Preimplantation Genetic Diagnosis

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Preimplantation genetic diagnosis (PGD) is a procedure to analyze the genetic make-up of embryos formed through in vitro fertilization (IVF). Based on this analysis embryos are selected for transfer to the uterus to establish a pregnancy. PGD was first accomplished by Alan Handyside in 1990 when his team performed embryo sex determination in families known to carry X-linked diseases \[1\]. By choosing to have only female embryos transferred to the uterus, these women ensured that their offspring would not be male and thereby eliminated the possibility of bearing an affected male child. Following this initial success, PGD has been used to test for a variety of chromosomal disorders \[2\], single-gene disorders \[3\], and recently human leukocyte antigen (HLA) typing of embryos to establish potential donor progeny for hemopoietic stem cell treatment of siblings in need of stem cell transplantation \[4\].

PGD represents an important adjunct to traditional prenatal testing through amniocentesis or chorionic villus sampling. Although traditional methods can accurately identify fetuses that have chromosome disorders and single-gene disorders during pregnancy, couples must make difficult choices when these tests find an affected fetus. PGD permits couples to avoid the issue of pregnancy termination by initiating pregnancies with unaffected embryos.

This article reviews key aspects of patient management, relevant IVF and PGD procedures, methods used in the genetic analysis, technical difficulties that can affect test results, and indications for PGD. It also examines some of the emerging technologies being introduced into PGD.
Patient management

The practice of PGD has evolved significantly over the 15 years since the original reports. During that time many clinical and laboratory procedures have been developed to improve the accuracy and reliability of PGD and to expand the number of conditions that can be tested [5]. The complexity of PGD requires a multidisciplinary team approach that includes reproductive endocrinologists, geneticists, nurses, genetic counselors, embryologists, cytogeneticists, and molecular biologists.

The first step for patients considering PGD is reproductive and genetic counseling. Consultation with a reproductive endocrinologist and nursing staff before embarking on treatment focuses on a discussion of the details of IVF, the risks of medical complications that can occur during ovarian stimulation and oocyte retrieval [6,7], the medications that will be used, the patient experience during an IVF stimulation cycle (often referred to as a “cycle”), the treatment timeline, and the cost. Medical history and laboratory testing of the patient and her male partner before a cycle are used to help predict the probability of success.

Consultation with a geneticist or genetic counselor focuses on an assessment of the genetic risk faced by the patient based on her history, her partner’s history, and family history. This information provides the basis for a discussion of the nature, severity, and recurrence risk of the genetic disorders in the family as well as age-related risks. This discussion indicates whether PGD can be helpful for a particular patient’s situation and the probability that PGD can identify an unaffected embryo. PGD requires that a DNA test or cytogenetic test exists that can identify the relevant abnormality in a cell derived from an embryo. Details of the test determine the chance that a normal embryo can be identified, the reliability of the test, and the chance of misdiagnosis. It is important that patients weigh PGD against other reproductive options and alternatives such as prenatal diagnosis, gamete donation (use of an egg or sperm donor), remaining childless, accepting the genetic risk without further testing, and adoption.

By combining patient-specific reproductive and genetic information, the reproductive medicine staff can discuss how many oocytes might be retrieved, how many embryos are likely to result, and the outcome of embryo biopsy (removal of one or more cells for analysis). Not all embryos are suitable for biopsy, and some may not survive biopsy. Biopsied cells may not yield a result, may yield ambiguous result, or, rarely, may give inaccurate results. Patients are made aware of the possibility that all embryos may be affected. They are also informed of the probability of a live birth for a given number of transferred embryos, the rate of pregnancy loss, and the risk of multiple births. Prenatal diagnosis is generally recommended to confirm the results of PGD. These discussions take place before and during the cycle to provide patients with a realistic expectation of their chance of success at every step in the process.
Before IVF is started, there is a discussion of the fate of embryos that are not transferred to the uterus. Patients generally cryopreserve appropriate embryos for use in a future cycles should the current cycle fail. Affected and nonviable embryos that are not transferred are discarded, although some couples choose to donate these embryos for research. The central principle that guides this and all other aspects of the PGD process is respect for patient autonomy [8]. At present, patients continue to be able to make these reproductive and genetic decisions despite increasing government regulation of assisted reproductive technologies [9,10].

In vitro fertilization and intracytoplasmic sperm injection

Patients who proceed with PGD are given medications to stimulate the ovaries to produce oocytes and other medications to make the endometrium receptive to embryos transferred into the uterine cavity [11]. During ovarian stimulation ultrasound examination and serum estradiol levels generally are used to assess follicular development [12]. Human chorionic gonadotropin is administered approximately 36 hours before retrieval to complete oocyte maturation [13]. The mature oocytes are retrieved with ultrasound guidance under conscious sedation [14]. Conventional IVF is performed by combining an egg with about 50,000 to 100,000 motile sperm for 12 to 18 hours on the day of retrieval [15], although a smaller number of sperm and a shorter time interval can be successful [16,17].

When PGD is performed following conventional IVF, many sperm cells often are present at the time of embryo biopsy on the third day after retrieval. As a result there is a chance that DNA from sperm cells will contaminate the biopsy and potentially produce erroneous results. This problem has been circumvented by the use of intracytoplasmic sperm injection (ICSI). The ICSI procedure takes a single sperm cell and injects it into the egg to fertilize it, thereby eliminating the risk of contamination with spermatozoan DNA at biopsy [18].

Embryo biopsy

Three methods have been developed to carry out PGD. The most widely used approach tests individual cells (blastomeres) obtained on the third day after in vitro fertilization of the egg at the cleavage stage (approximately eight cells). One or two blastomeres are removed through a hole created in the zona pellucida, and the cells are analyzed (Fig. 1) [19]. Based on this analysis, selected embryos are subsequently transferred to the uterus on day 4 or day 5 after IVF.

An alternative method for carrying out PGD examines the genetic material within the first and second polar bodies, the by-products of meiosis I.
and meiosis II, respectively. The polar bodies contain the genetic material that is absent from the female pronucleus that will combine with the male pronucleus to become the zygote. By establishing whether there is an abnormal gene or chromosome arrangement in the polar bodies, it is possible to infer the maternal genetic contribution to the embryo. This method can be used in cases of maternally derived dominant mutations, translocations, and aneuploidy. It cannot be used when paternally derived genetic information is critical to the diagnosis, such as paternally derived dominant mutations, translocations, and aneuploidy [20]. In cases of recessive disorders, it can provide information about the maternal contribution, but not the paternal contribution, and therefore is helpful when the polar body biopsy shows that the embryo received the normal copy of the gene in question. Unlike blastomere biopsy, in which two cells can be studied to replicate data, polar body biopsy data cannot be replicated unless the polar body biopsy is followed by blastomere biopsy.

The third and latest method is blastocyst-stage biopsy [21]. This technique is performed at approximately 5 to 6 days after insemination. The embryo at this stage has differentiated into the trophectoderm, which gives rise to the placenta, and the inner cell mass, which gives rise to the fetus. Laser-assisted biopsy of the human blastocyst using a noncontact infrared laser for drilling of the zona pellucida enables removal of several cells from the trophectoderm layer [22]. It does not expose the embryos to chemicals and does not invade the inner cell mass destined for fetal development. Because several cells can be removed from the trophectoderm for analysis, the accuracy and reliability of PGD is improved. Results are available for a day-6 transfer. In one recent study of 1050 biopsied blastocysts, 93% gave unambiguous results [23]. As blastocyst culture and cryopreservation improve, it is expected that this technique will supplant cleavage stage biopsy and polar body biopsy.
Analysis of genetic material from biopsy

There are a number of different approaches to testing the genetic material derived from PGD. The most commonly used are fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR). FISH is performed by fixing the cells derived from the biopsy to slides and hybridizing with fluorescently labeled chromosome-specific DNA probes. Individual cells are probed with a mixture of probes (Fig. 2) and then are stripped and probed with a second mixture (Fig. 3), permitting the diagnosis of abnormal cells (Fig. 4) [5]. This approach is used to identify aneuploidy and chromosome rearrangements such as translocations and for sex determination in the setting of X-linked disease and family balancing.

PCR is a DNA-amplification process performed on individual cells derived from the embryo biopsy that results in millions of copies of a few carefully chosen short sequences (loci) within the genome, generally 100 to 500 base pairs in length. To increase the signal from a single cell, the PCR product from the initial amplification can be used in a second PCR reaction to permit the detection of DNA mutations or polymorphic short tandem repeats (STRs) used in linkage analysis. The use of two rounds of PCR is referred to as “nested PCR” [5].

Accuracy of preimplantation genetic diagnosis

PGD is associated with a number of potential pitfalls that can result in misdiagnosis. Single-cell FISH is limited by the number of probes that

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Fig. 2. Fluorescence in situ hybridization of a blastomere. Normal result. Chromosome 13, red; chromosome 18, aqua; chromosome 21, green; chromosome X, blue; chromosome Y, gold. (Courtesy of Peter vanTuinen, PhD, Milwaukee, Wisconsin.)
can be applied simultaneously because the risk of hybridization failure and FISH artifacts increases with increasing numbers of probes [24]. At present, FISH cannot test for aneuploidy of all chromosomes; therefore most programs test for numeric abnormalities involving chromosomes 13, 18, 21, X, and Y, because abnormalities in these chromosomes can result in an affected live-born infant, and also for abnormalities in chromosomes 15, 16,
and 22, which are found commonly in spontaneous abortions. Some programs test for additional chromosomes involved in miscarriage or IVF failure [25,26]. Aneuploidy testing with a limited number of FISH probes is often referred to as “aneuploidy screening” or “preimplantation genetic screening” (PGS).

One source of difficulty intrinsic to the early embryo is the presence of mosaicism (presence of two cell lines with different genotypes or karyotypes). Chromosomally normal and abnormal blastomeres can coexist within the same embryo at the early-cleavage stage. In one recent study 28% of embryos had both normal and abnormal cells within the embryo [27]. This study analyzed the copy numbers of 10 chromosomes (1, 7, 13, 15, 16, 18, 21, 22, X, and Y). It is likely that the rate of mosaicism detected would be higher if all chromosomes were analyzed. Mosaicism also can affect the results of testing in single-gene disorders (eg, when the cell studied is trisomic for the chromosome that contains the gene of interest in a single-gene disorder).

Another potential source of error is the PCR used to amplify the targeted loci in the genomic DNA from a single cell. Common artifacts of single-cell PCR include allele dropout (ADO) and preferential amplification (PA) at heterozygous loci leading to misdiagnosis [28,29]. In single-cell PCR, ADO occurs when one allele fails to be amplified. PA occurs when one allele is poorly amplified. In either instance a heterozygous embryo would appear homozygous. In the case of a dominant disorder, an affected embryo will appear normal when the mutant allele is not detected. The use of a fluoroscencely labeled primer in the second-round PCR reaction has greatly enhanced the sensitivity of PCR product detection, reducing the rate of PA [30]. Blastocyst biopsy is another approach to overcoming ADO and PA. PCR analysis of two to five cells in a tube together markedly reduces rates of PA and ADO [31].

The accuracy of embryo diagnosis also can be improved by combining mutation detection with linkage analysis using STRs [3,32]. The locus containing the mutation along with several closely linked STRs are coamplified in the first-round PCR. This coamplification is followed by a separate amplification of the mutation-containing locus and each of the linked STRs in the second-round PCR. Mutations can be detected by minisequencing [3,33,34], restriction enzyme analysis of PCR products [35], or sequence analysis of single-cell PCR products [29,34,36]. Simultaneous analyses of STRs that are closely linked to the mutation provide independent verification of the results of mutation testing. To avoid meiotic recombination events between the mutation and linked markers, STRs located within or close to the mutation are selected for linkage analysis.

When PCR is performed on a single cell, there is a risk of contamination by previous PCR products (amplicons) or extraneous genomic DNA in the laboratory. Contamination can be minimized by following good laboratory practices as outlined by Thornhill and colleagues [5] and by setting up
reagents, first PCR reactions, second PCR reactions, and product analysis in different biosafety cabinets in different rooms of a dedicated PGD laboratory. Despite the aforementioned precautions, there remains a small risk for inaccurate results. Therefore, confirmation of PGD results by amniocentesis or chorionic villus sampling is recommended.

Indications for preimplantation genetic diagnosis

It is known that the rate of aneuploidy rises with maternal age, as has been shown in live-born children [37], in the midtrimester of pregnancy [38], and in embryos [39]. These data argue strongly for chromosome analysis of embryos in women of advanced maternal age who are using IVF to achieve a pregnancy. Aneuploidy screening in IVF patients of advanced maternal age is the most frequent indication for PGD using FISH [40]. This rationale for PGD extends also to couples who have had a previous child born with a chromosome aneuploidy [41].

IVF patients who have had recurrent miscarriages have been shown to have higher rates of chromosomally abnormal embryos. Aneuploidy screening by PGD reduces the rate of pregnancy loss [25]. Rubio and colleagues [42] found that 26.9% of couples with recurrent miscarriage had chromosomal aberrations in all the embryos in a given cycle and that the percentage of abnormal embryos was similar in subsequent cycles. This finding suggests that once other causes of recurrent miscarriage, such as a chromosome translocation in one member of the couple, have been evaluated, PGD to test for aneuploidy is a valuable diagnostic test in patients experiencing unexplained recurrent miscarriage. Patients who have high rates of aneuploidy can be offered donor eggs rather than proceeding with more IVF cycles using their own eggs.

Among the many causes of male infertility [43] some men who have a severe male-factor condition and a normal karyotype can have chromosomally abnormal sperm. In one such study of 27 men who had oligoasthenoteratozoospermia and 11 who had nonobstructive azoospermia who underwent testicular sperm extraction, 79% had a significantly increased rate of aneuploidy compared with controls [44]. Aneuploidy screening of embryos has obvious utility in this situation.

Constitutional chromosome abnormalities, notably Klinefelter’s syndrome and translocations, can be found in approximately 5% of infertile males [45]; 1.25% women who had secondary infertility [46] and 6% of women who had recurrent miscarriage [47] were found to harbor a chromosome abnormality. Although PGD using aneuploidy screening can be applied to the cases with Klinefelter’s syndrome, translocations and other chromosomal rearrangement often require FISH probes that are not among the probes in the typical aneuploidy screening. Translocations, inversions, and other chromosomal rearrangements require the use of FISH probes
that are specific to the rearrangement. Despite the technical challenge of customizing the FISH probes to each patient’s situation, PGD has been shown to be highly successful in this setting. In one recent study of 45 carriers of balanced translocations, the use of PGD reduced the rate of spontaneous abortions from 87.8% to 17.8% and improved the rate of live births from 11.5% to 81.4% for this cohort [48].

Single-gene disorders represent a large and diverse group of disorders that have been approached with PGD using PCR. Some disorders, such as spinal muscular atrophy [49], are the result of a single mutation that occurs in most affected families, allowing one assay to be used repeatedly. Unfortunately many families harbor mutations that are unique to that family. As a result a customized assay must be developed for each of these families. To validate a diagnostic single-cell PCR protocol before clinical application, extensive preclinical validation on single lymphocytes is necessary to evaluate single-cell amplification efficiencies and ADO rates for all the primers to be used in the procedure. Consequently, some couples requesting PGD must wait several months to permit test development before beginning the cycle. Currently PGD is available for more than 100 different single-gene disorders [20].

PGD testing has been applied to childhood-onset recessive and dominant inherited genetic disorders, such as spinal muscular atrophy [35,49], cystic fibrosis [50], neurofibromatosis types I and II [51], β-thalassemia syndromes [52], myotonic dystrophy [53], spinocerebellar ataxia [54], and retinoblastoma [55], to name a few.

PGD testing also has been developed for a variety of X-linked inherited genetic disorders, including Duchenne/Becker muscular dystrophy [56], hemophilias A and B [33], and fragile X syndrome [57]. When a specific assay for the mutation is not available, PGD to identify female embryos for transfer can be used to eliminate the risk of an affected male. This selection requires that half of the embryos be discarded. The percentage of discarded embryos can be greatly reduced by employing MicroSort (Genetics and IVF Institute, Fairfax, Virginia) to shift the X:Y ratio in the fertilizing sperm population. MicroSort is flow cytometric sperm sorting based on the detection of differential fluorescence emitted by fluorescently stained X and Y chromosome–bearing spermatozoa. Currently in clinical trial, the method averages approximately 90% X-bearing sperm or 75% Y-bearing sperm depending on the sort parameters [58]. When appropriately sorted sperm are used with IVF, a high percentage of the resulting embryos are female [59]. To date, approximately 75% of sorts have been for X-bearing sperm, both to avoid X-linked and X-limited disease and to balance the sex ratio among a family’s children. The use of MicroSort sperm has resulted in the birth of more than 700 babies to date. The major congenital malformation rate observed in birth records reviewed to date is 2.1% (David Karabinus, PhD, and Joseph Schulman, MD, personal communication, 2006). The rate of congenital malformations in the general population is 3% to 4% [60].
In recent years late-onset inherited disorders and highly penetrant cancer-predisposition mutations have also been approached using PGD. Because such diseases can present in later life and are not expressed in all cases, the application of PGD to this group of disorders has been controversial [61,62]. Nevertheless, it is an important option for at-risk couples who wish to give birth to unaffected children, because most of these couples would not consider prenatal diagnosis for the disorder. PGD testing has been undertaken for a variety of late-onset inherited genetic disorders including familial adenomatous polyposis coli [63], \textit{BRCA1} and \textit{BRCA2} gene mutations associated with breast and ovarian cancers [61], autosomal dominant polycystic kidney disease (\textit{PKD1} mutations) [64], amyloid precursor protein (\textit{APP}) gene mutations associated with early-onset Alzheimer disease [65], familial amyloid polyneuropathy (\textit{TTR} mutations) [66] and Huntington disease [67].

PGD also permits patients to test for late-onset genetic disorders without knowing their own genotype [68]. Patients at 50% risk of inheriting the autosomal dominant disorder Huntington disease have undertaken PGD to select embryos without knowing whether they have inherited the genetic mutation for this disorder. This nondisclosing Huntington disease PGD permits families to ensure that they will have an unaffected child when they do not wish to know whether they have inherited the mutation [69]. This non-disclosure testing (or exclusion testing) for Huntington disease has raised a number of ethical issues [70–72].

Another novel indication for PGD involves blood group incompatibility such as Kell or Rhesus (Rh) D alloimmunization. Although these disorders can be detected by prenatal diagnosis and treated with intrauterine blood transfusion, the potential complication for the fetus cannot be completely eliminated even after transfusion. Seeho and colleagues [73] describe the first report of an Rh-negative child born to an Rh-sensitized mother after PGD for Rh disease.

When a child is in need of an HLA-matched hematopoietic progenitor cell transplant (HPCT), but no match is available, the affected child’s parents can use PGD to conceive a child who is an HLA-matched sibling [4]. The sibling then can act as an umbilical cord blood donor for the child in need of the transplant. The first successful PGD-HLA matching for HPCT involved treatment of a child who had Fanconi anemia in the United States [74], followed by a second successful treatment of Fanconi anemia by an international collaboration [75]. Since then a number other international groups have performed PGD for HLA matching [76,77]. In the United States, PGD testing for HLA matching is available in a number of centers (eg, Reproductive Genetics Institute, Chicago, Illinois; Genesis Genetics Institute, Detroit, Michigan; Children’s Hospital & Research Center, Oakland, California; Medical College of Wisconsin/Froedert Lutheran Memorial Hospital, Milwaukee, Wisconsin).

Dinucleotide repeat microsatellites are the most frequent type STRs in the human genome and are the most commonly used linked markers for
PGD-HLA haplotype analysis [76–78]. Dinucleotide repeat microsatellite markers, however, suffer from PCR artifacts known as repeat slippage, generating multiple extra stutter bands when analyzed. Thus, polymorphic tetra- and tri-nucleotide STRs within and flanking the MHC have been developed as linked probes for HLA haplotype analysis (David Bick, MD, Eduardo Lau, PhD, Milwaukee, Wisconsin, unpublished data, 2005). To exclude the transfer of embryos with recombinant HLA haplotypes, which are generated by meiotic recombination within the HLA region, a panel of STR probes in the flanking regions and within the HLA region are selected for HLA matching.

PGD for HLA matching has been provided for families that have children affected with inherited genetic disorders such as Fanconi anemia, thalassemia, Wiscott-Aldrich syndrome, X-linked adrenoleukodystrophy, X-linked hyper-IgM syndrome, and X-linked hypohidrotic ectodermal dysplasia with immune deficiency [79], as well as sporadic diseases such as aplastic anemia and leukemia [80].

Although PGD for HLA matching can be life saving, the number of families helped through this procedure is small because of the probability of success associated with IVF and the chance of finding a matching embryo [81]. According to published data, 12 clinical pregnancies resulted from 78 transferred embryos [80], and 7 clinical pregnancies resulted from 46 transferred embryos [30]. These data suggest an implantation rate of approximately 15.32% per embryo biopsied on day 3 after conception. In 68 PGD cycles for HLA matching in 49 families, there were only five live births of matched siblings [3].

There is another limitation to the use of PGD in HLA matching. If the affected child inherits a recombinant HLA haplotype from a parent, it is extremely unlikely that that parent will transmit an identical recombinant allele to a potential sibling. The probability of this situation may be as high as 1 in 23 cases [77,78].

Emerging preimplantation genetic diagnosis technologies

FISH analysis of cells from PGD has been used successfully to identify and transfer embryos with normal numbers of the chromosomes assessed. The usefulness of FISH is limited, however, because only a few chromosomes can be detected simultaneously in a single biopsied cell. Complete karyotyping at the single-cell level has been achieved by comparative genomic hybridization (CGH) [82]. CGH detects aneuploidy of any chromosome and can detect partial aneuploidy as well. One study showed that FISH for nine chromosomes would fail to detect 25% of the aneuploidies that were detected using CGH [83]. CGH is a technically challenging method. At the present time embryo biopsy and analysis cannot be completed in time for a blastocyst transfer on day 5 or 6. Therefore, embryos must be
cryopreserved after biopsy. Once CGH is completed, appropriate embryos are thawed and transferred. Approximately 75% of embryos survive biopsy, freezing, and thawing, a rate similar to that for unbiopsied embryos [84]. Although this loss of embryos is a drawback to the current process, more rapid CGH procedures that provide a result in time for a blastocyst transfer can be anticipated.

For many couples an IVF cycle will result in more embryos than will be transferred to the uterus at one time. These surplus embryos are cryopreserved and then used in subsequent transfers should the initial fresh transfer fail. It would be reasonable to perform CGH on these embryos before freezing to identify chromosomally normal embryos, because doing so should improve the probability of a pregnancy from cryopreserved embryos. CGH may prove useful in other ways. FISH analysis has not been successful in identifying the cause in women who have experienced recurrent implantation failure. If this failure is related to the particular chromosomes chosen in the published FISH studies, CGH could provide an answer by testing for abnormalities in all chromosomes.

Whole-genome amplification (WGA) from single cells or small numbers of cells by multiple displacement amplification (MDA) [85,86] has started a new era for PGD [31,87,88]. WGA by MDA generates higher uniformity in sequence representation in the amplified DNA than previous methods for WGA [89,90], and the sizes of amplified fragments generated by MDA are greater than 10 kilobases in length. With this approach the first round of amplification from a single cell would be the same for all single-gene disorders, because the entire starting genome is greatly amplified by MDA. The second round of amplification would use PCR designed to detect the particular disorder in question, employing standard molecular methods and conditions because there is abundant DNA resulting from the first round [88]. A discrepancy in genotyping results has been reported for DNA before and after MDA [86,91]. As with PCR analysis of single cells, some preferential amplification or ADO was detected at heterozygous loci using the MDA-based method for single-cell analysis [31,87,88].

Other steps can be used to improve the accuracy and speed the process of PGD. The transition to faster PCR systems (eg, the 9800 PCR System, Applied Biosystems, Foster City, CA) will decrease the time of PCR amplification significantly. Microfluidic chips (eg, Bioanalyzers, Agilent Technologies, Palo Alto, California; LabChip 90 system, Caliper Life Sciences, Hopkinton, Massachusetts) can simplify the analysis by combining DNA separation, sizing, and genotyping of mutations, STRs and insertion/deletion polymorphisms [92–94]. Pyrosequencing, widely used in pharmacogenomics because of its capability for quantitative genotyping of single-nucleotide polymorphisms (SNPs) and highly accurate detection of mutations [95–97], should be able to generate reliable sequencing data for PGD. A more speculative approach could involve gold nanoparticle probes (nanospheres). These probes have enabled direct SNP identification
in unamplified human genomic DNA targets [98] as well as direct gene-expression analysis using unamplified total human RNA [99].

In developing human embryos, appropriate gene expression is vital for the regulation of metabolic pathways and key developmental events. It recently was shown that altered gene expression is associated with abnormal morphology in early embryos [100]. It may be possible to select healthy embryos according to gene-expression profiles that can predict viability and implantation potential [100]. Gene-expression profiling using DNA microarrays also can identify aneuploidy in eukaryotic cells [101]. With the invention of highly sensitive molecular detectors (eg, the Trilogy Single Molecule Analyzer, US Genomics, Woburn, Massachusetts), single RNA molecules can be analyzed directly, without amplification [102]. This technique could be applied to the analysis of gene transcription in single embryonic cells.

Use and outcome of preimplantation genetic diagnosis

Although there are no published data detailing the number of centers performing PGD, it is estimated that more than 7000 PGD cycles have been performed worldwide [20]. In the United States, among 101 assisted reproductive medicine programs that carry out 200 or more stimulation cycles per year, 65 offered PGD. Thirty of these programs carry out the analysis of the cells in their own laboratory; the remaining programs send the cells by rapid delivery to another laboratory for analysis (Estil Strawn, MD, David Bick, MD, Kelly Charles, unpublished data, 2005).

The European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium has undertaken a systematic assessment of PGD outcome. The consortium was established in 1997 to collect data concerning reasons for referrals, PGD cycles performed, resultant pregnancies, and outcome of babies born. Although the 66 reporting programs are primarily European, there also are reporting centers in Australia, Argentina, Israel, Korea, Taiwan, and the United States [40]. An analysis of data for the most recently available reporting period, 2002, examines 2219 PGD cycles. Cycle data were divided into PGD for inherited disorders (including chromosome abnormalities such as translocations, sexing for X-linked disease, and monogenic disorders), PGS, and PGD for social sexing (embryo sex determination for nonmedical reasons), called “PGD-SS.”

The pregnancy rates per cycle that reached oocyte retrieval for PGD, PGS, and PGD-SS were 18%, 16%, and 21% respectively. These numbers are lower in PGD than would be expected in a routine IVF cycle because embryos diagnosed as affected or abnormal cannot be transferred, resulting in fewer cycles reaching transfer than expected. Further, the PGS rate reflects a mixture of indications for testing. For example, when PGS was used in couples who have severe male-factor infertility, the pregnancy rate was 33%; when PGS was used in couples with advanced maternal age, the pregnancy rate was 12%.
The pregnancy rates per cycle in which embryos were transferred for PGD, PGS, and PGD-SS are 25%, 23%, and 25% respectively [40]. These numbers also seem lower than expected, raising the question of whether biopsy affects an embryo’s ability to result in an ongoing pregnancy. Evaluation of embryo survival after biopsy suggests that the removal of one or two cells does not have a significant impact on embryo viability [103]. One possible explanation for a lower pregnancy rate can be found by examining the number of embryos transferred when a transfer is performed. Examination of the ESHRE PGD Consortium data for 2002 [40] finds that there was an average of 1.8 embryos per transfer. The most recent assisted reproductive technology outcome data gathered by the Centers for Disease Control (CDC) [104] indicate that the pregnancy rate after a transfer is 43% for a maternal age comparable to the Consortium data. In the CDC data, however, three or more embryos were transferred in 56% of the transfers. The same CDC data show that the number of embryos transferred dramatically affects that pregnancy rate.

ESHRE PGD Consortium data [40] show that the course and outcome of pregnancies after PGD are comparable to those for pregnancies after IVF with ICSI but without PGD [105]. Embryo biopsy does not seem to affect the course of pregnancy, the baby’s characteristics at birth (birth weight, length, gestational age at delivery), or the rate of malformations at birth.

Summary

PGD is an important alternative to standard prenatal diagnosis for genetic disorders. It also can afford families a special opportunity in certain clinical settings such as HLA matching. Low pregnancy and birth rates and the high cost of the procedure, however, make it unlikely that PGD will replace the more conventional methods of prenatal testing. PGD remains a complex combination of different technologies that requires the close collaboration of a team of specialists.

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