A prospective multi-site evaluation of the intra-menstrual cycle variability of Anti-Müllerian Hormone (AMH) using an automated AMH immunoassay


*Beckman Coulter, Inc., Carlsbad, CA, **Beckman Coulter, Inc., Chaska, MN

ABSTRACT

Background and Objective: Research studies indicate that AMH may be useful for evaluating response to controlled ovarian stimulation in women undergoing in vitro fertilization procedures. Published results for intra-menstrual cycle variability lack agreement. The purpose of this study is to determine whether or not AMH levels vary significantly across the normal menstrual cycle.

Methods: 24 apparently healthy women were prospectively enrolled from 2 sites with IRB-approved informed consent. Blood samples were collected 2 times per week throughout each complete menstrual cycle (21 to 35 days) starting with baseline (day 2 to 4). Eligibility criteria: ≥18 years to ≤45 years, both ovaries present, no polycystic ovary syndrome (PCOS), no history of ovarian surgery, no exposure to cytotoxic drugs or pelvic radiation therapy, no recent contraceptive use, and no other recent hormonal therapy. Serum samples were tested on the Beckman Coulter Access 2 immunoassay. Age-adjusted mixed-effects models were constructed to estimate variability of AMH levels across each complete menstrual cycle (21 to 35 days) starting with baseline (day 2 to 4). Fixed effect variables: age, day of cycle. No trend in AMH results was observed throughout normal menstrual cycles. Random effect variable: subject. Both ovaries present. AMH measured using the automated Access AMH assay. AMH levels measured on a fixed day of the menstrual cycle would foster standardization and comparison between individual AMH test results.

Results: 191 specimens were collected from 24 women (mean age 35 years; range 24 to 45 years). Older age was significantly associated with lower mean AMH values (p-value = 0.004). The estimated ICC was 0.94 (95% confidence interval, 0.89-0.96), indicating that 6% of the overall variability in AMH was due to within-subject variability.

Conclusion: No trend in AMH results was observed throughout a normal menstrual cycle. Fluctuations in AMH results during the menstrual cycle accounted for only 6% of the overall variability.

BACKGROUND

• Research studies indicate that Anti-Müllerian Hormone (AMH) may be useful for evaluating response to controlled ovarian stimulation
• AMH has lower within-menstrual cycle variation compared to inhibin B or follicle-stimulating hormone (FSH)
• However, the degree of daily AMH variability across the normal menstrual cycle has yet to be fully described
• AMH levels measured on a fixed day of the menstrual cycle would foster standardization and comparison between individual AMH test results

OBJECTIVE

To describe the variability of AMH levels measured by the automated Beckman Coulter AMH immunoassay across the normal menstrual cycle

MATERIALS AND METHODS

• Prospective cohort study at two sites
• Study population
  • Women with regular menstrual cycle (21-35 days)
  • 18 to 45 years of age
  • Both ovaries present
  • Informed consent signed
• Exclusion criteria
  • Pregnant or pregnancy planned during the study period
  • Previous history of ovarian surgery
  • Recent hormonal contraceptives use
  • Subjects enrolled consecutively at each site
• Sample collection and testing
  • Serum and lithium heparin plasma specimens collected every 3 – 5 days after the start of the menstrual cycle
  • Blood samples processed on the same day and stored at -20°C or colder until the testing dates
  • AMH measured using the automated Access AMH assay
• Statistical Analysis
  • Mixed-effects models constructed to estimate variance components and intraclass correlation (ICC)
  • Dependent variable: AMH
  • Random effect variable: subject
  • Fixed effect variables: age, day of cycle

RESULTS

Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Site A</th>
<th>Site B</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Age (Mean ± SD)</td>
<td>38.4 ± 5.8</td>
<td>32.0 ± 4.7</td>
<td>35.2 ± 6.1</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>39.0 (25.0-45.0)</td>
<td>31.0 (24.0-40.0)</td>
<td>36.5 (24.0-45.0)</td>
</tr>
<tr>
<td>Age in yrs, n (%)</td>
<td>21 - 25</td>
<td>1 (8.3)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td></td>
<td>26 - 30</td>
<td>0 (0)</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td></td>
<td>31 - 35</td>
<td>1 (8.3)</td>
<td>6 (50.0)</td>
</tr>
<tr>
<td></td>
<td>36 - 40</td>
<td>5 (41.7)</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td></td>
<td>41 - 45</td>
<td>5 (41.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>No. of visits (Mean ± SD)</td>
<td>8.3 ± 0.9</td>
<td>7.7 ± 0.9</td>
<td>8.0 ± 0.9</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>8.5 (7.0-9.0)</td>
<td>8.0 (6.0-9.0)</td>
<td>8.0 (6.0-9.0)</td>
</tr>
<tr>
<td>AMH (ng/ml) (Mean ± SD)</td>
<td>3.7 ± 3.2</td>
<td>4.1 ± 4.1</td>
<td>3.9 ± 3.6</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>3.1 (0.0-9.7)</td>
<td>3.1 (0.6-18.7)</td>
<td>3.1 (0.6-18.7)</td>
</tr>
</tbody>
</table>

Distribution of mean AMH values per subject

<table>
<thead>
<tr>
<th>AMH (ng/ml)</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 ng/mL</td>
<td>12 96</td>
</tr>
<tr>
<td>≥3 ng/mL</td>
<td>12 96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>F Value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>8.48</td>
<td>0.004</td>
</tr>
<tr>
<td>Cycle day</td>
<td>0.97</td>
<td>0.325</td>
</tr>
</tbody>
</table>

Source of variability

<table>
<thead>
<tr>
<th>AMH Category</th>
<th>Number of Subjects</th>
<th>Number of Samples</th>
<th>Mean AMH (ng/mL)</th>
<th>Within Subject SD</th>
<th>CV</th>
<th>Between Subject SD</th>
<th>CV</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>24 191</td>
<td>3.9</td>
<td>0.76</td>
<td>19.4%</td>
<td>3.05</td>
<td>78.0%</td>
<td>0.941</td>
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</tr>
<tr>
<td>≥3 ng/mL</td>
<td>12 96</td>
<td>6.6</td>
<td>1.05</td>
<td>15.8%</td>
<td>2.93</td>
<td>44.1%</td>
<td>0.885</td>
<td></td>
</tr>
<tr>
<td>&lt;3 ng/mL</td>
<td>12 95</td>
<td>1.1</td>
<td>0.22</td>
<td>19.3%</td>
<td>0.79</td>
<td>69.3%</td>
<td>0.930</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS

• AMH values decreased with increasing age
• No trend in AMH results was observed throughout normal menstrual cycles
• Fluctuations in AMH results among individual subjects during the menstrual cycle accounted for only 6% of the overall variability
• Our results suggest that AMH can be reliably measured any day during the menstrual cycle