Non-invasive metabolomic profiling using near infrared spectroscopy

Thorir Hardarson

FERTILITETSCENTRUM, GÖTEBORG
POTENTIAL CONFLICT OF INTEREST

FERTILITETSCENTRUM HAS COLLABORATED WITH MOLECULAR BIOMETRICS FOR SEVERAL YEARS

NO COMMERCIAL INTEREST

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CONTENT OF THE LECTURE

BACKGROUND TO METABOLOMICS

BRIEF OVERVIEW OF RETROSPECTIVE STUDIES

RCT AT FERTILITETSCENTRUM

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From the Morula to the Blastocyst stage
SELECTING THE MOST VIABLE EMBRYO

MORPHOLOGY ALONE IS NOT ENOUGH

BETTER TECHNIQUES NEEDED TO SELECT THE OPTIMAL EMBRYO
Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploid in couples with advanced maternal age: a prospec randomised controlled trial

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BACKGROUND: It is generally accepted that the age-related increased aneuploidy rate is in implantation and a higher abortion rate. Therefore, advanced maternal age (AMA) cohort is to assess the possible benefit of preimplantation genetic diagnosis for aneuploidy screening in Embryos. RESULTS: Positive predictive value was 100% and per embryo transfer (per cycle), 25% (33.3%) and 11.5% in the control group (P = 0.00). We observed a normal diploid status in 36.8% of the embryos. CONCLUSIONS: Preimplantation genetic screening for improving clinical outcome per initiated cycle in patients with advanced maternal age suggests a benefit in terms of clinical pregnancy rate in IVF, in particular owing to the increased embryo aneuploidy rate.
New non-invasive methods

Time-lapse?

Genomics?

Proteomics?

Metabolomics?
Non-invasive studies of human embryo viability

*Pyruvate, lactate and glucose metabolism*

<table>
<thead>
<tr>
<th>Study</th>
<th>Embryo stage examined</th>
<th>Altered metabolite associated with improved outcome</th>
<th>Technology used</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardy et al. 1989</td>
<td>Day 2-4</td>
<td>↑ pyruvate uptake&lt;br&gt;No association with glucose uptake</td>
<td>Ultramicrofluorescence assay</td>
<td>Blastocyst development</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>↑ pyruvate uptake&lt;br&gt;↑ glucose uptake</td>
<td>Ultramicrofluorescence assay</td>
<td>Blastocyst development</td>
</tr>
<tr>
<td>Gott et al. 1990</td>
<td>Day 2-4</td>
<td>↑ pyruvate uptake&lt;br&gt;↑ lactate production&lt;br&gt;No association with glucose uptake</td>
<td>Ultramicrofluorescence assay</td>
<td>Blastocyst development</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>↑ pyruvate uptake&lt;br&gt;↑ glucose uptake&lt;br&gt;↑ lactate production</td>
<td>Ultramicrofluorescence assay</td>
<td>Blastocyst development</td>
</tr>
<tr>
<td>Conaghan et al., 1993</td>
<td>Day 2 – 3</td>
<td>↓ pyruvate uptake</td>
<td>Ultramicrofluorescence assay</td>
<td>Clinical pregnancy</td>
</tr>
<tr>
<td>Turner et al., 1994</td>
<td>Day 2</td>
<td>Intermediate pyruvate uptake</td>
<td>Ultramicrofluorescence assay</td>
<td>Clinical pregnancy</td>
</tr>
<tr>
<td>Gardner et al., 2001</td>
<td>Day 4</td>
<td>↑ pyruvate uptake&lt;br&gt;↑ glucose uptake</td>
<td>Ultramicrofluorescence assay</td>
<td>Blastocyst development</td>
</tr>
<tr>
<td>Seli et al.</td>
<td>Day 2-3</td>
<td>↑ pyruvate uptake&lt;br&gt;↑ glucose uptake</td>
<td>Proton NMR</td>
<td>Pregnancy and delivery</td>
</tr>
</tbody>
</table>

From Botros *et al.* 2008
Non-invasive studies of human embryo viability

Amino acid uptake and secretion

Table II. Amino acid uptake and secretion by the embryo as a predictor of embryo development viability—human studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Embryo stage examined</th>
<th>Altered metabolite associated with outcome</th>
<th>Technology used</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Houghton et al., 2002</td>
<td>Day 2 – 3</td>
<td>↓ amino acid turnover (sum of depletion and appearance) ↓ glutamine, arginine, methionine uptake ↓ alanine and asparagine release</td>
<td>HPLC</td>
<td>Blastocyst development</td>
</tr>
<tr>
<td></td>
<td>8 cell-Monula</td>
<td>↓ asparagine uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Britson et al., 2004</td>
<td>Day 2</td>
<td>↓ glycine and leucine in culture media</td>
<td>HPLC</td>
<td>Clinical pregnancy and live birth</td>
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<tr>
<td></td>
<td></td>
<td>↑ asparagine levels in culture media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seli et al., 2008</td>
<td>Day 3</td>
<td>↑ glutamate levels in culture media</td>
<td>Proton NMR</td>
<td>Clinical pregnancy and live birth</td>
</tr>
</tbody>
</table>

From Botros et al. 2008
### Metabolomics studies so far….

**Table IV.** Studies of non-invasive metabolomic profiling of embryo culture media to assess embryo viability in IVF.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>$n$</th>
<th>Day of transfer</th>
<th>Number of embryos transferred</th>
<th>Analytical technique</th>
<th>Center</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seli et al. (2007)</td>
<td>Algorithm development</td>
<td>36</td>
<td>Day 3</td>
<td>MET</td>
<td>Raman</td>
<td>YFC</td>
<td>A</td>
</tr>
<tr>
<td>Scott et al. (2008)</td>
<td>Blinded analysis</td>
<td>41</td>
<td>Day 3 and 5</td>
<td>MET</td>
<td>Raman</td>
<td>RMANJ</td>
<td>B</td>
</tr>
<tr>
<td>Seli et al. (2007)</td>
<td>Algorithm development</td>
<td>33</td>
<td>Day 3</td>
<td>MET</td>
<td>NIR</td>
<td>RMANJ</td>
<td>A</td>
</tr>
<tr>
<td>Seli et al. (2007)</td>
<td>Blinded analysis</td>
<td>16</td>
<td>Day 3</td>
<td>MET</td>
<td>NIR</td>
<td>YFC</td>
<td>B</td>
</tr>
<tr>
<td>Seli et al. (2008b)</td>
<td>Algorithm development</td>
<td>121</td>
<td>Day 2</td>
<td>SET</td>
<td>NIR</td>
<td>KLC</td>
<td>A, C</td>
</tr>
<tr>
<td>Seli et al. (2008b)</td>
<td>Blinded analysis</td>
<td>60</td>
<td>Day 2</td>
<td>SET</td>
<td>NIR</td>
<td>KLC</td>
<td>B, D</td>
</tr>
<tr>
<td>Vergouw et al. (2008)</td>
<td>Algorithm development</td>
<td>29</td>
<td>Day 2</td>
<td>SET</td>
<td>NIR</td>
<td>VUMC</td>
<td>A, C</td>
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<tr>
<td>Vergouw et al. (2008)</td>
<td>Algorithm development</td>
<td>304</td>
<td>Day 3</td>
<td>SET</td>
<td>NIR</td>
<td>VUMC</td>
<td>A, C</td>
</tr>
<tr>
<td>Hardarson et al. (2008)</td>
<td>Algorithm development</td>
<td>137</td>
<td>Day 5</td>
<td>SET</td>
<td>NIR</td>
<td>FCG, SG</td>
<td>A, C, D</td>
</tr>
</tbody>
</table>

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Metabolomics

WHY?

MB (James Posillico) - 4 years ago
New device - fast - promising

SET - spent media
Collaboration

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Metabolomics
ViaMetrics-E™ Methodology

■ Clinically
  ■ How the embryo modifies its environment.

■ Thesis
  ■ A viable embryo has a different metabolome than a non-viable embryo and this can be assessed by sampling the culture media.

■ Biologically
  ■ Changes in concentrations of:

<table>
<thead>
<tr>
<th>Functional Groups</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>•CH</td>
<td>•Albumin</td>
</tr>
<tr>
<td>•NH</td>
<td>•Lactate</td>
</tr>
<tr>
<td>•OH</td>
<td>•Pyruvate</td>
</tr>
<tr>
<td>•SH</td>
<td>•Glutamate</td>
</tr>
<tr>
<td>•C=C</td>
<td>•Glucose</td>
</tr>
</tbody>
</table>

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ViaMetrics-E™ Methodology

Step 1: Single embryo culture

Step 2: NIR Spectral analysis of media sample ratio against the blank

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Absorption of NIR wavelengths by vibrating bonds

Absorption is proportional to concentration

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Wavelength (nm)
Step 3: Calculate score for each embryo

ViaMetrics-E Score = $\alpha(W_{\alpha}) + \beta(W_{\beta}) + \gamma(W_{\gamma}) + \delta(W_{\delta})$

Step 4: Transfer embryo with highest Viability score

<table>
<thead>
<tr>
<th>Embryo</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>0.45</td>
</tr>
<tr>
<td>3</td>
<td>0.58</td>
</tr>
<tr>
<td>4</td>
<td>0.62</td>
</tr>
<tr>
<td>5</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Embryo 4 is the one with the highest Viability score.
We have previously shown…

ASRM 2007 – Hardarson et al. 2007 (Fertil. Steril. 88, S 307-308)
NIR predicts the outcome of day 2 embryos

ASRM 2008 - Hardarson et al. 2007 (Fertil. Steril. 90, S 77)
NIR predicts the outcome of day 5 embryos
Viability score correlated to implantation rate

*Day 2 transfers, (n= 75)*
Viability score correlated to *implantation rate* for sET (n=137) on Day 5
Morphology correlated to implantation rate for sET (n=137) on Day 5

- **Morphology**
- **Implantation rate** (%)

**Morphology grades**

- A
- B
- C
- D

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Morphology correlated to *viability score* for sET (n=137) on Day 5

NB! Only Good Quality Embryos!
Viability score correlated to implantation rate

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SHADY GROVE CLINIC, USA
47 SET spent media collected and analysed (SAGE)

The algorithm trained using the SGC data

The trained algorithm used to blindly predict outcome at FC

FERTILITETSCENTRUM GOTHENBURG, SWEDEN
42 SET spent media collected and analysed (Vitrolife CCM)

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NIR spectroscopy and embryo selection

Cross-validation and predictive value of near-infrared spectroscopy algorithms for day-5 blastocyst transfer

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doi: 10.1016/j.rbmo.2011.01.009
Conclusions

These pilot studies indicate that viability score improves prediction of implantation compared with morphology.

To show that the viability score truly is an independent predictor of clinical pregnancy a randomized controlled trial is needed.
**NIR - RCT**  
*At Fertility Centre Gothenburg, Sweden*

**Aim:** To study if NIR spectroscopy of spent culture media combined with morphology better predict an embryo's potential than embryo morphology alone.

**Primary end-point:** Ongoing pregnancy rate after SET.

**Inclusion criteria:** Patients seeking treatment at the clinic having two or more GQE

**Duration:** Estimated 2 years with 752 patients randomized.

**Interim analysis performed**  
January 2011

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Interim analysis

287 patients randomized

Conditions:
< 3% in favor of the NIR study group = stop the study
➢ 3% but less than 10% in favor of the NIR group => continue, but add more patients
➢ 10% or more in favor of the NIR group => continue as planned

The study was stopped
In total, 327 patients randomized, results being analyzed and will be published
Preliminary results

Data will be provided at course (unpublished data)
Future for metabolomics
Thank you for your attention!