Preimplantation genetic diagnosis

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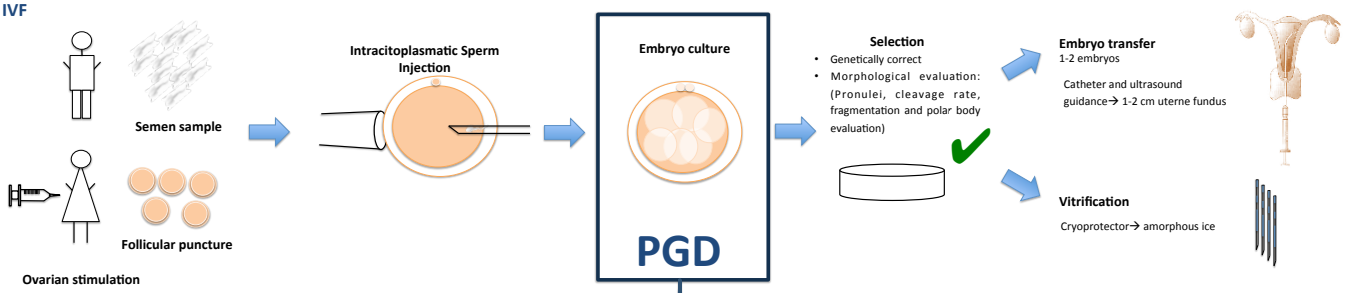
INTRODUCTION

Preimplantation Genetic Diagnosis (PGD) is the process of screening embryos for genetic abnormalities prior to be transferred to the uterus going through an in vitro fertilisation process. Firstly this technique was developed for use in fertile patients to avoid birth of an affected child with single gene disorder or chromosomal abnormality. Nowadays, while a common use is in subfertile patients to improve chance of successful pregnancy, it is also used to cure a sibling with a serious problem of compatibility or to avoid some late onset diseases, such as cancer.

IVF



1. BIOPSY

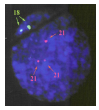
	Polar body	Blastomere	Trophectoderm
Day	0-1	3	5-6
Cells	1	6-8	Inner mass and trophectoderm cells
Removed cells	Polar body	1 blastomere	Some trophectoderm cells
Disease	Maternal	Maternal/paternal	Maternal/ paternal
Mosaicism	No	Yes	Yes, but less
IVF cycle	Same	Same	cryopreservation

2. GENETIC ANALYSIS

FISH

Cytogenetic analysis in an interphase nuclei. DNA probe + fluorophore.

Some chromosomes can only be tested (8,9, 15, 16, 17, 22, 13, 18, 21, X, Y)

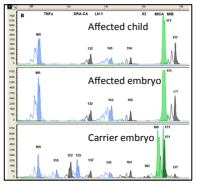


Down's syndrome

PCR

Molecular analysis
DNA amplification
→ Endonuclease digestion
→ Gel electrophoresis
→ SSCP/DGGE
→RFLP/ARMS test/VNTR/OLA

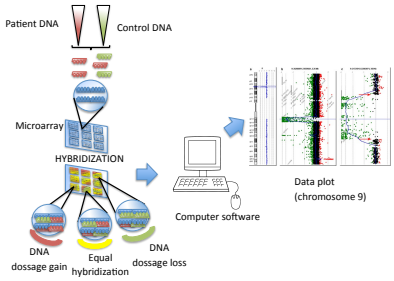
Amplification errors and allele drop out must be considered.



Capillary electrophoresis of fluorescent PCR products after analysis of 6 markers flanking the congenital adrenal hyperplasia gene.

aCGH

Patient and control DNA are amplified and labelled with fluorescent dyes. Both are applied to the microarray which has immobilised probes. The microarray results are analysed with a scanner and a computer software generates the plot.



TYPES OF PGD

ANEUPLOIDY SCREENING

A different number of chromosomes:

- Monosomy (a missing chromosome): X missing→ Turner's syndrome
- Trisomy (an extra chromosome): 21 extra→ Down's syndrome

- Polyploidy (more than two copies of each chromosome)

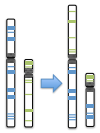
✓ Implantation failures→ Miscarriages (the risk rises with the age of the women)

✓ Birth diseases

STRUCTURAL REORGANIZATIONS

Translocations: exchange of material between two chromosomes

- Reciprocal



- Robertsonian (acrocentric chromosomes)

✓ Balanced translocations→ unaffected individuals but risk to create unbalanced gametes and have miscarriages or affected offspring.

SINGLE- GEN DISORDERS

Gene mutations associated to monogenic diseases

- 10 most common indications:

Cystic fibrosis *B- thalassemia* *Sickle cell disease* *Spinal muscular atrophy*	Autosomal recessive
Steinert myotonic distrophy *Hungtington disease* *Charcott Marie Tooth disease*	Autosomal dominant
Haemophilia *Fragile X syndrome* *Duchenne/ Becker muscular distrophy*	X-linked

CANCER

Specific gene mutations associated with some cancers:

- BRCA1/2→ breast cancer
- APC→ familial adenomatous polyposis

HLA matching

Selection of a histocompatible sibling to facilitate a bone marrow transplant.

Ab recognise non compatible HLA markers and destroy the cells.

CONCERNS

Is PGD safe?
PGD has been practised for many years without problems, however, it is not 100% reliable, so a prenatal test must be done after getting pregnant.

Where are the limits of PGD?
PGD is regulated in each country by the law. In Spain, 14/2006 law says it can be performed to detect serious, early and not curable diseases and to solve some problems of infertility, many times related with chromosomal disorders. Other indications, such as HLA compatibility and late onset diseases, must be approved by the "Comisión Nacional de Reproducción Humana Asistida".

Are the non viable embryos destroyed?
Not always, according to the law 14/2006, in Spain, embryos can be either cryopreserved and used by the couple, given to reproductive purpose or to science research or simply destroyed.

Does the end justify the means when having a soon to cure the other?
PGD allows to find the compatible embryo with his previous soon. The problems to find HLA compatibility makes PGD an optimism technique to get allogeneic hematopoietic stem cells.

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