Biomarkers to Redirect Response to Ovulation Induction

Pablo Diaz-Spindola, MD, FACOG
Medical Director IECH Fertility Center Monterrey
Professor of Endocrinology and Reproductive Medicine
Fellowship of UNAM
Monterrey, Mexico
Objectives

- Background
- What is AMH
- iCOS Biomarkers
- Protocols
- Discussion of advantages
- Conclusions
Decrease of the Female Fertility Based on Age

Decrease of the Female Fertility Based on Age

Risk Factors that Decrease the Ovarian Reserve

Reproductive older (over 35 years).
Family history of early menopause.
Genetic conditions (eg. Mosaic 45 X)

Permutation of the FMR1 (fragile X)
Conditions that can cause ovarian damage
(eg. Endometriosis, pelvic infection)
after ovarian surgery

Oophorectomy

Medical history of use of medicinal
gonadotoxics
smoking

Wait... What is all this about.

Redirect Response to Ovulation Induction
Biomarker: Definition

- NIH “A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention”

Palmer SS et al. Biomarkers in reproductive medicine: The promise, and can it be fulfilled?. Fertil Steril 2013 March 15;99(4):954-962
Objective for Having an Ovarian Biomarker

- Avoid excessive response and reduce ovarian responses.
- Identified poor responders to diminish cycles cancelation.
- Facilitate an optimal treatment strategy, potentially minimizing complications and the risk of treatment failure.
- Reduce the cost.
Women Always Want to be a Little Different....

So, we need to act differently.
How Easy is the IVF Treatment?

The way we would like it. The way it really is.
Our Focus are Biomarkers, but There are so Many Factor…
Which one is the best Biomarker to define ovarian response?
20 years later it was isolated and his sequence was known.

- Molecular weight 140 kDa
- 4 times heavier than FSH and LH
- Gene on chromosome 19 p13.3

It belongs to the super family of beta transforming growth factor

Discovered 1940 by Alfred Jost

Müllerian inhibiting substance

Müllerian Inhibiting Substance: An Instructive Developmental Hormone with Diagnostic and Possible Therapeutic Applications

Jose Teixeira, Shyamala Maheswaran, and Patricia K. Donahoe

Pediatric Surgical Research Laboratories, Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114
Physiology of AMH

Secreted by the primordial and prenatal follicle granulosa cells.

- They lose this ability from antral follicles (6-8 mm), mediated by FSH.
- The number of follicles in growth are those that produce the AMH.
- Effect on the primordial follicles remaining, inhibiting their recruitment.

Stability of the AMH

Factors that could influence on the serum concentration of AMH:

Reduce it:
- Age
- BMI
- Administration of chemotherapy and radiotherapy
- Oophorectomy

Increase it:
- PCOS

Have no influence:
- Menstrual cycle
- GnRh agonists
- oral contraceptives
- pregnancy

AMH Assays

## Characteristics of the Most Commonly Used Markers of Ovarian Reserve

<table>
<thead>
<tr>
<th>Test</th>
<th>Details</th>
</tr>
</thead>
</table>
| FSH plus estradiol | • Serum level on cycle day 2–3  
|                | • Variation between cycles possible  
|                | • High FSH value is associated with poor response to ovarian stimulation  
|                | • Does not predict failure to conceive                                    |
| AMH           | • No specific timing for the test  
|                | • Stable value within and between menstrual cycles  
|                | • Low AMH value is associated with poor response to ovarian stimulation  
|                | • Does not predict failure to conceive                                     |
| AFC           | • Number of visible follicles (2–10 mm) during transvaginal ultrasound  
|                | • Performed on cycle days 2–5  
|                | • Number of antral follicles correlates with ovarian response to stimulation  
|                | • Does not predict failure to conceive                                     |

*Abbreviations: AFC, antral follicle count; AMH, antimüllerian hormone; FSH, follicle-stimulating hormone.*
## Markers of Ovarian Reserve and its Influence on Pregnancy

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Outcome</th>
<th>Favourable</th>
<th>Unfavourable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior COH cycle</td>
<td>Delivery</td>
<td></td>
<td>No pregnancy</td>
</tr>
<tr>
<td>Age (years)</td>
<td>&lt;35</td>
<td></td>
<td>≥35</td>
</tr>
<tr>
<td>FSH (IU/l)(^a)</td>
<td>&lt;10</td>
<td></td>
<td>≥10</td>
</tr>
<tr>
<td>d3 E(_2) (pg/ml)</td>
<td>&lt;75</td>
<td></td>
<td>≥75</td>
</tr>
<tr>
<td>d10 P(_4)(^b)</td>
<td>&lt;0.9</td>
<td></td>
<td>≥1.1</td>
</tr>
<tr>
<td>AMH (pmol/l)(^c)</td>
<td>15.7–48.5(^*)</td>
<td></td>
<td>&lt;15.7</td>
</tr>
<tr>
<td>d3 inhibin-B (pg/ml)(^d)</td>
<td>≥45</td>
<td></td>
<td>≤45</td>
</tr>
<tr>
<td>AFC(^e)</td>
<td>≥5</td>
<td></td>
<td>&lt;5</td>
</tr>
<tr>
<td>Ovarian vascularity</td>
<td>Lower Pl</td>
<td></td>
<td>Higher Pl</td>
</tr>
<tr>
<td>Ovarian volume (cm(^3))(^f)</td>
<td>≥3</td>
<td></td>
<td>&lt;3</td>
</tr>
<tr>
<td>CCCT [FSH only (IU/ml)](^g)</td>
<td>&lt;12</td>
<td></td>
<td>≥12</td>
</tr>
<tr>
<td>GAST</td>
<td>Early E(_2) flare</td>
<td></td>
<td>Persistent E(_2)↑ or no response</td>
</tr>
</tbody>
</table>

Comparative Table of More Common Characteristics of the Ovarian Reserve Markers

<table>
<thead>
<tr>
<th>Characteristics for a Good Marker</th>
<th>Age</th>
<th>AMH</th>
<th>FSH</th>
<th>AFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediction of poor response</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Prediction of hyper response</td>
<td></td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Low inter-cycle variability</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Low intra-cycle variability</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Applicable to all patients</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Economic</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

-, not appropriate; +, not very appropriate; +++, very appropriate. AFC, antral follicle count; AMH, anti-Mullerian Hormone.

Both Technologies are Improving and Increasing Accurate

Markers of Ovarian Reserve of Today

### Normal Values Ovarian Reserve

<table>
<thead>
<tr>
<th>Response Type</th>
<th>AMH Range (pmol/l)</th>
<th>E2 Range (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-response</td>
<td>&lt; 1.1</td>
<td></td>
</tr>
<tr>
<td>Poor responder</td>
<td>1 - 5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Normal responder</td>
<td>5 - 15</td>
<td>1.0 - 3.5</td>
</tr>
<tr>
<td>High responder</td>
<td>&gt; 15</td>
<td>&gt; 3.5</td>
</tr>
</tbody>
</table>

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Fleming R. Et cols. Can Anti-Mullerian hormone concentrations be used to determine gonadotrophins treatment protocol for ovarian stimulation? Reproductive BioMedicine Online (2013) 26, 4311-439

AMH Conversion Factor

1 ng/ml = 7.143 pmol/lit

Somebody explain me, please.
Strategic Modelling of Controlled Ovarian Stimulation on the Basis of Ovarian Reserve Markers

Is This Nomogram Useful?

Can We Relate AMH to COS Protocols?

High Responder Patient

- Definition >15-20 oocytes
- AMH > 20 pmol/lit
- PCOS type
- History of OHSS
- Therapy suggested:
  - Antagonist protocol
  - rFHS alone
  - 100-125 IU/d
  - Eventually agonist triggering
  - Freeze all strategy

Fleming R. Et cols. Can Anti-Mullerian hormone concentrations be used to determine gonadotrophins treatment protocol for ovarian stimulation? Reproductive BioMedicine Online (2013) 26, 431f 439
Normal Responder Patient

- Definition 8 - 15 oocytes
- AMH 7-19.9 pmol/Lt
- History of normal response
- Therapy suggested:
  - Antagonist protocol
  - rFHS alone
  - 150-225 IU/d
  - hCG triggering

Low Responder Patient

- Definition: 5 - 8 oocytes
- AMH: 1.1-6.9 pmol/L
- Women <40 years of age
- History of Low response
- Therapy suggested:
  - Antagonist protocol
  - rFHS and LH
  - 300 IU/d FSH + 75-150 IU/d
  - hCG triggering

Poor Responder Patient

- Definition <5 oocytes
- AMH < 1.1 pmol/Lt
- Negligible chance of response
- Therapy suggested:
  - Antagonist or flare-up agonist protocol
  - 300 IU/d FSH plus 150 LH
  - hCG triggering

Fleming R. Et cols. Can Anti-Mullerian hormone concentrations be used to determine gonadotrophins treatment protocol for ovarian stimulation? Reproductive BioMedicine Online (2013) 26, 431-439
Not all agree
Predictor of Response in ART

Most useful tool when evaluating the ovarian reserve (fertile and infertile).

More specific prediction than FSH

Inter- and intra-ciclo variability and stability make handy tool. 6-8 months

AMH serum levels correlate more with CFA than other markers in day 3 of cycle (FSH, LH, estradiol and inhibin B).

Predictor of Response in ART

Predictive markers response in ovarian stimulation:
- FSH, LH
- Estradiol
- Inhibin B
- AMH
- AFC

Only the AHM was the determinant of the number of mature oocytes obtained

Low ovarian response cut-off point: 2.97ng/ml

ESHRE: AMH 0.5-1.1 ng/ml

100% Sensitivity
90% Specificity

Kunt C, et al. Anti-Mullerian hormone is a better marker than inhibin B, follicle stimulating hormone, estradiol or antral follicle count in predicting the outcome of in vitro fertilization. Arch Gynecol Obstet. 2011;283:1415-21
Predictor of Pregnancy

AMH is a good predictor of ovarian response
It does not predict who will become pregnant
Ovarian reserve tests: do not evaluate the quality of the oocytes.

Factors involved in the prediction of pregnancy
- Age
- Time of infertility
- Cause of infertility

Extremely low levels of HAM are associated to not pregnancy.
Low AMH should not be the only factor for refusing the opportunity to ART.
Most obvious limitations when HAM 0.4 ng/dl.

Predictor of Ovarian Response

HAM as ovarian predict response sensitivity 82% and specificity 76%
- AFC sensitivity 82% y Specificity 80%.

Low response <1.0 ng/ml
- 2-30% will be low responders.
- Lower pregnancy rates compared with normoresponders of the same age.
- Premature ovarian failure

(OHSS) ovarian hyperstimulation syndrome. >3.75 ng/ml
- Moderate: 15-20%
- Severe: 1-3%.
- Poor quality eggs
- Hiperestimulados cycles can cause multiple organ failure.
- AMH predicts OHSS
  - sensitivity of the 90.5%
  - specificity of the 81.3%

## HAM Values Used in Mexico as Predictors of Response

**Cuadro 1. Características demográficas y bioquímicas de la población**

<table>
<thead>
<tr>
<th></th>
<th>Normorrespondedora (6-15 OC, n=20)</th>
<th>Hiporrespondedora (≤5 OC, n=10)</th>
<th>Hiperrespondedora (≥16 OC, n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edad</td>
<td>35 ± 6</td>
<td>37 ± 7</td>
<td>35 ± 5</td>
<td>0.667</td>
</tr>
<tr>
<td>AMH 8ng/ml</td>
<td>1.46 ± 1.43</td>
<td>0.355 ± 0.33</td>
<td>3.10 ± 1.86</td>
<td>0.015*</td>
</tr>
<tr>
<td>FSH (UI/L)</td>
<td>7.40 ± 3.53</td>
<td>10.37 ± 9.23</td>
<td>5.53 ± 1.62</td>
<td>0.146</td>
</tr>
<tr>
<td>AFC en FFT</td>
<td>7.8 ± 2.4</td>
<td>1.6 ± 1.1</td>
<td>16 ± 2.2</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

Media ± desviación estándar (DE).

*Normorrespondedoras vs hiperrespondedoras
**Normorrespondedoras e hiperrespondedoras vs Hiporrespondedoras

### AMH Values Used in the IECH as Predictors of Response


<table>
<thead>
<tr>
<th>TABLA No. 1</th>
<th>0-5 OVOCITOS (n=10)</th>
<th>6-16 OVOCITOS (n=26)</th>
<th>17 O MÁS (n=12)</th>
<th>Valor P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edad</td>
<td>38.8±3.58</td>
<td>27.69±6.94</td>
<td>27.6±7.67</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>IMC</td>
<td>22.53±4.33</td>
<td>24.64±4.31</td>
<td>23.47±2.95</td>
<td>0.344</td>
</tr>
<tr>
<td>FSH</td>
<td>8.78±3.43</td>
<td>5.22±1.65</td>
<td>4.55±1.70</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Estradiol día 10</td>
<td>555.10 ±489.64</td>
<td>2416.27±1428.44</td>
<td>3564.75±1678.39</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Folículo &gt; 15 mm</td>
<td>2.30±1.89</td>
<td>9.54±3.89</td>
<td>11.58±2.31</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>HAM</td>
<td>0.59±0.40</td>
<td>2.27±1.36</td>
<td>4.74±1.97</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Ovocitos Totales</td>
<td>2.50 ±2.17</td>
<td>11.73±1.19</td>
<td>20.08±2.64</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Ovocitos Maduros</td>
<td>2.30±2.11</td>
<td>9.15±3.45</td>
<td>15.17± 3.83</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>
Applications

- Ovarian reserve: age of menopause after surgery or radiation therapy.
- Predict IVF response: Low response, hyperresponse, cancellation and ovarian hyperstimulation Syndrome
- Future in reproductive medicine: Pregnancy predictor
- Impact of chemotherapy
- Polycystic ovary Syndrome PCOS

Conclusions

• There are so many information about AMH as a biomarker for ovulation induction
• Today we can standardized level of AMH
• There are many protocols depends of AMH levels, and we need to avoid HOSS, and cancellation for poor responder patient.
• We would like to have always the “perfect patient with the perfect protocol” to help them to get pregnant.
Anti-Müllerian Hormone is an excellent biomarker of follicular reserve, however cannot be considered as a predictor of pregnancy.
Biomarkers to Redirect Response to Ovulation Induction

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