PCOCheck AMH ELISA: A Clinical Case Report to Resolve Mis-Matched Antral Follicle Counts (AFC) to Serum AMH.*

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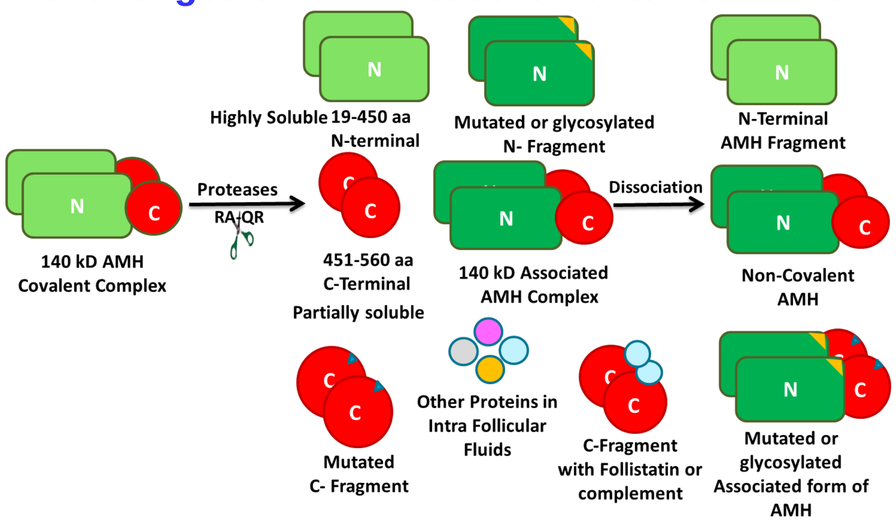
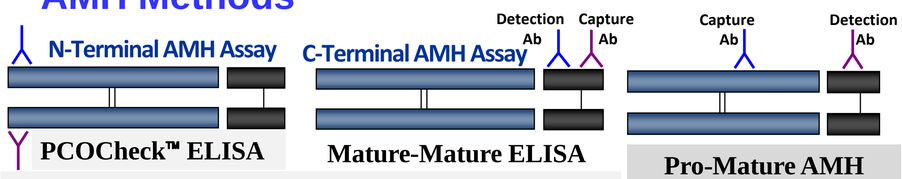
INTRODUCTION

At times clinicians wonder why a subject's Anti-Mullerian Hormone (AMH) does not correlate to the antral follicle count (AFC). AMH is secreted as a full-length protein and undergoes proteolytic cleavage at amino acid 451 to become biologically active. Additional proteolytic processing may take place at aa229. This processing, which may differ between individuals with different clinical conditions, exposes new antigenic sites which affect AMH measurements. Moreover, AMH epitopes might be masked by protein interaction in the circulation.

Challenges of AMH Measurements: Isoforms



AMH Methods



- PCOCheck AMH ELISA uses a two-sided Linear Epitope antibody with binding epitope **away from the glycosylation sites.**
- Assay designed to avoid antibody **binding to AMH mutation sites.**
- No interference to **Biotin or Follistatin in sample.**
- Very precise and clinically accurate results (**correlates to Antral Follicle Count, R>0.89**).
- Excellent sample stability. **Sample shipping and storage at ambient temperature for 72 hours**.

Pro-Mature AMH ELISA

AMH Gen-II ELISA/ BCI-Access/ Roche ECL uses conformational epitope antibodies, Ansh Labs US AMH ELISA uses linear epitope antibodies (Capture 358-369aa, Detection 491-502aa)

Clinical Cases:

Subject JE: Woman, Age 24 years, ovulatory cycle, facial acne, but no hirsutism, BMI 24 kg/m2, Testosterone 27 ng/mL, DHEAS 64 mcg/dL, Prolactin 1.1 ng/mL, Thyroid function tests normal, hCG negative, Progesterone 12 ng/mL, FSH 6.6 IU/L, LH 6.7 IU/L, Estradiol 51 pg/mL, Inhibin B 139 pg/mL, Ovarian volumes 8 and 6 mL, AFC 15 per ovary.

Subject 4660: Mediterranean woman, Age 24.57 years, BMI 24.3 kg/m2, PCOS by Rotterdam criteria, Follicle number per ovary (FNPO) L=22, R=37, Labs: LH 15, FSH 3.4, E2 465 pg/ml, Inhibin B 41.0 pg/ml, Hyperandrogenic, Oligomenorrhoea.

Subject 3063: Non-Hispanic Caucasian women, Normal weight, PCOS characterized by NIH criteria.

Subject 3028: Caucasian woman, Age 29.5 years, BMI 22.3 kg/m2, PCOS by Rotterdam criteria, FNPO L=15, R=19, Labs: LH 5, FSH 6.7, E2 250 pg/mL, Inhibin B 160.6 pg/mL, Oligomenorrhoea, Biochemical Hirsutism, PCOS Phenotype-A.

Subject 7328: Caucasian woman, BMI 41.8 kg/m2, PCOS by Rotterdam criteria, FNPO L=22, R=37, Labs: LH 17, FSH 8.0, E2 249 pg/mL, Inhibin B 76.48 pg/mL, Oligomenorrhoea, Hyperandrogenic, PCOS Phenotype-A.

RESULTS	Pro-Mature AMH ELISA (ng/mL)	Mature-Mature AMH ELISA (ng/mL)	PCOCheck™ AMH ELISA (ng/mL)
Pro + Mature (Associated)	✓	✓	✓
N-Fragment	✗	✗	✓
C- Fragment	✗	✓	✗
Subject-3063	**0.01**	**0.056**	**5.73**
Subject-JE	**0.1**	**0.14**	**4.99**
Subject-4660	**0.11**	**0.07**	**6.95**
Subject-3028	**0.06**	**2.61**	**6.37**
Subject-7328	**0.07**	**3.41**	**11.11**

DISCUSSION

We would have predicted a mid to high AMH levels in all these subjects. However, AMH levels were repeatedly found to be low (≤ 0.11 ng/mL) using the pro-Mature AMH ELISA and Mature-Mature AMH ELISA (≤ 3.42 ng/mL). All subjects AMH results matched the expected AMH levels with respects to AFC by PCOCheck AMH ELISA.

Subject 3063: Homozygous AMH gene mutation, RS16417628, 515Valine to 515Alanine. The recombinant AMH preparation with 515Alanine is not detected in the pro-mature assay.

Subject JE & 4660: The patient may have mutation in the C-terminus. This may result in circulating C-truncated AMH isoform in plasma. AMH in circulation here is completely fragmented and has unaltered N terminal-fragment.

Subject 3028 & 7328: AMH measurements suggest that the circulating pro-mature AMH form is completely dissociated. Glycosylation or interfering protein interaction may change the 3-D structure that restricts the re-association. This results in complete loss of AMH measurement by a pro-mature ELISA and a partial loss by the C-terminus, Mature-Mature ELISA.

CONCLUSIONS

Tests such as PCOCheck ELISA, targeted to specific regions of AMH will provide clinicians better tools for patient management. Such tests will also help resolve the observed AMH discrepancies with AFC using legacy AMH tests. PCOCheck ELISA can be a valuable tool for assessment of ovarian reserve or in polycystic ovary syndrome.

ACKNOWLEDGEMENTS

Authors would like to thank Professor Joop Laven, Erasmus, MC., Professor Daniel Dumesic, UCLA., and Ansh Labs PCOCheck team for their scientific support.