J Assist Reprod Genet (2016) 33:1129–1134
DOI 10.1007/s10815-016-0750-0

REVIEW

Recent advances in preimplantation genetic diagnosis and screening

Lina Lu[1,2] · **Bo Lv**[1] · **Kevin Huang**[3] · **Zhigang Xue**[1] · **Xianmin Zhu**[2] · **Guoping Fan**[2,3]

Received: 29 November 2015 / Accepted: 25 May 2016 / Published online: 7 June 2016

Abstract Preimplantation genetic diagnosis/screening (PGD/PGS) aims to help couples lower the risks of transmitting genetic defects to their offspring, implantation failure, and/or miscarriage during in vitro fertilization (IVF) cycles. However, it is still being debated with regard to the practicality and diagnostic accuracy of PGD/PGS due to the concern of invasive biopsy and the potential mosaicism of embryos. Recently, several non-invasive and high-throughput assays have been developed to help overcome the challenges encountered in the conventional invasive biopsy and low-throughput analysis in PGD/PGS. In this mini-review, we will summarize the recent progresses of these new methods for PGD/PGS and discuss their potential applications in IVF clinics.

Capsule PGD/PGS has become a routine clinical procedure in many IVF clinics worldwide. New techniques have been quickly adopted as embryo selection strategies in hopes of improving live birth outcomes in human ARTs.

Lina Lu and Bo Lv contributed equally to this work.

✉ Guoping Fan
eicjarg@gmail.com

[1] Translational Center for Stem Cell Research, Tongji Hospital, Department of Regenerative Medicine, Tongji University School of Medicine, Shanghai 200065, China

[2] School of Life Sciences and Technology, Advanced Institute of Translational Medicine, Tongji University, 1239 Siping Road, Shanghai 200092, China

[3] Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA

Keywords Preimplantation genetic diagnosis (PGD) · Preimplantation genetic screening (PGS) · In vitro fertilization (IVF) · Embryo biopsy · Non-invasive testing

Introduction

Preimplantation genetic diagnosis (PGD) is a procedure in infertility clinics whereby cells from oocytes or in vitro fertilized (IVF) embryos are examined for molecular anomalies such as chromosomal abnormalities, mutations in the genomic DNAe, and human leucocyte antigen (HLA) matching [1]. The first PGD/PGS detection of X-chromosome linked diseases was by Handyside et al. in 1990 [2, 3]. Preimplantation genetic screening (PGS) utilizes the methods in PGD to screen IVF embryos for their potential success in uterine implantation to achieve a high pregnancy rate. PGD/PGS are particularly relevant in cases of male infertility, advanced maternal age, and recurrent miscarriage [4]. In addition, PGD/PGS reduces the risk of conceiving a child with genetic disorders and thus lowering the rates of elective pregnancy termination [5, 6]. However, conventional PGD/PGS procedures require the invasive removal of the cells from preimplantation embryos, which may intervene with embryonic development. In this review, we summarize recent technologies in PGD/PGS and highlight the new non-invasive biopsy technologies including time-lapse technology, blastocoele fluid (BF) sampling, and cell-free nucleic-acid collection, as well as new breakthroughs in diagnosis methods. We also discuss the recent and potential clinical application of these new technologies in IVF clinic.

Recent perspectives on sampling approaches

In PGD/PGS, there are three major biopsy methods: blastocyst biopsy, blastomere biopsy, and polar body (PB) biopsy. Blastocyst biopsy has been more widely used than PB biopsy and blastomere biopsy, especially in the past 5 years, due to its low misdiagnosis rate [7, 8] and cost-effectiveness [9, 10]. Compared to the conventional biopsy methods, the newly developed non-invasive sampling mehtods have many advantages with regard to the ethical, legal, and economic issues.

BF sampling

BF is the liquid substance within the blastocyst cavity sealed in by the TE epithelium. Palini et al. showed, for the first time, the presence of genomic DNA in the BF by whole-genome amplification (WGA), quantitative PCR (qPCR), and analyzed multicopy genes such as TSPY1 and TBC1D3. This implies the possibility of using this method in sex-linked disorders screening. [11, 12]. Gianaroli et al. used aCGH to evaluate BF biopsies for ploidy prediction in comparison with the conventional biopsy methods, i.e., blastocyst, blastomere, and PB biopsy, and concluded that BF biopsy was comparable to conventional biopsy materials for chromosomal analysis [13]. Nevertheless, discordance of ploidy prediction was observed between BF and blastocyst biopsy possibly due to the embryo quality [14], suggesting that more experiments and clinical trials should be performed before BF sampling is ready for PGD/PGS.

Non-invasive technology—cell-free nucleic-acid collection in culture medium and time-lapse technology

Inspired by BF sampling, Assou et al. collected cell-free nucleic acids released from embryos in the culture medium and successfully determined the embryo sex by PCR [15]. Similarly, DNA isolated from media culturing α-thalassemias^{-SEA} carrier embryos had significantly higher diagnosis efficiency compared to blastomere biopsy [16]. However, this method still needs to be further validated by eliminating potential contamination and providing more robust evidences in PGD/PGS.

Another non-invasive technology, time-lapse imaging, was developed to select the best embryos for single-embryo transfer (SET) by correlating cellular morphology and morphokinetic parameters [17, 18]. Combining time-lapse imaging with comparative genomic hybridization (CGH), Chawla et al. found that some significant morphokinetic parameters can be used as markers to predict aneuploidies [19]. Meanwhile, different assessment assays and statistical models were applied to refine the morphokinetic variables and increase the chance of predicting a top-quality blastocyst [20]. However, considering the potential technical and statistical error in time-lapse imaging, one should be cautious in over-interpreting the results [21–24].

Advanced techniques of genetic analysis in PGD/PGS

After biopsy, PGD/PGS can be performed at either DNA or chromosome level. Polymerase chain reaction (PCR) is the first technique in PGD and had been developed to detect many genetic abnormalities such as single-gene mutations [3, 25–27], chromosomal imbalances [28], and mitochondrial mutations [29]. Likewise, fluorescence in-situ hybridization (FISH) had been performed to screen aneuploidy and chromosomal translocation for many years [30–36]. However, these two methods become obsolete due to their limitations, e.g., incapability of detecting de-novo genetic mutations, contamination, and sensitivity issues that lead to the false positive or negative. New diagnosis methods, such as array-comparative genomic hybridization (aCGH), single-nucleotide polymorphism (SNP) microarray, multiplex quantitative PCR (qPCR), karyomapping, and next generation screening (NGS) are developed to improve clinical efficiency and outcomes [37–39]. We will elaborate these new methods in detail below.

Multiplex qPCR

Recently, PCR has been adapted for chromosome copy number analysis [40]. This technique requires a high-order multiplex reaction after pre-amplification is conducted to amplify at least two sequences on each arm of each chromosome for rapid quantification and comparison of each product across the genome within 4–6 h [41, 42]. This qPCR technology has been investigated in PGS and shown improvement in implantation and live birth rates in IVF cycles [41]. It is reliable in determining aneuploidy, but not ideal for detecting structural chromosomal aberrations or uniparental disomy [43].

Microarray-based methods

Microarray-based CGH has higher resolution, throughput, and speed than conventional CGH [44, 45] and has been successfully adopted in the field of PGD/PGS [46]. With the differential labelled DNA hybridized onto the microarray which contains BAC DNA probes or long oligonucleotides [47], aCGH can assess chromosomal abnormalities such as copy number and unbalanced translocations [48]. It is compatible with sampling methods, i.e., blastocyst biopsy [49–53], blastomere biopsy [37, 46, 48, 52–54], and PB biopsy [54, 55], as well as BF sampling [56]. Most importantly, two independent clinical studies were conducted almost at the same time and both achieved healthy offsprings after PGD by aCGH [48, 57].

SNP array was initially designed for genome-wide association studies (GWAS) before its first application in PGD/PGS in 2011 [58]. With millions of probes covering the whole genome, the SNP array has relatively high resolution in detecting single-gene disorders, balanced or imbalanced translocation [59–61], as well as aneuploidy including triploidy and uniparental disomy [61]. Li et al. utilized a dense SNP microarray that detected approximately 300,000 genetic markers which made them capable of identifying all parental translocation imbalances in embryos [61]. These two array platform-based methods could strongly increase the reliability and stability in PGS, but they still need to be optimized for their future applications.

To expand and improve the diagnosis spectrum of the well-established aCGH and SNP array methods, Handyside et al. first proposed the term "karyomap," which, in contrast to "karyotype," identifies the offspring's SNP genotypes from four possible inherited haplotypes in all the chromosomes and thus reveals potential chromosomal abnormalities, CNVs, and single-gene mutations [62]. In the study of two families carrying cystic fibrosis transmembrane receptor (*CFTR*) mutations, Handyside et al. reported that karyomapping has the power to determine CFTR mutation and even distinguish monosomy and uniparental disomy [62]. Karyomapping is compatible with many different biopsy methods such as blastocyst biopsy [62, 63] and blastomere biopsy [62, 64, 65]. It was successfully adapted to a 24-h clinical timeframe in PGD of Marfan syndrome [64] and was performed to screen embryos of the parent with a de-novo mutation related to tuberous sclerosis [66]. The technical improvement resulted in the success of live births in several reports via aCGH, SNP array, and DNA fingerprinting [63–66].

Next generation sequencing (NGS)

NGS is based on ultra-high throughput parallel DNA sequencing that achieves genome-scale sequencing within days, or even within 24 h. It can detect genetic mutations at single-nucleotide level, with the capacity of detecting aneuploidy such as triploidy and uniparental disomy. Many studies also showed that NGS has become an efficient and robust technology for PGD/PGS [67–69]. For example, one study reported 24 healthy births after NGS testing [70] and another one resulting in a healthy birth to the parents carrying Robertsonian translocation after NGS-based PGD [71]. It has been tested following different biopsy methods in PGD/PGS, e.g., blastocyst biopsy [70, 72], blastomere biopsy [55, 69, 73], and PB biopsy [74]. We expect that it will soon be combined with the new non-invasive sampling methods in the near future. With advent of the third generation sequencing that generates longer, and more accurate single reads in even faster mode, NGS will bring new excitement and revolutionize the clinical application of PGD/PGS.

The application of single-cell genomics in PGD/PGS

Recently, we have witnessed fast development of single-cell genomics which offers higher resolution for PGD/PGS with limited sample size. Single-cell genomics consists of two major steps: whole-genome amplification (WGA) and high-throughput analysis. WGA aims to amplify DNA from a single cell and generate sufficient template for either microarray or NGS assays. Many WGA methods were developed based on the principles of PCR, including primer extension PCR (PEP) [75], degenerate oligonucleotide primed PCR (DOP-PCR) [76] and multiple displacement amplification (MDA) which employs ϕ29 DNA polymerase instead of *Taq* DNA polymerase to increase enrichment of genes and reduce PCR bias [77]. However, these WGA methods were limited by the technical obstacle of non-linear amplification. To solve this issue, a new approach called multiple annealing and looping-based amplification cycles (MALBAC) was recently introduced. This approach achieves quasi-linear amplification by initiating the reaction with random primers evenly binding to the template [78]. Thus, MALBAC showed a significant higher coverage of genome than that of the prevailing MDA [78]. Furthermore, the same group successfully examined the crossovers and aneuploidy in single sperm cells by MALBAC [79]. Similarly, Hou et al. also reported MALBAC-based sequencing could simultaneously detect maternal aneuploidy and monogenic disorders of oocytes and polar bodies with higher consistency and resolution than MDA-based aCGH [77]. Though it is not totally free of amplification bias [80], MALBAC-based sequencing is widely used in single-cell genomics tailored for PGD/PGS [81, 82] and shown to be highly sensitive, specific, and reproducible [77–79, 83].

Single-cell genomics has been applied to comprehensively study the genome and transcriptome of individual cells to select an optimal embryo in IVF. Since Tang et al.'s seminal work [84], single-cell RNA sequencing (RNA-seq) has been developed, by many groups including our labs [82, 84–87], to study the transcriptional regulation of IVF embryos at the single-cell level. Combined with exome sequencing of parental genotype, we showed that single-cell RNA-seq was capable of uncovering monoallelic expression patterns and screening for single-nucleotide variants (SNVs), which would be useful for future PGS/PGD [87]. More recently, Dey et al. reported the method of conducting genome DNA and mRNA sequencing simultaneously in single cells and discovered the transcriptional variation related to copy number variations (CNVs) [88]. The so-called gDNA-mRNA sequencing (DR-seq) showed consistent results with genomic DNA sequencing by MALBAC-seq and RNA-seq via Cell Expression by Linear amplification and sequencing (CEL-seq). Meanwhile, Macaulay et al. developed another new approach called genome and transcriptome sequencing (G&T-seq) which can detect both genetic alteration and subsequent

perturbation in transcriptional regulation [89]. Together, these new techniques offer promising tools for detecting the genetic variants and elucidating the regulatory mechanisms in preimplantation embryos, therefore, improving the quality of PGD/PGS. The successful use of NGS-based PGD/PGS in IVF clinic is reported in 2014 in China after MALBAC-based NGS PGD/PGS [90]. Yan et al. reported that an NGS-based PGD/PGS procedure simultaneously detected a single-gene disorder and aneuploidy by low coverage whole-genome sequencing for euploidy validation and targeted single allele deep-sequencing of amplicons [91].

Summary

Over the years, PGD/PGS has been increasingly performed in IVF clinic, which helps thousands of patients give healthy births. With the fast development in biotechnologies, new methods will reduce adverse impact on embryo development and increase the accuracy and efficiency of PGD/PGS. As discussed, blastocyst biopsy method is significantly improved and considered as the best choice among the three conventional biopsy methods, i.e., blastocyst, blastomere, and PB biopsy. Meanwhile, several new non-invasive sampling methods were introduced into the field, including time-lapse imaging technology, BF sampling, and cell-free nucleic-acid collection. However, these methods inevitably have limitations despite their advantages and would require further examination and validation for their clinical use. On the other hand, state-of-the-art technologies including aCGH, SNP array, and NGS analyzing genetic materials have revolutionized PGS/PGD in the post-genomic era. Because of the quick emergence of new technologies, there are still very limited randomized controlled trials (RCTs) to evaluate the clinical efficacy such as implantation and pregnancy rates [4]. So the level of evidence should be further thoroughly assessed before the application of these new technologies. Nevertheless, we anticipate that the combination of non-invasive sampling and powerful genomic analysis will bring PGD/PGS to higher levels in the near future.

References

1. Harper JC. Preimplantation genetic diagnosis. 2nd ed. Cambridge: Cambridge University Press. viii; 2009. p. 294 p.
2. Simpson JL. Preimplantation genetic diagnosis at 20 years. Prenat Diagn. 2010;30(7):682–95.
3. Handyside AH et al. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature. 1990;344(6268):768–70.
4. Dahdouh EM, Balayla J, Garcia-Velasco JA. Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials. Reprod Biomed Online. 2015;30(3):281–9.
5. Harton GL et al. ESHRE PGD consortium best practice guidelines for amplification-based PGD. Hum Reprod. 2011;26(1):33–40.
6. Brezina PR, Kutteh WH. Clinical applications of preimplantation genetic testing. BMJ. 2015;350:g7611.
7. Munne S et al. Chromosome mosaicism in human embryos. Biol Reprod. 1994;51(3):373–9.
8. Novik V et al. The accuracy of chromosomal microarray testing for identification of embryonic mosaicism in human blastocysts. Mol Cytogenet. 2014;7(1):18.
9. Schoolcraft WB et al. Clinical application of comprehensive chromosomal screening at the blastocyst stage. Fertil Steril. 2010;94(5):1700–6.
10. Greco E et al. Successful implantation and live birth of a healthy boy after triple biopsy and double vitrification of oocyte-embryo-blastocyst. Springerplus. 2015;4:22.
11. Palini S et al. Genomic DNA in human blastocoele fluid. Reprod Biomed Online. 2013;26(6):603–10.
12. Cohen J, Grudzinskas G, Johnson MH. Embryonic DNA sampling without biopsy: the beginnings of non-invasive PGD? Reprod Biomed Online. 2013;26(6):520–1.
13. Gianaroli L et al. Blastocentesis: a source of DNA for preimplantation genetic testing. Results from a pilot study. Fertil Steril. 2014;102(6):1692–9. **e6**.
14. Tobler KJ et al. The potential use of blastocoel fluid (BF) from expanded blastocysts as a less invasive form of embryo biopsy for preimplantation genetic testing. Fertil Steril. 2014;102(3):e183–4.
15. Assou S et al. Non-invasive pre-implantation genetic diagnosis of X-linked disorders. Med Hypotheses. 2014;83(4):506–8.
16. Wu H et al. Medium-based noninvasive preimplantation genetic diagnosis for human alpha-thalassemias-SEA. Medicine (Baltimore). 2015;94(12):e669.
17. Lemmen JG, Agerholm I, Ziebe S. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. Reprod Biomed Online. 2008;17(3):385–91.
18. Meseguer M et al. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2011;26(10):2658–71.
19. Chawla M et al. Morphokinetic analysis of cleavage stage embryos and its relationship to aneuploidy in a retrospective time-lapse imaging study. J Assist Reprod Genet. 2015;32(1):69–75.
20. Storr A et al. Morphokinetic parameters using time-lapse technology and day 5 embryo quality: a prospective cohort study. J Assist Reprod Genet. 2015;32(7):1151–60.
21. Swain JE. Could time-lapse embryo imaging reduce the need for biopsy and PGS? J Assist Reprod Genet. 2013;30(8):1081–90.
22. Friedman BE et al. Non-invasive imaging for the detection of human embryonic aneuploidy at the blastocyst stage. Fertil Steril. 2012;98(3, Supplement):S38.
23. Ottolini C, Rienzi L, Capalbo A. A cautionary note against embryo aneuploidy risk assessment using time-lapse imaging. Reprod Biomed Online. 2014;28(3):273–5.
24. Rienzi L et al. No evidence of association between blastocyst aneuploidy and morphokinetic assessment in a selected population of poor-prognosis patients: a longitudinal cohort study. Reprod Biomed Online. 2015;30(1):57–66.
25. Dreesen J et al. Evaluation of PCR-based preimplantation genetic diagnosis applied to monogenic diseases: a collaborative ESHRE PGD consortium study. Eur J Hum Genet. 2014;22(8):1012–8.
26. Muggleton-Harris AL et al. Genetic diagnosis using polymerase chain reaction and fluorescent in-situ hybridization analysis of biopsied cells from both the cleavage and blastocyst stages of individual cultured human preimplantation embryos. Hum Reprod. 1995;10(1):183–92.

27. Findlay I et al. Fluorescent PCR: a new technique for PGD of sex and single-gene defects. J Assist Reprod Genet. 1996;13(2):96–103.
28. Fiorentino F et al. Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal translocations. Fertil Steril. 2010;94(6):2001–U117.
29. Sallevelt SCEH et al. Preimplantation genetic diagnosis in mitochondrial DNA disorders: challenge and success. J Med Genet. 2013;50(2):125–32.
30. Griffin DK et al. Fluorescent in-situ hybridization to interphase nuclei of human preimplantation embryos with X and Y chromosome specific probes. Hum Reprod. 1991;6(1):101–5.
31. Munne S et al. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod. 1993;8(12):2185–91.
32. Grifo JA et al. Pregnancy after embryo biopsy and coamplification of DNA from X and Y chromosomes. JAMA. 1992;268(6):727–9.
33. Grifo JA et al. Preimplantation genetic diagnosis. In situ hybridization as a tool for analysis. Arch Pathol Lab Med. 1992;116(4):393–7.
34. Munne S et al. Spontaneous abortions are reduced after preconception diagnosis of translocations. J Assist Reprod Genet. 1998;15(5):290–6.
35. Munne S et al. First pregnancies after preconception diagnosis of translocations of maternal origin. Fertil Steril. 1998;69(4):675–81.
36. Conn CM et al. Infertile couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. Hum Genet. 1998;102(1):117–23.
37. Keltz MD 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):1333–9.
38. Treff NR et al. Single-cell whole-genome amplification technique impacts the accuracy of SNP microarray-based genotyping and copy number analyses. Mol Hum Reprod. 2011;17(6):335–43.
39. Forman EJ et al. Comprehensive chromosome screening alters traditional morphology-based embryo selection: a prospective study of 100 consecutive cycles of planned fresh euploid blastocyst transfer. Fertil Steril. 2013;100(3):718–24.
40. Treff NR, Scott Jr RT. Four-hour quantitative real-time polymerase chain reaction-based comprehensive chromosome screening and accumulating evidence of accuracy, safety, predictive value, and clinical efficacy. Fertil Steril. 2013;99(4):1049–53.
41. Scott Jr RT et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril. 2013;100(3):697–703.
42. Treff NR et al. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. Fertil Steril. 2012;97(4):819–24.
43. Brezina PR, Kearns WG. The evolving role of genetics in reproductive medicine. Obstet Gynecol Clin North Am. 2014;41(1):41–55.
44. Solinas-Toldo S et al. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer. 1997;20(4):399–407.
45. Pinkel D et al. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet. 1998;20(2):207–11.
46. Hellani A et al. Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening. Reprod Biomed Online. 2008;17(6):841–7.
47. Ramos L et al. Oligonucleotide arrays vs. metaphase-comparative genomic hybridisation and BAC arrays for single-cell analysis: first applications to preimplantation genetic diagnosis for Robertsonian translocation carriers. PLoS One. 2014;9(11):e113223.
48. Fiorentino F et al. PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. Hum Reprod. 2011;26(7):1925–35.
49. Capalbo A et al. 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):2298–307.
50. Nayot D et al. Live birth following serial vitrification of embryos and PGD for fragile X syndrome in a patient with the premutation and decreased ovarian reserve. J Assist Reprod Genet. 2013;30(11):1439–44.
51. Fragouli E 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):480–90.
52. Tobler KJ 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):418–25.
53. Fragouli E et al. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. Mol Hum Reprod. 2014;20(2):117–26.
54. Capalbo A 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):509–18.
55. Fishel S et al. Live birth after polar body array comparative genomic hybridization prediction of embryo ploidy-the future of IVF? Fertil Steril. 2010;93(3):1006 e7–1006 e10.
56. Tobler KJ 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):418–25.
57. Alfarawati S et al. First births after preimplantation genetic diagnosis of structural chromosome abnormalities using comparative genomic hybridization and microarray analysis. Hum Reprod. 2011;26(6):1560–74.
58. Treff, N.R., et al., *Single nucleotide polymorphism microarray-based concurrent screening of 24-chromosome aneuploidy and unbalanced translocations in preimplantation human embryos.* Fertil Steril, 2011. **95**(5): p. 1606–12 e1-2.
59. Brezina, P.R., et al., *Single-gene testing combined with single nucleotide polymorphism microarray preimplantation genetic diagnosis for aneuploidy: a novel approach in optimizing pregnancy outcome.* Fertil Steril, 2011. **95**(5): p. 1786 e5-8.
60. Tobler KJ et al. 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):843–50.
61. Treff NR et al. Use of single nucleotide polymorphism microarrays to distinguish between balanced and normal chromosomes in embryos from a translocation carrier. Fertil Steril. 2011;96(1):e58–65.
62. Li G et al. Increased IVF pregnancy rates after microarray preimplantation genetic diagnosis due to parental translocations. Syst Biol Reprod Med. 2014;60(2):119–24.
63. Handyside AH 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):651–8.
64. Konstantinidis M et al. Live births following karyomapping of human blastocysts: experience from clinical application of the method. Reprod Biomed Online. 2015;31(3):394–403.

65. Thornhill AR 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):347–56.
66. Natesan SA 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):600–5.
67. Gimenez, C., et al., *Karyomapping allows preimplantation genetic diagnosis of a de-novo deletion undetectable using conventional PGD technology.* Reprod Biomed Online, 2015.
68. Zheng H et al. Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos. Mol Cytogenet. 2015;8:38.
69. Fiorentino F 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):2802–13.
70. Fiorentino F et al. Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril. 2014;101(5):1375–82.
71. Tan Y et al. Clinical outcome of preimplantation genetic diagnosis and screening using next generation sequencing. Gigascience. 2014;3(1):30.
72. Lukaszuk K et al. Healthy baby born to a Robertsonian translocation carrier following next-generation sequencing-based preimplantation genetic diagnosis: a case report. AJP Rep. 2015;5(2):e172–5.
73. Yin X et al. Massively parallel sequencing for chromosomal abnormality testing in trophectoderm cells of human blastocysts. Biol Reprod. 2013;88(3):69.
74. Lukaszuk K et al. Routine use of next-generation sequencing for preimplantation genetic diagnosis of blastomeres obtained from embryos on day 3 in fresh in vitro fertilization cycles. Fertil Steril. 2015;103(4):1031–6.
75. Hou Y et al. Genome analyses of single human oocytes. Cell. 2013;155(7):1492–506.
76. Zhang L et al. Whole genome amplification from a single cell: implications for genetic analysis. Proc Natl Acad Sci U S A. 1992;89(13):5847–51.
77. Telenius HK et al. Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics. 1992;13(3):718–25.
78. Dean FB et al. Comprehensive human genome amplification using multiple displacement amplification. Proc Natl Acad Sci U S A. 2002;99(8):5261–6.
79. Zong C et al. Genome-wide detection of single-nucleotide and copy-number variations of a single human cell. Science. 2012;338(6114):1622–6.
80. Lu SJ et al. Probing meiotic recombination and aneuploidy of single sperm cells by whole-genome sequencing. Science. 2012;338(6114):1627–30.
81. Huang L et al. Single-cell whole-genome amplification and sequencing: methodology and applications. Annu Rev Genomics Hum Genet. 2015;16:79–102.
82. Huang J et al. Validation of multiple annealing and looping-based amplification cycle sequencing for 24-chromosome aneuploidy screening of cleavage-stage embryos. Fertil Steril. 2014;102(6):1685–91.
83. Shi J et al. Dynamic transcriptional symmetry-breaking in preimplantation mammalian embryo development revealed by single-cell RNA-seq. Development. 2015;142(20):3468–77.
84. Zhang, C.-Z., et al., *Calibrating genomic and allelic coverage bias in single-cell sequencing.* Nat Commun, 2015. **6**.
85. Tang F et al. mRNA-Seq whole-transcriptome analysis of a single cell. Nat Methods. 2009;6(5):377–82.
86. Yan L et al. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol. 2013;20(9):1131–9.
87. Blakeley P et al. Defining the three cell lineages of the human blastocyst by single-cell RNA-seq. Development. 2015;142(18):3151–65.
88. Xue Z et al. Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. Nature. 2013;500(7464):593–7.
89. Dey SS et al. Integrated genome and transcriptome sequencing of the same cell. Nat Biotechnol. 2015;33(3):285–9.
90. Macaulay IC et al. G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. Nat Methods. 2015;12(6):519–22.
91. Yan L et al. Live births after simultaneous avoidance of monogenic diseases and chromosome abnormality by next-generation sequencing with linkage analyses. Proc Natl Acad Sci U S A. 2015;112(52):15964–9.