Embryo Biopsy

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1. Biopsy procedures
   ♦ Opening the zona pellucida
   ♦ Removal of the cellular material

2. Developmental stage to perform the biopsy
Zona opening

1- MECHANICAL OPENING
   - Direct Puncture
   - Partial Zona Dissection

2- CHEMICAL OPENING (Ac. Tyrode’s ph=2.3)

3- PHOTOTHERMOLYSIS (Laser)
Zona opening: Mechanical opening

**Direct Puncture**: Performed with the use of a bevelled pipette. Not clinically applied in the human for blastomere biopsy but used for polar body biopsy Verlinsky and Cieslak, 1993

**Partial Zona Dissection**
Described for human oocytes to facilitate sperm penetration
Also useful for Assisted Hatching

**PZD**: Involves making a slit in the ZP by a sharp closed microneedle

**3-D PZD**: Cross shaped slit *(Cieslak, 1999)*
Zona opening: Chemical opening

**Acid Tyrode’s solution (pH 2.3)** Gordon and Talansky, 1983

- **The most widely used approach** (cheap option)
- Human ZP is more resistant to AT than mouse ZP
- Larger, rounder hole than with PZD. Size of the hole not always easy to control
- **Two separate** pipettes are usually used (double holder). Drilling pipette with an inner ø of 5-7 µm plus the aspiration pipette.
- Limiting the extent and duration of AT exposure is necessary to avoid acidification of medium and cell lysis
- Target site: between two blastomeres
- Embryo wash after AT exposure are recommended
- Useful for early cleavage stages but **inappropriate for oocytes**

Chen et al, 1998 described **a simplified technique** using only a single, larger pipette to perform zp drilling and blastomere aspiration
Zona opening: Phototermolysis

“Microdissection of mouse and human zona pellucida using a 1.48 µm diode laser beam: efficacy and safety of the procedure.”

**GERMOND ET AL, 1995.** Fertil. Steril 64:604-611

- Non contact laser: easily adapted to the microscope. Laser is transmitted trough a 45X objective
- Laser technique reduces time of biopsy procedure
- Quick, simple, safe and efficient procedure with no need for micropipette changes
- A direct relationship between the hole diameter (µm) and the exposure time (ms)
- Effective and focalised without dispersion. Minimal absorption by the culture dish and the medium
- Safe, with no mechanical, thermal or mutagenic effects

**Germond et al, 1996:** Drilled mouse embryos give rise to normal, fertile offspring and a healthy F2 generation was obtained
Zona opening: Ac. Tyrode’s versus Laser

<table>
<thead>
<tr>
<th></th>
<th>24-30 h CAVITATION</th>
<th>48h BLASTOCYST</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>67.6%</td>
<td>100%</td>
</tr>
<tr>
<td>LASER</td>
<td>54.3%</td>
<td>87.5%</td>
</tr>
<tr>
<td>ACID TYRODE’S</td>
<td>43.3%</td>
<td>62.5%</td>
</tr>
</tbody>
</table>

Zona-drilled embryos cavitated consistently later than non-drilled controls

<table>
<thead>
<tr>
<th></th>
<th>TROPHECTODERM CELLS</th>
<th>ICM CELLS</th>
<th>TOTAL REDUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>25.2</td>
<td>10.8</td>
<td>------</td>
</tr>
<tr>
<td>LASER</td>
<td>23.2</td>
<td>9.4</td>
<td>9.4%</td>
</tr>
<tr>
<td>ACID TYRODE’S</td>
<td>23.3</td>
<td>8.0</td>
<td>13%</td>
</tr>
</tbody>
</table>

There were significantly fewer cells in the zona-drilled embryos compared with non-drilled controls. The AT’s drilled embryos had significantly smaller ICM.
Zona opening: Ac. Tyrode’s versus Laser

**Blastocyst development rates in sibling embryos: a prospective randomized trial**

*Jones et al, 2006*

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th><strong>Day 3 and 5 embryo quality in terms of cell stage and blastocyst development.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Embryos by characteristics eligible for biopsy</strong></td>
<td><strong>ATH</strong></td>
</tr>
<tr>
<td>Embryos with &gt;5 cells on day 3 (n)</td>
<td>59</td>
</tr>
<tr>
<td>Average cell stage on day 3</td>
<td>7.37</td>
</tr>
<tr>
<td>Blastocyst development, grades A and B combined (%)</td>
<td>47.5%</td>
</tr>
</tbody>
</table>

**Note:** All P values comparing the two types of zona drilling were statistically nonsignificant.


Laser hatching did not impair embryonic development to the blastocyst stage.

It did not produce additional risks for embryonic development beyond the blastocyst stage.
Zona opening: Ac. Tyrode’s versus Laser

CHEMICAL ZONA OPENING

LASER ZONA PELLUCIDA DRILLING

(G. Nikas)

(Germond et al, 1995)

Preimplantation Genetic Diagnosis: a celebration of 20 years
Rome, 1st July 2010. 26th ESHRE Post Congress Workshop
LASER ASSISTED BIOPSIES

• EARLY CLEAVAGE EMBRYO BIOPSY
  
  (J.Assist Reprod Genet 15:302-307)

  
  Successful Use of a Laser for Human Embryo Biopsy in Preimplantation Genetic Diagnosis: Report of Two Cases
  
  M. BOADA,1,1  M. CARRERA,2 C. DE LA IGLESIA,2 M. SANDALINAS,1 P. N. BARRI,1 and A. VEIGA1

• BLASTOCYST BIOPSY
  

• POLAR BODY BIOPSY
  

Preimplantation Genetic Diagnosis: a celebration of 20 years  
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Blastomere Removal

1- ASPIRATION

2- EXTRUSION

3- DISPLACEMENT
Blastomere Removal: Aspiration

Ac. Tyrode’s & Aspiration

1. Dril the zona pellucida
2. Extrud the blastomere through the hole by pushing against the zp with a microneedle at some distance of the hole

Extrusion:

Preimplantation Genetic Diagnosis: a celebration of 20 years

Rome, 1st July 2010. 26th ESHRE Post Congress Workshop
### Blastomere Removal: Displacement

**TABLE 1**

<table>
<thead>
<tr>
<th>Biopsy methods</th>
<th>Aspiration</th>
<th>Displacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Age of patients</td>
<td>31.6</td>
<td>37.8</td>
</tr>
<tr>
<td>Total No. of eggs retrieved</td>
<td>69</td>
<td>197</td>
</tr>
<tr>
<td>Mean No. of eggs</td>
<td>13.8</td>
<td>14.1</td>
</tr>
<tr>
<td>No. (%) of M-II</td>
<td>59 (85.5%)</td>
<td>171 (86.8%)</td>
</tr>
<tr>
<td>No. (%) of 2PN</td>
<td>51 (86.4%)</td>
<td>166 (91.2%)</td>
</tr>
<tr>
<td>No. (%) of embryos biopsied</td>
<td>51 (100%)</td>
<td>151 (96.8%)</td>
</tr>
<tr>
<td>No. (%) of blastocysts</td>
<td>29 (58.8%)</td>
<td>84 (55.8%)</td>
</tr>
<tr>
<td>No. of patients with transfer</td>
<td>4*</td>
<td>10*</td>
</tr>
<tr>
<td>No. of live birth/ongoing pregnant</td>
<td>2 (50%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Total No. of embryo transferred</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Mean No. of embryos for transfer</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>Total No. of embryo implanted</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>25*</td>
<td>64.7*</td>
</tr>
</tbody>
</table>

* Patients without normal embryos or blastocysts for transfer were not included.

*P* < .005

Wei-Hua Wang et al, 2008
2. Developmental stage to perform the biopsy

- **Polar bodies from oocytes**
  (Day 0/ Day 1)

- **Blastomeres from early cleavage stage embryos**
  (Day 3)

- **Trophectoderm cells from blastocysts**
  (Day 5)
Polar body biopsy

- Verlinsky et al. Hum Reprod, 1990

- **1st polar body**: 2-3 hours after oocyte pick up (<6h). Degeneration or fragmentation of the 1st pb

- **Small hole** of 18-25 µm. (not less than 15 µm)

- **Ac. Tyrode’s**: is not recommended. It could be harmful and compromise the viability of the oocyte. **Mechanical zona opening** and **laser technology** (Montag et al, 1997) are the best options.

- **Pipettes for pb biopsy**: bevelled or not. Inner ø of 12-15 µm.

- **Sperm Microinjection** (ICSI). Not IVF.

- **Analysis of 1st and 2nd polar bodies:**
  - Simultaneous (6-14 h after fertilization; pb s at the 12 o’clock position)
  - Sequential with ICSI between. (Cytoplasmic bridges)
Polar body biopsy

- **Preconceptional manipulation in first pb biopsy** Advantage when ethical objections or legal restrictions to embryo manipulation exist

- **Removal of extra-embryonic material**
  Polar bodies often degenerate. They are expected to have no biological role in the embryo development. No embryonic cells are removed. No reduction of the cellular mass. No effects on the embryo development

- **Indirect Method**
  The chromosomal constitution of the oocyte will be complementary to what is observed in the 1st pb. Each chromosomal set should have two paired chromatids


- **Only genetic maternal contribution can be evaluated** Limitation for detecting the paternal influence and the errors that occurred post-fertilisation.

- **Useful for maternal structural and numerical chromosome aberrations and in certain monogenetic diseases**

- **Recombination of homologous chromosomes**
  If it occurs, the 1\textsuperscript{st} pb will be heterozygous and the complement of the oocyte cannot be derived. The additional information from the 2\textsuperscript{nd} pb will be required.

- **Premature division of chromatids** (Angell et al, 1991) could also lead to a difficult interpretation of the results.
Polar body biopsy

**PGD1**- 1\(^{st}\) pb (day 0)
**PGD2**- 1\(^{st}\) and 2\(^{nd}\) pbs (day 1)
**PGD3**- 1\(^{st}\) pb + 2\(^{nd}\) pb + blastomere biopsy (D+3)

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**Figure 1**

Effect of one to three micromanipulations for PGD on oocytes and embryos on blastocyst development by female age. Bars indicate the percentage of blastocysts in each PGD group and in the control group (blue bars, patient ages <35 y; yellow bars, patient ages ≥35 y). No significant differences were found between groups.

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*Cieslak-Janzen et al., 2006*
Blastomere biopsy

Early cleavage stage embryos (Day 3)
- The most widely used biopsy procedure

ESHRE PGD Consortium data Collection IX
Goossens et al, 2009

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar body biopsy</td>
<td>1089</td>
</tr>
<tr>
<td>Blastomere biopsy:</td>
<td></td>
</tr>
<tr>
<td>Cleavage aspiration</td>
<td>12836</td>
</tr>
<tr>
<td>Cleavage extrusion</td>
<td>1077</td>
</tr>
<tr>
<td>Cleavage flow displacement</td>
<td>38</td>
</tr>
<tr>
<td>Blastocyst biopsy</td>
<td>57</td>
</tr>
<tr>
<td>Polar body + Cleavage</td>
<td>5</td>
</tr>
<tr>
<td>Unknown</td>
<td>68</td>
</tr>
</tbody>
</table>

- It allows the detection of maternal, paternal and early post-fertilisation defects
- It gives enough time for the genetic diagnosis if it is performed on day 3 and transfer on day 5
Blastomere biopsy

- Place the embryo with the chosen cell to biopsy at the 3 o’clock position. The cell should contain a single, clearly visible nucleus.
- Zona opening: mechanical, Acid Tyrode’s, Laser. Small hole of approx. 40 µm.
- Cell removal: Aspiration is the most widely used.
- Special pipettes for blastomere aspiration. Inner Ø of 35-40 µm.
- The cell should be partially aspirated and pulled out rather than completely aspirated.
- Place the biopsied cell far from the embryo.
- Keep the embryo immediately in the incubator.
**Compaction:** On day 3, blastomeres show a strong tendency to adhere to each other but cells are not yet compacting.

Gap junctions are first detected at the 4 cell stage and the blastomeres adhere to each other (Hardy et al, 1996).

Full compaction does not occur before the 16-32 cell stage

- **Ca\(^{2+}\) & Mg\(^{2+}\) free culture medium** facilitates embryo biopsy with no detrimental effect on embryo development and pregnancy rates (Veiga et al 1994; Santaló et al, 1996; Dumoulin et al, 1998)

**Limit exposure time:** maximum 10 min. After biopsy, gently flush the embryo repeatedly
Blastomere biopsy

Day 3

Biopsy should be performed on the morning of Day 3 (68-72 h. after insemination)
Three cleavage cycles: approx. 60 h. Eight-cell stage. (6-10 cells)

- **Cells are still totipotent**
  It is thought that at this stage, blastomeres are allocated to, but not committed to, specific pathways (ICM, Trophectoderm)

- **1 or 2 blastomeres**
  Preferable to remove only one cell. If it is necessary to remove two, the same hole has to be used.

- **The removal of too many blastomeres can be detrimental**
  Risks of formation of a small ICM (Hardy and Handyside, 1993)
Blastomere biopsy

• **Fragmented embryos** (>35%) or **embryos with a low development rate** should not be biopsied.

• **Multinucleated embryos** should be considered for embryo biopsy?
  - Births after transfer of Mn embryos have been reported.
  - Decision could vary depending on:
    - The number of available embryos
    - The proportion of Mn blastomeres within the embryo
    - The multinucleation pattern
    - The day of its appearance

### Representativeness of a Mn blastomere of the sibling blastomeres

<table>
<thead>
<tr>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Inconclusive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Abnormal</td>
<td>7</td>
<td>82</td>
<td>9</td>
<td></td>
<td>98</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>16</td>
<td>86</td>
<td>14</td>
<td><strong>116</strong></td>
<td></td>
</tr>
</tbody>
</table>

87% abnormals 6% discordance

Parriego et al 2007 PGDIS- Melbourne
Blastomere biopsy

• **Mosaicism**

Up to 70% of mosaicism have been reported in preimplantation embryos (Munné, 1995, 1997; Voullaire, 2000; Wells and Delhanty, 2000; Bielanska, 2002)

*Coulam et al, 2007*

![Table 2](#)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>X</th>
<th>Y</th>
<th>13</th>
<th>15</th>
<th>16</th>
<th>18</th>
<th>21</th>
<th>22</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant</td>
<td>80</td>
<td>89</td>
<td>76</td>
<td>72</td>
<td>72</td>
<td>74</td>
<td>73</td>
<td>81</td>
<td>617</td>
</tr>
<tr>
<td>Discordant</td>
<td>22</td>
<td>13</td>
<td>26</td>
<td>30</td>
<td>30</td>
<td>28</td>
<td>29</td>
<td>21</td>
<td>199</td>
</tr>
<tr>
<td>% Discordant</td>
<td>21.6</td>
<td>12.8</td>
<td>25.5</td>
<td>29.4</td>
<td>29.4</td>
<td>27.5</td>
<td>28.4</td>
<td>20.6</td>
<td>24.4</td>
</tr>
</tbody>
</table>

- 74.5% rate of discordance when 2 blastomeres biopsied from the same embryo on D+3 were analyzed for 8 chromosomes

- Both technical and biological contributions
Blastomere biopsy

Mosaicism

Probabilities of normal and abnormal results of 1 or 2-cell biopsies taken from 8-cell embryos with different levels of mosaicism

1 cell biopsy
- 8 cell-stage embryos
- 7 cells after biopsy

2 cells biopsy
- 8 cell-stage embryos
- 6 cells after biopsy

Blastomere biopsy

Mosaicism

Self repair mechanisms

Hypothesis:

- Apoptosis of abnormal cells during early development
- Confination of certain anomalies to extra-embryonic tissues such as placenta, chorion, or amnios, later during pregnancy

Verjaal et al, 1987 found karyotypic differences between cells from placenta and other fetal issues

Evsikov and Verlinsky, 1998; Magli, 2000

There is no evidence to support the fact of a preferential allocation of euploid cells to the ICM and aneuploid cells to the trophectoderm

Blastocysts derived from aneuploid embryos revealed a high incidence of mosaicism of ICM cell lineages
Trophectoderm biopsy

Is the trophectoderm representative of the embryo itself?

<table>
<thead>
<tr>
<th>ICM</th>
<th>embryo (ectoderm, mesoderm and endoderm)</th>
<th>viteline vesicle</th>
<th>amnion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trophectoderm</td>
<td>→ non-embryonic tissues (chorion, placenta, umbilical cord)</td>
<td></td>
</tr>
</tbody>
</table>
Trophectoderm biopsy

- Blastocyst biopsy is an emerging technique
- Provides more cells to analyse
- Interesting in monogenic diseases (more DNA available)
- Lower degree of mosaicism
- ICM remains fully intact
- Requires a high blastocyst rate, an optimized culture system and specific laboratory expertise
- Genetic results should be obtained in <24 hours in order to avoid cryopreservation

Double selection by genetic diagnosis and culture to blastocyst stage may lead to high pregnancy and implantation rates
Laser blastocyst biopsy for preimplantation diagnosis in the human

A. Veiga¹, M. Sandalinas¹, M. Benkhalifa², M. Bouda¹, M. Carrera³, J. Santaló⁴, P.N. Barri¹ and Y. Ménézo²
Institut Universitari Dexeus and UAB, Barcelona, Spain and Laboratoire Marcel Mérieux, Lyon, France

More cells available to analyze
Trophectoderm biopsy

- Zona Pellucida drilling. A small gap 25-30 µm directly opposite the ICM (morning of day 5/ day 3-4)
- Incubation 4 h to allow blastocoele expansion and spontaneous herniation of trophectoderm cells
- Dissection of 3-10 trophectoderm cells using laser pulses
- Blastocyst incubation and transfer on late day 5 or morning day 6 (hatched blastocysts).

**First cases reported needed cryopreservation**
Trophectoderm biopsy

- Blastocyst biopsy and cryostorage and later transfer of biopsied blastocysts
  
  *Mc Arthur et al, 2005*

- Blastocyst biopsy on day 5 and transfer on day 6
  
  *De Boer et al, 2004*  
  *Mc Arthur et al, 2005*

  *Kokkali et al, 2007*

### Table 1: Overall cycle data for Groups A and B for the diagnosis of β-thalassaemia syndromes

<table>
<thead>
<tr>
<th>Indication</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cycles</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Female age (years)</td>
<td>36.8 ± 2.82</td>
<td>35 ± 2.94</td>
</tr>
<tr>
<td>Day of biopsy</td>
<td>D3</td>
<td>D3</td>
</tr>
<tr>
<td>Day of embryo transfer</td>
<td>D5</td>
<td>D5</td>
</tr>
<tr>
<td>Biopsy procedure</td>
<td>Laser</td>
<td>Laser</td>
</tr>
<tr>
<td>Biopsy method</td>
<td>Blastocyst</td>
<td>Trophectoderm</td>
</tr>
<tr>
<td>Embryology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilized</td>
<td>131</td>
<td>128</td>
</tr>
<tr>
<td>Biopsied</td>
<td>101</td>
<td>63</td>
</tr>
<tr>
<td>Diagnosed</td>
<td>76</td>
<td>50</td>
</tr>
<tr>
<td>Unaffected</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>Transferable at blastocyst</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>Transferred</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Average number transferred</td>
<td>3 ± 1.05</td>
<td>21 ± 0.99</td>
</tr>
<tr>
<td>Frozen</td>
<td>5</td>
<td>5 (±7)</td>
</tr>
<tr>
<td>Total blastocyst developed</td>
<td>66</td>
<td>60</td>
</tr>
<tr>
<td>Blastocysts affected</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Blastocyst not diagnosed</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Clinical outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycles to embryo transfer</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HCG positive</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Ectopic</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Miscarriage</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Number of fetal sacs</td>
<td>6 (±2)</td>
<td>10</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>26.7%</td>
<td>42.6%</td>
</tr>
</tbody>
</table>

*Preimplantation Genetic Diagnosis: a celebration of 20 years*  
*Rome, 1st July 2010. 26th ESHRE Post Congress Workshop*
Thank you for your attention!

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Silvia Mateo
Anna Veiga