Preimplantation diagnosis: a realistic option for assisted reproduction and genetic practice

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Purpose of review

Preimplantation genetic diagnosis (PGD) allows genetically disadvantaged couples to reproduce, while avoiding the birth of children with targeted genetic disorders. By ensuring unaffected pregnancies, PGD circumvents the possible need and therefore risks of pregnancy termination. This review will describe the current progress of PGD for Mendelian and chromosomal disorders and its impact on reproductive medicine.

Recent findings

Indications for PGD have expanded beyond those used in prenatal diagnosis, which has also resulted in improved access to HLA-compatible stem-cell transplantation for siblings through preimplantation HLA typing. More than 1000 apparently healthy, unaffected children have been born after PGD, suggesting its accuracy, reliability and safety. PGD is currently the only hope for carriers of balanced translocations. It also appears to be of special value for avoiding age-related aneuploidies in in-vitro fertilization patients who have a particularly poor prognosis for a successful pregnancy; the accumulated experience of thousands of PGD cycles strongly suggests that PGD can improve clinical outcome for such patients.

Summary

PGD would particularly benefit poor prognosis in-vitro fertilization patients and other at-risk couples by improving reproductive outcomes and avoiding the birth of affected offspring.

Keywords

expanding indications, improving reproductive outcome, preimplantation genetic diagnosis (PGD), PGD accuracy, preimplantation HLA typing

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Abbreviations

FISH	fluorescent in-situ hybridization
HLA	human leukocyte antigen
PB1, PB2	first and second polar bodies
PCR	polymerase chain reaction
PGD	preimplantation genetic diagnosis

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Introduction

Introduced 14 years ago, preimplantation genetic diagnosis (PGD) permits genetic testing before the transfer of embryos to the mother, which has distinct advantages in establishing pregnancies unaffected by tested disorders and without the potential need for pregnancy termination. Approximately 7000 PGD cases have been performed worldwide, which have resulted in the birth of more than 1000 healthy children [1•]. PGD can be used for the diagnosis of late-onset diseases with genetic predisposition, preimplantation HLA typing, and other non-traditional prenatal testing; thus, PGD complements other methods used in prenatal diagnosis [2]. By permitting selection of euploid embryos for transfer, PGD improves reproductive outcome, which has resulted in its extensive use in assisted reproduction practices [1•, 2–5]. This review will describe the current progress of PGD for Mendelian and chromosomal disorders and its impact on reproductive medicine.

Approaches to preimplantation genetic diagnosis

PGD involves genetic testing either of oocytes in the case of maternally derived genetic abnormalities or of single cells derived from preimplantation embryos. Normally screened embryos are subsequently transferred to the patient, which ensures the establishment of an unaffected pregnancy [6]. These methods have not been shown to have detrimental effects on embryonic development, even after sequential polar body and embryo biopsy [7,8]. Blastocyst biopsy, which is becoming more common, may be used to confirm the diagnosis based on polar body or blastomere biopsy [9•]. Each of these methods has advantages and disadvantages, and the choice depends on clinical circumstances. Embryo biopsy is associated with reduced embryo cell number with a possible negative impact on viability. Despite this potential drawback, embryo biopsy is the method of choice for paternally derived dominant conditions, translocations, gender determination, and HLA typing.

Oocyte testing, which is performed by analysis of the first and second polar bodies (PB1 and PB2), permits an assessment of the maternal genetic contribution to the subsequent embryo. This technique is the method of choice for maternally derived genetic abnormalities. Although information on gender and paternal mutations is not provided, oocyte testing can be used for autosomal

recessive disorders and for maternally derived dominant mutations and translocations. It can be particularly useful in the diagnosis of X-linked diseases, while avoiding the 50% discard of healthy male fetuses associated with gender-based determinations by blastomere evaluation [10]. Over 90% of chromosomal abnormalities originate from maternal meiosis; thus, the polar body approach has particular relevance for PGD of aneuploidies related to maternal age. The high rate of mosaicism at the cleavage stage is a major limiting factor in blastomere-based PGD, which, of course, does not impact on oocyte testing.

Each of the PGD approaches, which are often complementary, should be available to ensure an accurate diagnosis. As will be described below, the current standard of PGD may require testing for a causative gene and HLA typing along with aneuploidy testing, which in some cases cannot be done without a sequential polar body and embryo biopsy. Further testing by blastocyst biopsy may be necessary for confirmation of the polar body or blastomere diagnosis.

Accuracy of preimplantation genetic diagnosis

The accuracy and reliability of PGD are key issues, because of the limitations of single-cell DNA analysis. Critical to the design of any PGD strategy are methods to detect and avoid misdiagnosis. A key contributor to misdiagnosis is preferential amplification, known as allele-specific amplification failure (allele drop out), so special protocols are required to ensure the highest possible allele drop-out detection rate [6,11–15]. Allele drop-out rates in single-cell polymerase chain reaction (PCR) analysis have been shown to be around 10–20%, depending on the type of cell tested [16], which would lead to misdiagnosis, especially in compound heterozygous embryos [17,18].

Simultaneous detection of the mutant gene with up to three highly polymorphic markers that are closely linked to the gene being tested effectively guards against misdiagnosis due to preferential amplification [6,11]. The embryos should be transferred only if the polymorphic sites and the mutation tests agree. Frequently utilized for selecting mutation-free embryos in dominant conditions and normal or heterozygous embryos in autosomal recessive disorders, PGD should also include the detection of normal genes. Only multiplex amplification allows detection of allele drop out, which provides critical protection against the transfer of affected embryos.

Diagnostic errors of preferential amplification can also be reduced by fluorescence PCR, which allows detection of some heterozygous cells erroneously diagnosed as homozygous by conventional PCR [11]. This technology also permits simultaneous gender determination, DNA fingerprinting, and detection of common aneuploidies. Real-time PCR, which reduces the allele drop-out rate by almost half compared to conventional or fluorescent PCR [6], can further improve diagnostic accuracy and be of particular value in cases with an insufficient number of informative markers.

Aneuploidy testing, at least for the chromosome with the target gene, may be of special value in PGD for single-gene disorders in women with advanced reproductive age, because of the associated high aneuploidy rates in oocytes and embryos, and high prevalence of mosaicism at the cleavage stage [3,4,19]. Without such information, the lack of a mutant allele due to chromosomal monosomy in the biopsied blastomere or oocyte cannot be excluded; thus, the single-cell biopsy should ideally be tested simultaneously for the causative gene and specific chromosome number. This can be achieved by adding primers for chromosome-specific microsatellite markers to the multiplex PCR reaction designed for each genetic disorder.

The accuracy of PGD for chromosomal aneuploidies can also be improved. Biopsied single blastomeres may not represent the actual karyotype of the embryo, because up to half of the cleavage stage embryos have been shown to have chromosomal mosaicism [3,4,19]. Misdiagnosis due to mosaicism can be avoided by a fluorescent in-situ hybridization (FISH) analysis in two or three steps, with initial testing for maternally derived aneuploidies (which contribute at least 90% of all aneuploidies) by PB1 and PB2 analysis, and subsequent removal and testing of single blastomeres to exclude paternally derived abnormalities. Depending on the test results, a diagnosis may be established from a blastocyst biopsy on day 5.

Expanding indications for preimplantation genetic diagnosis

Initially, indications for PGD, which were similar to those for prenatal diagnosis, generally included at-risk couples (25–50% chance of a transmitted genetic disorder) that could not accept pregnancy termination for an affected fetus. Recently, the indications for PGD have been extended to include conditions with low-penetrance, late-onset disorders with genetic predisposition, and HLA typing with or without testing for the causative gene. PGD has now been applied to more than 100 conditions, with the most frequent indications still being cystic fibrosis and hemoglobin disorders. The choice between prenatal diagnosis and PGD mainly depends on the patient's views on pregnancy termination, which are, of course, strongly influenced by social and religious factors.

In about one half of cases, PGD of single-gene disorders has been performed by gender determination for X-linked conditions with either a PCR or FISH technique. The

reason for this was that the sequence information was frequently unknown for identification and that transfer of female embryos identified by DNA analysis or FISH was technically more straightforward, even though it involved discarding healthy male embryos. Because X-linked disorders are maternally derived, a more attractive option may involve preselection of mutation-free oocytes through testing for specific causative mutations in PB1 and PB2. In this method, subsequent testing of the embryos would not be needed because transfer can occur irrespective of gender or other paternal genetic contribution [10].

PGD is of particular value for couples with one affected partner, who have only a 50% chance of having an unaffected child. In these cases, the method of choice for PGD is sequential PB1 and PB2 analysis for the maternal mutation in order to preselect the mutation-free oocytes, rather than testing for two or three mutations following embryo biopsy, unless the mother is homozygous affected [6].

PGD generally requires knowledge of sequence information for Mendelian diseases, although PGD may be performed even when the exact mutation is unknown. Linkage analysis has been recently used to diagnose autosomal dominant polycystic kidney disease caused by either the PKD1 or PKD2 gene, for which direct testing is still unavailable [20].

Identification of those predisposed to genetically related disorders has not traditionally been an indication for prenatal diagnosis. PGD has the distinct advantage of selecting embryos for transfer that are free of genetic predisposition to disease, which obviates concerns over pregnancy termination. PGD may now be performed for a number of disorders, including p53 tumor suppressor gene mutations, ataxia telangiectasia, familial adenomatous polyposis coli, von Hippel–Lindau syndrome, retinoblastoma, neurofibromatosis types I and II, and familial posterior fossa brain tumor [21,22]. Such diseases present beyond early childhood and may not even occur in all cases; and, thus, PGD for this group of disorders is controversial [23–25], particularly when performed for a genetic predisposition to Alzheimer's disease [26]. The initial experience of offering PGD for these indications shows that many couples undergo the procedure who would otherwise not consider pregnancy an option.

Of special interest is the application of PGD for blood-group incompatibility, including rhesus disease and Kell (*KI*) genotype. PGD permits transfer of compatible embryos, which totally eliminates the potential risks involved in intrauterine transfusion. Some couples have been so traumatized by poor outcomes related to hemolytic disease of the newborn that they regard PGD as their only option for future pregnancies. Thus, PGD is attractive for patients at risk of alloimmunization, a condition for which prenatal diagnosis would only be rarely performed [27].

Another novel application for PGD is for inheritable forms of congenital malformations. For example, PGD has been successfuly performed for Sonic Hedgehog gene mutation [28], Crouzon [29] and Holt–Oram syndromes [30], and Currarino triad [31].

Finally, PGD has most recently been used for HLA matching, which obviates ethical issues of pregnancy termination for a HLA mismatch detected by prenatal diagnosis. PGD allows the transfer of a limited number of embryos with a 100% match for siblings. The application to stem-cell transplantation [32••] has evolved from earlier use of PGD to detect the causative gene for a number of disorders, including Fanconi anemia, thalassemia, hyperimmunoglobulin M syndrome, X-linked adrenoleukodystrophy, and Wiscott–Aldrich syndrome [33,34•, 35–37]. Although not without controversy [38–40], PGD has been used for approximately 150 cases of preimplantation HLA typing with successful treatment of children with Fanconi anemia, thalassemia and Diamond–Blackfan anemia [41].

Impact of preimplantation genetic diagnosis on assisted reproduction

PGD provides an attractive and probably the only solution for the poor reproductive outcome of couples carrying translocations. PGD for maternal translocations initially involved PB1 removal and FISH analysis with whole-chromosome painting probes, with the later addition of PB2 and blastomere testing [4,42–44]. Interphase FISH analysis using chromosome-specific probes has proven extremely efficient and reliable, despite its limitations in identifying some translocations and inability to distinguish balanced translocations from normal karyotype. Advances in the visualization and cytogenetic analysis of single blastomeres have improved the accuracy of PGD for both maternally and paternally derived translocations [6,44]. The accumulated experience of more than 500 cases of PGD for translocation carriers indicates that this method can reduce by at least four-fold the spontaneous abortion rate in such carriers [2,43,44]. Thus, PGD is preferable to prenatal diagnosis for carriers of chromosomal translocations.

One of the major indications for PGD in women of advanced reproductive age undergoing in-vitro fertilization (IVF) is the detection of aneuploidies. In this context, PGD has been performed in approximately 5000 cases for advanced reproductive age, repeated IVF failures, and repeated spontaneous abortions. The accumulated experience suggests that PGD improves reproductive outcomes [1•,2–5,45,46•,47,48].

More than half of the oocytes and embryos from poor prognosis IVF patients have chromosomal abnormalities, as determined by PGD [3,4,19,49,50]. Thus, the detection of chromosomal aneuploidy by PGD can prevent the transfer of chromosomally abnormal embryos and the attendant reproductive losses related to implantation failures and spontaneous abortion.

At least one half of chromosomally abnormal embryos are mosaics [3,4,19,51,52], which is a major limitation in the accurate detection of aneuploidies by embryo biopsy. The prevalence of aneuploidies in oocytes and embryos appears to be comparable, which suggests that mosaic embryos in most cases originate from aneuploid oocytes. Future advances in PGD will likely involve testing of both oocytes and embryos, which might be achieved by a sequential biopsy of both PB1 and PB2 and the single blastomere from the resulting embryo, to exclude both meiotic and mitotic errors. Information from both the oocyte and embryo chromosome sets may also help detect potential uniparental disomies (e.g. one-third of apparently disomic embryos originate from trisomic oocytes), and may explain syndromes recently reported in association with assisted reproductive technology [53–57].

Conclusion

Current data show PGD to be a realistic option for at-risk couples to avoid the birth of children with single-gene and chromosomal disorders. PGD may be performed by embryo biopsy or polar body analysis, both of which have no detectable deleterious effect on development before and after implantation. Up to now, the use of PGD for the detection of single-gene or chromosomal disorders has resulted in the birth of more than 1000 unaffected children, indicating that it is a safe, accurate and reliable technique for the prevention of genetic and chromosomal disorders. PGD is of particular value for couples who would not consider pregnancy termination to be an option.

PGD can be applied to genetic diseases that are currently detected by prenatal diagnosis, to other disorders, such as late-onset and complex disorders, congenital malformations, and blood-group incompatibility, and to preselection of unaffected and HLA-matched embryos. This latter application extends PGD to the treatment of siblings who require HLA-compatible stem-cell transplantation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Verlinsky Y, Cohen J, Munne S, *et al.* Over a decade of preimplantation genetic • diagnosis experience – a multi-center report. Fertil Steril 2004; 82:292– 294.
The largest PGD series (4748 cases) performed by the three most active PGD centers is reported, evidencing the birth of the first 1000 apparently healthy babies after PGD. The data suggest the safety, accuracy and reliability of the procedure and its impact on assisted reproduction and genetic practices.

2 Kuliev A, Verlinsky Y. Thirteen years' experience of preimplantation diagnosis: report of the Fifth International Symposium on Preimplantation Genetics. Reprod Biomed Online 2004; 8:229–235.

3 Gianaroli L, Magli MC, Ferraretti AP. The in vivo and in vitro efficiency and efficacy of PGD for aneuploidy. Mol Cell Endocrinol 2001; 183:S13–S18.

4 Munne S. Preimplantation genetic diagnosis of numerical and structural chromosome abnormalities. Reprod Biomed Online 2002; 4:183–196.

5 Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y. Nuclear abnormalities in series of 6733 human oocytes. Reprod Biomed Online 2003; 6:54–59.

6 Verlinsky Y, Kuliev A. Atlas of preimplantation genetic diagnosis. 2nd ed. London: Taylor & Francis; 2005.

7 Magli CM, Gianaroli L, Ferraretti AP, *et al.* The combination of polar body and embryo biopsy does not affect embryo viability. Hum Reprod 2004; 19: 1163–1169.

8 Cieslak J, Ilkevitch Y, Bernal A, *et al.* Developmental potential of embryos after 1 to 3 biopsy procedures for preimplantation genetic diagnosis (PGD). Fertil Steril 2004; 82(Suppl 2):S30.

9 De Boer KA, Catt JW, Jansen RPS, *et al.* Moving to blastocyst biopsy for • preimplantation genetic diagnosis and single embryo transfer at Sydney IVF. Fertil Steril 2004; 82:295–298.
The report of the first and largest series of PGD performed by blastocyst biopsy.

10 Verlinsky Y, Rechitsky S, Verlinsky O, *et al.* Polar body based preimplantation diagnosis for X-linked genetic disorders. Reprod Biomed Online 2002; 4: 38–42.

11 Rechitsky S, Verlinsky O, Amet T, *et al.* Reliability of preimplantation diagnosis for single gene disorders. Mol Cell Endocrinol 2001; 183:S65–S68.

12 Sermon K, Seneca S, De Rycke M, *et al.* PGD in the lab for triplet repeat diseases – myotonic dystrophy, Huntington's disease and Fragile-X syndrome. Mol Cell Endocrinol 2001; 183:S77–S85.

13 De Vos A, Sermon K, De Rijcke M, *et al.* Preimplantation genetic diagnosis for Charcot–Marie–Tooth disease type 1A. Mol Hum Reprod 2003; 9:429–435.

14 Goossens V, Sermon K, Lissens W, *et al.* Improving clinical preimplantation genetic diagnosis for cystic fibrosis by duplex PCR using two polymorphic markers or one polymorphic marker in combination with the detection of the delta F508 mutation. Hum Reprod 2003; 9:559–567.

15 Piyamongkol W, Bermudez G, Harper J, 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; 7:411–420.

16 Rechitsky S, Strom C, Verlinsky O, *et al.* Allele drop out in polar bodies and blastomeres. J Assist Reprod Genet 1998; 15:253–257.

17 International Working Group on Preimplantation Genetics. Preimplantation genetic diagnosis – experience of three thousand clinical cycles. Report of the 11th Annual Meeting International Working Group on Preimplantation Genetics, in conjunction with 10th International Congress of Human Genetics, Vienna, May 15, 2001. Reprod Biomed Online 2001; 3:49–53.

18 ESHRE Preimplantation Genetic Diagnosis Consortium. Data Collection III, May 2002. Human Reprod 2002; 17:233–246.

19 Kuliev A, Verlinsky Y. Meiotic and mitotic nondisjunction based on preimplantation genetic diagnosis experience. Hum Reprod Update 2004; 10: 401–407.

20 Verlinsky Y, Rechitsky S, Verlinsky O, *et al.* Preimplantation genetic diagnosis for polycystic kidney disease. Fertil Steril 2004; 82:926–929.

21 Rechitsky S, Verlinsky O, Chistokina A, *et al.* Preimplantation genetic diagnosis for cancer predisposition. Reprod Biomed Online 2002; 5:148–155.

22 Hellani A, Lauge A, Ozand P, *et al.* Pregancy after preimplantation genetic diagnosis for ataxia telangiectasia. Mol Hum Reprod 2002; 8:785–788.

23 Towner D, Loewy RS. Ethics of preimplantation diagnosis for a woman destined to develop early-onset Alzheimer disease. JAMA 2002; 287:1038–1040.

24 Robertson JA. Extending preimplantation genetic diagnosis: the ethical debate. Ethical issues in new uses of preimplantation genetic diagnosis. Hum Reprod 2003; 18:465–471.

25 Vastag B. Merits of embryo screening debated. JAMA 2004; 291:927–929.

26 Verlinsky Y, Rechitsky S, Verlinsky O, *et al.* Preimplantation diagnosis for early onset Alzheimer disease caused by V717L mutation. JAMA 2002; 287: 1018–1021.

27 Verlinsky Y, Rechitsky S, Seckin O, *et al.* Preimplantation diagnosis for Kell genotype. Fertil Steril 2003; 80:1047–1051.

28 Verlinsky Y, Rechitsky S, Verlinsky O, *et al.* Preimplantation diagnosis for Sonic Hedgehog mutation causing familial holoprosencephaly. New Engl J Med 2003; 348:1449–1454.

29 Abou-Sleiman PM, Apessos A, Harper JC, *et al.* Pregnancy following preimplantation genetic diagnosis for Crouzon syndrome. Mol Hum Reprod 2002; 8:304–309.

30 He J, McDermont DA, Song Y, *et al.* Preimplantation genetic diagnosis of human congenital heart disease and HoltOram syndrome. Am J Med Genet 2004; 126A:9398.

31 Verlinsky Y, Rechitsky S, Schoolcraft W, Kuliev A. Preimplantation diagnosis for homeobox gene HLXB9 mutation causing Currarino syndrome. Am J Med Genet (in press).

32 •• Verlinsky Y, Rechitsky S, Sharapova T, *et al.* Preimplantation HLA typing. JAMA 2004; 291:2079–2085.
The first report of preimplantation HLA typing as a primary indication, without involving actual PGD for a specific genetic disease, resulting in the birth of HLA-matched babies. One sibling with Diamond–Blackfan anemia received the first transplantation of stem cells and was no longer dependent on transfusion of red blood cells.

33 Verlinsky Y, Rechitsky S, Schoolcraft W, *et al.* Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA 2001; 285:3130–3133.

34 • Rechitsky S, Kuliev A, Tur-Kaspa I, *et al.* Preimplantation HLA typing with preimplantation genetic diagnosis. Reprod Biomed Online 2004; 6:488–493.
Presentation of the largest series of PGD combined with HLA typing, demonstrating high degree of accuracy and practical relevance of the procedure.

35 Fiorentino F, Biricik A, Karadayi H, *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; 10:445–460.

36 Van deVelde H, Georgiou I, De Rycke M, *et al.* Novel universal approach for preimplantation genetic diagnosis of β-thalassemia in combination with HLA matching of embryos. Hum Reprod 2004; 19:700–708.

37 Kahraman S, Karlilaya G, Sertyel S, *et al.* Clinical aspects of preimplantation genetic diagnosis of single gene disorders combined with HLA typing. Reprod Biomed Online 2004; 9:529–532.

38 Damewood MD. Ethical implications of a new application of preimplantation diagnosis. JAMA 2001; 285:3143–3144.

39 Fost NC. Conception for donation. JAMA 2004; 291:2125–2126.

40 Edwards RG. Ethics of PGD: thoughts on the consequences of typing HLA in embryos. Reprod BioMed Online 2004; 9:222–224.

41 Kuliev A, Verlinsky Y. Preimplantation HLA typing for stem cell transplantation. Reprod BioMed Online 2004; 6:488–493.

42 Scriven PN, Handyside AH, Mackie Ogilvie C. Chromosome translocations: segregation modes and strategies for preimplantation genetic diagnosis. Prenat Diagn 1998; 18:1437–1449.

43 Munné S, Sandalinas M, Escudero T. Outcome of preimplantation genetic diagnosis of translocations. Fertil Steril 2000; 73:1209–1218.

44 Verlinsky Y, Cieslak J, Evsikov S, *et al.* Nuclear transfer for full karyotyping and preimplantation diagnosis of translocations. Reprod BioMed Online 2002; 5:302–307.

45 Munne S, Cohen J, Sable D. Preimplantation genetic diagnosis for advanced maternal age and other indications. Fertil Steril 2002; 78:234–236.

46 • Munne S, Sandalinas M, Escudero T. Improved implantation after preimplantation genetic diagnosis of aneuploidy. Reprod BioMed Online 2003; 7: 91–97.
By studying 138 patients undergoing PGD for aneuploidy because of advanced reproductive age, matched to 138 controls, subgroups of infertile couples were identified that benefit from PGD for aneuploidies, demonstrating the improved implantation rate in patients with eight or more embryos available for analysis.

47 Ferraretti AP, Magli C, Kopcow L, Gianaroli L. Prognostic role of preimplantation genetic diagnosis for aneuploidy in assisted reproductive technology outcome. Hum Reprod 2004; 19:694–699.

48 Gianaroli L, Magli MC, Ferraretti AP. PGD for aneuploidies: clinical outcome. In: Abstracts of the Fifth International Symposium on Preimplantation Genetics; 5–7 June 2003; Antalya, Turkey. Istanbul, Turkey: International Working Group on Preimplantation Genetics (http://www.preimplantationgenetics.org) and Istanbul Memorial Hospital ART and Genetics Centre (http://www.pgdturkey.org), 2003; p. 25.

49 Montag M, van der Ven K, Dorn C, van der Ven H. Outcome of laser-assisted polar body biopsy and aneuploidy testing. Reprod BioMed Online 2004; 9:425–429.

50 Munne S, Bahce M, Sandalinas M. Differences in chromosome susceptibility to aneuploidy and survival to first trimester. Reprod BioMed Online 2004; 8:81–90.

51 Munne S, Sandalinas M, Escudero T, *et al.* Chromosomal mosaicism in cleavage-stage human embryos: evidence of maternal age effect. Reprod BioMed Online 2002; 4:223–232.

52 Baart RB, Van Opsal D, Los FL, *et al.* Fluorescence in situ hybridization analysis of two blastomeres from day 3 frozen–thawed embryos followed by analysis of the remaining embryo on day 5. Hum Reprod 2004; 19:685–693.

53 DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. Am J Hum Genet 2003; 72:156–160.

54 Gicquel C, Gaston V, Mandelbaum J, *et al.* In vitro fertilization may increase the risk of Beckwith–Wiedemann syndrome related to the abnormal imprinting of the KCN1OT gene. Am J Hum Genet 2003; 72:1338–1341.

55 Maher ER, Brueton LA, Bowdin SC. Beckwith–Wiedemann syndrome and assisted reproduction technology (ART). J Med Genet 2003; 40:62–64.

56 Halliday J, Oke K, Breheny S, *et al.* Beckwith–Wiedemann syndrome and IVF: a case-control study. Am J Hum Genet 2004; 75:526–528.

57 Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: a call for investigation. Am J Hum Genet 2004; 74:599–609.