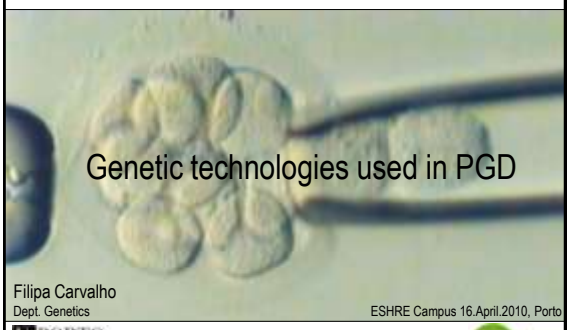

Genetic technologies used in PGD

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Dept. Genetics

ESHRE Campus 16.April.2010, Porto

Preimplantation Genetic Diagnosis

"High risk PGD" – for couples at risk of transmitting a genetic disease to the offspring

"Low risk PGD" – PGS (Preimplantation Genetic Screening) – for infertile couples in order to increase the pregnancy rates after IVF:

- Advanced maternal age
- Recurrent miscarriages
- Repetitive IVF failures
- Severe male factor
- Combined factors

Debate on usefulness of PGS is still ongoing- RCTs shows that it does not work.
ESHRE PGS task force – pilot study on polar body biopsy- 24 sure BlueGnome platform

Preimplantation Genetic Diagnosis

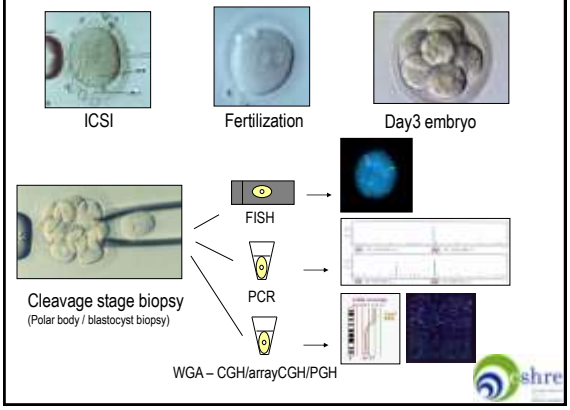
Referrals for chromosomal disorders

- Structural chromosomal abnormalities
  - Reciprocal translocations
  - Robertsonian translocations
  - Inversions
  - Deletions
- Numerical chromosomal abnormalities

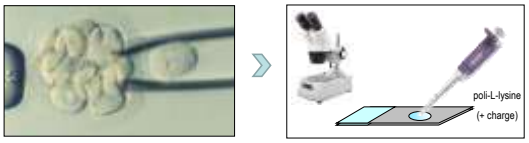
Preimplantation Genetic Diagnosis

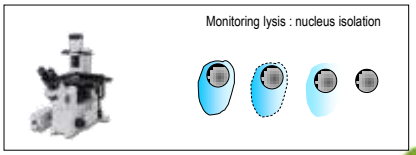
Referrals for monogenic diseases (examples)

- Autosomal recessive
  - Cystic fibrosis
  - B-Thalassemia
  - Spinal muscular atrophy
  - Tay-Sachs disease
  - Sickle cell anaemia
- Autosomal dominant
  - Myotonic dystrophy
  - Huntington's disease
  - Charcot-Marie-Tooth disease 1A
  - Marfan syndrome
  - Osteogenesis imperfecta type I
  - Familial Amyloidotic Polyneuropathy
- X-linked
  - Duchenne/Becker's muscular dystrophy
  - Haemophilia A
  - Fragile-X syndrome
  - Wiskott-Aldrich syndrome
  - Retinitis pigmentosa
  - Fabry Disease
- Other indications
  - HLA typing
  - Late-onset diseases
  - Mitochondrial diseases



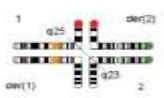
PGD for Chromosomal disorders - FISH





PGD for structural chromosomal abnormalities - FISH

Reciprocal translocation
t(1;2)(q25;p23)



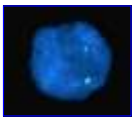
CEP1
Tel1q
Tel2p

Alternate

Adjacent 1

Adjacent 2

- Suitable probes to detect all viable combinations
- Probe combinations optimised on lymphocytes
- Polymorphisms need to be known
- 2 cells biopsy if only 1 informative probe



Normal/balanced translocation

PGD for structural chromosomal abnormalities - FISH

Robertsonian translocation
der(13;14)(q10;q10)

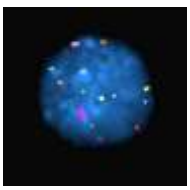
Tel14q
LSI13

Alternate

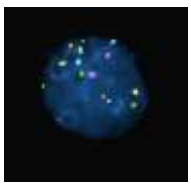
Adjacent

Normal/balanced translocation

Preimplantation Genetic Screening - FISH

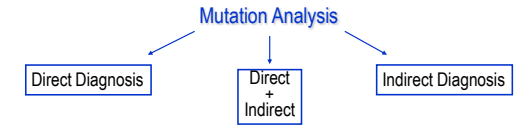


X Y (2x)13 (2x)15 (2x)16 (2x)18 (2x)21 (2x)22



XX (3x)13 (2x)15 (2x)16 (3x)18 (2x)21 (2x)22

PGD for Monogenic disorders



- PCR products sizing
- Restriction enzyme digestion
- Minisequencing
- Sequencing
- ...

- Direct mutation testing + linked STR markers

- Linkage analysis
- Exclusion testing
- Whole genome amplification + STR haplotyping

How to choose a strategy ?

✓ Rapid diagnosis (biopsy on D3 and transfer on D5)

✓ Sensitive to analyse only one cell

✓ Powerful to distinguish affected / unaffected embryos

The methodology used depends on the type of mutation.

PGD for Monogenic disorders

FACTORS INFLUENCING PGD

- Efficiency and accuracy of PCR at single cell level
- ADO – Allele dropout
- Contamination (maternal/paternal/external DNA)
- Mosaicism

PGD for Monogenic disorders

PGD SET-UP FOR SINGLE CELL

1 – Test the DNA from the progenitors and choose suitable informative markers

2 – Set-up the reactions to a reduced amount of DNA (100 pg)

3 – Study of single cells (lymphocytes/buccal cells/fibroblasts):

- amplifications efficiency (>90%) – at least 50 cells
- allele drop-out (ADO) (<5-10%)
- contamination (<5%)

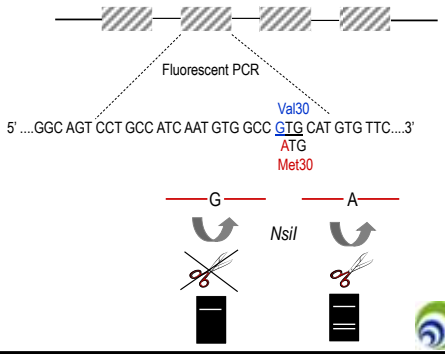
PGD for Monogenic disorders

Mutation detection: direct diagnosis

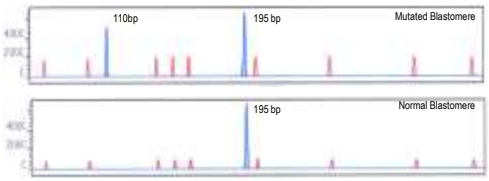
1. Flanking PCR (Fluorescent PCR)

2. Different types of mutation:
   - point mutations
   - triplets expansion
   - small insertions/deletions
   - large insertions/deletions

Mutation detection: direct diagnosis



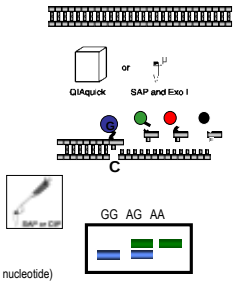
Mutation detection: direct diagnosis – PCR + RFLP



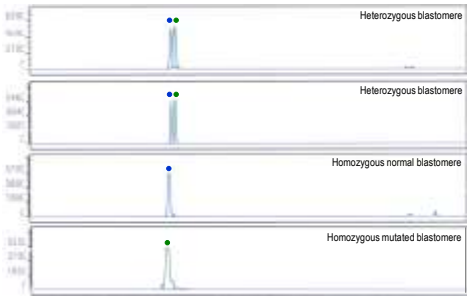
Normal: normal size
Mutation: different size

Mutation detection: direct diagnosis – Minisequencing (SNaPshot)

1. PCR
2. Purification of PCR product
3. SNaPshot™ reaction
   (primer extension-1 nucleotide added)
4. Purification [F]ddNTP
5. Electrophoresis
   (fragment labeled according to the added nucleotide)



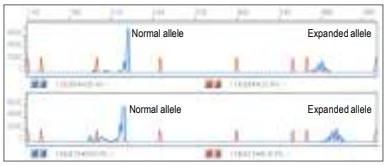
Mutation detection: direct diagnosis – Minisequencing (SNaPshot)



Normal: blue fluorocrome
Mutation: green fluorocrome

Mutation detection: direct diagnosis – Triplet expansion

- Precycle workup: informativity testing on parental DNA and in one affected relative
- PCR restricted to informative couples



Normal: Normal range allele
Small expansion: expanded allele
Large expansion: no amplification

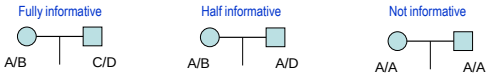
Mutation detection: indirect diagnosis

Indications

- Direct mutation testing is not possible if
  - The mutation is unknown or
  - The mutation is a large deletion/insertion
- Direct testing is not efficient if
  - The gene region is refractory to PCR or
  - Presence of a pseudogene
- Genes with a wide spectrum of mutations
  - Indirect diagnosis as a general protocol for different couples
- Preimplantation HLA typing
  - Flexible indirect HLA typing protocol applicable to a wide spectrum of possible HLA genotypes
- Exclusion testing

Mutation detection: indirect diagnosis

- The same protocol can be applied to many couples
- The couples need to be informative for the studied genetic markers and affected family members are needed
- Criterious selection of the genetic markers that should be preferable fully informative

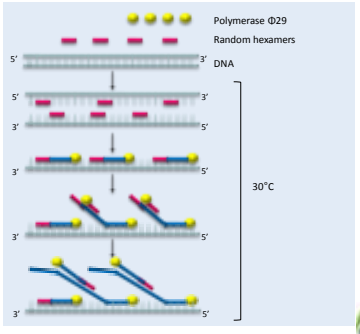


- Identification of the alleles associated with the mutation/disease
- Determination of the haplotypes

WGA - Whole genome amplification

- PEP (*Primer Extension Preamplification*)
- DOP (*Degenarete Oligonucleotide Primed*)
- OmniPlex WGA
- MDA (*Multiple Displacement Amplification*)

MDA – Multiple Displacement Amplification



Metaphase-CGH vs Array-CGH

Metaphase-CGH

- Template: metaphase chromosomes
- Full karyotyping
- Analysis of every chromosome – detects total or partial aneuploidy
- Time consuming and labour intensive
- Requires embryo cryopreservation if biopsy is performed on cleavage stage embryos

Array-CGH

- Same principle as the metaphase CGH
- Template is a solid support, spotted with sequences of DNA (BAC- or Oligo-Array)
- Loss or gain of genetic material is identified by relative fluorescence ratio
- Rapid and automated analysis

MDA and PGD

•Use MDA for array-CGH for PGS or chromosomal abnormalities

- Shorter protocol (less time required for hybridization), better resolution, automated

• Use MDA for haplotyping in PGD for monogenic disease

PGH (Preimplantation Genetic Haplotyping)

- High ADO rate, so many markers need to be analysed

• Combination of both

Conclusions

• Many different tests available for mutation detection

• The strategy depends on the mutation

• Direct diagnosis with mutation detection only

- risk of misdiagnosis in case of ADO or contamination
- amplification failure

• One cell vs two cells biopsy

- lower diagnostic efficiency / likelihood of blastocyst formation
- diagnosis based on only 1 cell requires a robust PGD protocol

Gold standard: Multiplex PCR combining
- mutation detection and analysis of linked markers
- or linkage with several markers

WGA and arrays

Conclusions