Associations of different molecular forms of antimüllerian hormone and biomarkers of polycystic ovary syndrome and normal women

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Objective: To study different antimüllerian hormone (AMH) isoforms in women with polycystic ovary syndrome (PCOS) and healthy regularly cycling women and to investigate whether levels of AMH isoforms combined with baseline characteristics can predict PCOS.
Design: Cross-sectional study.
Setting: Fertility clinic.
Patient(s): Eighty-eight women with PCOS and 24 age- and body mass index (BMI)–matched normal control subject women recruited from April 2010 to February 2013. AMH isoforms were analyzed in biobanked serum samples collected at Holbaek Fertility Clinic, Denmark. All study participants went through a baseline examination including gynecologic history, objective examination, transvaginal ultrasound, and blood sampling. Each woman was characterized by measurement of total T, free T, SHBG, A, DHEAS, FSH, LH, E_2, PRL, TSH, serum insulin, plasma glucose, and C-peptide.
Interventions(s): None.
Main Outcome Measure(s): Serum levels of three different AMH isoforms.
Result(s): Levels of AMH measured with each of three AMH ELISAs were significantly higher in women with PCOS compared with control women. The ratio between AMH isoforms showed significant associations with metabolic parameters (BMI, SHBG, C-peptide, cholesterols, triglycerides, and the modified homeostasis-model assessment). Prediction of PCOS showed a high precision with areas under the receiver operating characteristic curve of 97% when AMH measurements were combined with androgens and BMI.
Conclusion(s): Three ELISAs detecting different parts of the AMH molecule all detected significantly higher levels in women with PCOS compared with control women. The relative distribution of AMH isoforms did not differ between women with PCOS and control women. AMH isoforms alone and in combination with baseline characteristics predicted PCOS with close to 100% area under the receiver operating characteristic curve. (Fertil Steril® 2019;112:149–55. ©2019 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: PCOS, AMH assays, prediction of PCOS, AMH isoforms

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Antimüllerian hormone (AMH) is exclusively produced by granulosa cells and has gained widespread clinical use to monitor the ovarian reserve and follicular activity, to diagnose polycystic ovary syndrome (PCOS) (1, 2), and to predict response to ovarian stimulation. AMH is accumulated in the follicular fluid of especially smaller antral follicles and has been shown to exhibit a maximum in follicles around the time of selection at a diameter of 7–9 mm (3). AMH is secreted as a 140-kD homodimeric precursor, consisting of two identical monomers. Each monomer consists of two parts: 1) a 25-kD mature C-terminal region, which becomes bioactive after proteolytic cleavage and binds the AMH receptor II (AMHRII), inducing intracellular SMAD and downstream signaling (4); and 2), a proregion, which is important in AMH synthesis and extracellular transport. AMH is susceptible to proteolytic cleavage in various different positions within the molecule, and it has recently been demonstrated in immature granulosa cells that a number of different AMH isoforms can be detected by Western blot analysis (5). The proteolytic cleavage exposes new antigenic sites that affects AMH measurements and may induce protein interactions in the circulation that may affect measurements (6). The processing of AMH takes places within the follicle but also when AMH is released to circulation, and the isoform profile may differ from person to person.

PCOS is a common endocrine condition in which levels of AMH in circulation are increased due to the augmented number of follicles at the stage of maximal AMH production (i.e., 5–9 mm) and the lack of dominant follicle selection, but some studies have also suggested an enhanced AMH production within each follicle (7). Recent studies have found an increased cleavage of AMH in the follicular fluid of women with PCOS (8) and a higher proportion of receptor-competent AMH in the serum of women with PCOS (9). These findings suggest that a specific AMH isoform profile could characterize women with PCOS or that specific isoforms of AMH may provide additional diagnostic input to the diagnosis of PCOS.

The aim of the present study was to use three highly specific AMH assays that detect different isoforms of AMH in a group of normal women and in a group of women with PCOS who were characterized by a detailed profile of hormonal and baseline levels of markers normally used in the diagnosis of PCOS to determine whether diagnostic precision could be increased.

MATERIALS AND METHODS

Study Subjects

Study participants were recruited consecutively from April 2010 to February 2013 as part of the PICOLO study (10). The study was approved by the Danish Scientific Ethical Committee (SJ-156). All participants gave informed consents before inclusion in the study. Sixty-two women with PCOS, 26 metformin-treated women with PCOS, and 24 regularly cycling women without known disease (control subjects) were included. Exclusion criteria were diabetes type 1 or 2, impaired thyroid, renal, or hepatic function, congenital adrenal hyperplasia, endometriosis, poor ovarian reserve, premature ovarian failure, hypothalamic amenorrhea, or age >36 years.

According to the Rotterdam Consensus Criteria (American Society for Reproductive Medicine/European Society for Human Reproduction and Embryology Consensus Work Group on PCOS, 2004) (11), the diagnosis of PCOS was based on the presence of at least two of three following criteria: oligomenorrhea/amenorrhea, hyperandrogenaemia, and hyperandrogenism and polycystic ovaries.

All of the women receiving metformin had been treated for ≥4 months before baseline. The daily dosage was 1,500 mg and average treatment time before entering the study was 9 months.

Baseline examination involved focused gynecologic history and objective examination including transvaginal ultrasound of the ovaries and uterus. Blood samples were drawn after an overnight fast at 8:30–9:00 a.m. on cycle day 3–5 for regularly cycling women and on a random day for anovulatory women.

The biochemical tests were performed in the central biochemistry department of the hospital using routine assays. The specific assays and according reference intervals for normal values are listed in Supplemental Table 1 (Supplemental Tables 1–4 are available online at www.fertstert.org).

Modified homeostasis-model assessment (HOMA) was calculated as: 20/(fasting C-peptide × fasting glucose) (12).

AMH Assays

AMH is synthesized as two 70-kD nonactive homodimer precursor molecules covalently linked. After proteolytic cleavage AMH becomes biologically active. The biologically active C-terminal part of AMH may or may not remain associated with the biologically inactive N-terminal part. The N- and C-terminal complex also possesses biologic activity. Thus, at least four different forms of AMH may in theory be present in circulation: 1) nonactive AMH-precursor dimer (covalent); 2) active pro–mature dimer (associated); 3) nonactive N-fragment proregion; and 4) active mature C-terminal.

The proteolytic processing of AMH takes place at different sites in the molecule (i.e., amino acid at position 229 and 451 [13–15]) and introduces conformational changes which expose new antigenic determinants and change its affinity to antibodies. The mechanisms that govern AMH processing in circulation is known to only a limited extent and is illustrated by the presence of different molecular forms of AMH across the menstrual cycle (16); in addition it is likely that individual variations exist. The samples were analyzed with the use of ELISA assays targeting three different compositions of AMH according to the localization of the epitope (Supplemental Fig. 1, available online at www.fertstert.org).

The AMH assays were performed by Anshlabs, Webster, Texas, with the use of Anshlabs ELISAs AL-124, AL-132, and AL-145. The AMH ELISA method AL-124 (24/32) has been previously validated in a methodologic article by Robertson et al. (17), and the AL-132 uncleaved AMH ELISA (24/32, sample pretreated with sodium dodecyl sulfate) was

TABLE 1

Baseline parameters and AMH isoforms in women with PCOS and healthy regularly cycling women (control).

Parameter	PCOS	Control	*P* value
n	88	24	
Age (y)	28.2 ± 0.42	28.4 ± 0.86	NS
BMI (kg/m^2)	26.8 ± 0.55	24.8 ± 0.82	NS
No. of antral follicles	23.9 ± 0.89	15.8 ± 1.11	< .0001
No. of menstrual bleedings per year	4.9 ± 0.45	11.3 ± 0.50	< .0001
Total T (nmol/L)	2.34 ± 0.11	1.16 ± 0.08	< .0001
Free T (nmol/L)	0.0382 ± 0.0024	0.0177 ± 0.0018	< .0001
SHBG (nmol/L)	65.8 ± 4.1	66.7 ± 5.4	NS
A (nmol/L)	7.4 ± 0.3	4.4 ± 0.3	< .0001
DHEAS (μmol/L)	5811 ± 305	4674 ± 465	NS
FSH (IU/L)	5.8 ± 0.2	6.2 ± 0.4	NS
LH (IU/L)	12.1 ± 1.7	5.8 ± 0.5	.05
LH/FSH	1.9 ± 0.1	1.1 ± 0.1	.0006
E_2 (nmol/L)	0.25 ± 0.02	0.21 ± 0.04	NS
PRL (IU/L)	229 ± 12	244 ± 23	NS
TSH (IU/L)	2.0 ± 0.1	2.1 ± 0.2	NS
Serum insulin (μU/mL)	74.0 ± 6.1	53.5 ± 4.1	NS
Plasma glucose (mmol/L)	5.2 ± 0.1	5.1 ± 0.1	NS
C-Peptide (nmol/L)	0.67 ± 0.04	0.58 ± 0.03	NS
Modified HOMA	7.74 ± 0.53	7.37 ± 0.55	NS
HOMA-IR	17.2 ± 1.6	11.7 ± 1.1	NS
ALT (U/L)	25 ± 2	22 ± 5	NS
Total cholesterol (mmol/L)	4.7 ± 0.1	4.5 ± 0.2	NS
LDL cholesterol (mmol/L)	2.8 ± 0.1	2.6 ± 0.2	NS
HDL cholesterol (mmol/L)	1.5 ± 0.04	1.6 ± 0.1	NS
Triglycerides (mmol/L)	1.0 ± 0.07	0.7 ± 0.04	NS
Total (pro-mature) 24/32 AMH ELISA (pg/mL)	10,790 ± 711	4,056 ± 557	< .0001
10/24 AMH ELISA (pg/mL)	6,217 ± 337	2,479 ± 349	< .0001
24/32 uncleaved AMH ELISA (pg/mL)	417 ± 44	145 ± 28	< .0001
Ratio of 24/32 uncleaved AMH to 10/24 AMH (×100)	6.0 ± 0.3	5.11 ± 0.4	NS
Ratio of 24/32 uncleaved AMH to 24/32 total pro–mature AMH (×100)	3.4 ± 0.1	3.0 ± 0.2	NS
Ratio of 10/24 AMH to 24/32 total pro–mature AMH (×100)	60.2 ± 1.1	60.8 ± 1.3	NS

Note: Data are presented as mean ± SEM. *P* values were estimated by means of one-sided analysis of variance. A = androstendione; ALT = alanine transaminase; AMH = antimüllerian hormone; BMI = body mass index; HOMA = homeostasis-model assessment; HDL = high-density lipoprotein; HOMA-IR = homeostasis-model assessment of insulin resistance; LDL = low-density lipoprotein.

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previously validated in methodologic article by Mamsen et al. (18). The AL-145 (10/24) ELISA uses a pair of monoclonal antibodies both directed to the N-terminal. The assay recognizes pro-AMH, AMH-N/C, and dissociated profragment, but not the mature fragment. The interassay variations for the 24/32 and 10/24 ELISAs on two serum pools at 70 and 221 pg/mL run over 15 runs were 6.4% and 6.8% and 4.1% and 5.0%, respectively. The interassay variations for the AL-132 ELISA on two serum pools at 70 and 213 pg/mL over eight runs were 4.5% and 6.4%, respectively.

Statistical Analysis

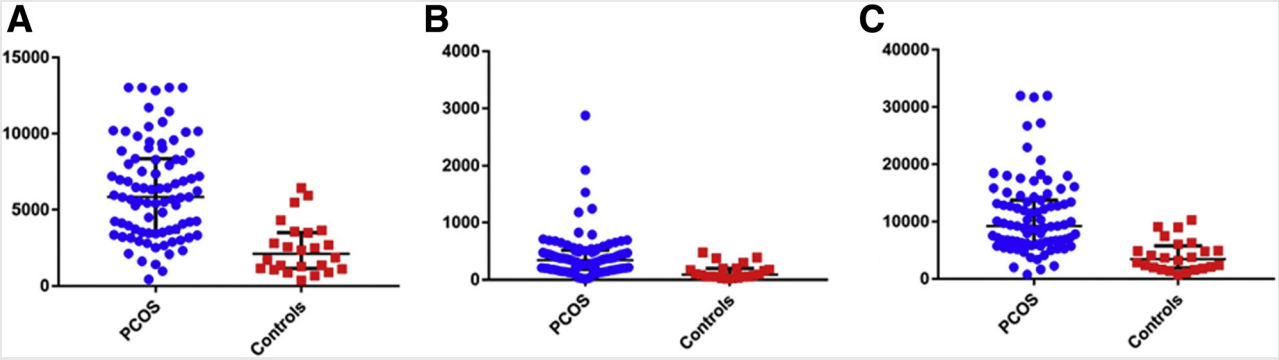
Statistical analyses were performed with the use of Graphpad Prism 7. Groups were compared with the use of one-way analysis of variance or Student *t* test where appropriate. Correlations between AMH isoforms and baseline parameters were calculated as Spearman correlation. receiver operating characteristic (ROC) curves were generated by means of maximum likelihood estimation of a binormal ROC curve from continuously distributed test results. ROC curves were calculated with the use of R version 3.4.3. The parameters androgen, body mass index (BMI), DHEAS, and antral follicle count (AFC) were chosen to maximize the discriminative value, because all of these are known indicators of PCOS. A *P* value of $< .05$ was considered to be significant.

RESULTS

This study included 62 women with PCOS, 26 women with PCOS receiving metformin, and 24 age- and BMI-matched normal control women. The PCOS group and the PCOS group receiving metformin were similar in terms of different AMH isoforms and other clinical parameters measured (Supplemental Table 2). Therefore, we decided to combine the two PCOS groups. As expected, the combined PCOS group differed significantly from the normal control group in number of antral follicles, number of menstrual bleedings per year, levels of total T and A, levels of free T, and levels of LH and the ratio of LH/FSH, whereas a number of other hormonal and biochemical characteristics did not differ (Table 1).

Each of the three AMH ELISAs showed highly significant differences between the group of control women and women

FIGURE 1



Scatter plots of antimüllerian hormone (AMH) values (pg/mL) determined with the use of three ELISAs detecting different epitopes. Data shown as median with interquartile range. **(A)** 10/24 AMH; **(B)** 24/32 uncleaved AMH; **(C)** total pro–mature 24/32 AMH. PCOS = polycystic ovary syndrome.

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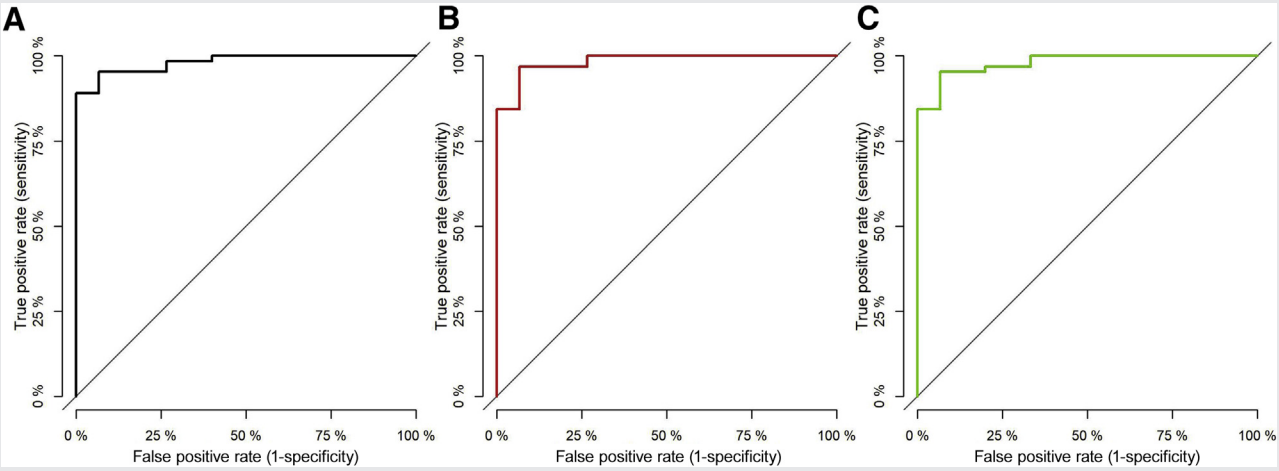
with PCOS (Table 1; Fig. 1). In contrast, the ratios between different AMH isoforms did not overall differ between the two groups (Table 1).

Combining the two groups of women, the associations between the different AMH isoforms and the baseline parameters showed highly significant differences for a number of parameters. Although the level of significance differed only slightly between the different assays for any given parameter, the 24/32 uncleaved AMH assay showed significant associations with levels of SHBG and C-peptide (borderline), which was not observed with the two other assays (Supplemental Table 3).

The prediction of the PCOS condition was high for each AMH assay in itself, showing ROC curves with areas under the curves of 90%–92% (Fig. 2; Supplemental Table 4). Combinations with various other parameters monitored increased the area under the ROC curve to as high as 97%–98% (Fig. 2; Supplemental Table 4). It is noticeable that based on circulatory androgens, BMI, and AMH measurements, the ROC area reached 97% without measurement of AFC.

In each woman the ratio between the different AMH measurements was calculated and associated with the baseline characteristics of the two groups (Table 2). Combining the two groups of women, the ratio between AMH levels measured according to 24/32 uncleaved AMH in relation to the 10/24 AMH assay and the ratio between AMH levels measured according to 24/32 uncleaved AMH in relation to the 24/32 pro–mature AMH assay showed significant associations with a number of baseline characteristics. In contrast, the ratio between AMH levels measured with the 10/24 AMH assay in relation to the 24/32 total promature assay showed only a few significant associations with the baseline characteristics (Table 2).

FIGURE 2



Receiver operating characteristic (ROC) curves of prediction of PCOS with the use of body mass index, total androgen level, and total number of antral follicles combined with measurement of AMH using one of three different ELISAs. **(A)** 10/24 AMH: area under the curve (AUC) 96.7%, confidence interval (CI) 93.5%–99.9%; **(B)** 24/32 uncleaved AMH: AUC 97.0%, CI 93.8%–100%; **(C)** 24/32 total pro–mature AMH: AUC 96.9%, CI 93.8%–100%. Abbreviations as in Figure 1.

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TABLE 2

Associations between baseline parameters and different ratios of AMH isoforms.

Parameter	Ratio of 24/32 uncleaved AMH to 10/24 AMH	Ratio of 24/32 uncleaved AMH to 24/32 pro–mature AMH	Ratio of 10/24 AMH to 24/32 total pro–mature AMH
BMI	0.0015 (−0.30)	0.0018 (−0.30)	NS
Antral follicle count	0.05 (0.26)	0.0002 (0.35)	NS
No. of menstruations per year	0.09 (−0.16)	NS	0.01 (0.24)
Waist circumference (cm)	0.0028 (−0.29)	0.0023 (−0.29)	NS
Total T (nmol/L)	0.0019 (0.30)	0.0007 (0.33)	NS
Free T (nmol/L)	NS	NS	NS
SHBG (nmol/L)	0.0025 (0.30)	0.0034 (0.29)	NS
A (nmol/L)	0.09 (0.17)	0.06 (0.19)	NS
DHEAS (μmol/L)	NS	NS	NS
FSH (IU/L)	0.07 (−0.18)	NS	NS
LH (IU/L)	NS	NS	NS
LH/FSH	NS	0.04 (0.21)	NS
PRL (IU/L)	NS	NS	NS
TSH (IU/L)	NS	NS	NS
Glucose (mmol/L)	NS	NS	NS
Insulin (μU/mL)	NS	NS	NS
C-Peptide (nmol/L)	0.0038 (−0.28)	0.0059 (−0.27)	NS
Total cholesterol (mmol/L)	NS	NS	NS
LDL cholesterol (mmol/L)	0.09 (−0.17)	NS	0.04 (0.20)
HDL cholesterol (mmol/L)	0.0044 (0.27)	0.006 (0.27)	0.04 (−0.20)
Triglycerides (mmol/L)	0.0008 (−0.32)	0.0051 (−0.27)	0.02 (0.22)
Modified HOMA	0.0039 (0.28)	0.0043 (0.27)	NS
HOMA-IR	NS	NS	NS

Note: Spearman correlation. Data presented as *P* (two-tailed) and *r*. Abbreviations as in Table 1.

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DISCUSSION

This study clearly confirms and extends that AMH is a strong predictor of PCOS. Each of the three different assays used in the present study were strongly associated with the PCOS condition with almost equal efficacy. Furthermore, this study shows on a relatively large well documented dataset that combining information from a single blood sample including AMH and androgens in combination with BMI collectively raises the area under the ROC curve to 97%, demonstrating that parameters monitored in circulation and BMI are sufficient to provide a very reliable prediction of the PCOS condition. There was almost no difference in the predictive power of each of the AMH assays tested in the present study, although a slightly better prediction was obtained with the use of the 24/32 total pro–mature AMH ELISA alone and in combination with other parameters. The clinical implication of this information is that these parameters should be available to the physician before seeing the patient. Monitoring of AFC with the use of ultrasound could be used as a confirmatory test during consultation and for assessment of potential pathologies on the uterus, salpinges, and ovaries.

A number of other combinations of parameters from this well characterized cohort of patients also reaches areas under the ROC curves well above 90% when including AMH measurements, showing an excellent predictive power to distinguish women with PCOS from normal women. However, neither one of the three AMH ELISAs stands out, showing almost identical ROC curves. This may reflect that cleavage of AMH in circulation is of limited importance and that the profile released from follicles to some extend persists in circulation.

The high precision in identifying PCOS women by means of AMH levels in circulation serves as a validation test in the present study that needs to be confirmed on an independent test sample set to show the full potential in diagnosis of PCOS. We are currently collecting a test sample set to validate these preliminary results.

The ratios between the different AMH assays did not improve the prediction of the PCOS condition. This suggests that processing of AMH in circulation is similar between normal women and women with PCOS. The ratios of the AMH isoforms did not differ between PCOS and control subjects, meaning that the relative distribution of inactive (uncleaved) AMH and active (cleaved) AMH were the same in PCOS and control subjects. In contrast, Pankhurst et al. (9) found that the ratio between pro-AMH (inactive) and receptor-competent AMH was decreased in PCOS women, indicating a higher conversion of non–receptor-competent AMH to receptor-competent AMH in women with PCOS; however, that study involved a limited number of patients. The AMH isoforms tested in the Pankhurst et al. study were not similar to the ones tested in the present study, and furthermore the study subjects were not matched by age and BMI and the women with PCOS were significantly younger than the control women and had significantly higher BMI, waist circumference, and HOMA insulin resistance. The lower ratio of pro-AMH (inactive) to receptor-competent AMH in PCOS found in the Pankhurst et al. study might reflect metabolic disturbances in the PCOS group and not the PCOS condition per se. Our finding of a correlation between uncleaved AMH–10/24 AMH and uncleaved AMH–24/32 AMH and several metabolic parameters indicated that this might

actually be the case. Our findings indicate that, regardless of PCOS, the relative distribution of inactive AMH to active AMH is associated with metabolic parameters. Increased BMI, waist circumference, C-peptide, low-density lipoprotein cholesterol and triglycerides, and decreased high-density lipoprotein cholesterol, SHBG, and modified HOMA were associated with a lower ratio of uncleaved (inactive) AMH to cleaved (active) AMH in the present study, which is in line with the findings in the Pankhurst et al. study (9). Some studies have found lower levels of total AMH in obese PCOS women (19), whereas others have found no correlation between AMH and BMI (20).

The three AMH assays used in the present study use different epitopes on the AMH molecule, and the concentrations monitored is dependent on antibody affinity for the specific pair of antibodies used. The same recombinant AMH calibrator was used in all assays, and epitope exposure on the recombinant AMH molecule and the antibody combination used affected the concentration monitored and explains why the monitored concentration of AMH is approximately one-half with the specific antibody combination of 10 and 24 compared with 24 and 32. In addition, some epitopes on the AMH molecule are conformational and become exposed only after the molecule is processed proteolytically. It is therefore difficult to directly compare between assays from different manufactures, especially if some of the antibodies used are conformational, which may not be information that is readily available.

In conclusion, this study showed that AMH concentrations in combination with well known clinical parameters of PCOS increased the precision of diagnosis substantially. However, each of the three different AMH assays showed almost similar efficacy and no one of them proved better than the others, showing that each one of them qualifies to be used clinically.

REFERENCES

1. Lauritsen MP, Bentzen JG, Pinborg A, Loft A, Forman JL, Thuesen LL, et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-müllerian hormone. Hum Reprod 2014;29:791–801.
2. Sathyapalan T, Al-Qaissi A, Kilpatrick ES, Dargham SR, Atkin SL. Anti-müllerian hormone measurement for the diagnosis of polycystic ovary syndrome. Clin Endocrinol (Oxf) 2018;88:258–62.
3. Jeppesen JV, Anderson RA, Kelsey TW, Christiansen SL, Kristensen SG, Jayaprakasan K, et al. Which follicles make the most anti-müllerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. Mol Hum Reprod 2013;19:519–27.
4. di Clemente N, Jamin SP, Lugovskoy A, Carmillo P, Ehrenfels C, Picard J-Y, et al. Processing of anti-müllerian hormone regulates receptor activation by a mechanism distinct from TGF-β. Mol Endocrinol 2010;24:2193–206.
5. Mamsen LS, Petersen TS, Jeppesen JV, Møllgård K, Grøndahl ML, Larsen A, et al. Proteolytic processing of anti-müllerian hormone differs between human fetal testes and adult ovaries. Mol Hum Reprod 2015;21:571–82.
6. Kawagishi Y, Pankhurst MW, Nakatani Y, McLennan IS. Anti-müllerian hormone signaling is influenced by follistatin 288, but not 14 other transforming growth factor beta superfamily regulators. Mol Reprod Dev 2017;84:626–37.
7. Pellatt L, Hanna L, Brincat M, Galea R, Brain H, Whitehead S, et al. Granulosa cell production of anti-müllerian hormone is increased in polycystic ovaries. J Clin Endocrinol Metab 2007;92:240–5.
8. Pierre A, Racine C, Rey RA, Fanchin R, Taieb J, Cohen-Tannoudji J, et al. Most cleaved anti-müllerian hormone binds its receptor in human follicular fluid but little is competent in serum. J Clin Endocrinol Metab 2016;101:4618–27.
9. Pankhurst MW, Shorakae S, Rodgers RJ, Teede HJ, Moran LJ. Efficacy of predictive models for polycystic ovary syndrome using serum levels of two antimüllerian hormone isoforms (proAMH and AMH N,C). Fertil Steril 2017;108:851–7.e2.
10. Aziz M, Naver KV, Wissing MLM, Mikkelsen AL, Nilas L, Skouby SO. Polycystic ovary syndrome: infertility, cardiovascular, metabolic and obstetrical risks, laboratory and clinical outcomes—the PICOLO study. Gynecol Endocrinol 2012;28:253–8.
11. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41–7.
12. Ohkura T, Shiochi H, Fujioka Y, Sumi K, Yamamoto N, Matsuzawa K, et al. 20/(fasting C-peptide × fasting plasma glucose) is a simple and effective index of insulin resistance in patients with type 2 diabetes mellitus: a preliminary report. Cardiovasc Diabetol 2013;12:21.
13. Pepinsky RB, Sinclair LK, Chow EP, Mattaliano RJ, Manganaro TF, Donahoe PK, et al. Proteolytic processing of müllerian inhibiting substance produces a transforming growth factor-beta–like fragment. J Biol Chem 1988;263:18961–4.
14. Donahoe PK. Müllerian inhibiting substance in reproduction and cancer. Mol Reprod Dev 1992;32:168–72.
15. Nachtigal MW, Ingraham HA. Bioactivation of müllerian inhibiting substance during gonadal development by a kex2/subtilisin–like endoprotease. Proc Natl Acad Sci U S A 1996;93:7711–6.
16. Pankhurst MW, Chong YH. Variation in circulating antimüllerian hormone precursor during the periovulatory and acute postovulatory phases of the human ovarian cycle. Fertil Steril 2016;106:1238–43.e2.
17. Robertson DM, Kumar A, Kalra B, Shah S, Pruysers E, Vanden Brink H, et al. Detection of serum antimüllerian hormone in women approaching menopause using sensitive antimüllerian hormone enzyme-linked immunosorbent assays. Menopause 2014;21:1277–86.
18. Mamsen LS, Munthe-Fog L, Petersen TS, Jeppesen JV, Møllgård K, Grøndahl ML, et al. Reply: methodological considerations in measuring different AMH splice forms using ELISA: validity of proAMH ELISA. Mol Hum Reprod 2016;22:374–5.
19. Lefebvre T, Dumont A, Pigny P, Dewailly D. Effect of obesity and its related metabolic factors on serum anti-müllerian hormone concentrations in women with and without polycystic ovaries. Reprod Biomed Online 2017;35:325–30.
20. Simões-Pereira J, Nunes J, Aguiar A, Sousa S, Rodrigues C, Sampaio Matias J, et al. Influence of body mass index in anti-müllerian hormone levels in 951 nonpolycystic ovarian syndrome women followed at a reproductive medicine unit. Endocrine 2018;61:144–8.

Asociaciones de diferentes formas moleculares de hormona antimülleriana y biomarcadores de síndrome de ovario poliquístico y mujeres normales

Objetivo: Estudiar diferentes isoformas de hormona antimülleriana (HAM) en mujeres con síndrome de ovario poliquístico (SOP) y mujeres sanas con ciclos regulares e investigar si los niveles de isoformas de HAM combinados con características basales pueden predecir SOP.

Diseño: Estudio transversal.

Ámbito: Clínica de fertilidad.

Paciente(s): Ochenta y ocho mujeres con SOP y 24 mujeres controles normales pareadas por edad e índice de masa corporal (IMC) reclutadas desde abril de 2010 hasta febrero de 2013. Las isoformas de HAM fueron analizadas en muestras séricas de biobanco recogidas en la Clínica de Fertilidad Holbaek, Dinamarca. Todas las participantes del estudio pasaron por un examen de base incluyendo historia ginecológica, examen objetivo, ecografía transvaginal, y muestra de sangre. Cada mujer fue caracterizada por la medición de T total, T libre, SHBG, A, DHEAS, FSH, LH, E_2, PRL, TSH, insulina sérica, glucosa plasmática, y péptido C.

Intervención(es): Ninguna.

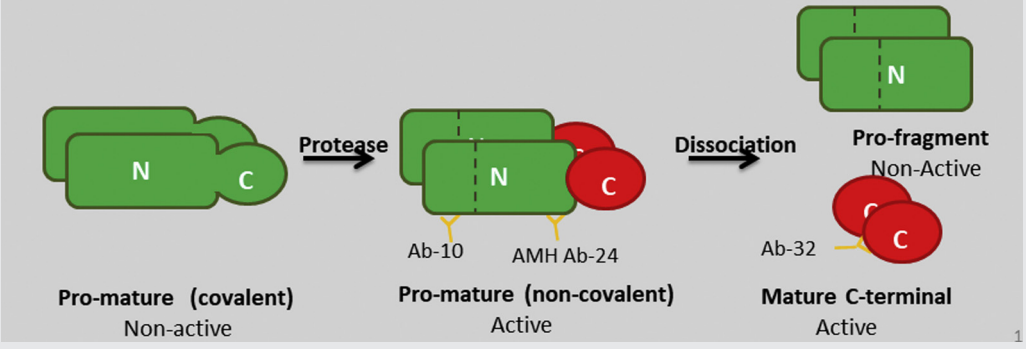
Principal(es) variable(s) de resultado(s): Niveles séricos de tres isoformas diferentes de HAM.

Resultado(s): Niveles de HAM medidos con cada uno de tres ELISAs para HAM fueron significativamente más altos en mujeres con SOP comparados con mujeres controles. La proporción entre isoformas de HAM mostró asociaciones significativas con parámetros metabólicos (IMC, SHBG, péptido C, colesterol, triglicéridos, y la evaluación del modelo de homeostasis modificado). La predicción de SOP mostró una alta precisión con áreas bajo la curva característica operativa del receptor del 97% cuando las mediciones de HAM fueron combinadas con andrógenos e IMC.

Conclusión(es): Tres ELISAs detectando diferentes partes de la molécula de HAM detectaron niveles significativamente más altos en mujeres con SOP comparadas con mujeres controles. La distribución relativa de isoformas de HAM no difirió entre mujeres con SOP y mujeres controles. Las isoformas de HAM solas y en combinación con características basales predijo SOP con un área bajo la curva característica operativa del receptor cercana al 100%.

SUPPLEMENTAL FIGURE 1

T=SDS Treated	24/32 pico AMH (Cat # AL-124)	24/32 T (Cat # AL-132)	*10/24 (Cat # AL-145)
Pro+Mature (Associated)			
Pro-Mature (Covalent)			
Pro Fragment			
Mature Fragment			



Antimüllerian hormone (AMH) isoforms in serum. Diagram showing the processing of AMH and the binding sites of the tested AMH antibodies. Yellow IgG molecules indicate the binding sites of the tested antibodies.

Wissing. AMH isoforms in PCOS and normal women. Fertil Steril 2019.