AFC/AMH and ovarian reserve: are they competitors or complementary?

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Head: ART/PGD Division
Director: INSERM U 1203

ART/PGD Department
Arnaud de Villeneuve hospital
University-hospital of Montpellier, INSERM U 1203
Montpellier-34295, France
de Bruin et Velde 2004
Societal and Behavioral Shifts...

Reproductive Delay

Ovarian Aging

Advancing Technology

Demand for "Female Fertility Testing"
Oh no !!!

Is there hope?

What can we do?
Pre- ART assessment of female partner

• Ovarian reserve (OR)
• Bio markers of OR
• Tow major competitors - **AFC** and **AMH**
Conventional bio markers of OR

- **Clinical**:  
  - age: oocyte quality++

- **Serum assays (D2-3)**:  
  - FSH, Estradiol, Inhibine B  
  - Anti-Müllerian Hormone (AMH)

- **US measurements**:  
  - Antral follicle count (AFC)  
    (2-10 mm)  
  - Ovarian volume

*Normal si > 10 follicles*  

Kwee et al, FS 2008
Others factors affects OR

BMI

Age

Ethnicity

Smoking
Does **AMH** and **AFC** for OR assessment will be competitors or complementary?
Our Tools

Circulating AMH

TVS AFC

- Primordial pool
- Primary follicles
- Pre-antral follicles
- 0.1-2 mm
- 2-7 mm
- 8-10 mm

Jeppesen, MHR 2013
Broer, COOG 2009
I- Antral follicle count (AFC)

Sonographic Evaluation of OR
Antral follicle count (AFC)

Sonographic Evaluation of OR

- Antral follicle counts (AFC)
- Ovarian volume (not recommended by ASRM, 2015)
- Doppler imaging techniques
Sonographic Evaluation of OR

Improving US resolution

Follicle number per ovary

Year of data collection

Max TransducerFreq (MHz)

Dewailly, et al HRU 2013
Antral Follicle Counts (AFC)

- 2-10 mm antral follicles
- Typically measured on Day 2 or 3
- Correlates with d3 FSH, amount of total gonadotropin used, peak E2 level and # oocytes
  
  Chang et al. 1998
- Predictor beyond patient age, d3 FSH level or inhibin B level
  
  Beckers, et al. 2000
  Fauser, 2000; Laszlo et al. 2002
- Normal responders typically have > 10 antral follicles vs. < 5 for poor responders
  
AFC normal ranges are just being established

Infertility population

Oocyte donor population

9,978 women

5,724 women

Iliodromiti et al JCEM, 2016
Excessive Age-Related Decline in Functional Ovarian Reserve in Infertile Women: Prospective Cohort of 15,500 Women

Stamatina Iliodromiti, Carlos Iglesias Sanchez, Claudia-Martina Messow, Maria Cruz, Juan Garcia Velasco,* and Scott M. Nelson*

Table 2. AFC Stratified by Age in Potential Oocyte Donors and Infertility Patients

<table>
<thead>
<tr>
<th>Age Bands, y</th>
<th>No. of Donors</th>
<th>AFC in Oocyte Donors, Median (IQR)</th>
<th>No. of Infertility Patients</th>
<th>AFC in Infertility Patients, Median (IQR)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>≤25</td>
<td>3100</td>
<td>21.0 (17.0, 25.0)</td>
<td>61</td>
<td>20.0 (12.0, 27.0)</td>
<td>.338</td>
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<tr>
<td>26–27</td>
<td>779</td>
<td>20.0 (16.0, 24.0)</td>
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<td>17.0 (12.0, 22.0)</td>
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<tr>
<td>28–29</td>
<td>749</td>
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<td>&lt;.001</td>
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<tr>
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<td>610</td>
<td>18.0 (15.0, 22.0)</td>
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<td>16.0 (11.0, 21.2)</td>
<td>&lt;.001</td>
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<tr>
<td>32–33</td>
<td>389</td>
<td>18.0 (14.0, 22.0)</td>
<td>1023</td>
<td>13.0 (9.0, 19.0)</td>
<td>&lt;.001</td>
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<tr>
<td>34–35</td>
<td>95</td>
<td>18.0 (14.0, 22.0)</td>
<td>1452</td>
<td>11.0 (8.0, 16.0)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
MRI determined AFC strongly correlates with AMH
Why is AFC not reliable?  
the variability is huge

Deb et al, 2009
Individualized dosing based on AFC

- does not improve LBR
- may improve safety for high responder patients
Antral follicle count (AFC)

- Widely used ultrasound measure of OR
  - Transvaginal, early follicular phase
- Probably the best predictor of ovarian response to COS
  - Thresholds for a low AFC definition vary
- Most use the combined number of 2-9 or 2-10 mm follicles on each ovary
- There is an age-related decline in AFC
II- Anti Müllerian Hormone - AMH -

- Produced only in ovarian GC
- Its serum concentration reflect the pool of ovarian follicules (preantral and small antral)
- AMH levels are 2 to 3 times higher in PCOS patients compared to normal adult women
Anti Müllerian Hormone - AMH -

• Technical issues with AMH measurement are now resolved
• RCT now confirm AMH better than all other biomarkers for ovarian response prediction
Anti Müllerian Hormone - AMH -

Measuring AMH

Global reference range

Different labs gave different results

Multicentre study confirms Roche automated AMH assay reproducible

Nelson et al FS 2011
Nelson et al RBMO 2012
Nelson et al FS 2013

Zuvela, et al Reprod Biol 2013

Iliodromiti et al, HRU 2015
Anderson et al FS 2015
Nelson et al FS 2016
Anti Müllerian Hormone - AMH -

Measuring AMH

Roche or BioMerieux release their automated test VIDAS® AMH

VIDAS® AMH quantitatif measurement of circulating AMH using ELFA (Enzyme Linked Fluorescence Assay)
Anti Müllerian Hormone - AMH - Measuring AMH

<table>
<thead>
<tr>
<th>Competitor assay Currently on the market</th>
<th>VIDAS® AMH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay type</strong></td>
<td>Automate</td>
</tr>
<tr>
<td><strong>Tests / kit</strong></td>
<td>30</td>
</tr>
<tr>
<td><strong>Sample type</strong></td>
<td>Serum, plasma (Li Hep)</td>
</tr>
<tr>
<td><strong>Minimum sample volume</strong></td>
<td>200 µl</td>
</tr>
<tr>
<td><strong>Incubation time</strong></td>
<td>35 min</td>
</tr>
<tr>
<td><strong>Limit of detection</strong></td>
<td>0.02 ng/mL</td>
</tr>
<tr>
<td><strong>Units</strong></td>
<td>ng/ml – pmol/L</td>
</tr>
<tr>
<td><strong>Measuring range</strong></td>
<td>0.02 – 9.00 ng/mL</td>
</tr>
<tr>
<td><strong>Calibrations and controls frequency</strong></td>
<td>28 days</td>
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</table>

BioMérieux Values
Anti Müllerian Hormone - AMH -

Factors that affect AMH

- 3 major SNPs for AMH identified
  Perry and Nelson 2015

- AMH is dynamic across the lifecourse
  Dewailly et al, HRU 2014, 2018
AMH can be measured on any day of the cycle

Kissell et al HR 2014
Ethnic differences may not exist in OR relate to AMH

Geometric Mean AMH

Age (years)

Bleil et al FS 2013
Bhide et al BJOG 2014
Smoking can reduce AMH

Median (p50) AMH values in subgroups

Median AMH values in subgroups

- Regular cycle + non smoking
- Regular cycle + smoking
- OC-use + non smoking
- OC-use + smoking

Dolleman et al JCEM 2013
PCOS women have higher AMH

PCOS patients ovary has x6 the density of pre-antral follicles compared with normal patients ovary.
• AMH decreases throughout reproductive life
• Undetectable AMH levels after spontaneous menopause
• Stable throughout the menstrual cycle

La Marca et al, 2006a
AMH decreases with age

Age-Specific Normal Reference Range for Serum AMH in Healthy Chinese Han Women: A nationwide Population-Based Study.

2055 women, aged 20 to 55 years, from 6 different regions in China

- Serum AMH concentrations declined progressively with age
- Median AMH levels were 6.23, 5.65, 4.55, 3.74, 2.78, and 1.09 ng/mL for the 20 ≤ age < 25, 25 ≤ age < 30, 30 ≤ age < 33, 33 ≤ age < 37, 37 ≤ age < 40, and 40 ≤ age < 55 groups, respectively
- AMH levels were positively correlated with AFCs and T, LH, PRL and PRG levels and negatively correlated with BMI and FSH levels and were not significantly correlated with E2
How does AMH compare to its competitors?

The major competitor - AFC
MRI determined AFC strongly correlates with AMH

Hagen et al JCEM 2014
Are AMH and AFC equivalent in COS?
Why no consensus with either US or AMH?

• Population issues
  – Patient populations
  – Control populations

• Technical issues
  – Different ultrasound machines (≥8MHz)
  – Different ways of counting/measuring
How AMH can inform clinical practice?
AMH predicted oocyte yield in antagonist RCT

Arce et al FSI 2013
at each clinic **AMH** better than **AFC**

<table>
<thead>
<tr>
<th>Clinic</th>
<th>AMH&lt;sup&gt;b&lt;/sup&gt;</th>
<th>AFC</th>
<th>MEGASET Trial (n=749)</th>
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<td>0.51</td>
<td>0.33</td>
<td>Highest</td>
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<tr>
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<td>0.57</td>
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<td>0.45</td>
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<td>0.51</td>
<td>Highest</td>
</tr>
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<td>0.59</td>
<td>0.44</td>
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<tr>
<td>16</td>
<td>0.67</td>
<td>0.75</td>
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<tr>
<td>17</td>
<td>0.71</td>
<td>0.49</td>
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<tr>
<td>18</td>
<td>0.75</td>
<td>0.26</td>
<td>Lowest</td>
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<table>
<thead>
<tr>
<th>Clinic</th>
<th>AMH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AFC</th>
<th>MERIT Trial (n=623)</th>
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<tr>
<td>19</td>
<td>0.77</td>
<td>0.34</td>
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</tbody>
</table>

Correlation coefficient

**AMH assay:**
- aBeckman Coulter ImmunoTech
- bBeckman Coulter Gen II

Nelson et al. FS 2015
ESTHER Trial

Individualised dosing:

- Not more oocytes in low responder range
- Less oocytes in the high responder range
- Not more GQ blastocysts...

Anderson et al, 2017
Algorithms that incorporate anti-Müllerian hormone

### Predictors of number of retrieved oocytes per IU of FSH

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate (p value)</th>
<th>Multivariate (p value)</th>
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<tbody>
<tr>
<td>Age</td>
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<td>0.009</td>
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<tr>
<td>Day 3 FSH</td>
<td>0.0093</td>
<td>0.02</td>
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<td>AMH</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>BMI</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.01</td>
<td>NS</td>
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</tbody>
</table>

Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 1: The predicted poor responder

Theodora C. van Tilborg1,*, Helen L. Torrance1, Simone C. Oudshoorn1, Marinus J.C. Eijkemans2, Carolien A.M. Koks3, Harold R. Verhoeve4, Annemiek W. Nap5, Gabrielle J. Scheffer6, A. Petra Manger7, Benedictus C. Schoot8,9, Alexander V. Sluijter10, Arie Verhoef11, Henk Groen12, Joop S.E. Laven13, Ben Willem J. Mol14,15, and Frank J.M. Broekmans1, on behalf of the OPTIMIST study group†

Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 2: The predicted hyper responder

Simone C. Oudshoorn1,*, Theodora C. van Tilborg1, Marinus J.C. Eijkemans2, G. Jur E. Oosterhuis3, Jaap Friederich4, Marcel H.A. van Hooff5, Evert J.P. van Santbrink6, Egbert A. Brinkhuis7, Jesper M.J. Smeenk8, Janet Kwee9, Corry H. de Koning10, Henk Groen11, Cornelis B. Lambalk12, Ben Willem J. Mol13,14, Frank J. M. Broekmans1, and Helen L. Torrance1, on behalf of the OPTIMIST study group†

Individualized FSH dosing based on ovarian reserve testing in women starting IVF/ICSI: a multicentre trial and cost-effectiveness analysis

Theodora C. van Tilborg1,*, Simone C. Oudshoorn1, Marinus J.C. Eijkemans2, Monique H. Mochtar3, Ron J.T. van Golde4,5, Annemieke Hoek6, Walter K.H. Kuchenbecker7, Kathrin Fleischer8, Jan Peter de Bruin9, Henk Groen10, Madelon van Wely9, Cornelis B. Lambalk11, Joop S.E. Laven12, Ben Willem J. Mol13,14, Frank J.M. Broekmans1, and Helen L. Torrance1, on behalf of the OPTIMIST study group†
AMH as a Tool to choice ART!

- AMH = good quantitative bio marker of OR
  - Can not be used alone (age, ovulatory status, AFC, BMI....)

- AMH = bad qualitative bio marker to predict conception
  - Low AMH = elevated FSH

- Age = best criteria for oocyte quality

- Normal AMH = non prognosis value
The Future

Circulating Nucleic Acids for evaluating Ovarian Reserve!
New markers in ART
Applying Genomics medicine to ART

Ovarian reserve and Stress Predicting

IW Detecting

Ovarian Response Predicting

Viable Embryo Selection
OR conventional biomarkers:

**AMH**...**CFA**...**FSH**...**LH**...**E2**...
Which one more reliable?

Discrepancies between AMH and AFC?

Which one to choice for choosing COS and predict protocols?

Possibilities and limits of ovarian reserve testing in ART

La Marca et al., Curr Pharm Biotechnol., 2012

Identification of new biomarkers for OR and COS response
Cell-free nucleic acids as non-invasive biomarkers of gynecological cancers, ovarian, endometrial and obstetric disorders and fetal aneuploidy

S. Traver1, S. Assou3, E. Scalici1,2, D. Haouzi1, T. Al-Edani1,3, S. Belloq2, and S. Hamamah1,2,4

MicroRNAs: new candidates for the regulation of the human cumulus–oocyte complex

S. Assou1, T. Al-Edani1,3, D. Haouzi1, N. Philippe1, C-M. Laccelier3, D. Pigemon1, T. Commes1, O. Al-Ahmed2, M. Dechaud1,2, and S. Hamamah1,2

Cell-free DNA in human follicular fluid as a biomarker of embryo quality

E. Scalici1,2,3, S. Traver1, N. Molinari4, T. Mullet2,3, M. Monforte4, E. Vinteaux3, and S. Hamamah1,2,3

Circulating microRNAs in follicular fluid, powerful tools to explore in vitro fertilization process

E. Scalici1,2, S. Traver1, T. Mullet2,3, N. Molinari4, A. Ferrière1, C. Brune1, S. Belloq2, and S. Hamamah1,2,4

Cell-free DNA in Human Follicular Microenvironment: New Prognostic Biomarker to Predict in vitro Fertilization Outcomes

Sabine Traver, Elodie Scalici, Tiffany Mullet, Nicolas Molinari, Claire Vincens, Samir Hamamah
• cfDNA level in serum was significantly higher in women with LFOR compared with normal OR (p=0.045).

• cfDNA level in serum tended to be higher in PCOS patients compared with normal OR (p=0.05).
Cell-free DNA level in serum according to patient’s age

- By including only patients with normal OR (N=40)
- Linear Regression
  $R^2=0.27$, $p=0.001$
- By using cut off of 38 years: **Significant higher cfDNA levels for women with ≥38 years** compared to women with < 38 years ($p=0.03$)

**Significant and positive association between cfDNA levels in serum at day 3 and patient’s age**
Cell-free DNA level in serum at day 3 related to ovarian reserve status

Prediction of LOR (n=38) according to cfDNA at D3

- AUC=0.71 (0.59-0.81)
- P<0.001
- Se=68%
- Sp=73%

Prediction of PCOS (n=30) according to cfDNA at D3

- AUC=0.81 (0.69-0.90)
- P=0.01
- Se=70%
- Sp=68%

OR status based on AMH level and AFC at day 3.
cfDNA according to AMH level

cfDNA level was significantly higher in serum samples from women with AMH ≤1 ng/ml or ≤ 2 ng/ml compared to those with AMH>1 ng/ml or > 2 ng/ml, respectively (p=0.004)
Cell-free DNA levels in serum at D3 and COS

Ovarian response to stimulation based on number of oocytes collected at oocyte retrieval day

- By including patients undergoing IVF/ICSI procedure (N=31)
- Linear Regression
  \[ R^2=0.15, \quad p=0.03 \]
- Logistic regression: Prediction of retrieved oocytes by using multivariate model including AMH and cfDNA levels at day 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Coeff.</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH</td>
<td>0.18</td>
<td>0.22</td>
<td>0.4 (NS)</td>
</tr>
<tr>
<td>CfDNA</td>
<td>-0.01</td>
<td>0.006</td>
<td>0.03</td>
</tr>
</tbody>
</table>

CfDNA levels at day 3 can predict significantly the number of retrieved oocyte independently of AMH levels.
Cell-free DNA versus AMH levels in serum at D3 for prediction of oocyte collection

Cut off of 6 oocytes

- Patients undergoing IVF/ICSI procedure

**Cell-free DNA**
- AUC = 0.79 (0.60-0.91)
- P = 0.001
- Se = 73%
- Sp = 82%

**AMH**
- AUC = 0.73 (0.54-0.87)
- P = 0.02
- Se = 67%
- Sp = 81%

CfDNA levels at D3 can predict significantly a poor ovarian response (<6 oocytes) to stimulation with higher Se and Sp than of AMH levels.
Comparison of cell-free DNA levels between women with normal OR, and PCOS

A. Comparison of cDNA levels in women with normal ovarian reserve and ovarian reserve disorders.

B. Comparison of cDNA levels in women with different AFC values (< 10 and ≥ 10).

C. Comparison of cDNA levels in women with different AMH values (≤ 1 and > 1).

Infertility length (years)
Significant associations between intra-follicular cell-free DNA levels and follicle size

High cell-free DNA levels

Low cell-free DNA levels

Significant and negative correlation between intra-follicular cfDNA levels and follicle size

Relationship with dynamic and functionnal follicle state.

Scalici...Hamamah et al., HR 2014
Significant associations between intra-follicular cfDNA levels and ovarian response to COS

Traver...Hamamah et al., Plos One 2015
ROC curve for obtaining a clinical pregnancy according to cfDNA level

ROC curve to evaluate the predictive value of FF cfDNA level for clinical pregnancy outcome in a multivariate model:
area under the curve = 0.73 [0.66–0.87],
sensitivity = 60%, specificity = 88%

Traver - Hamamah et al 2015
Schematic model summarizing the significant relationships between cf-DNA levels and:
infertility duration, OR status, ovarian stimulation, ovarian response to COS, embryo development and clinical pregnancy outcomes

- **Ovarian reserve disorders**
  - Follicular maturity abnormalities
  - Increased or accelerated apoptosis process

- **Long infertility length**
  - Increased stress in infertile couples

- **Long and strong stimulation**
  - Poor ovarian reserve and/or effect of stimulation treatment by gonadotropin (r-FSH)

- **Poor ovarian response**
  - Poor quality of follicular micro-environment

- **High cell-free DNA levels in human follicular fluid**

- **Poor embryo quality**
  - Negative effect on early embryo development (Scalici et al., 2014)

- **Clinical pregnancy prediction**
  - Negative effect on conception

References:
Scalici - Hamamah et al., HR 2014
Traver - Hamamah et al., Plos One 2015
Conclusions

• We now have a robust automated AMH assay from Roche, BioMerieux...
• RCTs confirm that AMH is superior to AFC biomarker
• AMH can be used for prediction hypo/hyper ovarian response
• In case of discrepancies between AMH and AFC, cfDNA should be investigated...