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Precision Reproductive Medicine: The Next Era of PGT

Main principles for establishing a clinical genetic (PGT) test

ANALYTICAL VALIDITY

how accurately the test measures the **genetic variant(s)** of interest

(PGT-A: aneuploidy detection in known bx samples)

CLINICAL VALIDITY

Clinical performance in detecting the **phenotype** of interest

(for PGT-A embryo lethality)

CLINICAL UTILITY

The extent to which the test improves health outcomes

(for PGT-A: LBR, miscarriage)

Go to clinic!

GENETIC FINDINGS SHOULD BE REPORTED ONLY IF THEY FACILITATE INFORMED DECISIONS AND IMPROVMENT OF MEDICAL CARE

acgs
Association for Clinical Genetic Science
Part of the British Society for Genetic Medicine

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]

ESHG www.nature.com/ejhg

ARTICLE OPEN

Check for updates

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 Õunap[7,8], Melody Tabiner[9], Rebecca Treacy[1] and Christi J. van Asperen[10]

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© American College of Medical Genetics and Genomics 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[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Elai

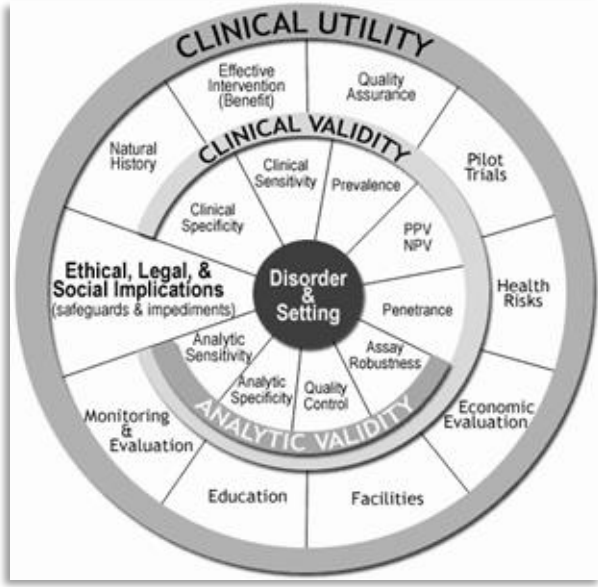
European Journal of Human Genetics (2016) 24, 2–5
© 2016 Macmillan Publishers Limited All rights reserved 1018-4813/16
www.nature.com/ejhg

EJHG Open

POLICY

Guidelines for diagnostic next-generation sequencing

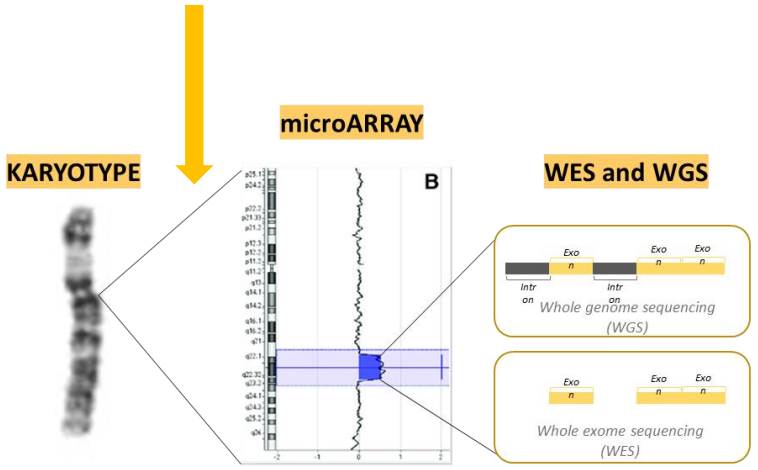
Gert Matthijs[*,1,8], Erika Souche[1,8], Mariëlle Alders[2], Anniek Corveleyn[1], Sebastian Eck[3], Ilse Feenstra[4], Valérie Race[1], Erik Sistermans[5], Marc Sturm[6], Marjan Weiss[5], Helger Yntema[4], Egbert Bakker[7], Hans Scheffer[4] and Peter Bauer[6]



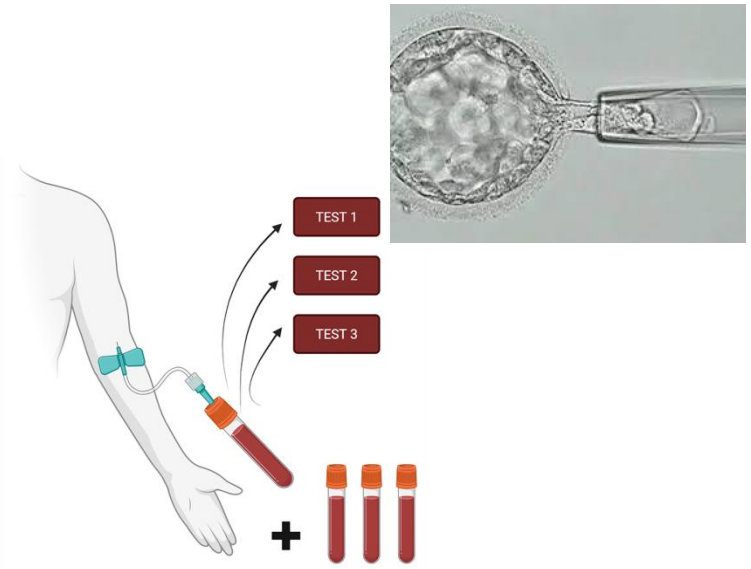
Rethinking the limits of genetic testing in embryos: **Achieving optimal analytical accuracy is particularly important in PGT**

CRUCIAL POINTS TO KEEP IN MIND

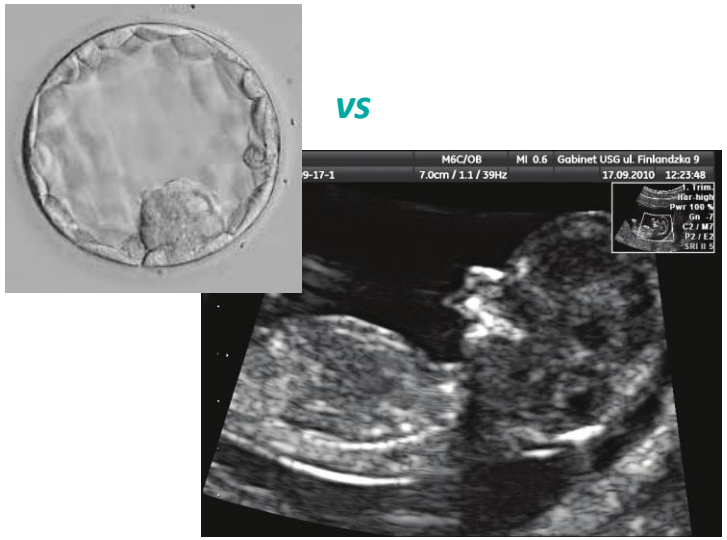
Resolution: 3-7 Mb (according to the platform/genomic position) PGT can detect aneuploidies and gross structural unbalances



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



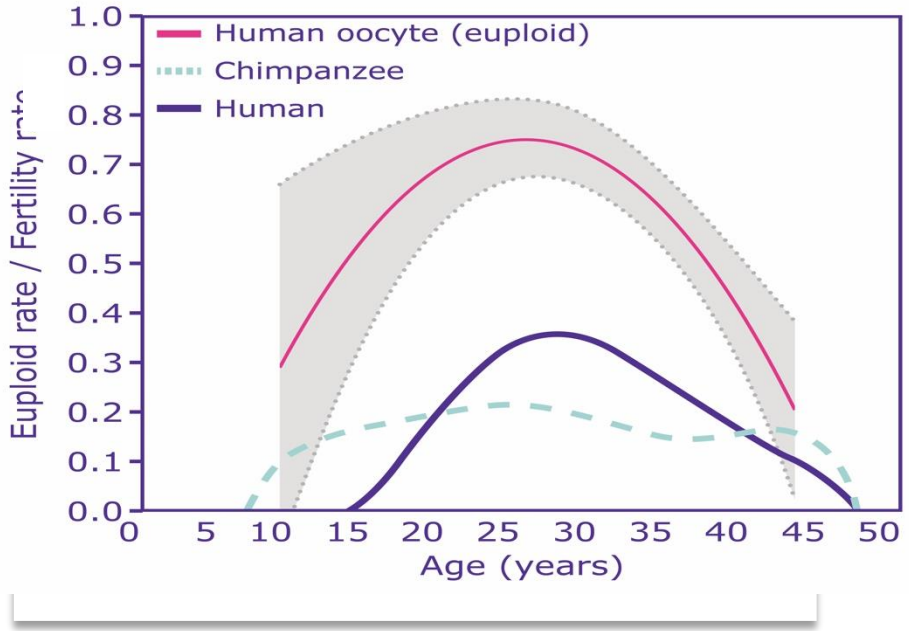
Meiotically derived aneuploidies from egg shape natural fertility

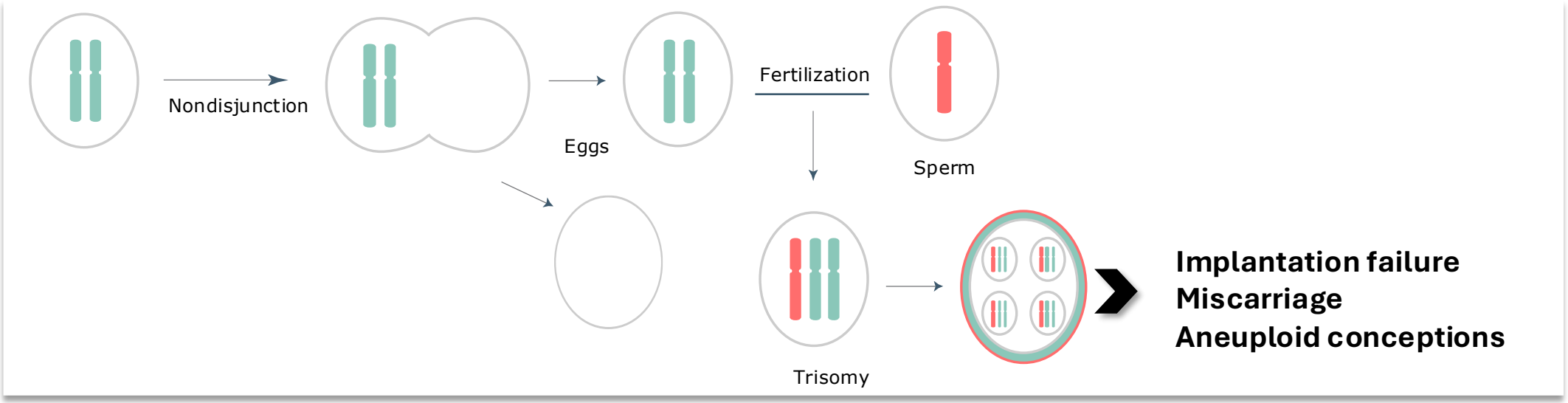
RESEARCH

REPRODUCTIVE BIOLOGY

Chromosome errors in human eggs shape natural fertility over reproductive life span

Jennifer R. Gruhn[1*], Agata P. Zielinska[2*], Vallari Shukla[1*], Robert Blanshard[3,4*], Antonio Capalbo[5], Danilo Cimadomo[6], Dmitry Nikiforov[7,8], Andrew Chi-Ho Chan[1], Louise J. Newnham[3], Ivan Vogel[1], Catello Scarica[9], Marta Krapchev[10], Deborah Taylor[11], Stine Gry Kristensen[7], Junping Cheng[7], Erik Ernst[12], Anne-Mette Bay Bjørn[12], Lotte Berdiin Colmorn[13], Martyn Blayney[14], Kay Elder[14], Joanna Liss[10,15], Geraldine Hartshorne[11], Marie Louise Grøndahl[16], Laura Rienzi[6], Filippo Ubaldi[6], Rajiv McCoy[17], Krzysztof Lukaszuk[10,18,19], Claus Yding Andersen[7], Melina Schuh[2], Eva R. Hoffmann[1†]

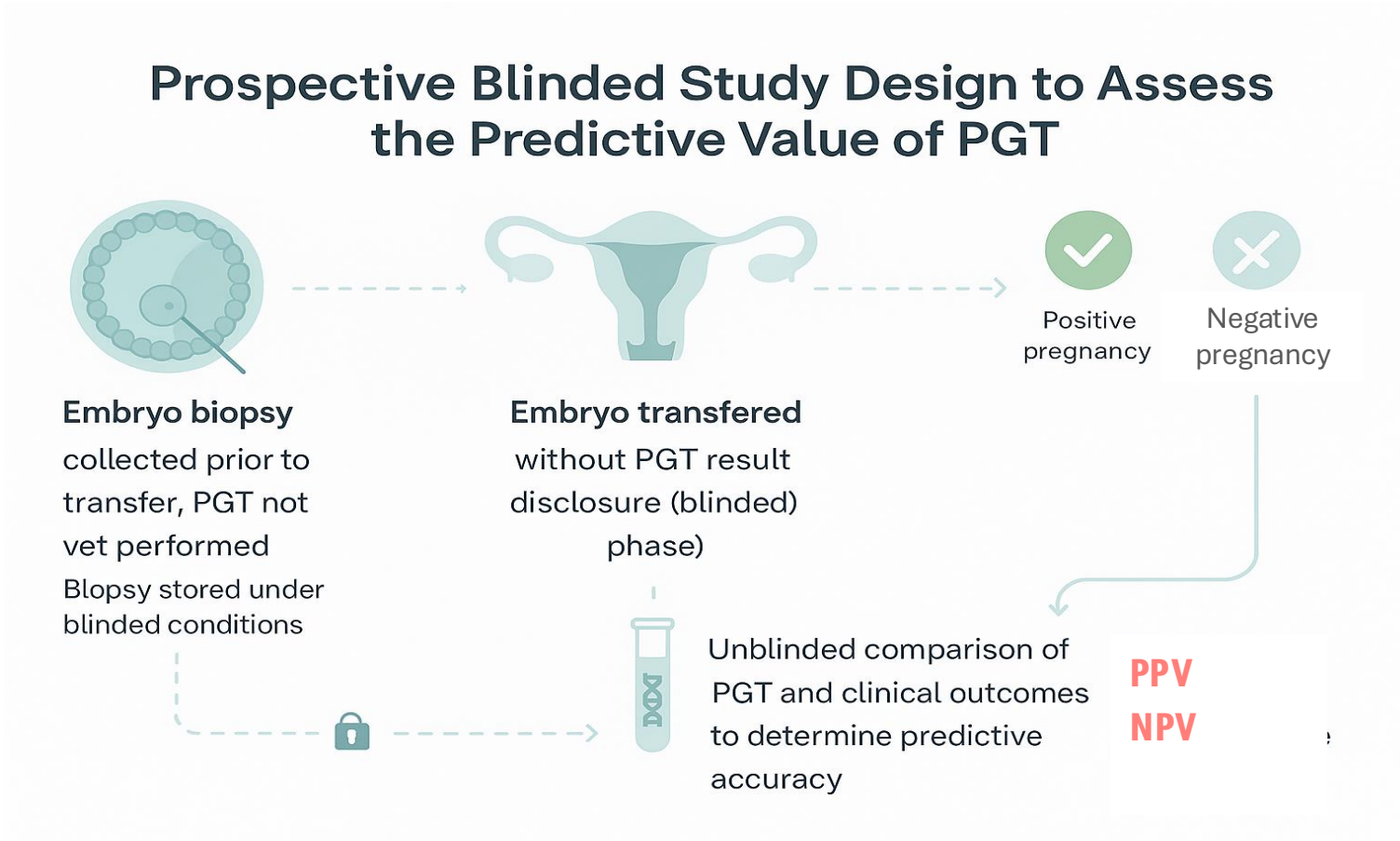


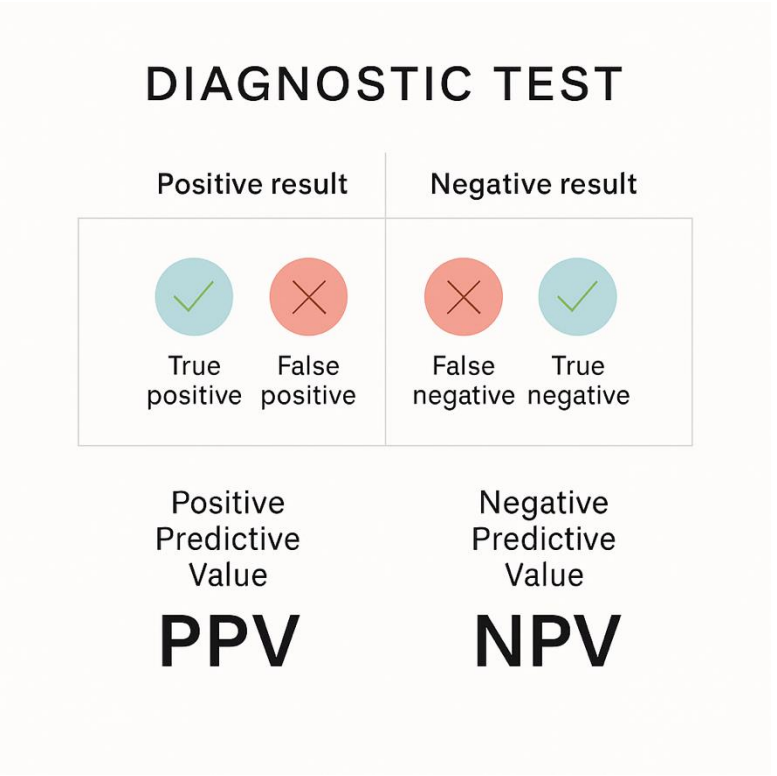


1. Gruhn JR, et al., Science. 2019; 2. Capalbo A, et al., Hum Reprod Update. 2017; 3. Ottolini CS, et al., Nat Protoc. 2016; 4. Ottolini CS, et al., Nat Genet. 2015; 5. McCoy RC, et al., Hum Mol Genet. 2018; 6. Hassold TJ & Hunt PA. Nat Rev Genet. 2001.

Evaluating clinical validity: why non-selection 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.



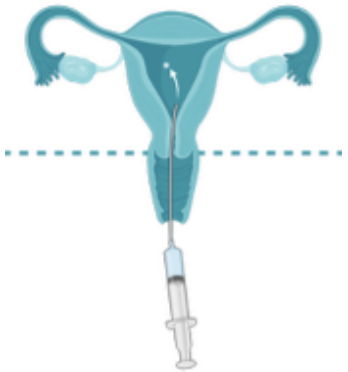


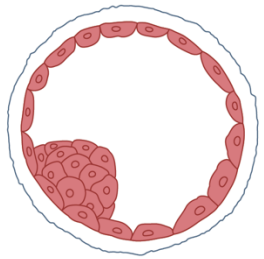
Uniform aneuploid embryos do not make babies!

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

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]

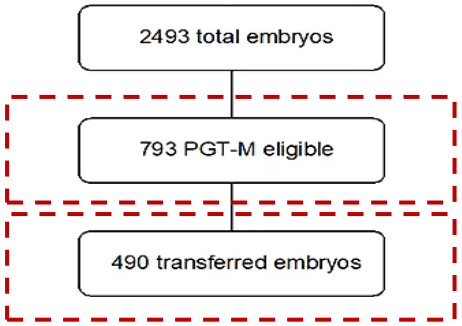


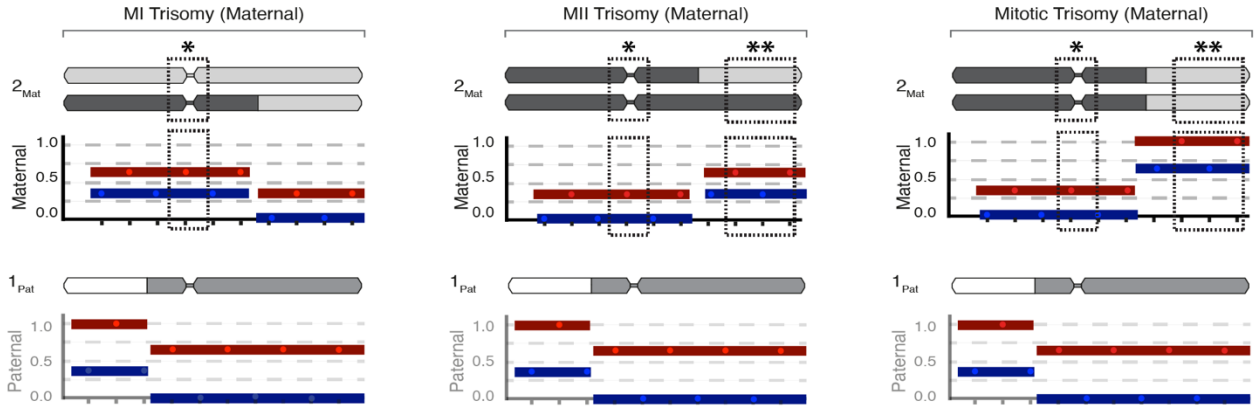


Study	Design	Transfers of Uniformly Aneuploid Embryos n	Miscarriage rate % (n, 95%CI)	Lethality rate % (n, 95%CI)
Scott et al. 2012	blinded	95	33.3% (2/6) (4.3%-77.7%)	95.8% (91/95) 84.5%-99.4%)
Tiegs et al. 2021	blinded	102	100% (24/24) (85.8%-100%)	100% (102/102) (96.5%-100%)
Wang et al. 2021	blinded	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%)**

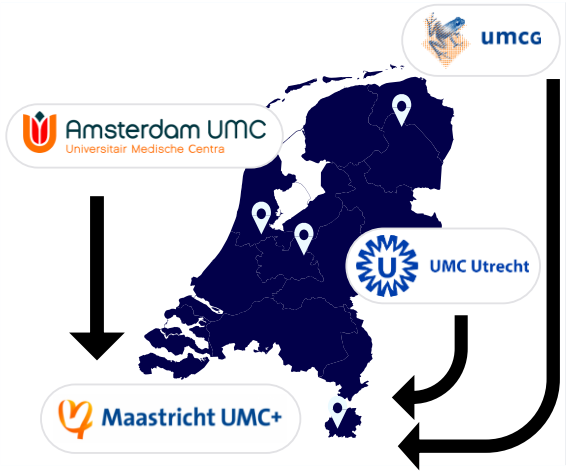
Embryos with meiotic aneuploidies fail to make babies….also in Maastricht!

PGT-AO (Aneuploidy Origin)





PGT-M Nov 2019 – Sept 2024

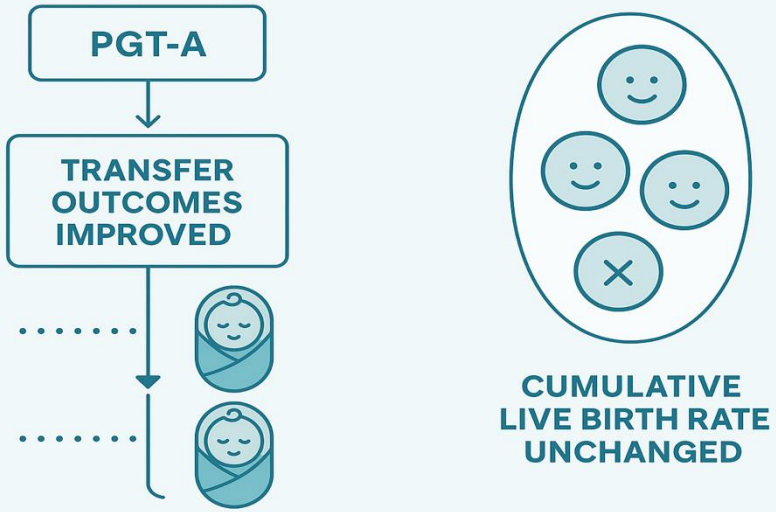


No Live Births in 83 meiotically trisomic Ets!

Essers et al. ESHRE 2024; PGT Consortium 2026

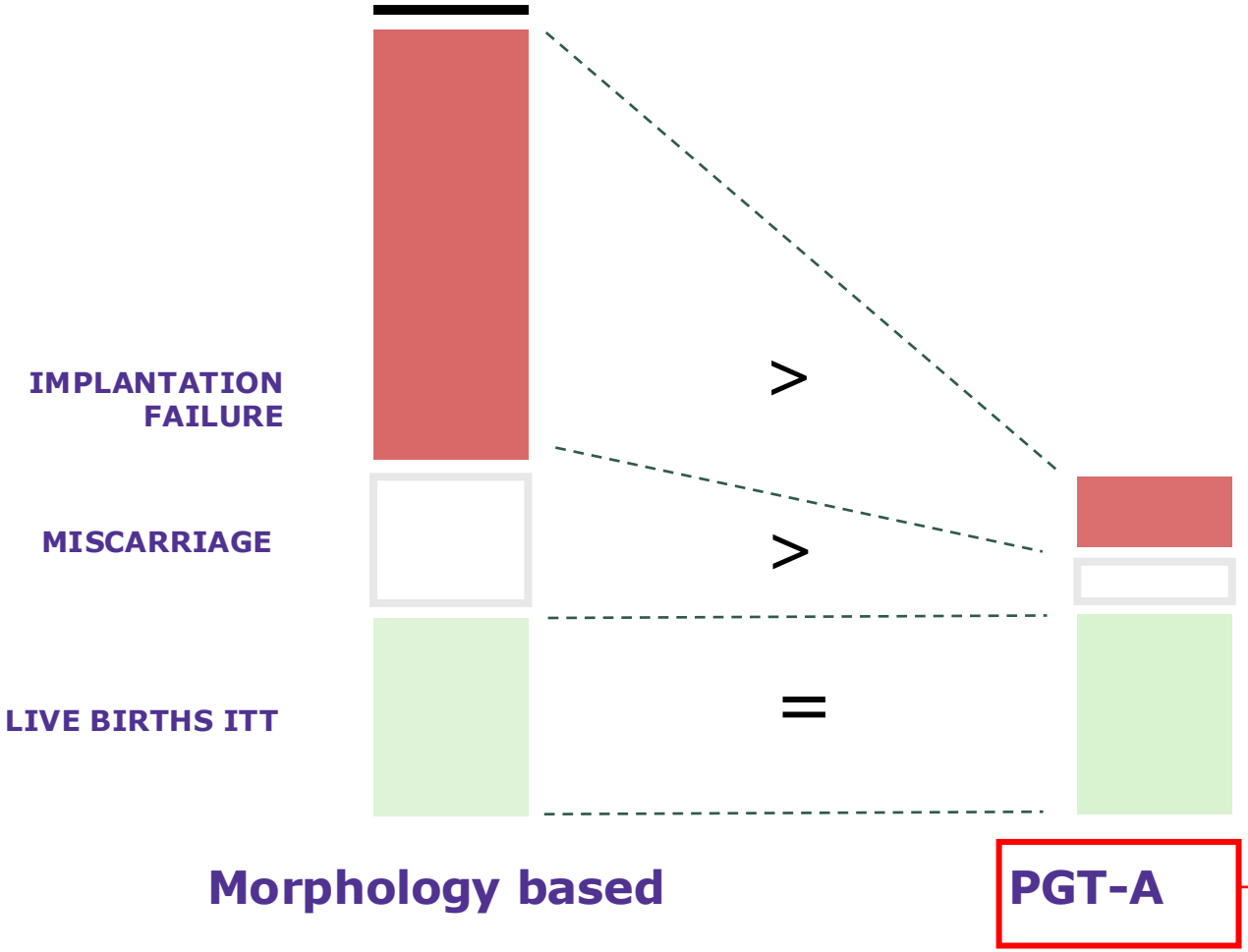
Why there still is debate?

1. Wrong outcome measure and patients population



The goal of PGT-A:

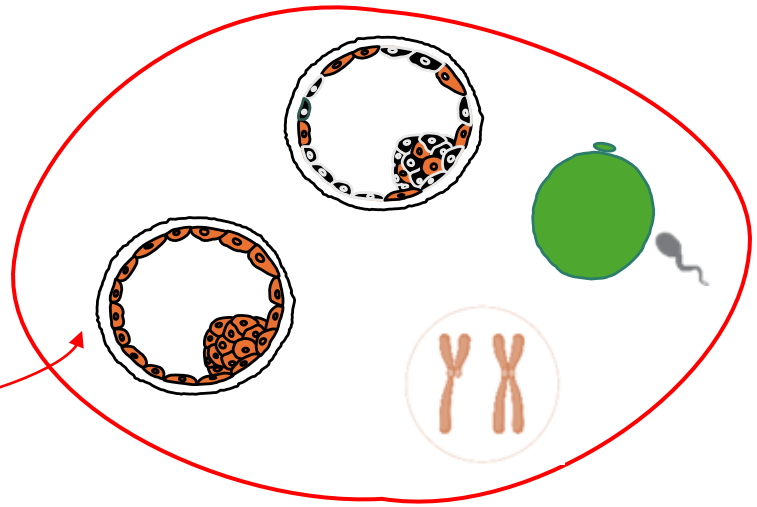
Identify and deselect embryos with uniform aneuploidies that have no reproductive potential.[1]



BEST CASE:

⇧ **ET outcomes**

= LBR per ITT



PGT-A: Preimplantation genetic testing for aneuploidies; ET: Embryo transfer; LBR: Live birth rate; ITT: Intention to treat

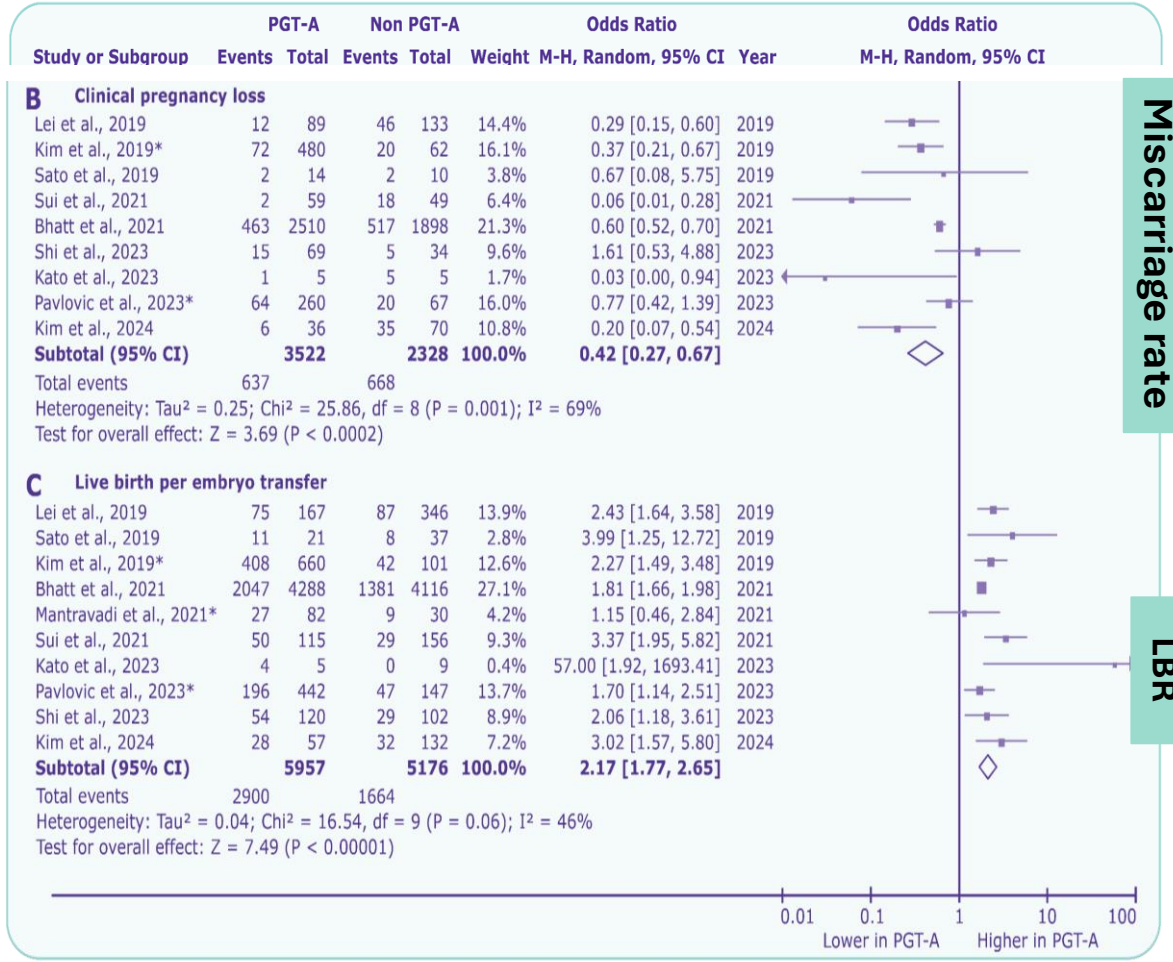
1. HFEA. Pre-implantation genetic testing for aneuploidy (PGT-A). 2025;
2. Image courtesy of Dr. Capalbo based on own insights

Clinical Utility in case-control studies

Cost-effectiveness
Somigliana and Capalbo 2019;
Collins SC et al., 2017;
Neal 2017.

Reduced time to pregnancy
Eliner et al., 2025;
Panatu et al., 2022;
Neal et al., 2018.

Similar LBR per ITT
Kasaven et al., 2023;
Sanders et al., 2021;
Yan et al., 2021.



Forest plot comparing reproductive outcomes per embryo transfer in patients with unexplained recurrent pregnancy loss undergoing in vitro fertilization treatment with and without preimplantation genetic testing for aneuploidy (PGT-A); (**A**) clinical pregnancy rates, (**B**) dinical pregnancy loss rates, (**C**) live birth rates. CI=confidence interval; M-H=Mantel-Haenszel; RPL=recurrent pregnancy loss. *Indicate meeting abstract.

Mumusoglu. PGT-A for unexplained RPL. Fertil Steril 2025.

1. Scott, et al., Fertil Steril. 2012; 2. Tiegs, et al., Fertil Steril. 2021; 3. Wang, et al., Prenatal Diagnosis. 2021; 4. Yang, et al., Nat Cell Biol. 2021; 5. Barad, et al., Hum Reprod. 2022; 6. Kasaven, et al., J Assist Reprod Genet. 2023; 7. Unpublished 8. Mumusoglu et al., Fertil Steril. 2025

Why RCTs failed: wrong definition of outcome measures
Cumulative LBR used as primary endpoint!

Live Birth with or without Preimplantation Genetic Testing for Aneuploidy

Yan J et al. DOI: 10.1056/NEJMoa2103613

CLINICAL PROBLEM

Selecting the best embryos for transfer during in vitro fertilization (IVF) optimizes the live-birth rate per transfer. Aneuploidy (i.e., missing or extra chromosomes) is associated with implantation failure and spontaneous abortion. Whether using preimplantation genetic testing for aneuploidy (PGT-A) in the embryo selection process might increase the rate of successful treatment is unclear.

CLINICAL TRIAL

Design: A multicenter, randomized, controlled, noninferiority trial evaluated whether conventional IVF is noninferior to PGT-A with respect to the cumulative live-birth rate.

Intervention: 1212 subfertile women 20 to 37 years of age with three or more good-quality blastocysts were assigned to conventional IVF, with embryos selected for transfer by morphologic criteria alone, or to PGT-A, with embryos selected by morphologic criteria and then tested for aneuploidy.

RESULTS

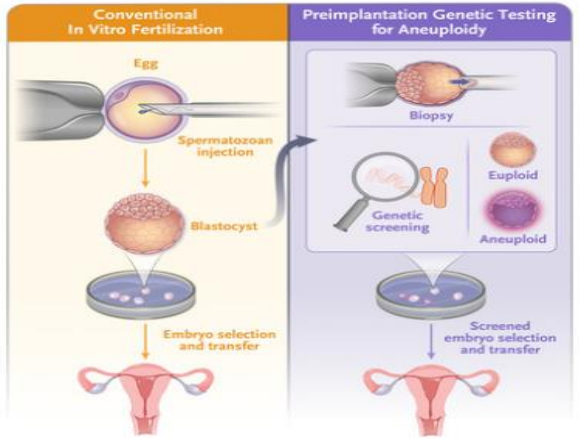
Efficacy: The cumulative live-birth rate after up to three embryo transfers within 1 year (the primary outcome) in the conventional IVF group was noninferior to that in the PGT-A group, with higher cumulative live-birth rates in the conventional IVF group.

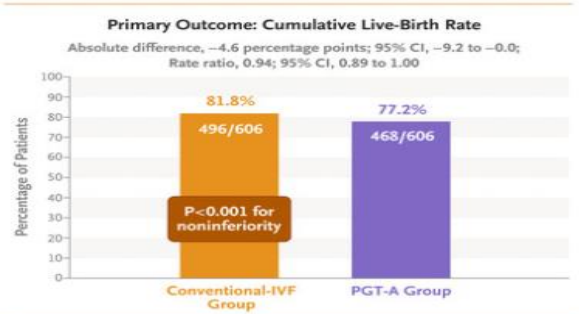
Safety: The incidences of adverse events, including moderate or severe ovarian hyperstimulation syndrome, ectopic pregnancy, and obstetrical or perinatal complications, were similar in the two groups.

LIMITATIONS AND REMAINING QUESTIONS

- Only women with a good prognosis for a live birth were included in the study, and no more than three transfers were performed, limiting the generalizability of the results to women with poorer prognoses or to situations in which more embryos are available.
- Intracytoplasmic sperm injection was performed in all patients in both groups but is not routinely performed or warranted as part of standard IVF programs.

Links: Full Article | NEJM Quick Take





CONCLUSIONS

Among women with a good prognosis for a live birth, conventional IVF resulted in a cumulative live-birth rate that was noninferior to that with PGT-A.

Study weaknessess:

- Only 3 embryos received biopsy in PGT group
- «Mosaic» embryos not trasnferred by policy!!!
- Mean female age **29 years**!!!!
- Primary outcome: **non-inferiority of control group for the cumulative LBR!!!**

RESULTS: Similar number of babies with fewer transfers and ~30% fewer miscarriages (8.7% vs. 12.6%)

CONCLUSIONS

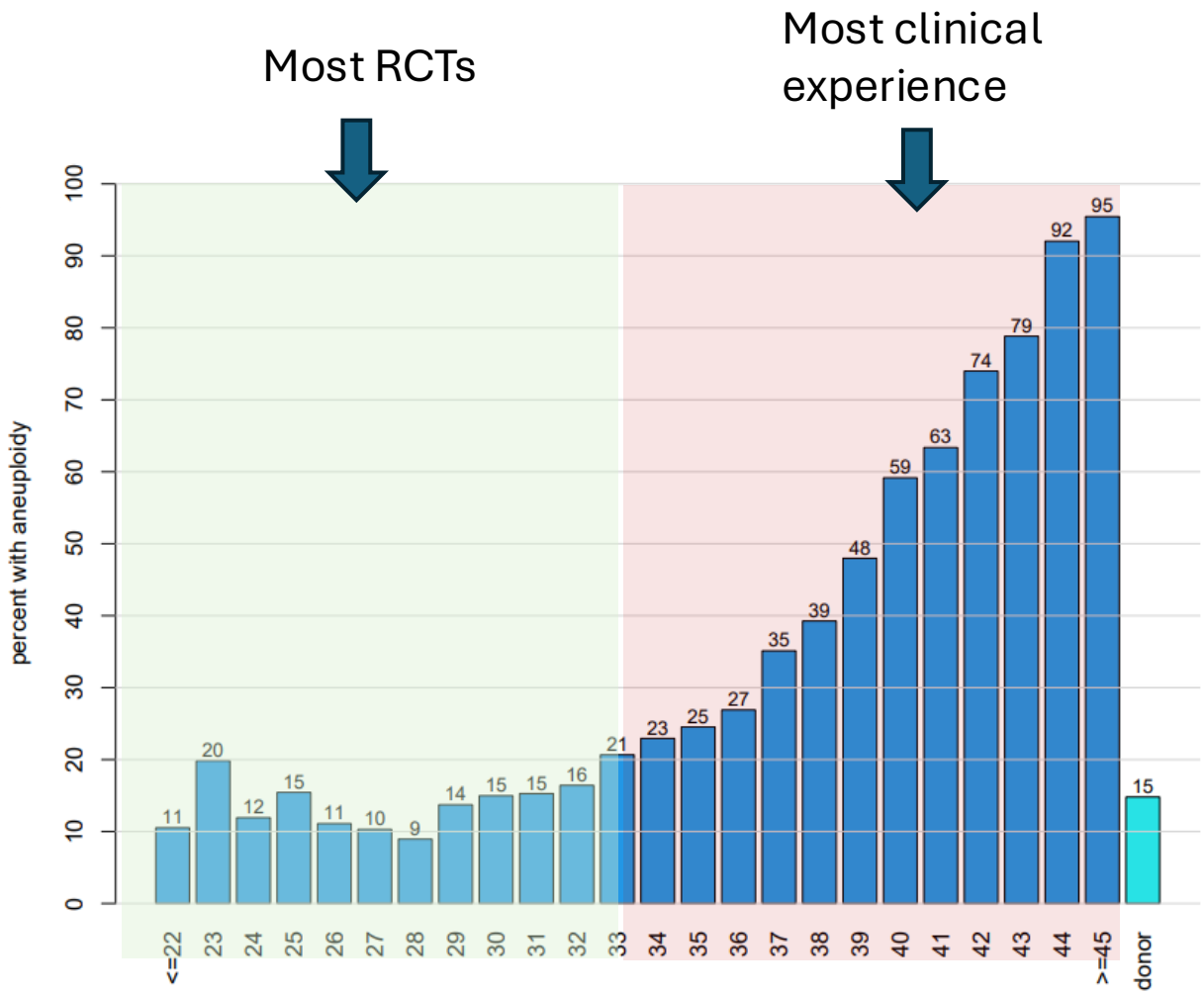
Among women with a good prognosis for a live birth, conventional IVF resulted in a cumulative live-birth rate that was noninferior to that with PGT-A.

More appropriate DEFINITION OF PATIENT POPULAITON FOR PGT-A

AMA: >35 years

RPL: avoid recurrent aneuploidies

RIF: reduced TTP



aneuploidy rate in blastocysts by female age

Journal of Clinical Medicine

MDPI

Article

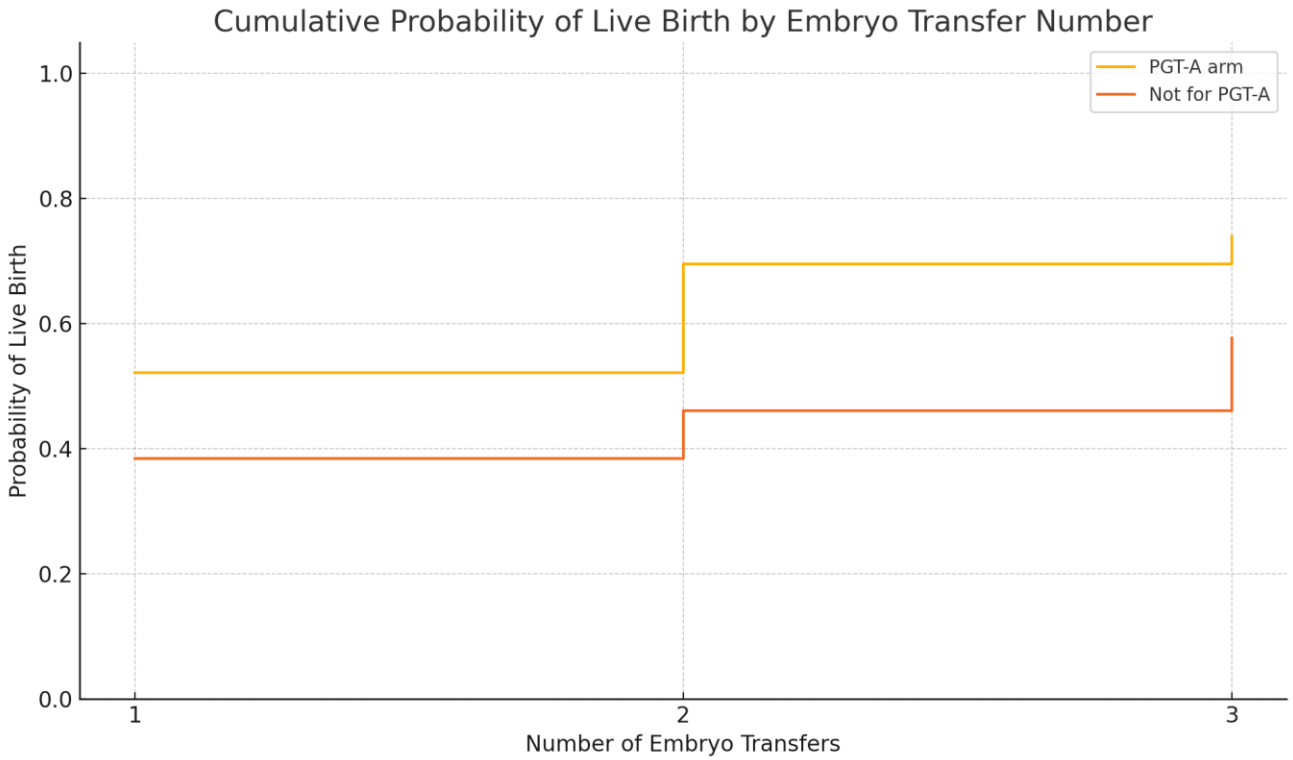
Preimplantation Genetic Testing for Aneuploidy Versus Morphological Selection in Women Aged 35–42: Results of a Pilot Randomized Controlled Trial

Yusuf Beebeejaun [1,2,*], Daniela Bakalova [3], Anastasia Mania [1], Timothy Copeland [4], Ippokratis Sarris [1,2], Kypros Nicolaides [2,5], Antonio Capalbo [6] and Sesh K. Sunkara [1,2]

Published: 21 July 2025

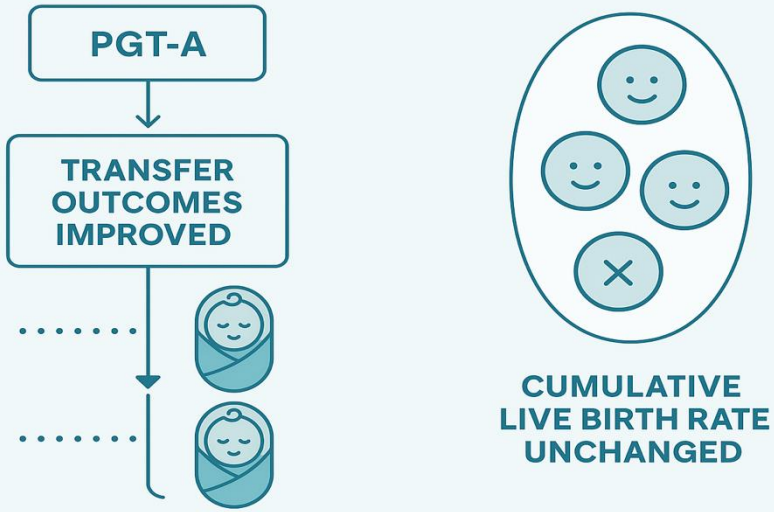
The only RCT where mosaicism was not used to inform embryo selection showed improved LBR with PGT-A

	PGT-A	Contr	
Cumulative live birth (Total of 3 ET)	**36/50 (72.0%)**	**26/50 (52.0%)**	**OR 2.37, 95% CI: 1.04–5.44; p-value = 0.04**



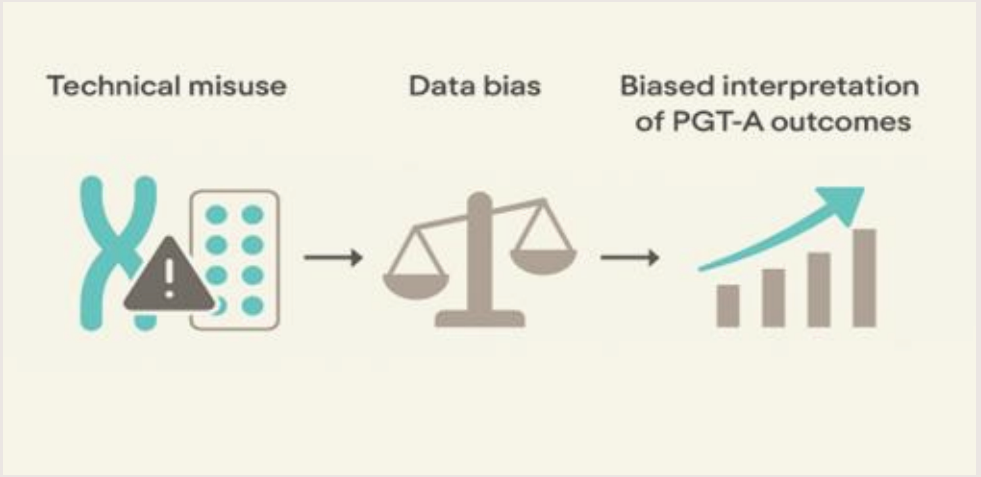
Why there still is debate?

1. Wrong outcome measure



2. Wrong use of technology

i.e. mosaicism reporting



The clinical utility of a technology should not be dismissed simply because it's inconsistently applied

Lack of clinical utility of mosaicism reporting in prospective blinded studies

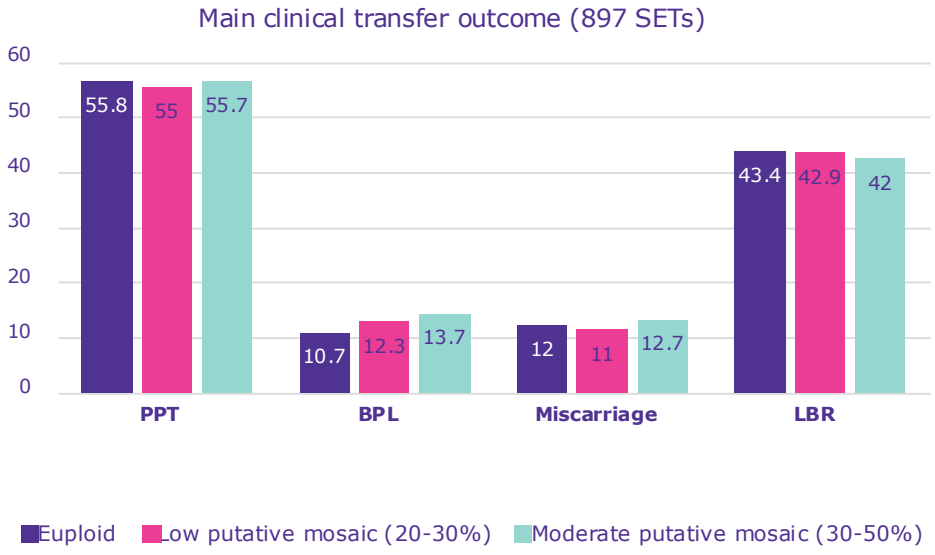
1st prospective blinded study

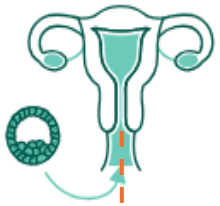
AJHG 2022

ARTICLE

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

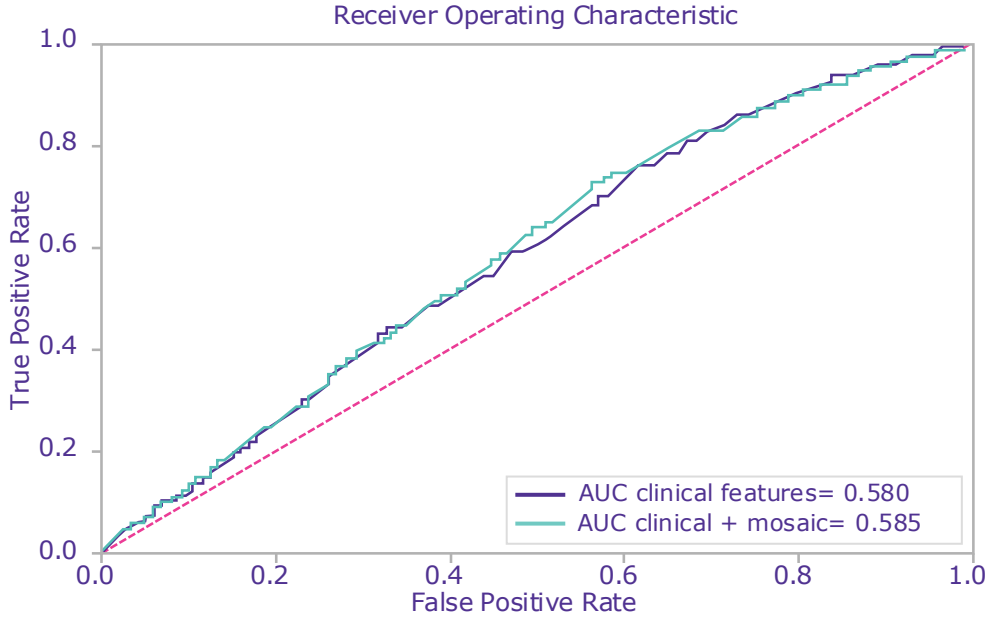
Antonio Capalbo,[1,*] Maurizio Poli,[1] Laura Rienzi,[2] Laura Girardi,[1] Cristina Patassini,[1] Marco Fabiani,[1] Danilo Cimadomo,[2] Francesca Benini,[3] Alessio Farcomeni,[4] Juliana Cuzzi,[5] Carmen Rubio,[6,7] Elena Albani,[8] Laura Sacchi,[8] Alberto Vaiarelli,[2] Matteo Figliuzzi,[1] Necati Findikli,[9,10] 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]





2nd prospective blinded study

Primary cohort, US clinics: **9828** SETs
Validation cohort, European clinics: **5,487** SETs



PGT-A Mosaicism Reporting Lacks Clinical Predictive Value: Evidence from a Multisite, Double-Blinded Study with Independent Validation Across 15,315 Single-Embryo Transfers

P. Gill, X. Tao, Y. Zhan, F. Mulas, C.S. Ottolini, L. Picchetta, S. Caroselli, D. Babariya, D. Wells, G. Clark, E. Fernandez Marcos, C. Marin Vallejo, V. Jobanputra, M. Werner, R. Scott, T. Molinaro, J. Pla, V. Vergara Bravo, A. Requena Miranda, Juan A. García Velasco, A. Pellicer, E. Mounts, C. Jalas, A. Capalbo

AJOG 2025

1. Capalbo A et al., Am J Hum Genet. 2022;
2. Gill P et al., MedRxiv. 2025 (preprint)

The Lack of an Evidence-Based Framework for Reporting Mosaicism Has Challenged PGT-A

REDUCED PGT-A ACCURACY

If an ongoing pregnancy should result, further discussion of prenatal diagnostic testing options should be offered.

GENETIC COUNSELLING AND INCREASED ANXIETY

Any patient considering transfer of an embryo diagnosed as mosaic should receive genetic counselling before and after the transfer.

INCREASED ADOPTION OF AMNIOCENTESIS

If an ongoing pregnancy should result, further discussion of prenatal diagnostic testing options should be offered.

POTENTIAL WASTAGE OF EMBRYOS

<3% transferred 143/4,932; (Munnè 2017)

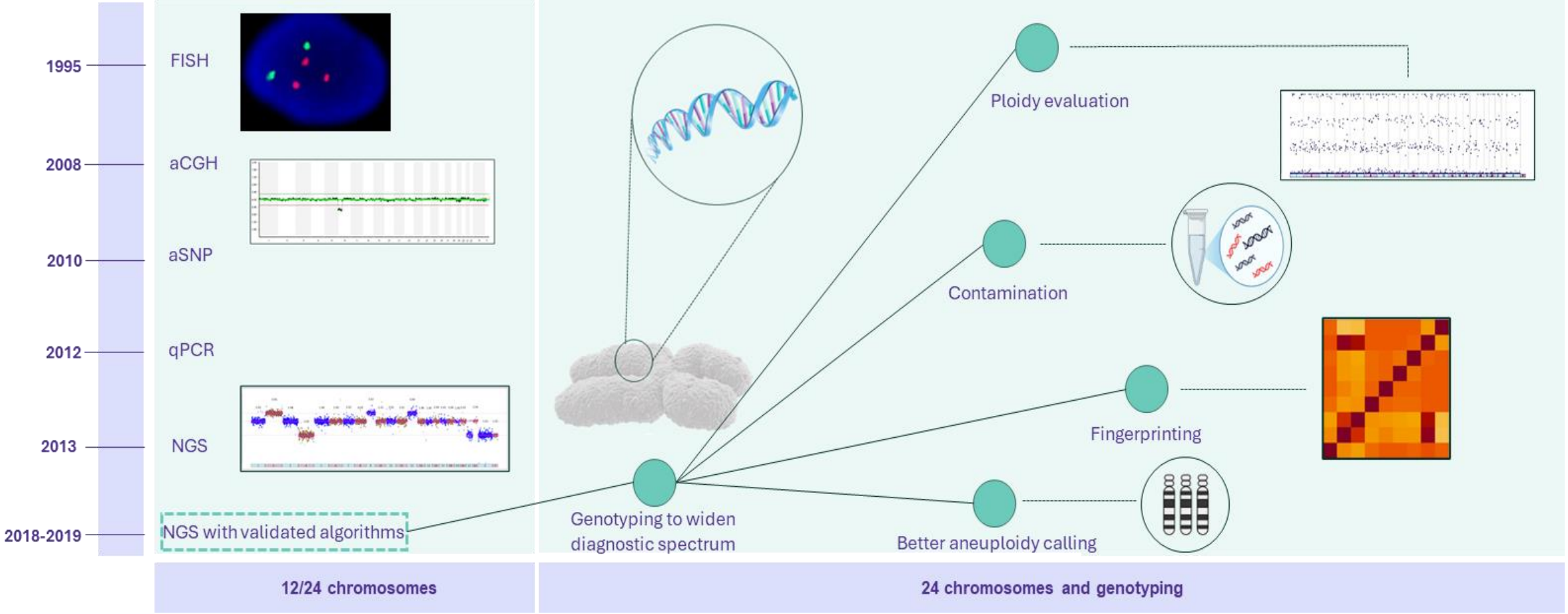
"Over half of the clinics surveyed do not permit mosaic embryo transfer" Kim 2018; Besser 2019

ESHRE SURVEY: 75% consider discarding or donating to research mosaic embryos!

PGT-A: Preimplantation genetic testing for aneuploidy; ESHRE: European Society of Human Reproduction and Embryology

Speaker's own insights; Munné S et al., Fertil Steril. 2017; McGowan et al., Fertil Steril. 2020; De Rycke et al., Hum Reprod. 2022

New technological developments in PGT-A hold potential for further improving clinical validity and utility[1–4]



1. Capalbo A, et al. Fertil Steril. 2016; 2. Caroselli S, et al. Human Reproduction. 2023;
3. Iturriaga A, et al. Fertility and Sterility. 2024; 4. Janssen AEJ, et al. Nature Communications. 2024

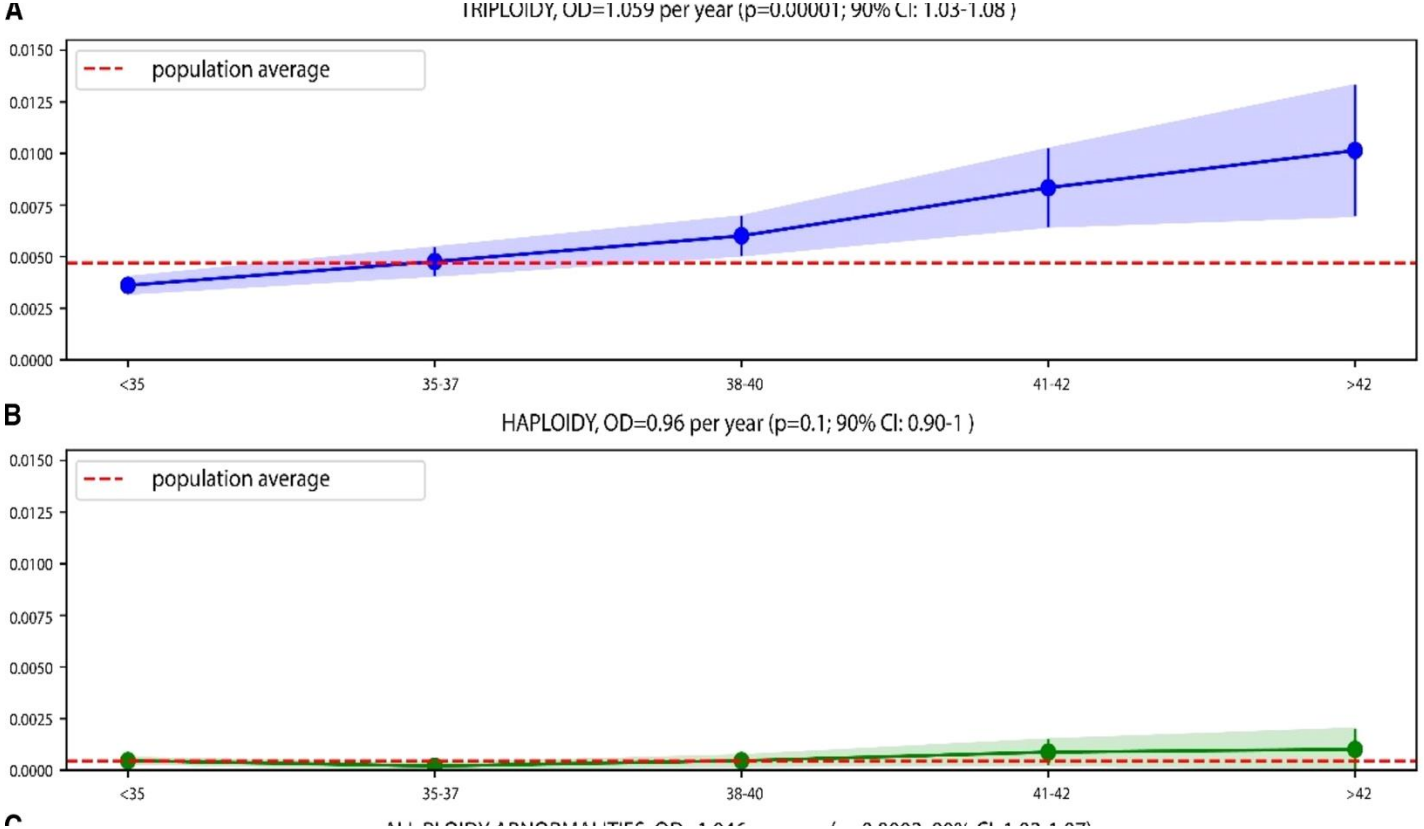
Spindle instability: tripolar spindles-triploid conceptions in humans

The American Journal of Human Genetics *112*, 1–14, November 6, 2025

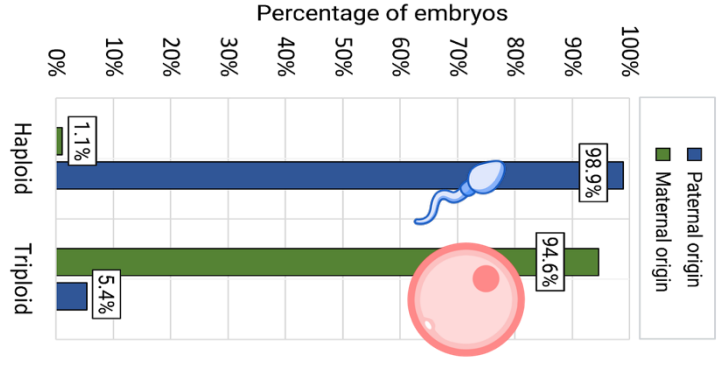
ARTICLE

Maternal age and genome-wide failure of meiotic recombination are associated with triploid conceptions in humans

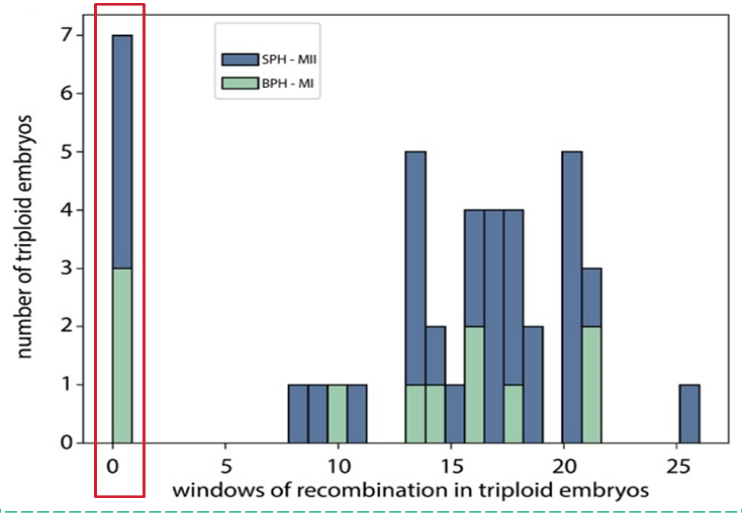
Ludovica Picchetta,[1,2] Christian Simon Ottolini,[1,3] Xin Tao,[4] Yiping Zhan,[4] Vaidehi Jobanputra,[4] Carlos Marin Vallejo,[5] Francesca Mulas,[1] Elvezia Maria Paraboschi,[1] Maria José Escribá Pérez,[6,7] Thomas Molinaro,[8] Christine Whitehead,[9] Pavan Gill,[8] Emily Mounts,[4] Dhruti Babariya,[10] Laura Francesca Rienzi,[11,12] Filippo Maria Ubaldi,[11] Juan Antonio Garcia-Velasco,[13] Antonio Pellicer,[14] Shai Carmi,[15] Eva R. Hoffmann,[16,18,*] and Antonio Capalbo[1,6,17,18,*]



Almost all haploid samples lack the paternal genome

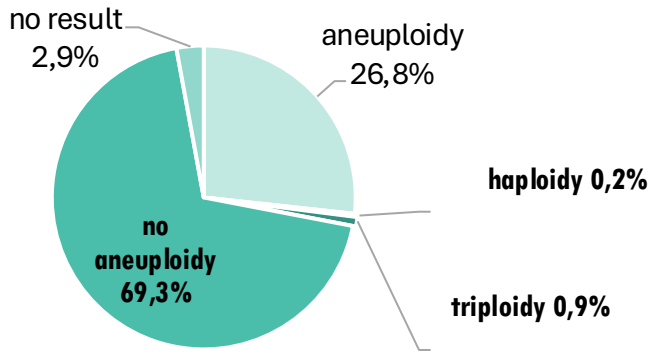


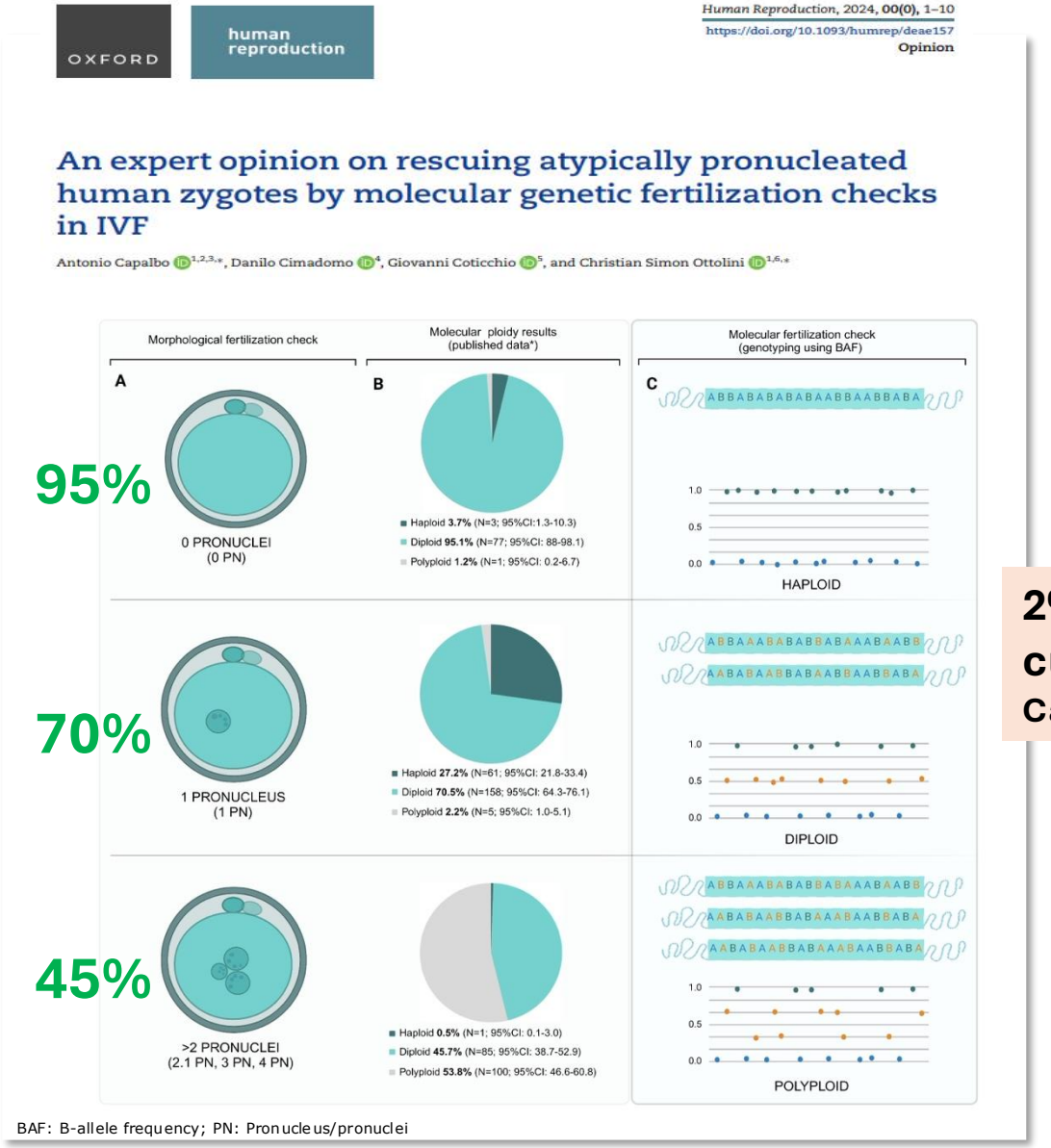
Lack of meiotic recombination explain 1/3 of triploid conceptions



New technological developments in PGT-A can further boost clinical and utility[1–4]

1% of 2PN embryos are ploidy abnormal

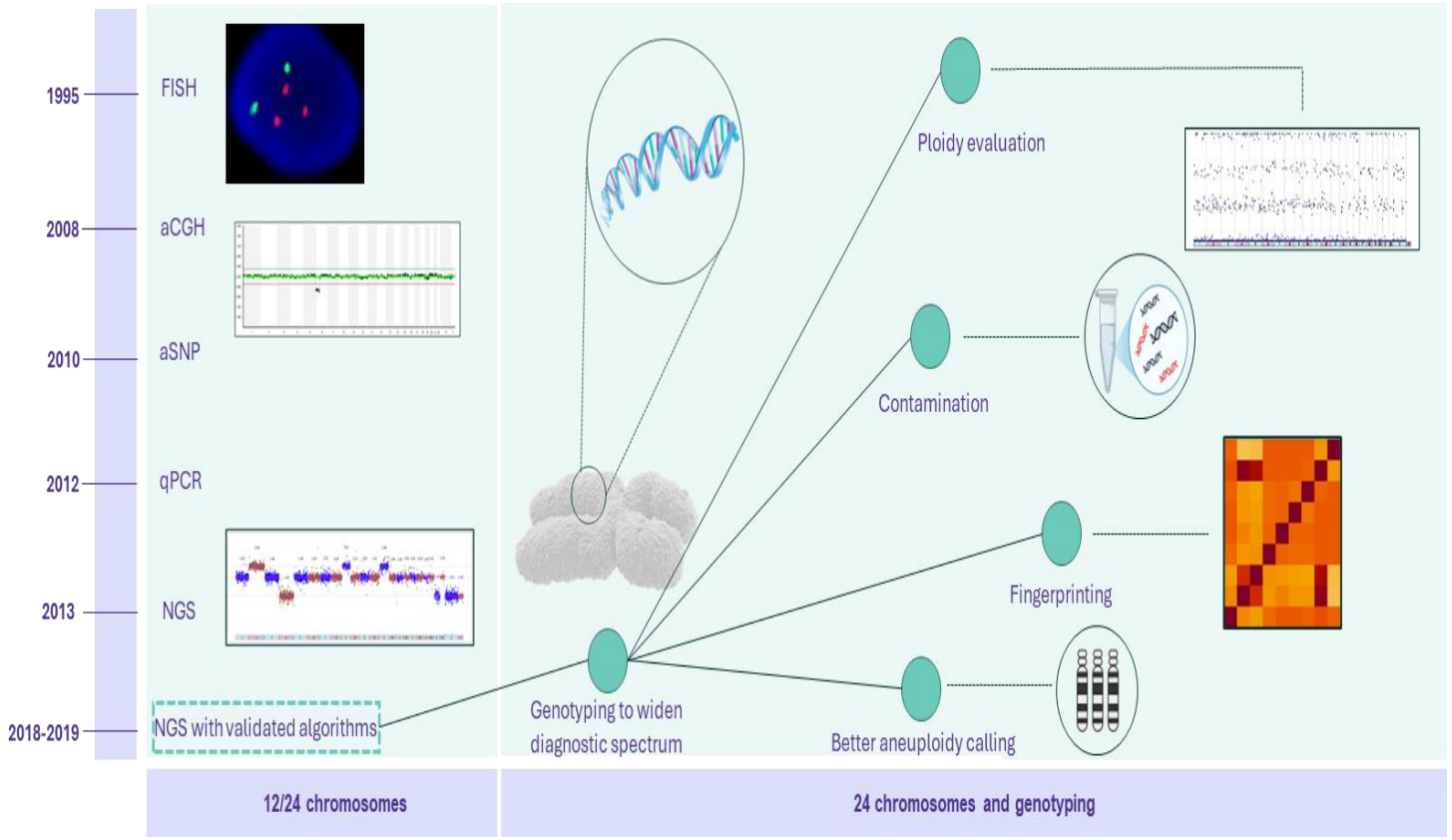




2% increase cumulative LBR,
Capalbo et al., und rev

1. Capalbo A, et al. Fertil Steril. 2016; 2. Caroselli S, et al. Human Reproduction. 2023;3. Iturriaga A, et al. Fertility and Sterility. 2024; 4. Janssen AEJ, et al. Nature Communications. 2024

New technological developments in PGT-A hold potential for further improving clinical validity and utility[1–4]



Embryo clinical genome sequencing

Types of Genomic Variations

Single Nucleotide Variants (SNVs or SNPs)
A change in a single DNA base (e.g., an A replaced by a G).
→ Most common type of variation.

Insertions and Deletions (Indels)
Small segments of DNA that are added or lost

Copy Number Variations (CNVs)

Structural Variations
Larger rearrangements such as inversions, translocations, or duplications of chromosomal segments

Repeat Expansions
Repetitive DNA sequences (e.g. trinucleotide repeats) that expand beyond normal ranges

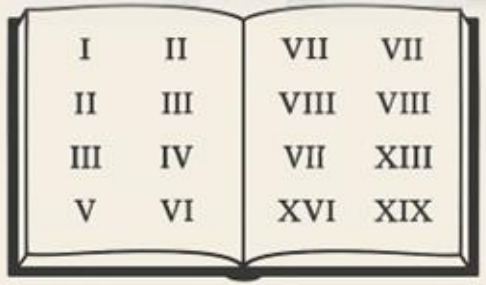
Mitochondrial Variations
Differences in mitochondrial DNA inherited maternally

1. Capalbo A, et al. Fertil Steril. 2016; 2. Caroselli S, et al. Human Reproduction. 2023;
3. Iturriaga A, et al. Fertility and Sterility. 2024; 4. Janssen AEJ, et al. Nature Communications. 2024

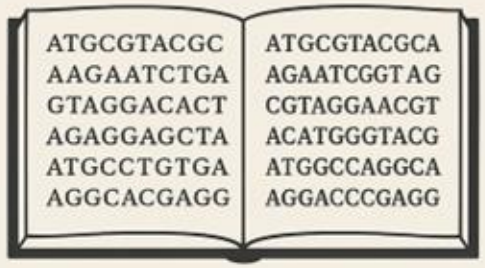
From standard PGT to clinical WGS of embryos to unravel genomic diversity of human embryos

STANDARD PGT-A

Gross chromosomal abnormalities



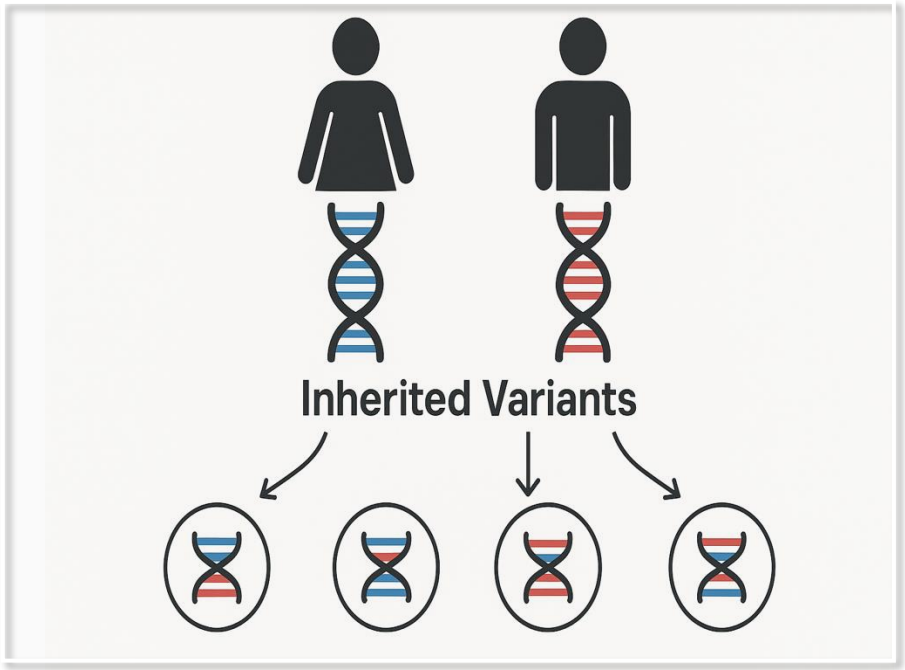
Skimming through a book to see if any chapters are missing or in the wrong order

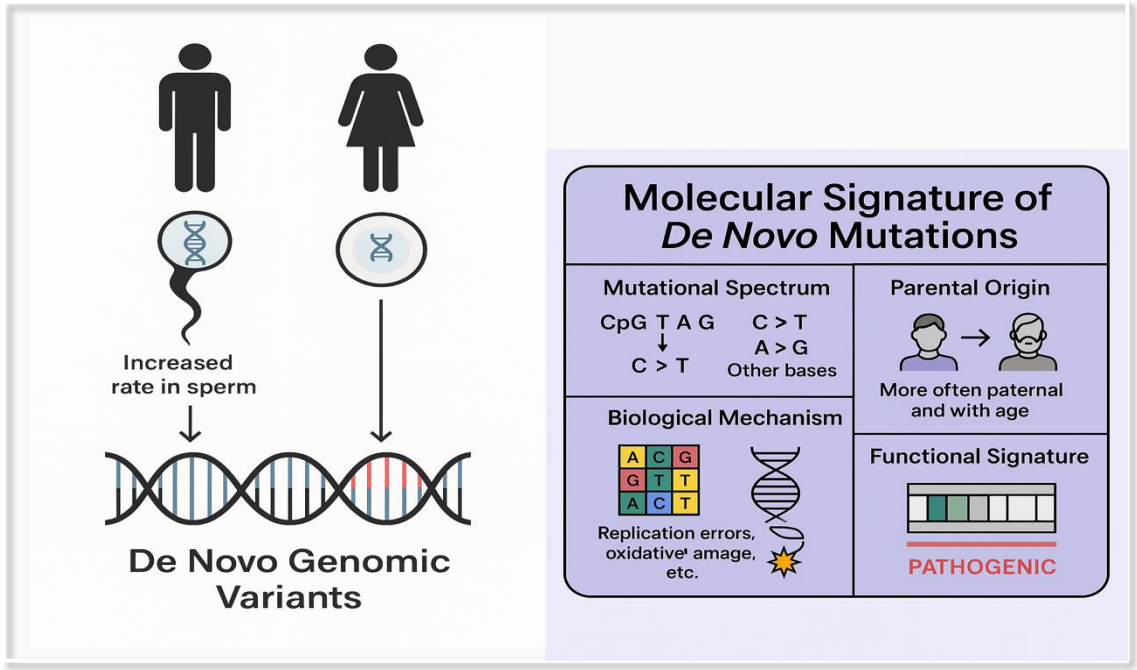


Reading every single letter of every word in the book, catching a typo

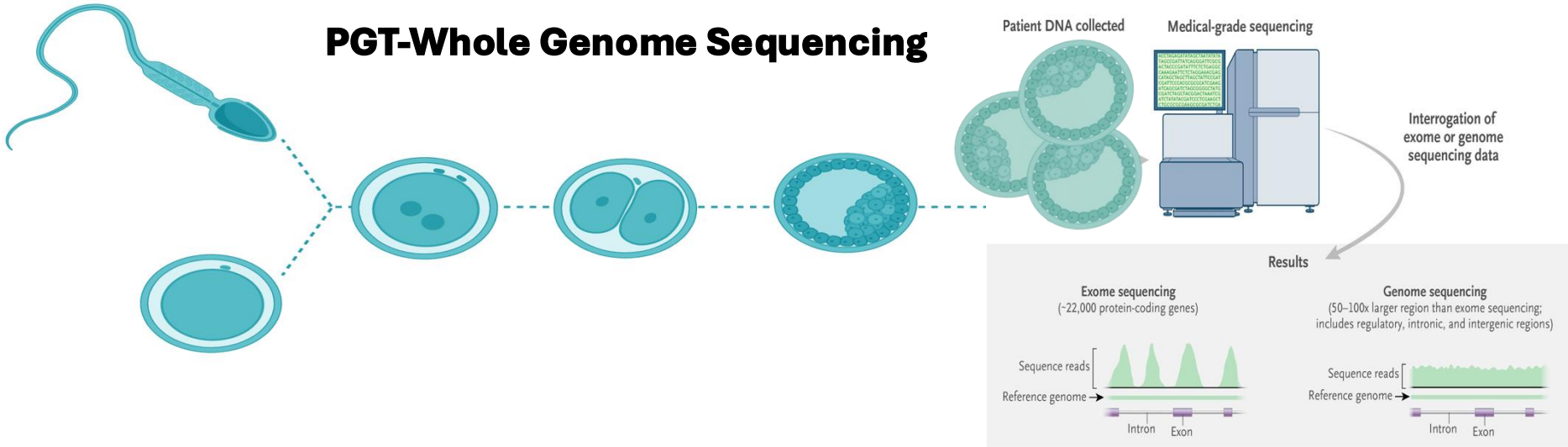
Clinical WGS in PGT-A

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





PGT-Whole Genome Sequencing

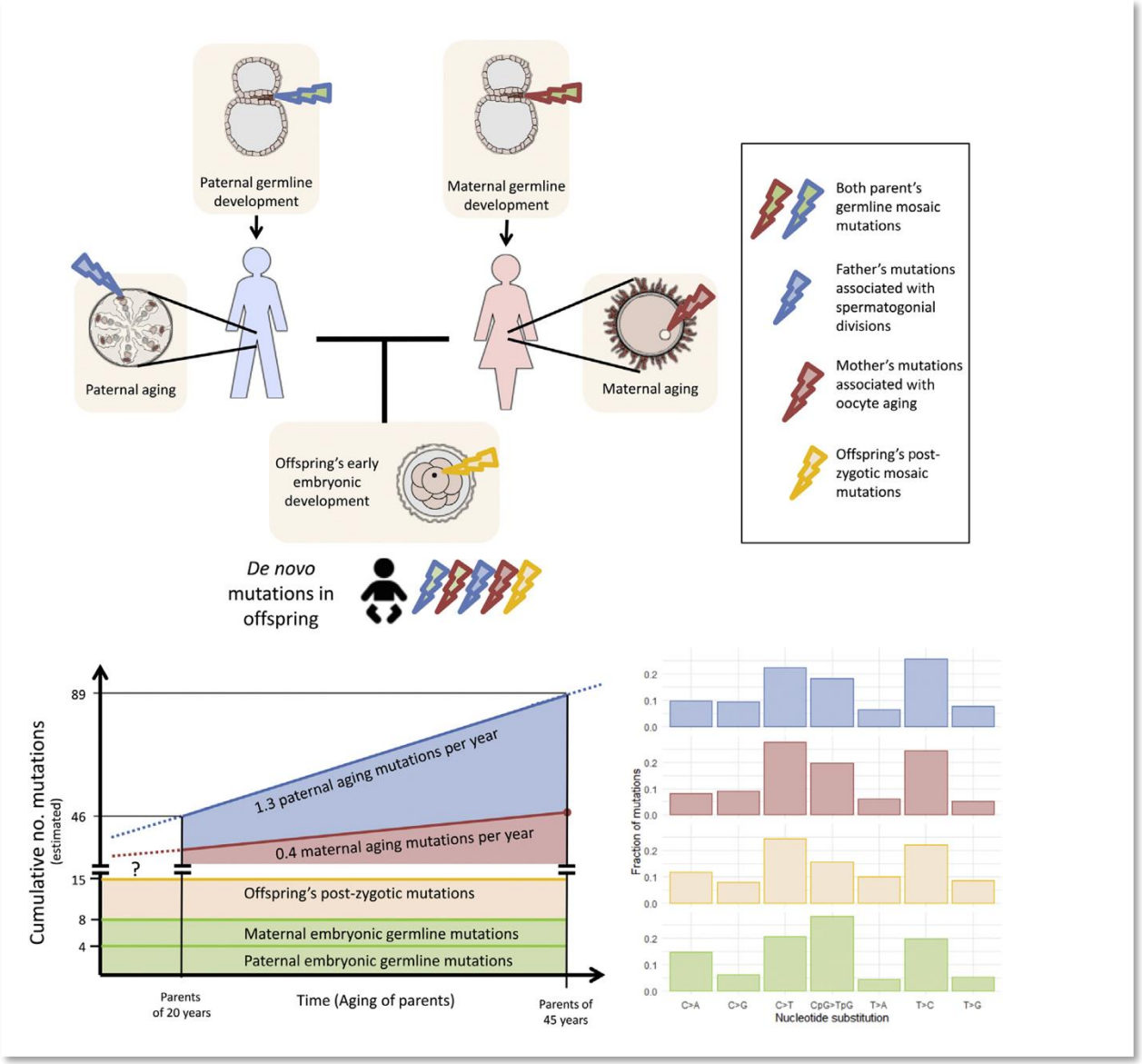


Germline De Novo Mutations (DNM) can explain a fraction of implantation failure and negative pregnancy outcomes

100 Novel SNVs, Indels or CNVs per genome per generation

Additional DNMs per year of life of the father at the time of conception **2**

60% Newborns with intellectual disability



Goldman et al., 2019; Veltman et al., 2012; Conrad, D. et al., Nat Gen 2011

Article

Sperm sequencing reveals extensive positive selection in the male germline

https://doi.org/10.1038/s41586-025-09448-3

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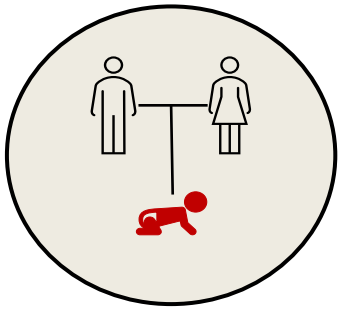
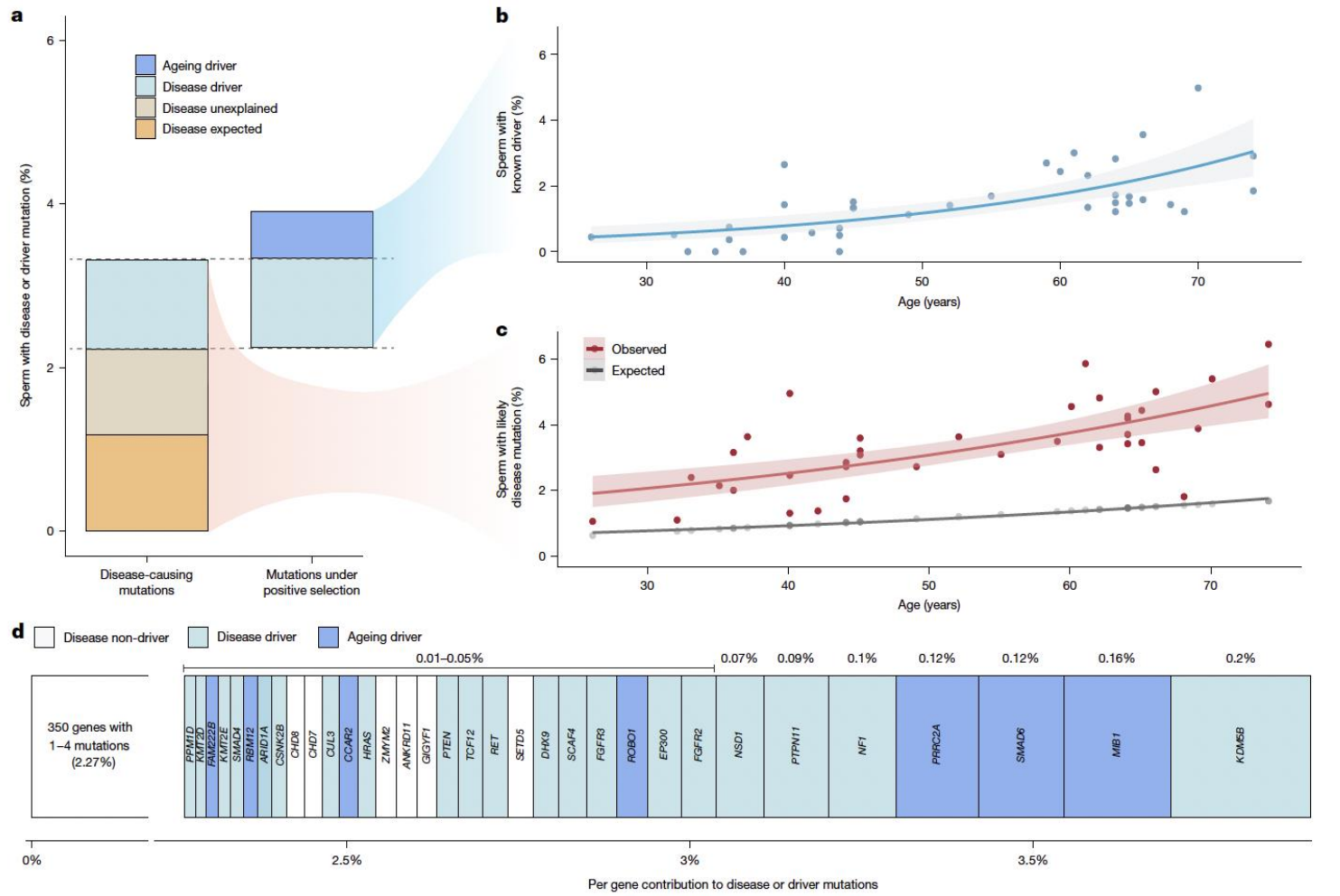
Matthew D. C. Neville[1], Andrew R. J. Lawson[1], Rashesh Sanghvi[1], Federico Abascal[1], My H. Pham[1], Alex Cagan[1], Pantelis A. Nicola[1], Tetyana Bayzetinova[1], Adrian Baez-Ortega[1], Kirsty Roberts[1], Stefanie V. Lensing[1,2], Sara Widaa[2], Raul E. Alcantara[1,3], María Paz García[4], Sam Wadge[4], Michael R. Stratton[1], Peter J. Campbell[1], Kerrin Small[4], Iñigo Martincorena[1], Matthew E. Hurles[1] & Raheleh Rahbari[1]



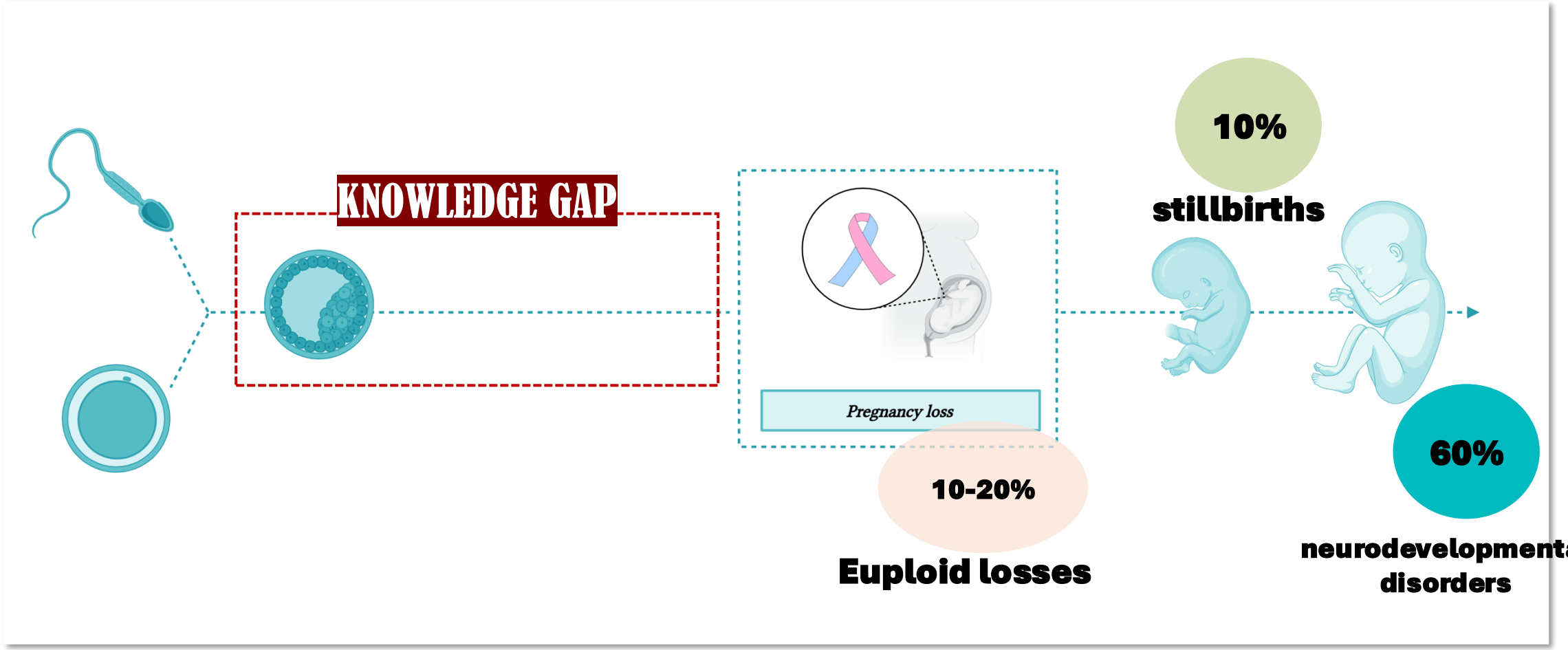


40 genes under significant positive selection in the male germline, involved in diseases

positive selection during spermatogenesis drives a 2–3-fold increased risk of known disease-causing mutations.

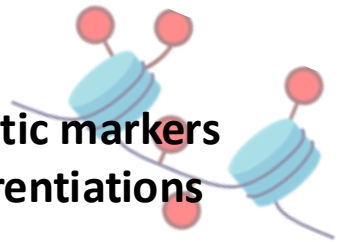
3–5% of sperm from middle-aged to older individuals with a pathogenic mutation across the exome.

The unkwown genomic diversity of human perimplantation embryos



❖ Small CNVs

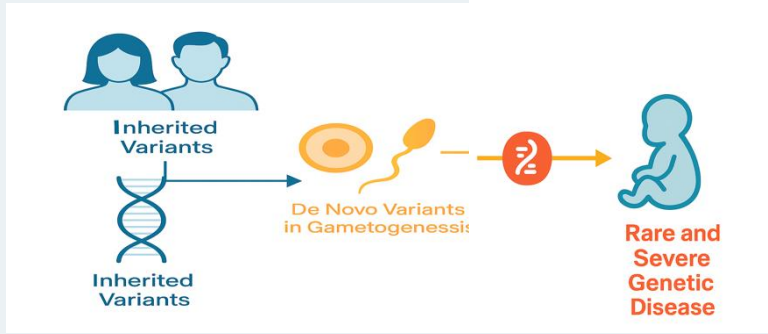
❖ SNVs



❖ Epigenetic markers of differentiations

Clinical whole genome sequencing-based PGT in pratical terms

- **Universal approach for all PGT indications**
- **Improve assessment of genetic embryonic lethality beyond aneuploidies (understand why euploid embryos fail)**
- **Mitigate the burden of genetic diseases for the next generations (de novo mutations/CNVs for severe developmental disorders)**



PGT: Preimplantation genetic testing; CNVs: Copy number variations

POINTS TO CONSIDER BEFORE IMPLEMENTATION OF WGS

- Clinical grade analytical performance!
- What is the clinical gain?
- Data Interpretation & Reporting (VUS, mild penetrance)
- Cost-effectiveness?
- How to manage Incidental/Secondary findings?
- Consent, privacy, counseling, and equitable access.

Clinical trials required

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

Take home

- Meiotically aneuploid embryos without reproductive potential can be accurately detected and safely deselected for clinical use.
- Information about PGT-A should be ready available and provided to all couples planning IVF, with AMA most appropriate

- Scientifically driven comunication to patients and public! Avoid overstatments or miscomunication
- Skilled IVF and genetic labs and teams are critical!

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