Preimplantation Genetic Diagnosis (PGD) for Fanconi Anemia and HLA matching

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Outline

- PGD overview
- In vitro fertilization (IVF) and PGD process
- Accuracy of PGD
- Cost
- Frequently asked questions
- RGI’s PGD experience
Definitions

• In Vitro Fertilization (IVF):
  ◦ Assisted reproductive technology where eggs are removed from a woman’s ovaries and fertilized by a man’s sperm outside the body in a laboratory
  ◦ The fertilized eggs develop into embryos which can be transferred into a woman’s uterus, with the hopes of implantation and pregnancy

• Preimplantation Genetic Diagnosis (PGD):
  ◦ Diagnosis of a genetic disease before pregnancy
  ◦ Embryos are created through IVF and tested prior to transfer/implantation
Common indications for PGD

- Autosomal recessive, dominant and X-linked genetic disorders
  - Childhood and adult-onset disorders
  - Cancer predisposition genes
  - Maternal-fetal blood incompatibility
- HLA matching
- Inherited chromosomal rearrangements
- Spontaneous chromosomal aneuploidies (incorrect chromosome number)
Getting started

- **PGD**
  - Submit FA and HLA genetic reports to PGD laboratory to determine feasibility
  - Consultation with genetic counselor (phone or in-person)
    - Discuss process, next steps, accuracy, limitations, costs and timeline
  - PGD set-up

- **IVF**
  - Consultation with fertility center/physician (Reproductive Endocrinologist or REI)
  - Evaluation (blood tests, ultrasounds, semen analysis) to evaluate feasibility of IVF
    - Required even if couple is fertile!
PGD set-up

- What is needed to begin?
  - Genetic reports on couple & children
  - DNA samples (blood or cheek swabs) from couple & children
  - Consent forms
  - Set-up fee

**Takes 4-8 weeks to complete**

Cannot start IVF medications/cycle until notified that set-up is complete!
“Establishing linkage”

PGD set-up for Fanconi Anemia
“Establishing linkage”

PGD set-up for HLA
Markers in HLA complex

- **Centromere**
  - D6S439
  - D6S291
  - D6S1583
  - D6S1610

- **Telomere**
  - D6S461
  - D6S276*
  - D6S299
  - D6S464*
  - D6S105
  - D6S306*
  - D6S1624*
  - D6S1615
  - D6S258
  - D6S248*
  - MOG a,b,c,d
  - RF
  - D6S265
  - D6S510
  - MIB
  - MICA

  - **Class I**
    - TNF a,b,c,d
    - 62
    - 82-1
    - 9 N2
    - D6S273*
    - D6S1666

  - **Class II**
    - D6S1629
    - DN
    - LH I
    - DQ-CAR II
    - DQ-CAR
    - G51152
    - D6S2447
    - D6S2443
    - D6S2444
    - TAP I
    - Ring 3CA
    - D6S1568
    - D6S1560
    - D6S1618

  - **Class III**
    - 6p21.3
IVF/PGD timeline overview

1. Downregulation
2. Stimulation
3. HCG
4. Egg retrieval
5. Embryo biopsy
6. Embryo transfer
7. Freeze all embryos
8. Thaw embryo(s) and transfer
9. Pregnancy test
IVF/PGD timeline

**Downregulation (~3+ weeks)**
- Shut off ovaries
- Birth control, Lupron

**Stimulation (~1.5 weeks)**
- Stimulate ovaries to produce many eggs
- Hormone injections

**HCG (one time)**
- “Trigger shot”
- Causes eggs to mature
Day 0

- 36 hours after HCG shot
- Egg retrieval
  - Outpatient procedure (~20 min)
  - “Twilight” anaesthesia
- Fertilization of eggs with partner’s sperm (ICSI)
  - Required: reduces contamination
IVF/PGD timeline

Option #1: Day 3 (blastomere) biopsy

Day 3

- Each embryo is ~4-8 cells in size
- Remove (biopsy) one cell (blastomere) from each embryo
- Biopsied cells undergo genetic testing

Day 5

- Results from genetic testing are available
- Embryo development checked by laboratory
- 1-3 healthy/developing embryos transferred into woman’s uterus
IVF/PGD timeline

Option #2: Day 5 (blastocyst) biopsy

Day 5/6
- Embryos are ~100 cells in size
- Remove (biopsy) trophectoderm cell(s) from each embryo
  - Trophectoderm: part of embryo that will become placenta during pregnancy
- Biopsied cells undergo genetic testing
- Embryos are frozen to provide enough time to obtain results

6+ weeks later
- Medications are taken to thicken the lining of the uterus
- Healthy embryo(s) thawed and transferred
Embryo (Day 3) biopsy
PGD results

$\frac{3}{4}$ (75%) chance of embryo to be unaffected with FA $\times \frac{1}{4}$ (25%) chance of embryo to be HLA match = $\frac{3}{16}$ embryos to be healthy matches

$\sim$19% of embryos will be a healthy match
## PGD results

<table>
<thead>
<tr>
<th>Embryo number</th>
<th>FA status</th>
<th>HLA status</th>
<th>Embryo transfer recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carrier</td>
<td>Non-match</td>
<td>Can be frozen</td>
</tr>
<tr>
<td>2</td>
<td>Affected</td>
<td>Non-match</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Affected</td>
<td>Match</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Carrier</td>
<td>Non-match</td>
<td>Can be frozen</td>
</tr>
<tr>
<td>5</td>
<td>Carrier</td>
<td>Match</td>
<td>YES</td>
</tr>
<tr>
<td>6</td>
<td>N/A</td>
<td>N/A</td>
<td>No, re-biopsy if possible</td>
</tr>
<tr>
<td>7</td>
<td>Non-carrier</td>
<td>Non-match</td>
<td>Can be frozen*</td>
</tr>
<tr>
<td>8</td>
<td>Carrier</td>
<td>Non-match</td>
<td>Can be frozen</td>
</tr>
</tbody>
</table>

*reduced accuracy
IVF/PGD timeline

~1.5 weeks after transfer
- Blood test to determine if pregnancy occurred
- If positive, follow up every few days with additional blood tests (make sure hormone levels are increasing)

~3.5 weeks after transfer
- 1st ultrasound to confirm presence of amniotic sac (4 weeks gestation)

~5.5 weeks after transfer
- Ultrasound to confirm heartbeat (6 weeks gestation)

~7-9 weeks after transfer
- Released to regular OBGYN (8-10 weeks gestation)
- Followed the same as a natural pregnancy
IVF/PGD timeline summary

- **Downregulation**
- **Stimulation**
- **HCG**
- **Day 0: egg retrieval, fertilization**
  - Day 3: blastomere biopsy
  - Day 5: embryo transfer
  - Day 5: blastocyst biopsy
  - Freeze all embryos
  - Thaw and transfer
- **Pregnancy test**
# Sample IVF/PGD cycles

<table>
<thead>
<tr>
<th>Patient #1</th>
<th>Patient #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 eggs retrieved</td>
<td>25 eggs retrieved</td>
</tr>
<tr>
<td>6 mature</td>
<td>18 mature</td>
</tr>
<tr>
<td>4 fertilize</td>
<td>13 fertilize</td>
</tr>
<tr>
<td>3 biopsied</td>
<td>10 biopsied</td>
</tr>
<tr>
<td>1 healthy HLA match – developing</td>
<td>3 HLA matches</td>
</tr>
<tr>
<td>1 embryo transferred</td>
<td>2 affected with FA</td>
</tr>
<tr>
<td></td>
<td>1 healthy – arrested</td>
</tr>
</tbody>
</table>

Positive pregnancy test!  
No embryo transfer

Statistics are often skewed with small sample sizes
Additional testing options (chromosomes)
Percentage of aneuploid eggs by age
Why screen for chromosome problems?

- Reduce chance of live birth with chromosome disorder
- Reduce risk of miscarriage
  - 50% of first trimester miscarriage caused by incorrect chromosome number
- Improve effectiveness of IVF
  - Failed implantation often associated with incorrect chromosome number
Aneuploidy/chromosome testing

- Can be considered by women of any age, ethnicity, family history
  - Chromosome problems **NOT** related to family history!
- Testing options
  - Common chromosomes (5-7)
  - All chromosomes
    - 24-chromosome microarray
- Limitations
  - Imperfect test
  - Rule out more embryos
PGD accuracy

- FA/HLA
  - Typically 95-98%
  - May be reduced for some embryos

- Chromosomes
  - 90-98% depending on type of test and sample type
Factors affected accuracy of PGD

- Single cell testing
  - Cell type being tested & quality of cell
  - Allele drop-out (ADO)
  - Failed amplification of DNA
  - Chromosomal mosaicism
- DNA contamination
- Human error

PGD involves a modification of risk – not the elimination of risk.

PGD does not replace prenatal diagnosis (CVS, amniocentesis)
PGD cost

**IVF**
- Initial evaluations ($3,000 - $4,000)
- Procedures/monitoring ($9,000 - $13,000)
- Medications ($2,000 - $5,000)

**PGD**
- Set-up ($3,500 - $5,000) – one-time
- Testing ($2,500 - $3,000)
- Biopsy ($1,500) – if at RGI
- Embryologist travel ($1,500 - $2,000) – if needed
- Chromosomes ($2,000 - $3,500) – optional
FAQ

1. How to choose an IVF center?
   ◦ Location
   ◦ Can they do their own biopsies?
   ◦ Will they work with any PGD lab?
   ◦ Exclusion criteria
   ◦ Day 5 embryo culture success, pregnancy rates

2. How to choose a PGD lab?
   ◦ Experience with FA/HLA testing
   ◦ Any misdiagnoses?
   ◦ Availability of chromosome testing options
   ◦ Is prenatal testing required?
   ◦ Availability of genetic counselors
FAQ

3. How many embryos are transferred?
   ◦ Typically 1 or 2, sometimes 3
   ◦ Up to patient and physician
   ◦ More embryos transferred = higher chance of multiple gestation

4. Is there a minimum number of embryos required?
   ◦ NO
   ◦ Possibility of batching cycles
FAQ

5. What if I have extra embryos?
   ◦ Freeze, discard, donate to research or other couples

6. Is there a risk to removing a cell from an embryo?
   ◦ Low risk of embryo arrest (<0.5%)
   ◦ Have not seen increased risk of birth defects, miscarriage, etc
FAQ

7. Do I have to come to Chicago?
   ◦ NO – we can work with any IVF center

8. What are the chances of getting pregnant?
   ◦ Age dependent, center dependent, does NOT depend on prior ability to conceive!
   ◦ If embryo transfer:
     • <35 ~50%
     • 35-37 ~40%
     • 38-42 ~30%
     • >43 ~10% or less
RGI’s experience with PGD

<table>
<thead>
<tr>
<th>Testing performed</th>
<th># Patients</th>
<th># Cycles</th>
<th>Pregnancies</th>
<th>Live births</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Gene</td>
<td>1206</td>
<td>2158</td>
<td>731</td>
<td>683</td>
</tr>
<tr>
<td>Aneuploidy</td>
<td>3205</td>
<td>4429</td>
<td>894</td>
<td>702</td>
</tr>
<tr>
<td>Translocation</td>
<td>367</td>
<td>539</td>
<td>150</td>
<td>119</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>4778</strong></td>
<td><strong>7126</strong></td>
<td><strong>1775</strong></td>
<td><strong>1504</strong></td>
</tr>
</tbody>
</table>

As of 06/2011

Over 250 genetic conditions tested in 22 years
# RGI’s experience with PGD for HLA

<table>
<thead>
<tr>
<th>Testing performed</th>
<th># Patients</th>
<th># Cycles</th>
<th>Pregnancies</th>
<th>Live Births</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA only</td>
<td>46</td>
<td>98</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>HLA with genetic disease</td>
<td>81</td>
<td>199</td>
<td>34</td>
<td>27</td>
</tr>
<tr>
<td>TOTAL</td>
<td>127</td>
<td>297</td>
<td>58</td>
<td>47</td>
</tr>
</tbody>
</table>

As of 06/2010
RGI's experience with PGD for FA/HLA

<table>
<thead>
<tr>
<th>Testing performed</th>
<th># Patients</th>
<th># Cycles</th>
<th># Embryo Transfers</th>
<th>Pregnancies</th>
<th>Live births</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA-A only</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>FA-C only</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>FA-A + HLA</td>
<td>11</td>
<td>39</td>
<td>22</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>FA-C + HLA</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FA-D2 + HLA</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FA-F + HLA</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FA-I + HLA</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FA-J + HLA</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>20</strong></td>
<td><strong>60</strong></td>
<td><strong>39 (65%)</strong></td>
<td><strong>10 (26%)</strong></td>
<td><strong>10</strong></td>
</tr>
</tbody>
</table>

As of 05/2012
Summary: Pros vs. Cons of IVF/PGD

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dramatically reduces risk of having affected offspring</td>
<td>Extremely expensive if insurance doesn’t cover (~$25,000/cycle)</td>
</tr>
<tr>
<td>Testing occurs prior to implantation in order to avoid difficult decision-making during pregnancy</td>
<td>Physically and emotionally difficult</td>
</tr>
<tr>
<td>Availability of HLA testing for couples with a sick child needing a stem cell transplant</td>
<td>Low pregnancy rates with IVF, regardless of prior fertility</td>
</tr>
<tr>
<td></td>
<td>Not a perfect technology (95-98% accurate, prenatal diagnosis is still recommended to confirm results</td>
</tr>
</tbody>
</table>
Thank you!

- Questions?
- RGI contact information:
  - 2825 N. Halsted St, Chicago, IL 60657
  - Phone: 773-472-4900
  - Fax: 773-871-5221
  - Email: rgiworld@gmail.com
  - www.reproductivegenetics.com