Trophectoderm Biopsy & Comprehensive Chromosome Screening

Dawn A. Kelk, PhD, HCLD
Audience Response

Question

Please use the response keypad.
Does your clinic biopsy for 24-chromosome PGS testing?

A. Yes

B. No
What do you currently biopsy in your lab?

A. Primarily polar body

B. Primarily blastomere

C. Primarily trophectoderm (TE)

D. Blastomere or TE depending on case

E. We are currently transitioning to TE

F. We don’t perform biopsies
Biopsy Background

- Biopsy >20 years, initially blastomere or polar body
- Improvements in blastocyst culture
- Improvements in blastocyst cryopreservation
- Limited evaluation of impact of biopsy
- Until relatively recently, no well controlled studies
Limitations to a well controlled IVF study:

- Variability in stimulation dynamics
- Cycle to cycle variability in oocyte quality
- Batch to batch variability in media
- Day to day variation in laboratory environment
- Embryo transfer techniques
- Endometrial receptivity
- Implantation window
- Hormonal milieu in luteal phase & early gestation
Impact of Biopsy Study

Fingerprint to determine if “biopsy” or “no biopsy” implanted

Treff et al. Fertil Steril. 2010; 94:477-484. (blastomere or trophectoderm)
Impact of Biopsy Study

![Graph showing implantation rate](image)

- Blastomere: Implanted Biopsy (p=0.0348), Implanted Unbiopsied (p=0.796)
- Trophectoderm: Implanted Biopsy, Implanted Unbiopsied

**34% reduction**

Treff *et al*, ASRM 2011
Day 3 Biopsy & Fresh BT vs TE Biopsy & FBT

Transfer of euploid blastocysts

Harton et al. ASRM 2011; (Euploid blastocysts by aCGH)
Blastocyst Biopsy

- Biopsy TE cells
- More cells available (3-10 cells)
- 3-5% vs 10-25% of embryo removed on Day 3
Reflections...

2008 Abstracts
*D3 vs D5 eSET*
- No 24-chrom
- Single gene PGD bx D3
- Blasts were slow frozen
- 2PN & Day 3 Cryo
- >50% of ET’s were D3

2012 Abstracts
*TE Biopsy & CCS Vitrification*
- All D3’s are hatched
- 100% Day 5 ET
- Vit everything at blast
- ~33% of cases are CCS
Reflections...

What hasn’t changed:

- Global culture media
- MINC incubators
- Group culture
- Sort into like cell numbers on Day 2
Trophectoderm Biopsy

- Laser zona breach on Day 3
  - Allows embryo to herniate out of zona on Day 5/6
  - If hole is too small, embryo pinches, cells bleb off, biopsy is difficult
  - If hole is too large, the blastocyst pulls out of the zona, biopsy is more difficult
  - 400 μsec pulse
Zona Breaching on Day 3:
Trophectoderm Biopsy – Embryo Selection

- The more cellular the better
- If too early/too few cells, the embryo pulls apart
- Hatching/herniating blastocysts ideal
- Hatched blastocysts can also be biopsied
Ideal for biopsy:
Embryo selection for TE biopsy:
Loose cells/fragments extruding:
Too early to biopsy:
Early for biopsy:
Borderline for biopsy:
Hatching blastocysts for biopsy:
Hatching blastocysts for biopsy:

Pull out and biopsy as hatched blastocyst
Cells/fragments frequently remain in zona:
Cells/fragments frequently remain in zona:
Cells/fragments frequently remain in zona:
Hatched Blastocyst (Day 6):
Hatched Blastocyst (Day 6):
Hatched Blastocyst (Day 6):
Hatched Blastocysts:
Collapsed Blastocyst:
Which of these embryos made a baby?

- Neither
- Embryo A
- Embryo B
- Both A & B
Which of these embryos made a baby?

A

B

BOTH!

Twin Girls Delivered
Twins delivered (boy & girl):
Twin girls delivered:
Delivered CCS normal singleton gestation:
Micromanipulator Set up

- Many labs still mouth pipetting
- Not necessary, Eppendorf Cell Tram Vario
- Prime with heavy silicone oil right through
Biopsy Dish Setup

- Lid of 35mm dish
- 10 μl drops
- 2.5ml oil overlay
- Inverted 60mm dish
**TE Biopsy Technique**

- Rotate the embryo so ICM is on holding pipet side
- Strong suction on zona or TE away from ICM
- **PULL & s-t-r-e-t-c-h** (more than you think you should)
- Cut with laser hits on cell junctions
- Do not cut through thick cytoplasmic areas
- When close to finishing cutting, as cells pull apart, lighten suction so biopsy doesn’t shoot up biopsy pipet
- Release the biopsy away from the embryo
Trophectoderm Biopsy:
Trophectoderm Biopsy (Hatched blastocyst):
Trophectoderm Biopsy (ICM extruded):
Trophectoderm biopsies:
If the biopsy sticks to the biopsy pipet...

- Move the pipet down to firmly touch the bottom of dish
- Move the stage north & south (quick & firm)
- Stay near the center of the drop
- Do not contact the oil interface
Loading the biopsy into the tube

- Drag drop of buffer slightly up the side of the tube
- 1-2μl of buffer depending on the testing lab
- Rinse the biopsy in buffer using 130μm stripper tip
- Angle the tube under the stereoscope to focus on the drop
- View the biopsy going into the drop
Loading 1µl Buffer in PCR Tube:

Note: This technique does not work for all PCR tubes.
Loading 1μl Buffer in PCR Tube:
Loading 1µl Buffer in PCR Tube:
Loading Biopsy in PCR Tube:
Loading Biopsy in PCR Tube:
Handling Biopsied Hatched Blastocysts

- When moving embryos, they occasionally come into contact with air or oil bubbles.

- For zona intact embryos, generally a nuisance, requiring the embryo be knocked off the bubble.

- If a hatched blastocyst contacts an air or oil bubble, the surface tension causes the embryo to closely adhere to the bubble and the embryo is easily lost.
Handling Biopsied Hatched Blastocysts

- Biopsied, zona-free blastocysts are sticky and may be more likely to stick inside a handling pipet.

- When handling hatched blastocysts, minimize the possibility of the zona-free blastocyst touching an air/oil bubble or sticking inside a handling pipet.
Handling Biopsied Hatched Blastocysts

- Rinse & prime the handling pipet cleanly with culture medium prior to aspirating the hatched blastocyst (no air/oil bubbles).

- Visualize embryo at all times as it moves in & out of the pipet.

- Work quickly. Do not allow the embryo to settle in the pipet.

- Special attention to not touch the hatched blastocyst to the tip of the handling pipet. Draw the embryo from a short distance with fluid/media aspiration.
Nonconcurrent results (a warning):

Very poor embryo quality = nonconcurrent chrom. data
We learned the hard way...

<table>
<thead>
<tr>
<th>Problem:</th>
<th>Solution:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garbage in = Garbage out</td>
<td>Practice on junk, but don’t test junk</td>
</tr>
<tr>
<td>Biopsy too early, cells</td>
<td>Be patient, more cellular is better</td>
</tr>
<tr>
<td>pull apart</td>
<td></td>
</tr>
<tr>
<td>Biopsy sticks to holding</td>
<td></td>
</tr>
<tr>
<td>Handling hatched blasts</td>
<td>Drag on bottom or flick</td>
</tr>
<tr>
<td></td>
<td>Avoid air &amp; oil bubbles</td>
</tr>
</tbody>
</table>
Key Hints to Success:

- Pull and stretch when cutting biopsy
- Hit the cell junctions/membranes (not cytoplasm)
- Draw buffer up side of PCR tube
- Visualize biopsy expelled into buffer
- Good vitrification is key to success of CCS
Audience Response

Question

Please use the response keypad.
If you do 24-chromosome testing, do you transfer any fresh tested embryos?

A. Yes

B. No, but we wish we could logistically

C. No, we do fine or better with FET
What percentage of your caseload do you estimate you do 24-chromosome testing?

A. 0%
B. 1-2%
C. ~5%
D. ~10%
E. ~20%
F. >25%
Where are your PGS biopsies currently tested?

A. Primarily by Reprogenetics
B. Primarily by Genesis Genetics
C. Primarily by RMA Genetics
D. Other or in-house testing lab
E. We send to multiple testing labs
F. We don’t perform biopsies for PGS
Advantages of 24-chromosome testing

- Transfer normal embryos
- Increase pregnancy rates
- Decrease miscarriage rates
- Decrease births of trisomies vs elective termination
  - Safer
  - Psychological benefit
  - More socially acceptable
Considerations for 24-chromosome testing

- More work, frequently need to biopsy Day 5 & Day 6
- Possible damage to embryo during biopsy
- No normal embryos for transfer
- Expense (~$5000 for biopsy & testing)
- In most cases, requires cryopreservation of embryos
Blastocysts Available for Biopsy by Age

Average # of Blastocysts Biopsied

Maternal Age

<35: 8.3
35-37: 5.5
38-40: 4
41-42: 3.7
43+: 2.5

Kelk et al. ASRM 2012; O-198
Comprehensive Chromosome Screening Data

% Aneuploid Embryos

Maternal Age

<35  35-37  38-40  41-42  43+

Kelk et al. ASRM 2012; O-198
Comprehensive Chromosome Screening Data

Maternal Age

Kelk et al. ASRM 2012; O-198
How CCS Affects # of Embryos Transferred:

Patients $\geq 38$ Years

Average # Embryos Transferred

- No CCS: 3.0
- CCS: 1.4
Chemical Pregnancy Rate With & Without CCS

Patients ≥38 Years

Chemical Pregnancy Rate/ET

- No CCS: 52%
- CCS: 72%
Clinical Pregnancy Rate With & Without CCS

Patients ≥38 Years

Clinical Pregnancy Rate/ET

No CCS: 39%
CCS: 60%
Implantation Rate With & Without CCS

Patients ≥38 Years

- No CCS: 22%
- CCS: 54%

Implantation Rate
Miscarriage Rate With & Without CCS

Patients $\geq$ 38 Years

- No CCS: 33%
- CCS: 6%
**Patient #1: (3/13/12 – 2/27/13; 40.7-42 yrs)**

<table>
<thead>
<tr>
<th>Cycle</th>
<th># Eggs</th>
<th># Mature</th>
<th># 2PN</th>
<th>Bx’d</th>
<th># Abnormal</th>
<th># Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>11</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>18</td>
<td>13</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>149</strong></td>
<td><strong>90 (60%)</strong></td>
<td><strong>69 (77%)</strong></td>
<td><strong>17 (25%)</strong></td>
<td><strong>13</strong></td>
<td><strong>1</strong>*</td>
</tr>
</tbody>
</table>
Patient #2: (10/8/12 – 4/30/13; 40.6-41.2 yrs)

<table>
<thead>
<tr>
<th>Cycle</th>
<th># Eggs</th>
<th># Mature</th>
<th># 2PN</th>
<th>Bx’d</th>
<th># Abnormal</th>
<th># Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>13</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>12</td>
<td>9</td>
<td>7</td>
<td>4</td>
<td>2 (1)</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>13</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>22</td>
<td>18</td>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>93</td>
<td>63 (68%)</td>
<td>42 (67%)</td>
<td>34</td>
<td>7</td>
</tr>
</tbody>
</table>

+1 nonconcurrent

2 CCS NORMAL FET’d → Ongoing Twins
Timing of Vitrification Post-Biopsy

- Most blastocysts seal & begin to re-expand in ~20 min
- Vitrify 30-90 minutes post-biopsy
- Before complete re-expansion
# Results – Fresh vs Frozen CCS Cycles

<table>
<thead>
<tr>
<th></th>
<th>Fresh Day 6</th>
<th>Frozen</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>44</td>
<td>---</td>
</tr>
<tr>
<td>Mean Maternal Age</td>
<td>37.4</td>
<td>38.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Positive βhCG</td>
<td>70.5%</td>
<td>75.0%</td>
<td>0.63</td>
</tr>
<tr>
<td>Clinical Pregnancy Rate</td>
<td>56.8%</td>
<td>63.6%</td>
<td>0.51</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>46.3%</td>
<td>53.7%</td>
<td>0.43</td>
</tr>
</tbody>
</table>
CCS Vitrification Results

- 44 CCS patient thaws in 2012
- 97.1% thaw survival rate
- 1.51 embryos transferred
- 48.8% of CCS FET’s are single ET
2013 CCS with Vitrification Outcomes – All Ages

- 2 Euploid ET’d $\rightarrow$ 80% clinical preg rate $\rightarrow$ 40% twin rate

- 1 Euploid ET’d $\rightarrow$ 59% clinical preg rate
Biopsy, Vit, Thaw & FET of single CCS normal

<table>
<thead>
<tr>
<th>Pre-Biopsy</th>
<th>Post-Biopsy</th>
<th>At Vit</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Singleton Delivered
Special Thank you to Embryology Team:

Jonathan Lo*
Karen Reyes*
Erica Paganetti
EJ Testa
Questions???

Comments???