

Chapter 2

Preimplantation Genetic Screening and Diagnostic Testing

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Introduction

Since preimplantation genetic diagnosis (PGD) was first accomplished 25 years ago, thousands of in vitro fertilization (IVF) cycles with PGD have been performed worldwide (www.pgdis.org). The first 1000 PGD births occurred by 2004 (Verlinsky et al. 2004), and since then the pace has accelerated. PGD has evolved from simply an extension of traditional prenatal genetic diagnosis to a method with additional, novel, indications. In this update, we shall consider traditional and novel indications, approaches to obtaining cell(s) for PGD, diagnostic accuracy and methods to maximize accuracy, given the small sample available for diagnosis. That PGD obviates certain ethical dilemmas, yet poses novel controversies, will be discussed.

History

Although usually considered a recent idea, PGD has actually long been envisioned (see Harper (2009) for detailed history). In 1968, Gardner and Edwards biopsied a rabbit blastocyst and performed X-chromatin analysis, suggesting application to human X-linked recessive traits. Over the next decades, mouse geneticists demonstrated the ability to obtain metaphase chromosomes from murine blastomeres (Dyban 1991). However, progress in human PGD was unavoidably delayed until IVF, was successful in 1978 (Steptoe and Edwards 1978). Thereafter, animal studies paved the way for human PGD. Monk and various colleagues biopsied mouse

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blastomeres, showing feasibility for detecting a single-gene disorder (Monk and Handyside 1988). Pioneering work by Wilton and Trounson in Melbourne (1989) and by Nijs and Van Steirteghem (1987) in Brussels was especially noteworthy.

With the molecular diagnostic era of the late 1980s, polymerase chain reaction (PCR) made PGD practical. In Chicago, Verlinsky and colleagues developed polar body biopsy and showed clinical application, reporting in 1987 at an international IVF Congress (Verlinsky et al. 1987); their peer publication of this PGD diagnosis (alpha-1 antitrypsin) was delayed until 1990 (Verlinsky et al. 1990). In 1990, PGD for cystic fibrosis was reported by the same group (Strom et al. 1990), again using polar body biopsy. In the UK, Handyside and colleagues pursued blastomere biopsy on cleavage stage (3 day) embryos, in 1990 determining sex in a pregnancy at risk for ornithine transcarbamylase deficiency (OTC), an X-linked disorder (Handyside et al. 1990). This was soon followed by detection of cystic fibrosis, using nested primer PCR (Handyside et al. 1992).

Progress in detecting chromosomal abnormalities awaited development of fluorescence in situ hybridization (FISH) and chromosome-specific probes. In the UK, Griffin successfully performed FISH on blastomeres (Griffin et al. 1991), and in the USA, Grifo did so (Grifo et al. 1990, 1992a). Working with Cohen at Cornell Medical College (New York), a blastomere was subjected to X and Y FISH (Grifo et al. 1992b) to determine sex. Munné applied multicolor FISH to a single blastomere, setting the stage for aneuploidy testing (Munné et al. 1993). Munné also applied FISH for PGD of chromosomal translocations (Munné et al. 1998a, 2000). In the UK, Delhanty and Harper were performing rapid FISH (Harper et al. 1994), and in the USA, Verlinsky et al. (1995) independently applied FISH to polar bodies. These initial approaches permitted concurrent analysis of only a limited number of chromosomes (5–7). At present, chromosomal abnormalities are detected by array CGH or other 24 chromosome approaches to be discussed below.

Obtaining Cells for Preimplantation Genetic Diagnosis

PGD requires a nucleus (DNA) from gametes or from embryos prior to implantation (6 days after conception). There are three potential approaches: (1) polar body biopsy, (2) blastomere biopsy (aspiration) from the 3 day six- to eight-cell cleaving embryo and (3) trophectoderm biopsy from the 5- to 6-day blastocyst.

Polar Biopsy

The oocyte genome—chromosomes or genes—can be deduced by analysis of the first and second polar biopsy (Kuliev et al. 2014). If the first polar body from a heterozygous individual with a mutant allele is abnormal, it must be complemented by a primary oocyte having the normal allele. In such a situation, oocytes deduced to

be genetically normal can be allowed to fertilize in vitro; the resulting embryo can be transferred for potential implantation. Conversely, a normal first polar body indicates an abnormal oocyte; thus, fertilization would not proceed. The same principle applies to cytogenetic analysis. If the first polar body does not show a chromosome 21, the oocyte must be presumed to have two number 21 chromosomes. If allowed to be fertilized by a normal (23 chromosomes) sperm, the result would be a trisomic zygote. The principle of complementation also dictates that if the first polar body were to have one number 21 chromosome, the oocyte would also have only one and, hence, be suitable for fertilization and transfer.

The obvious disadvantage of polar body diagnosis is the inability to assess paternal genotype, thus precluding application if the father has an autosomal dominant disorder. Polar body analysis is also less efficient in managing couples at risk for offspring with autosomal recessive disorders because even if the maternal allele is transmitted, the paternal allele might not be (normal sperm). In that case, the embryo would have been normal (heterozygous) like its parents but, not knowing the status of the paternal allele, this embryo would not be eligible for transfer. On the other hand, because chromosomal trisomy usually originates in maternal meiosis, in 90–95 % of all cases PGD is applicable for chromosome diagnosis.

PGD by polar body analysis must take into account recombination. At least one recombinational event occurs on each pair of chromosomes, thus maintaining orderly disjunction. If recombination were not to occur in the region or chromosome in question, the second polar body would be identical to the oocyte. If, however, crossing over were to involve the region containing the gene in question, the two chromatids of the single chromosome in the first polar body would differ and in aggregate show both alleles (heterozygosity). Given recombination, genotype of the oocyte could not be predicted without either biopsy of the second polar body or biopsy of the embryo *itself*. Actually, biopsy of polar bodies followed by biopsy of the embryo at the cleavage stage does not seem to decrease pregnancy rates compared to either alone (Cieslak-Janzen et al. 2006). In practice, both first and second polar bodies are typically biopsied unless a specific reason exists to the contrary. See below for one such an indication.

Cleavage Stage Embryo (8-Cell Blastomere Biopsy)

In the 3-day (8-cell embryo), a glycoprotein layer surrounds the embryo. This zona must be traversed by mechanical, laser or chemical means in order to extract a cell(s). The usual approach is to remove only one cell (blastomere). However, even one cell less reduces embryo survival, as manifested by a reduction in pregnancy rate; removal of two cells reduces the pregnancy rate further (Cohen et al. 2007). Practicing in an experienced center (Brussels) that once routinely removed two cells, DeVos et al. (2009) reported live birthrates of 37.4 % and 22.4 % after removal of one versus two cells, respectively.

Although beyond the scope of this chapter, a key diagnostic impediment in cleavage stage analysis is now recognized—mosaicism. This phenomenon can occur in any tissue, but is especially prone at this stage of embryogenesis. Presumably, the developing embryo is undergoing self-correction of some nature. The single blastomere removed may unwittingly be atypically aneuploid, unrepresentative of the remaining normal cells. However, because the euploid status of the remaining cells is unknown, the opportunity to transfer a normal embryo is lost.

Blastocyst (Trophectoderm Biopsy)

The trophectoderm can be biopsied in the 5- to -6 day, 120-cell blastocyst. More cells can be removed at this stage, potentially facilitating diagnosis. Because the trophectoderm is destined to form the placenta, embryonic cells per se are not removed.

Trophectoderm biopsy and analysis of blastocysts have long been envisioned (Dorkas et al. 1990; Carson 1991). Prior to development of PGD, Buster et al. (1985) recovered human blastocysts by uterine lavage. Lavage for blastocyst recovery was at that time considered an approach to obtain embryos for trophectoderm biopsy for genetic diagnosis. However, lavage and PGD were not pursued because of fear of retained (unexamined) embryos.

Decades later, blastocyst transfer in non-PGD assisted reproductive technology (ART) has become routine and shows high pregnancy rates. If biopsy is envisioned, the blastocyst is now seen as the preferred stage, facilitated by the use of laser for trophectoderm biopsy. The additional 2 or 3 days in culture beyond that required for transfer of an 8-cell embryo allows some selection against non-thriving embryos, but two-thirds of aneuploid embryos still continue until day 5 or 6. PGD is thus required to exclude remaining aneuploidies.

PGD for Traditional Prenatal Genetic Diagnostic Indications

Chromosomal Aneuploidy

The most common indication for PGD is detection of chromosomal abnormalities, most often aneuploidies. Although possible, it has never been possible to obtain a karyotype on a single cell reliably (Verlinsky and Evsikov 1999; Shkumatov et al. 2007). Therefore, PGD for cytogenetic analysis began by relying on FISH with chromosome-specific probes (Handyside et al. 2010; Simpson 2010). This was initially applicable for only a limited number of chromosomes, usually 5–9. No more than five different chromosome-specific probes could be subjected to hybridization per cycle, meaning 2–3 cycles were necessary even for interrogating 10–12 chromosomes. This required genuine expertise, which few laboratories were able to provide. FISH for PGD aneuploidy testing has now been largely replaced by genome-wide

molecular approaches, namely single nucleotide polymorphisms (SNPs) from all chromosomes (Handyside et al. 2010) or array CGH (chromosomal microarrays) (Munné et al. 2010).

Array CGH is based on comparative genomic hybridization (CGH), a molecular cytogenetic technique that allows comprehensive analysis of the entire genome. The basis is the ability of single-stranded DNA from one source to anneal (hybridize) with a complementary single-stranded DNA from another source. Typically, normal (control) DNA is labeled with a fluorochrome of one color (e.g., green); test (patient) DNA is labeled with a fluorochrome of a different color (e.g., red). Once both test and control DNA are denatured (single stranded), hybridization can occur between the two. Provided that equal amounts of control and test DNA are present, the color of the hybridized mixture should be yellow if fluorochromes of the above two colors are used. If test DNA originates from a trisomic individual, the DNA of that chromosome is present in excess. Thus, the array would show more of the color used to connote test (patient) DNA.

In practice, small amounts of single-stranded DNA of known sequence are placed by photolithography onto a platform (array) in ordered fashion. The amount of DNA in each “spot” is small (i.e., micro=microarray). The number of sequences is chosen in advance but is expected to encompass the entire genome, with one sequence overlaying the adjacent one (“tiling”). Again, “control” DNA embedded by photolithography is labeled with a fluorochrome of one color, and exposed to single-stranded test DNA (e.g., patient) labeled with a fluorochrome of a different color as reasoned above. If control and test DNA are equal in quantity for a given sequence, or given chromosomes, the result is yellow. If test (patient) DNA is in excess (trisomy), that color predominates: If test DNA is deficient, the other color predominates.

Various commercial platforms all interrogate/sequences of DNA along every chromosome; however, sensitivity (coverage) varies. Arrays used to interrogate blood from liveborn infants or chorionic villi or amniotic fluid cells are designed to be more sensitive than those used for analysis in PGD. The latter are designed to detect only aneuploidies or large duplications or deficiencies. It is reasoned that the small amount of DNA available for PGD (6 pg per cell) precludes robust, more sensitive, diagnostic attempts. In addition, analysis based on a single cell (blastomere) shows a very high number of variants of uncertain significance. Given the high proportion of the latter, it can be assumed that not all convey untoward clinical significance because, if so, very few conceptions would yield viable pregnancies.

The array CGH typically used for PGD deserves special comment because it is based on bacterial artificial chromosomes (BACs) (BlueGnome)TM. Only large chromatin segments are therefore interrogated because BAC arrays are large; thus only large (5 or 10 Mb: 5,000,000–10,000,000 base pairs) abnormalities are detected, much like a karyotype. The dilemma of disclosing or not disclosing smaller copy number variants (200 kb), which have increased likelihood of variants uncertain clinical significance (VOUS), does not arise in PGD diagnostics. Thus, PGD differs from prenatal genetic diagnosis; VOUS arises more often when analyzing chorionic villi or amniotic fluid cells. Of course, the downside is that microdeletions and other abnormalities smaller in magnitude are not sought in PGD.

Chromosomal Rearrangements

Chromosomal rearrangements (translocation or inversion) may result in unbalanced gametes and, hence, an unbalanced zygote. Many couples with balanced rearrangements are diagnosed after repeated spontaneous abortions, reflecting lethality often conferred by unbalanced gametes. Unbalanced rearrangements can be recognized by array CGH gains and/or losses involving two or more chromosomes. PGD not only reduces the number of abnormal liveborns but also obviates increased spontaneous abortions due to chromosomal imbalance. Reproductive efficiency is improved by transferring only cytogenetically normal or balanced embryos (Munné et al. 1998b, 2000). Because not all embryos are normal or balanced, clinical success requires a sufficient number of embryos from which the relatively few normal embryos can be identified. If pregnancy is attempted by natural conception, multiple attempts might be required to achieve conception with a normal embryo and, hence, continuing pregnancy (Fritz and Schattman 2008). Without PGD the necessary time required to achieve a pregnancy with a normal or balanced embryo might, for certain women, extend beyond the age realistic to achieve pregnancy. The mean time for translocation couples to achieve pregnancy naturally is 4–6 years (Goddijn et al. 2004; Stephenson and Sierra 2006; Sugiaura-Ogasawara et al. 2004). Using PGD, Otani et al. (2006) observed only 5.3 % abortions after PGD for translocations, far fewer than expected for women of comparable age. The lifetime cumulative pregnancy rate using PGD was 57.6 % involving an average of only 1.24 cycles.

One shortcoming of array CGH in couples with a balanced translocation is that it is not possible to distinguish between clinically normal embryos with or without the balanced translocation. Both have the same amount of DNA, the end point of array CGH. Breakpoint-specific probes could accomplish this, and were indeed employed in the early years of PGD translocation analysis (Munné et al. 1998a); however, costs were prohibitive. At present, haplotyping using SNPs could theoretically be applied. Current practice is simply to perform array CGH to exclude unbalanced embryos, transferring genetically normal embryos that may or may not be translocation carriers.

Single-Gene Disorders

Approximately one fourth of PGD cases are currently performed to detect a single mutant gene (ESHRE 2014). It is estimated that about 12,000 PGD cycles have been performed worldwide for this indication. The most frequent indications are hemoglobinopathies, cystic fibrosis, fragile X syndrome, and Duchenne muscular dystrophy. See Kuliev et al. (2014) and supplements to the ESHRE PGD Consortium (2014) for updated lists of disorders tested in US and European labs, respectively.

PGD can be performed whenever the chromosomal location of a given disease-causing gene is known. This holds even if the molecular basis of a causative mutation at the nucleotide level is not known. In that case, linkage analysis can be performed

using polymorphic loci. Initially the polymorphisms were short terminal repeats or STRs, but SNPs can now also be used. Linkage analysis should, in fact, *always* be employed in PGD for single-gene disorders because of the phenomenon of allele drop out (ADO), to be discussed below.

In detecting single-gene disorders, one must amplify the small amount of DNA, using a technique called whole genome amplification (WGA). Efficiency does not exceed 90–95 % (Guidelines for Good Practice in PGD: programme requirements and laboratory quality assurance 2008). When amplification does not occur, ADO exists and no information (no result) is obtained. This presumably reflects stochastic phenomena by which probes fail to locate patient DNA, precluding hybridization and, hence, diagnosis. This is probably exacerbated when embryo damage has occurred in biopsy, resulting in loss of embryonic DNA. Diagnostic problems arise if ADO is not recognized. If only one allele is detected, it would not be known whether both alleles (identical) are in fact present or if one of two discordant alleles failed to amplify. If the mutant allele failed to amplify in a dominant disorder, a false negative result would exist. However, one can recognize ADO if linked markers both 5' and 3' to the mutant allele are interrogated. Linkage analysis is thus required in all PGD single-gene cases. Using this approach, Reproductive Genetics Innovation (RGI) has observed in a 20-year period only three errors in 2300 single-gene PGD cycles that resulted in over 500 babies (Kuliev et al. 2014). Liebaers and colleagues (2010) reported 0.6 % misdiagnosis in 581 single-gene PGD pregnancies studied. In the most recent ESHRE PGD Consortium report (December 2009–October 2010), there were no single-gene misdiagnoses in 1597 oocyte retrievals for single-gene disorders.

Prenatal Diagnosis Practical Only by PGD

Multiple At-Risk Single-Gene Conditions

PGD may be the only practical reproductive genetic diagnosis option when two different single-gene disorders are segregating in a given family, especially if the couple is older and has a limited interval to conceive. If a couple is at 50 % risk for an autosomal dominant disorder and independently for a non-linked autosomal recessive disorder (25 %), the likelihood that any given offspring (embryo) will be genetically normal is only $1/2 \times 3/4$ or $3/8 = 37.5$ %. Choosing from among multiple embryos enables the minority of embryos that are not affected to be identified and transferred.

Avoidance of Knowledge of Parental Genotype

PGD is the only practical approach if a person at risk for an adult-onset autosomal dominant disorder wishes to remain unaware of his/her status but nonetheless does not wish to burden offspring with a similar dilemma. This occurs when an ostensibly normal individual is the offspring of a mother or father who is affected

with a disorder manifested only after reproductive age; risk is 50 % for the parent to be affected; and 25 % for each offspring ($1/2 \times 1/2 = 1/4$). The prototypic indications are Huntington disease and early onset autosomal dominant Alzheimer disease. With PGD, multiple embryos can be tested to identify unaffected embryos suitable for transfer. If provider and patient agree *not* to disclose, the couple will be told no information in a given cycle other than that an embryo will or will not be available. The couple will not know the number of embryos retrieved or reason for no transfer if that situation arises. Failure to transfer could thus be speculated to be failure of embryonic development, aneuploid embryos (even if they lack the mutation), or embryos having the mutation for which they are at risk. Given these plausible options, a sham transfer is not necessary. Traditional prenatal genetic diagnosis using chorionic villus sampling (CVS) or amniocentesis theoretically could accomplish the same goal, but would involve terminations or subterfuge (e.g., claiming a trisomic fetus when the real diagnosis was the presence of the mutant allele).

A caveat in nondisclosure is that the scenario must be repeated in subsequent cycles, even if studies during the initial cycle proved the at-risk patient was actually unaffected. Otherwise, any at-risk patient could readily deduce his/her genotype (e.g., if they were told PGD was no longer indicated). The number of PGD cases performed for non-disclosure constitutes about 5–10 % of single-gene disorders in the ESHRE data collection and in two large US centers. With the new option of biopsies of multiple embryos, vitrification, and then transfer of a single embryo in subsequent natural cycles, a less unwieldy protocol is possible.

Selection of HLA-Compatible Embryos

One in four sibs is human leukocyte antigen (HLA)-compatible (identical), barring recombination that occurs in 5–10 % of cases. Having an HLA-compatible sibling could be invaluable if an older, moribund sibling with a lethal disease—genetic or nongenetic—could potentially benefit from stem cell transplantation to repopulate his/her bone marrow. Stem cell transplantation of HLA-compatible sibs is very successful (90–95 %), but much less so (60 %) if the individuals are not HLA-compatible. The ideal source of stem cells from a healthy sibling is umbilical cord blood. Given 25 % risk for an autosomal recessive disorder (e.g., β -thalassemia or Fanconi anemia), the obvious strategy for a couple wishing to avoid another genetically abnormal child is not only using PGD to exclude an affected embryo but also selecting an HLA-compatible embryo. Doing so would allow harvesting at birth of otherwise discarded umbilical cord blood to use for generating stem cells. If the latter are transplanted successfully into the older, moribund sib, he/she should survive and thrive.

Given that the pregnancy is also at risk for an autosomal recessive disorder, the likelihood of a genetically normal HLA-compatible embryo is low: $1/4$ HLA-compatible \times $3/4$ unaffected with the disease = $3/16$. If recombination occurs within the HLA locus, it is not possible to have a 100 % compatible match. Recombination found in a tested embryo indicates lack of suitability for transfer.

PGD for the purpose of transferring HLA-compatible embryos was first performed by Verlinsky and colleagues in a couple at risk for Fanconi anemia (Verlinsky et al. 2001). By 2004, 45 cycles for HLA typing had been performed (Kuliev and Verlinsky 2004a, 2004b, 2006; Verlinsky et al. 2004); 17.5 % of embryos were genetically suitable for transfer, very near the expected 18.7 % (3/16). In their 2014 report, Kuliev et al. (2014) tabulated 374 PGD cycles for HLA testing on 163 different patients.

In the USA and Turkey, PGD to obtain HLA-compatible embryos is performed not only for genetic indications but for nongenetic cases. The most common indication is leukemia in an older sib; transplantation of umbilical cord stem cells from a younger sib could be lifesaving. This was first shown by Kuliev and Verlinsky (2004a). PGD for HLA typing alone accounts for approximately one third of HLA matching PGD cases in the USA. This indication is much less common in the ESHRE PGD Consortium, save Turkey.

Aneuploidy testing is also recommended in couples desiring HLA-compatible embryos, as it is for all single-gene PGD. This is in particular true when a couple is relatively older. Rechitsky et al. (2009) reported that aneuploidy testing concurrent with HLA typing in 57 cycles yielded a 48.5 % pregnancy rate, twice that of age-matched HLA cases (<35 years) not undergoing aneuploidy testing.

Preconceptional PGD

The first polar body is extruded *before* fertilization. The second polar body, by contrast, is not extruded until the oocyte is fertilized (syngamy). Biopsy of the first polar body can, uniquely, provide *preconceptional* information. This is the only option for prenatal diagnosis in couples who do not wish to discard an abnormal embryo. First polar body PGD is also the only option if one must, for statutory reasons, limit the number of oocytes fertilized or embryos transferred. Biopsy of the first polar body allows, in the absence of recombination, normal oocytes to be identified. Thus, fertilizing only euploid oocytes can yield reasonable pregnancy rates, despite restrictive legislation as in Italy (Law 40) (Gianaroli et al. 2009).

Other Indications for PGD Aneuploidy Testing

Repeated Spontaneous Abortions

At least 50 % of first-trimester spontaneous abortions have numerical chromosomal abnormalities (aneuploidy). This is positively correlated with maternal age. A corollary, given that so many aneuploidies persist until clinical recognition of pregnancy, is that 50 % of *morphologically normal* embryos in women >35 years old are

chromosomally abnormal and perhaps 25 % in younger women. Selecting an embryo optimal for transfer cannot be based solely upon morphology. Nonrandom distribution occurs in successive miscarriages; abortuses tend to be either successively aneuploid or successively euploid (Warburton et al. 2004). This stratification also occurs in preimplantation embryos tracked in successive cycles (Rubio et al. 2003).

Given the above, the rationale for performing PGD aneuploidy testing and transferring only euploid embryos in couples having experienced repeated abortions is unassailable. The rationale is strongest if at least one loss has been confirmed to be aneuploid. However, this information is not always known. If information regarding the chromosomal status of prior losses is not available, one can perform array CGH on archived specimens embedded in paraffin. If this also is not possible, the assumption should be that only half of couples with recurrent first trimester pregnancy losses will have experienced recurrent aneuploidy.

Randomized clinical trials (RCTs) have not been performed, but PGD in this circumstance has been repeatedly shown beneficial in descriptive studies (Gianaroli et al. 1999; Munné et al. 1999, 2005; Verlinsky et al. 2005; Verlinsky and Kuliev 2005a). One good surrogate involves comparison to objective criteria using the Brigham formula (Brigham et al. 1999), which takes into account maternal age and the number of prior abortions to derive the likelihood of a pregnancy loss. Munné et al. (2005) observed losses in only 13 % of couples who used PGD, compared to the expected rate (Brigham) of 33 %. Benefit was greatest for women older than 35 (expected 39 % vs observed 13 %; $p < 0.001$). That is, the increased loss rate due to aneuploidy in older women is obviated by PGD aneuploidy testing.

PGD Aneuploidy Testing to Improve ART Pregnancy Rates

Ability to detect and transfer euploid embryos in ART should increase pregnancy rates in women who otherwise have no genetic indications for PGD. In Europe this is typically termed preimplantation genetic screening or PGS. Pregnancy rates in ART decline markedly beginning late in the fourth decade of a woman's life, primarily as a result of high embryonic losses due to aneuploidy. That endometrial factors are not paramount is evident by women in their fifth decade having successful pregnancies following transfer of donor embryos or use of a donor oocyte. Not only does aneuploidy increase with increasing maternal age, but miscarriage rates do as well. The decreasing ART pregnancy rate per maternal age is thus a mirror image of the increasing miscarriage rate. Based on success rates prior to and after PGD, favorable results were reported from experienced centers worldwide beginning in the late 1990s (Gianaroli et al. 1999, 2005; Munné et al. 1999, 2003; Verlinsky et al. 2005; Verlinsky and Kuliev 2005b, c). The same held when compared to historical expectations for age-matched women not undergoing PGD. Two smaller RCTs conducted in the USA (Mersereau et al. 2008; Werlin et al. 2003) showed improved pregnancy rates.

By the early 2000s, most larger PGD and ART centers in the USA and Europe were offering PGD to improve pregnancy rates in older women. However, the largest centers in the USA and Italy could never complete a RCT. Not only could such “embryo research” not be funded federally (National Institutes of Health) in the USA, but patients universally declined to participate when RCTs were attempted (Munné 2008, Study NCT 006646893). In the USA, the reality of self-funding and lack of adequate insurance coverage will doubtless continue to impede such trials, few patients agreeing to being assigned to the control arm given plausible rationale and now data favoring benefit of the PGD arm.

Given inability to conduct RCTs in larger centers, other European centers did conduct RCTs. With one exception, these centers had relatively little experience before initiating their RCT. None showed significant improvement in pregnancy rates (Debrock et al. 2010; Hardarson et al. 2008; Mastenbroek et al. 2007; Mersereau et al. 2008; Schoolcraft et al. 2010; Staessen et al. 2004, 2008). This author elsewhere has critiqued the RCTs of this era.

Since the RCTs mentioned above were performed (~2007), diagnostic approaches have greatly improved. The preferred way now is to obtain embryo DNA from trophectoderm biopsy of the 5–6 day blastocyst. This minimizes the pitfall of mosaicism that may occur if a single (unrepresentative) cell is analyzed in a cleavage stage 8-cell, 3-day embryo. Trophectoderm biopsy of the 5–6 day blastocyst also seems less difficult technically than blastomere biopsy of the 3-day cleavage stage embryo. Further, more than a single cell is obtained.

Irrespective of biopsy, the pivotal advance has been ability to interrogate all 24 chromosomes and to do so accurately, using either array CGH or a SNP-based method.

Recent RCTs have been performed by experienced labs, all showing statistically significant increased pregnancy rates. Schoolcraft and Katz-Jaffe (2013) reported the pregnancy rate to be 61 % for a single euploid blastocyst undergoing PGD but 41 % with an untested embryo; Yang et al. (2012) reported pregnancy rates of 69 % and 42 %. Scott et al. (2013) reported sustained implantation rates (leading to delivery) to be 66 % with quantitative PCR-based PGD and 48 % without PGD ($p=0.001$).

Of great public health impact, blastocyst PGD aneuploidy testing allows such good pregnancy rates that single embryo transfer can be defended with its benefit of avoiding multiple gestation. Forman et al. (2013) performed a RCT in which a single morphologically normal blastocyst embryo was subjected to PGD aneuploidy testing to confirm euploidy; the comparison arm involved two morphologically normal embryos that were transferred without PGD. Pregnancy rates in the two groups were not significantly different (61 % vs 65 %), but twin rates were strikingly different: 0 vs 53 %. (It should be remembered that not all embryos reach blastocyst stage; thus, the rates cited above are not per initiated cycle but per blastocyst transfer.) Multiple gestations carry significant morbidity due to preterm births with high economic costs (Chambers et al. 2014). Thus, it can be confidently predicted that interest in single embryo transfer will grow in order to decrease the frequency of multiple gestations and their neonatal complications, and PGD to select euploid embryos will be increasingly utilized.

Adult-Onset Disorders in Which One Parent Is Already Affected

Prenatal genetic diagnosis for adult-onset single-gene conditions was once not considered appropriate (“not ethical”). The reasoning was that prenatal diagnosis should not be offered and, hence, a diagnosis provided, if it could lead to termination for a disorder not associated with mental retardation nor manifested at birth. Given preimplantation genetic diagnosis, the situation has changed (Simpson 2002). Certainly this applies in the USA, although reticence still exists in much of Europe. Presumably the reason is that PGD to exclude transmission of an embryo with an autosomal dominant trait is more acceptable to at-risk families than first—or second—trimester prenatal diagnosis with its potential for clinical termination. Greater acceptance seems to exist if embryos and not fetuses are being tested and their results acted upon before a clinical pregnancy.

The first PGD case performed for a familial cancer syndrome involved Li–Fraumeni syndrome, a multisystem cancer syndrome due to a p53 perturbation (Verlinsky et al. 2001). Detection of other disorders rapidly followed (Rechitsky et al. 2002). BRCA1, multiple endocrine neoplasia, familial adenomatous polyposis (FAP), retinoblastoma, and von Hippel–Lindau (VHL) syndrome are the most common indications.

Safety of PGD

Removal of embryonic cells as required for PGD might logically be expected to adversely affect implantation or decrease survival and, hence, decrease ART pregnancy rates. However, the totipotential nature of embryonic cells at this stage of embryogenesis offers safety against organ-specific anomalies arising in liveborns. Loss of one or more cells prior to commitment to a given developmental pathway is considered mitigated by another cell with the biological capacity to accomplish that same purpose. Thus, the malformation rate should not differ from that in the general population, as shown conclusively in many animal experiments.

In humans, a confounder in assessing safety of embryo biopsy is that the rate of birth defects in non-PGD ART pregnancies is 30 % greater than in the general (non-ART) population (Hansen et al. 2005). It is unclear whether this increase is due to the ART protocol itself or rather due to the underlying infertility that necessitated ART. The latter seems the likely explanation, as best shown by Zhu et al. (2006) who observed a 20 % increase in birth defects in infertile couples who after 12 months of infertility eventually became pregnant without ART. Similar results were reported by Jacques et al. (2010) and Davies et al. (2012). The latter showed the odds ratio to be 1.2 for birth defects in offspring of women who underwent ART in one cycle but conceived spontaneously in a later cycle.

Irrespective of the background rate against which anomalies in PGD offspring should be compared, there seems to be no increased rate in anomalous liveborns after PGD. Liebaers et al. (2010) performed physical exams 2 months after birth in 563 PGD liveborns, 18 stillborns and 9 neonatal deaths. Anomalies were compared to those in a previously reported cohort study of intracytoplasmic sperm injection (ICSI) offspring who did not undergo PGD (Bonduelle et al. 2003). In approximately half the cohort studied by Liebaers et al. (2010), the indication for PGD was a single-gene disorder; in the other half the indication was aneuploidy testing. Structural malformations were found in 2.13 % in the PGD group and 3.38 % in the ICSI group. No differences were observed between the single-gene PGD group and the aneuploidy PGD group. In a smaller matched pair study ($N=102$ in each arm), more in depth clinical assessment was performed, but still no statistical difference between PGD and ICSI offspring (Desmyttere et al. 2009) was observed.

In conclusion, it can be confidently stated that at present PGD seems safe (Simpson 2010). Given its efficacy and the increasing number of indications for which it is appropriate, it is reasonable to expect that PGD will become a common and desired component of IVF.

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