Preimplantation Genetic Diagnosis (PGD) for Fanconi Anemia and HLA

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Chicago, IL USA
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OBJECTIVES

- Preimplantation Genetic Diagnosis (PGD)
- In Vitro Fertilization (IVF)
- Fees / Insurance
- Common Questions
- Success / RGI experience with FA
- Advantages / Disadvantages / Limitations
Definitions:

**Preimplantation Genetic Diagnosis (PGD):** Diagnosis of Genetic Disease Before Pregnancy

**In Vitro Fertilization (IVF):** Assisted Reproductive technology procedure where oocytes (eggs) are removed from a woman’s ovary and are fertilized by the male’s sperm outside the body in a controlled setting. The fertilized eggs develop into embryos which can be implanted in a woman’s uterus to achieve pregnancy.
Current indications for PGD:

* Autosomal Recessive, Dominant and X liked Disorders
* HLA matching
* Cancer predisposition genes
* Adult-onset disorders
* Infertility-causing genes
* Maternal-fetal incompatibility
* Chromosomal Aneuploidy
* Combined Single gene, HLA, and aneuploidy testing
Testing Techniques utilized in PGD:

- **PCR** (polymerase chain reaction)
  - Single gene (Mendelian) disorders like FA
  - Mutation(s) with linked markers and HLA matching

- **FISH** (fluorescent in-situ hybridization)
  - Aneuploidy (abnormal # of chromosomes)
  - Translocations/structural rearrangements
Getting started....

- PGD Set-up
  - Create a testing system for the specific FA mutations and HLA

- Prepare for IVF
PGD SET-UP for FA/HLA

- **MEDICAL RECORDS**: FA mutation reports, HLA reports
- **PGD CONSULT** (phone or in person) with a PGD genetic counselor. Discuss the benefits, risks, limitations, accuracy (95-98% for FA and HLA) etc.....
- **DNA SAMPLES** (blood or cheek swabs) from parents and children
- **CONSENT FORMS**
- **FEES**
- **SET-UP SYSTEM** takes 4-6 weeks to complete
  - Testing for mutations and **linked markers**
- **TESTING STRATEGY** (PB/BB vs. BB)
- **IVF CYCLE DATES!!!!!!**
FANCONI ANEMIA-A
PGD SET-UP

LINKED MARKERS
1. D16S520
2. D16S3026
3. FANCA Intron 1
4. FANCA (Paternal Mutation :M)
5. FANCA (Maternal Mutation: M)
6. D16S3407

Husband (carrier)

Wife (carrier)

Child with FA

1. 10 / a
2. 20 / b
3. 30 / c
4. M / N
5. N / N
6. 60 / f

1. g / 70
2. h/ 80
3. i / 90
4. N / N
5. N / M
6. K / 120
Updated (June 2007) List of Markers in HLA Area for Preimplantation HLA Typing
Why are Linked Markers so important?

* Detection of Allele Drop Out (ADO)
* Detection of Recombination
* Detection of contamination
* Detection of chromosomal aneuploidy
* Embryo identification
In vitro fertilization (IVF)

- SELECT IVF CENTER
- IVF CONSULTATION
- FERTILITY WORKUP (required even if otherwise fertile)
  - **Female**: infectious disease panel, day 3 hormone levels, uterine cavity assessment, other genetic screening if needed, etc
  - **Male**: infectious disease panel, semen analysis, other genetic screening, etc
- IVF CYCLE DATES (PGD SET-UP MUST BE COMPLETE)
- BEGIN IVF CYCLE
IVF CYCLE

Menstrual cycle begins

BCP for ~14 days

Start Lupron injections

~10-14 days

Baseline ultrasound

Start Stimulation Medication injections

~10-14 days

with monitoring throughout

Egg Retrieval (Day 0)

PGD Testing (PB and/or BB)

Embryo Transfer (ET) on (Day 5)
Chronology of PGD Procedures

- **Day 0**: Egg Retrieval. 1st Polar Body removal. ICSI (fertilization)
- **Day 1**: Check Fertilization. 2nd Polar Body removal.
- **Day 2**: Embryos undisturbed. Polar Body testing in progress.
- **Day 3**: PB results. Blastomere Biopsy
- **Day 4**: Embryos undisturbed. Blastomere testing in progress.
- **Day 5**: Final Results and Embryo Transfer
- **Day 6**: Freezing of Residual Normal Embryos
Microsurgical Techniques

First Polar body removal

Second Polar body removal

Embryo biopsy

First and Second Polar body removal
Chances for Disease Free and HLA matched embryo

Chance of normal or healthy carrier of FA: \( \frac{3}{4} \)

Chance for HLA match: \( \frac{1}{4} \)

\( \frac{3}{4} \times \frac{1}{4} = \frac{3}{16} \)
Sample PGD/IVF Cycles

CYCLE #1

- 8 eggs aspirated
- 6 mature
- 4 fertilize
- 1 FA Normal, HLA match
- Transfer 1 embryo
- Positive Pregnancy test!

CYCLE #2

- 25 eggs aspirated
- 18 mature
- 13 fertilize
- 2 HLA matches
- 1 FA Affected, HLA match
- 1 FA Carrier, HLA match
- Both embryos arrested
- No embryo transfer
# Sample Results

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**Blastomere Analysis for Fanconi Anemia A and HLA**

<table>
<thead>
<tr>
<th>Embryo #</th>
<th>D11S 1759</th>
<th>Fanconi A Mutation</th>
<th>D11S 915</th>
<th>Predicted Embryo Genotype</th>
<th>ET</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10/70</td>
<td>M/M</td>
<td>20/80</td>
<td><strong>AFFECTED NON-MATCH</strong></td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>FA</td>
<td>FA</td>
<td>FA</td>
<td>NA</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14/70</td>
<td>N/M</td>
<td>FA</td>
<td><strong>CARRIER NON-MATCH</strong></td>
<td>NO</td>
<td>Can be frozen</td>
</tr>
<tr>
<td>4</td>
<td>14/70</td>
<td>N/M</td>
<td>12/80</td>
<td><strong>CARRIER NON-MATCH</strong></td>
<td>NO</td>
<td>Can be frozen</td>
</tr>
<tr>
<td>5</td>
<td>14/13</td>
<td>N/N</td>
<td>12/16</td>
<td><strong>NORMAL MATCH</strong></td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>FA 58</td>
<td>10/14</td>
<td>M/N</td>
<td>20/16</td>
<td>Husband</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA 59</td>
<td>13/70</td>
<td>N/M</td>
<td>12/80</td>
<td>Wife</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA 60</td>
<td>14/13</td>
<td>N/N</td>
<td>12/16</td>
<td>Normal child</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA 61</td>
<td>10/70</td>
<td>M/M</td>
<td>20/80</td>
<td>Affected child</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Factors Affecting PGD Accuracy

- Cell quality/cell type
- Allele-drop-out
- DNA contamination
- Failed amplification -> inconclusive results
- Human error

PGD involves a modification of risk – not the elimination of risk. PGD does not replace prenatal diagnosis.
Common Questions

1) How many embryos are transferred?
   1-3 (typically 2)

2) What are the chances of getting pregnant?
   Age dependent, center dependent, per ET!!
   <35 ~50%, 35-37 ~40%, 38-42 ~30%, >43 ~10% or less

3) What is the accuracy?
   ~95-98% (NOT 100%). Prenatal testing recommended.

4) Is there a minimum number of embryos required?
   No (RGI)
Common Questions

5) How often are results “inconclusive”?  
Small %. RGI can do blastocyst biopsy in these cases

6) What if I have “extra” embryos?  
Freeze, stem cell research, discard, donation

7) Can PGD be done on frozen embryos?
Usually

8) Do I have to have IVF with RGI in Chicago?  
No, RGI will work with any IVF center
Common Questions

8) Does the biopsy harm the embryo?
   Risk is low. 0.3-0.5% risk of harming the embryo.

9) Does PGD lower the pregnancy rates?
   No, similar pregnancy rates for IVF alone IF there is an ET

10) Can I use my younger sister as an egg donor when looking for an HLA match?
    Possibly. If you are looking for an HLA match your sister would first have to be tested to see if she is a ½ match to the child with FA.
RGI IVF/PGD Fees

**IVF**
- IVF (aspiration, monitoring, transfer etc)
  - $9,500-$12,000**
- IVF Medications
  - $2,000-$5,000

**PGD**
- Set up fees (one time fee)
  - $5,000
- PGD testing fees (per IVF cycle)
  - $2,500-$3,000 for FA (waived 1st cycle if IVF at RGI)
  - $1,000-1,500 for HLA in conjunction with FA (waived 1st cycle if IVF cycle at RGI)
- Other possible fees
  - $1,500 Biopsy fee (if performed by RGI)
  - $2,000 Embryologist travel fee (if IVF at outside center)
  - $2,000 Chromosome aneuploidy testing (optional)
Will insurance cover IVF/PGD?

- Rarely. ~90% of the time insurance does NOT cover PGD/IVF. Coverage varies from plan to plan. Some states have IVF coverage, but only if the couple is infertile.

- PGD set-up and testing for FA and HLA unlisted CPT code 84999, aneuploidy is 88299

- Biopsy codes are 89290-89291

- Can submit letter requesting coverage, then go through appeal process
Present RGI Experience -

2028 PGD cycles for 221 CONDITIONS

297 HLA cases
## Overall Results and outcome of PGD for Single Gene Disorders & Preimplantation HLA testing

<table>
<thead>
<tr>
<th>Testing</th>
<th>Patient/Cycle</th>
<th># of Transfer</th>
<th># Embryos Transferred</th>
<th>Pregnancy/Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA</td>
<td>127/297</td>
<td>194</td>
<td>301</td>
<td>58/47 (3)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30% PR per ET</td>
</tr>
<tr>
<td>Single Gene Disorders</td>
<td>1012/1731</td>
<td>1490</td>
<td>2958</td>
<td>619/592 (51)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41.5% PR per ET</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1139/2028</td>
<td>1684</td>
<td>3259</td>
<td>677/639 (54)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40.2% PR per ET</td>
</tr>
</tbody>
</table>

*Ongoing pregnancies*
Preimplantation HLA Typing in Overall RGI PGD Experience (1999-2007)

- 1999: 38 (10.6%), 4 (6.6%)
- 2000: 45 (18.4%), 3 (6.6%)
- 2001: 76 (19.4%), 14 (18.4%)
- 2002: 113 (21.2%), 23 (21.2%)
- 2003: 123 (25.7%), 28 (22.4%)
- 2004: 195 (24.8%), 50 (25.7%)
- 2005: 220 (24.9%), 52 (23.6%)
- 2006: 241 (24.9%), 41 (17.1%)
- 2007: 267 (24.8%), 40 (14.9%)

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- TOTAL
- HLA
# PGD for Single Gene Disorders & Preimplantation HLA testing

<table>
<thead>
<tr>
<th>Testing</th>
<th>Patient/Cycle</th>
<th># of Transfer</th>
<th># Embryos Transferred</th>
<th>Pregnancy/Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA + Mutation</td>
<td>81/199</td>
<td>129</td>
<td>202</td>
<td>34/27(3)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28% PR per ET</td>
</tr>
<tr>
<td>HLA</td>
<td>46/98</td>
<td>65</td>
<td>99</td>
<td>24/19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39% PR per ET</td>
</tr>
<tr>
<td>TOTAL</td>
<td>127/297</td>
<td>194</td>
<td>301</td>
<td>58/47(3)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30% PR per ET</td>
</tr>
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</table>

* Ongoing pregnancies
<table>
<thead>
<tr>
<th>Condition</th>
<th>Patient</th>
<th>Cycle</th>
<th>ET</th>
<th># embryos</th>
<th>Pregnancy</th>
<th>Birth</th>
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</thead>
<tbody>
<tr>
<td>Thalassemia</td>
<td>48</td>
<td>113</td>
<td>74</td>
<td>115</td>
<td>17</td>
<td>11 (2)*</td>
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<tr>
<td>Sickle cell anemia</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FANCA,FANCC, FANCD2,FANCF, FANCI, FANCJ</td>
<td>15</td>
<td>48</td>
<td>30</td>
<td>48</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>WAS</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>ALD</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Hyper IgM</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>HED+ID; IP</td>
<td>2</td>
<td>9</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>DBA</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Krabbe</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DM</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chronic Granulomatous Disease</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>(1)*</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>81</strong></td>
<td><strong>199</strong></td>
<td><strong>129</strong></td>
<td><strong>202</strong></td>
<td><strong>34</strong></td>
<td><strong>27 (3)</strong></td>
</tr>
</tbody>
</table>
### RGI Experience in Fanconi Anemia/HLA Testing

<table>
<thead>
<tr>
<th>Disease</th>
<th>Patient / Cycle</th>
<th>ET/ Cycles</th>
<th>Pregnancy / Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCA / HLA</td>
<td>9 / 34</td>
<td>19 / 34</td>
<td>5 / 3</td>
</tr>
<tr>
<td>FANCC / HLA And FANCC ONLY</td>
<td>4 / 8</td>
<td>7 / 8</td>
<td>2 / 3</td>
</tr>
<tr>
<td>FANCF / HLA</td>
<td>1 / 3</td>
<td>2 / 3</td>
<td>0</td>
</tr>
<tr>
<td>FANCJ / HLA</td>
<td>1 / 3</td>
<td>1 / 3</td>
<td>0</td>
</tr>
<tr>
<td>FANCI/HLA</td>
<td>1/2</td>
<td>2/2</td>
<td>0</td>
</tr>
<tr>
<td>FAND2/HLA</td>
<td>1/1</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>17/51</strong></td>
<td><strong>32 / 51</strong></td>
<td><strong>7 /6</strong></td>
</tr>
</tbody>
</table>

- 63% ET per cycle
- 22% PR per ET
- 14% PR per cycle
- 12% THBR/cycle
How do I choose a PGD center?

- Experience with FA and FA/HLA testing? Any misdiagnosis?
- Do they test linked markers or just mutations?
- Can they also test for chromosome aneuploidy?
- Can they test polar bodies and blastomeres?
- How often are there inconclusive results? Can they perform Day 5 blastocyst biopsy?
- Is prenatal testing (CVS or amnio) required?
- Who is the contact person? Do they have genetic counselors?
How do I choose an IVF center?

- Does the center have experience working with PGD centers?
- Will they work with any PGD center?
- Can they do their own biopsies?
- Location
- Pregnancy rates
- Exclusion criteria
- Day 5 embryo culture success
% Aneuploid Oocytes in relation to Maternal Age

AGE
% Abnormal Oocytes

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Objectives of PGD for Chromosomal Disorders

- Prevent Chromosomal Disorders
- Reduce Spontaneous Abortions
- Improve Effectiveness of IVF
**Sequential Polar Bodies Analysis**

<table>
<thead>
<tr>
<th>Oocyte #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
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</thead>
<tbody>
<tr>
<td>R951W / N</td>
<td>161</td>
<td>160</td>
<td>161</td>
<td>158</td>
<td>FA</td>
<td>161</td>
<td>160</td>
<td>158</td>
<td>161</td>
<td>158</td>
<td>161</td>
<td>160</td>
<td>158</td>
<td>161</td>
<td>160</td>
<td>158</td>
</tr>
</tbody>
</table>

**Predicted Genotype:**

- **FANCA**
  - N / R1080L
  - R951W / N

**PGD**

**Embryo #**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
</table>

**Predicted Genotype:**

- **FANCA**
  - N / R1080L
  - R951W / N

**Monosomy 6, 13, 16, 21 & Y**

- **Affected, Non-match**
- **Carrier, Normal**
- **Affected, Non-match**
- **Carrier, Normal**
- **Affected, Normal**
- **Carrier, Non-match**

**Trisomy X**

- **Affected, Non-match**
- **Monosomy 6, 13, 16, 21 & X**

**Normal, Non-match**

- **Affected, Non-match**
- **Carrier, Monosomy 6**

**HLA Markers:**

- D6S1568
- D6S1560
- TAP1
- MIB
- D6S265
- RF
- D6S306

**FANCA Markers order:**

- D16S520
- D16S3026
- FANCA Intron 1
  - FANCA Arg951Try
- FANCA Intron 27 SNP
  - D16S3407

**Blutomerces Analysis**

**HLA Markers:**

- D6S1568
- D6S1560
- TAP1
- MIB
- D6S265
- RF
- D6S306

**Blutomerces Analysis**

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- D6S1568
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- D16S520
- D16S3026
- FANCA Intron 1
  - FANCA Arg951Try
- FANCA Intron 27 SNP
  - D16S3407

**PGD for Fanconi A, HLA & Aneuploidy Testing**
Drawbacks/limitations of PGD

- Risk of micromanipulation
- Fewer embryos for transfer
- Patient response can’t be predicted
- Pregnancy rates (no guarantee of pregnancy)
- Cost, availability
- ICSI required
- PGD involves a modification of risk – not the elimination of risk
- PGD does not replace prenatal diagnosis
- Fanconi Anemia E is on the same chromosome as HLA
Advantages of PGD

- An acceptable option for at-risk couples that would not consider terminating an affected pregnancy after CVS or Amniocentesis.
- Increase the chance of a successful pregnancy through IVF for women with fertility problems, especially those who are AMA.
- Can test for HLA matched/unaffected HLA matched embryos for couples with a sick child needing a stem cell transplant.
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