Preimplantation Genetic Diagnosis (PGD)

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Pre-natal vs Pre-implantation diagnosis
Pre-natal Diagnosis

- Amniocentesis
- Chorionic Villus Sampling (CVS)
Pre-implantation Diagnosis

- Introduced initially in 1990
- Biopsy of a single cell per embryo, followed by its genetic diagnosis through different techniques (FISH, PCR, aCGH), and the subsequent replacement to the patient of those embryos classified by genetic diagnosis as normal.
PGD Indications

Procedure is offered to couples:

- With known **single gene disorders** that can be detected by PGD
- With known **chromosomal abnormalities** that can be detected by PGD
- requesting sex selection for **X-linked disorders**
PGD Indications

- The procedure has also been offered to couples: undergoing IVF at risk for aneuploidy
  - maternal age > 35 yo
  - Prior trisomic conception
- with recurrent pregnancy losses
- Prior failed IVF cycles (>3 prior embryo transfers with high quality, morphologically normal embryos)
- Requesting PGD for HLA-typing (to allow selection of embryos that are histocompatible with live siblings)
- Requesting sex selection for “family balancing”
Single Gene Disorders

Unaffected mother

Eggs: \(dd\)

Sperm: \(Dd\)

Children:
- \(dd\): unaffected
- \(Dd\): affected or predisposed

2 out of 4 chances: 50%
PGD Process

- Ovulation Induction
- Retrieval
- Fertilization
- Embryo Bx on Day-3
- Genetic Analysis
- Embryo Transfer
Ovulation induction
Oocyte Retrieval
Fertilization
Fertilization

- Conventional Insemination
- Intracytoplasmic Sperm Injection (ICSI)
Day 3/Cleavage Stage Embryo
Day 5/Blastocyst
Cleavage Stage Biopsy

- Most widely used technique
- Day 3, 6-8 cell stage
Cleavage Stage Biopsy
Genetic Analysis/PCR

- **DNA amplification**
  - sequence harboring the mutation)
  - billions of copies in several hrs

- **Mutation Characterization**
  - (by using mutation specific primers
  - by digestion with restriction enzymes
  - by heteroduplex analysis
Fluorescence in situ hybridization (FISH)

Chromosome 1 in a normal cell, “painted” with red fluorophore
Aneuploidy is the most frequent cause of spontaneous abortions.
Detecting a chromosome translocation

The Philadelphia Chromosome and Chronic Myelogenous Leukemia (CML)

Normal Chromosomes

*ABL* gene

*BCR* gene

9

22

Translocated Chromosomes

9 (elongated)

22 (Philadelphia chromosome)

The translocated *ABL* gene inserts into the *BCR* gene. The two genes fuse. The altered *ABL* gene functions improperly, resulting in CML.
Translocations may be:

- “Balanced” if chromosome material merely switches locations with no net loss or gain; or
- “Unbalanced” if switch is accompanied by a net loss or net gain of genetic material

Balanced translocations may reduce fertility but otherwise are generally less likely to cause serious health problems.

Unbalanced translocations often are harmful or lethal.
Genetic testing for specific disease loci (PCR or gene chips)

Polymerase chain reaction (PCR)
- amplification of DNA specific to a gene of interest (family history guides choice of genes)
Examples of genetic disorders detectable via PCR-based tests:

- Tay Sachs (autosomal recessive; ~98% accuracy)
- Cystic fibrosis (autosomal recessive; ~85% for common allele mutation)
- Huntington’s disease (autosomal dominant)
- Thalassemias (autosomal recessive blood disorder)
- Duchenne muscular dystrophy (X-linked recessive)
- Spinal muscular atrophy

As more genetic tests are developed as diagnostic tools, more will be used for predictive purposes in PDG.
Figure 1 is an image of a 24Sure analysis showing results from an embryo with the correct number of chromosomes. In this case there are two copies of each chromosome except the Y chromosome which is missing (image from Fishel et al J Fertiliz In Vitro 2011).
Figure 2 is an image of a 24sure analysis showing results from an embryo with extra chromosomes 2 and 4 and a 15 and 17 chromosome missing (image from Fishel et al J Fertiliz In Vitro 2011).
- Such chips allow cystic fibrosis accuracy of nearly 100% because all possible mutations can be screened.
Gene chip array showing expression results

• Both alleles may not amplify equally, leading to misdiagnosis or inconclusive results

• PCR-based tests only detect disorders at target loci; other mutations may exist elsewhere

• To accommodate these limitations, prenatal amniocentesis or chorionic villus sampling is usually recommended as a supplement to PGD.
Embryo Transfer
Embryo Implantation
Early Pregnancy
Can Mistakes Happen?
- **Single Gene Disorders**
  - 14 cases (0.3%), 86% PCR
  - 8 babies born, 78% Prenatal Diagnosis

- **Translocations (FISH)**
  - 3 cases, (0.08%)
  - No live births

- **PGS (FISH)**
  - 10 cases, (0.08%)
  - One baby born with T-21

- **SS**
  - One case (0.2%), 46 XX, pregnancy terminated
Causes of Misdiagnosis

- **Human Error**
  - Unprotected sex
  - mislabeling, misidentification, misinterpretation
  - wrong embryo transfer
  - incorrect probes or primers

- **Technical**
  - Probe or primer failure
  - contamination (maternal, paternal, operator, carry-over)

- **Intrinsic (embryo)**
  - Mosaicism
  - Allele drop out
  - Uniparental Disomy
Pre-natal Diagnosis
ESHRE-Delivery Outcomes

- 3163 deliveries
- 3841 children born
- 24% multiples, 96% twins
- 28% preterm births
  - 17% singletons
  - 67% twins
  - 74% triplets
- Mean Birth Weight:
  - 3215 grs
  - 2400 grs (twins)
- Mean Length: 50.0 cm
PGD & Malformations

ESHRE PGD Consortium, 2003

**Major malformations:** 2.6%
- Phocomelia and pulmonary deficiency, chylothorax, congenital hip dislocation, abdominal cystic mass, pes equinovarus, exencephaly

**Minor malformations:** 1.4%
- Syndactyly, hydrocele testis, ASD, mongolian spot, sacral dimple

Liebaers et al, Belgium 2010

**Major malformations:** 2.1% vs ICSI: 3.4%
- Chylothorax, VSD, oesophageal atresia, cataract, umbilical hernia, ichthyosis, cardiopathy
Most common autosomal **recessive** disorder
- β-thalassemia/sickle cell anemia, CF, SMA

Most common autosomal **dominant** disorder
- Myotonic Dystrophy, Huntington Disease, NF-1, Charcot-Marie-Tooth

Most common **X-linked** disorder
- Fragile X, DMD, and Becker Muscular Dystrophy
- Hemophilia A and B

3530 Cycles, dx: 85.8% of the biopsied embryos

CPR: 23% per OR, 29.5% per ET
PGD and Age

![Graph showing the relationship between age and pregnancy rates, live birth rates, and singleton live birth rates. The graph indicates a decline in these rates as age increases.]

*For consistency, all rates are based on cycles started.*
PGD and Age
Conclusions

- For couples at risk for producing offspring with either debilitating monogenetic disorders or chromosomal abnormalities, IVF/PGD represents a major scientific advance.
Conclusions

- Complications, both before and after birth, are no different in type or number from those found in a comparable ICSI population.
- Other parameters such as birth weight and length, are also similar to an ICSI population.
- PGD appears to be a safe method to avoid the birth of children with genetic defects.
Conclusions

- Before PGD is performed, genetic counseling must be provided to ensure that patients fully understand the
  - risk for having an affected child
  - the impact of the disease
  - the available options
  - the multiple technical limitations including the possibility of an erroneous result
- Prenatal diagnostic testing is strongly encouraged to confirm the results of PGD
What’s in the Future?

- With the advent of the microarray techniques for the analysis of the genome, transcripts of thousands of genes can be tested at one time, and the combination of both might dramatically change our future.
Thank You!