

Circulating antimüllerian hormone and steroid hormone levels remain high in pregnant women with polycystic ovary syndrome at term

Terhi T. Piltonen, M.D., Ph.D.,^a Paolo Giacobini, Ph.D.,^{b,c} Åsa Edvinsson, M.Sc.,^d Steinar Hustad, Ph.D.,^{e,f} Susanne Lager, Ph.D.,^d Laure Morin-Papunen, M.D., Ph.D.,^a Juha S. Tapanainen, M.D., Ph.D.,^{a,g} Inger Sundström-Poromaa, M.D., Ph.D.,^d and Riikka K. Arffman, Ph.D.^a

^a Department of Obstetrics and Gynecology, PEDEGO Research Unit, Medical Research Center, Oulu University Hospital, University of Oulu, Oulu, Finland; ^b Jean-Pierre Aubert Research Center, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Institut National de la Santé et de la Recherche Médicale, Lille, France; ^c Fédération Hospitalière Universitaire, School of Medicine, University of Lille, Lille, France; ^d Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden; ^e Department of Clinical Science, University of Bergen, Bergen, Norway; ^f Core Facility for Metabolomics, University of Bergen, Bergen, Norway; and ^g Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

Objective: To investigate plasma antimüllerian hormone (AMH) concentration and its relation to steroid hormone levels in pregnant women with polycystic ovary syndrome (PCOS) and controls at term.

Design: Case-control study.

Setting: University-affiliated hospital.

Patient(s): A total of 74 pregnant women at term: 25 women with PCOS (aged 31.6 ± 3.9 years [mean \pm standard deviation], body mass index 24.0 ± 3.9 kg/m², mean gestational length 279 ± 9 days) and 49 controls (aged 31.7 ± 3.3 years, body mass index 24.0 ± 3.3 kg/m², mean gestational length 281 ± 9 days).

Intervention(s): None.

Main Outcome Measure(s): Plasma AMH and steroid hormone levels.

Result(s): Antimüllerian hormone, T, and androstenedione levels were higher in women with PCOS at term compared with controls, whereas estrogen and P levels were similar. The differences were pronounced in women carrying a female fetus. Testosterone and AMH levels correlated positively in both groups, but E₂ levels only in women with PCOS.

Conclusion(s): Pregnant women with PCOS present with elevated AMH and androgen levels even at term, suggesting a hormonal imbalance during PCOS pregnancy. Differences were detected especially in pregnancies with a female fetus, raising the question of whether female pregnancies are more susceptible to AMH and steroid hormone actions. (Fertil Steril® 2019;111:588–96. Copyright ©2018 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)).

El resumen está disponible en Español al final del artículo.

Key Words: Antimüllerian hormone, androgens, polycystic ovary syndrome, pregnancy

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Reprint requests: Terhi T. Piltonen, M.D., Ph.D., University of Oulu, Oulu University Hospital, Medical Research Center, PEDEGO Research Unit, Department of Obstetrics and Gynecology, Aapistie 5, Box 5000, 90014 Oulu, Finland (E-mail: terhi.piltonen@oulu.fi).

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In females, ovarian granulosa cell antimüllerian hormone (AMH) expression can be detected from the second trimester onward until late fertile age (1, 2). Antimüllerian hormone is a transforming growth factor- β family protein that is mainly secreted from developing follicles of 5–8 mm, and secretion extends up to small antral stage (3, 4). Previous studies have demonstrated a role for AMH

in sustaining the ovarian follicle pool by restricting early recruitment of primordial follicles and FSH-dependent preantral follicle growth (4–6). Given that in clinical settings AMH correlates well with the ovarian antral follicle count, it has been used as a marker of ovarian follicle reserve and to guide clinical practice, especially in the IVF setting (2, 7, 8). During pregnancy, levels of circulating AMH decline as a result of ovarian down-regulation and cessation of folliculogenesis brought about by placental steroids, but a rapid postpartum increase can be detected (9). Until recently AMH was considered to be simply a marker of ovarian reserve; however, AMH receptor 2 (AMHR2) expression has been detected in human endometrium and placenta and recently also in GnRH neurons (10–12).

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of fertile-aged women, affecting approximately 10%–18% of the population when Rotterdam criteria are applied (13, 14). According to the consensus, PCOS can be diagnosed if at least two of the following criteria are fulfilled: oligo- or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries (PCOs) (15). Given that women with PCOS have higher AMH levels until their late reproductive years compared with women without PCOS, it has been suggested that high AMH levels could be used as a surrogate for PCOs detected in ultrasonography (2, 16–18). As for phenotypic alterations, levels of AMH are highest in women with PCOS fulfilling all three diagnostic criteria (19, 20). Moreover, *in vitro* granulosa cells from anovulatory polycystic ovaries show higher AMH expression than granulosa cells from ovulatory polycystic ovaries (21). In addition, obesity seems to reduce AMH levels, and in clinical practice high AMH levels are particularly detected in lean women with PCOS showing high LH levels (19). Indeed, a recent study revealed AMHR2 expression in hypothalamic GnRH neurons both in mice and in humans, and AMH was able to modify GnRH-a-neuron pulse frequency and cause alterations in LH pulsatility, leading to a PCOS-like phenotype in mice and raising the question of whether AMH could be involved in the early development of PCOS in humans as well (11).

Even though PCOS is strongly hereditary, known genetic factors explain only a small fraction of the cases (22). Genetic studies specifically focused on AMH or AMHR2 have also failed to unravel the origins of PCOS (23, 24). There is, however, evidence from animal models that gestational hyperandrogenism is able to induce a PCOS-like phenotype in female offspring, although the mechanism behind hyperandrogenism arising during pregnancy remains unsolved (25–27). Interestingly, a recent study in mice showed that high gestational AMH exposure at late pregnancy resulted in a PCOS-like phenotype in female offspring, suggesting a possible role for AMH in the transgenerational pathogenesis of PCOS (28). Antimüllerian hormone was able to reduce placental aromatase activity, thus resulting in high androgen levels in the dams as well as in the offspring later in life. The investigators also reported high AMH levels in second-trimester serum samples from women with PCOS compared with controls without PCOS (28). The aim of the present study was to assess serum AMH and steroid hormone levels in pregnant women with

PCOS in term pregnancies. We also planned on investigating differences in these hormonal parameters according to sex of the offspring, because it has been shown that daughters of women with PCOS have elevated levels of AMH (29, 30).

MATERIALS AND METHODS

Study Population

The study population consisted of 74 pregnant women: 25 women with PCOS and 49 healthy controls. The samples were derived from the “biology, affect, stress, imaging and cognition in pregnancy and puerperium” cohort (BASIC), concerning biological correlates of mood and anxiety disorders during pregnancy and the postpartum period (31). All pregnant women in Uppsala County, Sweden, are invited to participate in the study at their routine ultrasound screening at gestational week 16–18. Eligible women are older than 18 years, speak Swedish, and have no blood-borne diseases. The ultrasonographic examinations were carried out at Uppsala University Hospital. Placental tissues were collected between April 2012 and September 2013, and in all, 957 placental samples have been collected. Among these, women with PCOS were identified in the hospital patient register by International Classification of Diseases, 10th Revision diagnosis of polycystic ovary syndrome (E282). Altogether 25 women with a confirmed PCOS diagnosis and uncomplicated term pregnancies were identified. Six of the women had polycystic ovaries, hyperandrogenism, and oligoamenorrhea, whereas 19 had polycystic ovaries and oligoamenorrhea. Two healthy controls with no record of PCOS or anovulatory infertility were chosen for each woman with PCOS and matched for age, body mass index (BMI), and gestational length. Detailed characteristics of the study populations are shown in [Supplemental Table 1](#) (available online). All women were Caucasian except for one control subject, who was South American. Because the subject with the South American background did not present as an outlier in any of the analyses, she was included in the final analyses. The study and sample collection were approved by the research ethics committees of the University of Uppsala, Sweden and Oulu University Hospital, Finland.

Blood Samples

In all women, a venous blood sample was drawn upon admission to the delivery ward. Serum, ethylenediaminetetraacetic acid plasma, or lithium-heparin plasma samples were stored at -70°C .

Laboratory Methods for AMH, Steroid Hormone, and LH Analysis

Antimüllerian hormone concentrations were assayed in lithium-heparin plasma, ethylenediaminetetraacetic acid plasma, or serum samples, according to sample availability, at HUSLAB (Helsinki, Finland) using the Elecsys AMH Plus method (Roche Diagnostics). The availability for the sample type was similar between the study groups, and further analysis showed similar median AMH values between different sample types, and in nonparametric Kruskal-Wallis test no

differences were observed in the distributions of AMH between the sample types in either of the groups (data not shown).

Concentrations of P 17-hydroxoprogesterone (17OHP), androstenedione (A), DHEAS, T, E₂, estrone (E₁), and E₁-3-sulfate were measured in lithium-heparin plasma at the Core Facility for Metabolomics, University of Bergen, by using liquid chromatography–tandem mass spectrometry.

Serum LH was measured by immunoassay (LH ELISA; Demeditec Diagnostics).

Statistical Methods

Differences in variables in the two study groups were analyzed by using the independent-samples *t* test of the Mann–Whitney *U* test. For correlation and linear regression analysis, nonnormally distributed parameters were square root transformed. Correlations between steroid hormones and AMH levels were assessed by using Pearson's correlation. The impact of BMI, age, and gestational length was standardized by multiple linear regression analysis. Differences between slopes were assessed by ANCOVA. Risk ratios were estimated for the highest AMH quartile (cutoff value of >1.84 ng/mL, based on the highest quartile in the whole data set). The limit of statistical significance was set to $P \leq .05$. Statistical analyses were performed using IBM-SPSS Statistics (version 24.0.0.1) and GraphPad Prism (version 7.03) software. Because patient data were missing in some cases and different analyses required different blood sample types, the number of subjects varied between analyses.

RESULTS

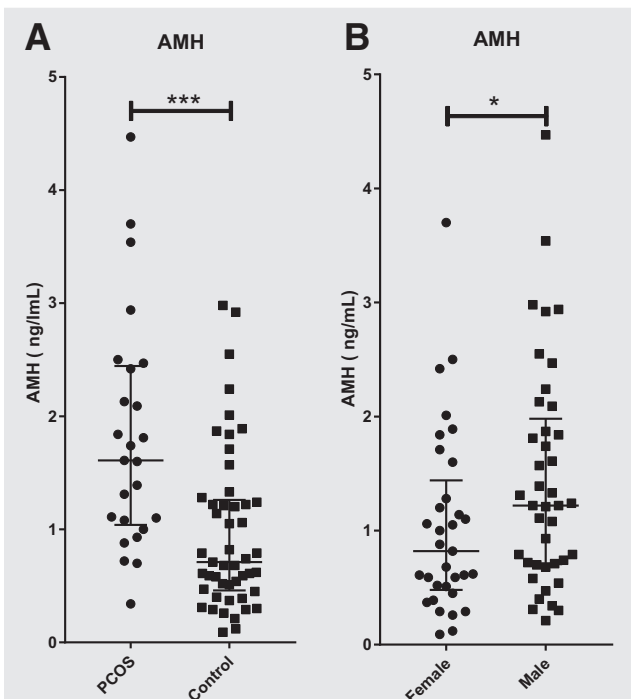
Women with PCOS Have Higher Circulating AMH and Steroid Hormone Levels at Term

Circulating AMH levels in women with PCOS at term were twofold higher than the levels in control women (median levels 1.61 vs. 0.71 ng/mL, $P < .001$; Fig. 1A). In the present dataset, 16 of 25 women with PCOS (64%) had AMH values higher than the interquartile range for the controls (Fig. 1A). In addition, 14% of controls had AMH values in the highest quartile of the whole dataset, compared with 40% in the PCOS group. Similarly to AMH, plasma A and T levels were higher in women with PCOS than in controls ($P < .05$; Supplemental Fig. 1C, D). Levels of P, 17OHP, DHEAS, E₂, E₁, and E₁-3-sulfate levels were comparable in the PCOS and control groups, whereas LH levels were undetectable at term (Table 1).

Correlation of AMH with Steroid Levels and with Maternal Age and Gestational Length

When analyzing the whole study population, AMH levels had positive correlations with those of T ($r = 0.452$, $P < .001$) and A ($r = 0.524$, $P < .001$), and adjustment for age and BMI had no effect on the coefficients (data not shown). After separating the data into cases and controls, the correlation between AMH and T was moderate but statistically significant in control women ($r = 0.378$, $P = .012$) and nearly significant in women with PCOS ($r = 0.403$, $P = .051$). After adjustment

FIGURE 1



(A) Levels of AMH were significantly higher in women with PCOS ($n = 25$) than in control women ($n = 49$) at term. (B) AMH levels of women carrying a female fetus ($n = 34$) were higher than those of women carrying a male fetus ($n = 41$). The horizontal line in each plot corresponds to the median value. The vertical line represents the 25th–75th percentile range. Comparisons between groups were performed using unpaired nonparametric two-tailed Mann–Whitney *U* tests. * $P < .05$; *** $P < .001$.

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for age, BMI, and gestational length, moderately significant correlations were observed in both groups, without any statistically significant difference between them (Table 2). For A, the unadjusted correlation was significant only in the control group ($r = 0.501$, $P = .001$), whereas in women with PCOS the correlation was weaker and did not reach statistical significance ($r = 0.361$, $P = .083$). However, after adjusting for age, BMI, and gestational length, A levels correlated with those of AMH in both groups, with no significant difference between them. Before adjustment, E₂ levels did not correlate with those of AMH in either group, although a positive trend was observed in the PCOS group ($r = 0.378$, $P = .068$). After adjusting for BMI and age, E₂ levels correlated moderately with those of AMH in women with PCOS ($r = 0.445$, $P = .048$), and after additional adjustment for gestational length the trend remained ($r = 0.412$, $P = .069$). There was no correlation between AMH and E₂ levels in the control group before or after adjustments. A difference in slopes was detected between the groups as regards E₂ and E₁ (Table 2). When correlations were studied only within the highest AMH quartile in the data set (>1.84 ng/mL), E₂ correlated strongly with AMH in the PCOS group ($r = 0.752$, $P = .012$), but no correlations could be detected in controls ($r = -0.371$, $P = .412$) (data not shown). In the PCOS group

TABLE 1

Hormonal parameters in the study groups.

Parameter	n	Mean/median	SD/IQR	P value
AMH (ng/mL)				<.001*
PCOS	25	1.61	1.04–2.45	.080
Control	49	0.71	0.46–1.26	
P (nmol/L)				
PCOS	24	465	134	
Control	43	402	147	
17OHP (nmol/L)				.234
PCOS	24	21.3	8.04	
Control	43	19.2	8.60	
A (nmol/L)				.019**
PCOS	24	8.77	5.82–11.29	
Control	43	6.25	3.84–8.98	
T (nmol/L)				.027**
PCOS	24	3.64	2.47–4.55	
Control	43	2.57	1.69–3.82	
DHEAS (μmol/L)				.844
PCOS	24	1.30	0.84–1.97	
Control	43	1.22	0.87–2.05	
E ₂ (nmol/L)				.101
PCOS	24	82.1	31.5	
Control	43	70.7	33.8	
E ₁ (nmol/L)				.073
PCOS	24	38.9	21.3–56.0	
Control	43	27.4	15.9–36.1	
Estriol (nmol/L)				.266
PCOS	24	39.8	24.6–67.2	
Control	43	33.9	22.1–48.9	
ES (nmol/L)				.756
PCOS	24	461	300–790	
Control	43	437	225–910	
LH (mIU/mL)				ND
PCOS	6	<1.27	ND	
Control	24	<1.27	ND	

Note: Normally distributed data are presented as mean and standard deviation (SD) and skewed data as median and interquartile range (IQR). Differences between groups were determined by using independent-samples *t*-test or the nonparametric Mann–Whitney *U* test. ES = E₁-3-sulphate; ND = not detectable.
* *P* < .001.
** *P* < .05.

Piltonen. AMH and steroids in term PCOS pregnancies. *Fertil Steril* 2018.

AMH levels did not correlate with maternal age or gestational length, whereas in controls AMH levels correlated negatively with maternal age ($r = -0.427, P = .004$) but not with gestational length (Supplemental Fig. 1). The coefficients for AMH vs. age were significantly different between groups ($P = .013$).

Risk Factors for High AMH Levels in Term Pregnancy

The cutoff value for high AMH levels was set on the basis of the highest quartile in the data set (>1.84 ng/mL). Seventeen women (23%) had AMH values higher than the cutoff value: 10 women with PCOS (40%) and 7 controls (14%). Women with PCOS had a 3.67-fold greater risk of being in the highest AMH quartile compared with controls, and the risk was only slightly reduced after adjusting for BMI and age (odds ratio 3.35; 95% confidence interval 1.04–10.82). Elevated T and A levels increased the likelihood of being in the highest AMH quartile. Adjustment for BMI and age did not affect the risk (Supplemental Table 2). Steroid hormones other than T or A did not show risk association with high AMH levels (Supplemental Table 2).

Differences in the Levels of AMH, A, T, and E₂ can only be Detected in Women with a Female Fetus

When all women with female fetuses were compared with all women carrying a male fetus, the levels of AMH, A, and T were significantly lower in women with female fetuses (Fig. 1B). In the sample set we had 8 pregnancies with female fetus in women with PCOS and 24 pregnancies in controls. After analyzing AMH and steroid levels according to fetal gender in the different study groups, we observed that in women with a female fetus, the levels of AMH, A, T, and E₂ were significantly higher in women with PCOS compared with controls (Fig. 2). When comparing women with PCOS and controls carrying a male fetus, the hormones levels did not differ between the groups.

TABLE 2

Correlation of AMH and steroid hormones in the study populations.

Parameter	Pearson coefficient	P value	Standardized coefficient ^a	P value ^a	Standardized coefficient ^b	P value ^b
A						
PCOS	0.361	.083	0.370	.088	0.464	.035*
Control	0.501	.001***	0.398	.009**	0.386	.009**
T						
PCOS	0.403	.051	0.447	.040*	0.663	.003*
Control	0.378	.012*	0.361	.047*	0.342	.027*
E ₂						
PCOS	0.378	.068	0.445 ^c	.048*	0.412 ^c	.069
Control	0.123	.430	−0.028 ^c	.868	−0.034 ^c	.829
E ₁						
PCOS	0.351	.093	0.351 ^c	.113	0.377 ^c	.088
Control	0.052	.739	−0.019 ^c	.898	−0.026 ^c	.857

Note: Pearson's correlation was run to assess the relationship between AMH concentration and study parameters. Adjustment for age, BMI, and gestational length was carried out by linear regression analysis.

^a Standardized by age and BMI at the start of pregnancy.

^b Standardized by age, BMI at the start of pregnancy, and gestational length.

^c Significant difference between slopes.

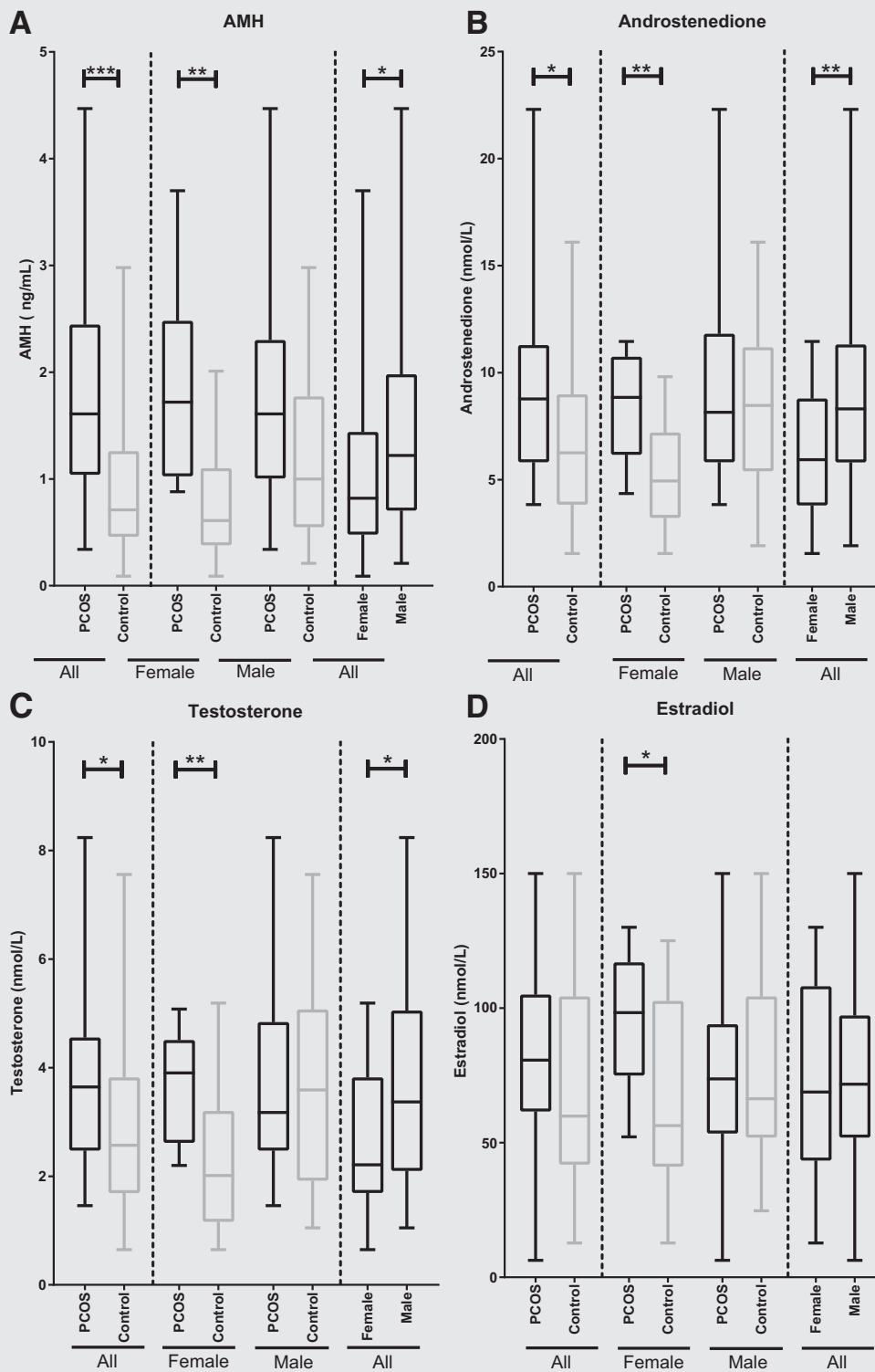
* *P* < .05.

** *P* < .01.

*** *P* < .001.

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FIGURE 2



Differences in levels of AMH, A, T, and E_2 between women with PCOS and controls, according to gender of the offspring. The data showed significant differences in AMH and steroid hormone levels, especially in pregnancies with a female fetus. Numbers of subjects in groups for AMH analysis (A): all (PCOS = 25, control = 49), women with a female fetus (PCOS = 8, control = 25), women with a male fetus (PCOS = 17, control = 24). Numbers of subjects in groups for steroid analysis (B–D): all (PCOS = 23, control = 45), women with a female fetus (PCOS = 8, control = 24), women with a male fetus (PCOS = 16, control = 19). The box represents the 25th–75th percentile range and the horizontal line the median. The whiskers represent minimum and maximum values. Differences between groups were determined by using nonparametric Mann–Whitney U tests. * $P < .05$; ** $P < .01$.

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DISCUSSION

Recently AMH has been shown to have extragonadal functions: AMHR2 was shown to regulate GnRH neuron activity and LH pulsatility, contributing to high LH levels, ovarian cysts, and high androgen levels in mice (11). Moreover, new data also suggest a possible role for AMH in transgenerational PCOS pathogenesis because high gestational AMH levels in mice resulted in a hyperandrogenic PCOS-like phenotype in adult female offspring (28). The present study shows for the first time that elevated AMH and androgen levels can be detected at term pregnancies in women with PCOS. We also report novel data on the correlation between gestational AMH and androgen levels in humans in late pregnancy in a time window sensitive to trigger PCOS-like phenotype in offspring in animal models (25, 28).

High AMH levels have been reported in nonpregnant women with PCOS in several previous studies (2, 19, 32). This also applies to PCOS pregnancies during the second trimester (28) and here at late third trimester, because the women at term presented with two-fold greater AMH levels compared with controls, although the levels were significantly lower than reported in nonpregnant women with PCOS. Indeed, AMH levels have been shown to decrease as gestation advances, and this has been postulated to be a result of suppressed gonadotropin release (33) and inhibition of follicular recruitment due to placental estrogen and P secretion (34), although some follicular recruitment seems to occur even during pregnancy (9). There was overlap in AMH levels between the PCOS and control groups. Whether high AMH levels are more detrimental in PCOS pregnancies cannot be concluded from these data.

This study reveals for the first time that at term, circulating AMH levels correlate with those of T and A both in women with PCOS and in controls when adjusted for age, BMI, and gestational length. A recent study by Tata et al. (28) showed decreased placental aromatase activity in mice exposed to high AMH levels during late pregnancy, resulting in high androgen levels in the dams and in female offspring in adulthood. In humans, AMH has been shown to modify the enzymatic activity of steroid hormone synthesis (35), and women with PCOS have been reported to have reduced placental aromatase activity and increased steroidogenic activity (36), giving support to the findings of Tata et al. also in humans. Because gestational AMH levels correlated with those of androgens in the controls as well, it would be of interest to investigate whether women with PCOS are especially sensitive to AMH-related androgen modulation in the placenta during pregnancy, resulting in high androgen exposure in the fetus.

Besides androgens, we also detected a positive correlation between AMH and E_2 levels in pregnant women with PCOS that was not observed in the control group. We also noticed that in the highest AMH quartile, E_2 levels correlated with AMH only in the PCOS group. In nonpregnant women with PCOS, E_2 has been reported to correlate with AMH both negatively (37) and positively (38), but in most studies no correlation has been detected (24, 32, 39). Previously an *in vitro* study demonstrated a lack of E_2 -mediated AMH down-

regulation in granulosa cells from women with PCOS (40). Thus, it is plausible that high E_2 levels would not inhibit AMH production in the ovaries of women with PCOS, as they would do in controls, and this could also be the case during pregnancy, again supporting the idea of an existing ovarian activity during pregnancy. On the other hand, peripheral conversion of androgens to estrogens in fat tissue might be more pronounced in women with PCOS also presenting with high AMH levels, and this might explain the correlation (9). In the present study AMH levels correlated negatively with maternal age only in the control group, but not in PCOS, in line with previous data (41). Even though AMH levels also decrease during aging in women with PCOS, they remain high until late reproductive years, and the correlations may not be as obvious as in controls during these years (2, 42). The nonexistent correlation between AMH levels and gestational length in the two groups most likely results from the fact that the samples were all from term pregnancies, with a maximum difference in length of 6 weeks.

Interestingly, when the whole study population was analyzed, AMH levels were lower in pregnancies with a female fetus, in line with a previous report (43), although contradictory data also exist (33, 41). Moreover, the levels of AMH, T, A, and E_2 were significantly higher in women with PCOS carrying a female fetus when compared with controls with a female fetus, and no differences were found between groups in pregnancies with male fetuses. In line with this, Caanen et al. (44) have also detected higher T and A concentrations in maternal circulation throughout the pregnancies of women with PCOS who are carrying a female fetus, but no fetal gender-specific differences in maternal serum androgen concentrations in women with PCOS were detected in a third study (36). Additional studies that support the idea of fetal gender influencing maternal steroid levels also exist: Maliqueo et al. (45) reported fetal gender-specific differences in placental aromatase expression, and Kallak et al. (27) detected a maternal AMH genotype correlating with maternal T levels in pregnancies with a male fetus. In any case, it is worth noting that the existing studies, including ours, have concerned limited populations, and larger studies are warranted in the future to address these questions.

Our study has some limitations, and some matters remain unsolved. Even though women with PCOS were more than three times more likely to have AMH values in the highest quartile, some of the values overlapped with those of control women. The range of AMH values was broader in controls, even though the median AMH value was lower. Thus, similarly to comparison in AMH levels between nonpregnant women with PCOS and controls, there are pregnant women without PCOS who have relatively high circulating AMH concentrations but who have no current reproductive dysfunction or PCOS symptoms. It is possible that tissue-specific AMH responses are altered in women with PCOS compared with counterparts without PCOS. Indeed, it has been shown that granulosa cells of oligoanovulatory women with PCOS display altered AMH and AMHR2 expression after steroid or LH exposure compared with normo-ovulatory women,

supporting this theory (21, 40). Whether this applies also to placental tissue remains to be investigated in future studies.

Owing to the limited number of participants with available samples and clinical data, we were not able to analyze the material according to specific PCOS phenotypes or measure sex hormone-binding globulin or FSH levels. The limited number of samples applies especially on the analysis related to offspring gender-specific differences between the study groups, warranting confirmation by larger studies. Moreover, because obesity has been associated with lower AMH levels (19) and higher gestational T levels (45), and because only a few of the participants in the present study were obese, further studies are needed to investigate whether AMH correlates with androgen levels in obese pregnant women with PCOS. In addition, all the samples were from uncomplicated term pregnancies, thus the association of AMH with pregnancy complications remains to be analyzed in other studies. Furthermore, it would be of interest to study AMH and androgen levels throughout PCOS pregnancy and determine whether AMH and androgen levels correlate with the levels in the offspring after birth.

To conclude, the present study shows for the first time that pregnant women with PCOS have higher AMH and androgen levels at term compared with controls without PCOS, and the levels of AMH correlate with those of A and T. The differences between the study groups were observed especially in pregnancies carrying female offspring, possibly implying that that these pregnancies are more susceptible to steroid hormone-related changes. In light of these findings, it is possible that a link between high AMH levels and gestational hyperandrogenism exists in some women. Future studies are warranted to investigate the role of AMH in placental function and transgenerational pathogenesis of PCOS in humans.

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REFERENCES

- Kuiri-Hänninen T, Kallio S, Seuri R, Tynväinen E, Liakka A, Tapanainen J, et al. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab* 2011;96:3432–9.
- Piltonen T, Morin-Papunen L, Koivunen R, Perheentupa A, Ruokonen A, Tapanainen JS. Serum anti-Müllerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with polycystic ovary syndrome. *Hum Reprod* 2005;20:1820–6.
- 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.
- Weenen C, Laven JSE, von Bergh ARM, Cranfield M, Groome NP, Visser JA, et al. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod* 2004;10:77–83.
- Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of primordial follicle recruitment by anti-Müllerian hormone in the mouse ovary. *Endocrinology* 1999;140:5789–96.
- Durlinger AL, Grijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, et al. Anti-Müllerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. *Endocrinology* 2001;142:4891–9.
- van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 2002;17:3065–71.
- Visser JA, Schipper I, Laven JS, Themmen AP. Anti-Müllerian hormone: an ovarian reserve marker in primary ovarian insufficiency. *Nat Rev Endocrinol* 2012;8:331–41.
- McCredie S, Ledger W, Venetis CA. Anti-Müllerian hormone kinetics in pregnancy and post-partum: a systematic review. *Reprod Biomed Online* 2017;34:522–33.
- Novembri R, Funghi L, Voltolini C, Belmonte G, Vannuccini S, Torricelli M, et al. Placenta expresses anti-Müllerian hormone and its receptor: sex-related difference in fetal membranes. *Placenta* 2015;36:731–7.
- Cimino I, Casoni F, Liu X, Messina A, Parkash J, Jamin SP, et al. Novel role for anti-Müllerian hormone in the regulation of GnRH neuron excitability and hormone secretion. *Nat Commun* 2016;7:10055.
- Carrarelli P, Rocha AL, Belmonte G, Zupi E, Abrao MS, Arcuri F, et al. Increased expression of antimüllerian hormone and its receptor in endometriosis. *Fertil Steril* 2014;101:1353–8.
- March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010;25:544–51.
- Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod* 2016;31:2841–55.
- Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25.
- Fraissinet A, Robin G, Pigny P, Lefebvre T, Cateau-Jonard S, Dewailly D. Use of the serum anti-Müllerian hormone assay as a surrogate for polycystic ovarian morphology: impact on diagnosis and phenotypic classification of polycystic ovary syndrome. *Hum Reprod* 2017;32:1716–22.
- Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. *Hum Reprod* 2011;26:3123–9.
- Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Müllerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. *J Clin Endocrinol Metab* 2013;98:3332–40.
- Piouka A, Farmakiotis D, Katsikis I, Macut D, Gerou S, Panidis D. Anti-Müllerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels. *Am J Physiol Endocrinol Metab* 2009;296:E238–43.
- Jacob SL, Field HP, Calder N, Picton HM, Balen AH, Barth JH. Anti-Müllerian hormone reflects the severity of polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2017;86:395–400.
- Pierre A, Peigné M, Grynberg M, Arouche N, Taieb J, Hesters L, et al. Loss of LH-induced down-regulation of anti-Müllerian hormone receptor expression may contribute to anovulation in women with polycystic ovary syndrome. *Hum Reprod* 2013;28:762–9.
- Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch twin-family study. *J Clin Endocrinol Metab* 2006;91:2100–4.
- Gorsic LK, Kosova G, Werstein B, Sisk R, Legro RS, Hayes MG, et al. Pathogenic anti-müllerian hormone variants in polycystic ovary syndrome. *J Clin Endocrinol Metab* 2017;102:2862–72.
- Kevenaar ME, Laven JSE, Fong SL, Uitterlinden AG, de Jong FH, Themmen APN, et al. A functional anti-Müllerian hormone gene polymorphism is associated with follicle number and androgen levels in polycystic ovary syndrome patients. *J Clin Endocrinol Metab* 2008;93:1310–6.
- Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum Reprod Update* 2005;11:357–74.
- Mannerås L, Cajander S, Holmang A, Seleskovic Z, Lystig T, Lonn M, et al. A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. *Endocrinology* 2007;148:3781–91.
- Kallak TK, Hellgren C, Skalkidou A, Sandelin-Francke L, Ubhayasekhara K, Bergquist J, et al. Maternal and female fetal testosterone levels are

- associated with maternal age and gestational weight gain. *Eur J Endocrinol* 2017;177:379–88.
28. Tata B, El Houda Mimouni N, Barbotin A, Malone SA, Lovens A, Pigny P, et al. Elevated prenatal anti-Müllerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. *Nat Med* 2018;24:834–46.
 29. Crisosto N, Echiburú B, Maliqueo M, Perez V, Ladron de Guevara A, Preisler J, et al. Improvement of hyperandrogenism and hyperinsulinemia during pregnancy in women with polycystic ovary syndrome: possible effect in the ovarian follicular mass of their daughters. *Fertil Steril* 2012;97:218–24.
 30. Sir-Petermann T, Ladrón de Guevara A, Codner E, Preisler J, Crisosto N, Echiburú B, et al. Relationship between anti-Müllerian hormone (AMH) and insulin levels during different Tanner stages in daughters of women with polycystic ovary syndrome. *Reprod Sci* 2012;19:383–90.
 31. Iliadis S, Sylven S, Jocelien O, Hellgren C, Hannefors AK, Elfström D, et al. Corticotropin-releasing hormone and postpartum depression: a longitudinal study. *Psychoneuroendocrinology* 2015;61:61.
 32. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, et al. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. *J Clin Endocrinol Metab* 2003;88:5957–62.
 33. La Marca A, Giulini S, Orvieto R, De Leo V, Volpe A. Anti-Mullerian hormone concentrations in maternal serum during pregnancy. *Hum Reprod* 2005;20:1569–72.
 34. Kuijper EAM, Ket JCF, Caanen MR, Lambalk CB. Reproductive hormone concentrations in pregnancy and neonates: a systematic review. *Reprod Biomed Online* 2013;27:33–63.
 35. Maliqueo M, Lara HE, Sánchez F, Echiburú B, Crisosto N, Sir-Petermann T. Placental steroidogenesis in pregnant women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* 2013;166:151–5.
 36. Cook CL, Siow Y, Brenner AG, Fallat ME. Relationship between serum müllerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *Fertil Steril* 2002;77:141–6.
 37. Peigne M, Catteau-Jonard S, Robin G, Dumont A, Pigny P, Dewailly D. The numbers of 2-5 and 6-9 mm ovarian follicles are inversely correlated in both normal women and in polycystic ovary syndrome patients: what is the missing link? *Hum Reprod* 2018;33:706–14.
 38. Matsuzaki T, Munkhzaya M, Iwasa T, Tungalagsuud A, Yano K, Mayila Y, et al. Relationship between serum anti-Mullerian hormone and clinical parameters in polycystic ovary syndrome. *Endocr J* 2017;64:531–41.
 39. Pierre A, Taieb J, Giton F, Grynberg M, Touleimat S, El Hachem H, et al. Dysregulation of the anti-Müllerian hormone system by steroids in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2017;102:3970–8.
 40. Pellatt L, Rice S, Mason HD. Anti-Müllerian hormone and polycystic ovary syndrome: a mountain too high? *Reproduction* 2010;139:825–33.
 41. Vanky E, Carlsen SM. Androgens and antimullerian hormone in mothers with polycystic ovary syndrome and their newborns. *Fertil Steril* 2012;97:509–15.
 42. Minooee S, Ramezani Tehrani F, Rahmati M, Mansournia MA, Azizi F. Prediction of age at menopause in women with polycystic ovary syndrome. *Climacteric* 2018;21:29–34.
 43. Empey R, Santillan D, Santillan M, Tuler E, Hunter S, Smith E, et al. The influence of fetal sex on patterns of change in anti-Mullerian hormone during pregnancy. *Proc Obstet Gynecol* 2012;2:4.
 44. Caanen MR, Kuijper EA, Hompes PG, Kushnir MM, Rockwood AL, Meikle WA, et al. Mass spectrometry methods measured androgen and estrogen concentrations during pregnancy and in newborns of mothers with polycystic ovary syndrome. *Eur J Endocrinol* 2016;174:25–32.
 45. Maliqueo M, Cruz G, Espina C, Contreras I, Garcia M, Echiburú B, et al. Obesity during pregnancy affects sex steroid concentrations depending on fetal gender. *Int J Obes (Lond)* 2017;41:1636–45.

Los niveles de hormona antimülleriana y esteroidea circulantes se mantienen altos en mujeres embarazadas a término con síndrome de ovario poliquístico

Objetivo: Investigar la concentración plasmática de hormona antimülleriana (AMH) y su relación con los niveles de hormonas esteroides en mujeres embarazadas a término con síndrome de ovario poliquístico (PCOS) y controles.

Diseño: Estudio de casos y controles.

Entorno: Hospital afiliado a la universidad.

Paciente(s): Un total de 74 mujeres embarazadas a término: 25 mujeres con PCOS (edad $31,6 \pm 3,9$ años [media \pm desviación estándar], índice de masa corporal $24,0 \pm 3,9$ kg/m², duración media de la gestación 279 ± 9 días) y 49 controles (edad $31,7 \pm 3,3$ años, índice de masa corporal $24,0 \pm 3,3$ kg/m², duración media de la gestación 281 ± 9 días).

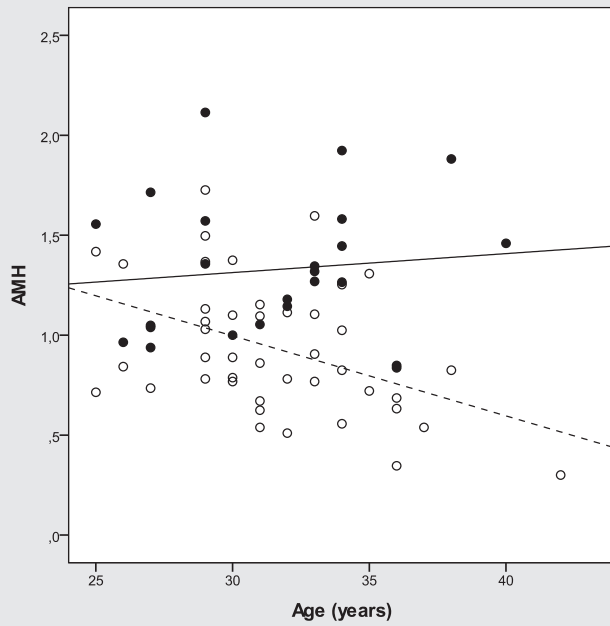
Intervención(es): Ninguna.

Principales variables: Niveles plasmáticos de AMH y de hormonas esteroides.

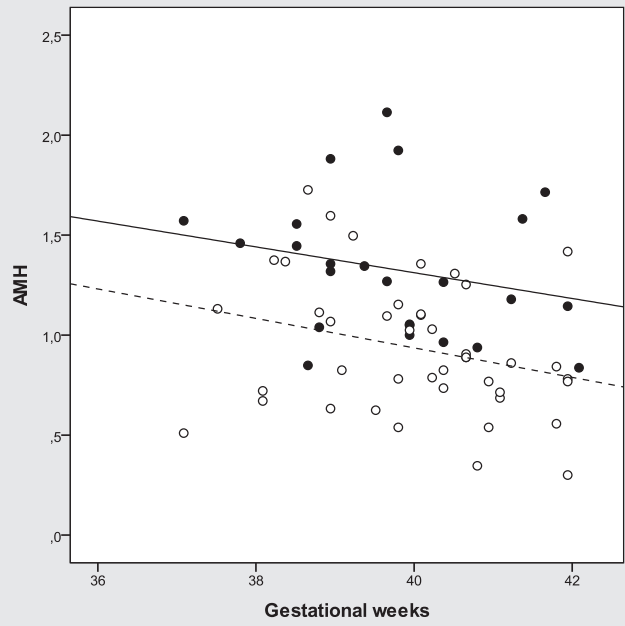
Resultado(s): Los niveles de hormona antimülleriana, T, y androstenediona fueron mayores en mujeres con SOP a término comparados con los controles, mientras que los niveles de estrógeno y P fueron similares. Las diferencias fueron pronunciadas en mujeres portadoras de un feto femenino. Los niveles de testosterona y AMH se correlacionaron positivamente en ambos grupos, pero los niveles de E₂ sólo en mujeres con PCOS.

Conclusión(es): Las mujeres embarazadas con PCOS presentan niveles elevados de AMH y andrógenos incluso a término, sugiriendo un desequilibrio hormonal durante el embarazo con PCOS. Las diferencias fueron detectadas especialmente en los embarazos con un feto femenino, lo que plantea la cuestión de si los embarazos femeninos son más susceptibles a los efectos de la AMH y de las hormonas esteroides.

SUPPLEMENTAL FIGURE 1



r (PCOS): 0.101, p = 0.613
 r (control): -0.427, p = 0.004
 p (difference) = 0.028



r (PCOS): -0.244, p = 0.251
 r (control): -0.277, p = 0.72
 p (difference) = 0.904

Maternal age correlated with square-root-transformed AMH levels only in the control group and not in women with PCOS. There was a significant difference between the slopes (studied by analysis of covariance). The correlations between AMH and gestational length were nonsignificant and equal in both groups. PCOS, black circles (●), continuous line; controls, white circles (○), dotted line.

Piltonen. AMH and steroids in term PCOS pregnancies. Fertil Steril 2018.