

Preimplantation Genetics Technology

An overview of recent publications featuring Illumina® technology



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This document highlights recent publications that demonstrate the use of Illumina technologies in preimplantation genetics. To learn more about the platforms and assays cited, visit www.illumina.com.

INTRODUCTION

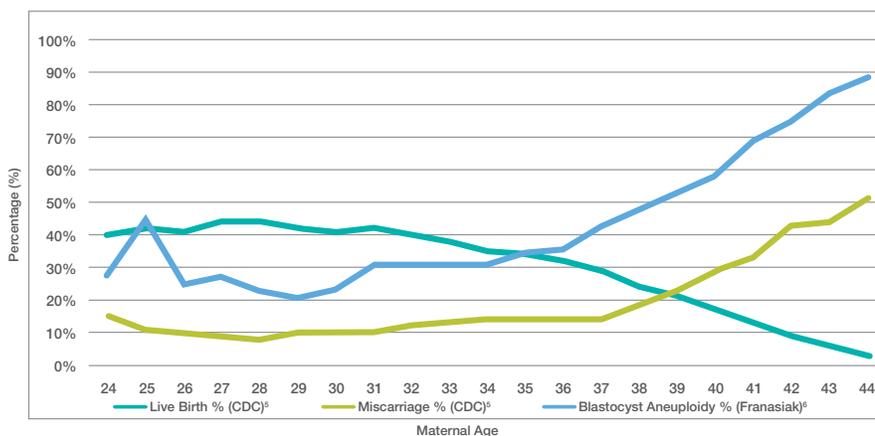
In vitro fertilization (IVF) success rates are low, with approximately 1 in 3 cycles resulting in a pregnancy.¹ One of the major causes of IVF failure is aneuploidy²; that is, embryos that have an incorrect number of chromosomes. Most aneuploidies are derived from errors during maternal meiosis. Since women are born with all of their eggs, these errors are likely to occur at higher frequencies as women age, therefore, the incidence of aneuploidy increases³ (Figure 1), and IVF success rates begin to fall as a woman reaches her late 30s or early 40s. However, even in younger patients, the aneuploidy rate can be significant enough to impact the success of IVF.⁴

Scientists developed preimplantation genetic screening (PGS) to help improve IVF success rates. The 24sure[®] assay and the more recent VeriSeq[™] PGS technology have enabled aneuploidy screening of embryos so that only embryos with the greatest likelihood of resulting in a live birth are considered for transfer. All 24 chromosomes (22 autosomes and the sex chromosomes, X and Y) can be screened within a 24-hour period, giving IVF clinics and/or patients the option of a fresh transfer after aneuploidy screening.

Many publications, conference presentations, and posters have demonstrated the potential of the 24sure assay to improve IVF success rates and discover more about the early stages of embryo development. Recent publications have also highlighted the accuracy and reliability of the VeriSeq PGS assay, which uses next-generation sequencing (NGS) technology. This overview highlights some of the key studies that have used 24sure array and VeriSeq PGS technology. It also provides background information for those interested in PGS and preimplantation genetic diagnosis (PGD).

- Centers for Disease Control and Prevention. Assisted Reproductive Technology: Fertility Clinic Success Rates Report. <ftp://ftp.cdc.gov/pub/Publications/art/ART-2013-Clinic-Report-Full.pdf>. Updated June 21, 2016. Accessed August 08, 2016.
- Fragouli E, Wells D. Aneuploidy in the human blastocyst. *Cytogenet Genome Res.* 2011;133(2-4):149-159. doi:10.1159/000323500.
- Ata B, Kaplan B, Danzer H, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. *Reprod Biomed Online.* 2012;24(6):614-620. doi:10.1016/j.rbmo.2012.02.009.
- Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol Hum Reprod.* 2000;6(11):1055-1062. doi:10.1093/molehr/6.11.1055.
- Centers for Disease Control and Prevention, 2013 ART National Summary Report, Figures 14-16. http://www.cdc.gov/art/pdf/2013-national-summary-slides/art_2013_graphs_and_charts_final.pdf. Updated February 10, 2016. Accessed June 30, 2016.
- Franasiak JM, Forman EJ, Hong KH, et al. The nature of aneuploidy with increasing age of the female partner: A review of 15,169 consecutive trophoctoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril.* 2014;101(3):656-663.e1. doi:10.1016/j.fertnstert.2013.11.004.

Figure 1: Percent of Live Birth, Miscarriage, and Aneuploidy vs Maternal Age in IVF Cycles^{5,6}



TECHNOLOGY

24sure array technology is based on bacterial artificial chromosome (BAC) microarrays. Each microarray consists of a glass slide with thousands of single-stranded DNA probes bound to it. Each DNA probe hybridizes to a specific place on a specific chromosome. The patient's DNA is fluorescently labeled and added to the glass slide. The probes bind to their specific targets in the patient DNA, and excess unbound DNA is washed off. An imaging device scans the glass slide and detects how much fluorescence is present at each probe. The computer software uses the positional information for each probe to produce a chart, plotting the intensity and wavelength of fluorescence along each chromosome. Much lower fluorescence in one area of a chromosome compared with other areas corresponds to a loss or deletion, and an increased amount of fluorescence indicates a gain or duplication.⁷

BAC microarray technology is sufficiently robust for applications such as the 24sure assay. It uses long probes, to which multiple fragments of sample DNA bind. This feature averages out the data for each region and reduces the effect of artifacts inherent in single-cell samples. 24sure array technology produces a reliable and scalable test, with high accuracy and a fast turn-around time.⁷

In 2014, Illumina introduced VeriSeq PGS technology. DNA extracted from either a single cell (cleavage-stage biopsy) or a few cells (trophectoderm (TE) biopsy) is subjected to whole-genome amplification, followed by sequencing library preparation. During the library preparation step, input DNA is tagged and fragmented, adding unique adapter sequences to the ends of the DNA. These adapter sequences are used to amplify the insert DNA in a limited-cycle polymerase chain reaction (PCR). In this step, index sequences are added to both ends of the DNA to allow dual-indexed sequencing. Prepared libraries are then sequenced using Illumina sequencing-by-synthesis chemistry. The data are analyzed, sequencing reads are aligned to a reference genome, and software is used to call copy number for each chromosome.^{8, 9, 10}

The accuracy and reliability of VeriSeq PGS has generally been compared with array comparative genome hybridization (aCGH), considered by some to be the gold standard for PGS. NGS can have a higher dynamic range than aCGH and therefore provide a greater level of resolution.^{11, 12} Before the introduction of aCGH and NGS, early attempts at PGS were carried out using fluorescence *in situ* hybridization (FISH). This technique suffers from inherent problems when analyzing single cells, including difficulty interpreting signals that may be overlapping or split, and the inability to analyze all chromosomes.^{13, 14, 15}

7. Handyside AH. 24-chromosome copy number analysis: a comparison of available technologies. *Fertil Steril.* 2013;100(3):595-602. doi:10.1016/j.fertnstert.2013.07.1965.
8. Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. *Mol Hum Reprod.* 2014;20(2):117-126. doi:10.1093/molehr/gat073.
9. Mertzaniidou A, Spits C, Nguyen HT, Van de Velde H, Sermon K. Evolution of aneuploidy up to Day 4 of human preimplantation development. *Hum Reprod.* 2013;28(6):1716-1724. doi:10.1093/humrep/det079.
10. Fragouli E, Alfarawati S, Daphnis DD, et al. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. *Hum Reprod.* 2011;26(2):480-490. doi:10.1093/humrep/deq344.
11. Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod.* 2014;29(12):2802-2813. doi:10.1093/humrep/deu277.
12. Zheng H, Jin H, Liu L, Liu J, Wang W-H. Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos. *Mol Cytogenet.* 2015;8(1):38. doi:10.1186/s13039-015-0143-6.
13. Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril.* 2013;100(3):624-630. doi: 10.1016/j.fertnstert.2013.04.039.
14. Fragouli E, Wells D. Aneuploidy screening for embryo selection. *Semin Reprod Med.* 2012;30(4):289-301. doi:10.1055/s-0032-1313908.
15. Hens K, Dondorp W, Handyside AH, et al. Dynamics and ethics of comprehensive preimplantation genetic testing: a review of the challenges. *Hum Reprod Update.* 2013;19(4):366-375. doi:10.1093/humupd/dmt009.

Key Publication Summaries

Zheng H, Jin H, Liu L, Liu J, Wang W-H. Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos. *Mol Cytogenet.* 2015;8(1):38. doi:10.1186/s13039-015-0143-6.

This validation study used NGS with VeriSeq PGS and an aCGH technology to screen for abnormalities in 43 embryo samples. The VeriSeq PGS assay identified all abnormalities that were identified by the aCGH technology, and it gave a successful result in 100% of samples. This preliminary preclinical evaluation indicates that NGS technology is both accurate and reliable for screening of human embryos.

Fiorentino F, Biricik A, Bono S, et al. Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. *Fertil Steril.* 2014;101(5). doi:10.1016/j.fertnstert.2014.01.051.

This retrospective, blinded study compared the results from NGS-based PGS (VeriSeq PGS) to results obtained from both karyotyping (n = 18) and biopsied single-cell products with consistent 24sure array results (n = 190). Sensitivity and specificity of aneuploidy calls for NGS were 100% and 99.98%, respectively.

Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod.* 2014;29(12):2802-2813. doi:10.1093/humrep/deu277.

This prospective study examined the level of correlation between the 24sure assay and the VeriSeq PGS assay. Overall, the study screened 192 embryos from 55 patients for aneuploidy at the blastocyst stage, and determined 99.5% concordance between the 2 assays. After analysis, 50 embryos were transferred. Those embryos classified by the lab as euploid based on VeriSeq PGS results led to a clinical pregnancy rate per embryo transfer of 63.8%. The study reported VeriSeq PGS assay sensitivity and specificity of $\geq 99.98\%$ with the ability to detect segmental aneuploidies as small as 14.7 Mb. The authors conclude that NGS using VeriSeq is an accurate, reliable, and potentially cost-effective technology for comprehensive chromosome screening of embryos.

Greco E, Bono S, Ruberti A, et al. Comparative genomic hybridization selection of blastocysts for repeated implantation failure treatment: a pilot study. *Biomed Res Int.* 2014; article ID 457913. doi:10.1155/2014/457913.

This prospective study aimed to show whether comprehensive chromosome screening (CCS) by 24sure array analysis and transfer of a single euploid blastocyst would improve outcomes in patients with 3 or more failed IVF transfers (repeated implantation failure; RIF) compared to those without a prior IVF failure. The study enrolled a total of 121 couples (76 RIF and 45 no prior failure/good prognosis) and all women were <36 years of age. The 76 RIF couples were divided into a treatment group (PGS, n = 43) and control group (no PGS, n = 33). All 45 good prognosis patients had PGS. The clinical pregnancy rate was significantly higher in the RIF PGS group compared to the RIF control group. Rates were similar between the RIF PGS group and the good prognosis group. The authors conclude that the combined use of aCGH and single blastocyst transfer can improve IVF outcomes in patients with RIF.

Handyside AH. 24-chromosome copy number analysis: a comparison of available technologies. *Fertil Steril.* 2013;100(3):595-602. doi:10.1016/j.fertnstert.2013.07.1965.

This review summarizes the key technologies that can be used for PGS and describes how they work. It discusses the advantages and disadvantages of each technology, including cost, scalability, and biopsy stage. aCGH is widely considered to be reliable, accurate, and robust, which is why it is used extensively around the world. This review builds on issues discussed previously as well as explores newer technologies, including NGS and karyomapping.

Additional References

Grifo JA, Hodes-Wertz B, Lee HL, Amperloquio E, Clarke-Williams M, Adler A. Single thawed euploid embryo transfer improves IVF pregnancy, miscarriage, and multiple gestation outcomes and has similar implantation rates as egg donation. *J Assist Reprod Genet.* 2013;30(2):259-264. doi:10.1007/s10815-012-9929-1.

Gutiérrez-Mateo C, Colls P, Sánchez-García J, et al. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil Steril.* 2011;95(3):953-958. doi:10.1016/j.fertnstert.2010.09.010.

Ma GC, Chen HF, Yang YS, et al. A pilot proof-of-principle study to compare fresh and vitrified cycle preimplantation genetic screening by chromosome microarray and next generation sequencing. *Mol Cytogenet.* 2016;9:25. doi: 10.1186/s13039-016-0238-8.

Rechitsky S, Verlinsky O, Kuliev A. PGD for cystic fibrosis patients and couples at risk of an additional genetic disorder combined with 24-chromosome aneuploidy testing. *Reprod Biomed Online.* 2013;26(5):420-430. doi:10.1016/j.rbmo.2013.01.006.

Yang Z, Lin J, Zhang J, et al. Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study. *BMC Med Genomics.* 2015;8:30. doi: 10.1186/s12920-015-0110-4.

Yang Z, Zhang J, Salem SA, et al. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. *BMC Med Genomics.* 2014;7:38. doi: 10.1186/1755-8794-7-38.

16. Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. *Mol Hum Reprod.* 2014;20(2):117-126. doi:10.1093/molehr/gat073.
17. Cimadomo D, Capalbo A, Ubaldi FM, et al. The impact of biopsy on human embryo developmental potential during preimplantation genetic diagnosis. *Biomed Res Int.* 2016; article ID 7193075. doi:10.1155/2016/7193075.

Table 1: Advantages and Disadvantages of Embryo Biopsy for PGS at Each Stage of Embryo Development

Biopsy Stage	Advantages	Disadvantages
Polar body (PB)	<ul style="list-style-type: none"> • Does not involve removal of any material from the embryo itself • May be the only legal option in some countries • Provides sufficient time for analysis before fresh transfer 	<ul style="list-style-type: none"> • Will not detect aneuploidies from paternal contribution • Will not detect mitotic errors • Requires large numbers of samples tested per cycle
Cleavage stage (3 day)	<ul style="list-style-type: none"> • Tests both maternal and paternal contribution • Provides sufficient time for analysis before fresh transfer 	<ul style="list-style-type: none"> • Higher rates of aneuploidy^{8, 9, 16} • Mosaicism is most common⁸ • Viability of embryo may be affected in some cases
Blastocyst stage (5 day)	<ul style="list-style-type: none"> • Less mosaicism than cleavage stage • Tests more than one cell, reducing failure rate and improving sensitivity in mosaic samples • Tests both maternal and paternal contribution • Has little or no impact on embryo viability^{13, 17} 	<ul style="list-style-type: none"> • More time pressure in the context of a fresh transfer • May not be appropriate for patients who are not expected to produce many blastocysts

EVIDENCE AND TRIALS

A growing number of researchers have studied the impact of the 24sure assay on IVF success. This body of evidence incorporates studies that have tested embryos at various stages of development and includes patients of different ages, demonstrating that the 24sure assay can be applied in many scenarios.

The publication of 2 randomized controlled trials (RCTs) represents a landmark addition to the growing collection of evidence demonstrating the benefits of 24sure technology. These 2 RCTs are summarized in the next section. Researchers in both studies performed 24sure screening on a cohort of young patients with good prognoses undergoing IVF. A large RCT (clinicaltrials.gov: NCT02268786) is underway to compare ongoing pregnancy rates for embryos screened using either VeriSeq PGS or morphology.

In addition to RCTs, there have been many retrospective and case-controlled studies comparing data from patients who underwent PGS to those whose embryos were selected based on morphology alone. These studies have consistently demonstrated the ability of 24sure technology to raise measures of IVF success, such as implantation rate and ongoing pregnancy rate, and to reduce negative outcomes, such as miscarriage and multiple births. Recent publications have also demonstrated the reliability and effectiveness of VeriSeq PGS technology for identifying aneuploidies in embryos.^{18,19}

Key Publication Summaries—RCTs

Yang Z, Salem SA, Liu X, Kuang Y, Salem RD, Liu J. Selection of euploid blastocysts for cryopreservation with array comparative genomic hybridization (aCGH) results in increased implantation rates in subsequent frozen and thawed embryo transfer cycles. *Mol Cytogenet.* 2013;6(1):32. doi:10.1186/1755-8166-6-32.

These data demonstrate the efficacy of 24sure array technology when combined with vitrification in patients with good prognoses. The study randomized 103 patients into embryo morphology and CCS groups with aCGH (n = 55) or embryo morphology only (n = 48). Results showed that CCS by aCGH before vitrification significantly increases the implantation rate per embryo (65% vs 33% in embryos screened by morphology only; P=0.038). The study also showed that aCGH screening may decrease miscarriage rates (no miscarriages occurred in the group screened using the 24sure assay), and reduces the cost associated with embryo storage, as aneuploid embryos would not be stored for future transfer.

Yang Z, Liu J, Collins GS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis patients: results from a randomised pilot study. *Mol Cytogenet.* 2012;5(1):24. doi:10.1186/1755-8166-5-24.

This publication represents the first randomized study comparing CCS with aCGH to standard morphology assessment. In addition, this study focused on increasing the use of fresh single-embryo transfer (SET) and whether pregnancy rates are improved when using 24sure array technology and fresh SET in IVF patients with good prognoses (age < 35 years, no prior miscarriage). The study randomized 112 patients into 2 groups. After drop-out, the final group breakdown included 48 patients in the morphology-only group and 55 patients in the group receiving both morphology and aCGH using the 24sure assay. All patients had elective single embryo transfer (eSET). Results showed a significant increase in clinical pregnancy rates with 24sure array compared with the morphology-only control group (70.9% and 45.8%, respectively; P = 0.017). Ongoing pregnancy rates at 20 weeks per cycle were 41.7% in the control group and 69.1% in the 24sure array analysis group (P = 0.009). The authors conclude that an improvement in IVF outcomes with eSET cycles can be seen when aCGH is integrated into the clinical program.

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18. Fiorentino F, Biricik A, Bono S, et al. Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. *Fertil Steril.* 2014;101(5). doi:10.1016/j.fertnstert.2014.01.051.
 19. Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod.* 2014;29(12):2802-2813. doi:10.1093/humrep/deu277.
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Key Publication Summaries—Retrospective Analysis

Rodrigo L, Mateu E, Mercader A, et al. New tools for embryo selection: comprehensive chromosome screening by array comparative genomic hybridization. *Biomed Res Int.* 2014; article ID 517125. doi:10.1155/2014/517125.

This retrospective study evaluated CCS with the 24sure assay by analyzing 7210 day-3 embryo biopsy samples from 1420 cycles. After CCS, 1 or more embryos were available for transfer in 783 cycles. Per-transfer and per-cycle pregnancy rates were 53.4% and 29.4%, respectively. Implantation and pregnancy rates were similar across all indications (male-factor infertility, repeat miscarriage, repeat IVF failure, previous trisomy, and advanced maternal age) following embryo transfer, independent of the origin of the oocytes or embryos. Overall, pregnancy and implantation rates in this study were higher compared to the authors' previous study evaluating PGS using FISH. The authors conclude that aneuploidy is one of the major factors that affect embryo implantation and that CCS may be a promising solution for couples with male-factor infertility.

Key Publication Summaries—Case-Controlled

Keltz MD, Vega M, Sirota I, et al. Preimplantation genetic screening (PGS) with comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. *J Assist Reprod Genet.* 2013;30(10):1333-1339. doi:10.1007/s10815-013-0070-6.

This retrospective case-controlled study looked at pregnancy outcomes in 35 patients (39 cycles) who underwent aneuploidy screening using the 24sure array platform and compared them to a control group (n = 394 fresh, nondonor IVF cycles). In both age groups (< 35 and ≥ 35), 24sure array analysis resulted in a significantly higher implantation rate (52.6% vs 19.2%; P < 0.001), clinical pregnancy rate (69.2% vs 43.9%; P = 0.0002), and ongoing pregnancy rate (61.5% vs 32.5%; P < 0.0001).

Key Publication Summaries—Other

Hodes-Wertz B, Grifo J, Ghadir S, et al. Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos. *Fertil Steril.* 2012;98(3):675-680. doi:10.1016/j.fertnstert.2012.05.025.

This study explored the possibility of aneuploid embryos being a significant factor in unexplained recurrent pregnancy loss. It included 2282 embryos from 287 cycles in couples with idiopathic recurrent pregnancy loss. The researchers performed embryo biopsy and aCGH with 24sure array analysis on either day 3 or day 5. After biopsy, 35.2% of embryos were reported to be euploid (181 cycles) and considered for embryo transfer. The rate of euploid embryos was significantly higher on day 5 vs day 3 (47% vs 31.2%; p < 0.001). This study reported an overall 45% implantation rate and 55.2% clinical pregnancy rate. No significant differences in implantation and pregnancy rates were noted based on maternal age. The miscarriage rate was 6.9% compared with an expected rate of 33.5% for recurrent pregnancy loss (RPL) patients. Based on these results and previous studies, the authors suggest that selection of euploid embryos for transfer could be a huge benefit in patients with idiopathic recurrent pregnancy loss.

Liu J, Wang W, Sun X, et al. DNA microarray reveals that high proportions of human blastocysts from women of advanced maternal age are aneuploid and mosaic. *Biol Reprod.* 2012;87(6):148. doi:10.1095/biolreprod.112.103192.

This study evaluated the types of chromosome abnormalities present in human blastocysts and whether there is consistency between the TE cells and the inner cell mass (ICM). The researchers used 2 array methods to assess both cell types: 24sure BAC array and the NimbleGen oligonucleotide array. Amplification was successful in 244/258 blastocysts from 51 IVF cycles, 43% of which were euploid. Aneuploidy was higher in women ≥ 38 years compared to the younger age group. The aneuploidy rate was 82% in women ≥ 41 years of age. Of the 138 blastocysts with aneuploidy, complex aneuploidies were seen in 37%. Analysis of TE and ICM cells in 13 aneuploid blastocysts revealed mosaicism in 9 (69.2%) embryos. Implantation and pregnancy rates were 63.5% and 70.2%, respectively. The authors summarize that in women of increased maternal age, a high proportion of blastocysts are aneuploid and mosaic.

Key Publication Summaries—Meta-analyses

Dahdouh EM, Balayla J, Garcia-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. *Fertil Steril*. 2015;104(6):1503-1512. doi:10.1016/j.fertnstert.2015.08.038.

This is the first meta-analysis on PGS since Mastenbroek et al. published a review of PGS using only FISH analysis in 2011. Dahdouh et al. included a review of 3 RCTs and 8 observational studies. Overall, PGS with CCS improved both clinical implantation rate and sustained implantation rate. All studies performed on TE cells showed > 50% implantation rate and > 45% sustained implantation rate. The authors conclude that, if applied properly, PGS with CCS may shorten time to pregnancy and decrease miscarriage rates, although it is not suitable for every patient and every practice at this time.

Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. *Hum Reprod*. 2015;30(2):473-483. doi:10.1093/humrep/deu303.

This systematic review analyzed 19 studies, including 3 RCTs in good-prognosis patients, and 16 observational studies. A majority of these studies used TE biopsy. Improvements in live birth rates in women of advanced maternal age and history of recurrent implantation failure or pregnancy loss were seen in observational studies, although causality could not be inferred as RCTs included only young, good-prognosis patients. Overall, implantation, clinical pregnancy, and miscarriage rates were improved with PGS compared to morphology assessment only.

Rubio C, Rodrigo L, Mir P, et al. Use of array comparative genomic hybridization (array-CGH) for embryo assessment: clinical results. *Fertil Steril*. 2013;99(4):1044-1048. doi:10.1016/j.fertnstert.2013.01.094.

This review reported improved pregnancy rates for various indications with 24sure array-based screening compared to pregnancy rates following FISH. The authors conclude that most data regarding the controversy of day-3 biopsy comes from outcomes following aneuploidy screening using FISH, and that the utility of day-3 biopsy using array-based screening should be further validated through RCTs.

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Chang J, Boulet SL, Jeng G, Flowers L, Kissin DM. Outcomes of *in vitro* fertilization with preimplantation genetic diagnosis: an analysis of the United States Assisted Reproductive Technology Surveillance Data, 2011-2012. *Fertil Steril*. 2016;105(2):394-400. doi:10.1016/j.fertnstert.2015.10.018

Chen M, Wei S, Hu J, Quan S. Can comprehensive chromosome screening technology improve IVF/ICSI outcomes? A meta-analysis. *PLoS One*. 2015;10(10):e0140779. doi: 10.1371/journal.pone.0140779.

Ma GC, Chen HF, Yang YS, et al. A pilot proof-of-principle study to compare fresh and vitrified cycle preimplantation genetic screening by chromosome microarray and next generation sequencing. *Mol Cytogenet*. 2016;9:25. doi: 10.1186/s13039-016-0238-8.

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Yang Z, Zhang J, Salem SA, et al. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. *BMC Med Genomics*. 2014;7:38. doi: 10.1186/1755-8794-7-38.

ANEUPLOIDY AND MATERNAL AGE

IVF success rates decrease as maternal age increases, in large part due to increased aneuploidy incidence.²⁰

Further investigations have revealed that a significant proportion of embryos produced by younger patients are also aneuploid, with levels estimated at up to 50% for patients in the youngest age categories.^{20, 21} Other studies have looked at whether there are increased aneuploidy rates in patients who are affected by a particular type of infertility or have had a certain number of miscarriages, or whether aneuploidy rates correlate with the number of embryos produced. 24sure array and VeriSeq PGS technologies can have a significant impact on patients, in particular in those of advanced maternal age (≥ 35 years). By screening embryos before transfer and selecting only those with a euploid PGS result, ongoing pregnancy rates remained static across age groups up to approximately age 42.²²

Clearly, in patients of even greater maternal age, the chance of having no euploid embryos to transfer increases, which can be a potential disadvantage to PGS. This chance depends on the number of blastocysts produced, as demonstrated by a retrospective study.²⁰ Counseling must be provided to ensure that couples are informed of the possibility that no embryos will be available for transfer.²³ Learning that the IVF treatment is unlikely to have the desired outcome of a healthy liveborn child prior to transfer may help minimize the distress couples experience when an embryo fails to implant or miscarries during pregnancy.

In patients for whom euploid embryos do exist, the chance of achieving a successful pregnancy is increased. The cost of cryopreserving any remaining embryos would decrease as well, because only euploid embryos would be stored.²⁴

Key Publication Summaries

Harton GL, Munné S, Surrey M, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. *Fertil Steril.* 2013;100(6):1695-1703. doi:10.1016/j.fertnstert.2013.07.2002.

This study aimed to determine if identification of euploid embryos using aCGH could overcome the decrease in implantation rates observed during IVF as maternal age increases. The researchers screened embryos from patients undergoing PGS in fertility clinics across the US using the 24sure assay following biopsy on either day 3 (n = 3412 blastocysts) or day 5/6 (n = 2467 blastocysts). Despite observing the expected increase in aneuploidy with increasing maternal age, implantation rates and ongoing pregnancy rates were constant among age groups up to 42 years of age. However, the implantation rate was greater for day-5 embryos compared to day-3 embryos (49.2% vs 39.6%; $P < 0.005$). Loss rates were not significantly different, based on either maternal age or biopsy timing.

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20. Fragouli E, Wells D. Aneuploidy in the human blastocyst. *Cytogenet Genome Res.* 2011;133(2-4):149-159. doi:10.1159/000323500.
 21. Ata B, Kaplan B, Danzer H, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. *Reprod Biomed Online.* 2012; 24(6):614-620. doi:10.1016/j.rbmo.2012.01.009.
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Ata B, Kaplan B, Danzer H, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. *Reprod Biomed Online*. 2012;24(6):614-620. doi:10.1016/j.rbmo.2012.01.009.

This retrospective study analyzed existing 24sure array data from a single laboratory in North America to evaluate the frequency of aneuploidy in embryos and determine whether a relationship exists between the number of embryos generated during an IVF cycle and the rate of aneuploidy. The overall study cohort consisted of 7753 embryos from 990 women. Results showed a negative relationship between maternal age and embryo euploidy. In addition, the authors conclude that the rate of aneuploidy is unrelated to the number of embryos generated per cycle.

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Liu J, Wang W, Sun X, et al. DNA microarray reveals that high proportions of human blastocysts from women of advanced maternal age are aneuploid and mosaic. *Biol Reprod*. 2012;87(6):148. doi:10.1095/biolreprod.112.103192.

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Origins of Aneuploidy

PGS technologies have enabled investigation of the origins and frequency of aneuploidy, and provided insight into when, why, and how chromosomal abnormalities arise. Until recently, it was thought that aneuploidies most commonly arise maternally through chromosomal nondisjunction during meiosis. Through careful analysis of 24sure array data, it is possible to differentiate between the loss of a chromatid and the loss of a whole chromosome, by looking at the degree to which the log ratio shifts. As a result, it is becoming increasingly apparent that most aneuploidies arise through premature sister chromatid separation rather than chromosomal nondisjunction.²⁵ Also, other studies have investigated the paternal incidence of aneuploidies. As 24sure array technology is designed to analyze single cells, it can also be used to analyze the chromosomal constitution of sperm. A recent study looked at the number of chromosomal abnormalities in men who had undergone chemotherapy, compared with a control cohort.²⁶

Key Publication Summaries

Capalbo A, Bono S, Spizzichino L, et al. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. *Hum Reprod*. 2013;28(2):509-518. doi:10.1093/humrep/des394.

This study used 24sure array technology to analyze all PGS biopsy stages of an embryo (PB, cleavage stage, and blastocyst stage) and review the incidence of aneuploidies at each time point. This approach enabled determination of the origin of aneuploidies, calculation of the number of corrected aneuploidies, and confirmation of the mechanism (eg, trisomy rescue). The number of aneuploidies increased up to day 3 of embryo development, but then a proportion of these aneuploidies were corrected by the blastocyst stage. The study also demonstrated that maternally derived aneuploidies, in this cohort of women >40 years of age, resulted from chromatid mis-segregation at meiosis II.

Patassini C, Garolla A, Bottacin A, et al. Molecular karyotyping of human single sperm by array-comparative genomic hybridization. *PLoS One*. 2013;8(4):e60922. doi:10.1371/journal.pone.0060922.

This proof-of-principle study described the first use of CCS on single human sperm. Using 24sure array technology, the researchers analyzed 129 individual sperm from normozoospermic donors and compared them to 130 individual sperm from patients with Hodgkin lymphoma at the end of 3 months of chemotherapy. Sperm from postchemotherapy Hodgkin's lymphoma patients had 24% unbalanced gains or losses vs only 7.8% in sperm from normozoospermic donors. In addition, the study presented data from validation of the technique through the analysis of single sperm from a carrier of a balanced translocation.

Handyside AH, Montag M, Magli MC, et al. Multiple meiotic errors caused by pre-division of chromatids in women of advanced maternal age undergoing *in vitro* fertilisation. *Eur J Hum Genet*. 2012;20(7):742-747. doi:10.1038/ejhg.2011.272.

This study used data compiled during an aCGH proof-of-principle trial conducted by the European Society for Human Reproduction and Embryology (ESHRE) PGS taskforce to determine when meiotic-division errors occur. The study examined first and second PBs for aneuploidy, using 24sure array technology, in 34 couples undergoing IVF. In cases where aneuploidy was predicted in the zygote, the corresponding zygote was also analyzed. Comparisons of aneuploidy in the 3 products of female meiosis enabled study of the incidence of aneuploidy in human oocytes at conception for the first time. The study also identified in which meiotic division errors occurred. The authors conclude that premature predivision of chromatids causes most errors in the first division.

Gabriel AS, Thornhill AR, Ottolini CS, et al. Array comparative genomic hybridisation on first polar bodies suggests that non-disjunction is not the predominant mechanism leading to aneuploidy in humans. *J Med Genet*. 2011;48(7):433-437. doi:10.1136/jmg.2010.088070.

This study examined whether aneuploidy arises from nondisjunction (whole-chromosome segregation errors) or through precocious separation (sister-chromatid segregation errors). The researchers analyzed 164 first PB biopsies using 24sure array technology. The authors conclude that most aneuploidy arises from sister-chromatid separation errors (92%), not whole-chromosome segregation errors (8%), disputing that nondisjunction is the primary mechanism leading to human aneuploidy.

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Morphology, Morphokinetics, and Aneuploidy

Traditionally, embryos have been selected for transfer based on morphology alone. Characteristics such as the degree of blastocyst expansion, ICM appearance, fragmentation, rate of growth, and hatching status were scored to give each embryo a final grade.²⁷ These grades were used to select the “best” embryo for transfer and “good quality” embryos for freezing. These techniques are subjective, have multiple scoring systems, and exhibit high intrinsic interoperator and intraoperator variation. Also, morphology can be a poor predictor of embryo viability, and there is no guarantee that even the most morphologically perfect embryo will implant.^{28, 29}

With the use of 24sure array technology, it has become apparent that aneuploidy often does not manifest morphologically and that even chromosomally chaotic embryos can appear morphologically normal.²⁸ In fact, approximately 50% of top morphologically graded embryos are aneuploid.³⁰ Recent innovations have made time-lapse imaging a possibility for embryo selection.^{31, 32} Although this technique produces better predictive information than assessing the embryo at a single time point, studies indicate that it is still not indicative of aneuploidy.³³

Key Publication Summaries

Balakier H, Sojecki A, Motamedi G, Librach C. Impact of multinucleated blastomeres on embryo developmental competence, morphokinetics, and aneuploidy. *Fertil Steril*. 2016; doi: 10.1016/j.fertnstert.2016.04.041.

This retrospective study compared the association between embryo multinucleation and the rate of aneuploidy, morphokinetics and pregnancy outcome. The researchers performed time-lapse imaging using EmbryoViewer software and CCS using the 24sure array platform. Forty-three percent (1055/2441) of embryos showed multinucleation at the 2-cell stage, and 15% at the 4-cell stage. The frequency of multinucleation was equal between euploid and aneuploid embryos.

Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum Reprod*. 2014;29(6):1173-1181. doi:10.1093/humrep/deu033.

This retrospective, observational study evaluated whether conventional blastocyst morphology correlates with euploidy reported by PGS using aCGH with the 24sure assay. The study included 956 blastocysts obtained from 223 PGS cycles and graded morphology as excellent, good, average, or poor. Euploidy rates were highest in embryos graded excellent and lowest in embryos graded poor (56.4% vs 25.5%); however, implantation potential was similar across all morphological grades. The authors conclude that, when PGS is not available, blastocyst morphology can slightly reduce the risk of transferring aneuploidy embryos; however, it should not be considered a reliable alternative to CCS.

Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. *Mol Hum Reprod*. 2014;20(2):117-126. doi:10.1093/molehr/gat073.

This study aimed to determine whether aneuploidy results in any detectable differences in embryo morphology. The researchers tested 1213 embryos using the 24sure assay and compared morphological characteristics of euploid and aneuploid embryos at both the cleavage and blastocyst stages. The results showed virtually no difference in morphology grading between chromosomally normal and chromosomally abnormal cleavage-stage embryos (83% vs 72%). At the blastocyst stage, embryos with 3 or more aneuploidies tended to have poorer morphology and be less well-developed. Based on the results, the authors conclude that aneuploidy has minimal impact on embryo morphology, and that most clinically relevant abnormalities would be undetectable by morphology alone.

Cruz M, Garrido N, Herrero J, Pérez-Cano I, Muñoz M, Meseguer M. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. *Reprod Biomed Online*. 2012;25(4):371-381. doi:10.1016/j.rbmo.2012.06.017.

This retrospective cohort study evaluated the possible association between the timing of embryo division (morphokinetics) and the ability of an embryo to reach the blastocyst stage. Of 962 fertilized oocytes from donor cycles, the researchers analyzed 834 embryos using EmbryoViewer software. A significant difference ($P = 0.007$) was seen in the rate of blastocysts and good-morphology blastocysts when cleavage-stage cell division was symmetric vs asymmetric. After analysis, 274 embryos were transferred with an implantation rate of 49.6%. The authors conclude that the capability of an embryo to develop to the blastocyst stage is related to embryo morphokinetics.

SINGLE-EMBRYO TRANSFER

Risks associated with multiple gestation, for both the mother and babies, are well documented. Babies born as multiples are significantly more likely to be admitted to the neonatal intensive care unit after birth compared to singletons, and they are more likely to be premature and of low birth weight.^{34,35} Despite these risks, it has been routine to transfer more than 1 embryo in an IVF cycle to compensate for the lower implantation rate usually observed. 24sure array and VeriSeq PGS technologies permit more efficient single-embryo transfer for many couples because of the improved success rates afforded by these technologies.^{36,37} PGS increases the chance of success when a single screened blastocyst is transferred such that it is comparable to the chance of success with the transfer of 2 unscreened embryos.³⁶ Thus, complications associated with IVF-related multiple gestation can be reduced without compromising pregnancy rates.³⁸

Key Publication Summaries

Schoolcraft WB, Katz-Jaffe MG. Comprehensive chromosome screening of trophectoderm with vitrification facilitates elective single-embryo transfer for infertile women with advanced maternal age. *Fertil Steril.* 2013;100(3):615-619. doi:10.1016/j.fertnstert.2013.07.1972.

This review outlines the advantages of single-embryo transfer as opposed to double-embryo transfer and discusses how CCS techniques (such as the 24sure assay) can enable single-embryo transfer without a reduction in IVF success rates. The authors suggest employing a strategy of TE biopsy, CCS, embryo vitrification, and frozen-embryo transfer. In developing this strategy, the authors cite evidence that: i) blastocyst biopsy is not detrimental to the embryo (whereas there is evidence that cleavage-stage biopsy may be); ii) chromosome screening improves success rates; and iii) frozen-embryo transfer may result in higher clinical pregnancy rates.

Sills ES, Yang Z, Walsh DJ, Salem SA. Comprehensive genetic assessment of the human embryo: can empiric application of microarray comparative genomic hybridization reduce multiple gestation rate by single fresh blastocyst transfer? *Arch Gynecol Obstet.* 2012;286(3):755-761. doi:10.1007/s00404-012-2396-1.

This review article discusses improvements in array platforms and CCS that have improved the reliability and cost-effectiveness of IVF. The authors conclude that it will soon be standard practice to determine which (single) embryo to transfer in an IVF cycle by assessing the chromosome complement using aCGH.

34. Schoolcraft WB, Katz-Jaffe MG. Comprehensive chromosome screening of trophectoderm with vitrification facilitates elective single-embryo transfer for infertile women with advanced maternal age. *Fertil Steril.* 2013;100(3):615-619. doi:10.1016/j.fertnstert.2013.07.1972.
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POLAR-BODY BIOPSY

Many aneuploidies arise during meiotic errors in oocyte development and, therefore, can be detected at the earliest stages of embryo development through PB testing. To accomplish this detection, the 24sure assay analyzes both polar body 1 (PB1) and polar body 2 (PB2). The resulting copy-number information is used to deduce the likely chromosome constitution of the oocyte itself. For example, a gain of a chromosome in the second PB indicates an oocyte with a loss of the same chromosome. The technique of PB biopsy has been well described in the literature, and best practice guidelines have been drawn up by the ESHRE PGD consortium.³⁹

PB testing offers the advantage of being the biopsy method least likely to affect the embryo. PBs do not form any part of the resulting embryo and are byproducts of oocyte formation. Therefore, this method may be more ethically acceptable to the individual patient. In some countries, it may be the only legally available option due to local regulations. It also provides the most time for analysis before embryo transfer.

An inherent disadvantage in not testing the embryo itself is that any conclusions about the embryo have to be inferred. Also, PB testing will not identify aneuploidies arising from causes other than maternal meiotic errors, such as paternal meiotic errors or mitotic errors.

Key Publication Summaries

Christopikou D, Tsorva E, Economou K, et al. Polar body analysis by array comparative genomic hybridization accurately predicts aneuploidies of maternal meiotic origin in cleavage stage embryos of women of advanced maternal age. *Hum Reprod.* 2013;28(5):1426-1434. doi:10.1093/humrep/det053.

This retrospective analysis investigated concordance between PB1 and PB2 biopsies with cleavage-stage biopsies using aCGH analysis with 24sure arrays. The study included PB1 analysis of 92 mature oocytes. In 58/92 (63%) cases, normal fertilization occurred and biopsies were performed in 57/58 (98%). Whole genome amplification and aCGH were successful in 54/57 (93%) cases for both PB1 and PB2. Biopsies were performed in 30 cleavage-stage embryos predicted to have aneuploidy by PB1 and PB2 aCGH analysis. There was 100% concordance between embryos predicted to be aneuploid by PB biopsy vs. aneuploid by cleavage-stage biopsy. Of the 74 aneuploidies in 30 embryos, 69 (93%) were associated with copy number changes in polar bodies, supporting the reliability of PB biopsy for aneuploidy screening.

Fishel S, Craig A, Lynch C, et al. Assessment of 19,803 paired chromosomes and clinical outcome from first 150 cycles using array CGH of the first polar body for embryo selection and transfer. *J Fertiliz In Vitro.* 2011;1(101):1-8. doi:10.4172/2165-7491.1000101.

This proof-of-principle study by CARE Fertility Group presented their experience using the 24sure array on PBs since 2008, when they first assessed aCGH aneuploidy screening and achieved a live birth following use of the technology. The researchers analyzed a total of 861 PBs from 134 couples (mean maternal age of 41 years), including all 19,803 chromosome pairs. The results showed aneuploidy in 67.4% of PBs, nearly half of which were single chromosome aneuploidies. Chromosomes 15, 16, 21, and 22 were seen more frequently in aneuploid cells compared to other chromosomes.

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Geraedts J, Collins J, Gianaroli L, et al. What next for preimplantation genetic screening? A polar body approach. *Hum Reprod.* 2010;25(3):575-577. doi:10.1093/humrep/dep446.

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Magli MC, Montag M, Köster M, et al. Polar body array CGH for prediction of the status of the corresponding oocyte. Part II: technical aspects. *Hum Reprod.* 2011;26(11):3181-3185. doi:10.1093/humrep/der295.

Montag M, Köster M, Strowitzki T, Toth B. Polar body biopsy. *Fertil Steril.* 2013;100(3):603-607. doi:10.1016/j.fertnstert.2013.05.053.

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CLEAVAGE-STAGE BIOPSY

At day 3, the embryo is at the cleavage stage and consists of approximately 8 cells. Biopsy at this stage involves the removal of just 1 (or, at most, 2) cells. Traditionally, this was the most widely practiced biopsy method. The major advantage of testing at this stage is that the detection of all aneuploidies, including those resulting from a paternal meiotic errors or mitotic errors can enable a fresh embryo transfer. However, there is some evidence that performing biopsies at this stage may damage the embryo.⁴⁰ Also, embryos at this stage are more likely to be mosaic, making it less certain that the single cell being tested is representative of the whole embryo.⁴¹ Despite these drawbacks, research has demonstrated that the advantages of selecting euploid embryos outweigh the potential impact to the embryo at this stage.^{42, 43}

Key Publication Summaries

Cimadomo D, Capalbo A, Ubaldi FM, et al. The impact of biopsy on human embryo developmental potential during preimplantation genetic diagnosis. *Biomed Res Int.* 2016; article ID 7193075. doi:10.1155/2016/7193075.

This review presents published evidence regarding embryo biopsy at the PB, cleavage, morula, and blastocyst stages of human embryonic development. In summary, there is evidence that cleavage-stage biopsy results in potential embryo harm and decreased clinical outcomes, while blastocyst biopsy is safer and more accurate..

Keltz MD, Vega M, Sirota I, et al. Preimplantation genetic screening (PGS) with comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. *J Assist Reprod Genet.* 2013;30(10):1333-1339. doi:10.1007/s10815-013-0070-6.

This retrospective case-controlled study looked at pregnancy outcomes in 35 patients (39 cycles) who underwent aneuploidy screening using the 24sure array platform and compared them to a control group without CCS (n = 394 fresh, nondonor IVF cycles). In both age groups (< 35 and ≥ 35), 24sure array analysis resulted in significantly higher implantation rates (52.6% vs 19.2%; P < 0.001), clinical pregnancy rates (69.2% vs 43.9%; P = 0.0002), and ongoing pregnancy rates (61.5% vs 32.5%; P < 0.0001). For all patients, the mean number of embryos transferred was significantly lower in the aCGH group compared to the control group (1.5 vs. 3.3; p<0.0001). The multiple pregnancy rates (8.3% vs. 34.4%) and miscarriage rates (11.1% vs. 26.0%) were lower in the aCGH group compared to controls, however, neither difference met significance.

Rubio C, Rodrigo L, Mir P, et al. Use of array comparative genomic hybridization (array-CGH) for embryo assessment: clinical results. *Fertil Steril.* 2013;99(4):1044-1048. doi:10.1016/j.fertnstert.2013.01.094.

This review reports improved pregnancy rates for various indications when CCS with aCGH was performed, compared to pregnancy rates following FISH. Although the authors have published studies demonstrating a benefit of FISH analysis in patients with certain indications, they acknowledge that alternative methods to screen all 24 chromosomes exist. However, questions still remain about these newer PGS methodologies and RCTs are needed to assess various aCGH platforms and biopsy approaches.

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BLASTOCYST-STAGE BIOPSY

At day 5/6, the zygote has formed a blastocyst consisting of an ICM (which goes on to form the embryo), surrounded by the TE (a sphere of cells that form the supportive tissues, such as the placenta). At this stage, a proportion of the mosaicism seen in cleavage-stage embryos has been eliminated.^{44, 45}

Biopsy at the blastocyst stage involves removal of a few (5–10) TE cells. Since these cells go on to develop the supportive tissues and not the embryo, the risk of impact to embryo viability is reduced. Testing multiple cells increases the chance of detecting mosaicism present at a level that could affect the embryo.⁴⁶

As this biopsy is performed at day 5, it is possible for testing to occur before embryo transfer on day 6; albeit with more time pressure than at other developmental stages. Alternatively, embryos can be frozen for transfer in a later cycle, which may offer further improvements in success rates and obstetric outcomes.^{47, 48} A growing body of evidence suggests that TE biopsy at the blastocyst stage, may be more advantageous than cleavage-stage or PB biopsy, with larger improvements in implantation rate being observed in comparative studies.^{49, 50}

Key Publication Summaries

Capalbo A, Wright G, Elliott T, Ubaldi FM, Rienzi L, Nagy ZP. FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage. *Hum Reprod.* 2013;28(8):2298-2307. doi:10.1093/humrep/det245.

This retrospective cohort study further analyzed 79 blastocysts that were reported as abnormal by aCGH. After warming, 70 blastocysts underwent ICM isolation and TE partitioning (40 single aneuploidy, 10 double aneuploidy, and 20 unbalanced diploid embryos). The researchers analyzed samples using FISH for the 9 chromosomes involved in aneuploidy by aCGH and found that 51 embryos with either a single or double aneuploidy had a total of 66 chromosome abnormalities. The overall accuracies per embryo and per chromosome were 98.6% and 99.3%. Overall, mosaicism was detected in 11/71 (15.7%) blastocysts. The authors conclude that the 24sure assay accurately predicts the ploidy status of the embryo.

Mamas T, Gordon A, Brown A, Harper J, Sengupta S. Detection of aneuploidy by array comparative genomic hybridization using cell lines to mimic a mosaic trophectoderm biopsy. *Fertil Steril.* 2012;97(4):943-947. doi:10.1016/j.fertnstert.2011.12.048.

This study used a cell-culture system to simulate levels of mosaicism that may be present in an embryo. The researchers measured the level of aneuploidy using 24sure array technology in mixed-cell populations. 24sure array detected aneuploidy in cell populations where 25% of cells were aneuploid and confidently called where 50% of cells were aneuploid.

Fragouli E, Alfarawati S, Daphnis DD, et al. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: Scientific data and technical evaluation. *Hum Reprod.* 2011;26(2):480-490.

High rates of mosaicism in blastocysts have previously been reported as a problem that can lead to misdiagnosis. This study investigated whether the use of TE cells from blastocyst-stage embryos offers an optimal strategy for PGS. The researchers analyzed 52 good-quality blastocysts using either FISH and CGH using Abbot technology (n = 32) or FISH, CGH, and aCGH using 24sure array (n = 20). The study detected mosaicism in 33% of embryos. Performance was similar between CGH and aCGH with respect to concordance and the ability to provide results. Discrepancies were seen in 10% of embryos with both CGH and aCGH, likely related to mosaicism. The authors conclude that aCGH analysis of TE cells is an appropriate tool for determining chromosomal make-up of blastocysts.

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BLASTOCOEL FLUID BIOPSY

24sure array technology has been used to investigate whether embryonic DNA exists within the blastocoel fluid (BF) and whether it can be used to perform less invasive PGS. The fluid is sometimes removed before vitrification and would not require any further handling of the embryo to obtain a sample for PGS. However, only limited evidence exists so far. More validation work is required to determine the efficacy of this method.

Key Publication Summaries

Magli MC, Pomante A, Cafueri G, et al. Preimplantation genetic testing: polar bodies, blastomeres, trophoctoderm cells, or blastocoelic fluid? *Fertil Steril*. 2016;105(3):676-683.e5. doi:10.1016/j.fertnstert.2015.11.018.

This study aimed to verify the presence of DNA in BF and determine whether chromosomal status correlated with that of PB cells, blastomeres, or TE cells using 24sure or 24sure+ arrays. The study included 51 couples and obtained informative results in 82 of 116 blastocysts. The researchers successfully recovered DNA from BF. Ploidy concordance was 94.3% between BF and PBs and blastomeres, and 97.1% between BF and TE cells. Segmental aneuploidies were also successfully identified in BF when present in either PBs or blastomeres (n = 16).

Zhang Y, Li N, Wang L, et al. Molecular analysis of DNA in Blastocoel fluid using next-generation sequencing. *J Assist Reprod Genet*. 2016;33(5):637-645. doi:10.1007/s10815-016-0667-7.

This study aimed to explore the potential of BF as an alternative source of embryonic DNA for PGS/PGD analysis. The study included 11 sample pairs with both cleavage-stage blastomere and BF biopsies, of which 3 BF samples failed amplification. The researchers analyzed BF from the remaining 8 embryos using HiSeq® 2500 sequencing. The study found that overall sequencing profiles at the chromosome and gene level were very similar to blastomere profiles; therefore, BF whole-genome amplification could be suitable for 24-chromosome screening with both aCGH and NGS.

Palini S, Galluzzi L, De Stefani S, et al. Genomic DNA in human Blastocoel fluid. *Reprod Biomed Online*. 2013;26(6):603-610. doi:10.1016/j.rbmo.2013.02.012.

This study explored the feasibility of using the DNA isolated from BF in a PGS test. The study used real-time PCR to show that genomic DNA was present in approximately 90% of BF samples harvested during the vitrification procedure and to amplify specific genomic loci successfully. In addition, the researchers achieved whole-genome amplification of the isolated DNA. They also attempted aCGH to confirm the sex of embryos and to detect several aneuploidies. The authors conclude that further studies are needed to validate this approach and to confirm that the accuracy is sufficient for diagnostic purposes.

Additional References

Tobler KJ, Zhao Y, Ross R, et al. Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis. *Fertil Steril*. 2015;104(2):418-425. doi:10.1016/j.fertnstert.2015.04.028.

CHROMOSOMAL MOSAICISM

Chromosomal mosaicism, the presence of 2 or more chromosomally different cell lines, is common in early embryos. The results of PGS with aCGH and NGS indicate that mosaicism is common throughout day 3 and day 4; however, by day 5, its incidence is reduced.^{51, 52, 53} The reasons for this effect may include preferential growth of euploid cells, allocation of euploid cells to the inner cell mass (ICM), and/or activation of the embryo genome with resultant activation of cell-cycle checkpoints and permitting apoptosis of aneuploid cells.⁵¹ Given the higher resolution of VeriSeq (PGS) technology, the detection of embryo mosaicism has increased.^{54, 55} The significance of embryo mosaicism and the associated reproductive potential of presumed mosaic blastocysts is still largely unknown.^{56, 57, 58}

Key Publication Summaries

Vera-Rodriguez M, Michel C-E, Mercader A, et al. Distribution patterns of segmental aneuploidies in human blastocysts identified by next-generation sequencing. *Fertil Steril*. 2016;105(4):1047-1055. doi:10.1016/j.fertnstert.2015.12.022.

This validation study analyzed embryos from 70 couples previously screened using 24sure array technology, using the VeriSeq PGS assay, to determine the concordance between the 2 assays. The results showed an overall concordance rate of 99.8% for all analyzed chromosomes. This study demonstrates that VeriSeq PGS is a viable technique for detecting both segmental aneuploidies (as small as 10 Mb) and whole-chromosome aneuploidies, including those with mosaic patterns.

Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. *N Engl J Med*. 2015;373(21):2089-2090. doi:10.1056/NEJMc1511634

This pilot study evaluated outcomes in women who elected transfer of a mosaic embryo when no euploid embryos were available. The study detected mosaicism in 181/3802 (4.8%) blastocysts analyzed using 24sure array. Eighteen women with no euploid embryos underwent transfer of a mosaic embryo (mosaicism ranged from 35% to 50%). Of these, 8/18 (44.4%) resulted in a clinical pregnancy and 6/18 (33.3%) resulted in a live birth. All 6 pregnancies with a live birth had a normal fetal karyotype by chorionic villus sampling (CVS). Although this is a small pilot study, the authors conclude that "transfer of mosaic embryos with purportedly viable aneuploidies should be considered with extreme caution."

Novik V, Moulton EB, Sisson ME, et al. The accuracy of chromosomal microarray testing for identification of embryonic mosaicism in human blastocysts. *Mol Cytogenet*. 2014;7(1):18. doi:10.1186/1755-8166-7-18.

This study looked at establishing the log₂ ratio threshold for mosaicism detection by mixing amplified DNA from euploid and aneuploid cell lines to mimic mosaic samples, followed by CCS using a 24sure array. After establishing these thresholds, the researchers reanalyzed 551 blastocysts from previous IVF cycles and performed follow-up FISH analysis for all abnormal embryos. The results showed that the 24sure array could detect mosaic chromosome gains consistently (25%–37% mosaicism) and losses (37%–50% mosaicism) in TE cells. These findings confirm the diagnostic accuracy of array technology for both non-mosaic and mosaic embryo aneuploidies. There remains a challenge to detect low level mosaicism in embryos.

51. Mertzaniidou A, Spits C, Nguyen HT, Van de Velde H, Sermon K. Evolution of aneuploidy up to Day 4 of human preimplantation development. *Hum Reprod*. 2013;28(6):1716-1724. doi:10.1093/humrep/det079.
52. Fragouli E, Alfarawati S, Daphnis DD, et al. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. *Hum Reprod*. 2011;26(2):480-490. doi:10.1093/humrep/deq344.
53. Northrop LE, Treff NR, Levy B, Scott RT Jr. SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. *Mol Hum Reprod*. 2010;16(8):590-600. doi:10.1093/molehr/gaq037.
54. Zheng H, Jin H, Liu L, Liu J, Wang W-H. Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos. *Mol Cytogenet*. 2015;8(1):38. doi:10.1186/s13039-015-0143-6.
55. Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod*. 2014;29(12):2802-2813. doi:10.1093/humrep/deu277.
56. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. *N Engl J Med*. 2015;373(21):2087-2089. doi:10.1056/NEJMc1511634.
57. Munné S, Grifo J, Wells D. Mosaicism: "survival of the fittest" versus "no embryo left behind." *Fertil Steril*. 2016;105(5):1146-1149. doi:10.1016/j.fertnstert.2016.01.016.
58. Scott RT Jr, Galliano D. The challenge of embryonic mosaicism in preimplantation genetic screening. *Fertil Steril*. 2016;105(5):1150-1152. doi:10.1016/j.fertnstert.2016.01.007.

Liu J, Wang W, Sun X, et al. DNA microarray reveals that high proportions of human blastocysts from women of advanced maternal age are aneuploid and mosaic. *Biol Reprod.* 2012;87(6):148. doi:10.1095/biolreprod.112.103192.

This study evaluated the types of chromosome abnormalities present in human blastocysts and whether there is consistency between TE cells and the ICM. Two array methods were used to assess both cell types: 24sure BAC array and the NimbleGen oligonucleotide array. The researchers obtained successful amplification in 244/258 blastocysts from 51 IVF cycles, 43% of which were euploid. Aneuploidy was higher in women \geq 38 years compared to the younger age group. The aneuploidy rate was 82% in women \geq 41 years of age. Thirty-seven percent of aneuploid embryos had more complex aneuploidies. TE and ICM cells were analyzed in 13 aneuploid blastocysts, revealing mosaicism in 9 (69.2%) embryos. Implantation and pregnancy rates were 62.5% and 80.2%, respectively. The authors summarize that, in women of advanced maternal age, a high proportion of blastocysts are aneuploid and mosaic.

Fragouli E, Alfarawati S, Daphnis DD, et al. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. *Hum Reprod.* 2011;26(2):480-490. doi:10.1093/humrep/deq344.

High rates of mosaicism in blastocysts have previously been reported as a problem that can lead to misdiagnosis. This study investigated whether the use of TE cells from blastocyst-stage embryos offers an optimal strategy for PGS. The researchers analyzed 52 good-quality blastocysts using either FISH and CGH using Abbot technology ($n = 32$) or FISH, CGH, and aCGH using 24sure array ($n = 20$). The study detected mosaicism in 33% of embryos. Performance was similar between CGH and aCGH with respect to concordance and the ability to provide results. Discrepancies were seen in 10% of embryos with both CGH and aCGH, likely related to mosaicism. The authors conclude that aCGH analysis of TE cells is an appropriate tool for determining chromosomal make-up of blastocysts.

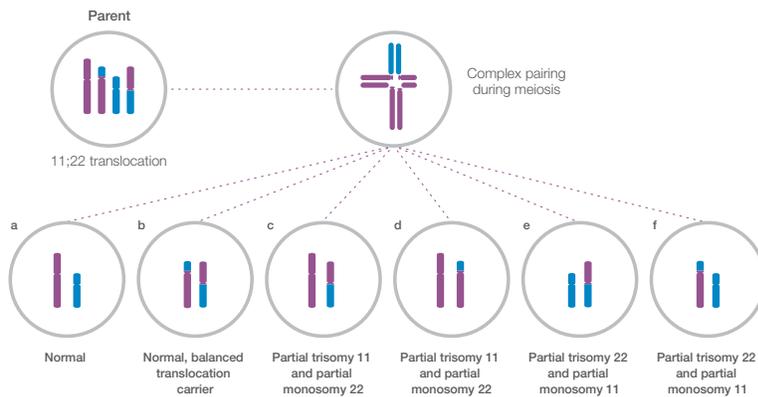
24SURE+ ARRAY: PGD FOR TRANSLOCATION CARRIERS

Reciprocal translocation involve the exchange of segments between non-homologous chromosomes.⁵⁹ Figure 2 provides an example of an 11;22 reciprocal translocation, where a portion of the long arm of chromosome 11 has exchanged segments with chromosome 22. Another type of translocation is a Robertsonian translocation. These translocations involve the fusion of 2 acrocentric chromosomes (i.e. 13, 14, 15, 21, 22). In both types of translocations, the chromosomes with exchanged material are known as “derivative” chromosomes.⁵⁹

59. Gardner RJM, Sutherland GR, Shaffer LG. Chromosome Abnormalities and Genetic Counseling. 4th ed. New York, NY: Oxford University Press; 2011

Individuals who carry a balanced translocation do not typically have any visible characteristics as there is no gain or loss of genetic material.⁵⁹ As a result, balanced translocations often go undetected in carriers until issues arise during reproduction. Balanced translocation carriers are at risk for producing sperm or egg cells with unbalanced versions of their rearrangement. Using the example of an 11;22 reciprocal translocation carrier, a sperm cell may be produced with one copy of the derivative chromosome 22 and one copy of the normal (unaffected) chromosome 11. (Figures 2c and 2d). This could result in an embryo with a partial trisomy 11 and partial monosomy 22.

Figure 2: Possible Chromosomal Segregation at Meiosis in a Carrier of a Reciprocal Translocation



Depending on the origin and size of the chromosome fragments involved in the translocation, the resulting unbalanced embryos may not be viable and would lead to infertility and/or recurrent miscarriage.⁵⁹ Alternatively, some unbalanced embryos can result in live birth, however, these babies may have varying degrees of physical abnormalities and/or intellectual disabilities.⁵⁹

Because the technology used in the 24sure assay permits high resolution, even when using single cells, it can—in some cases—detect subchromosomal imbalances as low as 5–6Mb, and it has been used successfully in PGD for reciprocal translocation

carriers.⁶⁰ However, the ability to detect small imbalances is not guaranteed. For these applications, Illumina recommends the 24sure+ array. The 24sure+ array is a BAC microarray-based platform that is similar to the 24sure array platform, but it is designed specifically to provide PGD for carriers of reciprocal translocations.

This array allows analysis at higher resolution than the 24sure assay, meaning that subchromosomal imbalances as small as approximately 2.5-2.8Mb can be detected, as well as whole-chromosome aneuploidy.^{60, 61} Higher resolution provides significantly higher sensitivity and specificity than previous PGD methods (usually FISH) for reciprocal translocation cases.⁵⁹ It also simultaneously provides aneuploidy screening to aid in the selection of embryos that are unaffected and euploid for all other chromosomes before transfer. This capability is particularly important, given recent evidence that the presence of reciprocal translocations may actually increase the incidence of aneuploidy for other chromosomes.⁶²

Key Publication Summaries

Colls P, Escudero T, Fischer J, et al. Validation of array comparative genome hybridization for diagnosis of translocations in preimplantation human embryos. *Reprod Biomed Online*. 2012;24(6):621-629.

This study validated the use of aCGH with the 24sure+ assay to assess embryos for structural chromosomal abnormalities. The authors aimed to determine the minimal resolution detectable and the error rate of detection in translocation cases, investigate mosaicism, and determine the number of historical cases in which aCGH could have improved detection. The researchers analyzed a total of 432 embryos from 47 cycles with a known translocation carrier parent (30 reciprocal and 17 Robertsonian). Fifty-four embryos (unbalanced = 44; normal = 10) analyzed by aCGH were reanalyzed using FISH for confirmation. The overall false-positive and false-negative rates were 2.3% and 0%, respectively, with an overall error rate of 1.9%. The authors recommend that aCGH is a better approach than FISH, due to the ability to simultaneously detect aneuploidy of other chromosomes not involved in the translocation.

Alfarawati S, Fragouli E, Colls P, Wells D. First births after preimplantation genetic diagnosis of structural chromosome abnormalities using comparative genomic hybridization and microarray analysis. *Hum Reprod*. 2011;26(6):1560-1574. doi:10.1093/humrep/der068.

In this study, conventional CGH and aCGH (24sure+ array) were applied to PBs, cleavage-stage embryos, and blastocysts to assess the chromosomes of embryos from 20 cycles, in 16 patients known to be carriers of reciprocal or Robertsonian translocations or inversions. The researchers obtained results for 121 out of 132 biopsy samples (91.7%). Overall, 77.7% of samples were abnormal and, in 4 cycles, no euploid embryos were available for transfer. Of 16 cycles with available embryos, 15 completed transfer with a 33% clinical pregnancy rate and live birth rate of 27% per embryo transfer.

Fiorentino F, Spizzichino L, Bono S, et al. PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. *Hum Reprod*. 2011;26(7):1925-1935. doi:10.1093/humrep/der082.

This study used the 24sure+ array to detect translocations and CCS simultaneously in day 3 embryos. The researchers then selected balanced embryos for transfer on day 5 of the same cycle. Of 200 embryos analyzed from 24 couples where 1 parent was a known translocation carrier (Robertsonian carriers = 8; reciprocal carriers = 16), sequencing was successful in 187 embryos. In cases where an unbalanced rearrangement was reported on day 3 (n = 57), reanalysis was performed on day 6 for confirmation with a concordance rate of 100%. Only 16% of embryos from 17 cycles were normal or balanced for the translocation and euploid for all other chromosomes, with a clinical pregnancy rate of 70.6% per embryo transfer.

Additional References

Handyside AH. Preimplantation genetic diagnosis after 20 years. *Reprod Biomed Online*. 2010;21(3):280-282. doi:10.1016/j.rbmo.2010.07.007.

Tobler KJ, Brezina PR, Benner AT, Du L, Xu X, Kearns WG. Two different microarray technologies for preimplantation genetic diagnosis and screening, due to reciprocal translocation imbalances, demonstrate equivalent euploidy and clinical pregnancy rates. *J Assist Reprod Genet*. 2014;31(7):843-850. doi:10.1007/s10815-014-0230-3.

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60. Colls P, Escudero T, Fischer J, et al. Validation of array comparative genome hybridization for diagnosis of translocations in preimplantation human embryos. *Reprod Biomed Online*. 2012;24(6):621-629. doi:10.1016/j.rbmo.2012.02.006.
 61. Alfarawati S, Fragouli E, Colls P, Wells D. First births after preimplantation genetic diagnosis of structural chromosome abnormalities using comparative genomic hybridization and microarray analysis. *Hum Reprod*. 2011;26(6):1560-1574. doi:10.1093/humrep/der068.
 62. Alfarawati S, Fragouli E, Colls P, Wells D. Embryos of Robertsonian translocation carriers exhibit a mitotic interchromosomal effect that enhances genetic instability during early development. *PLoS Genet*. 2012;8(10):e1003025. doi:10.1371/journal.pgen.1003025.
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KARYOMAPPING

PGD relies on the use of genetic markers within the genome to assess the likelihood of an embryo carrying a mutated version of a gene involved in a single-gene disorder. This technique has been performed traditionally through short tandem repeat (STR) analysis. Karyomapping is an alternative tool that enables PGD for single-gene disorders. This method can be used for virtually all disorders where the affected gene is known. It offers a number of advantages over traditional STR-based methodologies, such as virtually eliminating the risk of misdiagnosis due to allele dropout and the need for lengthy prior work-up for each patient.^{63, 64}

Karyomapping uses single-nucleotide polymorphism (SNP) genotyping data from the parents and a reference (close relative of a family member with the condition) to create a comprehensive map of the parental origin of chromosome segments inherited by the embryo. These chromosome segments are called haploblocks. By establishing the inheritance of haploblocks surrounding the region of interest, it is possible to infer the disease status of each embryo—affected, carrier, or unaffected—by comparing it to the disease status of a reference.

Key Publication Summaries

Giménez C, Sarasa J, Arjona C, et al. Karyomapping allows preimplantation genetic diagnosis of a de-novo deletion undetectable using conventional PGD technology. *Reprod Biomed Online*. 2015;31(6):770-775. doi:10.1016/j.rbmo.2015.08.017.

This case study demonstrated the simultaneous use of PGD with Illumina karyomapping technology and CCS using 24sure arrays in a patient with tuberous sclerosis type 2. The researchers analyzed 7 blastocysts with both methodologies and ultimately recommended 3 embryos for transfer. After 2 transfer cycles, the couple had a successful live birth. This study demonstrates that karyomapping is a valuable tool for PGD of single-gene disorders.

Thornhill AR, Handyside AH, Ottolini C, et al. Karyomapping—a comprehensive means of simultaneous monogenic and cytogenetic PGD: comparison with standard approaches in real time for Marfan syndrome. *J Assist Reprod Genet*. 2015;32(3):347-356. doi:10.1007/s10815-014-0405.

This proof of principle study evaluated the broader application of karyomapping in a couple where the father was affected with Marfan syndrome. The researchers performed direct mutation analysis on 6 embryos after targeted haplotyping with 3 STR markers. Results were successful in 7 of 8 single cells biopsied. The researchers performed SNP genotyping for all 7 single cells, followed by karyomapping analysis with high accuracy. The results demonstrate that karyomapping can be applied clinically for the autosomal dominant condition, Marfan syndrome. Additionally, unlike other methods, karyomapping does not require a preclinical work-up for STR determination.

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63. Natesan SA, Handyside AH, Thornhill AR, et al. Live birth after PGD with confirmation by a comprehensive approach (karyomapping) for simultaneous detection of monogenic and chromosomal disorders. *Reprod Biomed Online*. 2014;29(5):600-605. doi:10.1016/j.rbmo.2014.07.007.
 64. Handyside AH, Harton GL, Mariani B, 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. doi:10.1136/jmg.2009.069971.
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Natesan SA, Bladon AJ, Coskun S, et al. Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro. *Genet Med*. 2014;16(11):838-845. doi:10.1038/gim.2014.45.

This multicenter validation study compared the accuracy of family- or disease-specific targeted haplotyping. The researchers analyzed 213 embryos from 44 PGD cycles with 25 single-gene disorders, using genome-wide SNP genotyping, followed by blind, retrospective karyomapping. Karyomapping was concordant with targeted haplotyping in 208 (97.7%) samples. Consanguineous regions limiting haplotype analysis explained the 5 discordant samples. The authors conclude that karyomapping is highly accurate and can efficiently detect a wider range of genetic conditions that previously required gene customization for each family.

Natesan SA, Handyside AH, Thornhill AR, et al. Live birth after PGD with confirmation by a comprehensive approach (karyomapping) for simultaneous detection of monogenic and chromosomal disorders. *Reprod Biomed Online*. 2014;29(5):600-605. doi:10.1016/j.rbmo.2014.07.007.

This study used karyomapping technology to investigate a family at risk of Smith Lemli Opitz (SLO) syndrome, an autosomal recessive condition. The study aimed to demonstrate the feasibility of using karyomapping to test for a known monogenic disorder, while also potentially simultaneously screening for aneuploidy. aCGH for aneuploidy screening was performed on PB1 and PB2, followed by karyomapping at the cleavage-stage. Karyomapping enabled the diagnosis for SLO in a single assay and showed concordance with established STR tests. Additionally the parent of origin of the meiotic chromosome error was distinguished based on PB analysis. The authors conclude that karyomapping offers a comprehensive approach to PGD, offering screening for both single gene and chromosome disorders.

Additional References

Handyside AH, Harton GL, Mariani B, 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. doi:10.1136/jmg.2009.069971.

Handyside AH, Xu K. Preimplantation genetic diagnosis comes of age. *Semin Reprod Med*. 2012;30(4):255-258. doi:10.1055/s-0032-1313904.

Harton GL, De Rycke M, Fiorentino F, et al. ESHRE PGD consortium best practice guidelines for amplification-based PGD. *Hum Reprod*. 2011;26(1):33-40. doi:10.1093/humrep/deq231.

Preimplantation Genetic Diagnosis International Society (PGDIS). Guidelines for good practice in PGD: programme requirements and laboratory quality assurance. *Reprod Biomed Online*. 2008;16(1):134-147. doi:10.1016/S1472-6483(10)60567-6.

PRE-TEST AND POST-TEST CONSIDERATIONS

In order to ensure patients are able to make informed decisions regarding PGS and PGD, health care providers are tasked with conveying the risks, benefits, and limitations of available options to their patients. The Society for Assisted Reproductive Technology, in combination with the American Society for Reproductive Medicine (ASRM) as well as ESHRE, have published opinions on key components that should be included during the PGS and PGD counseling process.^{65, 66} Both publications discuss the importance of obtaining a detailed medical and family history, reviewing basic genetics and inheritance, and providing a risk assessment. Discussions may also include a comprehensive review of the risks, benefits, and limitations of PGS and PGD analysis, possible outcomes of both the biopsy and analysis, alternatives for reproduction (eg, donor gamete or adoption), and recommendations for follow-up prenatal and/or postnatal testing.^{65, 66, 67} In addition to these societal publications, other publications, as summarized below, have addressed counseling considerations for couples considering PGS or PGD.

Key Publication Summaries

Brezina PR, Kutteh WH, Bailey AP, Ke RW. Preimplantation genetic screening (PGS) is an excellent tool, but not perfect: A guide to counseling patients considering PGS. *Fertil Steril.* 2016;105(1):49-50. doi:10.1016/j.fertnstert.2015.09.042.

This article summarizes key components to consider as part of PGS-related counseling. The authors state that an important distinction to be made during counseling is that PGS does not improve the chances of live birth per retrieval; however, improved embryo selection can result in improved IVF efficiency. The diagnostic accuracy of PGS is not without limitations, and patients should be made aware of the possibility of misdiagnosis due to both biological and technical limitations, which can vary from laboratory to laboratory. Additional factors to address with patients include, but are not limited to, a review of test accuracy and data regarding whether embryo damage can occur during biopsy.

Gebhart MB, Hines RS, Penman A, Holland AC. How do patient perceived determinants influence the decision-making process to accept or decline preimplantation genetic screening? *Fertil Steril.* 2016;105(1):188-193. doi:10.1016/j.fertnstert.2015.09.022.

This was the first published study to identify patient-perceived determinants that contribute to the decision to accept or decline PGS. Cost was an overall important determinant of whether to have PGS. Support from society, family, and friends was also a significant determinant. Religious and ethical influences—as well as concerns regarding cryopreservation, delayed embryo transfer, and disposition of abnormal embryos—were not identified as statistically significant determinants but were used as part of the decision-making process. Past reproductive experiences should be taken into account during the decision-making process. The authors conclude that the decision-making process should be collaborative between the provider and the patient and that the development of decision-aids will benefit patients as they work through their options.

Hershberger PE, Gallo AM, Kavanaugh K, et al. The decision-making process of genetically at-risk couples considering preimplantation genetic diagnosis: initial findings from a grounded theory study. *Soc Sci Med.* 2012;74(10):1536-1543. doi: 10.1016/j.socscimed.2012.02.003.

This study describes the first research-based ethical model for the decision-making process of couples who had considered PGD use in the previous 3 months, or who were actively considering PGD due to a known genetic risk. The researchers conducted interviews with 22 couples by phone or email. Couples worked through 4 active stages of decision-making: i) identify (risk awareness, knowledge-seeking); ii) contemplate (explore, consider, deliberate all reproductive options); iii) resolve (intended decision is reached with emergence into acceptance, declination, or oscillation); and iv) engage (behaviors initiated to carry out a decision to accept or decline PGD). The decision to utilize PGD is a dynamic process, which unfolds over time in a series of choices. The authors summarize that clinicians can use these findings to better understand the different decision-making stages and tailor patient discussions accordingly.

Additional References

Beukers F, Houtzager BA, Paap MC, et al. Parental psychological distress and anxiety after a successful IVF/ICSI procedure with and without preimplantation genetic screening: follow-up of a randomised controlled trial. *Early Hum Dev.* 2012;88(9):725-730. doi:10.1016/j.earlhumdev.2012.03.001.

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65. Practice Committee of the Society for Assisted Reproductive Technology; Practice Committee of the American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril.* 2008;90(5 Suppl.):136-143. doi:10.1016/j.fertnstert.2008.08.062.
 66. Harton G, Braude P, Lashwood A, et al. ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening. *Hum Reprod.* 2011;26(1):14-24. doi:10.1093/humrep/deq229.
 67. Brezina PR, Kutteh WH, Bailey AP, Ke RW. Preimplantation genetic screening (PGS) is an excellent tool, but not perfect: A guide to counseling patients considering PGS. *Fertil Steril.* 2016;105(1):49-50. doi:10.1016/j.fertnstert.2015.09.042.
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RELATED SOCIETAL STATEMENTS

To date, several reproductive societies have published position statements, opinions, or guidelines pertaining to PGS and PGD. This list does not include all relevant societal guidelines and policy statements.

Practice Committee of the American Society for Reproductive Medicine (ASRM) and Practice Committee of the Society for Reproductive Technology (SART)

Practice Committee of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion. *Fertil Steril*. 2013;99(3):667-672. doi:10.1016/j.fertnstert.2013.01.087.

Practice Committee of the Society for Assisted Reproductive Technology and Practice Committee of the American Society for Reproductive Medicine. Elective single-embryo transfer. *Fertil Steril*. 2012;97(4):835-842. doi:10.1016/j.fertnstert.2011.11.050.

Practice Committee of the American Society for Reproductive Medicine. Multiple pregnancy associated with infertility therapy. *Fertil Steril*. 2006;86(5):S106-S110. doi:10.1016/j.fertnstert.2006.08.073

Practice Committee of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Preimplantation genetic testing: a committee opinion. *Fertil Steril*. 2008;90(5):S136-S143. doi:10.1016/j.fertnstert.2008.08.062.

European Society of Human Reproduction and Embryology (ESHRE)

De Los Santos MJ, Apter S, et al. Revised ESHRE Guideline Group on Good Practice in IVF Labs, guidelines for good practice in IVF laboratories. *Hum Reprod*. 2016;31(4):685-686. doi:10.1093/humrep/dew016.

Harton G, Braude P, Lashwood A, et al. ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening. *Hum Reprod*. 2011;26(1):14-24. doi:10.1093/humrep/deq229.

Harton GL, De Rycke M, Fiorentino F, et al. ESHRE PGD consortium best practice guidelines for amplification-based PGD. *Hum Reprod*. 2011;26(1):33-40. doi:10.1093/humrep/deq231.

Harton GL, Harper JC, Coonen E, et al. ESHRE PGD consortium best practice guidelines for fluorescence *in situ* hybridization-based PGD. *Hum Reprod*. 2011;26(1):25-32. doi:10.1093/humrep/deq230.

Harton GL, Magli MC, Lundin K, Montag M, Lemmen J, Harper JC. ESHRE PGD Consortium/Embryology Special Interest Group—best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). *Hum Reprod*. 2011;26(1):41-46. doi:10.1093/humrep/deq265.

Harper J, Coonen E, De Rycke M, et al. What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium Steering Committee. *Hum Reprod*. 2010;25(4):821-823. doi:10.1093/humrep/dep476.

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