

Anti-Müllerian Hormone and Ovarian Morphology in Women With Hypothalamic Hypogonadism

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Context: Different phenotypical features of women with hypothalamic hypogonadism (HH), also known as World Health Organization-1 anovulation, including ovarian morphology, have been scarcely described in large cohorts. Some studies have reported increased levels of anti-Müllerian hormone (AMH) in women with HH.

Objective: To assess whether women with HH, compared with healthy controls, have increased serum levels of AMH and what proportion of these women erroneously meet the Rotterdam Criteria for Polycystic Ovarian Syndrome (PCOS).

Design, Setting and Participants: Retrospective cohort study in a Dutch academic medical center including 83 women with neither anovulation nor menstrual cycle disorders (healthy controls), 159 women with HH and 3640 women with PCOS. Age matching was used between the HH and PCOS group (1:2 ratio) to create a second group consisting of 318 age-matched women with PCOS.

Intervention: None.

Main outcome measures: AMH levels and ovarian morphology.

Results: Median AMH serum levels for the HH group were 3.8 (<0.1–19.8), compared with 7.5 (<0.1–81.0) in the PCOS group and 1.9 (<0.1–21.5) in the control group ($P < 0.001$). In the HH group, 58 (36%) erroneously met the Rotterdam Criteria for PCOS (meeting 2 of 3 criteria).

Conclusions: AMH levels are increased in women with HH. We hypothesize that this increase, although there was no increase in follicle count, may be explained by the presence of a relatively large pool of antral follicles smaller than 2 mm in diameter, that are undetectable by transvaginal ultrasound. This study highlights the importance of measuring gonadotropins and estradiol before diagnosing a patient with PCOS. (*J Clin Endocrinol Metab* 105: e2008–e2015, 2020)

Keywords: anti-Müllerian hormone, hypothalamic hypogonadism, PCOS, ovarian function, ovarian morphology

Anovulation is the state in which a woman does not ovulate, often resulting in oligomenorrhea or amenorrhea (1). The World Health Organization

(WHO) identifies 3 distinct groups: WHO-1 is characterized by dysfunction of the hypothalamic-pituitary axis to produce gonadotropins (LH and FSH) resulting

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Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; E2, estradiol; FAI, free androgen index; FNPO, follicle number per ovary; HH, hypothalamic hypogonadism; PCOM, polycystic ovarian morphology; PCOS, polycystic ovary syndrome; WHO, World Health Organization

in anovulation and in low estrogens. This category of anovulation is also known as hypothalamic hypogonadism (HH) (2, 3). HH may be caused by excessive exercise or physical stress, or from eating disorders such as anorexia nervosa or bulimia (4). In about 5% to 10% of all anovulatory women, HH seems to be the cause (3). Patients with HH are at risk of osteoporosis (5, 6) and cardiovascular disease (7, 8), as a result of their persistent low estrogen levels (7).

By far the largest part of women with anovulation are classified in the WHO-2 category, which is characterized by normal FSH and estradiol (E_2) levels. Most women in this category can be classified as having polycystic ovary syndrome (PCOS). In these women, irregular cycles are accompanied by hyperandrogenism and polycystic ovarian morphology (PCOM) (9–11).

Anti-Müllerian hormone (AMH) is produced in the ovary by granulosa cells of small growing pre-antral and antral follicles. AMH inhibits the recruitment of primordial follicles out of the primordial follicle pool, decreases FSH sensitivity of individual follicles, and inhibits aromatase activity (consequently increasing androgen levels in the ovary) (12, 13). Because the ovarian follicle pool gradually diminishes over the course of a women's reproductive lifetime, a simultaneous decrease in AMH levels occurs (14, 15).

In the past, higher follicle number per ovary (FNPO) in women with HH compared with controls has been reported (16). This may lead to the suspicion that, when follicle count reaches the number required for the characterization of PCOM, women with HH may be misdiagnosed with PCOS, if gonadotropin levels FSH and estrogen serum levels are not taken into account (17).

The primary goal of this paper is to assess whether women with HH have increased AMH serum levels compared with women with regular cycles and compared with women with PCOS. The secondary goal of this paper is to assess whether PCOM occurs more often in women with HH than in women with regular cycles and in women with PCOS. Finally, we wanted to evaluate how often the negligence of measuring serum levels of FSH, LH, and E_2 would falsely lead to a PCOS diagnosis instead of the correct HH diagnosis.

Materials and Methods

Study design

In this retrospective single-center study, patients with cycle disorders and anovulation who visited the reproductive endocrinology and infertility clinic of the Erasmus University Medical Center in Rotterdam, The Netherlands, were consecutively selected between 1993 and 2018. Participants were not on fertility medication during this visit; in case of oral contraceptive

usage, they were seen at day 7 in their pill-free week. During their visit, extensive patient history, general health, family history, previous and current use of medication, and self-reported ethnicity was recorded. During physical examination, anthropometric measurements (e.g., weight, height, body-mass index [BMI]) were assessed. Transvaginal ultrasonography was performed to assess the number of antral follicles sized 2 to 10 mm in diameter, as well as to assess the ovarian volume in the left and right ovary. Laboratory blood tests were drawn randomly in the anovulatory cycles (fasting blood samples were taken with venipuncture) to collect data on endocrine and metabolic profiles. Blood samples were processed within 2 hours. Serum was produced and isolated by centrifuging blood samples at 3000 rpm for a duration of 10 minutes at 20°C.

Patient selection

HH was defined as having low levels of gonadotropins (FSH and LH <2.0 U/L), low levels of E_2 (<100 $\mu\text{mol/L}$), and either oligomenorrhea (menstrual periods being between 35 days and 6 months apart) or amenorrhea (no menstruation for at least 6 months).

PCOS was defined as having normal levels of FSH, LH, and estrogen in addition to meeting 2 of the 3 criteria in the Rotterdam Criteria (1) oligomenorrhea/amenorrhea, (2) PCOM (defined as ≥ 12 follicles measuring 2 to 9 mm in diameter in at least 1 ovary), and/or (3) signs of hyperandrogenism (17). In accordance to the Rotterdam Criteria for PCOS, hyperandrogenism was assessed and classified as either biochemical or clinical hyperandrogenism (hirsutism). Biochemical hyperandrogenism was defined by the free androgen index (FAI) on the calculation of (testosterone/SHBG) $\times 100$ (18). Biochemical hyperandrogenism was defined as having a FAI higher than 2.9. Clinical hyperandrogenism was assessed visually using the modified Ferriman-Gallwey score (19, 20), a standardized visual scale. Clinical hyperandrogenism was defined as a modified Ferriman-Gallwey score of 5 or higher (18).

Healthy control women were selected from ongoing cohort studies at our department where, in addition to having a regular cycle pattern and no oligomenorrhea or amenorrhea, having normal levels of LH, FSH and estrogen as well as testosterone were required (21). Laboratory blood tests for the control group were drawn at days 3 through 5 of their natural cycle or seventh day of their pill-free week. This study was approved by the Medical Ethics Review Committee (institutional review board protocol number 2016-216, EMC) and written informed consent was obtained from all participants.

Hormone assays

Patient samples were stored at -20°C before hormone levels were determined. AMH levels were determined using double-antibody ELISA (Beckman Coulter, Inc., AMH Gen II assay, Webster, TX) (3). In patients who had their AMH levels measured before 2011, determination of AMH levels was done with an ultrasensitive ELISA (Immunotech-Coulter, Marseilles, France) (15, 22). These measurements were batch by batch-adjusted for comparability to AMH levels measured with the newer ELISA technology.

FSH, LH, and SHBG were determined using the Immulite platform assay. E_2 was determined using radioimmunoassay (DPC). Androstenedione was determined using the Immulite 2000 assay and the liquid chromatography tandem mass

spectrometry assay. Testosterone was determined using liquid chromatography tandem mass spectrometry.

Intra-assay and inter-assay coefficient variations for the Beckman Coulter Inc. Gen II assay AMH assay were <5% and <10% (3), for the Immunotech-Coulter AMH assay, these were <5% and 8%, respectively (15, 22). Intra-assay and inter-assay coefficient variations for FSH were <3.0% and <6.2%, for LH <3.5% and <6.4%, for E₂ <11% and <11%, for testosterone <3% and <5%, for androstenedione <8% and <11%, and for SHBG, respectively, <4% and <5% (23).

Statistical analysis

IBM SPSS Statistics 25 was used for statistical analysis. Continuous variables were presented as mean and standard deviation. Independent *t*-tests were used when comparing continuous variables that were normally distributed. Continuous variables that were not normally distributed were assessed using the Mann-Whitney *U* test, where these variables were presented as median and range. Normality was assessed using the Kolmogorov-Smirnov test. Categorical variables were presented as numbers and percentages. Differences between categorical variables such as elevated hormone levels were assessed using a χ^2 test. If a cell contained less than 5 individuals, Fischer's exact test was used for assessing the difference in categorical variables. Rstudio version 1.1.463 in combination with the packages "Matchit" (<https://cran.r-project.org/web/packages/MatchIt/index.html>) and "foreign" (<https://cran.r-project.org/web/packages/foreign/index.html>) were used to age match every HH patient with 2 PCOS patients (1:2 ratio). *P* values <0.05 were considered statistically significant.

Results

Study population

We identified 159 women with HH and 3640 women with PCOS. The control group consisted of 83 women

with no cycle disorders or anovulation. We age-matched 318 PCOS patients to the 159 patients in the HH group; this group will be referred to as the age-matched PCOS group.

The HH group did not statistically differ in age from the control group (26.67 ± 6.67 vs 28.37 ± 5.86). The PCOS group (N = 3640) was significantly older (27.62 ± 5.43) compared with the HH group (*P* < 0.05), but did not statistically differ in age from the control group.

The HH group's BMI (23.06 ± 5.78) did not statistically differ from that of the control group (23.55 ± 3.74). The PCOS group had a higher BMI (26.95 ± 6.50) compared with both the HH (*P* < 0.001) and control group (*P* < 0.01). The increased BMI levels in the PCOS group persisted after age matching with the HH group (N = 318, BMI 27.07 ± 6.46) (Table 1).

AMH levels and ovarian morphology

Median AMH serum levels were significantly higher in the HH group compared with the control group (medians 3.8 vs 1.9 µg/L, *P* < 0.001), but lower than AMH levels in the PCOS group (median AMH 7.5 µg/L) (Fig. 1A and Table 2). After age-matching, the difference in AMH levels between the PCOS group and HH group decreased. In the age-matched PCOS group, median AMH levels were 6.7 µg/L. These AMH levels remained significantly higher compared with both the HH group and the control group (*P* < 0.001) (Table 2).

Antral follicle count was not statistically different in the HH group compared with the control group (20.5 [range 4.0–109.0] vs 21.5 [range 4.0–70.0]) (Table 2

Table 1. Patient's characteristics

	Controls	HH	Age-Matched PCOS	PCOS
N	83	159	318	3640
Age (years)	28.37 ± 5.86	26.67 ± 6.67	26.66 ± 6.63 ^a	27.62 ± 5.43 ^b
BMI (kg/m ²)	23.55 ± 3.74	23.06 ± 5.78	27.07 ± 6.46 ^{c,d}	26.95 ± 6.50 ^{c,d}
Height (cm)	170.67 ± 7.32	168.32 ± 8.06	167.02 ± 7.62 ^c	167.13 ± 7.56 ^c
Weight (kg)	69.00 ± 14.27	65.38 ± 17.73	75.55 ± 19.06 ^{a,d}	75.25 ± 18.76 ^{a,e}
Insulin (pmol/L)	54.25 ± 27.32	60.67 ± 67.52	84.14 ± 76.65 ^{e,f}	84.54 ± 147.39 ^b
FSH (IU/L)	6.33 ± 2.49	3.09 ± 2.23 ^e	5.25 ± 2.23 ^{d,f}	5.56 ± 2.52 ^{c,e}
LH (IU/L)	3.78 ± 1.60	0.84 ± 0.90 ^e	8.86 ± 7.07 ^{d,f}	9.58 ± 8.96 ^{d,f}
E ₂ (pmol/L)	169.60 ± 108.86	72.31 ± 119.09 ^e	270.08 ± 198.79 ^{d,f}	278.59 ± 225.07 ^{d,f}
Testosterone (nmol/L)	1.15 ± 0.51	0.78 ± 0.42 ^e	1.91 ± 0.93 ^{d,f}	1.95 ± 1.04 ^{d,f}
Androstenedione	3.48 ± 1.20	3.05 ± 1.46	6.16 ± 2.59 ^{d,f}	6.14 ± 2.70 ^{d,f}
SHBG (nmol/L)	71.43 ± 44.13	62.88 ± 48.75	45.37 ± 29.77 ^{d,f}	46.67 ± 29.85 ^{d,f}
Free androgen index	1.47 ± 0.78	1.67 ± 1.79	5.50 ± 4.38 ^{d,f}	5.65 ± 5.30 ^{d,f}
Ferriman-Gallwey score	3.45 ± 1.97	4.64 ± 3.85	6.92 ± 5.14 ^{a,e}	7.29 ± 5.55 ^{a,e}

Values represent the mean ± standard deviation.

BMI, body mass index; E₂, estradiol; PCOS, polycystic ovarian syndrome.

^a*P* < 0.05 compared with controls.

^b*P* < 0.05 compared with HH.

^c*P* < 0.01 compared with controls

^d*P* < 0.001 compared with HH *P* values obtained through independent-samples *t*-test.

^e*P* < 0.01 compared with HH.

^f*P* < 0.001 compared with controls.

and Fig. 1B). When looking at the follicle counts in each ovary separately, no differences were found in follicle counts. Similarly, no statistical difference was found for ovarian volume. However, the PCOS group showed increased antral follicle count (36.0, range 4.0–220.0) and total ovarian volume (17.52, range 1.26–200.62) compared with both the HH group and control group ($P < 0.001$). In the age-matched PCOS group, significantly higher antral follicle count and total ovarian volume were also found compared with both the HH and control group, but were not higher than found in the PCOS group.

Table 3 shows that PCOM did not occur more often in the HH group compared with the control group (31.4% vs 24.1%). In the PCOS group, PCOM (87.5%) occurred more often compared with both the HH group and control group ($P < 0.001$). After age-matching, the difference between these groups remained large; PCOM

occurred in 81.4% of the patients in the age-matched PCOS group.

Hyperandrogenism

Testosterone levels in the PCOS group and age-matched PCOS group (1.95 ± 1.04 nmol/L and 1.91 ± 0.93 nmol/L, respectively) were higher compared with both the HH group and control group (0.78 ± 0.42 and 1.15 ± 0.51 nmol/L, respectively, $P < 0.001$).

SHBG and androstenedione levels did not statistically differ in the HH group compared with the control group. In the PCOS and age-matched PCOS group, SHBG was significantly lower, whereas androstenedione levels were significantly higher compared with both the HH group and the control group (Table 1).

Biochemical hyperandrogenism did not occur more often in the HH group compared with the

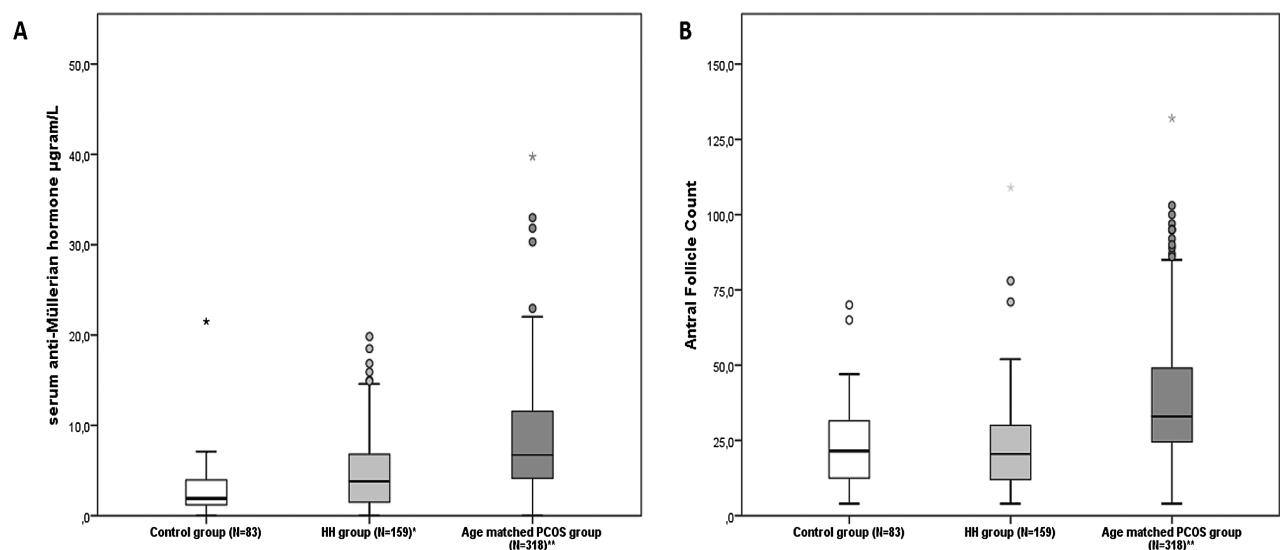


Figure 1. (A) Boxplot showing serum anti-Müllerian hormone levels in the control, HH, and age-matched PCOS group. (B) Boxplot showing antral follicle count in the control, HH, and age-matched PCOS group. * $P < 0.001$ compared with the control group. ** $P < 0.001$ compared with the control group and the HH group.

Table 2. Patient's characteristics

	Controls	HH	Age-Matched PCOS	PCOS
N	83	159	318	3640
AMH ($\mu\text{g/L}$) ^a	1.9 (<0.1–21.5)	3.8 (<0.1–19.8) ^b	6.7 (<0.1–39.8) ^{b,c}	7.5 (<0.1–81.0) ^{b,c}
Follicle count right ovary	10.0 (2.0–32.0)	9.0 (2.0–57.0)	17.0 (2.0–67.0) ^{b,c}	18.0 (2.0–110.0) ^{b,c}
Follicle count left ovary	9.5 (2.0–43.0)	9.0 (2.0–52.0)	16.0 (2.0–65.0) ^{b,c}	17.00 (2.0–115.0) ^{b,c}
Antral follicle count	21.5 (4.0–70.0)	20.5 (4.0–109.0)	33.0 (4.0–132.0) ^{b,c}	36.0 (4.0–220.0) ^{b,c}
Volume right ovary	4.95 (1.26–23.57)	3.71 (0.10–40.67)	8.90 (0.80–50.59) ^{b,c}	9.16 (0.53–131.33) ^{b,c}
Volume left ovary	4.31 (0.46–26.69)	3.60 (0.39–21.18)	7.64 (0.10–80.15) ^{b,c}	7.90 (0.10–182.21) ^{b,c}
Total ovarian volume	9.98 (2.03–50.25)	8.46 (0.78–53.45)	16.71 (2.89–130.74) ^{b,c}	17.52 (1.26–200.62) ^{b,c}

Values represent the median (range).

AMH, anti-Müllerian hormone; HH, hypothalamic hypogonadism; PCOS, polycystic ovary syndrome.

^aThe lower limit of detection for AMH was 0.1 $\mu\text{g/L}$.

^b $P < 0.001$ compared with controls.

^c $P < 0.001$ compared with HH P values obtained through Mann-Whitney U test.

Table 3. Biochemical and clinical hyperandrogenism given as numbers and percentages among the study groups

	Controls	HH	Age-Matched PCOS ^a	PCOS
N	83	159	318	3640
Biochemical hyperandrogenism	3 (3.6%)	8 (5.0%)	182 (57.2%) ^{c,d}	1972 (54.2%) ^{c,d}
Clinical hyperandrogenism score	2 (2.4%)	16 (10.1%) ^b	98 (30.8%) ^{c,d}	986 (27.1%) ^{c,d}
Polycystic ovarian morphology	20 (24.1%)	50 (31.4%)	259 (81.4%) ^{d,e}	3185 (87.5%) ^{d,e}

Values represent the number (percentage) of women. Biochemical hyperandrogenism was defined as Free-androgen-index > 2.9; clinical hyperandrogenism was defined as Ferriman-Gallwey score \geq 5; Polycystic ovarian morphology was defined as having 12 or more antral follicles in the left or right ovary.

^aAge matched with HH group.

^b $P < 0.05$ compared to controls (Fischer's Exact Test)

^c $P < 0.001$ compared to controls (Fischer's Exact Test).

^d $P < 0.001$ compared to HH (Pearson's χ^2 test).

^e $P < 0.001$ compared to controls (Pearson's χ^2 test).

control group. In the HH group, 5.0% had biochemical hyperandrogenism compared with 3.6% in the control group. Clinical hyperandrogenism did occur more often in the HH group compared to the control group: in the HH group, 10.1% had clinical hyperandrogenism compared with 2.4% in the control group ($P < 0.05$) (Table 3).

In our control group, we found that 3.6% (3 patients) had biochemical hyperandrogenism but the entire group had normal testosterone levels: this was caused by low SHBG levels in specifically these 3 patients resulting in a high FAI.

Rotterdam Criteria for PCOS in women with HH

In the HH group, 58 (36%) women met the Rotterdam Criteria for PCOS, if gonadotropin and estrogen levels were not taken into account. The phenotypes for meeting the Rotterdam Criteria for PCOS in the HH group were: 39 (24.5%) had PCOM and amenorrhea/oligomenorrhea, 11 (6.9%) had amenorrhea/oligomenorrhea and hyperandrogenism, and 8 (5.0%) had PCOM, amenorrhea/oligomenorrhea and hyperandrogenism.

As for the control group: only 1 patient met the Rotterdam Criteria for PCOS. This patient had both PCOM and hyperandrogenism.

Discussion

This large study shows that AMH serum levels are increased in women with HH compared with healthy regularly cycling ovulatory controls, but that their levels are lower than those of women with PCOS. Antral follicle count and total ovarian volume are, however, not increased in women with HH compared with healthy controls.

These findings are in discordance with results from a small study including 28 women with HH (16), which reported higher AMH levels and higher FNPO in women with HH compared with controls. However, of these 28 women, only 11 had validated data available for FNPO. This same group (24) also reported that women with HH had higher AMH levels and a higher mean ovarian volume compared with controls. These findings are also in discordance with results from a study published by Luisi et al (25), in which no significant difference in AMH was found between a group of women with hypothalamic amenorrhea and a group of women consisting of healthy controls.

We hypothesize that the increase in AMH levels in the HH group compared with the control group in the current study, whereas there was no increase in follicle count, can be explained by the presence of a relatively large pool of antral follicles <2 mm in diameter. These follicles are not counted during transvaginal ultrasonography although they do secrete AMH. Support for this explanation is found in the study performed by Weenen et al., (26) in which, after staining with AMH antibodies, histological specimens showed considerable AMH expression in antral follicles <2 mm in diameter. Moreover, although we did not find an increase in antral follicles in the HH group, increased numbers of antral follicles may not necessarily be required for elevated AMH levels. Pellatt et al. (27) suggest that AMH production per antral follicle may be increased in different pathological conditions leading to anovulation and could drive increased levels of AMH. This may also explain the higher AMH levels with similar follicle counts, as was also found in the study by Jonard et al. (28). On the 1 hand, the authors of this study suggest that increased AMH production per antral follicle might indeed be the cause for increased AMH levels

without a coincident increase in antral follicle count in women with hypothalamic amenorrhea. On the other hand, they also took into consideration the possibility of an increased number of small follicles undetectable on ultrasound because of their size.

Another explanation might be that the follicles that produce large amounts of AMH are the healthiest ones and therefore they will respond better to exogenous gonadotropins. Indeed, the predictive value of AMH levels in women with HH has been reported by van Elburg et al. They showed that as baseline levels of AMH increased, so did the probability of weight increase and recovery of menstrual cycles (29).

In line with the similar number of follicles in women from the HH group and control group, PCOM did not occur more often in women with HH compared with healthy ovulatory controls. Indeed, similar findings were reported in a study performed by Lie Fong et al. (3).

Biochemical hyperandrogenism did not occur more often in women with HH than in the control group, and testosterone levels were in fact, as to be expected (30), lower in women with HH. However, clinical hyperandrogenism did occur more often in women with HH than in healthy controls. Indeed, women with HH may experience, for example, acne, alopecia, and/or hirsutism (18), and physicians should be aware of these symptoms and discuss this with their patient. Moreover, treatment of these distressing complaints can be important steps in optimizing quality of life in these women. That clinical hyperandrogenism was observed more often in the HH group without an obvious increase in FAI may indicate disturbances at the level of the androgen receptor or androgen processing. Future research is warranted to explore this finding.

Interestingly, we found that more than one-third of the HH group met the Rotterdam Criteria for PCOS. Indeed, other studies (31) support this finding, showing that 86% women with HH met the Rotterdam Criteria for PCOS. This stresses the need for a correct distinction between HH and PCOS because false-positive diagnoses may lead to employment of treatment options that are suboptimal for these women.

In the latest guidelines for diagnosing PCOS (18), a cutoff criterion for PCOM is described as 20 antral follicles per ovary, instead of the 12 antral follicles per ovary we have used as a cutoff. Because our employed ultrasound had a capacity of 4 to 8 MHz, (and the guideline changed the threshold of PCOM because of improving technologies with improving bandwidth >8 MHz), we chose to maintain the analyses using the cutoff of 12 antral follicles per ovary. Regardless, when changing the cutoff to 20 antral follicles per ovary,

we found that 20.3% met the Rotterdam Criteria for PCOS. That is still more than one-fifth of women who have hypothalamic hypogonadism.

In light of recent debate whether the cumbersome ultrasound assessment of PCOM might be replaced in time by a single AMH measurement (12), the need for initial assessment of FSH, LH, and E₂ levels becomes even more important. In particular, those women with HH and elevated levels of AMH will be erroneously misdiagnosed as having PCOS.

In conclusion, we report increased AMH levels in women with HH compared with healthy controls, and we therefore stress the importance of measuring gonadotropins and E₂ in the diagnostic workup of anovulatory patients because AMH and sonographic data are not specific enough to distinguish between PCOS and HH.

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Additional Information

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Data Availability: Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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