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ORIGINAL ARTICLE ***Reproductive endocrinology***

The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Müllerian hormone

M.P. Lauritsen[1,*], J.G. Bentzen[1], A. Pinborg[1], A. Loft[1], J.L. Forman[2], L.L. Thuesen[1], A. Cohen[3], D.M. Hougaard[3], and A. Nyboe Andersen[1]

[1]The Fertility Clinic, Section 4071, Copenhagen University Hospital, Rigshospitalet, DK-2100 Copenhagen, Denmark [2]Department of Biostatistics, University of Copenhagen, DK-1014 Copenhagen, Denmark [3]Department of Clinical Biochemistry and Immunology, Statens Serum Institute, DK-2300 Copenhagen, Denmark

*Correspondence address. Tel: +45-3545-1861; Fax: +45-3545-4946; E-mail: mette.petri.lauritsen@regionh.dk

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STUDY QUESTION: What is the prevalence in a normal population of polycystic ovary syndrome (PCOS) according to the Rotterdam criteria versus revised criteria including anti-Müllerian hormone (AMH)?

SUMMARY ANSWER: The prevalence of PCOS was 16.6% according to the Rotterdam criteria. When replacing the criterion for polycystic ovaries by antral follicle count (AFC) > 19 or AMH > 35 pmol/l, the prevalence of PCOS was 6.3 and 8.5%, respectively.

WHAT IS KNOWN ALREADY?: The Rotterdam criteria state that two out of the following three criteria should be present in the diagnosis of PCOS: oligo-anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries (AFC ≥ 12 and/or ovarian volume >10 ml). However, with the advances in sonography, the relevance of the AFC threshold in the definition of polycystic ovaries has been challenged, and AMH has been proposed as a marker of polycystic ovaries in PCOS.

STUDY DESIGN, SIZE, DURATION: From 2008 to 2010, a prospective, cross-sectional study was performed including 863 women aged 20–40 years and employed at Copenhagen University Hospital, Rigshospitalet, Denmark.

PARTICIPANTS/MATERIAL, SETTING, METHODS: We studied a subgroup of 447 women with a mean (±SD) age of 33.5 (±4.0) years who were all non-users of hormonal contraception. Data on menstrual cycle disorder and the presence of hirsutism were obtained. On cycle Days 2–5, or on a random day in the case of oligo- or amenorrhoea, sonographic and endocrine parameters were measured.

MAIN RESULTS AND THE ROLE OF CHANCE: The prevalence of PCOS was 16.6% according to the Rotterdam criteria. PCOS prevalence significantly decreased with age from 33.3% in women < 30 years to 14.7% in women aged 30–34 years, and 10.2% in women ≥ 35 years ($P < 0.001$). In total, 53.5% fulfilled the criterion for polycystic ovaries with a significant age-related decrease from 69.0% in women < 30 years to 55.8% in women aged 30–34 years, and 42.8% in women ≥ 35 years ($P < 0.001$). AMH or age-adjusted AMH *Z*-score was found to be a reliable marker of polycystic ovaries in women with PCOS according to the Rotterdam criteria [area under the curve (AUC) 0.994; 95% confidence interval (CI): 0.990–0.999] and AUC 0.992 (95% CI: 0.987–0.998), respectively], and an AMH cut-off value of 18 pmol/l and AMH *Z*-score of −0.2 showed the best compromise between sensitivity (91.8 and 90.4%, respectively) and specificity (98.1 and 97.9%, respectively). In total, AFC > 19 or AMH > 35 occurred in 17.7 and 23.0%, respectively. The occurrence of AFC > 19 or AMH > 35 in the age groups < 30, 30–34 and ≥ 35 years was 31.0 and 35.7%, 18.8 and 21.3%, and 9.6 and 18.7%, respectively. When replacing the Rotterdam criterion for polycystic ovaries by AFC > 19 or AMH > 35 pmol/l, the prevalence of PCOS was 6.3 or 8.5%, respectively, and in the age groups < 30, 30–34 and ≥ 35 years, the prevalences were 17.9 and 22.6%, 3.6 and 5.6%, and 3.6 and 4.8%, respectively.

LIMITATIONS, REASON FOR CAUTION: The participants of the study were all health-care workers, which may be a source of selection bias. Furthermore, the exclusion of hormonal contraceptive users from the study population may have biased the results, potentially excluding women with symptoms of PCOS.

WIDER IMPLICATIONS OF THE FINDINGS: AMH may be used as a marker of polycystic ovaries in PCOS. However, future studies are needed to validate AMH threshold levels, and AMH *Z*-score may be appropriate to adjust for the age-related decline in the AFC.

STUDY FUNDING/COMPETING INTEREST(S): None.

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Key words: polycystic ovary syndrome / prevalence / Rotterdam criteria / diagnosis / anti-Müllerian hormone

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age. The key features of PCOS are ovulatory dysfunction, hyperandrogenism and the morphological appearance of polycystic ovaries (Balen and Michelmore, 2002). Prevalence estimates lie between 6 and 26% depending on the population and the diagnostic criteria applied (Diamanti-Kandarakis *et al.*, 1999; Michelmore *et al.*, 1999; Azziz *et al.*, 2004).

Over the last decades, three different classification systems have been used. The National Institutes of Health (NIH) criteria include only hyperandrogenism and oligo-anovulation in the diagnosis of PCOS (Zawadzki and Dunaif, 1992). According to the Androgen Excess and PCOS Society (AE-PCOS) criteria, hyperandrogenism is also central in the diagnosis in combination with oligo-anovulation and/or polycystic ovaries (Azziz *et al.*, 2006). The Rotterdam criteria define PCOS by the presence of at least two out of three criteria: oligo-anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries (≥12 follicles measuring 2–9 mm in diameter, or ovarian volume >10 ml in at least one ovary) (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

In recent years, the relevance of the antral follicle count (AFC) threshold in the Rotterdam classification has been challenged. With the development of more refined ultrasound equipment, it is now possible to visualize a higher number of antral follicles leading to a potential overdiagnosis of polycystic ovaries and PCOS, especially in younger women (Duijkers and Klipping, 2010; Johnstone *et al.*, 2010; Kristensen *et al.*, 2010).

To better standardize the criteria, anti-Müllerian hormone (AMH), a glycoprotein produced by the granulosa cells of small antral follicles (Durlinger *et al.*, 2002; Weenen *et al.*, 2004; Jeppesen *et al.*, 2013), has been proposed as a substitute for polycystic ovaries in the diagnosis of PCOS (Pigny *et al.*, 2006; Dewailly *et al.*, 2011; Eilertsen *et al.*, 2012). Serum AMH level seems to be highly correlated with the number of antral follicles (Weenen *et al.*, 2004; La Marca *et al.*, 2009), and the AMH concentration is therefore high in women with polycystic ovaries (Pigny *et al.*, 2003). The age-related decline in the AFC is also reflected in the AMH concentration (van Rooij *et al.*, 2005). Recently, data on AMH level during normal reproductive ageing have been published, allowing age-adjusted reference values of AMH to be created for clinical use (Hagen *et al.*, 2010; La Marca *et al.*, 2012; Bentzen *et al.*, 2013).

The aim of this study was to estimate the prevalence of PCOS according to the Rotterdam criteria in a large cohort of normal women, including prevalence estimates in different age groups. Furthermore, we aimed to evaluate the use of AMH in PCOS and to assess a revised version of the Rotterdam criteria in our study population, using AFC > 19 or AMH > 35 pmol/l as a substitute for polycystic ovaries in PCOS (Dewailly *et al.*, 2011).

Materials and Methods

Participants

Our data originate from a prospective, cross-sectional cohort study carried out at Copenhagen University Hospital, Rigshospitalet, Denmark, between 2008 and 2010. The study design has previously been described in detail (Bentzen *et al.*, 2013). Participants were recruited from a list of employed female health-care workers, aged 20–40 years, provided by the Human Resource Department at Copenhagen University Hospital, Rigshospitalet, Denmark. In all, 2696 women were invited by regular mail to participate in the study. The response rate was 52.1% and the inclusion rate was 32.0%. Among the 1092 women who agreed to participate, 229 completed the questionnaire but failed to undergo the clinical examination.

Out of the 863 participants, 447 were included in the present study. The majority were nurses (63.5%), and some were medical doctors (6.5%), academics (6.5%), laboratory technicians (6.5%), midwifes (4.0%), secretaries (4.0%) and other (8.9%). In all, 97.3% were Caucasian. Causes for exclusion were the use of the levonorgestrel-releasing intrauterine device (IUD) or progesterone-only contraceptives ($n = 64$), pregnancy ($n = 67$), lack of assessment of AFC or volume in both ovaries ($n = 28$), lactational amenorrhoea ($n = 13$), start of oral contraceptives (OCs) or contraceptive vaginal ring treatment at the time of screening ($n = 7$), lack of serum androgen measurement ($n = 3$), previous cancer and ovarian pathology ($n = 2$) and other ($n = 1$). Furthermore, users of OCs or contraceptive vaginal ring ($n = 231$) were excluded from the study population but included in a separate analysis of PCOS characteristics (Fig. 1).

Reproductive history

Before the clinical examination, an internet-based questionnaire was completed by all participants, gathering information about menstrual cycle length, gynaecological and reproductive history, use of hormonal contraceptives, pregnancies, deliveries and lactation, socio-economic status, life style factors, body weight and height, acne, alopecia and the degree of hirsutism according to self-reported modified Ferryman–Gallwey (m-FG) score (Hatch *et al.*, 1981).

Clinical assessment

On Days 2–5 of the menstrual cycle, or on a random day in the case of oligo- or amenorrhoea, a transvaginal ultrasound examination was performed and blood samples were drawn. At the time of the clinical examination, the investigator was unaware of the participants' questionnaire answers.

Sonography

The total number of antral follicles for each ovary was counted by scrolling through the ovary in two planes, longitudinal and transverse. All follicles measuring 2–10 mm were considered to be antral follicles. The volume of each ovary was estimated using the formula for an ellipsoid (Rosendahl *et al.*, 2010). Mean values of the volume and follicle count of the right and left ovary were calculated. All ultrasound examinations were performed by

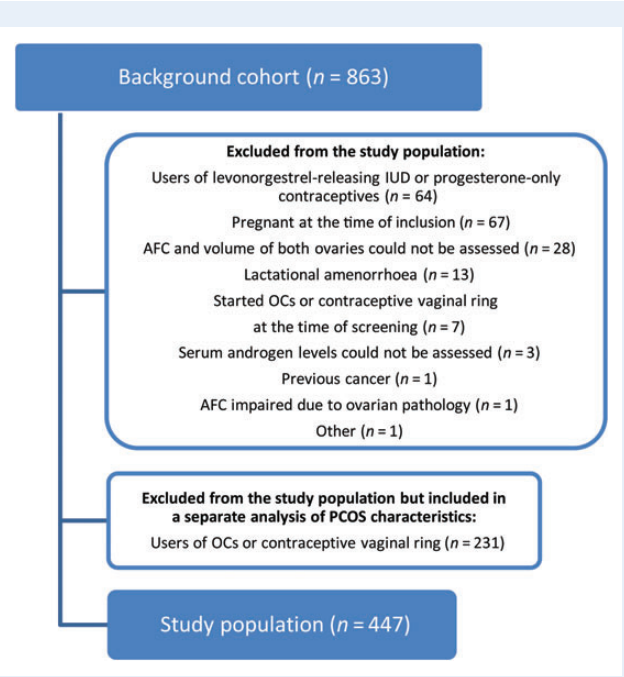

Figure 1 Flow chart showing the cohort of female health-care workers included in the study from 2008 to 2010 and the selection of the study population. *n*, number; IUD, intrauterine device; AFC, antral follicle count; OCs, oral contraceptives.

the same investigator (J.G.B.) using a BK Medical, Denmark, pro focus ultrasound scanner with a 4–9 MHz transducer.

Endocrine assessment

Serum levels of AMH, FSH, estradiol (E_2), LH, total testosterone (T) androstenedione, sex hormone-binding globulin (SHBG), free T, dihydroepiandrosterone sulphate (DHEAS) and 17-hydroxyprogesterone (17-OHP) were measured. Fresh blood samples were centrifuged at 3000*g* for 12 min, and serum samples were stored at −24°C until analysis. Analyses of AMH, FSH, E_2 and LH levels were performed at the Department of Clinical Biochemistry, Copenhagen University Hospital, Rigshospitalet, Denmark. Serum AMH levels were assessed in duplicate by an enzyme-linked immunosorbent assay using the AMH/MIS kit (Immunotech, Beckman Coulter, Marseilles, France). The sensitivity and the intra- and inter-assay coefficients of variation were 0.7 pmol/l, 12.3 and 14.2%, respectively. Serum levels of FSH, E_2 and LH were determined by electrochemiluminescence immunoassay using the E170 kit (Roche, Mannheim, Germany). The sensitivity and the intra- and inter-assay coefficients of variation were FSH < 0.1 IU/l, 2.8 and 4.5%, respectively; E_2 0.02 nmol/l, 3.3 and 4.7%, respectively, and LH 0.1 IU/l, 1.2 and 2.2%, respectively.

Analyses of total T, androstenedione, SHBG, free T, DHEAS and 17-OHP levels were performed at the Department of Clinical Biochemistry and Immunology, Statens Serum Institute, Copenhagen, Denmark. Total T, androstenedione, DHEAS and 17-OHP were on-line extracted from serum and separated by reversed phase chromatography and detected by tandem mass spectrometry. The sensitivity and the intra- and inter-assay coefficients of variation were total T 0.1 nmol/l, 9 and 10%, respectively; androstenedione 0.3 nmol/l, 8 and 9%, respectively; DHEAS 35 nmol/l, 9 and 10%, respectively, and 17-OHP 0.4 nmol/l, 9 and 10%, respectively. SHBG levels were determined by immunofluorimetric assays. For SHBG, the sensitivity and the intra- and inter-assay coefficients of variation were 0.1 nmol/l, 4 and 6%, respectively. Free T was calculated from the measurement of total T and SHBG (Bartsch *et al.*, 1980).

Defining PCOS

Oligo-anovulation was defined as a cycle length > 35 days or amenorrhoea. Clinical hyperandrogenism was defined as the presence of hirsutism recorded as m-FG score ≥ 8. Biochemical hyperandrogenism was defined as elevated free T levels (>0.034 nmol/l). The finding of polycystic ovaries was defined as ≥12 follicles measuring 2–10 mm in diameter or ovarian volume >10 ml in at least one ovary. We included 10 mm follicles in the definition of antral follicles according to our clinic's standard of practice.

Statistics

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences 19.0 software for Windows (IBM Corporation, New York, USA) and R version 2.13.2. (R Development Core Team 2011, Vienna, Austria). Statistical significance was defined as a probability value of $P < 0.05$. Differences in the means of normally distributed quantitative data were analysed using Student's *t*-test and presented as means with standard deviations (SDs). For non-normally distributed quantitative data, medians with interquartile ranges (IQR) were reported, and differences were analysed with Wilcoxon–Mann–Whitney test. Pearson χ^2 test was used to compare distributions between two (or more) groups. Correlations between AMH and other variables were assessed using Spearman's rank correlation coefficient (r_s). We used the estimated age-specific means and SDs from the previously published work on AMH level during normal reproductive ageing to generate age-adjusted AMH *Z*-scores (Bentzen *et al.*, 2013). The *Z*-score or standard normal deviate expresses the level of AMH relative to the expected mean and SD for the particular age. Thus, a *Z*-score of 2.0 corresponds to an AMH level two SDs above the mean level for the particular age or roughly the upper 95% population limit for the particular age. While AMH decreases with age, the *Z*-score can be taken as a relative measure of high versus low AMH which is independent of the individual woman's age. Receiver operating characteristic (ROC) curves and complete sensitivity/specificity reports were used to evaluate both serum AMH and age-adjusted AMH *Z*-score as a tool in the diagnosis of PCOS. Adjustment for BMI and age was performed in a multiple logistic regression model.

Ethical approvals

The study was approved by the Ethics Committee of the Capital Region of Denmark (no. H-B-2007-129), and all participants gave verbal and written consent prior to inclusion.

Results

Study population

Table I summarizes baseline characteristics of the entire study population ($n = 447$), the PCOS ($n = 74$) and the non-PCOS ($n = 373$) groups. The mean (±SD) age of the study population was 33.5 (±4.0) years. Women with PCOS were slightly but significantly younger than women without PCOS. There was no significant difference between the two groups with respect to parity or fertility treatment. The mean (±SD) BMI in the study population was 23.1 (±3.5) kg/m^2. Women with PCOS had significantly higher BMI and higher occurrence of obesity and hirsutism than women without PCOS. There was no between-group difference in waist–hip ratio, acne or alopecia. As expected, the LH/FSH ratio was significantly higher in the PCOS than in the non-PCOS group. Median (IQR) serum AMH in the study population was 20.3

Table I Age, fertility, clinical, hormonal and ultrasound characteristics in the entire study population and in the PCOS and non-PCOS groups according to the Rotterdam criteria.

Variables	All (n = 447)	PCOS (n = 74)	Non-PCOS (n = 373)	P-value[a]
Age (years)	33.5 ± 4.0	31.5 ± 3.9	33.9 ± 3.9	<0.001[b]
Previous childbirth[c]	241 (77.2)	29 (67.4)	212 (78.8)	0.10[d]
Previous fertility treatment[e]	46 (13.2)	9 (19.1)	37 (12.3)	0.20[d]
BMI (kg/m^2)	23.1 ± 3.5	24.2 ± 4.2	22.9 ± 3.4	0.02[b]
Overweight (BMI 25.0–29.9 kg/m^2)	61 (13.6)	12 (16.2)	49 (13.1)	0.48[d]
Obesity (BMI ≥ 30.0 kg/m^2)	32 (7.1)	11 (14.9)	21 (5.6)	0.01[d]
Waist circumference (cm)[g]	80.7 ± 11.9	83.1 ± 12.5	80.2 ± 11.8	0.30[b]
Hip circumference (cm)[h]	97.5 ± 13.1	100.1 ± 13.2	97.1 ± 13.0	0.06[b]
Waist/hip ratio	0.83 ± 0.1	0.84 ± 0.16	0.83 ± 0.09	0.93[b]
Hirsutism (mF-G score ≥ 8)	30 (6.7)	20 (27.0)	10 (2.7)	<0.001[d]
Acne	221 (49.4)	36 (48.6)	185 (49.6)	0.88[d]
Alopecia	32 (7.2)	7 (9.5)	25 (6.7)	0.40[d]
FSH (IU/l)	7.2 ± 3.4	6.4 ± 1.4	7.3 ± 3.6	0.02[b]
LH (IU/l)	5.9 ± 2.7	6.4 ± 2.3	5.8 ± 2.7	0.09[b]
LH/FSH ratio	0.87 ± 0.43	1.08 ± 0.72	0.83 ± 0.33	<0.001[b]
E_2 (nmol/l)	0.16 (0.13–0.21)	0.18 (0.12–0.19)	0.16 (0.13– 0.22)	0.11[f]
AMH (pmol/l)	20.3 (10.9–34.0)	35.6 (22.2–62.9)	17.8 (9.8– 29.4)	<0.001[f]
Free T (nmol/l)	0.017 (0.012–0.026)	0.037 (0.026–0.046)	0.010 (0.006–0.014)	<0.001[f]
SHBG (nmol/l)	64.1 ± 24.0	52.9 ± 21.3	66.3 ± 23.0	<0.001[b]
Androstenedione (nmol/l)	4.93 (3.41–7.01)	8.74 (6.05–11.40)	4.61 (3.14–6.26)	<0.001[f]
Total T (nmol/l)	1.15 (0.80–1.68)	2.10 (1.39–2.89)	1.11 (0.75–1.53)	<0.001[f]
DHEAS (nmol/l)	5128.6 (3322.0–8022.3)	8650.6 (5481.0–11404.5)	4945.1 (3123.8–7302.6)	<0.001[f]
17-OHP (nmol/l)	1.38 (0.94–1.96)	2.12 (1.41–2.90)	1.33 (0.91–1.73)	<0.001[f]
AFC 2–10 mm, total	22.8 ± 12.3	32.8 ± 11.8	20.8 ± 11.4	<0.001[b]
AFC ≥ 12 in at least one ovary	234 (52.3)	73 (98.6)	161 (43.2)	<0.001[d]
Ovarian volume, mean (ml)	5.4 ± 2.1	6.8 ± 2.1	5.1 ± 2.0	<0.001[b]
Ovarian volume >10 ml in at least one ovary	40 (8.9)	16 (21.6)	24 (6.4)	<0.001[d]

Data are presented as mean ± standard deviation, n (percentage) or median (interquartile range). mF-G, modified Ferryman-Gallwey; E_2, estradiol; AMH, anti-Müllerian hormone; T, testosterone; SHBG, sex hormone-binding globulin; DHEAS, dihydroepiandrosterone sulphate; 17-OHP, 17 hydroxyprogesterone; AFC, antral follicle count.
[a]P-value indicates the difference between the PCOS and non-PCOS groups.
[b]Student's t-test.
[c]Proportion among women previously trying to conceive (n = 312).
[d]Pearson χ^2 test.
[e]Proportion among women who have wished to become pregnant (n = 348).
[f]Wilcoxon–Mann–Whitney test.
[g]Missing values 4.
[h]Missing values 5.

(10.9–34.0) pmol/l. Women with PCOS had significantly higher median (IQR) AMH of 35.6 (22.2–62.9) pmol/l versus 17.8 (9.8–29.4) pmol/l in women without PCOS. Additionally, significantly higher free T, androstenedione, total T, DHEAS and 17-OHP levels and lower SHBG levels were found in the PCOS group. Women with PCOS had significantly higher total AFC (±SD) and mean ovarian volume (±SD) of 32.8 (±11.8) and 6.8 (±2.1) ml, respectively, versus 20.8 (±11.4) and 5.1 (±2.0) ml, respectively, in women without PCOS.

Characteristics of PCOS

In total, 20 women (4.5%) fulfilled the criterion for oligo-anovulation, 79 (17.7%) had clinical and/or biochemical hyperandrogenism and 239 (53.5%) had polycystic ovaries (Table II). Each of the three Rotterdam criteria was more frequently fulfilled in women below 30 years. Oligo-anovulation occurred in 13.1% of women below 30 years, 2.5% of women aged 30–34 years and 2.4% of women aged 35 years or over. Clinical and/or biochemical hyperandrogenism occurred in 28.6% of women below 30 years, 16.8% of women aged 30–34 years and 13.3% of women aged 35 years or over. The age-related decline in clinical and/or biochemical hyperandrogenism was due to free T level decreasing with age, whereas hirsutism did not differ significantly between age groups. The sonographic criterion for polycystic ovaries was fulfilled in 69.0% of women below 30 years compared with 55.8% of women aged 30–34 years and 42.8% of women aged 35 years or over. Table II also shows the occurrence of characteristics of clinical

Table II Characteristics of PCOS according to the Rotterdam criteria in the entire study population and in different age groups.

	All (n = 447)	<30 years (n = 84)	30–34 years (n = 197)	≥35 years (n = 166)	P- value[a]
Rotterdam criteria					
Oligo-anovulation	20 (4.5)	11 (13.1)	5 (2.5)	4 (2.4)	<0.001
Clinical and/or biochemical hyperandrogenism	79 (17.7)	24 (28.6)	33 (16.8)	22 (13.3)	0.01
Hirsutism	30 (6.7)	6 (7.1)	12 (6.1)	12 (7.2)	0.90
Elevated free T[b]	55 (12.3)	18 (21.4)	23 (11.7)	14 (8.4)	0.01
Polycystic ovaries	239 (53.5)	58 (69.0)	110 (55.8)	71 (42.8)	<0.001
AFC ≥ 12	234 (52.3)	57 (67.9)	107 (54.3)	70 (42.2)	<0.001
Ovarian volume > 10 ml	40 (8.9)	14 (16.7)	17 (8.6)	9 (5.4)	0.01
Clinical hyperandrogenism (other than hirsutism)					
Acne	221 (49.4)	46 (54.8)	92 (46.7)	83 (50.0)	0.46
Alopecia	32 (7.2)	5 (6.0)	12 (6.1)	15 (9.0)	0.50
Biochemical hyperandrogenism (other than elevated free T)					
Elevated total T[c]	97 (21.7)	28 (33.3)	39 (19.8)	30 (18.1)	0.02
Elevated androstenedione[d]	63 (14.1)	19 (22.6)	23 (11.7)	21 (12.7)	0.04
Low SHBG[e]	70 (15.7)	13 (15.5)	29 (14.7)	28 (16.9)	0.85

Data are presented as n (percentage). T, testosterone; AFC, antral follicle count; SHBG, sex hormone-binding globulin.
[a]P-value indicates the difference between age groups (Pearson χ^2 test).
[b]> 0.034 nmol/l.
[c]> 1.8 nmol/l.
[d]> 8.9 nmol/l.
[e]< 41 nmol/l.

and/or biochemical hyperandrogenism other than hirsutism and elevated free T. No age-related decline was observed in acne or alopecia. The occurrence of elevated total T (> 1.8 nmol/l) and elevated androstenedione (> 8.9 nmol/l) significantly declined with age, whereas low SHBG (< 41 nmol/l) did not differ with age.

PCOS phenotypes

A total of 74 women in the study population fulfilled two out of three Rotterdam criteria giving a PCOS prevalence of 16.6% (Table III). The PCOS prevalence was significantly higher in women below 30 years (33.3%) than in women aged 30–34 years (14.7%) and 35 years or over (10.2%). The phenotype of oligo-anovulation and clinical and/or biochemical hyperandrogenism only occurred in 4 women (0.9%), whereas the phenotypes of polycystic ovaries in combination with either oligo-anovulation or clinical and/or biochemical hyperandrogenism occurred in 16 (3.6%) and 62 women (13.9%), respectively. Only 4 women (0.9%) fulfilled all three criteria. All women who fulfilled the AE-PCOS or the NIH criteria for PCOS also fulfilled the Rotterdam criteria.

Users of hormonal contraception

Characteristics of PCOS according to the Rotterdam criteria in the excluded group of users of OCs or contraceptive vaginal ring (n = 231) were assessed in a separate analysis. Oligo-anovulation could not be assessed as data on menstrual cycle characteristics prior to the use of hormonal contraception were not available. In total, 20 women (8.7%) fulfilled the criteria for clinical and/or biochemical hyperandrogenism, and 94 (41.4%) had polycystic ovaries (4 missing values). Women using OCs or contraceptive vaginal ring had a significantly lower prevalence of both clinical and/or biochemical hyperandrogenism and polycystic ovaries than observed in the study population [8.7 versus 17.7% (P = 0.002) and 41.4 versus 53.5% (P = 0.003), respectively].

AMH in the diagnosis of polycystic ovaries and PCOS

The use of AMH as a predictor of polycystic ovaries according to the Rotterdam criteria was evaluated by constructing an ROC curve (Fig. 2a). Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular AMH cut-off value. The area under the curve (AUC) for AMH identifying polycystic ovaries was 0.906 [95% confidence interval (CI): 0.878–0.933]. The best compromise between sensitivity and specificity was found at an AMH cut-off level of 20 pmol/l, diagnosing 82.0% of women with polycystic ovaries and correctly identifying 84.6% as not having polycystic ovaries. When adjusting for BMI and age, the predictive power of AMH in identifying polycystic ovaries was essentially unchanged (AUC 0.905; 95% CI: 0.878–0.933) (curve not shown).

The AUC for AMH identifying AFC ≥ 12 was 0.909 (95% CI: 0.882–0.936) and the AUC for AMH identifying ovarian volume >10 ml was 0.804 (95% CI: 0.732–0.876). AMH could predict PCOS with an AUC of 0.783 (95% CI: 0.730–0.836), oligo-anovulation with an AUC of 0.788 (95% CI: 0.699–0.877) and clinical and/or biochemical

Table III Prevalence of PCOS and PCOS phenotypes (A–D) in the entire study population and in different age groups according to the Rotterdam, AE-PCOS and NIH criteria.

	All (n = 447)	< 30 years (n = 84)	30–34 years (n = 197)	≥ 35 years (n = 166)	P-value[a]
Rotterdam criteria					
Prevalence of PCOS[b]	74 (16.6)	28 (33.3)	29 (14.7)	17 (10.2)	<0.001
(A) Oligo-anovulation + clinical and/or biochemical hyperandrogenism	4 (0.9)	2 (2.4)	1 (0.5)	1 (0.6)	0.28
(B) Oligo-anovulation + polycystic ovaries	16 (3.6)	9 (10.7)	4 (2.0)	3 (1.8)	<0.001
(C) Clinical and/or biochemical hyperandrogenism + polycystic ovaries	62 (13.9)	21 (25.0)	26 (13.2)	15 (9.0)	<0.001
(D) Oligo-anovulation + polycystic ovaries + clinical and/or biochemical hyperandrogenism	4 (0.9)	2 (2.4)	1 (0.5)	1 (0.6)	0.28
AE-PCOS criteria (A + C)					
Prevalence of PCOS[b]	62 (13.9)	21(25.0)	26 (13.2)	15 (9.0)	0.002
NIH criteria (A)					
Prevalence of PCOS	4 (0.9)	2 (2.4)	1 (0.5)	1 (0.6)	0.28

Data are presented as n (percentage).
[a]P-value indicates the difference between age groups (Pearson χ^2 test).
[b]When adjusting for overlap between phenotypes.

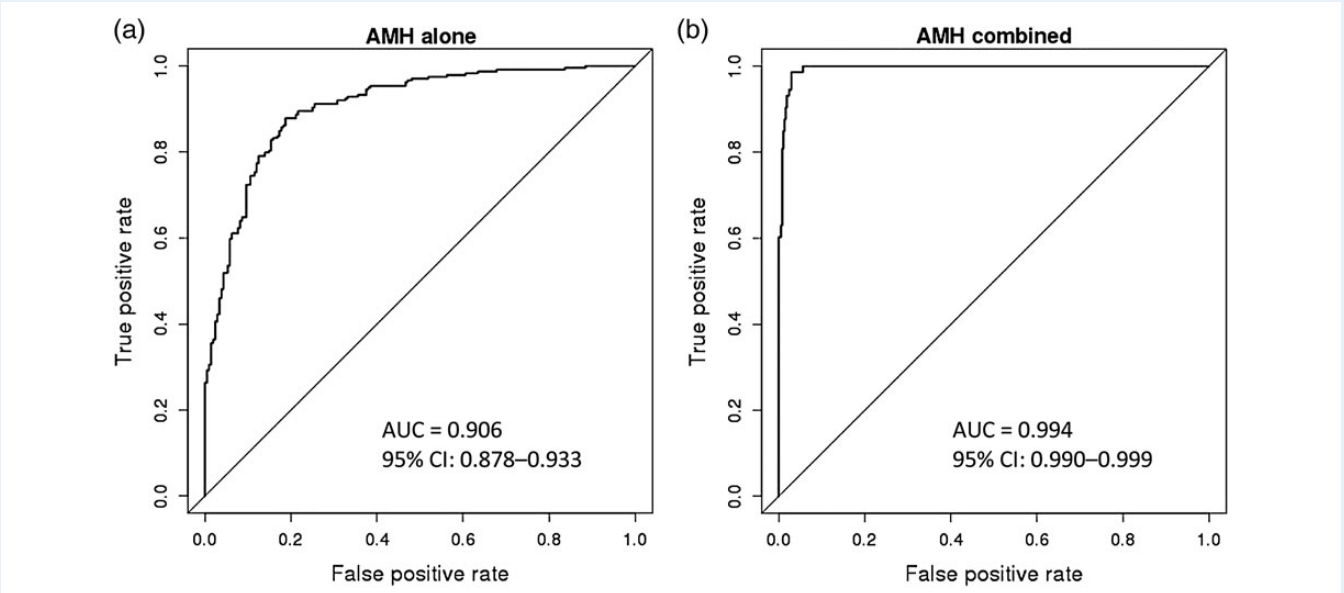

Figure 2 ROC curves with AUC and confidence interval (CI) for the area showing AMH (test variable) as a predictor of polycystic ovaries according to the Rotterdam criteria (state variable) (**a**) and AMH combined with clinical and/or biochemical hyperandrogenism and/or oligo-anovulation (i.e. AMH replaces the criterion for polycystic ovaries in the Rotterdam criteria; test variable) as a predictor of PCOS according to the Rotterdam criteria (state variable) (**b**).

hyperandrogenism with an AUC of 0.654 (95% CI: 0.587–0.722) (curves not shown).

Figure 2b shows the ROC curve obtained when different AMH cut-off values were used to replace the criterion for polycystic ovaries, and then combined with the other Rotterdam criteria for PCOS. The AMH-based diagnosis of PCOS thus requires the presence of at least two out of the following three criteria: oligo-anovulation, clinical and/or biochemical hyperandrogenism and/or AMH above different cut-off levels. The AUC was 0.994 (95% CI: 0.990–0.999), indicating an almost perfect fit of the AMH-based diagnosis of PCOS. The best compromise between sensitivity (91.8%) and specificity (98.1%) was found at an AMH cut-off level of 18 pmol/l (Table IV).

Table IV Results of ROC analysis showing the sensitivity and specificity of different AMH cut-off levels as predictors of polycystic ovaries in PCOS according to the Rotterdam criteria.

AMH cut-off (pmol/l)	PCOS (n = 73), sensitivity (%)	Non-PCOS (n = 374), specificity (%)
12	95.6	97.1
14	94.5	97.3
16	93.2	98.1
18	91.8	98.1
20	84.9	98.7
22	79.5	99.2
24	69.9	99.2

AMH Z-score

ROC curve analyses showed that the age-adjusted AMH Z-score could predict polycystic ovaries according to the Rotterdam criteria with an AUC of 0.884 (95% CI: 0.853–0.915) (curve not shown). When different AMH Z-score levels were used to replace polycystic ovaries in combination with clinical and/or biochemical hyperandrogenism and/or oligo-anovulation, the Rotterdam-based diagnosis of PCOS could be predicted with an AUC of 0.992 (95% CI: 0.987–0.998) (curve not shown). The best compromise between sensitivity (90.4%) and specificity (97.9%) was found at an AMH Z-score level of −0.2 (report not shown). Fig. 3 shows the AMH level according to age in the study population. The cut-off levels of AMH 18 pmol/l and AMH Z-score −0.2 replacing polycystic ovaries in the Rotterdam criteria are indicated. As shown, the AMH cut-off level tends to identify a higher proportion of women in the younger age group and a lower proportion in the older age group as having PCOS according to the Rotterdam criteria.

AFC > 19 or AMH > 35 pmol/l as predictors of polycystic ovaries

In line with the suggestion by Dewailly *et al.* (2011), we evaluated the use of AFC > 19 (in at least one ovary) or AMH > 35 pmol/l as a diagnostic marker of polycystic ovaries in the Rotterdam classification. In total, AFC > 19 occurred in 79 women (17.7%) and AMH > 35 pmol/l occurred in 103 (23.0%). As expected, both AFC > 19 and AMH > 35 pmol/l showed an age-related decline from 26 (31.0%) and 30 (35.7%) in women below 30 years to 37 (18.8%) and 42 (21.3%) in women aged 30–34 years and 16 (9.6%) and 31 (18.7%) in women aged 35 years or over, respectively (Table V). When applying the revised AFC > 19 or AMH > 35 pmol/l criteria to our study population, the prevalence of PCOS was 6.3 or 8.5%, respectively. The PCOS prevalence in different age groups was 17.9 and 22.6% in women below 30 years, 3.6 and 5.6% in women aged 30–34 years and 3.6 and 4.8% in women aged 35 years or over, respectively.

AMH correlations

AMH levels were positively correlated with LH ($r_s = 0.26$; $P < 0.001$), LH/FSH ratio ($r_s = 0.48$; $P < 0.001$), free T ($r_s = 0.25$; $P < 0.001$), total T ($r_s = 0.26$; $P < 0.001$), androstenedione ($r_s = 0.28$; $P < 0.001$) and 17-OHP ($r_s = 0.26$; $P < 0.001$). Additionally, there was a strong, positive correlation between AMH and total AFC (r_s 0.87; $P < 0.001$), and a positive correlation between AMH and mean ovarian volume ($r_s = 0.62$; $P < 0.001$). AMH was negatively correlated with age ($r_s = -0.29$; $P < 0.001$), FSH ($r_s = -0.32$; $P < 0.001$) and E_2 ($r_s = -0.16$; $P = 0.001$). There was no significant correlation between AMH and BMI, obesity (BMI ≥ 30.0 kg/m^2), waist/hip ratio, hirsutism, SHBG and DHEAS levels, respectively.

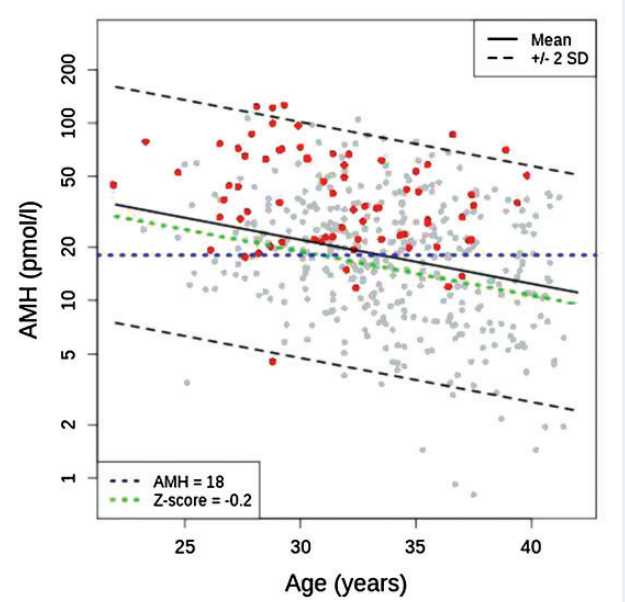

Figure 3 AMH level according to age in the study population (n = 447). The AMH and AMH Z-score cut-off levels identified in this study as markers of polycystic ovaries in women with PCOS according to the Rotterdam criteria are indicated by the blue and green dotted lines, respectively. Participants fulfilling the Rotterdam criteria for PCOS are indicated by red dots. Mean = geometric mean; SD, standard deviation.

Discussion

In this study population, the overall prevalence of PCOS was 16.6% according to the Rotterdam criteria. To our knowledge, this is the largest prevalence study in a Caucasian population including an assessment of endocrine and sonographic parameters in all participants.

March *et al.* (2010) conducted a community-based study of 728 women and reported a PCOS prevalence of 11.9% according to the Rotterdam criteria. However, only 108 women consented to a transvaginal ultrasound examination to confirm the presence of polycystic ovaries which may explain that the prevalence of PCOS was lower than observed in our study. In a study by Eilertsen *et al.* (2012), 21.4% of the participants fulfilled the Rotterdam criteria for PCOS. The study included 262 women with prior preterm birth and their controls with prior full-term birth, and all participants underwent an ultrasound examination. As also recognized by the authors, the results are biased by including only parous women who may have a milder form of PCOS. Still, the prevalence of

Table V Prevalence of PCOS and PCOS phenotypes (A–D) in the entire study population and in different age groups according to the Rotterdam criteria when replacing the criterion for polycystic ovaries by AFC > 19 or AMH > 35 pmol/l.

	All (n = 447)	< 30 years (n = 84)	30–34 years (n = 197)	≥ 35 years (n = 166)	P-value[a]
AFC > 19 criterion					
AFC > 19	79 (17.7)	26 (31.0)	37 (18.8)	16 (9.6)	<0.001
Prevalence of PCOS[b]	28 (6.3)	15 (17.9)	7 (3.6)	6 (3.6)	<0.001
(A) Oligo-anovulation + clinical and/or biochemical hyperandrogenism	4 (0.9)	2 (2.4)	1 (0.5)	1 (0.6)	0.28
(B) Oligo-anovulation + AFC > 19	10 (2.2)	7 (8.3)	1 (0.5)	2 (1.2)	<0.001
(C) Clinical and/or biochemical hyperandrogenism + AFC > 19	20 (4.5)	10 (11.9)	7 (3.6)	3 (1.8)	0.001
(D) Oligo-anovulation + AFC > 19 + clinical and/or biochemical hyperandrogenism	3 (0.7)	2 (2.4)	1 (0.5)	0 (0.0)	0.09
AMH > 35 pmol/l criterion					
AMH > 35 pmol/l	103 (23.0)	30 (35.7)	42 (21.3)	31 (18.7)	0.01
Prevalence of PCOS[b]	38 (8.5)	19 (22.6)	11 (5.6)	8 (4.8)	<0.001
(A) Oligo-anovulation + clinical and/or biochemical hyperandrogenism	4 (0.9)	2 (2.4)	1 (0.5)	1 (0.6)	0.28
(B) Oligo-anovulation + AMH > 35 pmol/l	11 (2.5)	7 (8.3)	2 (1.0)	2 (1.2)	0.001
(C) Clinical and/or biochemical hyperandrogenism + AMH > 35 pmol/l	31 (6.9)	14 (16.7)	10 (5.1)	7 (4.2)	<0.001
(D) Oligo-anovulation + AMH > 35 pmol/l + clinical and/or biochemical hyperandrogenism	4 (0.9)	2 (2.4)	1 (0.5)	1 (0.6)	0.28

Data are presented as n (percentage).
[a]P-value indicates the difference between age groups (Pearson χ^2 test).
[b]When adjusting for overlap between phenotypes.

PCOS was higher than observed in our study due to a higher prevalence of oligo-anovulation (12.2 versus 4.5%) and clinical and/or biochemical hyperandrogenism (22.9 versus 17.7%). Eilertsen *et al.* (2012) included both androstenedione, free T index and total T in biochemical hyperandrogenism which may have increased the prevalence. The prevalence of polycystic ovaries was comparable to our findings (43.1 versus 53.5%). In a study by Yildiz *et al.* (2012), including 392 female employees from a government-based institute and reporting ultrasound measures in all participants, the prevalence of PCOS was 19.9% according to the Rotterdam criteria. Again, several androgens were assessed, and a lower F-G score threshold level (≥6) was used to define hirsutism, leading to a prevalence of clinical and/or biochemical hyperandrogenism of 24.8%. Furthermore, a higher prevalence of oligo-anovulation was reported compared with our findings (15.3 versus 4.5%), whereas the prevalence of polycystic ovaries was lower (36.5 versus 53.5%). The mean age of participants in the studies by March *et al.*, Eilertsen *et al.* and Yildiz *et al.* was comparable to our findings, and the populations were all primarily of Caucasian ethnicity. However, none of the three studies excluded users of hormonal contraception which may have influenced the results.

In our study population, a very large proportion of women had polycystic ovaries on ultrasound, especially in the youngest age group. Thus, the Rotterdam criterion for polycystic ovaries was fulfilled in 69.0% of women below 30 years, leading to a more than 3-fold higher prevalence of PCOS in this age group than in the age group of 35 years or above. In a study including 171 female volunteers, 84% of the 25 women in the age group between 18 and 22 years had polycystic ovaries (Duijkers and Klipping, 2010). A larger study showed that 68% of 154 young women with a mean age of 20 years had polycystic ovaries according to the Rotterdam criteria (Kristensen *et al.*, 2010).

Our findings require three main considerations.

First, the AFC threshold ≥12 in the Rotterdam classification may, as previously suggested, need revision. In a recent study, Dewailly *et al.* (2011) proposed an AFC threshold > 19 (or AMH > 35 pmol/l) as substitute criteria for polycystic ovaries in PCOS. When applying the AFC > 19 criterion to our study population, the prevalence of polycystic ovaries was lowered from 69.0 to 31.0% in women below 30 years and the prevalence of PCOS was lowered from 16.6 to 6.3%.

Secondly, it should be considered whether the AFC and/or ovarian volume can be successfully substituted by another parameter like AMH. Even though a single, standardized AMH assay is not yet available, it is well established that the AMH concentration is correlated with the AFC and increased in women with polycystic ovaries (Laven *et al.*, 2004). The role of AMH as a diagnostic marker in PCOS was evaluated in a recent meta-analysis (Iliodromiti *et al.*, 2013). Reported AMH cut-off values ranged from 20 to 60 pmol/l due to variability in study design and selection of controls. In our study, we showed that AMH can be used as a substitute for the presence of polycystic ovaries in the Rotterdam classification for PCOS, but that it should not be used as a single marker of PCOS. AMH was not found to be a suitable marker of oligo-anovulation nor clinical and/or biochemical hyperandrogenism. An AMH cut-off level >18 pmol/l produced the best compromise between sensitivity

and specificity in the AMH-based diagnosis of PCOS according to the Rotterdam criteria. Similar results were found by Eilertsen *et al.* (2012), reporting an AMH cut-off level >20 pmol/l to be a reliable marker of polycystic ovaries in women with PCOS according to the Rotterdam criteria.

In 2011, Dewailly *et al.* (2011) proposed a cut-off value of AMH > 35 pmol/l with a sensitivity of 92% and a specificity of 97% (AUC 0.973) in the diagnosis of polycystic ovaries in PCOS. The results were based on data from 240 patients all referred for treatment of hyperandrogenism, menstrual disorders and/or infertility. Women with polycystic ovary morphology but no other symptoms of PCOS were excluded from the control group. When applying the AMH > 35 pmol/l criterion to our study population, the prevalence of polycystic ovaries was 23.0% and the overall prevalence of PCOS was 8.5%. These prevalence estimates of polycystic ovaries and PCOS seem, in our view, to be more biologically plausible in a normal population than the estimates according to the original Rotterdam criteria.

Thirdly, our study showed an age-related decline in all three Rotterdam criteria for PCOS, which was especially pronounced for the occurrence of polycystic ovaries. This underlines the need for age-adjusted criteria for polycystic ovaries as previously suggested (Duijkers and Klipping, 2010; Kristensen *et al.*, 2010). We found that an age-specific AMH *Z*-score could be substituted for polycystic ovaries in women with PCOS according to the Rotterdam criteria with a high accuracy. An acceptable sensitivity and specificity was found at an AMH *Z*-score of −0.2. It should, of course, be taken into account that the low cut-off levels of both AMH and AMH *Z*-score reported in this study reflect the AFC/ovarian volume threshold defined by the Rotterdam criteria. While AMH and AMH *Z*-scores largely identify the same amount of true positive and true negative PCOS cases, AMH tends to diagnose a higher proportion among the young women and a lower proportion among the elder. For this reason, we believe that the use of AMH *Z*-score as a potential diagnostic marker in PCOS should be considered. The AMH *Z*-score adjusts for the age-related decline in AMH and indirectly adjusts for the age-related decline in antral follicles. This may matter considerably if the currently suggested threshold values for AMH should be altered in the future.

It could be argued that PCOS is primarily a disease of the young and that the high prevalence reported in this study actually reflects the high level of women remaining undiagnosed in the general population (Eilertsen *et al.*, 2012). As the true occurrence of PCOS is still unknown, this question remains unanswered. In our view, the risk of overdiagnosis of PCOS in young women with normal ovaries and a high AFC should be carefully considered as the diagnosis of PCOS may lead to increased anxiety and impaired well-being and quality of life (Jones *et al.*, 2008). However, we acknowledge the importance of diagnosing the women who are at high risk of developing diseases associated with PCOS, such as diabetes mellitus, metabolic syndrome and cardiovascular disease (Moran *et al.*, 2010; Wild *et al.*, 2010).

The present study has potential limitations. It may be a source of selection bias that all participants were health-care workers, representing a population that tend to be healthier than the general population as reflected by the lower rate of obesity in our study (7.1%) compared with women aged 25–34 years in the general Danish population (11.4%) (National Board of Health, 2010). Furthermore, the study population was restricted to non-users of hormonal contraception. This was done to obtain the most accurate measurements of PCOS characteristics possible. A recent study including data from our population showed that users of hormonal contraception had up to 30% lower AFC and AMH levels than non-users (Bentzen *et al.*, 2012), and moreover, hormonal contraceptives are known to reduce hyperandrogenism, another key feature of PCOS (Mulders *et al.*, 2005). However, as hormonal contraceptives can mask the symptoms of PCOS and are indeed prescribed to treat symptoms such as hirsutism and menstrual cycle disorders, it is likely that some PCOS women were excluded in our population. Thus, a low prevalence of oligo-anovulation in our study compared with other population-based studies may reflect this exclusion criterion. On the other hand, we showed that the candidate participants we excluded because of hormonal contraceptive use displayed a lower prevalence of hyperandrogenism and polycystic ovaries in line with the known effect of hormonal contraception, making it less likely that the prevalence of PCOS has been underestimated in our study population.

Furthermore, the definition of clinical and/or biochemical hyperandrogenism in PCOS is still debated (Barth *et al.*, 2007). The use of self-reported assessment of hirsutism in our study may not have been optimal even though the scoring system was carefully illustrated to the participants. We did not include acne or androgenic alopecia in clinical hyperandrogenism as these parameters are even more difficult to standardize. As a marker of biochemical hyperandrogenism, we used the measurement of free T in line with the Rotterdam consensus (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Had we defined biochemical hyperandrogenism by elevated total T instead of free T, the overall prevalence of PCOS would have increased to 21.5%. If total T and/or androstenedione had been used in the definition, the PCOS prevalence would have further increased to 23.5%.

To conclude, our data confirm that AMH is a reliable marker of polycystic ovaries in PCOS. Furthermore, prevalence estimates in our study population indicate a need of revision of the Rotterdam criterion for polycystic ovaries. The AFC and AMH criteria proposed by Dewailly *et al.* diminished the prevalence of PCOS in our study population to a more appropriate figure. However, future studies are required to validate the AMH threshold level. A revision of the Rotterdam criteria should also include age adjustments to avoid overdiagnosis of PCOS in young women, and *Z*-score is a way to adjust for the age-related decline in the number of antral follicles.

Authors' roles

M.P.L., J.G.B., A.P., A.L., J.L.F., L.L.T. and A.N.A participated in the study design and analysis and in the interpretation of data. J.G.B. established the cohort and the database. M.P.L. drafted the manuscript. A.C. and D.M.H. measured the androgens and advised concerning androgen assays. All authors have critically reviewed the manuscript and approved the final version.

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Conflict of interest

None declared.

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