Reprod Med Biol (2016) 15:69–75
DOI 10.1007/s12522-015-0224-6

REVIEW ARTICLE

Preimplantation genetic diagnosis: an update on current technologies and ethical considerations

Kou Sueoka[1]

Received: 7 August 2015 / Accepted: 15 September 2015 / Published online: 14 November 2015

Abstract The aim of reproductive medicine is to support the birth of healthy children. Advances in assisted reproductive technologies and genetic analysis have led to the introduction of preimplantation genetic diagnosis (PGD) for embryos. Indications for PGD have been a major topic in the fields of ethics and law. Concerns vary by nation, religion, population, and segment, and the continued rapid development of new technologies. In contrast to the ethical augment, technology has been developing at an excessively rapid speed. The most significant recent technological development provides the ability to perform whole genome amplification and sequencing of single embryonic cells by microarray or next-generation sequencing methods. As new affordable technologies are introduced, patients are presented with a growing variety of PGD options. Simultaneously, the ethical guidelines for the indications for testing and handling of genetic information must also rapidly correspond to the changes.

Keywords Microarray · Polymerase chain reaction (PCR) · Preimplantation genetic diagnosis (PGD) · Preimplantation genetic screening (PGS) · Whole genome amplification

Introduction

Preimplantation genetic diagnosis (PGD) has been in use for more than two decades since its introduction by Handyside et al. in 1990 [1]. Since then, the indications and methods of diagnosis and biopsy have been a subject of discussion for ethicists [2]. The original aim of PGD was to prevent the inheritance of severe genetic diseases from carriers to their offspring. However, the application of PGD is expanding to include so-called preimplantation genetic screening (PGS) [3–7] and the concept of 'designer babies' [8]. PGS is mainly performed for the diagnosis of frequently occurring chromosomal aneuploidies [7, 9]. The concept of 'designer babies' developed for those seeking a donor with acceptable major compatibility for cord blood transplantation [8].

Genetic diagnostics have developed to the point where comprehensive genetic analysis is available. In addition to the advances in diagnostic technologies, the development of whole genome amplification (WGA) from single-cell DNA has provided a tremendous advantage for applications of PGD, which was traditionally performed by fluorescence in situ hybridization (FISH) [9, 10]. WGA, in turn, has enabled the use of microarray technology for PGD and PGS.

While the benefits of PGD for genetic carriers is well accepted, the use of PGS remains a controversial subject with regard to indications, diagnostic methods, biopsy stage, and efficiency of the treatment [2]. Ethical and legal auguments and strictures also vary by nation. Although PGD helps ensure that parents have a healthy conception and newborn, the moral justification for a given indication must be determined.

Embryo biopsy

Preimplantation genetic diagnosis procedures include assisted reproductive technology and genetic diagnosis. A basic embryo biopsy is performed at the eight-cell stage of

✉ Kou Sueoka

[1] Department of Obstetrics and Gynecology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

totipotent embryos on day 3 [11]. Typically, one or two blastomeres of a day 3 embryo are biopsied [12]. Although a two-blastomere biopsy provides better diagnostic accuracy than that provided by a single-blastomere biopsy, the risk of harm to the embryo development and implantation may increase with the collection of two cells [13].

On the other hand, one study reported no significant difference in accuracy between one- and two-blastomere biopsy specimens [14].

Blastocyst-stage biopsy is an alternative method [15, 16]. In this method, 5–10 trophectoderm cells are biopsied on day 5, after herniation from the zona pellucida. The benefits of blastocyst biopsy include the ability to collect more cells, improved amplification efficiency, and reduced misdiagnosis and cost.

Amplification from a single cell always carries the risk of amplification failure, contamination, and allele drop out (ADO), and many studies have sought to improve the diagnostic efficacy of these techniques [17–19]. However, greater sample sizes (more cells) are generally associated with more reliable and accurate results. In addition, it has been reported that the aneuploidy rate is significantly lower in blastocysts than in earlier-stage embryos [20].

Zona opening for blastocyst biopsy used to be performed on day 3 and the herniated trophectoderm used to be collected on day 5. However, the inner cell mass is sometimes herniated from the position of opened zona. To avoid biopsy of the inner cell mass, another option is day 5 zona opening away from inner cell mass and collecting herniated trophectoderm.

Polar body biopsy is another option for PGD [21]. The polar body itself does not contribute to embryo development, which is the reason why polar body biopsy is thought less invasive than embryonic cell biopsy. However, the first and second polar bodies are sometimes difficult to distinguish and technically difficult to collect. The primary limitation of polar body biopsy is that it yields only maternal genetic information; therefore, the primary aim of polar body analysis is to determine chromosomal aneuploidy.

PGD for monogenic diseases

For couples at high risk of transmitting an inherited disorder, PGD is a long-established reproductive alternative. The European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium reported that PGD was applied to >190 different monogenic disorders over the past decade [12].

Genetic analysis has primarily been based on PCR techniques applied to a single-cell sample obtained from the early embryonic cleavage stage. However, PGD accuracy and diagnostic efficiency have been limited, as single-cell diagnosis is technically the most challenging and difficult procedure to perform, with a high potential for producing results of sub-optimal quality. Single-cell PCR amplification has limited diagnostic efficacy due to amplification failures, ADO, mosaicism, and contamination. ADO occurs when only one of two alleles in a cell is amplified to a detectable level; this generally affects 5–20 % of single-cell amplifications [17–19].

During the decade of ESHRE PGD Consortium data collection, the outcomes of >4,700 cycles of PGD for monogenic disease were recorded, of which 12 resulted in adverse misdiagnosis. This is likely to be a low estimate by the reason of difficulty based on the diagnosis for single gene mutation of single cell. To prove the diagnostic accuracy, the reanalysis trial of PGD for monogenic diseases has been reported [22].

Since diagnosis from a single cell always has absolute limitations, a two-cell biopsy may provide greater diagnostic accuracy and a more confident diagnosis without reducing implantation efficiency. Blastocyst biopsy enables sampling of >5 cells, making diagnosis easier and reducing the risk of misdiagnosis [14, 15].

For improving diagnostic accuracy and expanding diagnostic variation, various DNA amplification options are also valuable. WGA is considered to have great potential to provide sufficient DNA templates for each independent PCR amplification, including detection of mutation and polymorphic markers [23].

When a disorder has been molecularly characterized, the mutation is principally analyzed in cells biopsied from the embryo. However, when the disease-causing mutation is unknown, diagnosis is performed by linkage analysis (or by gender analysis for sex-linked disorders) [24, 25]. The methodology is being currently developed to ensure the availability of single nucleotide polymorphism (SNP) arrays for characterization of individual mutations and for polymorphic analysis [26].

The number of unaffected transferable embryos also differs according to genetic disease type. The frequency of affected embryos is estimated to be one-quarter in autosomal recessive diseases, one-half in autosomal dominant diseases, and one-quarter in X-linked recessive diseases [13].

WGA

WGA technologies have many benefits, particularly for PGD. The greatest technical difficulty for PGD is the challenge of analyzing a limited number of DNA copies from one or only a few cells. WGA provides a sufficient supply of DNA templates for independent PCR amplifications including mutation detection and haplotype

analysis. Comprehensive microarray analysis also requires preliminary WGA. Another benefit of WGA is the ability to confirm the diagnosis by repeat analysis and haplotyping. WGA is considered to reduce the risk of misdiagnosis due to amplification failure or ADO [27].

The principles of WGA procedure are classified as PCR-based and non-PCR-based.

In the initial stages of WGA development, primer extension preamplification (PEP) using random primers of 15-base oligonucleotides, was exploited as a PCR-based WGA [28, 29]. The second reported procedure was degenerate oligonucleotide-primed PCR (DOP-PCR), which uses primers with partially degenerate sequences to achieve increased amplification efficiency [18, 30]. GenomePlex and PicoPlex of the PCR based-WGA have been currently used for PGD or PGS. It involves the use of a DNA polymerase from the thermophile bacterium *thermos aquatics* and repeated cycling between temperatures appropriate to sequentially denature and elongate the DNA. GenomPlex is the combined amplification technology of PEP and DOP-PCR using degenerate oligonucleotide primers coupled with universal adaptors for linker-adaptor PCR of a fragmented template. PicoPlex was developed later, following GenomPlex, and is well matched to BAC clone microarray analysis and at present widely used for aCGH analysis (24 sure, and 24 sure+) [7, 8].

A non-PCR-based WGA is based on multiple displacement amplification (MDA) with exonuclease-resistant primers and bacteriophage φ29 DNA polymerase [31]. MDA is performed in an isothermal reaction and yields amplified DNA products (10 kb in length) with a variety of structures. MDA products can be used for haplotype analysis by PCR and oligonucleotide array [32–34].

Comprehensive genetic diagnosis by microarray

WGA technology enables comprehensive genetic analysis in PGD in combination with clinical microarray technologies. In comparative genomic hybridization (CGH), the test and reference DNA are amplified by WGA and differentially labeled with fluorochromes (Cy3 and Cy5). The labeled DNA is mixed and applied to a microarray for competitive hybridization. In the history of this technology development, the array platform containing small DNA (aCGH) has been presented and also successfully analyzed from single cells [35–37]. To date, aCGH using BAC clone has been commonly used from the report of high detecting performance of chromosomal imbalance and aneuploidy [38, 39].

The shortfalls of aCGH technology include the inability to detect polyploidies such as triploidy, hemi-parental disomy, small gene mutations, and balanced chromosomal structural anomalies. aCGH by oligonucleotide array may enable detection of small gene mutations such as gene deletions or duplications; however, the other limitations are theoretically insurmountable [2].

In order to cover the shortfalls of aCGH, SNP arrays also provide comprehensive analysis for PGD and PGS [40, 41]. SNP arrays with oligonucleotides provide a genotype (i.e., AA, BB, or AB) for each marker. SNP arrays have been developed for PGD of single-gene disorders based on linkage analysis, chromosomal anomalies, and aneuploidy screening. In terms of detection ability for aneuploidy and parental reciprocal translocation, SNP and aCGH technologies provide equivalent diagnostic efficiencies [42]. However, SNP technology allows for the detection of polyploidies, hemi-parental disomy, and smaller mutations, unlike aCGH.

WGA is required prior to analysis to select for subsequent microarrays [40, 43, 44]. PCR-based and non PCR-based WGA are more matched to BAC clone array and oligonucleotide array of aCGH and SNP array, respectively.

While the genetic information obtained by SNP array was greater than FISH or aCGH, it may have some predisposition potential including occasional unexpected and unexplained gene mutations or information to suggest their characteristics [45, 46].

Evaluation of SNP array results also requires an informed understanding; the SNP array result from a small number of cells is relevant to amplification efficiency and also contains parental DNA copy number variation when diagnosis is aimed to linkage analysis.

Next-generation sequencing (NGS)

Recent advances in NGS technologies have been developing another stage of genetic analysis and also been introduced to PGD and PGS [47–49]. The potential analytical advantages of NGS include reduced cost of DNA sequencing, enhanced detection of partial or segmental aneuploidies, enhanced detection of mosaicism in multicellular samples, and potential for analytical automation [50, 51]. Complete concordance for transferable embryos has been demonstrated between NGS and aCGH [50].

However, the NGS panel for PGD is only for aneuploidy analysis with 5,000 reads. Furthermore, the NGS protocol for PGD/PGS still has controversial aspects at this stage with respect to the evaluation of gain or loss when the signal of the analyzing software is in the atypical range. While NGS provides high resolution and accurate detection of segmental imbalances <14 Mb in DNA size, it is still unable to detect balanced chromosomal rearrangements; in addition, the sequence coverage and real depth are

insufficient to enable allele detection [51]. Further data and the future improvement of the system are expected to define the higher potential of NGS-based PBD/PGS.

PGS

PGS is a subcategory of PGD. The primary aim of PGD is to identify genetically normal embryos for carriers of genetic disease. In contrast to PGD, the aim of PGS is to improve pregnancy outcome and delivery [52–55]. The majority of miscarriages in the first trimester are caused by aneuploidies; thus, the first aim of PGS is to reduce the miscarriage rate. However, to date, PGS does not have confidential evidence of efficiencies in respect of pregnancy outcome regarding indications and methodologies. PGS is typically performed for patients with advanced maternal age and repeated implantation failure and for patients with normal karyotypes experiencing repeated miscarriage. PGS is also sometimes used for couples with male infertility, for those who have delivered a baby with a chromosomal anomaly, or those with a history of radiotherapy and chemotherapy [12].

FISH analysis had been used for PGS since 1995 [52, 53], and aCGH and SNP array has also recently been used for this purpose. The numerous reports on PGS of cleavage-stage embryos (blastomere) using FISH that have been published suggested the expectation of increasing implantation rate per transfer and reducing miscarriage rate by transferring normally diagnosed embryo [52, 55, 56]. However, most PGS trials have been non-randomized studies with inadequate controls that were not well organized. The first randomized controlled study (RCT), published in 2007 by Mastenbroek et al., showed a significantly lower delivery rate in patients who had undergone PGS [57]. A number of RCTs of PGS have since been published [58–60] and suggest no evidence of improvement in delivery rates by FISH diagnosis.

The ESHRE PGD Consortium presented a position statement for PGS in 2010. They assert that PGS for advanced maternal age by using cleavage-stage biopsy and FISH analysis of a limited number of chromosomes is not a valid use of the technology and the significant increase in delivery rate should be demonstrated by other approaches with biopsy at different stages and microarray analysis [61].

The biopsy stage and diagnostic method may influence the rates of implantation and delivery. Day 3 cleavage-stage biopsy is considered more invasive than day 5 blastocyst biopsy for embryo development. Furthermore, day 5 trophectoderm of blastocyst may have less incidence of aneuploidy due to chromosomal rearrangement and/or developmental failure [20].

At present, comprehensive chromosomal analysis using aCGH or SNP array has been commonly used instead of FISH due to analytical limitation with numbers of chromosomes and only part of gene regions mainly for subtelomeres [62]. Information obtained by microarray is definitely more precise than by FISH. While an embryo with normal karyotype is expected to implicate higher pregnancy outcome per embryo transfer, the efficiency for pregnancy rate per oocyte retrieval cycle in each category of patients with various factors is not clear yet.

Indications for PGD and ethics

Preimplantation genetic diagnosis has been established as an option for people at high risk of having a child affected with a serious genetic disorder or handicap; however, PGD has a wide range of potential applications for genetic carriers and to ensure a reduction in miscarriages and other conditions. Genetic testing technologies have developed rapidly and enabled us to obtain comprehensive genetic profiles that have expanded the options for PGD indications, which has created controversies from the stand point of ethical and legal definitions. The indication for PGD should be decided from the view of reproductive autonomy not only regarding a person's request or right but also by consideration towards the wide variety of social discipline and mutual aid.

To date, PGD has been applied mainly to disorders caused by very high-penetrance mutations with a high risk of seriously affecting children; however, it is difficult to draw a line between serious and non-serious disorders and many discussions revolve around whether PGD indications should extend to low-penetrance mutations, late-onset diseases, or hereditary disabilities. Prior to PGD, genetic counseling is essential to support and ensure that the clients have clearly understood both the advantages and disadvantages before entry.

The discussions regarding PGD indications may not be able to reach a clear concensus. The ESHRE Task Force on Ethics and Law suggested PGD is morally acceptable if it meets the proportionality criterion, and psychological and relational factors should be taken into account when discussing possible indications for PGD [63], although controversial indications remain. PGD for sex selection may be morally acceptable if the aim is to avoid trans-generational transmission, while social sexing is viewed as unacceptable. PGD for mitochondrial diseases caused by higher heteroplasmy ratio of mitochondria mutation may be acceptable except when there is uncertainty regarding obtainability of the healthy embryo. In contrast, PGD to select for handicap or disability may not be socially acceptable.

According to technological development and stage status including socio-economic circumstances, population, demand, etc, the ethical discussion should be required continuously to select for entry to each stage always from the stand point of a healthy and safer life for human beings.

Conclusion

The value of PGD has been established in terms of protection against the transmission of severe hereditary diseases and avoidance of miscarriages caused by chromosomal imbalance. To address the concerns regarding PGD indications, the advantages for genetic carriers have been deemed ethically justified. However, the developing speed of technology is accelerating so fast and providing more precise and wide genetic information from the embryo. These technologies also present opportunities for more controversial and ethically questionable uses. Sometimes rapid technological advances outpace the establishment of ethical guidelines.

Moreover, the analyzed results sometimes take account of scientific complexities and uncertainties involved. In cases where there are obvious abnormalities for the baby, the embryo should not be transferred. In those situations, we always need to consider how unexpected mutations obtained by PGD should be handled and discussed with the clients prior to analyzing genetic information.

Compliances with ethical standards

Conflict of interest Kou Sueoka declares that he has no conflict of interest.

References

1. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature. 1990;344:768–70.
2. Harper JC, SenGupta SB. Preimplantation genetic diagnosis: State of the ART 2011. Hum Genet. 2012;131:175–86.
3. Verlinsky Y, Cieslak J, Freidine M, Ivakhnenko V, Wolf G, Kovalinskaya L, et al. Pregnancies following preconception diagnosis of common aneuploidies by fluorescent in situ hybridization. Hum Reprod. 1995;10:1923–7.
4. Staessen C, Platteau P, Van Assche E, Michiels A, Tourmaye H, Camus M, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod. 2004;19:2849–58.
5. Stevens J, Wale P, Surrey ES, Schoolcraft WB. Is aneuploidy screening for patients aged 35 or over beneficial? A prospective randomized trial. Fertil Steril. 2004;82:249.
6. Mersereau JE, Pergament E, Zhang X, Milad MP. Preimplantation genetic screening to improve in vitro fertilization pregnancy rates: a prospective randomized controlled trial. Fertil Steril. 2008;90:1287–9.
7. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet. 2012;5:24.
8. Fiorentino F, Biricik A, Karadayi H, Berkil H, Karlikaya G, Sertyel S, et al. Development and clinical application of a strategy for preimplantation genetic diagnosis of single gene disorders combined with HLA matching. Mol Hum Reprod. 2004;10:445–60.
9. Fiorentino F, Spizzichino L, Bono S, Biricik A, Kokkali G, Rienzi L, et al. PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. Hum Reprod. 2011;26:1925–35.
10. Wells D, Escudero T, Levy B, Hirschhorn K, Delhanty JD, Munné S. First clinical application of comparative genomic hybridization and polar body testing for preimplantation genetic diagnosis of aneuploidy. Fertile Steril. 2002;78:543–9.
11. Hardy K, Martin KL, Leese HJ, Winston RM, Handyside AH. Human preimplantation development in vitro is not adversely affected by biopsy at the 8-cell stage. Hum Reprod. 1990;5:708–814.
12. Moutou C, Goossens V, Coonen E, De Rycke M, Kokkali G, Renwick P, et al. ESHRE PGD Consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010. Hum Reprod. 2014;29:880–903.
13. Coco R. Reprogenetics: preimplantational genetics diagnosis. Genet Mol Biol. 2015;37(Suppl 1):271–84.
14. McArthur SJ, Leigh D, Marshall JT, de Boer KA, Jansen RP. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril. 2005;84:1628–36.
15. Kokkali G, Vrettou C, Traeger-Synodinos J, Jones GM, Cram DS, et al. Birth of a healthy infant following trophectoderm biopsy from blastocytes for PGD of beta-thalasssaemia major. Hum Reprod. 2005;20:1855–9.
16. Goossens V, De Rycke M, De Vos A, Staessen C, Michiels A, Verpoest W, et al. Diagnostic efficiency embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis. Hum Reprod. 2008;23:481–92.
17. Ray PF, Handyside AH. Increasing the denaturation temperature during the first cycles of amplification reduces allele dropout from single cells for preimplantation genetic diagnosis. Mol Hum Reprod. 1996;2:213–8.
18. Wells D, Sherlock JK. Strategies for preimplantation genetic diagnosis of single gene disorders by DNA amplification. Prenat Diagn. 1998;18:1389–401.
19. Piyamongkol W, Bermúdez MG, Harper JC, Wells D. Detailed investigation of factors influencing amplification efficiency and allele drop-out in single cell PCR: implications for preimplantation genetic diagnosis. Mol Hum Reprod. 2003;9(7):411–20.
20. Scott RT, Upham KM, Forman EJ, Zhao T. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013;100:624–30.
21. Verlinsky Y, Cieslak J, Ivakhnenko V, Eviskov S, Wolf G, White M, et al. Preimplantation diagnosis of common aneuploidies by the first- and second-polar body FISH analysis. J Assist Reprod Genet. 1998;15:285–9.
22. Dreesen J, Destouni A, Kourlaba G, Degn B, Mette WC, Carvalho F, et al. Evaluation of PCR-based preimplantation genetic diagnosis applied to monogenic diseases: a collaborative ESHRE PGD consortium study. Eur J Hum Genet. 2014;22:1012–8.
23. Hughes S, Arneson N, Done S, Squire J. The use of whole genome amplification in the study of human disease. Prog Biophys Mol Biol. 2005;88:173–89.

24. Abou-Sleiman PM, Aperssos A, Harper JC, Serhal P, Delhanty JDA. Pregnancy following preimplantation genetic diagnosis for Crouzon syndrome. Mol Hum Reprod. 2002;8:101–4.
25. Dhanjal S, Kakourou G, Mamas T, Saleh N, Doshi A, Gotts S, et al. Preimplantation genetic diagnosis for retinoblastoma predisposition. Br J Ophthalmol. 2007;91:1090–1.
26. Treff NR, Su J, Kasabwala N, Tao X, Miller KA, Scott RT Jr. Robust embryo identification using first polar body single nucleotide polymorphism, microarray-based DNA fingerprinting. Fertil Steril. 2010;93:2453–5.
27. Rechitsky S, Pomerantseva E, Pakhalchuk T, Pauling D, Verlinsky O, Kuliev A. First systematic experience of preimplantation genetic diagnosis for de novo mutations. Reprod Biomed Online. 2011;22:350–61.
28. Zhang L, Cui X, Schmitt K, Hubert R, Navidi W, Arnheim N. Whole genome amplification from a single cell: implications for genetic analysis. Proc Natl Acad Sci. 1992;38:5847–51.
29. Arneson N, Hughes S, Houlston R, Done S. Whole-genome amplification by improved primer extension preamplification PCR (I-PEP-PCR). CSH Protoc. 2008. doi:10.1101/pbd.prot4921 (**Online document**).
30. Telenius H, Carter NP, Bebb CE, Nordenskjöld M, Ponder BA, Tunnacliffe A. Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics. 1992;13:718–25.
31. Dean FB, Hosono S, Fang L, Wu X, Faruqi AF, Bray-Ward P, et al. Comprehensive human genome amplification using multiple displacement amplification using multiple displacement amplification. Proc Natl Acad Sci. 2002;99:5261–6.
32. Hellani A, Coskun S, Tbakhi A, Al-Hassan S. Clinical application of multiple displacement amplification in preimplantation genetic diagnosis. Reprod Biomed Online. 2005;10:376–80.
33. Burtt NP. Whole-genome amplification using φ29 DNA polymerase. Cold Spring Harbor. 2011. doi:10.1101/pbd.prot5552 (**Online document**).
34. Renwick P, Trussler J, Lashwood A, Braude P, Ogilvie CM. Preimplantation genetic haplotyping: 127 diagnostic cycles demonstrating a robust, efficient alternative to direct mutation testing on single cells. Reprod Biomed Online. 2010;20:470–6.
35. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, et al. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet. 1998;20:207–11.
36. Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, et al. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet. 1999;23:41–6.
37. 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;6:1055–62.
38. Thornhill A, Ottolini C, Harton G, Griffin D. Aneuploidy testing by array-CGH. In: Markus M, editor. A practical guide to selecting gametes and embryos. CRC Press; 2014. p. 255–68.
39. Fiorentino F, Rienzi L, Bono S, Capalbo A, Spizzichino L, Baroni E, et al. Preimplantation genetic screening on day 3 embryos using array comparative genomic hybridization in patients with advanced maternal age: a prospective double blinded randomized controlled trial. Hum Reprod. 2013;28:i49–50.
40. Treff NR, Su J, Mavrianos J, Bergh PA, Miller KA, Scott RT Jr. Accurate 23 chromosome aneuploidy screening in human blastomeres using single nucleotide polymorphism (SNP) microarrays. Fertil Steril. 2007;86:S217.
41. Handyside AH, Harton GL, Mariani B, Thornhill AR, Affara N, Shaw MA, et al. Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet. 2010;47:651–8.
42. 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;31:843–50.
43. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med. 2009;15:577–83.
44. Kearns WG, Pen R, Benner A, Kittai A, Widra E, Leach R. SNP microarray genetic analyses to determine 23-chromosome ploidy, structural chromosome aberrations and genome-wide scans to identify disease risks from a single embryonic cell. Fertil Steril. 2008;90(Suppl 1):S23.
45. Renwick P, Trussler J, Lashwood A, Braude P, Ogilvie CM. Preimplantation genetic haplotyping: 127 diagnostic cycles demonstrating a robust, efficient alternative to direct mutation testing on single cells. Reprod Biomed. 2010;20:470–6.
46. Natesan SA, Bladon AJ, Coskun S, Qubbaj W, Prates R, Munne S. Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro. Genet Med. 2014. doi:10.1038/gim.2014.45 (**Online document**).
47. Handyside AH, Wells D. Single nucleotide polymorphisms and next generation sequencing. In: Gardner DK, Sakkas D, Seli E, Wells D, editors. Human gametes and preimplantation embryos: assessment and diagnosis. New York: Springer Science Business Media; 2013. p. 135–46.
48. Treff NR, Fedick A, Tao X, Devkota B, Taylor D, Scott RT Jr. Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease. Fertil Steril. 2013;99:1377–84.
49. Fiorentino F, Biricik A, Bono S, Spizzichino L, Cotroneo E, Cottone G, et al. Development and validation of a next-generation sequencing (NGS)-based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril. 2014;101:1375–82.
50. Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, Cottone G, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod. 2014;29:2802–13.
51. Rubio C. Next-generation sequencing challenges in reproductive genetics. Fertil Steril. 2014;101:1252–3.
52. Verlinsky Y, Cieslak J, Freidine M, Ivakhnenko V, Wolf G, Kovalinskaya L, et al. Pregnancies following pre-conception diagnosis of common aneuploidies by fluorescent in situ hybridisation. Mol Hum Reprod. 1995;10:1923–7.
53. Munné S, Dailey T, Sultan KM, Grifo J, Cohen J. The use of first polar bodies for preimplantation diagnosis of aneuploidy. Hum Reprod. 1995;10:1015–21.
54. Munné S, Sultan KM, Weier HU, Grifo JA, Cohen J, Rosenwaks Z, et al. Assessment of numeric abnormalities of X, Y, 18, and 16 chromosomes in preimplantation human embryos before transfer. Am J Obstet Gynecol. 1995;172:1191–9.
55. Munné S, Escudero T, Colls P, Xuezhong Z, Oter M, Garrisi M, et al. Predictability of preimplantation genetic diagnosis of aneuploidy and translocations on prospective attempts. Reprod Biomed Online. 2004;9:645–51.
56. Wilton L. Preimplantation genetic diagnosis for aneuploidy screening in early human embryos. Prenat Diagn. 2002;22:512–8.
57. Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med. 2007;357:9–17.

58. Staessen C, Verpoest W, Donoso P, Haentjens P, Van der Elst J, Liebaers I, et al. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. Hum Reprod. 2008;23:2818–25.
59. Blockeel C, Schutyser V, De Vos A, Verpoest W, De Vos M, Staessen C, et al. Prospectively randomised controlled trial of PGS in IVF/ICSI patients with poor implantation. Reprod Biomed Online. 2008;17:848–54.
60. Hardarson T, Hanson C, Lundin K, Hillensjö T, Nilsson L, Stevic J, et al. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomised controlled trial. Hum Reprod. 2008;23:2806–12.
61. Harper J, Coonen E, De Rycke M, Fiorentino F, Geraedts J, Goossens V, et al. What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium steering committee. Hum Reprod. 2010;25:821–3.
62. Fiorentino F. Array comparative genomic hybridization: its role in preimplantation genetic diagnosis. Curr Opin Obstet Gynecol. 2012. doi:10.1097/GCO (**Online document**).
63. De Wert G, Dondrop W, Shenfield F, Devroey P, Tarlatzis B, Barri P, et al. ESHRE task force on ethics and Law22: preimplantation genetic diagnosis. Hum Reprod. 2014;29:1610–7.