PRE-IMPLANTATION GENETIC DIAGNOSIS AND ASSISTED REPRODUCTIVE TECHNOLOGY IN HAEMOPHILIA

DR PENELOPE FOSTER
WHAT IS PGD?

- early embryo diagnosis
- allows identification of gender or abnormal gene
- can select which embryos to transfer to patient
- an alternative to antenatal testing and termination of affected pregnancy
TECHNIQUE OF PGD

- standard IVF cycle
- biopsy of 1 or 2 cells from day 3 embryo
- diagnostic testing on biopsied cells
- selection of embryos for transfer
PGD IN HAEMOPHILIA

OPTIONS (1)

GENDER SELECTION
If male partner has haemophilia:
(all male offspring unaffected, all female offspring carriers)
Gender selection enables transfer of male embryos only,
  excludes all carriers
If female partner is carrier:
(50% male offspring affected, 50% female offspring carriers)
Gender selection enables transfer of female embryos only,
but 50% discarded male embryos unaffected, 50% of
  transferred female embryos carriers
PGD IN HAEMOPHILIA

OPTIONS (2)
SPECIFIC GENE DETECTION
(identifies embryos with the X chromosome mutation assoc with haemophilia)
- more embryos available for transfer (~60%)
- avoids discarding unaffected male embryos
- avoids transfer of carrier female embryos
GENDER SELECTION - FISH

FLUORESCENT IN-SITU HYBRIDISATION

detects the presence and number of particular chromosomes inc X and Y
single cell from embryo fixed to slide
apply FISH probes
labelled DNA probes which bind to complementary sequences on specific chromosomes
probes labelled with coloured fluorochromes
coloured spots indicate presence of sequence
8-probe FISH – chromosomes 4,13,16,18,21,22,X,Y
select euploid XX or XY embryos for transfer
only ~15% of embryos suitable for embryo transfer
60% patients will not have embryo transfer
FI SH ON BLASTOMERES

13, 16, 18, 21, 22

X, Y, 4
PGD FOR SPECIFIC GENE DETECTION

looking for the presence or absence of the haemophilia mutation in each embryo tested
2 cells from each embryo
DNA amplification (by PCR)
fragment analysis on DNA sequencer
analyse polymorphic markers along the F8 gene
inclusion of informative markers (belt and braces)
individualised tests for each couple
significant time and effort required for each test
Markers Alleles
DXS1073  124, 126, 128
DXS8061  139, 145, 147
Factor VIII wt, mut
AMEL X c. some

Unaffected female
Unaffected Male
Carrier Female
Affected male

Husband
Mother

128 147
wt

124 145
wt

124 145
wt

128 147
wt
126 139
mut

126 139
mut

111 =
X c. some

117 =
Y c. some
RESULTS OF PCR ANALYSIS FOR HAEMOPHILIA A

MOTHER

AFFECTED MALE
PGD options in Haemophilia

If man has haemophilia:

**gender selection** (for male embryos)
- but with 8-probe FISH
- only ~ 10 -15% embryos suitable for transfer
- in 60% cycles there will be no transfer

**gene detection**
- no value as all males unaffected, all females carriers

If female is carrier:

**gender selection** (for female embryos)
- but with 8-probe FISH
- only ~10 -15% embryos suitable for transfer
- in 60% cycles there will be no transfer
- ½ discarded males unaffected, ½ transferred females carriers

**gene detection**
- select unaffected male embryo or non-carrier female embryos
- 88% of cycles will have embryo transfer
## PGD OUTCOMES
(MIVF data 1997 - 2008)

<table>
<thead>
<tr>
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<th>GENDER SELECT</th>
<th>MONOGENIC</th>
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<tr>
<td>CYCLES</td>
<td>56</td>
<td>128</td>
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<tr>
<td>AGE</td>
<td>35.3</td>
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<tr>
<td>% genetically suitable EMBRYOS</td>
<td>11.9</td>
<td>47.6</td>
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<td>% NO ET</td>
<td>62.5</td>
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<tr>
<td>% CLIN PREG</td>
<td>33.3</td>
<td>24.8</td>
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<tr>
<td>IMP. RATE</td>
<td>30.8</td>
<td>21.0</td>
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</table>
Tests developed to date:
- Huntington disease (direct)
- Huntington disease (exclusion)
- Cystic fibrosis
- β-thalassaemia
- α-thalassaemia
- Duchenne muscular dystrophy
- α-1-antitrypsin deficiency
- Kennedy disease
- Fragile-X
- Motor neurone disease (exclusion)
- Neurofibromatosis type 1
- Hirschprung's disease
- X-linked hydrocephalus
- Myotonic dystrophy
- Chronic granulomatous disease
- Niemann-Pick type C
- Opitz syndrome
- Leigh syndrome
- Multiple exostosis
- Rapp-Hodgkin ectodermal dysplasia
- Tuberous sclerosis
- X-chromosome deletion
- Menkes disease
- Treacher-Collins syndrome
- Retinoblastoma
- WHIM syndrome
- Mucopolysaccharidosis IIIB

Tests in development:
- Complex 1 deficiency
- Spinal muscular atrophy (SMA)
- Ataxia telangiectasia
- Congenital amegakaryocytic thrombocytopenia
- TTR amyloidosis
- Generalised arterial calcification of infancy (GACI)
- HNPCC (hereditary non-polyposis colon cancer)

Multiple cases for many of these
Conditions that have been diagnosed by PGD – worldwide

- Cystic fibrosis
- Tay Sachs disease
- β-thalassaemia
- Sickle cell anaemia
- Rh blood typing
- Spinal muscular atrophy
- Adrenogenital syndrome
- Congenital adrenal hyperplasia
- Plakophilin-1 (PKP1)
- MCAD
- CDG1C
- Epidermolysis bullosa
- Gaucher’s disease
- Hyperinsulinemic hypoglycemia PHH1
- Fanconis anemia
- HLA matching
- Fragile X
- Myotonic dystrophy
- Huntington's
- Wiscott-Aldrich syndrome
- Incontinentia pigenti
- Ornithine transcarbamylase def.
- Myotubular myopathy
- Hunter syndrome
- Fabry disease
- Choroideraemia
- Kallman syndrome
- Coffin-Lowy syndrome
- Barth syndrome
- Hypospadias
- Golabi-Rosen syndrome
- Marfans syndrome
- Charcot-Marie-Tooth disease (type 1A)
- Amyloid polynephropathy
- Crouzon's syndrome
- NF2
- Osteogenesis imperfecta I and IV
- Stickler syndrome
- Tuberous sclerosis
- Central core disease
- Familial adenomatous polyposis coli
- Li Fraumeni syndrome
- Lesch Nyhan syndrome
- Duchenne muscular dystrophy
- Becker muscular dystrophy
- Haemophilia A
- Charcot-Marie-Tooth disease
- Retinitis pigmentosa
- Ornithine Transcarbamylase Deficiency
- Agammaglobulinemia
- Alport syndrome
- Hunter’s syndrome MPSII
- Oro-facial-digital syndrome type 1
- Adrenoleukodystrophy
- Chronic granulomatous disease
- Menkes disease
- Lowe syndrome
- Ectodermal dysplasia
- Epilepsy
- BRCA1
- Ataxia
- Renal agenesis
- Norrie disease
IVF Cycle

- Pituitary down – regulation with OCP & GNRH agonist (gonadotrophin – releasing hormone)
- Ovarian stimulation with r FSH (follicle stimulating hormone)
- hCG trigger
- Vaginal ultrasound – assisted OPU (ovum pick up)
- Embryo transfer (ET) 2 or 3 days after OPU

- Monitor follicular maturation with vaginal ultrasound
- Aim for cohort of “leading follicles” of 18-20mm diameter
- Average egg no / OPU = 11
- Fertilisation ~60%
Acid drilling

Pipette loaded with acidified culture media – pH 2.4
ACCESS TO PGD AT MELBOURNE IVF

Request for PGD submitted

PGD Committee
• Senior PGD scientists
• IVF doctor
• Clinical geneticist
• Genetics counsellor
• PGD nurse

PGD counselling with genetics counsellor
PGD counselling with clinical geneticist
PGD cycle starts

PGD refused

IVF counselling (mandatory in Victoria)
CONSENT TO PGD

- Although the degree of accuracy of these tests is high, all tests have a failure rate, and the test results could be wrong.

- A full genetic analysis is not being carried out and there are many other genetic conditions that are not being analysed or tested for.

- Finding a normal cell using FISH testing does not mean that a baby resulting from the embryo will have the normal number of chromosomes or be of the expected sex.

- In single gene defect testing, we cannot guarantee that the embryo will not have the disorder being tested for.
It is strongly recommended that all women with PGD pregnancy consider DNA testing in early pregnancy (CVS or amniocentesis) to confirm the early embryo diagnosis.

Spontaneous conception may occur during a PDG cycle, and all couples having PGD should avoid any form of unprotected sex during the treatment cycle.

Rarely, some embryos may be destroyed during the biopsy procedure.

Rarely, it may not be possible to obtain a result on an embryo.

Embryos that are very poor quality will not be subjected to embryo biopsy and will be discarded.
PGD - BENEFITS

RELIABLE
    97% embryos diagnosed

ACCURATE
    misdiagnosis rate ~ 2%

RAPID
    embryo biopsy and diagnostic testing completed
    8 – 30 hours

TREATMENT OPTION
    alternative to antenatal testing and TOP
PGD – PITFALLS

- Invasive
- Highly medicalised, requires IVF
- Expensive
- Specific feasibility testing can take months
- No guarantee of pregnancy
HIV AND ASSISTED REPRODUCTIVE TECHNOLOGY

• Chronic Viral Illness Clinic at Royal Womens Hospital Melbourne established 2002

• principle of harm minimisation (reduced risk of HIV transmission to partner and baby)

• Use of assisted reproductive technology (intra-uterine insemination or IVF)
HIV +ve MALE

- Good health
- Undetectable viral load (blood) for 2 months
- Semen screening for HIV
- 2 successive samples <50 copies
  = semen storage for IUI / IVF
  (all semen samples tested for HIV RNA and DNA)
- Risk of transmission to partner: <1/4000
CVI PROGRAMME RWH
2002 - 2009

~70 couples referred
~30 couples treated
(majority HIV-positive males)

Total number of babies born 16 inc 2 sets of twins
1 miscarriage
1 ectopic
4 ongoing

no of patients on treatment 8
no of patients pre-treatment 6

There have been no cases of transmission of HIV to partner or baby
ACKNOWLEDGEMENTS
MIVF PGD TEAM

Leeanda Wilton
Sharyn Stock-Myer
Pam Matthews
Mirjana Marti
Kay Oke
Angie Giasli
Rebecca Cameron
Sophie Falle

Peter Coleman
David Amor
Fleur Cattrall
Andrea Twomey
Paisu Tang
Celine Lawler
Riddhi Marfatia
Penelope Foster
ACKNOWLEDGEMENTS
RWH CVI COMMITTEE

Michelle Giles
Gordon Baker
Harold Bourne
Suellen Peak
Gary Clarke
Suzanne Crowe
Penelope Foster

Rachael Knight
Jenny Hoy
Sam Perna
Sepehr Tabrizi
Vicky Greengrass
Gayle de Bruyckere
Suzanne Garland