Testing for Ovarian Reserve

How do we measure it and what does it mean?

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Division of Reproductive Endocrinology and Infertility

Disclosures

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- Nora Therapeutics – grant support

Reproductive Aging - Quantity

The life history of a woman’s oocyte endowment

What is Reproductive Aging?
Quantity vs. Quality

- **Quantity**: decline in follicle number ultimately leads to menopause
- **Quality**: decreased implantation potential
  - Increase in meiotic non-disjunction
    - “Production-line” theory
    - Accumulated damage
    - Deficiencies of the granulosa cell function
Concurrent Loss in Quantity AND Quality

How do you measure quantity?

Quantity

- Direct measures
  - Histological

- Indirect measures
  - FSH
  - Inhibin B
  - AFC/ovarian volume
  - Anti-mullerian Hormone (AMH)

Primordial follicle decline

Faddy and Gosden
Fertil Steril 1995
Reproductive Aging
Indirect measures

- FSH
  - Indirect measure of follicular pool
  - Decrease in inhibin B (and estradiol) leads to increase FSH
  - Variable from cycle-to-cycle
  - Decreased predictive ability in populations with a low prevalence
  - Not associated with increased risk of aneuploidy (vanMontfrans, 2004; Thum, 2008)

Quantity – Antral follicle count (AFC)

AFC = 18
AFC = 3

Decline in AFC over time

Faddy and Gosden
Fertil Steril 1995
Scheffer GJ
Fertil Steril 1999

Is AFC a Genetic Trait?
Age and Antral follicle count

R² = 0.2071

Rosen MP, Menopause 2010
Rate of AFC decline with different ages of Maternal menopause

Reproductive Aging - Quantity

AFC and Age
Antral follicle count

AFC and Age

AFC as biomarker of ovarian aging

- Correlation with number of oocytes at retrieval for ART
  - Bansci 2002; Broekmans 2006; Hendriks 2007
- ? Correlation with increased aneuploidy risk
  - Haadsma 2009 – yes; Kline 2004 - no
- Correlation of oocyte (follicle) number with age at menopause
  - OMEGA study – Dutch cohort study (26,428)
    - Decreased response - < 4 associated with increased risk early menopause (<46years of age)
    - Small numbers (38) menopausal

Reproductive Aging
Indirect measures

- Antimullerian Hormone (AMH)
  - Member of the TGFβ family
  - Produced by granulosa cells of pre-antral and early antral follicles
  - Good intracycle stability (LaMarca 2006, Hehenkamp 2006)
  - Good intercycle stability (Fanchin R 2005)
Normative AMH data

AMH: conception to menopause

AMH: impact of lifestyle and reproductive factors

Markers of Ovarian Aging

- Altered feedback
  - Reduced inhibin B
  - Late rise in FSH
- Decline in AFC/AMH parallel histological decline

LaMarca A; European J Obstet Gynecol & Reprod Biol 2012

Kelsey TW; PLoS ONE 2011

Dolleman M: JCEM 2013

Rosen M; Fertil Steril 2010
AMH vs. AFC

\[
AMH = 0.2305 \times AFC - 0.0363
\]

But what is the real rate of decline?
Requires longitudinal study

Reproductive Aging – Rate
Longitudinal Analysis

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std Dev</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first visit</td>
<td>36.61</td>
<td>4.11</td>
<td>36.74</td>
<td>24.57-46.19</td>
</tr>
<tr>
<td>Age at last visit</td>
<td>39.10</td>
<td>3.98</td>
<td>39.23</td>
<td>26.67-51.98</td>
</tr>
<tr>
<td>Number of visits</td>
<td>3.94</td>
<td>2.13</td>
<td>3.0</td>
<td>2-16</td>
</tr>
<tr>
<td>Length of follow up (yrs)</td>
<td>2.49</td>
<td>1.35</td>
<td>2.22</td>
<td>0.66-10.99</td>
</tr>
</tbody>
</table>

Quantity – Longitudinal Analysis

1.2 AFC/yr (95% CI - 1.73, -0.87)

348 patients
Age 25-48
**Mixed effect repeated measure linear regression model of AFC decay**

**Low AFC vs. High AFC**

Changes in AFC/year from Initial Age

<table>
<thead>
<tr>
<th>Initial AFC</th>
<th>25-35yo</th>
<th>36-40yo</th>
<th>41-45yo</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFC ≥ 20</td>
<td>-2.50</td>
<td>-2.31</td>
<td>-2.00</td>
</tr>
<tr>
<td>AFC ≤ 10</td>
<td>-1.10</td>
<td>-1.25</td>
<td>-1.28</td>
</tr>
</tbody>
</table>

Rate of follicle loss increases at age 37-38 years or 25K primordial follicles

Dogma:

- Summary – based on *longitudinal* data
  - there is no acceleration of loss with increasing age
  - there is no acceleration of loss with decreasing follicle number
  - there is no acceleration of loss at a low, set follicle number

What does it mean in terms of fertility?
Fertility decline with age


Abnormalities in oocytes increase with age


Is the Infertile population a good model for study of ovarian aging?

- AFC and AMH approximate the number of oocytes retrieved in IVF cycles with ovarian stimulation
  Ng 2000; Frattarelli 2000; Huang 2001; Bancsi 2002; Hsieh 2001; Broer 2009; La Marca 2010

- AFC and AMH predict the timing of the last natural pregnancy and menopause
  Broekmans 2004; Giacobbe 2004; Doellman 2013; Tcherni 2013
Fertility declines with age

- But is this solely due to decline in follicle number?
- Is this due solely to a decline in oocyte quality with increasing age?
- Do quantity and quality decline together?

AFC Distribution: Infertile vs. Reference

- Why might women with lower follicle count be infertility?
  - Genetic errors
    - Known increase with age
    - Unclear risk with declining follicle number
  - Hormonal environment
    - What clinical factors might predict?
    - What could we do?
**Mean Daily PdG levels**

![Graph of Mean Daily PdG levels]

**Mean Daily E1G levels**

![Graph of Mean Daily E1G levels]

### Cycle Length and Ovarian Aging

<table>
<thead>
<tr>
<th>Age Group</th>
<th>25-27 days (N = 94)</th>
<th>28-32 days (N = 405)</th>
<th>33-35 days (N = 26)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37 ± 5.1</td>
<td>35.7 ± 5.2</td>
<td>35.0 ± 5.8</td>
<td>0.07</td>
</tr>
<tr>
<td>AMH (pmol/l)*</td>
<td>19.9 ± 19.1</td>
<td>26.8 ± 22.3</td>
<td>40.3 ± 25.6</td>
<td>0.001</td>
</tr>
<tr>
<td>FSH (mIU/ml) †</td>
<td>7.97 ± 5.39</td>
<td>6.86 ± 2.8</td>
<td>5.75 ± 1.58</td>
<td>0.002</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>48.8 ± 30.9</td>
<td>49.6 ± 27.9</td>
<td>44.8 ± 24.2</td>
<td>0.70</td>
</tr>
<tr>
<td>Total AFC*</td>
<td>12.2 ± 8.4</td>
<td>15.4 ± 9.4</td>
<td>21.2 ± 12.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Follicles 2-4.9 mm †</td>
<td>7.2 ± 6.5</td>
<td>9.0 ± 7.6</td>
<td>13.9 ± 11.1</td>
<td>0.0004</td>
</tr>
<tr>
<td>Follicles 5-8.9 mm†</td>
<td>4.6 ± 4.3</td>
<td>6.1 ± 4.7</td>
<td>7.2 ± 4.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Follicles 9-10 mm</td>
<td>0.45 ± 0.81</td>
<td>0.27 ± 0.70</td>
<td>0.15 ± 0.46</td>
<td>0.05</td>
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### Oocyte Quantity and Quality

- Factors that affect the size of the follicular pool may also affect oocyte quality.
- For spontaneous conceptions:
  - Lower pregnancy rate (? increased time to conception) with lower AFC and AMH
  - Elevated FSH associated with infertility and higher risk SAB
  - Earlier menopause associated with increased pregnancy loss
  - Kline et al; LaMarca et al: no correlation in FSH, inhibin, AFC between women with trisomic losses vs. normal losses or deliveries
### Impact of Age on conception with IUI

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<tr>
<th>Female Age</th>
<th># Cycles TMC ≥20M</th>
<th>% Ongoing Pregnancy TMC ≥20M</th>
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<td>35-37</td>
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<td>7.0</td>
<td>2.5-19.4</td>
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<td>41-42</td>
<td>330</td>
<td>8.2</td>
<td>130</td>
<td>3.1</td>
<td>2.8</td>
<td>1.0-8.2</td>
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<tr>
<td>43-50</td>
<td>255</td>
<td>1.6</td>
<td>93</td>
<td>0</td>
<td>N/A</td>
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Herndon C, unpublished

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### “Poor response”

What about impact of low follicle count on IVF?

Ferraretti AP, Hum Reprod 2011
Infertile women
Can ART compensate?

<table>
<thead>
<tr>
<th>N</th>
<th>&lt;35</th>
<th>35-37</th>
<th>38-40</th>
<th>41-42</th>
<th>&gt;42</th>
</tr>
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<tr>
<td>&lt; 6 eggs</td>
<td>25</td>
<td>47</td>
<td>85</td>
<td>68</td>
<td>35</td>
</tr>
<tr>
<td>≥ 6 eggs</td>
<td>675</td>
<td>377</td>
<td>343</td>
<td>125</td>
<td>36</td>
</tr>
</tbody>
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Average egg retrieved

| < 6 eggs | 3.9 | 3.9 | 3.8 | 3.8 | 3.5 |
| ≥ 6 eggs | 17.1 | 15.3 | 14.8 | 16.4 | 16.8 |

Embryos transferred

| < 6 eggs | 2.2 | 2.8 | 3.5 | 5.2 | 6.6 |
| ≥ 6 eggs | 2.2 | 1.9 | 2.1 | 2.4 | 2.1 |

Implantation

| < 6 eggs | 34.0% | 25.5% | 17.3% | 10.3% | 3.0% |
| ≥ 6 eggs | 38.0% | 26.0% | 23.0% | 12.1% | 5.2% |

Ongoing Pregnancy Rates

| < 6 eggs | 52.0% | 31.9% | 21.2% | 19.1% | 5.7% |
| ≥ 6 eggs | 53.5% | 43.2% | 42.9% | 32.0% | 30.1% |

Impact of AMH and AFC on pregnancy outcome

- AFC and AMH predict number of oocytes received
- they do NOT predict pregnancy rate with IVF
  - Broer SL, et al. 2013

Summary

- Best current measures of ovarian aging are AMH and AFC
- There is no acceleration in ovarian follicle decline with aging
- Neither AMH nor AFC correlate with aneuploidy risk
- AMH and AFC correlate with the number of remaining oocytes and thus likely predict age at menopause – but...

Clinically – How do manage the patient?

- What to be concerned about
  - Shortened inter-menstrual interval
  - Pre-cycle spotting
- What assessment matters?
  - AFC
  - AMH
Conclusions

- Limited longitudinal data to predict for individual patients
- Limited data regarding spontaneous pregnancy and AMH/AFC
- Counseling women with low ovarian reserve is complex
- When do you refer for egg freezing?