Endometrial BCL6 Testing for Prediction of In Vitro Fertilization Outcomes: A Cohort Study

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Disclosure

I have no meaningful conflicts of interest to declare for this educational activity.
Background

- Over 200,000 cycles of IVF annually in US
- Success rate for live birth remains <50%
- Endometriosis listed in only 3-4% of cases in SART database
  - Up to 50% of infertile women likely have endometriosis
  - Up to 70% of patients with endometriosis will not have a live birth
Background

- Endometriosis and IVF
  - Oocyte quality implicated
  - Endometrial receptivity
- Undiagnosed endometriosis
  - Laparoscopic treatment in 29 patients
    - 22 of 29 conceived compared to 13 of 35 control
  - Need a nonsurgical method to diagnose
Background

- BCL6 reported as biomarker for endometriosis
  - Proto-oncogene and transcriptional repressor contributing to cell-cycle control and differentiation, as well as apoptosis inhibition
  - Overexpression associated with increased cellular proliferation
  - BCL6 appears to be related to progesterone resistance
Objective

• To use BCL6 as a surrogate biomarker for endometriosis
• To determine whether aberrant BCL6 predicts IVF outcome in a population of women with otherwise unexplained difficulty conceiving
Hypothesis

- Endometrial BCL6 expression could be used as a prognostic biomarker for IVF outcome in women with unexplained infertility before embryo transfer
Methods
Methods

• Study Design
  – Cohort study

• Setting
  – Fertility Center of the Carolinas at GHS
Methods

- Participants
  - Ovulatory women with healthy male partners ≥ 1 year infertility
    - LH timed endometrial biopsy 7-10d after ovulation within 6 months before IVF
    - Fresh IVF cycles with embryo transfer
    - No surgical or medical suppression of endometriosis
- Exclusion criteria:
  - Significant fibroids (>4cm)
  - Male factor infertility
  - Endometritis on EMB
  - Lack of adequate tissue for analysis on EMB
Methods

• Immunohistochemistry
  – BCL6 expression
    • HSCORE = \( \sum P_i \frac{(i+1)}{100} \)
      – \( I \) = intensity of staining with a value of 1, 2, or 3, (weak, moderate or strong, respectively)
      – \( P_i \) = percentage of stained epithelial cells for each intensity, varying from 0-100%
    • Score assigned blinded to clinical history or outcome
## Methods

### Variables

- Age
- Body mass index
- Peak $E_2$
- Days of stimulation
- Number of oocytes retrieved
- Fertilization rate
- Number of embryos transferred
- Clinical pregnancy rate (PR)
- Live birth rate (LBR)
- Median BCL6 expression
Methods

• Data collection and bias
  – Data obtained from SART database and medical records
  – Two researchers verified independently
  – Pregnancy tests and ultrasounds performed without knowledge of BCL6 result

• Study size
  – Alpha error 5%, power of 80%
  – Expect 80% of patients to have abnormal BCL6
  – Need at least 65 subjects
Outcomes

• Clinical pregnancy rate (PR)
• Live birth rate (LBR) per transfer
Statistical Analysis

• Categorical data
  – Fisher’s exact test
  – Relative risk
  – 95% confidence interval

• Parametric data
  – Student’s t test or Mann-Whitney U test
Results
Results

Infertile couples n=70

Excluded for Cancelled cycle: 1

Normal BCL6 n=17
- Pregnant n=11
- Not pregnant n=6

Abnormal BCL6 n=52
- Pregnant n=9
- Not pregnant n=43
Table 1. Characteristics of the sample population based on outcome (clinical pregnancy rate)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pregnant n=20</th>
<th>Not Pregnant n=49</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age - years (mean ± SD)</td>
<td>36.3 ± 3.2</td>
<td>34.5 ± 3.9</td>
<td>0.05a</td>
</tr>
<tr>
<td>BMI - median (range)</td>
<td>24.3 (18.6 - 44.6)</td>
<td>23.9 (17.9 - 36)</td>
<td>0.8b</td>
</tr>
<tr>
<td>BCL6 expression median (range)</td>
<td>0.9 (0 - 4)</td>
<td>2.1 (0.5 - 4)</td>
<td>0.01b</td>
</tr>
</tbody>
</table>

Cycle characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pregnant</th>
<th>Not Pregnant</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of stimulation - mean ± SD</td>
<td>10.4 ± 1.9</td>
<td>10.4 ± 1.8</td>
<td>0.9a</td>
</tr>
<tr>
<td>Peak estradiol pg/mL - median (range)</td>
<td>1387(341 - 5000)</td>
<td>1945 (477 - 5000)</td>
<td>0.7b</td>
</tr>
<tr>
<td>Oocyte retrieved - median (range)</td>
<td>14 (2 - 35)</td>
<td>11 (3 -56)</td>
<td>0.3</td>
</tr>
<tr>
<td>Oocytes fertilized - median (range)</td>
<td>7 (1 - 24)</td>
<td>6 (1 - 21)</td>
<td>0.4</td>
</tr>
<tr>
<td>Embryos transferred - median (range)</td>
<td>2 (1 - 4)</td>
<td>2 (1-3)</td>
<td>0.2b</td>
</tr>
</tbody>
</table>
## Results

**Table 2.** Characteristics of the sample population based on BCL6 expression

<table>
<thead>
<tr>
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<th>Normal n=17</th>
<th>Abnormal n=52</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age - years (mean ± SD)</td>
<td>35.6 ± 3.1</td>
<td>34.8 ± 3.9</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI - median (range)</td>
<td>24.8 (18.6 - 36.4)</td>
<td>23.6 (17.9 - 44.6)</td>
<td>0.9</td>
</tr>
<tr>
<td>Clinical pregnancy rate (CPR)</td>
<td>11 (64.7%)</td>
<td>9 (17.3%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Relative risk (95% CI) for normal BCL6</td>
<td>0.26 (0.13 to 0.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live birth rate (LBR)</td>
<td>10 (58.8%)</td>
<td>6 (11.5%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Relative risk (95% CI) for normal BCL6</td>
<td>0.19 (0.08 to 0.45)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cycle characteristics**

<table>
<thead>
<tr>
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<tr>
<td>Days of stimulation - mean ± SD</td>
<td>10.4 ± 2.1</td>
<td>10.4 ± 1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Peak estradiol pg/mL - median (range)</td>
<td>1331(341 - 3096)</td>
<td>1650 (477 - 5000)</td>
<td>0.3</td>
</tr>
<tr>
<td>Oocytes retrieved - median (range)</td>
<td>11 (2 - 35)</td>
<td>12.5 (2 - 56)</td>
<td>0.8</td>
</tr>
<tr>
<td>Oocytes fertilized - median (range)</td>
<td>6 (1 - 24)</td>
<td>7 (1 - 21)</td>
<td>0.6</td>
</tr>
<tr>
<td>Embryos transferred - median (range)</td>
<td>2 (1 - 3)</td>
<td>2 (1 - 4)</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Results

Expression Patterns of BCL6

Percentage of Women with Clinical Pregnancy Based on BCL6 Expression
Discussion
Discussion

• Endometriosis may be commonly associated with IVF failure in unexplained infertility (UI)

• Abnormal expression of BCL6 strongly associated with poor reproductive outcomes in IVF

• Other alterations in endometriatual receptivity associated with endometriosis and UI
Discussion

• Progesterone essential for establishment of pregnancy
• Abnormal BCL6 expression in endometrium of women with UI associated with endometrial P resistance
• Reduction in progesterone action could explain association with poor IVF outcome
Conclusion

• Aberrant expression of endometrial BCL6 is associated with poor reproductive outcomes in subsequent IVF cycles

• High levels of BCL6 expression in this cohort suggests undiagnosed endometriosis may be a common factor that needs to be considered in women before undergoing IVF
Strengths

• Prospective cohort
• Clinically relevant outcome
• Complete and non-biased assessment
  – Objective outcome measured
  – Pregnancy tests/ultrasound performed without knowledge of biopsy results
  – Single GYN pathologist read biopsies without knowledge of IVF outcome
• Results similar to those published by SART
  – Mean clinical PR for UI 27.1% (SART) vs 28.5%
• BCL6 test validated in women with UI
  – previously shown to be associated with both endometriosis and P resistance
Weaknesses

- Small sample size
- Prevalence of aberrant endometrial BCL6 high in study population (75.3%)
  - External validity?
- Potential effect of endometrial scratching on pregnancy rates
  - Performed in all subjects
- Only fresh IVF cycles included
Future studies

- Randomized controlled trial
- Outcomes after treatment
Acknowledgement

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  – Dr. Lessey
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  – Easley Committee

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  – CAPES/PROAP (RFS).
References


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