Should AMH replace ultrasound PCO morphology as a diagnostic criteria?

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Basic facts

- Polycystic Ovarian Syndrome is a Multifactorial Polygenic Endocrine disorder.
- The underlying Endocrine dysfunction relates primarily to Androgen production and Metabolism.
- Heterogeneity of Clinical and Endocrine features.
- HI & Obesity – important etiological factors contribute to HA.
- Obesity – >IR, > severity of PCOS
Endocrine abnormalities in PCOS

The typical endocrine abnormalities of anovulatory women with PCOS are

• Serum Androgens - 50-80%
• LH - 60%
• Normal, or slightly low serum FSH levels
• Hyperinsulinaemia - >IR 60%

Franks, 1995
Prevalence

16.6% by Rotterdam criteria
Prevalence < with age
33.3% - <30
14.7% - 30-34yrs
10.2% - > 35 yrs. Lauritson et al 2014

South Asian population:

Caucasian population:
PCOS - importance of an Accurate Diagnosis

Associated with health risk in all age groups

Early Recognition – Prevention – Intervention

• **Adolescence - M. Irregularity, Hirsuitism, Acne.**

• **Reproductive age**: Infertility (Oligo/anovulation), Pregnancy complications.

• **Peri-menopause & Menopause - IGT, Dyslipidemia, Metabolic syndrome.** (DM 2, HT, CAD, Endometrial Ca).

Complications of infertility treatment - OHSS, MPR

Life threatening - severe OHSS
### Criteria for Diagnosis of PCOS

<table>
<thead>
<tr>
<th>PCOS definition</th>
<th>Rotterdam criteria 2003 (ESHRE/ASRM)</th>
<th>AE- PCOS society task force - 2009</th>
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<tbody>
<tr>
<td>NIH 1990</td>
<td>2/3 manifestations:</td>
<td>2 criteria</td>
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<td>Patient</td>
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<td>demonstrates</td>
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<td>both:</td>
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<tr>
<td>1. Clinical and/or biochem signs of HA</td>
<td>1. HA (clinical or biochemical)</td>
<td>1. Hirsutism and/or HA (fixed)</td>
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<tr>
<td>2. Oligo- or chronic anovulation</td>
<td>2. Irregular or absent ovulation (OA)</td>
<td>2. Oligo-anovulation and/or polycystic ovaries</td>
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</table>

PCOM - included in latest NIH recommendations (Johnson et al., 2012)
Exclude other etiologies of androgen excess - Late onset congenital adrenal hyperplasia, Androgen secreting tumours, Cushing’s syndrome
Phenotypes

- Phenotypes: four possible subcategories
  - Phenotype 1/A - IM/HA/PCOM,
  - Phenotype 2/B - IM/HA
  - Phenotype 3/C - HA/PCOM, and
  - Phenotype 4/D - IM/PCOM.

Sub-Categories
- Obese - Lean PCOS
- Ovulatory and Anovulatory PCOS
- Different metabolic profiles/
- Ovarian Response / Reproductive Outcome
PCOM only

Is it a form of PCOS?

• Prevalence 10-30%. Prevalent across ethnic groups.

• PCOM is not a morphological variant of ovaries but represent a functional entity that may be considered as a silent form of PCOS (Franks et al., 2008)

• These women have a slightly higher mean A level (Mortensen et al., 2009) or AMH levels (Johnstone et al., 2010), not sign in all studies (Johnstone et al., 2010).

• PCOM may be a fore runner to PCOS – should be monitored.

• AMH could be the best marker for identification. (Dewailly 2011)
PCOS - Diagnostic Criteria

Rotterdam Criteria - 2003

2/3 criteria have to be present.

- Oligo/anovulation – IM (irregular menses).
- Androgen excess – HA (clinical/biochem).
- Polycystic ovarian morphology (single ovary)

All other causes of HA & ovulatory dysfunction have to be excluded.
Establishing PCOM criteria

- First widely adopted criteria - Adams et al. (1985, 1986) arbitrary - 10 or > follicles (2–8 mm) in one cross section of ovary - using TAS.

- Jonard et al 2003 - established FNPO threshold at ≥12 follicles, 2–9 mm in diam (mean of both ovaries) using ROC .75% sensitivity & 99% specificity in distinguishing PCOS from controls by TVS.

Rotterdam criteria based on this 1study +

Expert opinion. (Balen et al 2003)
USG characteristics

- ≥ 10–12 follicles /ovary, 2–9mm measured in follicular phase.
- > Ovarian volume >10cm³
- Necklace sign
- > Stromal density.
Ultrasonography - 2003 consensus

- Presence of 12 or > follicles in each ovary measuring 2 - 9 mm in diameter (FNPO)
- Scan performed on D 3 - 5 (no DF- foll. ≥10)
- Increased ovarian volume [ > 10 ml ]

Follicular distribution omitted
Stromal echogenicity and volume omitted
Changing criteria

- Improved USG technology—possible to visualize a higher number of AF’s leading to a potential over diagnosis of polycystic ovaries and PCOS, especially in younger women (Duijkers and Klipping, 2010; Johnstone et al., 2010; Kristensen et al., 2010).
Impact of recent advancements in imaging technology on the variability in follicle counts

- Regression analysis confirmed sign effect of Max Transducer Frequency on FNPO \((P < 0.023)\), independent of the mean age of pts.

- Post hoc analysis of FNPO at different transducer frequencies revealed a significant increase in reported FNPO when the transducer frequency was \(\geq 8\) MHz \((P < 0.0001)\). (Dewailly et al 2014)
How do you define normal?
FNPO cut off in healthy women?

In selected populations 20-35 yrs with regular MC and no HA - median values of FNPO between 11-13 were established.
(Bentzen et al., 2013; Deb et al., 2013; Lujan et al., 2013)

These studies published after the 2003 Rotterdam consensus strongly suggest that the FNPO ≥12 threshold is no longer valid for defining PCOM.

Dewailly et al 2014
**PCOM - Establishing threshold - FNPO**

- Dewailly et al., 2011; Lujan et al., 2013 compared PCOS to controls by means ROC curve analysis - diagnostic threshold raised substantially to ≥19 and to ≥26 FNPO, resp.

- Dewailly et al. (2011) applied cluster analysis in order to exclude PCOM. Omitting this would have yielded a cut-off value of 25 follicles.

Dewailly et al., 2011
Why are we looking to change PCOM? Variability in the threshold for FNPO

- Differences in the methods of counting follicles
- Differences in transducer frequency.
- Observer variability in assessing follicle number—highly subjective, observer variability.
- Greater inter-cycle variation—overweight and obese women.
- 3D–cumbersome, =2D

Broekmans et al., 2010—initial a ‘scout sweep’ of in 2 planes define boundaries, caliper meas of foll. to exclude >10mm, longit sweep. Counts for both ov added & foll >10 mm subtr- total AFC.

Balens et al. (2003)—estimate follicles in multiple planes and report mean foll count of Lt & Rt. Ov when assessing PCOM (i.e. to generate an FNPO). Perform estimates in absence of DF.
**PCOS Definition**

What is the cut-off for ovarian volume?

- **Rotterdam consensus - threshold 10 ml.** (Balen et al. 2003)
- **Lower cut off values suggested.** 6.4 ml (Kosus et al., 2011), 7.0 ml (Jonard et al., 2005;), 7.5 ml (Carmina et al., 2005).

- **Ovarian Size variable - Clinical & metabolic characteristics, Ethnicity, BMI and insulin levels.** (> in >IR, BMI).
- **Mean ov size - high- USA & Canada, inter-mediate - Europe, low- East Asian countries** (chen et al 2008).
- **Ov size varies with age-** max during adolescence (1.3 - 3.8 yrs post-menarche), slowly decr during adulthood and rapidly shrinking after menopause.

Ov size not affected by improved transducer frequency

Min change between 20-39
Recommendations:

1. FNPO threshold for definition of PCOM at ≥25 follicles for most populations. Transducers frequency ≥8. (may change with improving technology)

2. OV – < sensitivity. Use when image quality is poor or TVS no possible. Use of in-house reference N values is highly recommended if unavailable, OV ≥10 ml threshold can be used conservatively.

3. TAS not suitable. Can be used to measure OV when TVS not possible

4. Insufficient data to recommend an FNPS threshold to define PCOM (cross sectional measurement)
Relevance of AFC threshold in the definition of Polycystic ovaries has been challenged

<table>
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<th>Table III Factors contributing to variations in thresholds for follicle number in polycystic ovaries.</th>
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<td><strong>Inclusion criteria for controls</strong></td>
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AMH has been proposed as a marker of polycystic ovaries in PCOS to standardize criteria
Ante Mullerian Hormone - AMH

AMH is a dimeric glycoprotein that belongs to the TGF β family.

Max expression <6mm AF

no longer expressed during FSH dependent final stages

No expression in atretic foll

Surrogate marker of ovarian reserve

60% of serum AMH is derived from follicles 5 – 8 mm in diameter (Jeppesen et al., 2013).
AMH - a marker of OR Levels birth - menopause

Strong & positive correlation ($r < 0.96$) between declining AMH & declining numbers of NGF.

A peak shortly after birth - 'mini puberty' of the neonate, a sustained rise to 9 years of age. An inflection with even a slight decline during the pubertal ages (9 - 15 yrs), a 2nd growth phase peak 25 years. Steady decline to undetectable levels at an average age of 50 - 51.
AMH levels in controls & PCOS

Exaggerated AMH tone could be involved in the follicular arrest seen in polycystic ovaries.

Mean values & 95% CI for AMH (pmol/l) controls, PCOM & PCOS. Homburg 2012
AMH, AFC & OR

- Both AMH and AFC were significantly raised in the high responders group and decreased in the poor responders group (P<.0001).
- Compared with FSH and AFC, AMH performed better in the prediction of excessive response to ovarian stimulation – AMH (ROCAUC) 0.81, AFC (ROCAUC) 0.69. Nardo et al 2009.
Cut-off value for basal serum AMH to predict OHSS by ROC curve analysis. The selected value was 3.36 ng/ml, with a sensitivity of 90.5% (95% CI 69.6–98.5) and a specificity of 81.3% (95% CI 75.8–86.0).

Advantage AMH

- **Deeper Probe**: more sensitive & specific than AFC as it also reflects pre antral & small AF’s (<2 mm), which are hardly seen in usg. (Dewailly 2014)
- **< variability - inter & intra-cycle**: DF & CL don’t secrete AMH.
Advantage AMH

- Can be measured on any day of the cycle.
- AMH is useful in obese, virgin or < echogenic pts.
- AMH level is rather independent from H-P axis and is not modified in pathologies - HPRL, FHA.
- Ethnicity does not influence AMH level (controversial).
- AMH level correlated severity of PCOS symptoms and is higher when HA or OA is present. (Eldar-Geva et al 2005, Pellat et al 2010)
Concerns

• **Accuracy/limitations of various tests available.**
  – **Manual, Automated**

• **Absence of international standard for AMH assays.**

• **What is the Cut off AMH value for Diagnosis?**
By ROC analysis each assay displayed similar efficiency for PCOS diagnosis.

Values obtained with GenII & AL-105i ELISA's were similar to EAI AMH/MIS values, whereas automatic assays generated (16%-20%) lower values.

After exclusion of PCOM, the 95th percentile of controls was 4.2 ng/mL (30 pmol/L) with the automatic assays and 5.6 ng/mL (40 pmol/L) with the manual assays.

Box and whisker plots showing the values of serum AMH in nanograms per milliliter (y-axis, log scale) with each assay in the two subgroups of patients: controls and PCOS patients with a full phenotype. Horizontal small bars represent the 5th to 95th percentile range, and the boxes indicate the 25th to 75th percentile range. The horizontal line in each box corresponds to the median; 1 ng/mL corresponds to 7.14 pmol/L.

**What is the Cut off for PCOS diagnosis?**

**Can Anti-Müllerian Hormone Predict the Diagnosis of Polycystic Ovary Syndrome? A Systematic Review and Meta-Analysis of Extracted Data**

*Iliodromati et al 2013*

**PCOS VS NON-PCOS**

### Table 1. Characteristics of the 10 Included Studies

| Author          | Year | Study                  | Diagnosis of PCOS | N (PCOS) per Rotterdam | Age, y     | Cutoff, ng/mL | Sensitivity | Specificity | AUC, 95% Cl | Selection Bias | Verification Bias | Assay |
|-----------------|------|------------------------|-------------------|------------------------|------------|---------------|--------------|-------------|-------------|---------------|-----------------|-------------------|-------|
| Homburg et al (47) | 2013 | Prospective case-control | Rotterdam         | 90                     | 32.1 ± 3.3 | 6.72          | 60.0         | 98.2        | 0.81         | Yes            | No                | DSL               |
| Woo et al (42)   | 2012 | Prospective cross-sectional | Rotterdam     | 87                     | 22.0–38.0  | 7.82          | 75.9         | 86.8        | 0.868        | 0.801–0.919     | No                | IBC               |
| Chao et al (45)  | 2012 | Case-control           | Rotterdam         | 45                     | 29.0–38.0  | 3.50          | 74.0         | 79.0        | NA           | No              | No                | DSL               |
| Eilertsen et al (25) | 2012 | Case-control           | Rotterdam AES    | 56                     | 33.3 ± 5.5 | 2.80          | 94.6         | 97.1        | 0.992        | Yes            | No                | DSL               |
| Lin et al (29)   | 2011 | Prospective case-control | Rotterdam        | 126                    | 27.7 ± 5.8 | 7.30          | 76.0         | 70.0        | 0.774        | Yes            | No                | DSL               |
| Dewailly et al (24) | 2011 | Prospective           | Rotterdam         | 62                     | 20.1–34.0  | 4.90          | 92.0         | 97.0        | 0.973        | Yes            | No                | IBC               |
| Li HWR et al (46) | 2011 | Retrospective          | Rotterdam         | 33                     | 25.0–31.0  | 5.88          | 79.0         | 96.0        | 0.913        | Yes            | No                | IBC               |
| Li L et al (22)  | 2010 | Cohort                 | Rotterdam         | 47                     | 17.0–25.0  | 8.00          | 61.7         | 70.0        | 0.564        | Yes            | No                | DSL               |
| Hart et al (43)  | 2010 | Prospective cohort     | Rotterdam NIH    | 64                     | 14.5–17.6  | 4.20          | 53.1         | 69.8        | 0.64         | No             | No                | IBC               |
| Pigny et al (23) | 2006 | Prospective cohort     | Rotterdam         | 73                     | 22.0–36.4  | 8.40          | 67.0         | 92.0        | 0.851        | Yes            | No                | IBC               |
Age matched PCOS/Controls

S. AMH was almost 4-fold higher in PCOS cf non-PCOS [median (25th to 75th percentile), 8.71 ng/mL (5.29–14.09 ng/mL) vs 2.36 ng/mL (1.52–4.24 ng/mL)].

For a Cutoff value of AMH of 4.7 ng/mL. The AUC was high [0.87, 95% CI 0.83, 0.92] suggesting that AMH is a good diagnostic test of PCOS.

Specificity & Sensitivity in diagnosing PCOS in symptomatic women by using AMH were 79.4% & 82.8%, resp.

Adding Age to AMH in the predictive model did not change the cutoff value of AMH.
Limitations

- Adolescents with PCOS - higher AMH levels, thresholds set at 30 pmol/L, similar to range in older PCOS. (Pinola et al 2014).

- Chilian study- AMH 60 pmol/l (8.5ng/ml) (with IOT assay) to diagnose PCOM in adolescents with regular menses- sensitivity & specificity of 64 & 90% (auc ROC curve < 0.873, CI 0.782 – 0.963) (Villarroel et al., 2011).

- For Australian adolescents with the same assay (area under the ROC curve < 0.67, CI 0.60 – 0.75).

- S.AMH concentrations were a questionable surrogate marker of PCOM in adolescents. (Dewailly et al 2014).
AMH & PCOS phenotypes

- AMH plays a pathophysiological & diagnostic role
- Cut off for diagnosis - 5ng/ml, OHSS -3.7ng/ml

| Phenotype 1 (ANOV + HA + PCOM) - highest LH, A, AMH (9.27 ± 8.17 ng/mL, P < .05 cf grp 2 & n controls) | Phenotype 2 (ANOV + HA), were hirsute, intermediate FAI value, low ov volume & < AMH levels (4.05 ± 4.12 ng/mL) | Phenotype 3 (HA + PCOM)- intermediate state of HA and slightly augmented AMH levels (5.87 ± 4.35 ng/mL). | Phenotype 4 (ANOV + PCOM)- resembled controls, except for > ov volume & AMH levels (7.62 ± 3.85 ng/mL; P < .05) |

Heterogeneity of association between >AMH levels, n PCOS phenotypes. Romualdi D 2015
Improving accuracy
Adding age, HA markers, LH

- Homburg et al 2013 - AMH + LH. LH>6 was added to this it would predict 85% of women with PCOS.
- Lauritson et al 2014 - age-specific AMH Z-score could be substituted for PCOM. An acceptable sensitivity & specificity was found at AMH Z-score of -0.2.
- Kim et al 2017 - A multivariate model including AMH (cutoff 6.26 ng/mL, auc .788) together with SHBG & total T exhibited 93.4% predictive power for diagnosing PCOS.

Table 1 Details of the 215 participants expressed as means (± SD). Between-group differences calculated by one way ANOVA or Kruskal–Wallis with a post hoc test, Bonferroni or Tamhane’s T2, depending on the distribution of the data.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age</th>
<th>BMI</th>
<th>FSH (IU/l)</th>
<th>LH (IU/l)</th>
<th>AMH (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>90</td>
<td>32.5</td>
<td>24.8</td>
<td>6.3 (2.0)</td>
<td>4.9 (3.0)</td>
<td>23.6 (15.0)</td>
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<tr>
<td>PCOM</td>
<td>35</td>
<td>32.1</td>
<td>24.7</td>
<td>5.6 (1.4)</td>
<td>5.3 (3.0)</td>
<td>52.2* (35.0)</td>
</tr>
<tr>
<td>PCOS</td>
<td>90</td>
<td>31.6</td>
<td>24.9</td>
<td>5.1* (1.4)</td>
<td>8.8* (5.2)</td>
<td>77.6*** (61.0)</td>
</tr>
</tbody>
</table>

AMH, anti-Müllerian hormone; PCOM, polycystic ovary morphology; PCOS, polycystic ovary syndrome.

*P < 0.001 versus control.

**P < 0.05 versus PCOM.
Conclusion

Till AMH assay is standardized and a cut-off level is established for adolescents and adults it is appropriate to use this marker in association with FNPO or where it is difficult to get a correct FNPO.
Proposed Classification

>19FNPO, AMH >35pmol/L

Table III: Adaptation of the previous classifications for the diagnosis of PCOS, proposing an excessive FN of >19 or serum AMH concentration >35 pmol/l or >5 ng/ml as a surrogate when either oligo-anovulation or HA is missing.

<table>
<thead>
<tr>
<th>Oligo-anovulation</th>
<th>Clinical and/or biological HA</th>
<th>FN &gt; 19 and/or serum AMH&gt; 35 pmol/l (5 ng/ml)</th>
<th>Diagnosis</th>
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<td>PCOS</td>
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<td>Normal woman with PCOM^c</td>
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<td>Idiopathic anovulation</td>
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<td>+</td>
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<td>Idiopathic hyperandrogenism</td>
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</tbody>
</table>

As with the previous classifications, other causes of oligo-anovulation and/or HA must be excluded before applying this classification.

^aTo be used preferentially.

^bNot necessary for the diagnosis.

^cConsider the risk for OHSS.

(Dewailly et al 2011)
Thank you