PRE-IMPLANTATION GENETIC DIAGNOSIS AND ASSISTED REPRODUCTIVE TECHNOLOGY IN HAEMOPHILIA

DR PENELOPE FOSTER
WHAT IS PGD?

- Early embryo diagnosis
- Allows selection of unaffected embryos for transfer to patient
- 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

Sex selection:
- if affected husband, all male offspring unaffected, all females carriers = select male embryos for transfer
- if carrier wife, 1/2 males affected, 1/2 females carrier = select female embryos for transfer
PGD IN HAEMOPHILIA

OPTIONS

Specific gene detection

- avoids discarding unaffected male embryos
- avoids transfer of carrier female embryos
SEX SELECTION - FISH

FLUORESCENT IN-SITU HYBRIDISATION
- detects chromosome number
- cell from embryo fixed to slide
- apply FISH probes
- DNA sequences complementary to small segment of particular chromosome
- 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
PGD FOR SPECIFIC GENE DETECTION

- DNA amplification by PCR
- 2 cells from embryo
- Fragment analysis on DNA sequencer
- Inclusion of informative markers
- 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 111 =
X c.some
117 =
Y c.some

Add title
RESULTS OF PCR ANALYSIS FOR HAEMOPHILIA A
MONOGENIC PGD AT MELBOURNE IVF

Tests developed to date:
- Cystic fibrosis
- β-thalassaemia
- α-thalassaemia
- Duchenne muscular dystrophy
- α-1-antitrypsin deficiency
- Kennedy’s disease
- Fragile-X
- Motor neurone disease (exclusion)
- Huntington’s disease (direct)
- Huntington’s disease (exclusion)
- Neurofibromatosis 1
- Hirschsprung’s disease
- X-linked hydrocephalus
- Myotonic dystrophy
- Chronic granulomatous disease
- Niemann-Pick type C
- Opitz syndrome
- Rapp-Hodgkin ectodermal dysplasia

Tests being developed:
- Congenital adrenal hyperplasia
- Tuberous sclerosis
- Multiple exostosis

Waiting:
- BRCA2
- Waardenburg syndrome
- Neurofibromatosis 2
- AR polycystic kidney disease
- Treacher-Collins syndrome
- Menkes disease
- Familial adenomatous polyposis
- Retinoblastoma
- WHIM syndrome
- Haemophilia A

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 pigментi
- 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 polyneuropathy
- Crouzons 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%
IVF Cycle

Period

Synarel

O C P

FSH

OPU

ET
Acid drilling

Pipette loaded with acidified culture media – pH 2.4

Hole digested in zona by acid
## PGD Outcomes – “Fertile” Patients (all data up to end 2006)

<table>
<thead>
<tr>
<th></th>
<th>SEXING</th>
<th>ROB TRANS</th>
<th>REC TRANS</th>
<th>MONOGENIC</th>
<th>MIVF TOTAL</th>
<th>ESHRE DATA V</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYCLES</td>
<td>43</td>
<td>29</td>
<td>49</td>
<td>62</td>
<td>183</td>
<td>730</td>
</tr>
<tr>
<td>AGE</td>
<td>34.5</td>
<td>35.2</td>
<td>33.5</td>
<td>35.4</td>
<td>34.4</td>
<td>~34</td>
</tr>
<tr>
<td>% “Normal” Embryos</td>
<td>14.3</td>
<td>21.4</td>
<td>11.5</td>
<td>52</td>
<td>25.3</td>
<td>36.0</td>
</tr>
<tr>
<td>% NO ET</td>
<td>58</td>
<td>52</td>
<td>51</td>
<td>9.7</td>
<td>44.2</td>
<td>19</td>
</tr>
<tr>
<td>% CLIN PREG</td>
<td>38.9</td>
<td>14.3</td>
<td>29.2</td>
<td>25.0</td>
<td>26.8</td>
<td>24</td>
</tr>
<tr>
<td>IMP. RATE</td>
<td>34.8</td>
<td>13.6</td>
<td>28.1</td>
<td>18.3</td>
<td>21.8</td>
<td>16</td>
</tr>
</tbody>
</table>
Access to PGD at Melbourne IVF

Request for PGD submitted

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

PGD refused

PGD counselling with genetics counsellor

PGD counselling with clinical geneticist

IVF counselling (mandatory in Victoria)

PGD cycle starts
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.
Consent to PGD

- 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 Women’s 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/ICSI)
HIV +ve MALE

- Good health
- Undetectable viral load 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 <1/2000
CVI PROGRAMME RWH

- 33 referrals
  - 27 male HIV+ve
  - 4 female HIV+ve
  - 1 couple both HIV+ve

- 20 patients treated
  - 16 male HIV+ve - 12 pregnancies
    - (7 delivered, inc 2 sets twins, 2 ongoing)
  - 3 female HIV+ve - 1 ongoing pregnancy
ACKNOWLEDGEMENTS
MIVF PGD TEAM

Leeanda Wilton
Sharyn Stock-Myer
Pam Matthews
Mirjana Martic
Greta Gillies
Kay Oke
Kate Pope
John McBain
Penelope Foster
Mac Gardner
David Amor
Stacey Roe
Peter Coleman
Riddhi Marfatia
ACKNOWLEDGEMENTS
RWH CVI COMMITTEE

Janell Archer          Rachael Knight
Gordon Baker          Hayley Matic
Harold Bourne         Anne Mijch
Kate Cherry           Sam Perna
Gary Clarke           Sepehr Tabrizi
Suzanne Crowe         Loreto Valent
Penelope Foster       Vicky Greengrass
Suzanne Garland       Michelle Giles