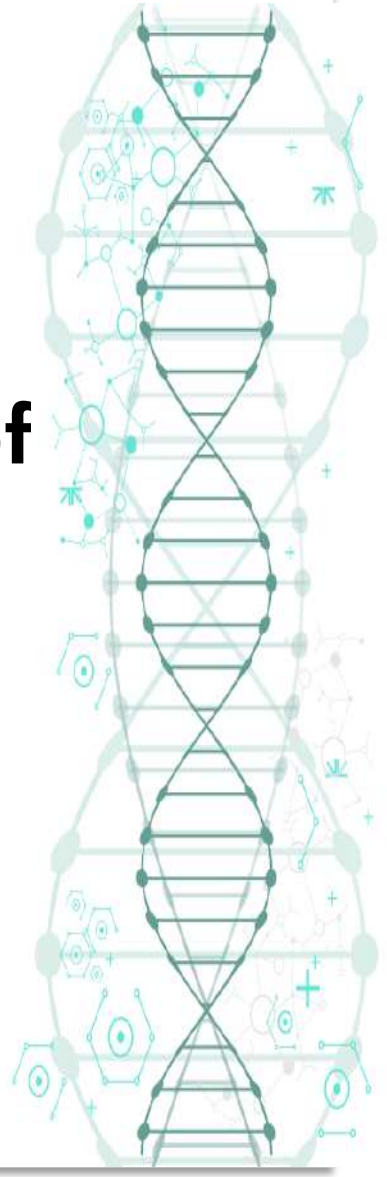
Antonio Capalbo

- Chief Scientific Officer, CSO, Juno Genetics;
- Director of Genomics Research IVIRMA Global Innovation Alliance
- Adjunct Professor, Unit of Medical Genetics, Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" School of Medicine and Health Sciences University of Chieti-Pescara.

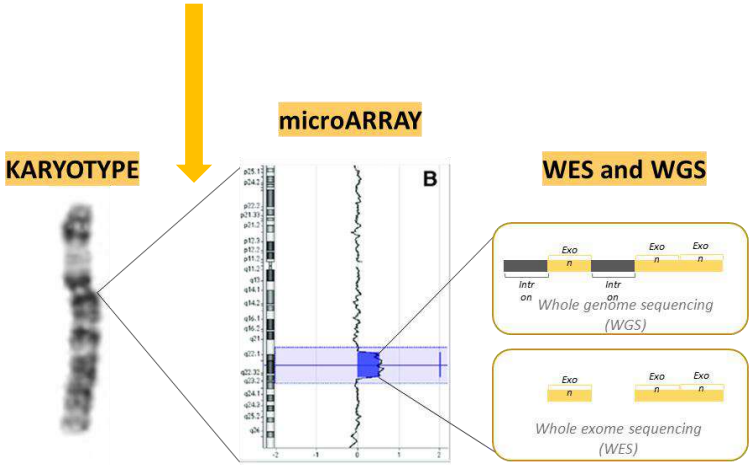
Whole genome sequencing of embryos: Laboratory challenges and learning from our mistakes



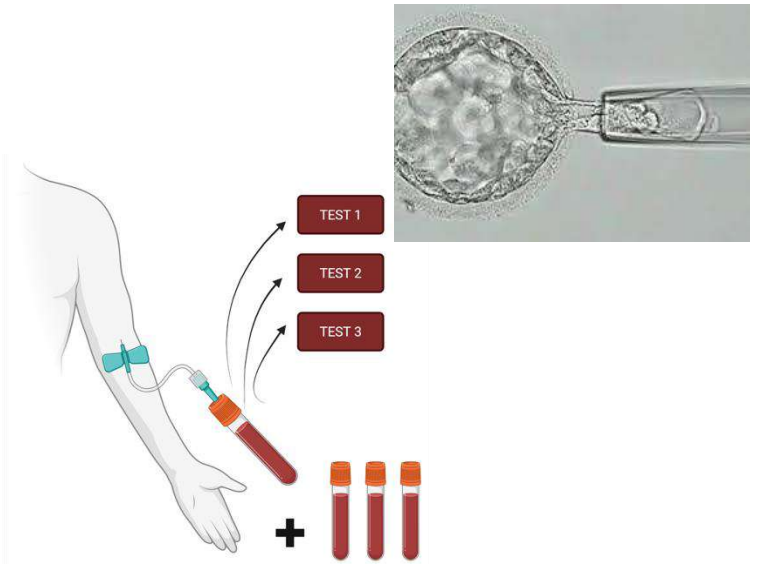
Achieve optimal analytical accuracy is particularly important in PGT

CRUCIAL POINTS TO KEEP IN MIND

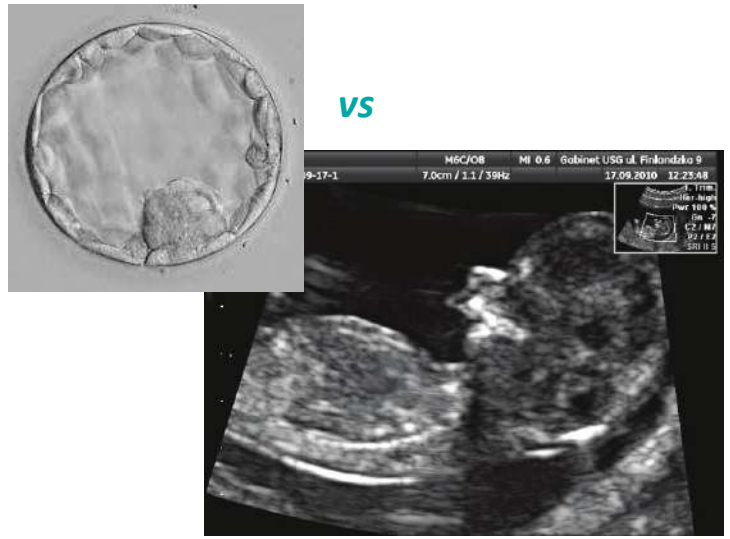
Resoltuion: 3-7 Mb (according to the platform/genomic position) PGT can detect aneuploidies and gross structural unbalances



One-time procedure: unlike blood tests, PGT is single shot, performed once without the possibility of repetition



Phenotype-agnostic: PGT is performed without prior knowledge clinical phenotype that may aid in the interpretation of findings



Fundamental question to ask to a PGT-A assay

ANALYTICAL VALIDITY

Consistency and reproducibility of aneuploidies detection in embryo biopsies

CLINICAL VALIDITY

Clinical performance in predicticting the phenotype (embryo lethality)

CLINICAL UTILITY

Improvement of IVF clinical outcomes (RCTs)

GENETIC FINDINGS SHOULD BE REPORTED ONLY IF THEY PROVIDE CLEAR AND UNAMBIGUOUS RESULTS IN TO FACILITATE INFORMED DECISIONS AND IMPROVMENT OF MEDICAL CARE

acgs
Association for Clinical Genetic Science

General Genetic Laboratory Reporting Recommendations

Kath Smith[1], Jo Martindale[1], Yvonne Wallis[2], Nick Bown[3], Natasha Leo[4], Lara Creswell[5], Graham Fews[2], Zandra Deans[6]

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,14], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

ESHG www.nature.com/ejhg

ARTICLE OPEN

Recommendations for reporting results of diagnostic genomic testing

Zandra C. Deans[1], Joo Wook Ahn[2], Isabel M. Carreira[3], Elisabeth Dequeker[4], Mick Henderson[5], Luca Lovrecic[6], Katrin Ounap[7,8], Melody Tabiner[9], Rebecca Treacy[1] and Christi J. van Asperen[10]

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European Journal of Human Genetics (2016) 24, 2–5

EJHG Open

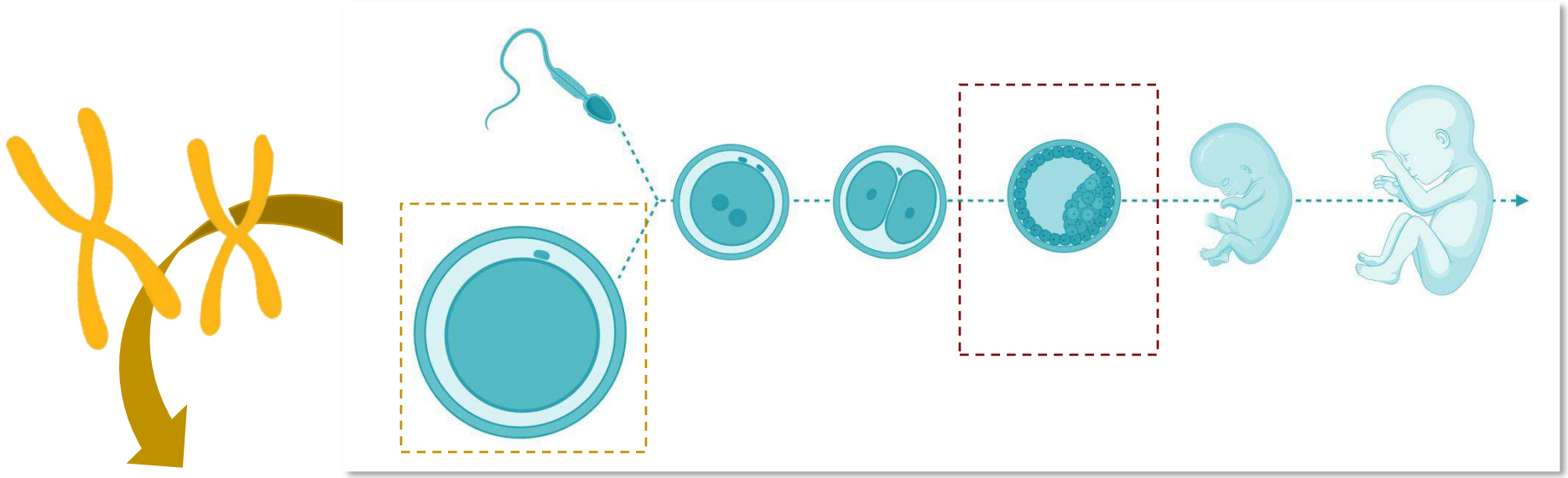
POLICY

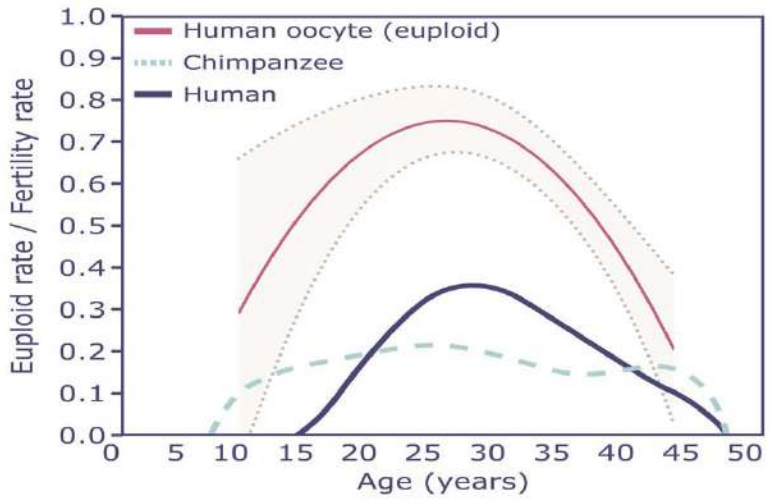
Guidelines for diagnostic next-generation sequencing

Gert Matthijs[1,2,8], Erika Souche[3,8], Marielle Alders[2], Anniek Corveleyn[3], Sebastian Eck[2], Ilse Feenstra[4], Valérie Race[3], Erik Sistermans[5], Marc Sturm[6], Marjan Weiss[5], Helger Yntema[4], Egbert Bakker[7], Hans Scheffer[4] and Peter Bauer[6]

Established Frameworks for evaluating genetic tests

Gametes fuel genomic diversity





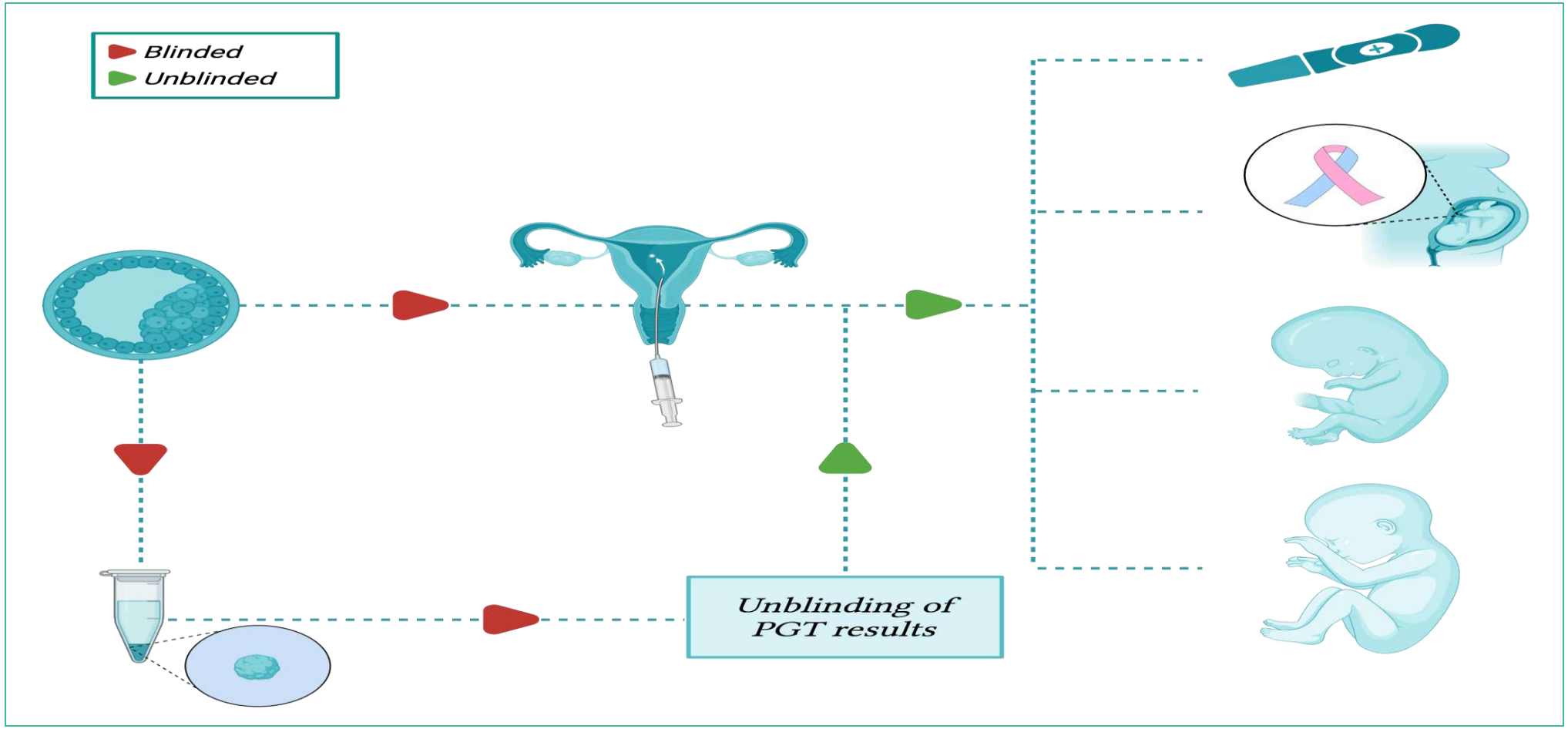
PGT and Embryo Assesment

-Improve efficiency and safety of IVF by deselecting fully aneuploid embryos

-Provide ground knowledge to develop new clinical application to counteract reproductive decline with female age

Evaluating clinical validity: why **non-selection blinded studies** offer the strongest evidence

Blinded studies **minimizes selection bias** by avoiding decisions based on test results, allowing for a **reliable assessment** of the relationship between genetic findings and pregnancy outcomes.



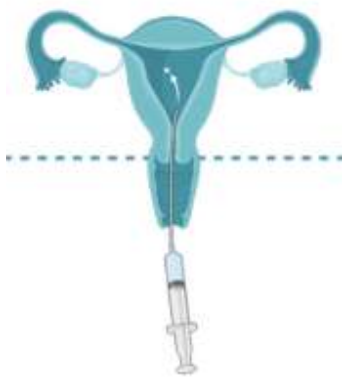
Uniform aneuploidy detection in preimplantation embryo is highly predictive of early lethality

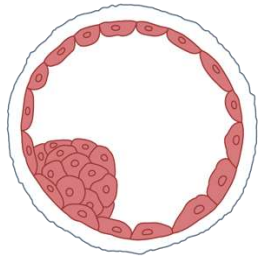
The American Journal of Human Genetics *109*, 1572–1581, September 1, 2022

PERSPECTIVE

On the reproductive capabilities of aneuploid human preimplantation embryos

Antonio Capalbo,[1,*] Maurizio Poli,[1] Chaim Jalas,[2] Eric J. Forman,[3] and Nathan R. Treff[4]





Study	Design	Transfers of Uniformly Aneuploid Embryos n	Miscarriage rate % (n, 95%CI)	Lethality rate % (n, 95%CI)
Scott et al. 2012	blinded	95	33.3% (2/6) (4.3%-77.7%)	95.8% (91/95) 84.5%-99.4%)
Tiegs et al. 2021	blinded	102	100% (24/24) (85.8%-100%)	100% (102/102) (96.5%-100%)
Wang et al. 2021	blinded	44	75.0% (6/8) (34.9%-96.8%)	95.5% (42/44) (84.5%-99.4%)
Yang et al. 2022	blinded	6	100% (6/6) (54.1%-100%)	100% (6/6) (54.1%-100%)
Barad et al. 2022	Unblinded	106	85.7% (6/7) (42.1%-99.6%)	99.1% (105/106) (94.9%-99.9%)
TOTAL		**353**	**86.3% (44/51) (73.7%-94.3%)**	**98.0% (346/353) (96.0%-99.2%)**

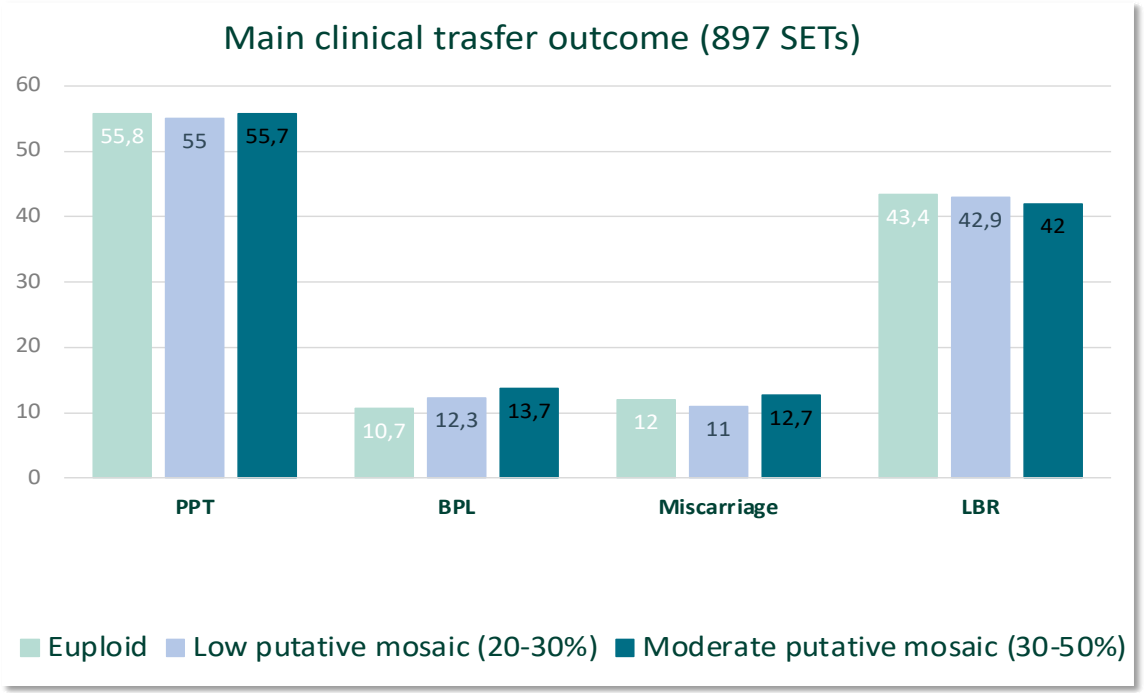
1° PROSPECTIVE BLINDED STUDY

AJHG 2022

ARTICLE

Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial

Antonio Capalbo,[1,*] Maurizio Poli,[1] Laura Rienzi,[2] Laura Girardi,[1] Cristina Patassini,[1] Marco Fabiani,[1] Danilo Cimadomo,[2] Francesca Benini,[3] Alessio Farcomeni,[4] Juliana Cuzzi,[5] Carmen Rubio,[6,7] Elena Albani,[8] Laura Sacchi,[8] Alberto Vaiarelli,[2] Matteo Figliuzzi,[1] Necati Findikli,[9,10] Onder Coban,[11] Fazilet K. Boynukalin,[12] Ivan Vogel,[13] Eva Hoffmann,[13] Claudia Livi,[3] Paolo E. Levi-Setti,[8] Filippo M. Ubaldi,[2] and Carlos Simón[6,7,14,15]

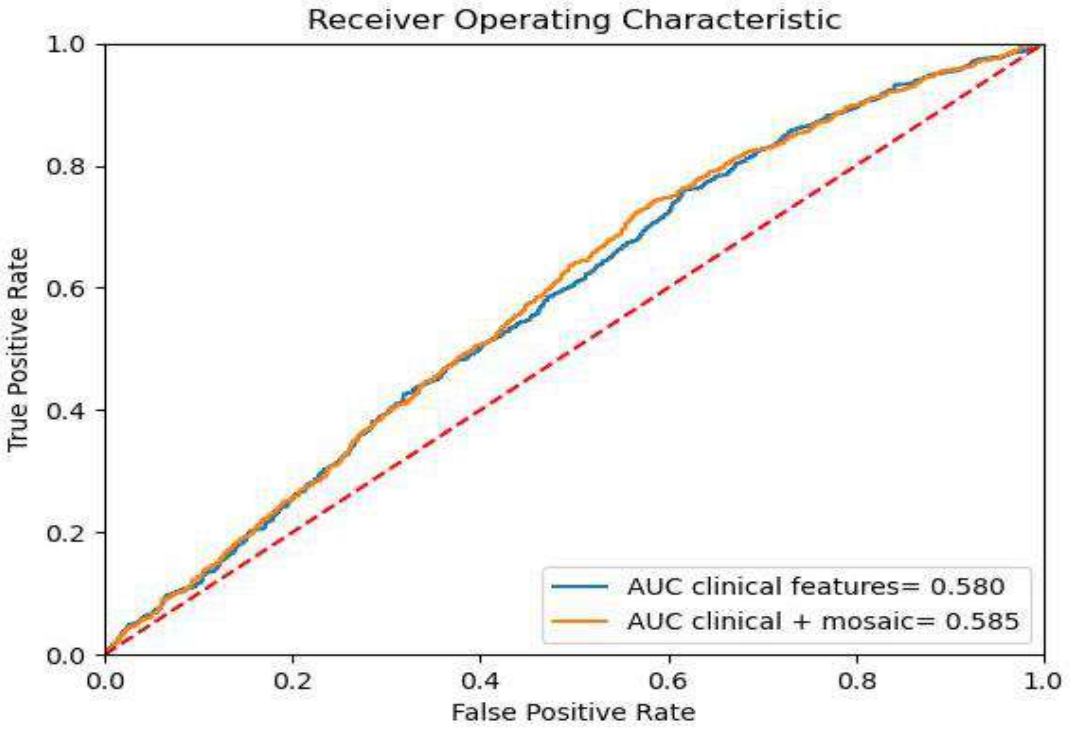


2° PROSPECTIVE BLINDED STUDY

JUNO GENETICS

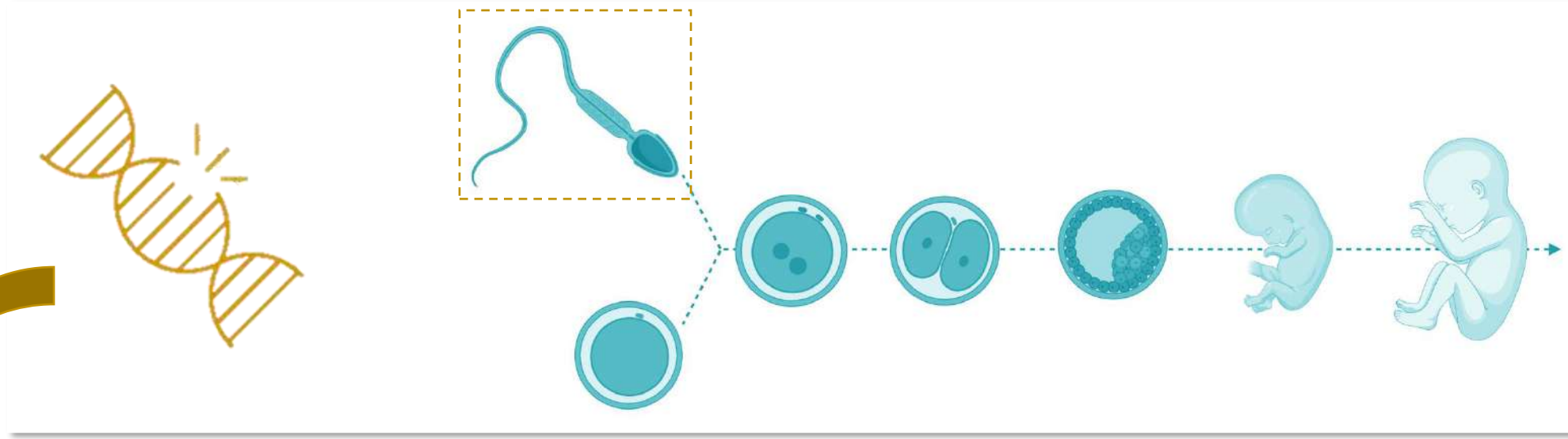
IVIRMA Global Research Alliance

15.324 single embryo transfers in non-selection/blinded design

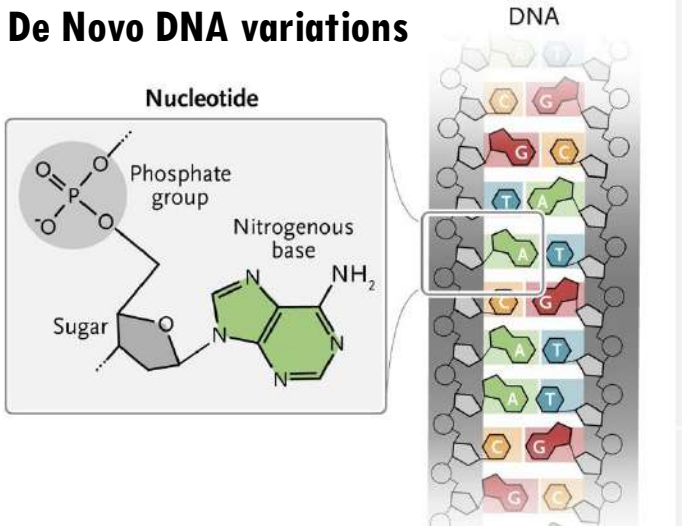


Gill et al., ASRM 2023; in submission

The positive or negative evolutionary stream of genomic diversity



De Novo DNA variations



PGT and Embryo Assesment

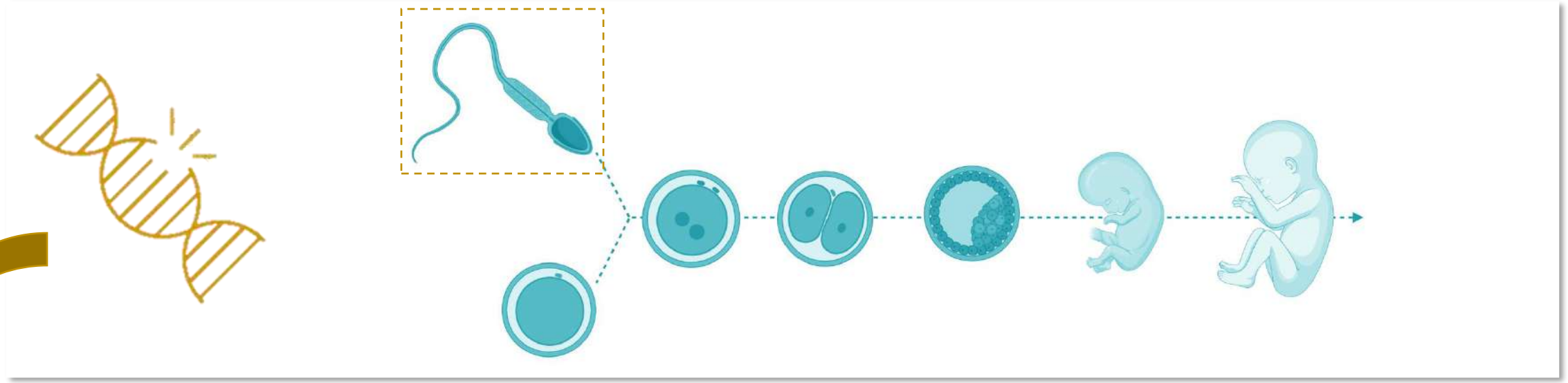
-Improve efficiency and safety of IVF treatment

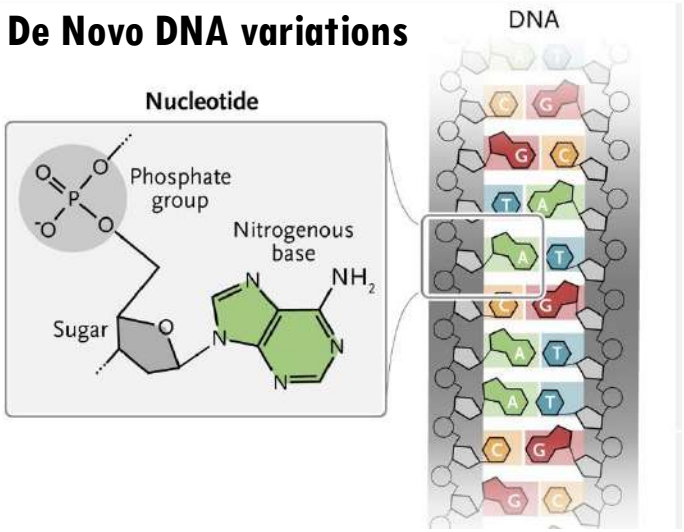
-Provide ground knowledge to develop new clinical application to counteract reproductive decline with female age

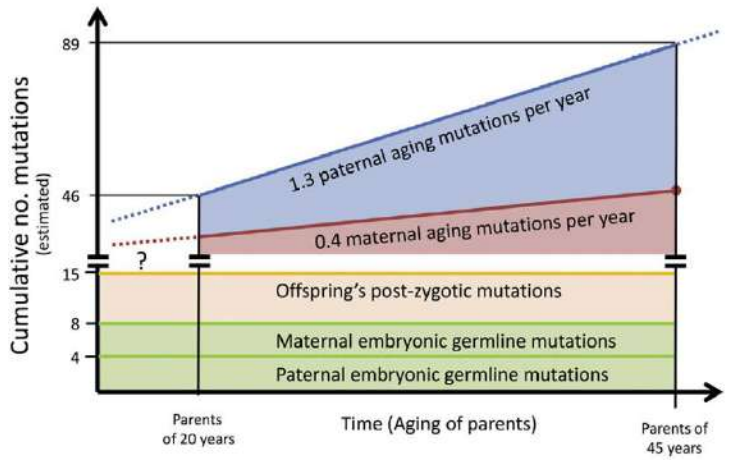
-Improve assessment of genetic embryonic lethality beyond aneuploidies

-Mitigate the burden of genetic diseases for the next generations

The positive or negative evolutionary stream of genomic diversity





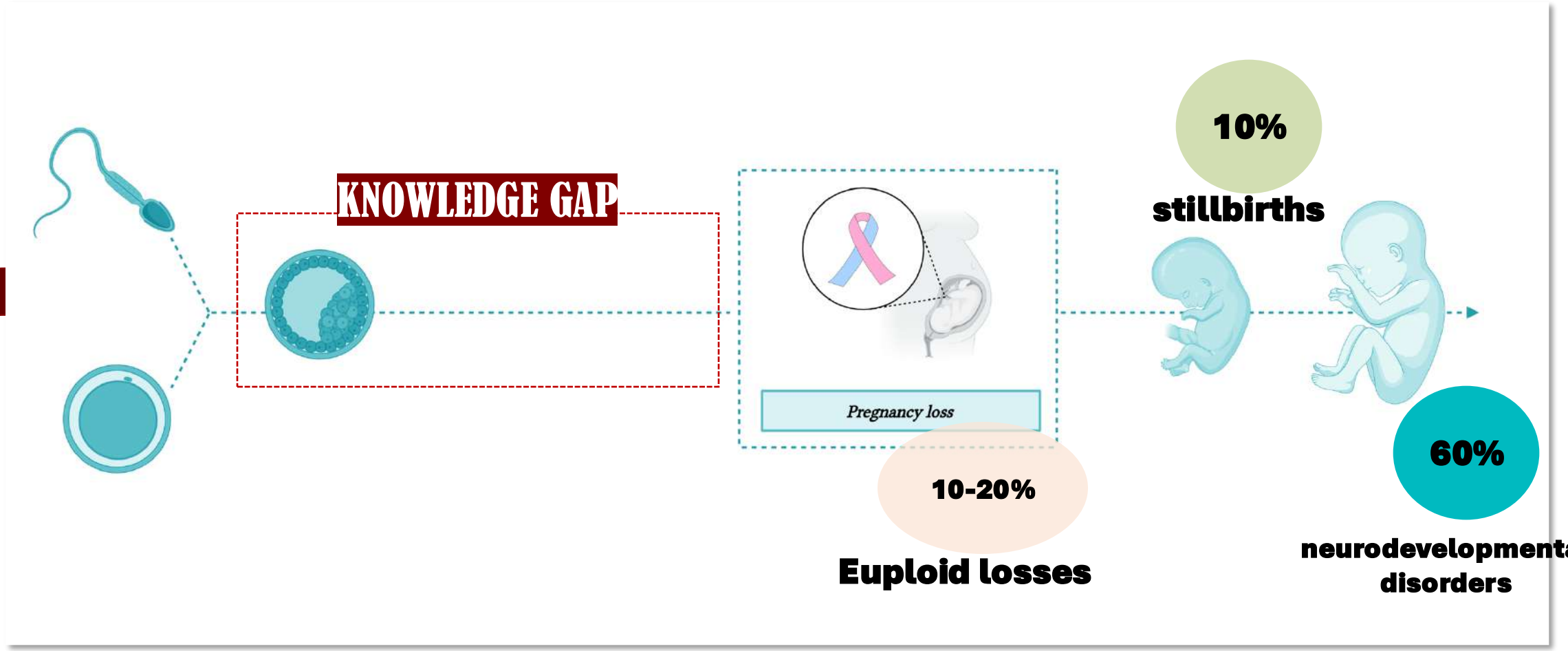


100 Novel SNVs, Indels or CNVs per genome per generation

Additional DNMs per year of life of the father 2

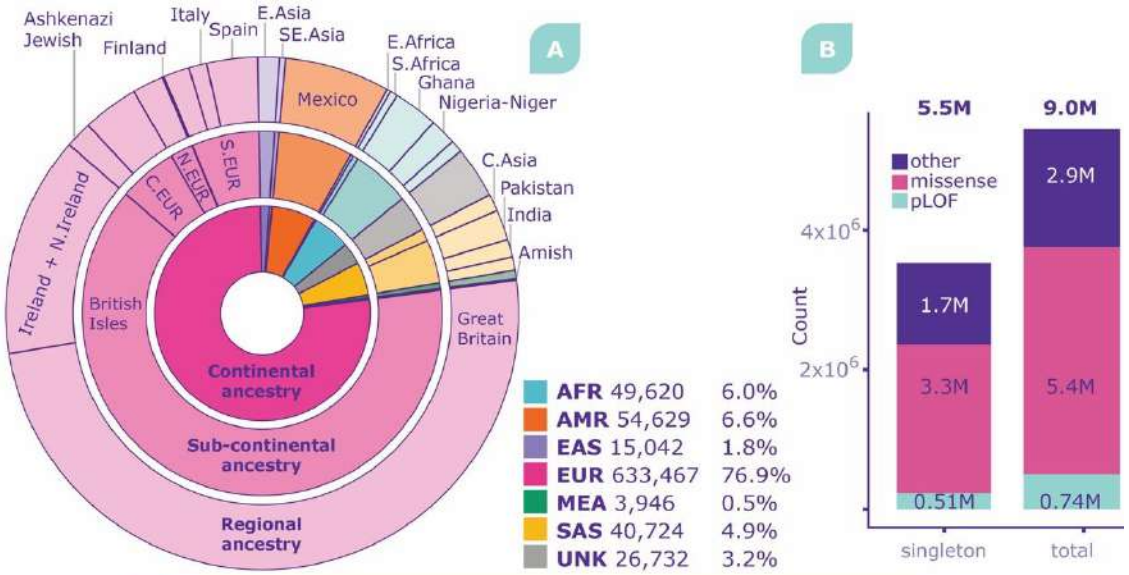
60% **Newborns with neurodevelopmental disorders**

The unkwown genomic diversity of human perimplantation embryos



- Small CNVs
- SNVs
- Epigenetic markers of differentiations

Evolutionary constraint and embryonic lethal genes



C

Functional category	Total variants	% singletons	% Per-sample counts med
frameshift	561,980	58%	71
splice acceptor	96,329	54%	10
pLOFs	**1,117,942**	**53%**	**137**
splice donor	119,447	51%	10
stop gained	340,191	47%	46
in-frame indel	185,021	46%	148
missense	10,462,959	40%	8,649
synonymous	4,652,474	36%	10,180

3,459 genes intolerant to loss-of-function

Infertility and early embryonic lethality

RGC-ME: Regeneron Genetics Center; pLOF: predicted loss-of-function; AFR: African; AMR: Admixed American; EUR: European; EAS: East Asian; MEA: Middle Eastern; SAS: South Asian; UNK: Unknown

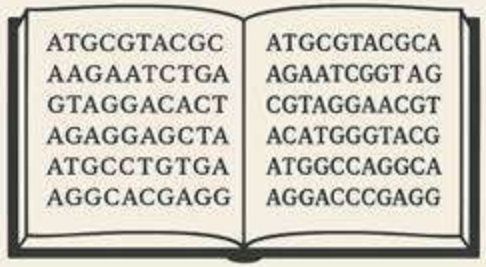
Sun KY, et al., bioRxiv [Preprint]. 2023

From standard PGT to clinical WGS of embryos to unravel genomic diversity of human embryos

STANDARD PGT-A

Gross chromosomal abnormalities

Skimming through a book to see if any chapters are missing or in the wrong order



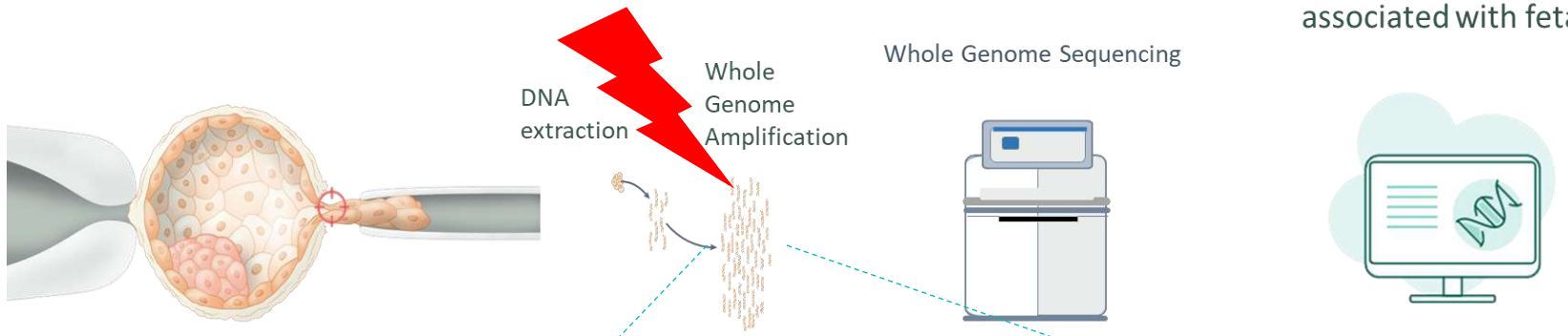
Reading every single letter of every word in the book, catching a typo

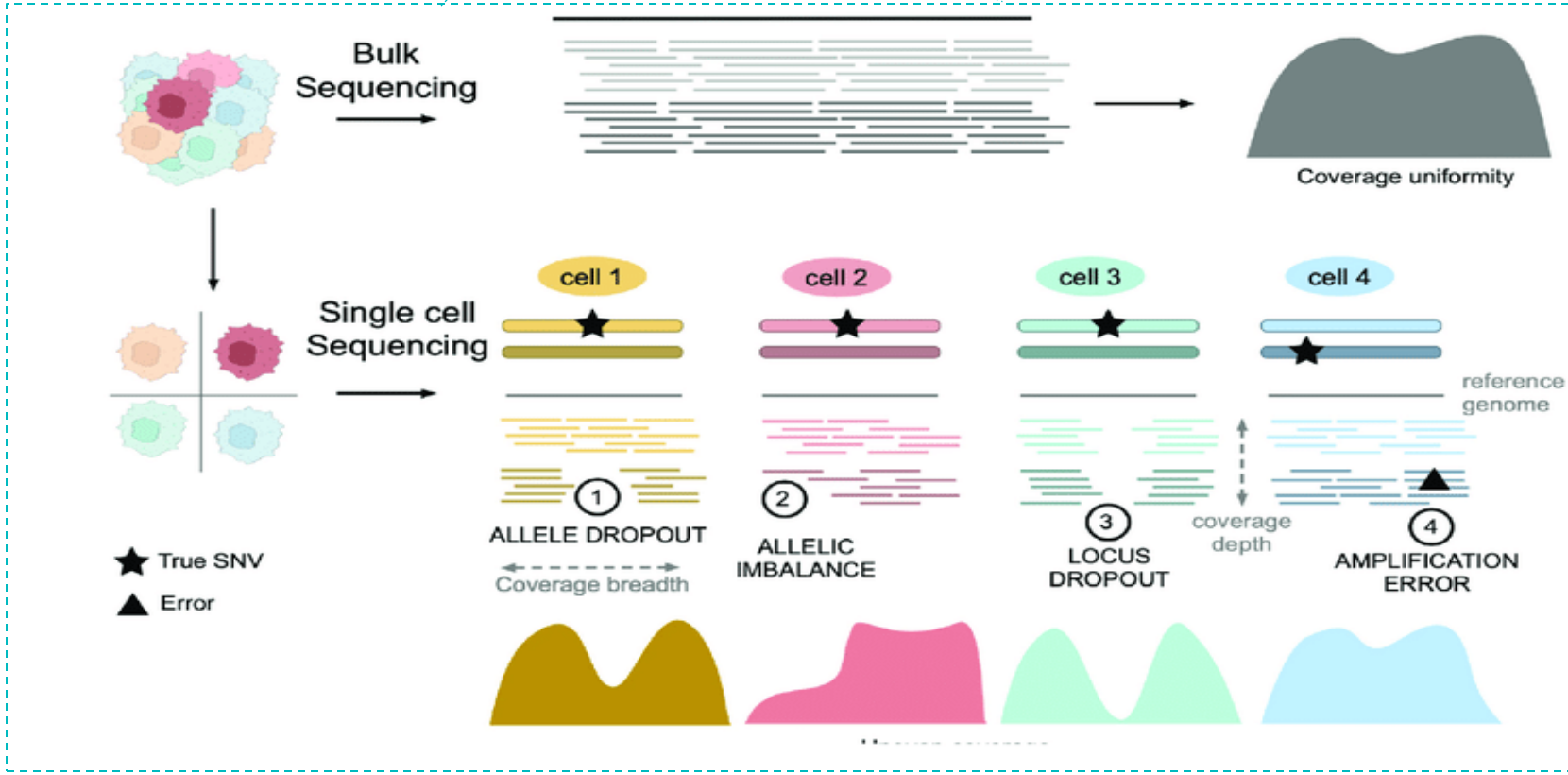
Clinical WGS in PGT-A

- SVs
- Indels
- Criptic structural rearrangements
- CNVs
- Repeat expansion
- mtDNA variants
- Gross chromosomal rearrangements

Limitation of current whole genome sequencing: whole genome amplification artifacts

SNV, Indels, CNVs
associated with fetal death





ref

"If you cannot measure it, you cannot improve it". Lord Kelvin

On average **441,608,608 autosomal SNVs x genome** (UK biobank analysis on 77 WGS trios)

Minimal analytical requirements

>90% genome covered

>99% small variants concordance

Table 3. Metrics for clinical whole-genome sequencing.

Metric	Description	Type (threshold) or typical expected value
Examples of pass/fail metrics		
Sample identity	Concordance with genotype (orthogonal and/or family structure when available).	Pass/fail (match)
Contamination[a]	The estimated level of sample cross-individual contamination based on a genotype-free estimation.	Pass/fail (≤2%)
Gb ≥ Q30[b]	Total aligned gigabases (Gb) of data with base quality score >Q30.	Pass/fail (≥80 Gb)
Autosome mean coverage[c]	The mean coverage across human autosomes, after all filters are applied.	Pass/fail (≥30)
% Callability[d]	Percent of non-N reference positions in autosomal chromosomes with a passing genotype call.	Pass/fail (>95%)
Examples of metrics to monitor		
%Q30 bases total	The percentage of bases that meet Q30 scores.	≥85%
20x%[e]	The fraction of non-N autosome bases that attained at least 20x sequence coverage in post-filtering bases.	≥90%
PF reads aligned %	The percentage of passing filter (PF) reads that align to the reference sequence.	>98%
PF aligned Q20 bases[f]	The number of bases aligned to the reference sequence in PF reads that were mapped at high quality and where the base call quality was Q20 or higher.	>1.0E+11
Adapter-dimer %	The fraction of PF reads that are unaligned and match to a known adapter sequence right from the start of the read.	<0.2%
Chimera %	The percentage of reads that map outside of a maximum insert size (usually 100 kb) or that have the two ends mapping to different chromosomes.	<1%
Duplication %	The percentage of mapped sequence that is marked as duplicate.	<10%
Median insert size[g]	The median insert size of all paired end reads where both ends mapped to the same chromosome.	>300 bp
Excluded total %	The percentage of aligned bases excluded due to all filters.	<15%

Reference samples and methodology

Benchmarking analytical performance of WGS on orthogonally validated genome in a bottle trios

$$\text{Precision} = \frac{TP}{TP + FP}$$

$$\text{Recall} = \frac{TP}{TP + FN}$$

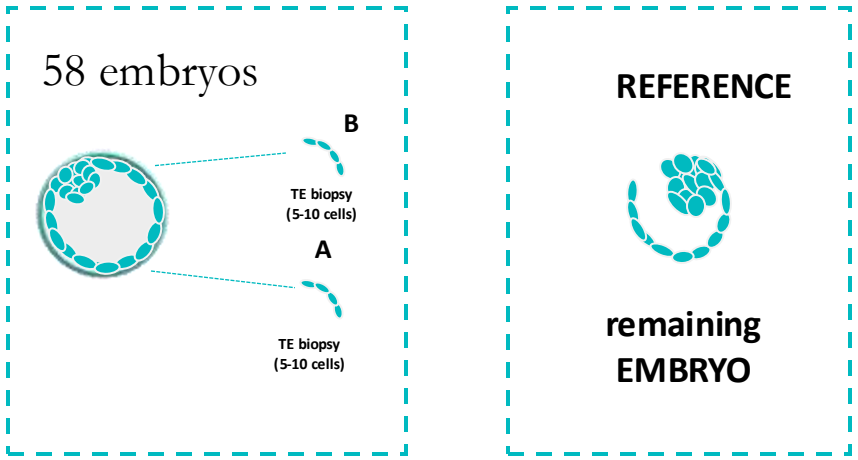
Marshall et al.,2021; Wang et al., 2021; Browning et al., 2023

Whole Genome Amplification based Whole genome sequencing: first validation

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] Dhruve Chandramohan, Ph.D.,[a] Elan Bechor, Ph.D.,[a] Benjamin Podgursky, M.Sc.,[a] Michael Hoxie, B.S.,[a] Qinnan Zhang, Ph.D.,[a] Willy Chertman, M.D.,[a] Jessica Kang, B.S.,[b] Edwina Blue, B.S.,[b] Justin Chen, B.S.,[b] Justin Schleede, Ph.D.,[a] Nathan R. Slotnick, M.D., Ph.D.,[a] Xiaoli Du, Ph.D.,[a] Robert Boostanfar, M.D.,[b] Eric Urcia, M.Sc.,[b] Barry Behr, Ph.D.,[c] Jacques Cohen, Ph.D.,[d] and Noor Siddiqui, M.Sc.[a]

[a] Laboratory Department, Orchid Health, Palo Alto, California; [b] HRC Fertility-Encino, Encino, California; [c] Department of Obstetrics and Gynecology - Reproductive Endocrinology and Infertility, Stanford University, Sunnyvale, California; and [d] A.R.T. Institute of Washington, Bethesda, Maryland



WGA and WGS *NovaSeq6000 at 30X*

Validation results of the whole genome screening. Comparison of biopsies and their embryos in terms of genomic coverage, total SNV called, accuracy, specificity, precision, and sensitivity. The whole embryos were used as references.

Sample	Genomic Coverage	Total SNV	Accuracy	Specificity	Precision	Sensitivity
1	99.7%	3343804	99.995%	99.997%	97.8%	98.4%
2	99.6%	3327631	99.995%	99.997%	98.0%	98.1%
3	99.6%	3312139	99.995%	99.997%	97.9%	98.1%
4	99.4%	3279468	99.993%	99.997%	97.8%	97.1%
5	99.6%	3404155	99.995%	99.998%	98.4%	98.1%
6	99.7%	3414277	99.996%	99.998%	98.4%	98.4%
7	99.6%	3360682	99.995%	99.998%	98.4%	98.1%
17	99.6%	3275227	99.995%	99.997%	97.7%	98.2%
18	99.6%	3254447	99.995%	99.998%	98.1%	98.4%
19	99.4%	3234717	99.995%	99.998%	98.2%	97.9%
20	99.7%	3274002	99.995%	99.997%	97.9%	98.5%
21	99.5%	3257361	99.995%	99.998%	98.1%	98.2%
22	99.6%	3270572	99.995%	99.998%	98.2%	97.9%
23	99.7%	3284833	99.995%	99.998%	98.2%	98.3%
24	99.6%	3237713	99.993%	99.997%	97.9%	97.0%
25	99.7%	3382545	99.996%	99.998%	98.3%	98.6%
Average	99.6%	3294417	99.995%	99.997%	98.0%	98.1%

Xia. First clinical validation of PGT-WGS. Fertil Steril Rep 2024.

With a precision of 98%, on 400.000.000 variants:
At least 8,000 FP variants called per sample.

- Recall not assessed because lack of parental DNA and TPs data
- 3294417 SNVs, loss of genomic representation (vs 441M SNVs)
- FPs potentially underestimated because of lack of parental DNA

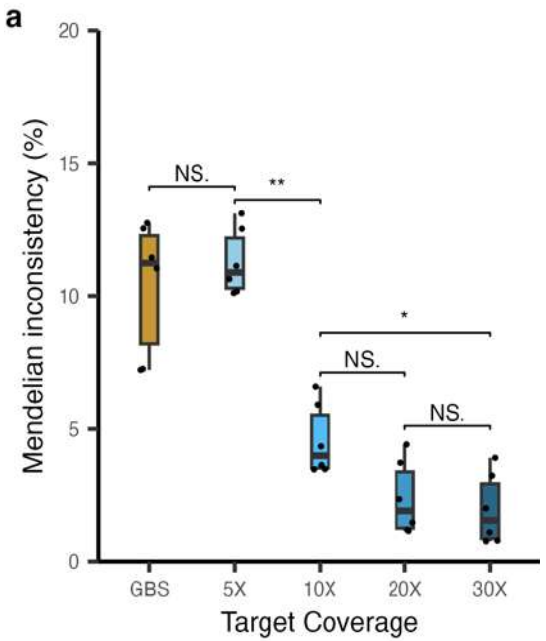
nature communications

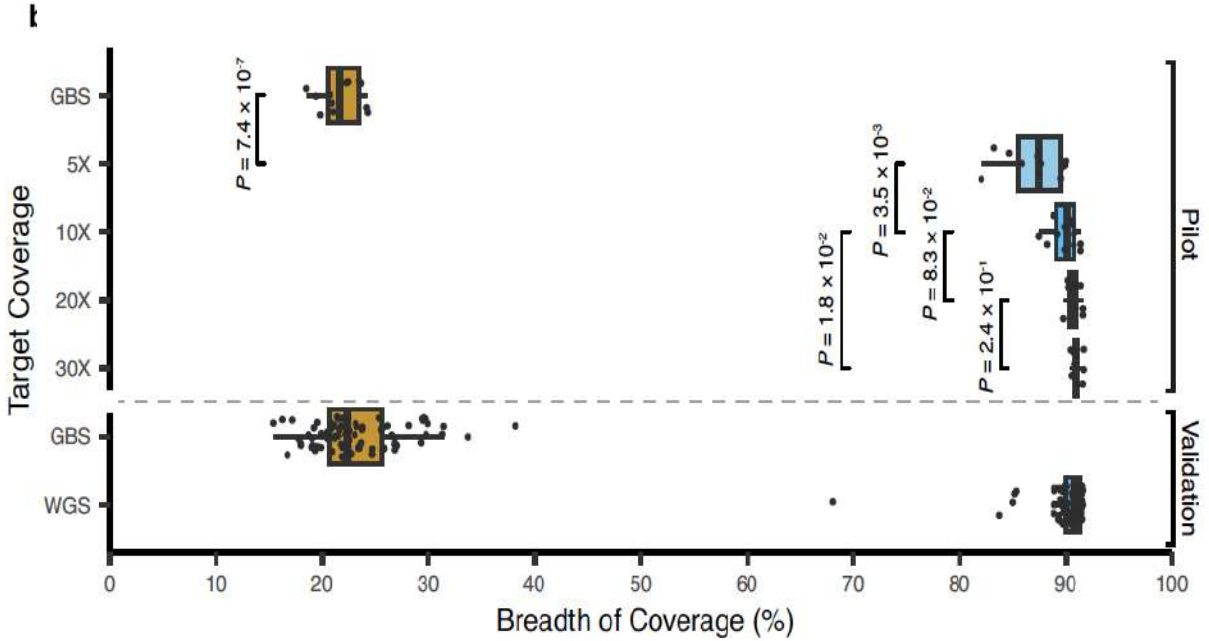
Article

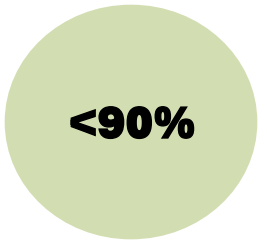
https://doi.org/10.1038/s41467-024-51508-1

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

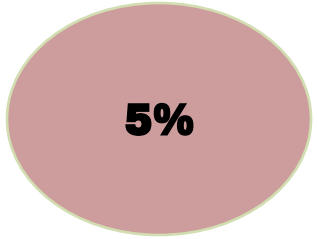
Anouk E.J. Janssen[1,2,*], Rebekka M. Koeck[1,2,*], Rick Essers[1,2,*], Ping Cao[1,2], Wanwisa van Dijk[1], Marion Drüsedau[1], Jeroen Meekels[1], Burcu Yaldiz[1], Maartje van de Vorst[1], Bart de Koning[1], Debby M.E.I. Hellebrekers[1], Servi J.C. Stevens[1], Su Ming Sun[1], Malou Heijligers[1], Sonja 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], 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[1,2,7]





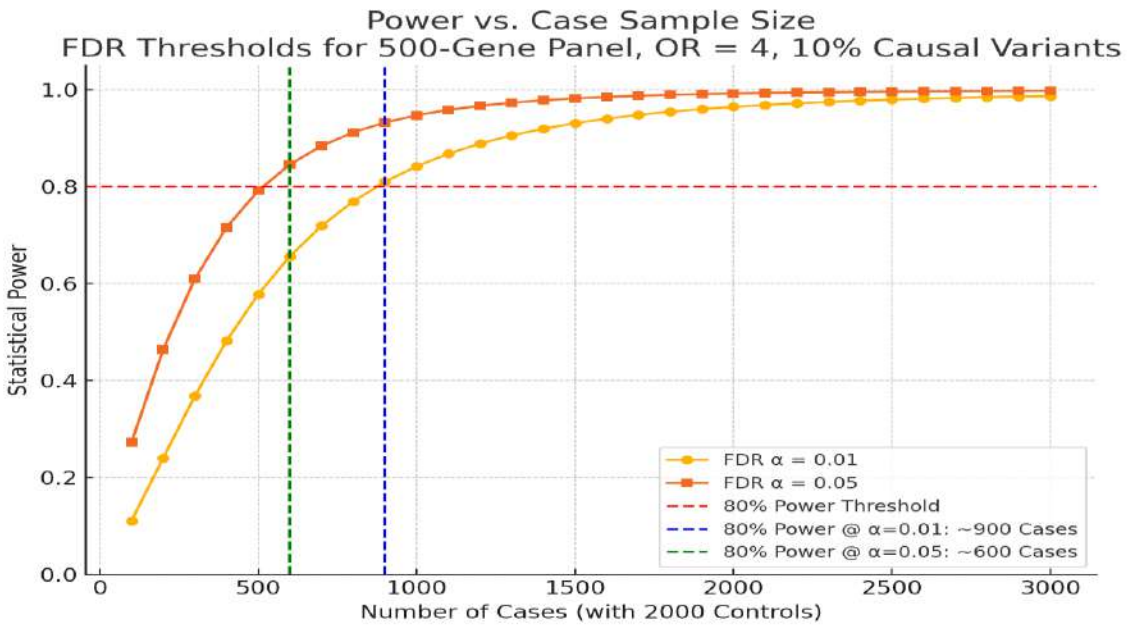


genome covered

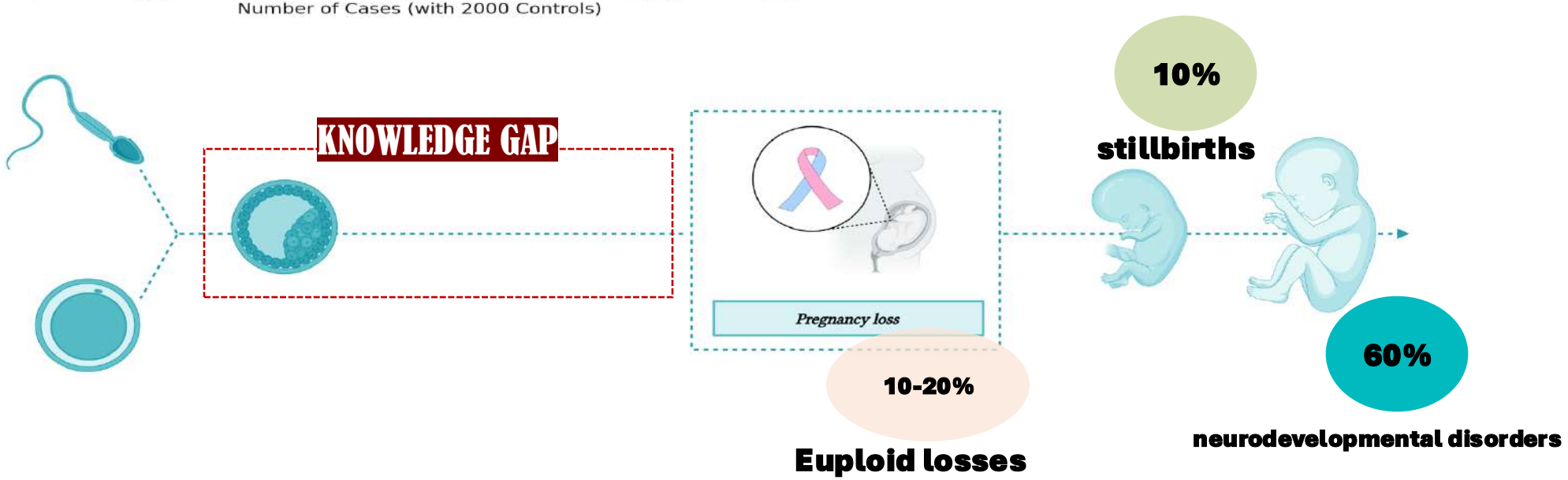


Mendelian inconsistency

Next step after analytical validation is established: clinical validity assessment in prospective blinded trials



Testing association of candidate genes to embryonic lethal phenotype will require powered prospective blinded studies



Advancing PGT Through Whole Genome Sequencing

- Genomic causes of developmental failure
- Make PGT equitable across All indications (mtDNA, Repeat expansions, Small translocations)
- Genomic health of future child (carrier screening and new-born screening)

*if analytical performance and clinical trials will confirm

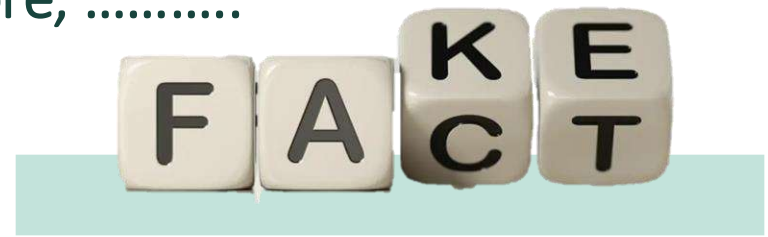
POINTS TO CONSIDER BEFORE IMPLEMENTATION OF WGS

- What is the clinical gain?
- Cost-effectiveness?
- How to manage incidental findings?

Clinical trials required

From research to clinic, there is no shortcut:

After niPGT, overcalling mosaicsism, mithochondrial DNA score, ...



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Reproductive Geneticists Gathered in Paris Present the First-Ever Whole Genome Sequencing Test in Embryos

Tuesday, April 18, 2023 5:05 PM

Claims without validations

It has been designed to:

- offer the **most comprehensive level of screening** currently available in preimplantation genetic testing field;
- screen for **5000+ severe genetic disorders, inherited** or due to ***de novo*** mutations;
- focus on the exonic regions of **4000+** disease-causing genes associated with known clinical phenotypes, thereby comprising the **clinical exome**;
- provide a more uniform and robust coverage and performance over **genes of clinical interest**;
- screen for **aneuploidies** and **structural chromosomal abnormalities**.

We MUST be careful NOT to prematurely adopt a technology just because of its potential

Grazie a tutti

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