Finding the best egg with old-fashioned molecular biology

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Dr. Gleicher is listed as co-inventor on a number of pending patent applications claiming diagnostic and therapeutic benefits from determination of CGG repeat numbers and ovarian FMR1 genotypes and sub-genotypes.

Dr. Gleicher is co-inventor of three awarded U.S. patents, claiming therapeutic benefits for supplementation of DHEA in women with diminished ovarian reserve, a topic discussed in this talk. Other patent applications in regards to DHEA and other fertility-related claims, with no relationship to this talk, are pending. Dr. Gleicher receives royalties from, and owns shares in Fertility Nutraceuticals, LLC, a distributor of a DHEA product.
Outline

- Outline some of the current difficulties with the concept of egg/embryo selection
- Point out age-dependency of egg/embryo selection
- Demonstrate the molecular changes in follicles with advancing age
- Demonstrate translational treatment consequences
Current emphasis is on embryo selection
- Blastocyst stage culture
- PGS
- Time lapse systems

But is this the correct emphasis?
Oocyte scoring system with better predictability of clinical IVF pregnancies than currently practiced embryo quality assessment

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• Total oocyte score related to odds of increased embryo cell numbers (OR 1.12, P=0.025), embryo grade (OR 1.19, P < 0.001) and clinical pregnancy [OR 1.58 (95% Cl 1.23 to 2.02), p < 0.001]. For 8-cell/good grade embryos, TOS predicted clinical pregnancy (OR 20.078 (95% Cl 1.2 to 3.44, P < 0.001).
• For 8-cell embryos, odds of clinical pregnancy were significantly increased with higher average PTOS (OR 2.08 (95% Cl 1.26 to 3.44; P=0.004);
• For high quality embryos, oocyte assessment appears thus, superior to embryo scoring.
Oocyte or Embryo Selection?

- The concept of “selection” only makes sense if there is a need for selection.
- In how many women/patients does such a need exist?
Benefit | Detriment | Egg numbers
---|---|---
Young with NFOR | | HIGH
Medium age with relative NFOR | | MODERATE
Older and/or with LFOR | | LOW
## Cleavage vs. Blastocyst Stage Transfer

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Days 5/6</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Day 1" /></td>
<td><img src="image2" alt="Day 2" /></td>
<td><img src="image3" alt="Day 3" /></td>
<td><img src="image4" alt="Days 5/6" /></td>
</tr>
</tbody>
</table>

- **Pregnancy/embryo**: Dashed line
- **Cumulative pregnancy chance**: Solid line

*Gluyovsky et al. Cochrane Database Sept.Rev 2012; 7:CD002118*

“Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology”
<table>
<thead>
<tr>
<th>Female Age (Years)</th>
<th>Clinical Pregnancy Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>50.0</td>
</tr>
<tr>
<td>30-35</td>
<td>52.0</td>
</tr>
<tr>
<td>36-37</td>
<td>37.9</td>
</tr>
<tr>
<td>38-39</td>
<td>30.2</td>
</tr>
<tr>
<td>40</td>
<td>26.3</td>
</tr>
<tr>
<td>41</td>
<td>25.7</td>
</tr>
<tr>
<td>42</td>
<td>20.0</td>
</tr>
<tr>
<td>43</td>
<td>17.9</td>
</tr>
<tr>
<td>≥44</td>
<td>14.5</td>
</tr>
</tbody>
</table>

2013 Patient Age Distribution

- <30 years: 18.30%
- 30-35 years: 5.30%
- 36-37 years: 16.70%
- 38-40 years: 9.70%
- 41-43 years: 29.30%
- 44+ years: 20.70%
We, therefore, wondered whether we could find out by molecular means what differentiates younger from older follicles.
<table>
<thead>
<tr>
<th></th>
<th>Group 1 Donors N=31</th>
<th>Group 2 Intermediate age infertility patients N=64</th>
<th>Group 3 Older infertility patients N=41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>24.4±3.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>34.1±3.0&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>44.3±1.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>6.3±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.6±1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.3±1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>3.1±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of follicles/cycle</td>
<td>22.5±8.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>10.5±7.1&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>6.8±5.1&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of oocytes retrieved/cycle</td>
<td>15.5±7.0&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.1±6.6&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>3.6±3.2&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of artretic oocytes retrieved/cycle</td>
<td>1.3±1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9±1.3</td>
<td>0.6±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of embryo ≥ 4 cells</td>
<td>15.5±6.5&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.1±6.5&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>3.6±3.1&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant rate/cycle</td>
<td>16 (51.6%)</td>
<td>22 (34.4%)</td>
<td>3 (7.3%)</td>
</tr>
<tr>
<td>Progesterone/estradiol ratio</td>
<td>0.26±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.96±0.47&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with a-d in their superscripts in same row differ significantly (P<0.05).
The P4/E2 ratio was significantly higher in the oldest group

A first hint at “premature luteinization”
Impact of Maternal Aging on Gene Expression in GCs

Confirmed by western blot

FSHR ↓
LHR ↑
Impact of Maternal Aging on Steroidogenic Activity in GC

Confirmed by western blot

- Aromatase: ↓
- 17b/HSD: ↓
- P450sc: ↑
Impact of Maternal Aging on Apoptosis

No differences were found. Confirmed by western blot.
Conclusion

Increased LHR expression
+
Reduced FSHR expression
+
Reduced Aromatase expression

with advancing age are supportive of premature luteinization of GCs in older women.
Progestosterone receptor (PR)

This was further confirmed by older women who also demonstrated higher PR expression.
For further confirmation, we now went into a GC culture system where cells were evaluated on days 1, 3, and 5 in presence/absence of FSH
Impact of Maternal Aging on GCs in Culture

- In absence of FSH, cell proliferation declines rapidly

- Growing patterns are distinctively different.
Impact of Maternal Aging on Apoptosis During Culture

Here older women demonstrated much more rapid increase in apoptosis
Conclusion

- FSH, thus at all ages demonstrates positive effects on GC proliferation and apoptosis of cultured GCs.
- This effect is, however, weaker in older women, going along with previously noted lower FSHR expression.
Impact of Maternal Aging on Gene Expression During Culture

- On Day-1 FSHR mRNA expression was low at all ages. It subsequently increased in younger but not in older women.

- FSH in culture enhanced the response but less so in older women.
Impact of Maternal Aging on Gene Expression During Culture (cont.)

- LHR mRNA expression increased much faster in older women.

- Though FSH in culture did not affect LHR expression, it did stimulate aromatase mRNA and protein expression.
Bcl-2 gene expression decreased at all ages, the fastest in oldest patients.

Concurring between protein expression by western blot and PCR results

FSH, by inhibiting this decline thus appears to inhibit apoptosis after all
In vivo as well as in vitro results suggest that premature luteinization represents a central feature of the “old” follicle
This raised the question whether early oocyte retrieval would improve outcome by avoiding exposure of oocytes to premature luteinization?
Changes in IVF protocol in Women older than 43

- Retrieval at 14-16 mm follicle size (from 19-21 mm)
- 30 hours instead of 36 hours hCG to retrieval interval
- ET on day-2 (from day-3)
<table>
<thead>
<tr>
<th></th>
<th>Early retrieval group N=39</th>
<th>Normal retrieval group N=91</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>45±1.9</td>
<td>44.3±1.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Number of follicles/cycle</td>
<td>7.2±4.7</td>
<td>7.3±5.6</td>
<td>0.947</td>
</tr>
<tr>
<td>Number of oocytes/cycle</td>
<td>6.3±5.1</td>
<td>5.9±4.9</td>
<td>0.703</td>
</tr>
<tr>
<td>Number of immature oocytes</td>
<td>2.4±2.5</td>
<td>1.1±1.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Number of arrested oocytes</td>
<td>0.3±0.5</td>
<td>0.8±1.4</td>
<td>0.037</td>
</tr>
<tr>
<td>Number of good embryos/cycle</td>
<td>3.0±2.8</td>
<td>2.8±2.3</td>
<td>0.551</td>
</tr>
<tr>
<td>Percentage of cycles resulting pregnancies</td>
<td>12.8 (5/39)</td>
<td>7.7 (7/91)</td>
<td>N/A</td>
</tr>
<tr>
<td>Percentage of transferred cycles resulting in pregnancies</td>
<td>16.7 (5/30)</td>
<td>8.9 (7/28)</td>
<td>N/A</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>4.8</td>
<td>3.3</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Molecular biology of follicles after early retrieval

![Graph showing molecular biology of follicles after early retrieval.](image-url)
Premature luteinization, a negative effect of aging on granulosa cells, avoided by early oocyte retrieval at smaller follicle sizes

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Summary

- This study reemphasizes the importance of age in all aspects of egg/embryo selection.
- It, however, also reemphasizes that in older women egg/embryo selection loses relevance and should be replaced by individualization of treatment.