# **Preimplantation genetic diagnosis**

#### Karen Sermon, André Van Steirteghem, Inge Liebaers

Preimplantation genetic diagnosis (PGD) was introduced at the beginning of the 1990s as an alternative to prenatal diagnosis, to prevent termination of pregnancy in couples with a high risk for offspring affected by a sex-linked genetic disease. At that time, embryos obtained in vitro were tested to ascertain their sex, and only female embryos were transferred. Since then, techniques for genetic analysis at the single-cell level, involving assessment of first and second polar bodies from oocytes or blastomeres from cleavage-stage embryos, have evolved. Fluorescence in-situ hybridisation (FISH) has been introduced for the analysis of chromosomes and PCR for the analysis of genes in cases of monogenic diseases. In-vitro culture of embryos has also improved through the use of sequential media. Here, we provide an overview of indications for, and techniques used in, PGD, and discuss results obtained with the technique and outcomes of pregnancies. A brief review of new technologies is also included.

Preimplantation genetic diagnosis (PGD) is an early form of prenatal diagnosis, in which embryos created in vitro are analysed for well-defined genetic defects; only those free of the defects are replaced into the womb.1 The technique is used mainly in two broad indication groups. The first group are individuals at high risk of having a child with a genetic disease-eg, carriers of a monogenic disease or of chromosomal structural aberrations, such as translocations-who have repeatedly opted to terminate their pregnancies on the basis of results of prenatal tests, have concurrent infertility (as in congenital bilateral absence of the vas deferens), have had recurrent miscarriages (as is often the case in translocation carriers), or have religious or moral objections to abortion. The second group are those being treated with in-vitro fertilisation (IVF), who might have a low genetic risk but whose embryos are screened for chromosome aneuploidies to enhance their chance of an ongoing pregnancy. PGD for aneuploidy screening (PGD-AS) is mainly applied when a low IVF success rate might be attributable to chromosomal aneuploidies in the embryos, as is sometimes the case in women older than age 37-40 years.2

PGD was first described in a clinical setting in a groundbreaking report published in 1990,<sup>3</sup> which had a great effect on UK legislation with respect to embryo research. Preliminary experiments had, however, been described several years earlier.<sup>4,5</sup> The first application for PGD was in patients who were carriers of an X-linked disease and had thus one chance in four of having an affected child.<sup>3</sup> Sequences on the Y-chromosomes were amplified by PCR to discriminate male from female embryos, and only female embryos were transferred. Since then, PCR has been used and refined for diagnosis of several of the more common monogenic diseases.<sup>6</sup> In the early 1990s, a method that allowed single-cell analysis at the chromosomal level was

Lancet 2004; 363: 1633-41

Centre for Medical Genetics (Prof K Sermon MD, Prof I Liebaers MD) and Centre for Reproductive Medicine (Prof A Van Steirteghem MD), University Hospital and Medical School, Dutch-speaking Brussels Free University, Brussels, Belgium

**Correspondence to:** Prof Karen Sermon, Centre for Medical Genetics, AZ-VUB, Laarbeeklaan 101, 1090 Brussels, Belgium (e-mail: Karen.sermon@az.vub.ac.be)

described; fluorescence in-situ hybridisation (FISH) has since replaced PCR as a reliable method for the sexing of embryos,<sup>7,8</sup> and has been widely used for PGD-AS and for detection of imbalanced forms of chromosomal aberrations.<sup>2,9,10</sup>

Here, we discuss methods used for diagnosis of genetic diseases, indications for PGD and PGD-AS, results obtained with the techniques, and subsequent outcomes of pregnancies. We then look ahead at some of the new methods being introduced.

#### In-vitro diagnostic procedures

Procedures used to obtain oocytes through ovarian hyperstimulation, IVF techniques such as intracytoplasmic sperm injection, and embryo culture conditions have been widely reviewed elsewhere<sup>11,12</sup> and are considered outside the scope of this review.

# Biopsy of polar bodies and embryos

# Polar-body biopsy

Polar bodies can be biopsied without affecting an egg's rate of fertilisation or eventual cleavage of the embryo, and can be used to deduce the genotype of the oocyte. To obtain polar bodies, a slit is made in the zona pellucida by mechanical means (with sharp needles) or with laser technology. The polar body is then gently drawn out of the egg with a biopsy pipette.

Verlinsky and colleagues<sup>13</sup> pioneered the approach, to begin with on the first polar body only and later on the second (extruded after fertilisation and completion of the second meiotic division) to increase accuracy of diagnosis.<sup>14</sup> In a large series of first and second polar-body analyses for single-gene disorders, Verlinsky and co-workers<sup>15</sup> correctly identified a genetic disorder in 98% (157 of 160) of oocytes tested.

### Search strategy and selection criteria

We did a PubMed search with the phrase "preimplantation genetic diagnosis" for articles published between 1989 and 2003. Other key phrases used were single-cell PCR, comparative genomic hybridisation, and any monogenic disease. Also included were a selection of the most important or trend-setting articles on the subject. We limited our search to articles published in English.

THE LANCET • Vol 363 • May 15, 2004 • www.thelancet.com



Figure 1: Cleavage-stage biopsy

A: hole in zona pellucida made with laser. B: removal of first blastomere from embryo. C: deposition of blastomere in medium. D: removal of second blastomere.

## Cleavage-stage biopsy

Cleavage-stage biopsy is a widely used technique,<sup>16</sup> for which embryos are grown in vitro until they reach their third division (when they have six or more cells), which occurs on the third day after insemination. The zona pellucida is then breached, using an acid solution (Tyrode's solution) or laser technology. A biopsy pipette is introduced through the hole into the embryo and one or two blastomeres are gently aspirated (figure 1). Alternatives to aspiration are extrusion, which involves making a hole in the embryo and squeezing the cells out, and fine-needle aspiration, though these techniques are less widely used.<sup>16,17</sup>

Results of a retrospective study<sup>18</sup> indicate no difference in implantation potential between embryos from which two cells are taken and non-biopsied embryos obtained after intracytoplasmic sperm injection. However, doubt remains about whether the biopsy of one cell would not be less detrimental to the embryo than biopsy of two cells. Randomised prospective studies to address this question are underway.

#### Blastocyst biopsy

The blastocyst is the latest stage at which an embryo can

be biopsied. The advantage of biopsy at this stage is that more cells can be obtained, but the disadvantages are that only about 36% of embryos mature this far and that little time is left for diagnosis, since embryos should be transferred before day 5 or  $6.^{19,20}$  Hence, blastocyst biopsy has been attempted by only one centre to date.<sup>21</sup>

# Analysis of cells obtained

PCR

PCR is used to amplify sufficient DNA from cells obtained from an oocyte or embryo to diagnose monogenic diseases.<sup>1</sup> In brief, a polar body or a blastomere is placed in a solution that lyses the cell and releases the DNA. A PCR reaction mix is then added and PCR begins. Because of its high sensitivity, contamination of the study sample with extraneous DNA is a danger and has led to the adoption of rigorous laboratory procedures and standards, such as the use of intracytoplasmic sperm injection.<sup>15</sup> Moreover, amplification of only one, rather than both, of the genes present in a cell can result in misdiagnosis of disease and the transfer and implantation of affected embryos.<sup>22</sup> To overcome this potential difficulty, dubbed allele-drop out, various techniques have evolved for the analysis of PCR fragments; fluorescent PCR and fragment analysis on automated sequencers were introduced first and, later, multiplex PCR was developed.<sup>1</sup> Since then, the introduction of automated sequencing,<sup>23</sup> minisequencing, and real-time PCR has further refined our diagnostic capabilities.<sup>24-28</sup>

#### FISH

FISH is the most frequently used method for analysis of the chromosomal complement of blastomeres.<sup>2,7-10</sup> Collected cells are spread on a glass microscope slide, after which DNA probes labelled with fluorochromes specific for the chromosomes of interest are applied to their nuclei (figure 2). The type and number of FISH probes used depends on the indication: for sexing, only two probes are needed (for the X and Y chromosomes), though probes for autosomal chromosomes are often added to check for aneuploidy; for identification of translocations, two or three probes are used as explained later; for aneuploidy screening, the number of chromosomes that can be analysed simultaneously is limited by the number of fluorochromes available-kits that contain probes for chromosomes X, Y, 13, 18, and 21 (figure 3), or for chromosomes X, Y, 13, 16, 18, 21, and 22 are available, but separate probes can only usually be obtained with a red or a green label. Mixing of two colours and the application of two, or even three, rounds of FISH can enhance the number of chromosomes analysed simultaneously to nine with acceptable efficiency. In large series, the approximate FISH error level is about 10%.<sup>2,29-31</sup>

## Comparative genomic hybridisation

Another way to identify chromosomal aberrations is to use comparative genomic hybridisation, a new technique introduced by two groups.<sup>32–35</sup> Here, pre-amplified DNA from a single test cell is labelled with one fluorochrome eg, red—and then mixed with pre-amplified DNA from a control sample labelled with a different fluorochrome eg, green—with which it is compared. The mixture is



Figure 2: Principle of FISH



Figure 3: **Result of five-colour FISH applied to nuclei of single blastomere** Different chromosomes are identified with their number. A: male nucleus (X and Y chromosome present). B: female nucleus (two X chromosomes present).

applied to a normal metaphase spread, and the colour ratio measured. Areas with more red indicate that the test sample contained more of this genetic material, whereas areas showing more green indicate that the test sample contained too little of this genetic material.

The advantage of comparative genomic hybridisation over FISH is that the whole chromosome complement is analysed, though polyploidy and balanced translocations cannot be detected. The disadvantage is that the whole procedure takes about 72 h, which is why the method is either applied to polar bodies, leaving 5 days for analysis, or to cells obtained at the cleavage stage of development, after which embryos are cryopreserved and transferred in a later cycle if identified as healthy.<sup>34,35</sup> The time taken to complete the procedure could be shortened by replacing the metaphase spread with microarrays of carefully chosen sequences from all 24 chromosomes, so-called microarray comparative genomic hybridisation.<sup>36</sup> This technique is described under future developments.

#### **Cryopreservation of biopsied embryos**

After PGD, few embryos usually remain for cryopreservation.<sup>6</sup> Although most centres do cryopreserve surplus embryos, the survival rate of the embryos is extremely low, and only one pregnancy has been described after standard procedures were followed.<sup>37-39</sup> Jericho and colleagues<sup>40</sup> have, however, adapted these standard procedures to make the freezing and thawing processes more gentle, and have successfully applied their new technique to embryos analysed with comparative genomic hybridisation. The group<sup>40</sup> noted a survival rate of 75% (by comparison with 43% with the standard protocol) and an implantation rate of 12%; 50 embryos were transferred in 36 transfer procedures, leading to six ongoing pregnancies.

# Indications for PGD and PGD-AS Monogenic diseases

PCR was the first technique developed for the analysis of DNA from single cells. That monogenic diseases were the first genetic abnormalities to be diagnosed in embryos is, therefore, not surprising. PGD in general and single-cell PCR in particular are demanding labour-intensive techniques however, so only the most common monogenic diseases have been studied extensively. One such disease is cystic fibrosis, which has a prevalence of one in 2500 births in white people and is caused, in most instances, by the same mutation—namely the  $\Delta$ F508 mutation. A multitude of reports describe protocols for preimplantation diagnosis of the disease,<sup>13,14,41-48</sup> which was the first to be identified by PGD.<sup>41</sup> Several methods of PGD have also been described for a group of autosomal recessive diseases with a high prevalence in certain populations—ie, defects in the  $\beta$  globin gene, such as sickle-cell anaemia (in African populations).<sup>49-53</sup> Spinal muscular atrophy has also received a lot of attention.<sup>54-56</sup>

Autosomal dominant diseases—eg, myotonic dystrophy, $^{57,58}$  Huntington's disease, $^{59,60}$  and Marfan's syndrome $^{61-63}$ —are less prevalent than autosomal recessive diseases. However, because the risk to their offspring is 50%, rather than 25% for recessive diseases, individuals with an autosomal dominant disease will more readily opt for PGD.

X-linked diseases, such as Duchenne muscular dystrophy and hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency, were among the first for which PGD was undertaken.3 With a simple technique, the sex of the embryos is ascertained and all male embryos disposed of. A drawback of this approach is that half of the discarded male embryos are healthy and half of the female embryos transferred are carriers of the condition. For more and more X-linked diseases, the specific genetic defect has now been identified, however, allowing a specific DNA diagnosis. Again, most reports are for PGD methods for the more frequent X-linked diseases, such as Duchenne muscular dystrophy and fragile X syndrome64-67 (which should not be diagnosed through sexing because girls can also be affected). Ethically, for X-linked recessive diseases, most would agree that to transfer non-carrier girls and healthy boys is preferable than to transfer carrier girls.68,69

The third report of the European Society for Human Reproduction and Embryology (ESHRE) PGD consortium,<sup>6</sup> which presents data from 26 different PGD centres, lists 33 monogenic diseases for which PGD had been applied. The list is comparable to the list of diseases for which prenatal diagnosis is undertaken, though it is shorter. We do not believe that the list will increase in

THE LANCET • Vol 363 • May 15, 2004 • www.thelancet.com

	Indication	Method	Number of treatment cycles	Number of clinical pregnancies (gestation >20 weeks)
Reference				
Ao et al, 1998 <sup>70</sup>	Familial adenomatous polyposis coli	Primer extension pre-amplification+PCR for mutation +PCR for marker	1	0
Strom et al, 199871	Autosomal dominant retinitis pigmentosa	First and second polar body, nested multiplex	1	0
Strom et al, 199814	Cystic fibrosis	First and second polar body, nested multiplex	3	3
Kuliev et al, 199951	β thalassaemia	First and second polar body, nested multiplex	17	7
Van de Velde et al, 199972	21-hydroxylase deficiency	Fluorescent PCR of mutation	2	1
Kanavakis et al, 199953	$\beta$ thalassaemia	Denaturing gradient gel electrophoresis for 12 different mutations	11	6
Sermon et al, 199963	Marfan's syndrome	Fluorescent PCR of marker	3	0
Ray et al, 200073	Ornithine transcarbamylase deficiency	Nested PCR (mutation+marker)	2	1
Dreesen et al, 200044	Cystic fibrosis	Fluorescent marker multiplex	NA	NA
Sermon et al, 2000 <sup>74</sup>	Medium-chain acyl-CoA dehydrogenase deficiency	Fluorescent PCR of mutation	1	1
De Rycke et al, 2001 <sup>50</sup>	β thalassaemia, sickle-cell anaemia	Fluorescent PCR of mutation	12	3
Ray et al, 200165	Duchenne muscular dystrophy	Nested multiplex	NA	NA
Georgiou et al, 200175	Spinal and bulbar muscular atrophy	Fluorescent PCR of mutation	1	1
Sermon et al, 2001 <sup>58</sup>	Myotonic dystrophy, Huntington's disease, fragile X syndrome	Nested and fluorescent PCR of mutation	92	19
Piyamongkol et al, 200157	Myotonic dystrophy	Fluorescent multiplex (mutation+marker)	3	2
Moutou et al, 200247	Cystic fibrosis	Fluorescent multiplex (mutation+marker)	3	0
Harper et al, 2002 <sup>76</sup>	Myotonic dystrophy, cystic fibrosis, fragile X syndrome and cystic fibrosis, neurofibromatosis 2, Crouzon	Fluorescent multiplex	13	4
Sermon et al, 2002 <sup>77</sup>	Huntington's disease exclusion	Fluorescent multiplex	7	1
Hussey et al, 2002 <sup>23</sup>	β thalassaemia	Fluorescent sequencing	8	2
Rechitsky et al, 2002 <sup>78</sup>	Familial adenomatous polyposis coli, von Hippel-Lindau disease, retinoblastoma, Li-Fraumeni syndrome, neurofibromatosis 1 and 2, familial posterios fossa brain tumour	First and second polar body, nested multiplex	20	5
De Vos et al. 200379	Charcot-Marie-Tooth disease type 1A	Fluorescent multiplex	13	3
Fiorentino et al. 2003 <sup>25</sup>	Cystic fibrosis. ß thalassaemia, sickle-cell	Fluorescent multiplex and minisequencing	55	15
	anaemia, haemophilia A, retinoblastoma, spinal muscular atrophy			10
Girardet et al, 2003 <sup>80</sup>	Retinoblastoma	Fluorescent multiplex	1	0
Moutou et al, 2003 <sup>81</sup>	Achondroplasia	Fluorescent PCR of mutation	4	0
Rechitsky et al, 2003 <sup>82</sup>	Familial dysautonomia	First and second polar body, nested multiplex	1	1
Goossens et al, 2003 <sup>83</sup>	Cystic fibrosis	Fluorescent multiplex (mutation+marker)	22	4

NA=not applied clinically.

Monogenic diseases for which PGD protocols have been assessed

length at a rapid rate, because only large PGD centres have the time and resources needed to research and develop the needed tests. The table gives an overview of monogenic diseases for which PGD has been assessed in individuals with up-to-date technologies.<sup>14,23,25,44,47,50,51,53,57,58,63,65,70-83</sup>

#### **Ethically difficult indications**

Since PGD can be viewed as an early form of prenatal diagnosis, many believed that the indications for both types of diagnosis would be similar. However, for some diseases, PGD but not prenatal diagnosis can be defended from an ethical point of view.

In non-disclosure PGD, which has been described for Huntington's disease but could also be applied to other late-onset diseases, patients do not wish to know their carrier status but want to have disease-free offspring. Their embryos are, therefore, tested without revealing any of the details of the cycle or diagnosis.<sup>60</sup> Because non-disclosure testing puts practitioners in a difficult ethical position—eg, having to undertake PGD cycles even when the results of previous cycles preclude the individual as a carrier, or having to do mock-transfers if no embryos are available the ESHRE ethics task force discourages non-disclosure testing, encouraging instead exclusion testing,<sup>68,84</sup> which involves assessment of embryos with linked markers for the presence of a chromosome from the affected grandparent; the mutation itself is not analysed.

Another new indication for PGD involves the selection of embryos, according to their HLA type, so that a child born out of a PGD cycle can be a stem-cell donor for a sick sibling. The first such case was described by Verlinsky and colleagues<sup>85</sup> and concerned a family with a child who had Fanconi anaemia. In this instance, PGD was undertaken to select embryos not affected with Fanconi anaemia, but which had the same HLA type as the affected sibling. This application of PGD is controversial.

The main ethical arguments against are that a child is being used as a treatment, hence breaching the Kantian imperative that a person should never be used as a means. Furthermore, how will the child feel when they are told that they were made for the purpose of saving a sibling? Pennings and colleagues,<sup>86</sup> however, argue that the Kantian imperative is not violated because the future donor child is not solely made for the purpose of donating stem cells, but will also be loved and cared for by the family as an individual. Careful counselling by a psychologist should be arranged before PGD is used for this purpose to ascertain the real motivations of the prospective parents. Pennings and co-workers also note that a child who is told that they were made to save a sibling will not experience feelings of low self-esteem but will, on the contrary, be proud to have contributed in such an important way to the well-being of a sibling. Furthermore, giving cord blood at birth could be considered something one family member would do naturally for another. Finally, they argued that parents would not hesitate to volunteer an existing child as a bonemarrow donor for a sick sibling, and that it is ethically acceptable to make a child for a reason if it is acceptable to use an existing child for the same reason. Several other authors have reached the same conclusion.68,69,87

REVIEW

The use of PGD to diagnose risk of late-onset diseases (such as Huntington's disease and Alzheimer's disease) and to search for genes that predispose for cancer (BRCA1, BRCA2, Li-Fraumeni, neurofibromatosis 1 and 2) is also ethically debatable.59,60,76,78,88 These two groups are distinct in pathology: late-onset genetic diseases have full penetrance, cancer predisposition genes do not; late-onset diseases are often not curable, whereas cancer predisposition syndromes can be treated, although the value of treatment needs to be demonstrated. However, the ethical arguments with respect to PGD for these diseases are quite similar. Many believe PGD is ethically acceptable for these indications because of the heavy burden imposed on patients who are carriers of one of these diseases, be it because of the burden imposed by the eventual fate in late-onset diseases, or because of the burden on the quality of life imposed by far-reaching preventive measures in cancer predisposition syndromes.68,69,87

Finally, several reports have been published on the use of sexing for social reasons and have provoked mixed reactions.<sup>6,68,89-91</sup> Opponents argue that sex selection for non-medical purposes is sexist and tantamount to discrimination against women. Those in favour argue that sex selection at the embryonic stage of development is preferable to sex selection after prenatal diagnosis, or even after birth. Moreover, they claim that overall sex distribution will not be skewed because only a few people will use PGD to choose their child's sex.<sup>89</sup> A distinction should be made, however, between sex selection for convenience and for so-called family balancing. In the latter instance, the family should have at least one or two children of one sex before they can ask for a child of the opposite sex.<sup>90</sup> In family balancing, the issues of sex discrimination and skewed sex ratios are avoided, giving the family an increased autonomy without conflicts with other ethical principles.84,87,90

# Sex-linked diseases

Women who are carriers of a recessive X-linked disease have an a-priori risk of 25% of having an affected boy, but are themselves, like their carrier daughters, healthy. Hence, sexing of embryos with FISH has been used extensively to avoid the birth of boys affected by X-linked disease.8,92 As noted previously, however, half of discarded male embryos are healthy and carrier girls cannot be distinguished from healthy girls with this technique. If available, individuals therefore prefer specific DNA diagnosis. Nonetheless, sexing remains a useful method when the gene responsible for a disease is unknowneg, non-fragile X, X-linked mental retardation-when the search for a specific mutation is judged too difficulteg, X-linked retinitis pigmentosa-or when individuals do not want to wait for the specific test to be developedeg, when the woman is already aged older than 37 years.

# **Chromosomal abnormalities**

# Reciprocal and Robertsonian translocations

Reciprocal translocations are characterised by the exchange of fragments of DNA between chromosomes, whereas in Robertsonian translocations a whole chromosome is translocated to another through centromeric fusion. Healthy carriers of such translocations are at risk of having children with congenital anomalies and mental retardation due to chromosomal imbalances, or are likely to have recurrent miscarriages or to be infertile (especially if the man is a carrier).

To offer PGD to female carriers of balanced translocations, Munné and colleagues<sup>9</sup> analysed the first

polar body of their eggs; if biopsied within a couple of hours after oocyte retrieval, first polar bodies are still in metaphase and can be assessed by chromosome painting—ie, a type of FISH in which chromosomes are identified over their entire length. A major drawback of this method is that only translocations carried by the mother can be analysed. The current most widely used method hence involves analysis of cleavage-stage embryos. Here, two FISH probes are used for Robertsonian translocations, one locus-specific probe centromeric to the breakpoint of one of the chromosomes involved, and one on the telomere of the other chromosome involved, and three FISH probes are used for assessment of reciprocal translocations-two on the centromeric side of the breakpoints of the chromosomes involved, and a third on one of the telomeres on the other side of the breakpoint. The two or three probes used have to carry a different fluorochrome, and it must be possible to use them together in one assay.<sup>10,93,94</sup> Identification of the probes needed for a particular couple can be difficult, since commercially available probes usually come in only two different fluorochromes (red and green). Once identified, the combination of probes centromeric and telomeric to the breakpoints allows embryos that carry an unbalanced chromosome complement to be distinguished from healthy ones, but cannot help to distinguish between embryos without the translocation or carrying the balanced translocation. Since reciprocal translocations are often different for each couple, a new probe mixture needs to be tested in each instance. Moreover, several centres have shown that a large proportion (over 80%) of embryos chromosomally abnormal and cannot be are transferred,6,93-97 leading to a low pregnancy rate per cycle (but not per transfer) in individuals with reciprocal translocations. However, because of the occurrence of multiple miscarriages or infertility in these patients, PGD is often the only way to achieve an ongoing healthy pregnancy.

# Aneuploidy screening

Results of investigations<sup>98,99</sup> with classic cytogenetic methods indicate that between 23% and 80% of embryos are aneuploid, and use of FISH is providing some information about the types of chromosomal abnormalities and why they arise. For example, Delhanty and colleagues<sup>100</sup> undertook FISH with probes for the X and Y chromosomes to assess embryos discarded after PGD, and noted a range of anomalies, such as monosomy X and mosaics for trisomy/triploidy, and Munné and co-workers<sup>101</sup> noted, after analysis of normally as well as abnormally developing embryos with five-colour FISH for chromosomes X, Y, 13, 18, and 21, that 70% of the abnormally developing embryos were abnormal for at least one of the chromosomes studied. Furthermore, analysing the chromosomal abnormalities in 1255 nonviable cleavage-stage embryos, Marquez and colleagues102 showed that aneuploidy increases with maternal age (from 1.4% in patients aged 20-34 years to 52.4% in patients aged 40-47 years), whereas polyploidy and mosaicism are related to poor embryo morphology. Considering these data, together with the well documented correlation between advanced maternal age and fetal aneuploidy and the recognition that 50-60% of all spontaneous abortions from clinically recognised pregnancies carry a grossly abnormal and lethal chromosome complement,103 researchers soon realised that embryo selection based on chromosome complement would improve IVF results in individuals with poor outcome and reduce the number of babies born with chromosomal defects.

PGD-AS has been used to identify problems in individuals of advanced maternal age,<sup>29,104</sup> in those in whom embryos have repeatedly not implanted, and in women who have recurrent miscarriages not due to translocations.<sup>2,100,105</sup> PGD-AS has, in fact, become the most widely used application of PGD, because of the relative ease of the technique compared with others and because of the large potential group of patients.6 The misdiagnosis rate after biopsy of one blastomere was estimated to be 7% in a large series;<sup>29</sup> nearly 6% of blastomeres are misdiagnosed due to mosaicism. Findings of large retrospective series<sup>106,107</sup> have indicated a higher implantation rate after polar-body biopsy than without selection through biopsy, and a nonsignificant increase in implantation rate with a significant decrease in spontaneous abortion rate after single blastomere biopsy. More recent data<sup>108</sup> indicate that the use of eight or nine different probes (X, Y, 13, 14, 15, 16, 18, 21, and 22) for diagnosis greatly increases implantation rates. However, prospective randomised studies are needed before the real efficiency of PGD-AS is known.<sup>2</sup>

# Outcome

#### Misdiagnosis

Reports of misdiagnoses are anecdotal, making an approximation of rates of misdiagnosis difficult. In the early days of PGD, one misdiagnosis for sexing, using PCR, and one misdiagnosis for cystic fibrosis in a compound heterozygote embryo were reported. Both misdiagnoses were due to the low efficiency of single-cell PCR, and technical developments—eg, FISH for sexing and multiplex fluorescent PCR—have ruled out the reoccurrence of such errors.<sup>22</sup> Munné and colleagues<sup>107</sup> reported one misdiagnosis (trisomy 21 after aneuploidy screening) in a series of 57 pregnancies. Pickering and co-workers<sup>109</sup> reported one misdiagnosis, for spinal muscular atrophy, in a series of 18 pregnancies.

The third report of the ESHRE PGD consortium<sup>6</sup> details the occurrence of eight misdiagnoses in 451 (2%) pregnancies (or of eight in 265 [3%] pregnancies if only those fetal sacs for which a control had been undertaken are counted): five of 145 (3%) pregnancies after PCR (two for sexing [one for Duchenne muscular dystrophy and one for retinitis pigmentosa], one each for  $\beta$  thalassaemia, cystic fibrosis, and myotonic dystrophy) and three of 305 (1%) after FISH (one each for sexing for social reasons, translocation [11;22], and trisomy 21 [after PGD-AS]). We believe the reporting of these misdiagnoses was an important step in the identification of technical flaws and shortcomings, which have led to the adoption of stringent diagnostic criteria.

# **Pregnancy outcome**

Because of the newness and technical complexity of PGD, large series on the practice and outcome of the various techniques involved are rare. Within ESHRE, therefore, the ESHRE PGD consortium was formed to obtain data on PGD cycles and their outcome.<sup>6</sup>

Of all the treatment cycles in couples whose embryos were tested by PGD in the 26 centres which are members and contribute their data, clinical pregnancies resulted in: 62 of 368 (17%) after testing for structural chromosomal abnormalities, including translocations, 41 of 254 (16%) after sexing, and 119 of 575 (21%) after testing for monogenic diseases. These numbers are lower than would be expected in a regular IVF cycle (20–25%), though it is noteworthy that many embryos are diagnosed as affected or abnormal and cannot therefore be transferred.

These findings contrast with the results for PGD-AS, in which instance patients are not at high risk of having

genetically abnormal offspring, but which involves the selection of embryos most likely to implant in the womb. Here, of the 799 cycles reported,6 199 or 25% led to a clinical pregnancy in individuals with a previously poor prognosis because of advanced maternal age, repetitive IVF failure, and recurrent miscarriages. Results of further analyses of these figures indicate that women of advanced age who have had recurrent miscarriages are particularly likely to become pregnant (28%) after PGD-AS, whereas patients who have little success after IVF are less likely to become pregnant (8%). Although the PGD-AS data are not compared with a suitable control group, most believe that PGD-AS increases the chances of pregnancy for patients with a poor prognosis. Wilton noted that most of the large studies published on PGD-AS and its outcome did not include an appropriate control group,<sup>2,29,102-104</sup> but large, multicentre, randomised studies are underway that will, hopefully, provide information on the efficiency of PGD-AS and the groups of individuals most likely to benefit from it.

The ESHRE PGD consortium is also interested in the outcomes of pregnancies after PGD. The most important cause of morbidity and mortality is multiple pregnancy. For this aspect and many others, such as birthweight and rate of congenital malformations, statistics for children born after PGD are comparable to those for children born after intracytoplasmic sperm injection.

In their report,<sup>110</sup> the International Working Group on Preimplantation Genetics noted that more than 3000 PGD cycles had been undertaken by mid-2001 since the early 1990s, resulting in a pregnancy rate of about 24%. Nearly 700 children were born as a result of the pregnancies and 5% of the babies had some kind of abnormality. Furthermore, when data for this period from the four most active centres worldwide were combined, a total of 2774 PGD cycles are shown to have resulted in 2265 transfers of embryos to the womb and 652 subsequent clinical pregnancies (29%).<sup>110</sup> In the report,<sup>110</sup> it is stressed that outcome of pregnancy was comparable to that for IVF populations. The authors of a study on another large cohort of 109 children,<sup>111</sup> not included in the ESHRE PGD consortium report, also conclude that children born after PGD are comparable to those born after intracytoplasmic sperm injection with respect to pregnancy and birth parameters, and that PGD is a safe method of avoiding the birth of children with genetic defects.

# **Future developments**

New methods for diagnosis of monogenic diseases are being developed at a rapid rate, some of which are very suitable for use with single-cell PCR.

#### **Real-time PCR**

In real-time PCR, the accumulation of PCR product is measured during cycling, when PCR is in its exponential phase, instead of after the process is complete. To differentiate between different PCR products-eg, a gene with a mutation and a wild-type gene—molecular beacons are added to the reaction mixture. These beacons consist of short oligonucleotides complementary to the region of interest within the PCR fragment. The oligonucleotides have complementary ends, one of which carries a fluorochrome and the other a quencher, so that if they do not anneal to the DNA and remain in solution the beacons are folded on themselves and do not fluoresce.27,28 Other methods are also commercially available-eg, fluorescence resonance energy transfer (FRET). In realtime PCR, short PCR fragments are generated, thus reducing the likelihood of allele drop-out. Moreover,

continuous measurement of PCR product during the exponential phase of the process gives a more accurate idea of the homozygous or heterozygous state of a single cell. Real-time PCR has been used for PGD of sex, by analysing the presence of a Y chromosome, and for diagnosis of Tay-Sachs disease.<sup>27,28</sup> However, its use has not been described in the diagnosis of mitochondrial diseases, despite the fact that real-time PCR allows for quantitative measurements.

#### Minisequencing

Minisequencing permits the quick and accurate detection of single nucleotide polymorphisms (SNPs). SNPs are widespread in the genome and can be either true polymorphisms, which do not affect functioning of genes, or mutations. SNPs can be used as linked polymorphisms to enhance the accuracy of the PGD, in the same way that microsatellites are used in duplex and multiplex PCR for PGD. The advantage of SNPs is that they are more common than microsatellites, thus giving a higher chance of finding a linked informative SNP, and that they can be detected with short PCR fragments. When starting from a single cell, the amplification of small stretches of DNA is much more efficient than for larger fragments; thus SNP analysis is ideally suited for single-cell PCR. In PGD, only a few loci need to be analysed simultaneously-eg, two mutations and two SNPs-so minisequencing on microcapillary systems are ideal.<sup>25</sup>

#### **Microarrays**

Mutation-specific primers can also be spotted on microarrays,<sup>112</sup> which are another promising development. First described in the analysis of expression in cells, microarrays have moved from RNA to DNA technology. Small glass slides or other supports are covered with well-defined DNA fragments, to which fluorescently labelled sample DNA is then allowed to hybridise. The full array can be read by an automated system.

Microarrays could be used in PGD in two ways. First, they could be used to diagnose common genetic diseases caused by more than one mutation-eg, cystic fibrosis (associated with more than 1000 mutations) or  $\hat{\beta}$  thalassaemia.<sup>112</sup> Because only small stretches of DNA need to be amplified, preliminary work-up should be simple and straightforward, and analysis of microarrays could be standardised for all patients. Second, microarrays could be used in comparative genomic hybridisation, where they would replace the metaphase spread to which the mixture of test and comparative DNA are hybridised. In this instance, the microarray would contain not sequences from one gene but sequences spread over the whole genome. The specificity of the sequences in the microarrays would lead to shorter hybridisation and computer analysis times than arise with comparative genomic hybridisation, obviating the need for polar-body analysis or cryopreservation. Other advantages of microarray comparative genomic hybridisation would be its greater resolution (100-200 kb instead of 2-10 Mb) compared with the use of metaphase spreads, while the important standardisation of the whole procedure by use of computers would allow more IVF centres to use microarray comparative hybridisation genomic routinely for aneuploidy screening.2,36

Conflict of interest statement None declared.

#### Acknowledgments

We thank Catherine Staessen, Hubert Joris, and Elvire Van Assche for their advice about cryopreservation and aneuploidy issues, and for their critical reading of the manuscript. K Sermon is supported by a postdoctoral fellowship from the Fund for Scientific Research, Flanders. The PGD team is supported by grants from the Fund for Scientific Research Flanders, the Research Council of the Dutch-speaking Brussels Free University, the Forton Fund, and the Concerted Action of the Brussels Free University. None of the sponsors had a role in the research or writing of this Review.

#### References

- Sermon K. Current concepts in preimplantation genetic diagnosis (PGD): a molecular biologist's view. *Hum Reprod Update* 2002, 8: 1–10.
- 2 Wilton L. Preimplantation genetic diagnosis for aneuploidy screening in early human embryos: a review. *Prenat Diagn* 2002; 22: 312–18.
- 3 Handyside A, Kontogianni E, Hardy K, Winston R. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990; **344:** 768–70.
- 4 Coutelle C, Williams C, Handyside H, Hardy K, Winston R, Williamson R. Genetic analysis of DNA from single human oocytes: a model for preimplantation diagnosis of cystic fibrosis. *BMJ* 1989; 299: 22–24.
- 5 Holding C, Monk M. Diagnosis of beta-thalassaemia by DNA amplification in single blastomeres from mouse preimplantation embryos. *Lancet* 1989; 2: 532–35.
- 6 ESHRE PGD Consortium Steering Committee. ESHRE Preimplantation Genetic Diagnosis Consortium: data collection III (May 2001). *Hum Reprod* 2002; 17: 233–46.
- 7 Griffin D, Wilton L, Handyside A. Dual fluorescent in situ hybridisation for simultaneous detection of X and Y chromosomespecific probes for sexing of human preimplantation embryonic nuclei. *Hum Genet* 1992; 89: 18–22.
- 8 Staessen C, Van Assche E, Joris H, et al. Clinical experience of sex determination by fluorescent in situ hybridisation for preimplantation genetic diagnosis. *Mol Hum Reprod* 1999; 5: 392–99.
- 9 Munné S, Sandalinas M, Escudero T, Fung J, Gianaroli L, Cohen J. Outcome of preimplantation genetic diagnosis of translocations. *Fertil Steril* 2000; 73: 1209–18.
- 10 Conn C, Harper J, Winston R, Delhanty J. Infertile couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. *Hum Genet* 1998; 102: 117–23.
- Thornton K. Advances in assisted reproductive technologies. Obstet Gynecol Clin North Am 2000; 27: 517–27.
- 12 Liebaers I, Sermon K, Staessen C, et al. Clinical experience with preimplantation genetic diagnosis and intracytoplasmic sperm injection. *Hum Reprod* 1998; **13** (suppl 1): 186–95.
- 13 Verlinsky Y, Rechitsky S, Evsikov S, et al. Preconception and preimplantation diagnosis for cystic fibrosis. *Prenat Diagn* 1992; 12: 103–10.
- 14 Strom C, Ginsberg N, Rechitsky S, et al. Three births after preimplantation genetic diagnosis for cystic fibrosis with sequential first and second polar body analysis. Am J Obstet Gynecol 1998; 178: 1298–306.
- 15 Rechitsky S, Strom C, Verlinsky O, et al. Accuracy of preimplantation diagnosis of single-gene disorders by polar body analysis of oocytes. *J Assist Reprod Genet* 1999; 16: 192–98.
- 16 De Vos A, Van Steirteghem A. Aspects of biopsy procedures prior to preimplantation genetic diagnosis. *Prenat Diagn* 2001; 21: 767–80.
- 17 Inzunza J, Iwarsson E, Fridström M, et al. Application of singleneedle blastomere biopsy in human preimplantation genetic diagnosis. *Prenat Diagn* 1998; 18: 1381–88.
- 18 Van de Velde H, De Vos A, Sermon K, et al. Embryo implantation after biopsy of one or two cells from cleavage-stage embryos with a view to preimplantation genetic diagnosis. *Prenat Diagn* 2000; 20: 1030–37.
- 19 Verheyen G, Van Landuyt L, Joris H, Devroey P, Van Steirteghem A. Different embryo developmental patterns in different sequential media appear to result in similar pregnancy rates. *Hum Reprod* 2003; 18 (suppl 1): xviii37.
- 20 Veiga A, Sandalinas M, Benkhalifa M, et al. Laser blastocyst biopsy for preimplantation diagnosis in the human. Zygote 1997; 5: 351–54.
- 21 Boada M, Carrera M, De la Iglesia C, Sandalinas M, Barri PN, Veiga A. Successful use of a laser for human embryo biopsy in preimplantation genetic diagnosis: report of two cases. *J Assist Reprod Genet* 1998; 15: 302–07.
- 22 Lissens W, Sermon K. Preimplantation genetic diagnosis: current status and new developments. *Hum Reprod* 1997; 12: 1756–61.
- 23 Hussey N, Davis T, Hall J, et al. Preimplantation genetic diagnosis for β-thalassemia using sequencing of single cell PCR products to detect mutations and polymorphic loci. *Mol Hum Reprod* 2002; 8: 1136–43.
- 24 Syvänen A-C. From gels to chips: "Minisequencing" primer extension for analysis of point mutations and single nucleotide polymorphisms. *Hum Mutat* 1999; 13: 1–10.

- 25 Fiorentino F, Magli MC, Podini D, et al. The minisequencing method: an alternative strategy for preimplantation genetic diagnosis of single gene disorders. *Mol Hum Reprod* 2003; 9: 399–410.
- 26 Szuhai K, Ouweland J, Dirks R, et al. Simultaneous A8344G heteroplasmy and mitochondrial DNA copy number quatification in myoclonus epilepsy and ragged-red fibers (MERFF) syndrome by a multiplex molecular beacon based real-time fluorescence PCR. *Nucleic Acids Res* 2001; 29: E13.
- 27 Pierce K, Rice J, Aquiles Sanchez J, Brenner C, Wangh L. Real-time PCR using molecular beacons for accurate detection of the Y-chromosome in single human blastomeres. *Mol Hum Reprod* 2000, 6: 1155–64.
- 28 Rice J, Aquiles Sanchez J, Pierce K, Wangh L. Real-time PCR with molecular beacons provides a highly accurate assay for detection of Tay-Sachs alleles in single cells. *Prenat Diagn* 2002; 22: 1130–34.
- 29 Munné S, Sandalinas M, Escudero T, Marquez C, Cohen J. Chromosome mosaicism in cleavage-stage human embryos: evidence of a maternal age effect. *Repr BioMed Online* 2003; 4: 223–32.
- 30 Munné S, Magli C, Bahçe M, et al. Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. *Prenat Diagn* 1998; 18: 1459–66.
- 31 Pehlivan T, Rubio C, Rodrigo L, et al. Impact of preimplantation genetic diagnosis on IVF outcome in implantation failure patients. *Reprod BioMed Online* 2002; 6: 232–37.
- 32 Voullaire L, Slater H, Williamson R, Wilton L. Chromosome analysis of blastomeres from human embryos by using CGH. *Hum Genet* 2000; 105: 210–17.
- 33 Wells D, Delhanty J. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative gnomic hybridisation. *Mol Hum Reprod* 2000; 6: 1055–62.
- 34 Wilton L, Williamson R, McBain J, Edgar D, Voullaire L. Birth of a healthy infant after preimlantation confirmation of euploidy by comparative genomic hybridisation. N Engl J Med 2001; 345: 1537–41.
- 35 Wells D, Escudero T, Levy B, Hirschhorn K, Delhanty J, Munné S. First clinical application of comparative genomic hybridisation and polar body testing for preimplantation genetic diagnosis of aneuploidy. *Fertil Steril* 2002; 78: 543–49.
- 36 Wells D, Brynn L. Cytogenetics in reproductive medicine: the contribution of comparative genomic hybridisation (CGH). *BioEssays* 2003; 25: 289–300.
- 37 Joris H, Van den Abbeel E, De Vos A, Van Steirteghem A. Reduced survival after human embryo biopsy and subsequent cryopreservation. *Hum Reprod* 1999; 14: 2833–37.
- 38 Magli M, Gianaroli L, Fortini D, Ferrarreti A, Munné S. Impact of blastomere biopsy and cryopreservation techniques on human embryo viability. *Hum Reprod* 1999; 14: 770–73.
- 39 Lee M, Munné S. Pregnancy after polar body biopsy and freezing and thawing of human embryos. *Fertil Steril* 2000; 73: 645–47.
- 40 Jericho H, Wilton L, Gook D, Edgar D. A modified cryopreservation method increases the survival of human biopsied cleavage stage embryos. *Hum Reprod* 2003; 18: 568–71.
- 41 Handyside A, Lesko J, Tarin J, Winston R, Hughes M. Birth of a normal girl after in vitro ferilization and preimplantation diagnostic testing for cystic fibrosis. N Engl J Med 1992; 327: 905–09.
- 42 Liu J, Lissens W, Silber S, Devroey P, Liebaers I, Van Steirteghem A. Birth after preimplantation diagnosis of the cystic fibrosis  $\Delta$ F508 mutation by polymerase chain reaction in human embryos resulting from intracytoplasmic sperm injection with epididymal sperm. *JAMA* 1994; **272**: 23.
- 43 Moutou C, Viville S. Improvement of preimplantation genetic diagnosis (PGD) for cystic fibrosis mutation ΔF508 by fluorescent polymerase chain reaction. *Prenat Diagn* 1999; 19: 1248–50.
- 44 Dreesen J, Jacobs L, Bras M, et al. Multiplex PCR of polymorphic markers flanking the CFTR gene: a general approach for preimplantation genetic diagnosis of cystic fibrosis. *Mol Hum Reprod* 2000; 6: 391–96.
- 45 Goossens V, Sermon K, Lissens W, et al. Clinical application of preimplantation genetic diagnosis for cystic fibrosis. *Prenat Diagn* 2000; 20: 571–81.
- 46 Eftedal I, Schwartz M, Bendtsen H, Andersen A, Zieve S. Single intragenic microsatellite preimplantation genetic diagnosis for cystic fibrosis provides positive allele identification of all CFTR genotypes for informative couples. *Mol Hum Reprod* 2001; 7: 307–12.
- 47 Moutou C, Gardes N, Viville S. Multiplex PCR combining delta F508 mutation and intragenic microsatellites of the CFTR gene for pre-implantation genetic diagnosis (PGD) of cystic fibrosis. *Eur J Hum Genet* 2002; 10: 231–38.
- 48 Vrettou C, Tzetis M, Traeger-Synodinos J, Palmer G, Kanavakis E. Multiplex sequence variation detection throughout the CFTR gene appropriate for preimplantation genetic diagnosis in populations with heterogeneity of cystic fibrosis mutations. *Mol Hum Reprod* 2002; 8: 880–86.

- 49 Xu K, Shi Z, Veeck L, Hughes M, Rosenwaks Z. First unaffected pregnancy using preimplantation genetic diagnosis for sickle cell anemia. *JAMA* 1999; **281:** 1701–06.
- 50 De Rycke M, Van de Velde H, Sermon K, et al. Preimplantation genetic diagnosis for sickle-cell anemia and for β-thalassemia. *Prenat Diagn* 2001; 21: 214–22.
- 51 Kuliev A, Rechistky S, Verlinsky O, et al. Birth of healthy children after preimplantation diagnosis of thalassemias. J Assist Reprod Genet 1999; 16: 207–11.
- 52 Vrettou C, Palmer G, Kanavakis E, et al. A widely applicable strategy for single cell genotyping of β-thalassemia mutations using DGGE analysis: application to preimplantation genetic diagnosis. *Prenat Diagn* 1999, **19**: 1209–16.
- 53 Kanavakis E, Vrettou C, Palmer G, Tretis M, Mastrominas M, Traeger-Synodinos J. Preimplantation genetic diagnosis in 10 couples at risk for transmitting  $\beta$ -thalassemia major: clinical experience including the initiation of six singleton pregnancies. *Prenat Diagn* 1999; **19**: 1217–22.
- 54 Dreesen J, Bras M, de Die-Smulders C, et al. Preimplantation genetic diagnosis of spinal muscular atrophy. *Mol Hum Reprod* 1998; 4: 881–85.
- 55 Fallon L, Harton GL, Sisson ME, et al. Preimplantation genetic diagnosis for spinal muscular atrophy type I. *Neurology* 1999; 53: 1087–90.
- 56 Moutou C, Gardes N, Rongières C, et al. Allele-specific amplification for preimplantation genetic diagnosis (PGD) of spinal muscular atrophy. *Prenat Diagn* 2001; 21: 498–503.
- 57 Piyamongkol W, Harper J, Sherlock J, et al. A successful strategy for preimplantation genetic diagnosis of myotonic dystrophy using multiplex fluorescent PCR. *Prenat Diagn* 2001; 21: 223–32.
- 58 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. *Moll Cell Endocrinol* 2001; 183 (suppl 1): 77–85.
- 59 Sermon K, Goossens V, Seneca S, et al. Preimplantation diagnosis for Huntington's disease (HD): clinical application and analysis of the HD expansion in affected embryos. *Prenat Diagn* 1998; 18: 1427–36.
- 60 Stern H, Harton G, Sisson M, et al. Non-disclosing preimplantation genetic diagnosis for Huntington disease. *Prenat Diagn* 2002; 22: 303–07.
- 61 Harton G, Tsipouras P, Sisson M, et al. Preimplantation genetic testing for Marfan syndrome. Mol Hum Reprod 1996; 2: 713–15.
- 62 Blaszczyk A, Tang Y, Dietz H, et al. Preimplantation genetic diagnosis of human embryos for Marfan's syndrome. J Assist Reprod Genet 1998; 15: 281–84.
- 63 Sermon K, Lissens W, Messiaen L, et al. Preimplantation genetic diagnosis of Marfan syndrome with the use of fluorescent polymerase chain reaction and the Automated Laser Fluorescence DNA Sequencer. *Fertil Steril* 1999; **71:** 163–65.
- 64 Hussey N, Donggui H, Froiland D, et al. Analysis of five Duchenne muscular dystrophy exons and gender determination using conventional duplex polymerase chain reaction on single cells. *Mol Hum Reprod* 1999; 5: 1089–94.
- 65 Ray P, Vekemans M, Munnich A. Single cell multiplex PCR amplification of five dystrophin gene exons combined with gender determination. *Mol Hum Reprod* 2001; 7: 489–94.
- 66 Sermon K, Seneca S, Vanderfaeillie A, et al. Preimplantation diagnosis for Fragile X syndrome based on the detection of the non-expanded paternal and maternal CGG. *Prenat Diagn* 1999; 19: 1223–30.
- 67 Apessos A, Abou-Sleiman P, Harper J, Delhanty J. Preimplantation genetic diagnosis of the fragile X syndrome by use of polymorphic linked markers. *Prenat Diagn* 2001; **21:** 504–11.
- 68 Shenfield F, Pennings G, Devroey P, Sureau C, Tarlatzis B, Cohen J. Taskforce 5: preimplantation genetic diagnosis. *Hum Reprod* 2003; 18: 649–51.
- 69 de Wert G. Ethics of assisted reproduction. In: Fauser B, Bouchard P, Hsueh A, Rutherford A, Simpson JL, Strauss J, Van Steirteghem A, eds. Reproductive medicine: molecular, cellular and genetic fundamentals. London: Parthenon Publishing, 2003: 645–65.
- 70 Ao A, Wells D, Handyside A, Winston R, Delhanty J. Preimplantation genetic diagnosis of inherited cancer: familial adenomatous polyposis coli. J Assist Reprod Genet 1998; 15: 140–44.
- 71 Strom C, Rechitsky S, Wolf G, Cieslak J, Kuliev A, Verlinsky Y. Preimplantation diagnosis of autosomal dominant retinitis pigmentosum using two simultaneous single cell assays for a point mutation in the rhodopsin gene. *Mol Hum Reprod* 1998; 4: 351–55.
- 72 Van de Velde H, Sermon K, De Vos A, et al. Fluorescent PCR and automated fragment analysis in preimplantation genetic diagnosis for 21-hydroxylase deficiency in congenital adrenal hyperplasia. *Mol Hum Reprod* 1999; 5: 691–96.
- 73 Ray PF, Gigarel N, Bonnefont JP, et al. First specific preimplantation genetic diagnosis for ornithine transcarbamylase deficiency. *Prenat Diagn* 2000; 20: 1048–54.

1640

- 74 Sermon K, Henderix P, Lissens W, et al. Preimplantation genetic diagnosis for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. *Mol Hum Reprod* 2000; 6: 1165–68.
- 75 Georgiou I, Sermon K, Lissens W, et al. Preimplantation genetic diagnosis for spinal and bulbar muscular atrophy (SBMA). *Hum Genet* 2001: 108: 494–98.
- 76 Harper J, Wells D, Piyamongkol W, et al. Preimplantation genetic diagnosis for single gene disorders: experience with five single gene disorders. *Prenat Diagn* 2002; 22: 525–33.
- 77 Sermon K, De Rycke M, Lissens W, et al. Preimplantation genetic diagnosis for Huntington's disease with exclusion testing. *Eur J Hum Genet* 2002; 10: 591–98.
- 78 Rechitsky S, Verlinsky O, Chistokhina A, et al. Preimplantation genetic diagnosis for cancer predisposition. *Reprod BioMed Online* 2002; 4: 148–55.
- 79 De Vos A, Sermon K, De Rycke M, et al. Preimplantation genetic diagnosis for Charcot–Marie-Tooth disease type 1A. *Molec Hum Reprod* 2003; 9: 429–35.
- 80 Girardet A, Hamamah S, Anahory T, et al. First preimplantation genetic diagnosis of hereditary retinoblastoma using informative microsatellite markers. *Mol Hum Reprod* 2003; 9: 111–16.
- 81 Moutou C, Rongieres C, Bettahar-Lebugle K, Gardes N, Philippe C, Viville S. Preimplantation genetic diagnosis for achondroplasia: genetics and gynaecological limits and difficulties. *Hum Reprod* 2003; 18: 509–14.
- 82 Rechitsky S, Verlinsky O, Kuliev A, et al. Preimplantation genetic diagnosis for familial dysautonomia. *Reprod BioMed Online* 2003; 6: 488–93.
- 83 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 ΔF508 mutation. *Molec Hum Reprod* 2003; 9: 559–67.
- 84 Sermon K, De Rijcke M, Lissens W, et al. Preimplantation genetic diagnosis for Huntington's disease with exclusion testing. *Eur J Hum Genet* 2002; 10: 591–98.
- 85 Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA 2001; 285: 3130–33.
- 86 Pennings G, Schots R, Liebaers I. Ethical considerations on preimplantation genetic diagnosis for HLA typing to match a future child as a donor of haematopoietic stem cells to a sibling. *Hum Reprod* 2002; 17: 534–38.
- 87 Robertson J. Extending preimplantation genetic diagnosis: the ethical debate—ethical issues in new uses of preimplantation genetic diagnosis. *Hum Reprod* 2003; 18: 465–71.
- 88 Verlinsky Y, Rechitsky S, Verlinsky O, Masciangelo C, Lederer K, Kuliev A. Preimplantation diagnosis for early-onset Alzheimer disease caused by V717L mutation. *JAMA* 2002; 287: 1018–21.
- 89 Malpani A. The use of preimplantation genetic diagnosis in sex selection for family balancing in India. *Reprod BioMed Online* 2002; 4: 16–20.
- 90 Pennings G. Personal desires of patients and social obligations of geneticists: applying preimlantation genetic diagnosis for non-medical sex selection. *Prenat Diagn* 2002; 22: 1123–29.
- 91 Ray P, Munnich A, Nisand I, Frydman R, Vekemans M, Viville S. Sex selection by preimplantation genetic diagnosis: should it be carried out for social purposes?: Is preimplantation genetic diagnosis for "social sexing" desirable in today's and tomorrow's society? *Hum Reprod* 2003; 18: 463–64.
- 92 Harper JC, Coonen E, Ramaekers FC, et al. Identification of the sex of human preimplantation embryos in two hours using an improved spreading method and fluorescent in-situ hybridization (FISH) using directly labelled probes. *Hum Reprod* 1994; 9: 721–24.

- 93 Coonen E, Martini E, Dumoulin J, et al. Preimplantation genetic diagnosis of a reciprocal translocation t(3;11)(q27.3;q24.3) in siblings. *Mol Hum Reprod* 2000; 6: 199–206.
- 94 Van Assche E, Staessen C, Vegetti W, et al. Preimplantation genetic diagnosis and sperm analysis by fluorescence in situ hybridisation for the most common reciprocal translocation t(11;22). *Mol Hum Reprod* 1999; 5: 682–90.
- 95 Evsikov S, Cieslak J, Verlinsky Y. Effect of chromosomal translocations on the development of preimplantation human embryos in vitro. *Fertil Steril* 2000; 74: 672–77.
- 96 Iwarsson E, Malmgren H, Inzunza J, et al. Highly abnormal cleavage divisions in preimplantation embryos from translocation carriers. *Prenat Diagn* 2000; 20: 1038–47.
- 97 Gianaroli L, Magli MC, Ferraretti AP, et al. Possible interchromosomal effect in embryos generated by gametes from translocation carriers. *Hum Reprod* 2002; 17: 3201–07.
- 98 Zenzes MT, Casper RF. Cytogenetics of human oocytes, zygotes, and embryos after in vitro fertilization. *Hum Genet* 1992; 88: 367–75.
- 99 Pellestor F, Girardet A, Andréo B, Arnal F, Humeau C. Relationship between morphology and chromosomal constitution in human preimplantation embryo. *Mol Reprod Dev* 1994; **39**: 141–46.
- 100 Delhanty J, Griffin D, Handyside A, et al. Detection of aneuploidy and chromosomal mosaicism in human embryos during preimplantation sex determination by fluorescent in situ hybridisation, (FISH). *Hum Mol Genet* 1993; 2: 1183–85.
- 101 Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. *Hum Reprod* 1993; 8: 2185–91.
- 102 Marquez C, Sandalinas M, Bahçe M, Alikani M, Munné S. Chromosome abnormalities in 1255 cleavage-stage human embryos. *Reprod BioMed Online* 2000; 1: 17–26.
- 103 Boué A, Boué J, Gropp A. Cytogenetics of pregnancy wastage. Adv Hum Genet 1985; 14: 1–57.
- 104 Verlinsky Y, Cieslak J, Ivakhnenko V, et al. Prevention of age-related aneupoidies by polar body testing of oocytes. *J Assist Reprod Genet* 1999; 16: 165–69.
- 105 Rubio C, Simon C, Vidal F, et al. Chromosomal abnormalities and embryo development in recurrent miscarriage couples. *Hum Reprod* 2003; 18: 182–88.
- 106 Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y. Chromosomal abnormalities in a series of 6733 human oocytes in preimplantation diagnosis for age-related aneuploidies. *Reprod BioMed Online* 2002; 6: 54–59.
- 107 Munné S, Magli C, Cohan J, et al. Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. *Hum Reprod* 1999; 14: 2191–99.
- 108 Munné S, Cohen J, Sable D. Preimplantation genetic diagnosis for advanced maternal age and other indications. *Fertil Steril* 2002; 78: 234–36.
- 109 Pickering S, Polidoropoulos N, Caller J, et al. Strategies and outcomes of the first 100 cycles of preimplantation genetic diagnosis at the Guy's and St Thomas' Center. *Fertil Steril* 2003; 79: 81–90.
- 110 Anon. Report of the 11<sup>th</sup> annual meeting of International Working Group on Preimplantation Genetics: preimplantation genetic diagnosis—experience of 3000 clinical cycles. *Reprod BioMed Online* 2001; **3:** 49–53.
- 111 Strom C, Levin R, Strom S, Masciangelo C, Kuliev A, Verlinsky Y. Neonatal outcome of preimplantation genetic diagnosis by polar body removal: the first 109 infants. *Pediatrics* 2000; **106:** 650–53.
- 112 Kurg A, Tonisson N, Georgiou I, Shumaker J, Tollett J, Metspalu A. Arrayed primer extension: solid-phase four-color DNA resequencing and mutation detection technology. *Genet Test* 2000; 4: 1–7.

For personal use. Only reproduce with permission from The Lancet.