Poor embryo development: biopsy or not biopsy?

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Bologna, May 8, 2016

Embryo biopsy

Day 1
Polar Body Biopsy

Day 2
Cleavage stage biopsy

Day 3
Morula stage biopsy

Day 4
Blastocyst biopsy
Trophectoderm

Day 5
Blastocoelic fluid
1. Polar Body Biopsy

- Polar bodies are by-products of meiosis which have no influence on further embryo development.
- Generally more accepted from a legal point of view.
- Minimally invasive.
- More time to carry out tests.
- Not under division.
- No mosaicism.

- No information about paternal genome.

## 1. Polar Body Biopsy

**DIAGNOSTIC EFFICIENCY**

2013-2015

- 122 cycles - 115 couples → PGS for advanced maternal age.
- 598 biopsied zygotes → 1196 polar bodies analyzed.

- **Positive Amplification**: 1118; 93%.
- **Pb1 Failed amplification**: 78; 7%.
- **Pb2 Failed amplification**: 47; 4%.
- **Failed amplification** per sample: 31; 3%.

**S.I.S.Me.R. data**
1. Polar Body Biopsy

**DIAGNOSTIC EFFICIENCY**

529 zygotes diagnosed on 598 (88.5%)

- **69 undiagnosed zygotes**
- **529 zygotes diagnosed**
- **41 embryos arrested on day 2/3 (65.2%)**
- **24 embryos (34.8%)**

**Zygotes/PBs’ morphology not related to positive results**

**Negative results mostly related to poor embryo quality**

**PB Storage and delivery can be an issue**

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1. Polar Body Biopsy

- **Flat profile**
  - **Pb1: euploid**

- **Noisy profile**
  - **Pb1: weak amplification-no result**

- **Clear call**
  - **Pb2: +20**

- **Pb2: -16,22**

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*S.I.S.Me.R. data*
1. Polar Bodies Biopsy

From the genetic lab point of view:

DO IT!

11.5% of zygotes do not have a complete diagnosis → 2/3 arrest
only 4% of developing zygotes undiagnosed.

Embryo biopsy

Day 1  Day 2  Day 3  Day 4  Day 5  Day 6

Polar Body Biopsy  Cleavage stage biopsy  Morula stage biopsy  Blastocyst biopsy  Trophectoderm  Blastocoelic fluid
2. Cleavage stage biopsy

- Performed on day 3 embryos (ESHRE 2010 guidelines: ≥6 cells with less than 30% of fragmentation)
- Historically most widely used method
- Information about paternal and maternal genomes
- Mitotic contribution to aneuploidy
- Multiple embryos available
- Fresh transfer

- Mosaicism and genetic instability
- Only 1 cell

514 embryos

- 6-8 cells: 80%
- 4-5 cells: 10%
- 9-12 cells: 10%

S.I.S.Me.R. data
2. Cleavage stage biopsy

- 4-5 cells: n=53
- 6-8 cells: n=411
- 9-12 cells: n=50

**Diagnosed**
- 96%
- 94%
- 92%

**Not Informative**
- 2%
- 3%
- 2%

**Failed Amplification**
- 2%
- 3%
- 6%

No statistically significant differences in terms of informative results between the three groups.

514 embryos

- Good Morphology: 79%
- Poor Morphology: 21%
- 6-8 cells: 80%
- 4-5 cells: 10%
2. Cleavage stage biopsy

514 embryos

- GOOD MORPHOLOGY: 79%
- POOR MORPHOLOGY: 21%

<table>
<thead>
<tr>
<th></th>
<th>Good morphology</th>
<th>Poor morphology</th>
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<tbody>
<tr>
<td>N. Biopsied embryos</td>
<td>407</td>
<td>107</td>
</tr>
<tr>
<td>Diagnosed</td>
<td>386 (95%)</td>
<td>91 (85%)</td>
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<tr>
<td>75% Not informative</td>
<td>8 (2%)</td>
<td>12 (11%) *</td>
</tr>
<tr>
<td>Failed amplification</td>
<td>13 (3%)</td>
<td>4 (4%)</td>
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</tbody>
</table>

p=0.001

2. Cleavage stage biopsy

- good morphology: 95%
- poor morphology: 85%

- DIAGNOSED: 95%
- NOT INFORMATIVE: 85%
- FAILED AMPLIFICATION: 95%

p=0.001
2. Cleavage stage biopsy

Good morphology embryo

Blastomere: loss 15

FLAT PROFILE

CLEAR CALL

Poor morphology embryo

Blastomere: not informative

NOISY PROFILE
2. Cleavage stage biopsy

From the genetic lab point of view:

Good morphology embryos: **DO IT!**

Poor morphology embryos: 11.2% of undiagnosed embryos

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Embryo biopsy

- **Polar Body Biopsy**
- **Cleavage stage biopsy**
- **Morula stage biopsy**
- **Blastocyst biopsy**
  - Trophectoderm
  - Blastocoelic fluid
3. Morula stage biopsy

- Performed on day 4 embryos
- Information about paternal and maternal genomes
- Multiple cells available for diagnosis
- Fresh transfer

More data needed to evaluate its feasibility

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Biopsy of Human Morula-Stage Embryos: Outcome of 215 IVF/ICSI Cycles with PGS

Elena E. Zolkerev, Victor Z. Zolevsky, Alexander S. Kirkitdevskh, Zoltan C. Falikovski

Abstract

Preimplantation genetic diagnosis (PGD) is currently performed on biopsies from 3-day-stage embryos in Antarctica. However, information about the postimplantation and embryonic development of embryos is critical for the selection of more fit embryos. In this study, we evaluated the feasibility of biopsying morula-stage embryos for PGS. A total of 215 cycles were included in this analysis. The results showed that the biopsy procedure was successful in 94% of the cycles, and the postimplantation development of the embryos was normal in 91% of the cycles. These results indicate that the morula-stage biopsy is a viable option for PGS.

Gynecology & Obstetrics

Day 4 Biopsy Improves Pregnancy Outcome Comparing to Day 3 Biopsy in Preimplantation Genetic Screening

Su-kyung Kim, Seunghoon Kim, Dong-Hyung Kim, Young-Jin Im, Hyeong-Kyu Kim

Abstract

Day 4 biopsy improves pregnancy outcome compared to day 3 biopsy in preimplantation genetic screening (PGS). The study design was prospective, and the data were collected from 116 patients who underwent PGS on day 3 or day 4 of embryo culture. The results showed that day 4 biopsy resulted in higher implantation rates (P = 0.01) and clinical pregnancy rates (P = 0.03) compared to day 3 biopsy. These findings suggest that day 4 biopsy may be a more effective strategy for PGS in clinical practice.
3. Morula stage biopsy

Biospy performed on embryos without fragmentation

- Biopsied embryos
- Diagnosed embryos
- Not diagnosed embryos

<table>
<thead>
<tr>
<th>3-7 cells</th>
<th>FISH</th>
<th>1-2 cells</th>
<th>SNP array</th>
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<tbody>
<tr>
<td>709</td>
<td>92%</td>
<td>58</td>
<td>100%</td>
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<tr>
<td>651</td>
<td></td>
<td>150</td>
<td>0</td>
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</table>

Elena et al.

Kim et al.

Blastomere: gain 1
3. Morula stage biopsy

Wait for more data!

Embryo biopsy

Day 1  Day 2  Day 3  Day 4  Day 5  Day 6

Polar Body Biopsy  Cleavage stage biopsy  Morula stage biopsy  Blastocyst biopsy  Trophectoderm  Blastocoelic fluid
4. Trophectoderm biopsy

- Performed on day 5/6 embryos
- Increasingly the preferred method
- Multiple cells available for diagnosis-removal of a low proportion of total blastocyst's cell number
- Fewer embryos available
- Less mosaicism

Training!

Over extended embryo culture-frozen transfer

4. Trophectoderm biopsy

- Widely demonstrated that there are no detrimental effects on the blastocyst after TE biopsy
- Diagnostic efficiency: 96-98% of diagnosed embryos (depending on the lab)

How many cells can be removed?

Number of biopsied trophectoderm cells is likely to affect the implantation potential of blastocysts with poor trophectoderm quality
4. Trophectoderm biopsy

Significant difference in the median number of cells in the biopsies obtained by different embryologists

More biopsied cells in embryos with a higher TE score

Zhang et al., Fert Ster 2016

DIAGNOSTIC EFFICIENCY

Diagnostic efficiency decreases with the number of biopsied cells

Zhang et al., Fert Ster 2016
Trophectoderm biopsy
IMPACT ON IMPLANTATION RATE

In embryos with a low TE score the number of biopsied cells negatively influences the IR

Zhang et al., Fert Ster 2016

* p<0.05
** p<0.01

Trophectoderm: loss 18, (loss 13,14)

Trophectoderm: gain 18, (loss 13,14)

FLAT PROFILE
CLEAR CALL
MOSAICISM
4. Trophectoderm biopsy

From the genetic lab point of view:

DO IT!

CONSIDER:
- TE quality
- number of biopsied cells

Embryo biopsy

Day 1  Day 2  Day 3  Day 4  Day 5  Day 6

Polar Body Biopsy  Cleavage stage biopsy  Morula stage biopsy  Blastocyst biopsy  Trophectoderm  Blastocoelic fluid
5. Blastocentesis

Blastocentesis: innovation in embryo biopsy
L. Gianaroli
Monday 9th May, 16:30

Conclusions

Day 1  Day 2  Day 3  Day 4  Day 5  Day 6

Polar Body Biopsy  Cleavage stage biopsy  Morula stage biopsy  Blastocyst biopsy  Trophectoderm  Blastocoelic fluid

DO IT!  DO IT!  DO IT!  MORE DATA NEEDED  DO IT!  ?

Morphology  TE score and number of cells

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