Journal of Human & Clinical Genetics

Mini Review Article Open Access

Recent Developments in Preimplantation Genetic Testing and Embryo Selection

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Article Info

Article Notes
Received: October 03, 2025
Accepted: October 21, 2025

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Current Preimplantation Genetic Testing (PGT) consists of taking a biopsy of human embryos, usually at blastocyst stage, on day 5-6 of development, to be genetically analyzed to select healthy embryos for transfer during the in vitro fertilization (IVF) process.

PGT-A is standard of care

The major use of PGT has been to detect aneuploidy and other chromosomal defects, which is known as PGT-A (PGT for Aneuploidy). The rationale for PGT-A, first developed by Munné et al. [1], is that aneuploidy in human embryos is very common and increases with advancing maternal age, from about 30% in young women to more than 80% after age 42[2] (Figure 1). Most aneuploid embryos fail to implant or lead to a miscarriage for this reason, selecting euploid embryos increases ongoing pregnancy rates per transfer, since euploid embryos mostly implant at the same rate irrespective of maternal age[2]. Several randomized clinical trials (RCTs) involving women 35 and older[3-8] show an improvement in ongoing pregnancy rates per transfer. For younger women, the results are not so clear, with some RCTs showing beneficial effect and others not[6,9-11]. However, these initial RCTs in young patients included fewer than 2,514 IVF cases. In contrast, a recent analysis of the compiled data of IVF cycles performed in the USA, comprising over 35,000 IVF cycles with a transfer, showed a significant improvement in ongoing live birth rates in young patients[2] (Figure 1). Reasons why some early

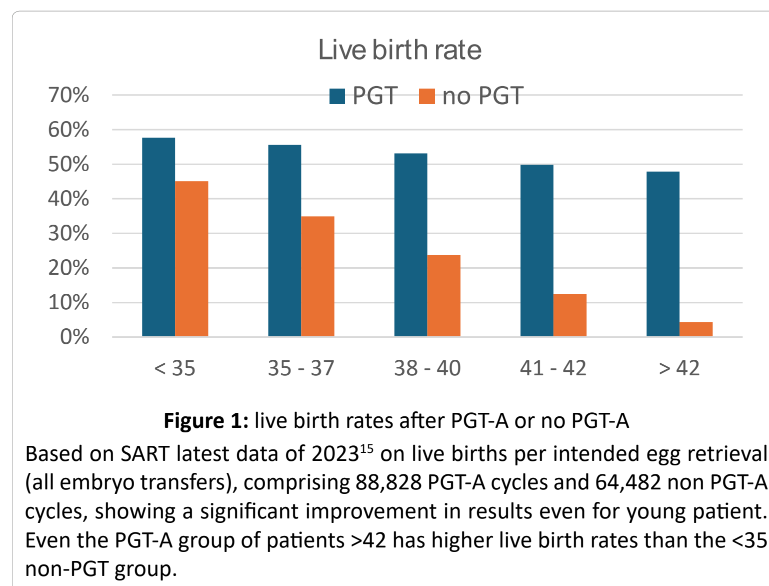

Figure 1: live birth rates after PGT-A or no PGT-A
Based on SART latest data of 2023[15] on live births per intended egg retrieval (all embryo transfers), comprising 88,828 PGT-A cycles and 64,482 non PGT-A cycles, showing a significant improvement in results even for young patient. Even the PGT-A group of patients >42 has higher live birth rates than the <35 non-PGT group.

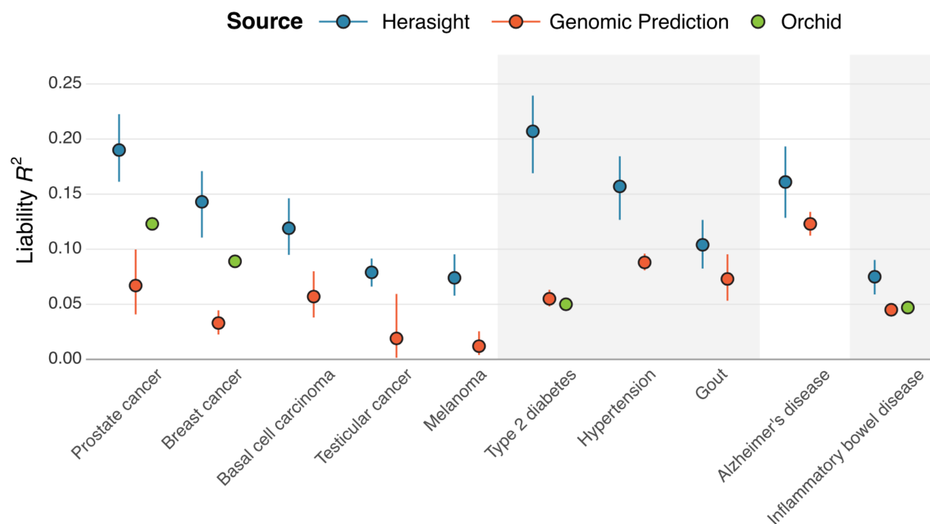

Figure 2: More et al 2025 method for PGT-P vs other PGT-P providers

Results are shown for three commercial entities, Herasight, which used the Moore et al[29] methods, Genomic Prediction and Orchid. Bands represent bootstrapped 95% confidence intervals, although these could not be determined for Orchid. Equivalent population prevalences were applied when converting to liability R^2. [30]

RCTs did not show beneficial effects in younger patients include the embryo biopsy techniques damaging embryos, and the fact that mosaic embryos were often discarded because it was not known at the time that they often have the potential to implant[12]. Most recent studies show that putative mosaic embryos detected by PGT-A and with 50% or fewer cells have a lower to similar chance of implanting than euploid embryos, and very little risk of producing a mosaic baby[13,14].

The most recent SART data[15] shows that PGT was performed in 233,073 cycles vs. 182,880 cycles that did not use PGT. Therefore, the procedure is now standard of care. In addition, SART data[15] shows that by performing PGT, about 15,000 miscarriages were prevented, and 11,000 more miscarriages would have been avoided if PGT was performed[16].

PGT-A Error Rate and Recent Improvements

PGT-A has evolved over the years, from using FISH to analyze only a handful of chromosomes to the currently most used next generation sequencing (NGS). Overall, most studies reanalyzing embryos or performing non-selection studies show <1% error rate[17,18] and therefore transferring fully aneuploid embryos results in fewer than 1% viable euploid pregnancies[18,19]. Some of these errors, as well as misclassifying embryos due to putative mosaicism, were due to artifacts of amplification. Newer and more precise methods of amplifying DNA, such as primary template-directed amplification (PTA)[20] have been recently introduced, potentially lowering further PGT-A error rates.

PGT-A using molecular techniques (SNP arrays, NGS, targeted NGS) can also be used to detect structural chromosome rearrangements, such as translocations, insertions, and deletions that would appear as gains or losses, also called PGT-SR (for 'structural rearrangements'). However, since using PGT-A does not differentiate between normal and balanced embryos ("balanced" embryos contain the entire genome but with a structural rearrangement), so if a balanced embryo is transferred the structural problem is passed along to future generations.

To illustrate this problem, consider the real case of a woman whose parents underwent PGT 30 years ago. She has a *balanced* translocation, and is perfectly healthy but at risk, again, of passing along an *unbalanced* translocation and is now seeking PGT.

Karyomapping or high-density SNP arrays have been used to differentiate balanced from normal embryos using **an unbalanced embryo as a reference** to phase parental haplotypes near the breakpoints[21]. An improved approach uses long-read sequencing (typically 10–100 kb), which enables direct identification of rearrangement breakpoints in parental DNA by spanning the breakpoint site and resolving it at single-base precision. Because long reads capture multiple heterozygous SNPs within a single fragment, they provide much more accurate phasing than short-read sequencing. This allows precise determination of which parental chromosome harbors the balanced translocation[22].

Other recent developments in PGT-A involve detecting not only aneuploidy but also triploidy and haploidy. About 1% of embryos classified by standard NGS methods as euploid are in fact haploid or triploid. By analyzing SNPs in addition to copy number variations and detecting A/B allele

frequencies of only 0% or 100% (haploidy), 0% or 50% or 100% (diploidy), or 0%-33%-66%-100% (triploidy) all numerical chromosome abnormalities (except tetraploidy) can be detected (23). This is important because in the past embryologists have usually discarded embryos derived from zygotes with abnormal number of pronuclei (0PN, 1PN, 3PN). But a significant proportion of these embryos are euploid when analyzed with PGT-A with SNPs and can be rescued[23]. Interestingly, the error rate of PGT is lower than the proportion of embryos derived from abnormal pronuclei zygotes that are euploid and would be discarded without PGT-A with SNPs.

Non-invasive PGT-A (niPGT-A)

Although several studies indicate there is no detrimental effect of blastocyst biopsy when done correctly, lack of expert embryologists and equipment in developing countries, or regulations that do not allow embryo biopsy, makes non-invasive selection of embryos attractive. niPGT-A[24] aims to avoid embryo biopsy by analyzing embryonic cell-free DNA (cfDNA) released into blastocoel fluid and spent culture medium (SCM). Initial reports confirmed that cfDNA could be reliably detected in these sources, though the quantity and integrity vary with embryo stage, freezing/thawing, and mechanisms of release (apoptotic versus necrotic pathways). Evidence suggests that aneuploid embryos may shed more DNA due to increased apoptosis and autophagy, potentially improving detection sensitivity. Research has focused on SCM, where results have been mixed. Small, well-controlled studies report concordance rates of 90–100%, but a large multicenter trial[25] observed only 78% agreement, primarily due to maternal DNA contamination from cumulus/corona cells[24]. Strategies to reduce this include stringent denudation, culture media changes, parent-of-origin sequencing to detect contamination, and prolonging culture to day 6, which increases embryonic DNA yield relative to maternal contamination. However, culturing to day 6 is not standard practice and makes embryo vitrification of expanded or hatched embryos more difficult. Another limitation of niPGT-A is that embryos cannot be group-cultured. Other limitations have been the need to culture embryos in smaller drops than usual (20uL or less).

Nonetheless, amplification failure, fragmented DNA, and culture variability remain challenges. Meta-analyses indicate that while niPGT-A can approximate biopsy-based results, error rates compared with whole embryo analysis are 8–12%, similar to or slightly lower than TE biopsy. Importantly, an RCT[8] demonstrates potential clinical benefit comparable to invasive PGT-A. They found cumulative live birth rates significantly higher with both niPGT-A (44.9%) and biopsy PGT-A (51.0%) compared with no testing (27.9%). Overall, niPGT-A shows strong promise as a less invasive alternative for embryo selection, but robust validation, standardization, and careful management of contamination and amplification issues are essential before routine clinical use.

PGT-M, Comprehensive PGT and Whole Genome Sequencing of Embryos (PGT-WGS)

There are 20,000 genes coding for proteins and over 10,000 genetic diseases identified, of which the majority are rare. Carriers of recessive monogenic diseases will produce 25% of embryos that are affected, 50% that are carriers and 50% that are non-carriers. A variety of techniques can detect embryos with monogenic diseases (called PGT-M for monogenic diseases). PGT-M currently represents about 10% of all PGT procedures. Compounding the risk of transmitting a monogenic disease with that of aneuploidy (30-80% abnormal embryos depending on age) makes analyzing all potential abnormalities at once, with the same platform, paramount.

PGT-M has been performed for over 1500 different genetic defects. First generation PGT-M involved using linked polymorphic markers such as short tandem repeats. It was prone to misdiagnosis if recombination occurred and the markers were not close enough to the mutation to detect the recombination. Second generation PGT-M, still widely used, uses SNP arrays and parental Haplophasing, that is, determining which SNPs are located together on the same maternal or paternal chromosome to reconstruct haplotypes, which are contiguous stretches of DNA sequence that are inherited together. Once the haplotypes are known, Haplophasing is performed, consisting of assigning alleles in an embryo biopsy to their parental origin by comparing them with the parents' haplotypes. This allows us to infer the mutation site by reconstructing which chromosomal segments came from which parent[26]. Its drawback is that some areas of the genome are not well covered. In contrast, third generation PGT-M can use either long-read sequencing of the area of interest plus haplophasing, or whole exome or whole genome sequencing (WGS) plus haplophasing, which allows us to detect chromosome abnormalities and monogenic conditions simultaneously.

Many techniques have been developed to perform comprehensive PGT involving PGT-M -SR and -A. Some strategies involve the use of high-throughput SNP arrays to genotype as well as perform copy number analysis, other strategies involve sequencing with enough depth to allow enough SNPs to be detected or combine sequencing plus targeted SNPs.

Genotyping strategies use high throughput SNP arrays (200K - 800K SNPs) of parents and embryos, which allows copy number quantitative analysis and genotyping by analyzing Log R and SNPs B-allele frequencies can achieve 99% accuracy and can detect all polyploidies[27]. Using parental DNA allows linkage analysis and detects

enough SNPs within 2Mb window of the gene defect. For PGT-SR purposes it can differentiate normal from balanced embryos with a 10Mb resolution.

Sequencing strategies involve either whole genome sequencing at high depth (>20x) without the need of parental DNA analysis or sequencing the parents at high depth to reconstruct the haplotypes and then sequencing the embryos at lower depth, which is more affordable.

These strategies allow copy number analysis enough to detect aneuploidy, polyploidy, structural abnormalities (≤10Mb), haplotyping for mutation detection, or direct mutation analysis of the inherited mutation. However, only whole genome sequencing (x30 depth or more) of the embryo can detect de novo mutations.

De novo mutations (DNV) DNV mutations accumulate in gametes with age and are not detectable by carrier screening of the parents. It is estimated that on average, embryos carry more than seven DNVs, 1–2 of which are pathogenic. DNV mutations increase with paternal age with fathers aged 45 having about 3.5 times the risk of conceiving an autistic child and 27 times the risk of conceiving a child who develops bipolar disorder compared to a 25-year-old father[28]. In fact, 1/488 children are born with a DNV mutation causing congenital abnormalities, more than from inherited genetic defects (parents being carriers).

In addition, in women <40 years old only 60% of implantation failure is explained by aneuploidy and the rest potentially by mutations that reduce viability. For example, over 50% euploid miscarriages have mutations incompatible with life. Therefore, to detect DNV mutations whole genome sequencing is necessary. Several approaches have been developed, one consisting of sequencing the parents at x30 depth and the embryos at x40 and applying extensive variant filtration to differentiate amplification artifacts from de novo mutations[29]. With this approach about 1.2 de novo mutations per embryo were detected. Another method is to use PTA amplification for more faithful amplification without then the need of parental sequencing[20,30].

PGT-WGS for Germ Line Gene Editing

Germ line gene editing has been banned in most countries due to the off-target effects produced by older methods such as CRISPR/cas-9. However, newer methods of gene editing are now more precise, such as base editing, and if off-target mutations were generated we can now perform PGT-WGS after the editing to check whether the embryo is free of genetic defects. In principle, germline gene editing could be used for carriers of recessive gene defects and chromosome structural abnormalities (with 25% risk of an affected embryo), as well as dominant late onset genetic defects (with 50% risk of an affected embryo), or for couples with a baby with a gene defect that would benefit from bone marrow transplant from a perfect HLA match sibling, which only 1/18 embryos would be. By editing the affected embryos, we would have a much higher chance of a pregnancy free of genetic disease. A limitation, to be assessed with newer techniques, is the generation of mosaic embryos if editing is not fully attained in all cells. These advances raise the question of whether the current ban should be reconsidered, especially once the genetic architecture of complex diseases is better understood.

PGT for Polygenic Diseases and Traits (PGT-P)

Most inherited diseases are not monogenic but polygenic, meaning that many genetic variants – often hundreds or thousands – add up to an overall risk of developing a disease like breast cancer, schizophrenia, or rheumatoid arthritis. Non-disease traits, such as intelligence, personality, and height, are also highly polygenic. Preimplantation genetic testing for *polygenic* traits (PGT-P) is the latest and most complex form of genetic testing for embryos.

PGT-P requires using either SNP-based arrays or whole-genome sequencing, which includes coverage from rare variants and non-coding regions of the genome. Whole-genome sequencing can improve the predictive power of polygenic scores over arrays. Instead of directly performing WGS on embryos it is also possible to impute the whole genome of each embryo (with varying degrees of accuracy) from embryo PGT-A genetic data plus WGS data from the parents.

PGT-P is made possible by polygenic risk scores, which measure the likelihood that a trait will emerge, given a particular genetic endowment. These scores are developed using biobanks, which contain genetic data from hundreds of thousands of people. A past limitation of polygenic risk scores was the use of data sets mostly representing Caucasian population, such as the UK Biobank, or its use in admixed populations.

Researchers with access to larger and more diverse biobanks can look for common genetic variants that are correlated with particular traits or diseases[31]. These scores are validated by testing how accurately they gauge the traits of adult siblings. Since embryos are essentially unrealized siblings, if polygenic scores can predict the traits of adult siblings, they should be able to predict the likelihood that embryos will develop those traits if they are implanted. Polygenic scores are best validated using 'within-family' methods which capture genetic variants that are *causally* associated with the relevant outcome, rather than signals that might come from sources that are irrelevant to genomic prediction[31].

PGT-P is only useful if the polygenic scores that make it possible have strong predictive power. One challenge for improving polygenic scores is the quality and quantity of

data to which researchers have access: accurate polygenic scores require genetic data from large biobanks with reliable measures of the traits being studied. They also require genetic data from diverse ancestry groups if they are to work well for all people.

PGT for Fertile Couples

An increasing number of fertile couples without known genetic diseases are undergoing IVF electively, so that they can use PGT to select embryos with a lower risk of polygenic diseases like diabetes, and for traits that they value, including intelligence, which a recent study can predict 16% differences between individual embryos[32]. With birth rates declining worldwide, many couples are putting more resources into making sure that every child has the best possible start in life. They spend money on education, health care, and a nurturing *social* environment. And now they have the option to invest in their children's *genetic* potential. Current PGT techniques extend earlier tests to enable parents to reduce a far greater number of diseases and influence other traits that they care about. PGT thereby enables parents to create future children who enjoy the best chance of the best life.

References

1. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. *Hum Reprod*. 1993;8(12):2185–2191.

2. Munné S, Griffin DK. Opinion: Contemporary insights into the efficacy of preimplantation genetic testing for aneuploidy (PGT-A) by mining the Society for Assisted Reproductive Technology (SART) database. *BMJ Conn Clin Genet Genomics*. 2024;1:e000003. doi:10.1136/bmjccgg-2024-000003.

3. Scott RT, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases IVF implantation and delivery rates: a randomized controlled trial. *Fertil Steril*. 2023;100(3):697–703.

4. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril*. 2013;100(1):100–107.

5. Rubio C, Bellver J, Rodrigo L, Castillon G, Guillen A, Vidal C, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized controlled study. *Fertil Steril*. 2017;107(5):1122–1129.

6. Munné S, Kaplan B, Frattarelli JL, Gysler M, Child T, Nakhuda G, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen embryo transfer in good prognosis patients: a multicenter randomized clinical trial. *Fertil Steril*. 2019;112(6):1071–1079.e7. doi:10.1016/j.fertnstert.2019.07.1346.

7. Sui YL, Lei CX, Ye JF, Fu J, Zhang S, Li L, et al. In vitro fertilization with SNP microarray-based preimplantation genetic testing for aneuploidy significantly improves outcomes in infertile women with recurrent pregnancy loss: a randomized controlled trial. *Reprod Dev Med*. 2020;4(1):32–41.

8. Sun Q, Xu J, Yao Y, Huang X, Zhao D, Lu S, et al. Efficacy of non-invasive chromosome screening, preimplantation genetic testing for aneuploidy, and morphological grading in selecting embryos of patients with advanced maternal age: a three-armed prospective cohort study. *BMC Pregnancy Childbirth*. 2024;24:545. doi:10.1186/s12884-024-06736-0.

9. Ozgur K, Berkkanoglu M, Bulut H, Yoruk GDA, Candurmaz NN, Coetzee K. Single best euploid versus single best unknown-ploidy blastocyst frozen embryo transfers: a randomized controlled trial. *J Assist Reprod Genet*. 2019;36(4):629–636. doi:10.1007/s10815-018-01399-1.

10. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, et al. Birth with or without preimplantation genetic testing for aneuploidy. *N Engl J Med*. 2021;385:2047–2058.

11. Katz-Jaffe M, Gassen C, Makloski R, Reed L, Schoolcraft WB. Impact of aneuploidy on reproductive success in young infertile women: prospective analysis. *Reprod Biomed Online*. 2024;49:103858. doi:10.1016/j.rbmo.2024.103858.

12. Pagliardini L, Viganò P, Alteri A, Corti L, Somigliana E, Papaleo E. Shooting STAR: reinterpreting the data from the 'Single Embryo TrAnsfeR of Euploid Embryo' randomized clinical trial. *Reprod Biomed Online*. 2020;40(4):475–478.

13. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. *Fertil Steril*. 2021;115(5):1212–1224. doi:10.1016/j.fertnstert.2020.11.041.

14. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective non-selection clinical trial. *Am J Hum Genet*. 2021;108(2):1–10.

15. Society for Assisted Reproductive Technology (SART). Clinic summary report 2023. Available from: https://sartcorsonline.com/CSR / PublicSnapshotReport? ClinicPKID=&reportingYear=2023 &from Disclaimer=true.

16. Griffin DK, McWilliams K, Munné S, Gordon T. Preimplantation genetic screening for aneuploidy (PGT-A) in the USA in 2023 avoided nearly fifteen thousand miscarriages and over six and a half thousand pre-term births. *Unpublished manuscript*. 2023.

17. Munné S, Spinella F, Grifo J, Zhang J, Parriego Beltran M, Fragouli E, et al. Clinical outcomes after the transfer of blastocysts characterized as mosaic by high-resolution next generation sequencing—further insights. *Eur J Med Genet*. 2020;63(3):103741. doi:10.1016/j.ejmg.2019.103741.

18. Tiegs AW, Tao X, Zhan Y, Whitehead C, Kim J, Hanson B, et al. A multicenter, prospective, blinded, nonselection study evaluating the predictive value of an aneuploid diagnosis using a targeted next-generation sequencing–based preimplantation genetic testing for aneuploidy assay. *Fertil Steril*. 2021;115(3):627–637. doi:10.1016/j.fertnstert.2020.07.052.

19. Barad DH, Albertini DF, Molinari E, Gleicher N. IVF outcomes of embryos with abnormal PGT-A biopsy previously refused transfer: a prospective cohort study. *Hum Reprod*. 2022;37(10):2131–2140. doi:10.1093/humrep/deac063.

20. Gonzalez-Pena V, Natarajan S, Xia Y, Klein D, Carter R, Pang Y, et al. Accurate genomic variant detection in single cells with primary template-directed amplification. *Proc Natl Acad Sci U S A*. 2021;118(24):e2024176118. doi:10.1073/pnas.2024176118.

21. Treff NR, Tao X, Schillings WJ, Bergh PA, Scott RT, Levy B. Use of single nucleotide polymorphism microarrays to distinguish between balanced and normal chromosomes in embryos from a translocation carrier. *Fertil Steril*. 2011;96(1):58–65.

22. Xia Q, Li S, Ding T, Liu Z, Liu J, Li Y, et al. Nanopore sequencing for detecting reciprocal translocation carrier status in preimplantation genetic testing. *BMC Genomics*. 2023;24:1.

23. Munné S, Kim H, Babu V, Prasetyo M, Costa-Borges N, Arrach N. Sensitivity and specificity of a new preimplantation genetic test for aneuploidy, polyploidy, haploidy, and parental origin and creation of haploid embryos as controls. *BMJ Conn Clin Genet Genomics*. In press, 2025.

24. Munné S, Horcajadas Almansa JA, Seth-Smith ML, Perugini M, Griffin DK. Non-invasive selection for euploid embryos: prospects and pitfalls of the three most promising approaches. *Reprod Biomed Online*. 2025. doi:10.1016/j.rbmo.2025.105077.

25. Rubio C, Navarro-Sánchez L, García-Pascual CM, Ocali O, Cimadomo D, Venier W, et al. Multicenter prospective study of concordance between embryo cell-free DNA and trophectoderm biopsies from 1,301 human blastocysts. *Am J Obstet Gynecol*. 2020;223(5):751.e1–751.e13. doi:10.1016/j.ajog.2020.04.035.

26. Handyside AH, Harton GL, Mariani B, Thornhill AR, Affara NA, Shaw MA, et al. Karyomapping: a universal method for genome-wide analysis of genetic disease based on mapping crossovers between parental haplotypes. *J Med Genet*. 2010;47(10):651–658.

27. Kratka C, Vadapalli PS, Mendola R, Garrisi J, Treff NR, Marin D. Accurate detection and frequency of abnormal ploidy in the human blastocyst. *F&S Sci*. 2023;4(1):27–35. doi:10.1016/j.xfss.2023.02.003.

28. Zweifel J, Woodward J. The risky business of advanced paternal age: neurodevelopmental and psychosocial implications for children of older fathers. *Fertil Steril*. 2022;118(6):1267–1278. doi:10.1016/j.fertnstert.2022.10.029.

29. Murphy NM, Samarasekera TS, Macaskill L, Mullen J, Rombauts LJF. Genome sequencing of human in vitro fertilization embryos for pathogenic variation screening. *Sci Rep*. 2020;10:3795. doi:10.1038/s41598-020-60704-0.

30. Xia Y, Katz M, Chandramohan D, Bechor E, Podgursky B, Hoxie M, et al. The first clinical validation of whole-genome screening on standard trophectoderm biopsies of preimplantation embryos. *F&S Rep*. 2024;5(1):63–71. doi:10.1016/j.xfre.2024.01.001.

31. Moore S, Davidson I, Anomaly J, Li JH, Ahangari M, Moissiy L, et al. Development and validation of polygenic scores for within-family prediction of disease risks. *bioRxiv*. 2025. doi:10.1101/2025.08.06.25333145.

32. Wolfram T, Moore S, Li JH, Anomaly J, Davidson I, Christensen M. Within-family validation of a new polygenic predictor of general cognitive ability. *bioRxiv*. 2025. doi:10.1101/2025.08.06.25333145.