Preimplantation Genetic Diagnostics: A Review

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Introduction: Preimplantation genetic diagnostics (PGS/PGD) refers to the "determination of the nature of a pathological condition or disease in the ovum; zygote; or blastocyst prior to implantation. Cytogenetic analysis is performed to determine the presence or absence of genetic disease"(MeSH). PGD evolved as an "alternative to prenatal diagnosis to reduce the transmission of severe genetic disease for fertile couples with a reproductive risk"(Braude). In comparison with prenatal testing—which includes such procedures as amniocentesis, chorionic villus sampling, and cordocentesis; all of which are various procedures that sample fetal tissue or blood from either the amniotic sac, placenta, or umbilical cord—clients can avoid issues regarding pregnancy termination since the embryo is screened prior to implantation in the uterus. Overall, the set of methods available for PGD involve techniques used for assisted reproductive technologies as well as general genetic testing; including PCR, FISH, karyotyping, and even genetic sequencing. This paper reviews selected methods involved in the process of PGD, current applications with regard to specific diseases, and ethical considerations.

Methods: Preimplantation genetic diagnostics involves three primary stages. First, embryos or gametes must be collected. In this way PIGD is inherently tied to the use of assisted reproductive technologies (ARTs) such as ovarian stimulation, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Second, the collected cell or cells must be biopsied in order to extract their genetic material; which can subsequently undergo various genetic tests. Generally fertilized embryos are biopsied for analysis, and a few different biopsy options will be described here. Lastly, the genetic material is analyzed using methods such as PCR, flourescent *in situ* hybridization (FISH), or karyotyping. Recent and rapid progress in genetic sequencing and bioinformatics pose the possibility for improved diagnostic techniques.

Assisted Reproductive Technologies: According to the CDC and 1992 Fertility Clinic Success Rate and Certification Act, ARTs refer to "procedures [that] involve surgically removing eggs from a woman's ovaries, combining them with sperm in the laboratory, and returning them to the woman's body or donating them to another

woman"(CDC). This is distinct from artificial insemination or other fertilization techniques that may just involve the handling of sperm. In order to collect oocytes, a woman generally undergoes ovary stimulation via exogenous gonadotrophins [which] leads to the recruitment of many follicles, and the process can be monitored by pelvic ultrasonography"(Braude). Following stimulation, oocytes are collected (transvaginally) and prepared for further procedures including fertilization.

Two principle techniques exist for the *in vitro* creation of embryos; the first being IVF. IVF began in 1965 with the work of british scientist Robert Edwards, which eventually culminated in the first live birth of an IVF conceived child in 1978 (PBS). For IVF to work, egg and sperm are prepared in culture such that other cells are stripped away and the oocyte and thousands of sperm are then left to incubate in a medium. This method depends on such things as high sperm count and sperm motility. In contrast, the other principle ART—ICSI—is a special type of IVF in which a single, isolated sperm is directly injected into the cytoplasm of an oocyte. An advantage to ICSI is that it can be used for cases of male infertility (eg. sperm with no motility or low numbers), which would have relatively less success with traditional IVF techniques (UK health centre). In combination with other PGS techniques, this provides far more control and selectivity in the embryo profiling process. In fact, "ICSI is also recommended in all cases in which PCR is required for PGD, as the presence of supernumerary sperm, buried in the zona pellucida after IVF, might lead to a contamination of PCR reactions with paternal DNA and, therefore, to a possible misdiagnosis"(Braude).

"According to CDC's 2012 preliminary ART Fertility Clinic Success Rates Report, 176,275* ART cycles were performed at 456 reporting clinics in the United States during 2012, resulting in 51,294 live births (deliveries of one or more living infants) and 65,179 live born infants." This accounts for >1% of live births within the US. (CDC). Truly, the rate of ART usage is on the rise; increasing 3.6% from 2007-2008 (Ginsburg). According to the Society for Assisted Reproductive Technology (SART), in that same time frame, "of 190,260 fresh, non-donor assisted reproductive technology (ART) cycles [...] 8,337 included PGT. Of 6,971 cycles with a defined indication, 1,382 cycles were for genetic diagnosis, 3,645 for aneuploidy

screening (PGS), 527 for translocation, and 1,417 for elective sex election"(Ginsburg). In concordance with the trend of ART usage, "PGD use for single-gene defects (+3.2%), elective sex selection (+5.3%), and translocation analysis (+0.5%) increased"(Ginsburg).

Biopsy Options: "The biopsy procedure always involves two steps: opening of the zona pellucida and then removal of the cellular material"(De Vos). Genetic material can be extracted and characterized at multiple stages during the PGD process. Amongst those options include polar body biopsy, cleavage-stage biopsy, and blastocyst biopsy. Polar body biopsy offers the advantage of only removing "extra- embryonic material and are expected to have no biological role in the development of the future embryo"(De Vos) and therefor has reduced risk of interfering with fertilization. The disadvantage is that only the genetic material of the mother is tested; therefore this biopsy is more appropriate when only risk of maternally inherited diseases or related problems is in question (De Vos).

Post fertilization, "in humans, the best moment for embryo biopsy [is] the eight-cell stage, which is normally on the morning of Day 3"(De Vos) when cells are still totipotent, and removal of cells does not significantly diminish the inner cell mass. This is known as cleavage-stage biopsy. Later biopsy at the blastocyst stage has the advantage of removing proportionally fewer cells but has the disadvantage of possible problems with non-representative sampling due to mosaicism or that the extracted cells (which come from the trophectoderm) do not adequately represent the embryo (De Vos). "So far, it has not been extensively used in humans because of the difficulty in culturing embryos to the blastocyst stage"(De Vos). That being said, "practice appears to be transitioning to blastocyst biopsy, with cryopreservation of the biopsied blastocysts if results [from cleavage-stage biopsies] are not available by the next day"(Berger). Additionally, recent guidelines on PGD have stated that the most recent consensus is that there is "no benefit

of cleavage stage biospy/PGS (possibly owing to the high levels of mosaicism at cleavage stages and the limitation of FISH)"(Harton).

Polymerase Chain Reaction: PCR is used to detect monogenetic disorders for which genetic sequences are known and primers are available. PCR has limitations for PGD in that there is a risk of allele drop out, which is "the failure to detect an allele in a sample or the failure to amplify an allele during PCR"(Braude). PCR could also miss cases of aneuploidy or issues arising from chromosomal translocations. Conversely, PCR generally has "high sensitivity, [and thus] contamination of the study sample with extraneous DNA is a danger"(Sermon). Consequently, investigators generally use nested PCR (primers are used that lie within the genetic sequence of another set of primers) as well as ICSI. ICSI is generally a prerequisite to avoid contamination by extraneous sperm (Sermon).

Flourescent *in situ* hybridization: FISH is used to detect chromosomal abnormalities and for sex determination (De Vos). The basic premise of FISH sex profiling is that flourescent tags can be hybridized to complementary sequences on the X or Y chromosome (Braude); thus, the sex of an embryo can be determined. Such methods can be used for X-linked disorders such as Duchenne muscular dystrophy. Embryos that are female (or unaffected embryos in either sex) can then be selectively transferred for implantation. One probe that has been used for FISH analysis has been biotinylated pHY2.1—which "recognizes a repeated sequence in the long arm of the Y chromosome"(Griffin et al.). Alternatively, probes like "pBamX7, which preferentially recognizes a repeated centromeric sequence on the X chromosome"(Griffin et al.) can be used in cases to highlight the X chromosome. Other unique probes can be used to identify autosomes.

Diagnostic Uses: The ultimate goal of preimplantation genetic screening is to identify embryos at risk for genetic diseases as well as increase the success rate of assisted reproduction. "After diagnosis, only the embryos [without genetic abnormalities] are selected for transfer to the uterus"(Braude). Currently, PIGD is used to winnow out embryos that are aneuploid, have monogenetic diseases, and/or have predispositions for adult onset diseases. Additionally, PGS can also be used to identify embryos that are HLA matches for relatives or other patients, to select embryos by sex,

identify embryos at risk for diseases like cancer, and theoretically for the selection of embryos based on general genetic makeup (the concept of the "designer baby"). In the past decade, "cystic fibrosis and spinal muscular atrophy have been the most common autosomal recessive disorders which have been screened by PGD"(Berger), and concerns regarding aneuploidy is another common indication for testing.

Aneuploidy occurs when "the chromosomal constitution of cells which deviate from the normal by the addition or subtraction of chromosomes, chromosome pairs, or chromosome fragments"(MeSH). Two common forms of aneuploidy are monosomy (a chromosome is missing) and trisomy (an extra chromosome is present); which may occur do to problems during meiosis, such as non-disjunction of homologues at the metaphase plate during meiosis I or of sister chromatids during meiosis II. Aneuploidy is of the utmost concern because it "is the most commonly identified chromosome abnormality in humans, occurring in at least 5% of all clinically recognized pregnancies. Most aneuploid conceptuses perish *in utero*, which makes this the leading genetic cause of pregnancy loss. However, some aneuploid fetuses survive to term and, as a class, aneuploidy is the most common known cause of mental retardation"(Hassold). One reason that aneuploid embryos are often inviable is because of dosage issues—having one too many or too few of a chromosome may lead to excessive or insufficient gene products. Cases of aneuploidy tend to be more common in embryos derived from older age women.

The use of PGD for the classification of aneuploid cells within embryos could potentially reduce the rate of miscarriages and viable pregnancies like cases of trisomy twenty-one. In one study to examine the benefits of PGD amongst IVF patients, "spontaneous abortions, measured as FHB aborted/FHB detected, decreased after PGD ($P < 0.05$), and ongoing pregnancies and delivered babies increased ($P < 0.05$) in the PGD group of patients. Two conclusions were

obtained: (i) PGD of aneuploidy reduced embryo loss after implantation; (ii) implantation rates were not significantly improved, but the proportion of ongoing and delivered babies was increased"(Munne). More recent studies like the concurrent cystic fibrosis and aneuploidy PGD tests done by Rechitsky et al. shows favorable pregnancy outcomes following screening (Rechitsky).

Monogenetic disorders refer to diseases caused by a single gene. For patients pursuing PGD, this means that embryos can be screened for disorders that are "either autosomal dominant, autosomal recessive or X-linked recessive — in which the specific mutation that is associated with the disease is known and can be amplified using PCR, or in which embryos that are likely to be unaffected can be identified using genetic linkage"(Braude). An exclusion diagnosis can help avoid pregnancies resulting in the birth of children positive for monogenetic disorders including: [Autosomal Recessive] cystic fibrosis, spinal muscle atrophy, beta-thalassemia; [Autosomal Dominant] myotonic dystrophy, Huntington disease, [X-linked] Duchenne muscular dystrophy, hemophilia, fragile X syndrome, etc. (Berger).

One example for the successful use of PGD has involved counseling for cystic fibrosis. One review conducted by the Reproductive Genetics Institute looked at 404 PGD cycles involving 265 patients that tested for 52 different CF mutations. 685 total embryo transfers were made (~2 per cycle), resulting in 172 pregnancies and 175 babies born (some pregnancies involving multiple births) (Rechitsky). Critically, only *one* misdiagnose was made during the 20 years that all of these PGD cycles occurred in. The low incidence of misdiagnosis is attributed to refined PCR techniques (including nested-PCR) that tests "at least three linked markers are currently amplified together with the gene in question"(Rechitsky). Ultimately, the authors conclude that "the clinical outcome of PGD for CF was com- parable or even more favourable

than routine IVF"(Rechitsky).

HLA matching: A newer application for PGD has been the selection of embryos based on Human Leukocyte Antigen (HLA) profiles with the purpose of preselecting "potential donor progeny for bone marrow transplantation"(Verlinsky). Generally, ten to twelve embryos are collected and analyzed with PGD following ovarian stimulation; but only two or three embryos are ever transferred back to the uterus with the hope of initiating a pregnancy (Verlinsky). Thus, authors Verlinsky et al. argue that "instead of a 'blind' selection of embryos for transfer, only those representing a match for an affected sibling needing a transplant [could be] preselected." In fact, those investigators demonstrated the successful use of PGD combined with HLA matching for a couple that were carriers for Fanconi Anemia and also had a previous child affected by Fanconi Anemia.

Fanconi Anemia (FA) is "disorder affecting all bone marrow elements, resulting in anemia; leukopenia; and thrombopenia"(MeSH). Treatment requires bone marrow transplantation; but this depends on adequate HLA matching to prevent graft versus host disease. Siblings of course are much more likely to share HLA profiles. Using nested PCR, Verlinsky et al. Were able identify "5 heterozygous unaffected embryos [(for FANC)] for transfer with HLA antigen match for the affected sibling" out of 24 FA unaffected embryos. While this proved successful, the prospect of combining HLA matching with PGD has important ethical implications. The ethics of this situation and others are briefly discussed below.

Ethical Implications: From a clinical standpoint, preimplantation genetic testing has many benefits, including some of those that were mentioned in this paper: it can help increase the rate of successful pregnancies; it can help parents prevent passing heritable genetic diseases to their potential offspring; it can be used for HLA matching; etc. However, these clinical

benefits must be understood within a greater context. In fact, PGD poses many ethical questions. For example, there exist concerns that PGD could be inappropriately used to promote sex/gender bias, could cause problems with disease disclosure (eg. Huntington's), or may lead to genetic discrimination and eugenics. In order to deal with such issues, various guidelines have been put in place to help guide the use of PGD. That being said, “PGD/PGS is still relatively unregulated and lacks standardization compared with other forms of diagnostic testing”(Harton).

One set of guidelines that is often referenced are the European Society for Human Reproduction and Embryology (ESHRE) guidelines. Among the first inclusion/exclusion criteria for patients seeking PGD referral is that “diagnosis is technically possible in principle and the reliability of the diagnosis is high [...] Current technology in most PGD centres allows for error rates as low as 1–2%”(Harton). The suggestion here seeks to avoid situations of misdiagnoses. Other inclusion criteria include testing for many basic monogenetic disorders, including diseases like Huntington's—which can be conducted in a way to avoid “presymptomatic testing of [a] partner with a family history of the disease”(Harton). A few other ethically interesting cases are mentioned below.

HLA typing: With regard to HLA matching, according to ESHRE, “PGD is acceptable for couples who already have a child affected with a malig- nant disorder or a genetic disorder, if the affected child is likely to be cured or life expectancy is substantially prolonged by stem cell trans- plantation with cord blood from a HLA-matched sibling”(Harton). That being said, HLA matching places huge moral obligations on the embryo which may be unfair. In fact, it is unknown what the “psychological impact [is on a] child being brought into the world to save a sibling’s life [...] Another criticism is that parents are bringing a child into the world that they may otherwise not desired if they did not have an already sick child in need of cure”(Berger).

Achondroplasia and Hearing disorders: Parents may seek PGD with the hope of not only avoiding potentially lethal diseases in their children, but also to select a child that would better fit their lifestyle. For instance, clients with achondroplasia, a heritable form of short-limbed dwarfism, “might request PGD for homozygous affected embryos, which are generally lethal *in utero*, but wish to select only heterozygous embryos (which would give rise to children with achondroplasia)”(Braude). Similarly, “in a recent high-profile case, a non-hearing child was deliberately conceived using donor insemination by a male with substantial genetic history of deafness, to be deaf like its lesbian parents”(Braude). In these cases, the issue seems to be about more than just evaluating the medical problems associated with heritable diseases; the logic choice of an embryo is not always clear depending on what perspective is taken.

Ultimately, most of the ethical problems with PGD boil down to the idea of “eggspectations.” PGD seems capable of accurately identifying various disorders and promoting healthy births, but how far can it go? And to what level is PGD actually predictive—what would be the E-value of any characteristic for a child conceived by PGD? Extrapolating this, many argue that PGD treads on eugenic territory; especially when considering precedence like egg donation in which clients will often seek donor oocytes from perceived high IQ individuals. The concept of “ARTistic” reproduction (the german term for this is *kunstliche befruchtung*, which literally translates into artistic reproduction) and “designer babies” doesn't seem to far fetched. And again, PGD could be considered to be itself in infancy, and therefore it would be especially errant to have expectations for potential children when the genetic testing for such things as IQ is really not present. And if it were available, would it be appropriate. Going forward, these are some of the questions that need to be addressed. In the meantime, the general consensus is that PGD is appropriate in selected cases that truly consider the welfare of the future child.

Bibliography

Berger, Victoria K. & MD Valerie L. Baker, MD. “Preimplantation Diagnosis for Single Gene Disorders.” DOI http://dx.doi.org/ 10.1055/s-0033-1363552. ISSN 1526-8004.

Betheseda: Huntington Disease
<http://www.ncbi.nlm.nih.gov/books/NBK22226/>

Braude, Peter, et al. "Preimplantation genetic diagnosis." *Nature Reviews Genetics* 3.12 (2002): 941-955.

CDC: Assisted Reproductive Technology
<http://www.cdc.gov/art/>

De Vos, Anick, and Andre Van Steirteghem. "Aspects of biopsy procedures prior to preimplantation genetic diagnosis." *Prenatal diagnosis* 21.9 (2001): 767-780.

Die-Smulders, C. E. M., et al. "Reproductive options for prospective parents in families with Huntington's disease: clinical, psychological and ethical reflections." *Human reproduction update* 19.3 (2013): 304-315.

Genetic Home Reference: Huntington Disease & HTT Gene
<http://ghr.nlm.nih.gov/condition/huntington-disease>
<http://ghr.nlm.nih.gov/gene/HTT>

Ginsburg, Elizabeth S., et al. "Use of preimplantation genetic diagnosis and preimplantation genetic screening in the United States: a Society for Assisted Reproductive Technology Writing Group paper." *Fertility and sterility* 96.4 (2011): 865-868.

Griffin, D. K., et al. "Fluorescent in-situ hybridization to interphase nuclei of human preimplantation embryos with X and Y chromosome specific probes." *Human Reproduction* 6.1 (1991): 101-105.

Harton, G., et al. "ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening." *Human reproduction* 26.1 (2011): 14-24.

Hassold, Terry, and Patricia Hunt. "To err (meiotically) is human: the genesis of human aneuploidy." *Nature Reviews Genetics* 2.4 (2001): 280-291.

MeSH: Aneuplodiy; PGD
<http://www.ncbi.nlm.nih.gov/mesh/68000782>
<http://www.ncbi.nlm.nih.gov/mesh/?term=preimplantation+genetic+diagnosis>

Munne, S. *et al.* Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. *Hum. Reprod.* **14**, 2191–2199 (1999).

PBS: The History of In Vitro Fertilization
http://www.pbs.org/wgbh/americanexperience/features/timeline/babies/2/

Rechitsky, Svetlana, Oleg Verlinsky, and Anver Kuliev. "PGD for cystic fibrosis patients and couples at risk of an additional genetic disorder combined with 24-chromosome aneuploidy testing." *Reproductive biomedicine online* 26.5 (2013): 420-430.

Sermon, Karen, André Van Steirteghem, and Inge Liebaers. "Preimplantation genetic diagnosis." *The Lancet* 363.9421 (2004): 1633-1641.

UK Health Centre: Advantages and Disadvantages of ICSI
<http://www.healthcentre.org.uk/fertility-treatment/icsi.html>

Verlinsky, Yury, et al. "Preimplantation diagnosis for Fanconi anemia combined with HLA matching." *Jama* 285.24 (2001): 3130-3133.

Wong, Kai Mee, Sjoerd Repping, and Sebastiaan Mastenbroek. "Limitations of Embryo Selection Methods." *Seminars in reproductive medicine*. Vol. 32. No. 02. Thieme Medical Publishers, 2014.

A second indication for PGD would be for monogenetic adult onset disorders like Huntington disease (HD). This is an interesting example because it also poses unique ethical considerations. In terms of the disease itself, adult onset Huntington disease "is a progressive brain disorder that causes uncontrolled movements, emotional problems, and loss of thinking ability (cognition)"(Genetic Home Reference) that usually surfaces between ages 30-40yrs. The disease is caused by a mutation in the HTT gene located on the short arm of chromosome 4p16.3. The mutation is a copy number variant of CAG nucleotide repeats; patients with HD may have 36-120 repeats such that the number of repeats is inversely correlated with age of disease onset (Die-Smulders). This ultimately results in "the production of an abnormally long version of the huntingtin protein. The elongated protein is cut into smaller, toxic fragments that bind together and accumulate in neurons, disrupting the normal functions of these cells"(Genetic Home Reference).

The reason HD is highly relevant to PGD is that not only is the disease heritable, but it is autosomal dominant (and therefore one faulty copy causes the disease) and because successive generations may be at increased for earlier disease onset as the number of CAG repeats increases across generations due to the unstable nature of the trinucleotide repeat (Betheseda). PGD is therefore indicated and can be done using PCR.