Controversies in the diagnosis of polycystic ovary syndrome

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Abstract: Polycystic ovary syndrome is a common endocrinological condition which is found to be prevalent in 5–10% of women of reproductive age. Historically, a combination of anovulation and androgen excess was considered a hallmark in the diagnosis of polycystic ovary syndrome. Addition of ultrasound features of polycystic ovary syndrome has improved the detection of variation in the polycystic ovary syndrome phenotype. Despite the widespread use of consensus diagnostic criteria, there remain several unresolved controversies in the diagnosis of polycystic ovary syndrome. Difficulty arises in methods of assessment and types of androgens to be measured to detect biochemical hyperandrogenism, setting a cut-off value for the diagnosis of clinical hyperandrogenism, setting an ultrasound threshold of antral follicle count to diagnose polycystic ovaries and also diagnosing this condition in adolescence where there is no clear definition for ‘irregular cycles’. This article looks at various controversies in the diagnosis of polycystic ovary syndrome.

Keywords: PCOS, diagnosis, polycystic ovary syndrome

Received: 31 May 2020; revised manuscript accepted: 13 February 2020.
Despite the consensus diagnostic criteria being in common use globally, there still exist several controversies in the diagnosis. This article aims to summarise the various controversies arising in the diagnosis of PCOS.

**Irregular cycles and ovarian dysfunction**

It has been observed that almost three quarters of patients diagnosed with PCOS have abnormal menstruation. In adults, a regular cycle indicates any woman who is menstruating between 24 and 35 days, which suggests an ovulatory cycle. Irregular cycles, which reflect ovarian dysfunction and oligo-anovulation, form one of the essential features of the Rotterdam criteria.

Serum progesterone levels can be measured by radioimmunoassay in the mid-luteal phase to confirm ovulation. In women with irregular cycles, the test might need to be measured later in the cycle depending on the length of the cycle. The test might also need to be measured every 7 days till menstruation commences. If the menstrual cycles are very irregular, measuring serum progesterone is futile as diagnosis is based on clinical features and ovulation induction therapy is indicated. The lower limit of serum progesterone level to confirm ovulation ranges from 16 to 28 nmol/L.

The greatest controversy hovering around this diagnostic criterion remains in defining ‘irregular cycles’ during pubertal transition. The accurate diagnosis is still challenging due to very limited evidence on this subject. During pubertal transition, distinguishing irregular cycles due to reproductive immaturity from those due to PCOS is quite challenging due to lack of a clear definition. In the first 2–3 years following menarche, the cycles may be irregular and most cycles range between 21 and 45 days. Studies show that the lower limit of a normal cycle is 21 days. The upper limit is somewhat variable at 40–45 days. Cycles more than 90 days represent 95th percentile for length, and this should warrant further assessment even if seen in the first gynaecological year where gynaecological age is conceptualised as number of years after menarche. Three years after menarche, most cycles are like adults.

Recent data suggest that in the first-year post menarche, about half of the cycles are anovulatory. Most of them occur in a range of 21–45 days,
lasting 2–7 days. Two years post menarche, 80% of irregular cycles tend to be ovulatory. By the third-year post menarche, 95% of cycles are regular and ovulatory and it is in these 5% of girls with irregular cycles that PCOS should be considered.19–22

The ESHRE international evidence-based guidelines recommendations around irregular cycles in adolescents are based on paediatric consensus opinion. This consensus recommends that if an adolescent girl has irregular periods (<21 days or >35 days) even after 3 years of menarche, she should be assessed for PCOS.22

If a girl has irregular cycles with many months of period-free intervals, particularly if she has signs of hyperandrogenism in the first few gynaecologic years, she should be assessed to rule out PCOS rather than reassuring it to be a normal phase of pubertal transition.20,23

Many adolescents may be considered or may already be on oral contraceptive pill (OCP) treatment. The ESHRE international guidelines recommend assessment of menstrual cycle patterns as well as assessment of clinical and biochemical hyperandrogenism before commencing OCP therapy in adolescents with irregular cycles after 1 year of menarche. If baseline assessment is abnormal, they must be explained about the risk of PCOS and might warrant further reassessment. If a baseline assessment has not been undertaken, it may be appropriate to stop the pills for 3 months and then assess to rule out PCOS.22

To summarise, it is still unclear when adolescent menstrual irregularity becomes pathophysiological. In adolescents, setting a clear-cut boundary between PCOS and normal physiological immaturity of hypothalamic pituitary axis is controversial. This could in turn potentially lead to over- or underdiagnosis which could lead to change in overall prevalence of the condition. Identifying the natural course of PCOS in young girls as well as early predictors by further longitudinal studies will allow appropriate and timely diagnosis.

Biochemical hyperandrogenism
It has been found that more than three quarters of women who have PCOS have increased circulating androgen levels.6 PCOS can be picked up by assessing biochemical hyperandrogenism especially in women who lack the signs of hirsutism or have unclear signs of hirsutism. Controversy arises on which androgens to measure, defining normal ranges for these, which assays to use, cost factors, access to high-quality tests and also overlap between control and PCOS patients.

What androgens to measure
The androgens that are often measured include total testosterone (TT), free testosterone (FT), calculated bioavailable testosterone (BA-T), calculated free testosterone (calculated FT) using the formula of Vermeulen and colleagues,24 free androgen index [FAI = 100 × (total testosterone/sex hormone-binding globulin [SHBG])], dehydroepiandrosterone sulphate (DHEAS) and androstenedione.

The use of TT can identify 20–30% of PCOS women as hyperandrogenemic. However, only 1–3% of testosterone is unbound to plasma proteins, thus raising concerns whether TT or FT is the most clinically useful measure. FT can identify 50–60% of such women with PCOS as hyperandrogenemic. The levels of SHBG are reduced in women with PCOS resulting in a further increase in FT. Androstenedione can be mild to moderately elevated in PCOS. Androstenedione can be elevated in PCOS, but marked rise indicates adrenal pathology especially 21-OH deficient nonclassic congenital adrenal hyperplasia (CAH). Elevated levels of 17 hydroxyprogesterone can indicate nonclassic CAH, 21-hydroxylase deficiency type, which is a milder and late onset form of CAH.

Another useful measure is FAI, which is the ratio of TT to SHBG multiplied by 100 and hence its measurement requires accurate measurement of testosterone and SHBG. FAI results can be biased by inaccuracies in measurements of testosterone and SHBG.25 Studies show an acceptable correlation between FAI and FT.25

Hahn and colleagues26 included 133 untreated PCOS patients and 54 healthy control women and measured androgens which included TT and SHBG, luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione, DHEAS and albumin. They concluded that PCOS patients had a significantly higher levels of
androgens responsible for biochemical hyperandrogenism than controls (all \( p < 0.0001 \)). The highest area under receiver operating characteristic curve (AUC-ROC) in decreasing order was found for BA-T (0.852), FAI (0.847) and FT (0.837). AUC-ROC was found to be lower for SHBG, TT and androstenedione. The correlation of FAI and BA-T with TT, androstenedione, LH/FSH ratio and DHEAS was found to be statistically significant with \( p \) value < 0.05 for all the parameters. In addition, FT, BA-T and FAI correlated well with other nonbiochemical parameters like hirsutism as well as ultrasound parameters of PCOS. Escobar-Morreale and colleagues recruited 114 women and measured various androgens as well as FSH, LH and estradiol. Based on ROC calculated for all, they strongly suggested that higher levels of FAI, FT and DHEA and reduced SHBG were effective measures for detection of PCOS. Villarroel and colleagues recruited 26 hirsute girls with irregular cycles and 63 nonhirsute girls with regular cycles as controls. They concluded that the best diagnostic tests were FAI \( \geq 6.1 \) and testosterone \( \geq 2.4 \text{ nmol/L} \). Follicle number \( \geq 12 \) and ovarian volume (OV) \( \geq 10 \text{ mL} \) provided similar diagnostic accuracy. Anti-Mullerian hormone (AMH) did not prove to have high diagnostic accuracy.

Measuring TT comes with their own limitations. TT values show variation depending on the time of the day they were taken and that many similar steroids present in the circulation tend to interfere with the assay. Also, age- and gender-corrected reference values are lacking and there is no universally accepted testosterone calibrating standard. Based on all the evidence, ESHRE PCOS guideline group 2018 concluded that though there is inadequate evidence to recommend which androgens to measure, they suggest that FT provides the best accuracy to detect biochemical hyperandrogenism. The other hormones that could be tested are TT, DHEAS and androstenedione. DHEAS and androstenedione on its own do not provide additional information regarding hyperandrogenemia in PCOS.

Many women are on OCPs when they are seen in the clinics. Measuring these hormones cannot reliably assess hyperandrogenism as inherent increase in SHBG and reduction in gonadotropin-dependent androgen production due to medication effect. Hence before testing these hormones, OCPs should be discontinued for at least 3 months.

**Hormonal assays**

The assays that are used to measure androgens to diagnose PCOS include liquid chromatography–tandem mass spectrometry (LC-MS/MS), gas chromatographic mass spectrometry (GCMS), radioimmunoassay (RIA), chemiluminescence immunoassays (CLIA) and enzyme-linked immunosorbent assay (ELISA). Based on the studies and data available, measuring FT provides the best ability to detect biochemical hyperandrogenism. Unfortunately, direct assays to measure FT are not entirely reliable. Direct assays like RIA, ELISA and CLIA are technically simple, relatively inexpensive and can be automated. These measure TT. The testosterone measurement using these assays is designed for males where the levels are higher compared with lower levels in women. Hence their accuracy is limited at testosterone levels <300 ng/dL. Also, testosterone levels are overestimated with these assays, they are not standardised, are of insufficient precision and also show poor sensitivity. RIA and chromatography is widely used with well-documented reference values among different populations and has better sensitivity than CLIA and ELISA but is rather labour intensive, costly and time consuming.

MS after extraction and liquid (LC) or gas chromatography (GC) are highly accurate when validated properly but are expensive and current standardisation is still lacking. High-quality assays (LC-MS/MS and extraction/chromatography immunoassays) to assess total or unbound (free) testosterone provide the best possible accuracy. Many of these assays have their limitations in that the reference ranges in different laboratories vary widely and are often based on an arbitrary percentile or variances of the mean of the values observed in a population.

**Clinical hyperandrogenism**

Mild-to-moderate androgen excess is represented by hirsutism, alopecia and acne. Women with PCOS can present with one or more signs of hyperandrogenism.
**Hirsutism**

Hirsutism is described as terminal hair in male-like pattern in women. An overestimation of hirsutism can easily be made if body and facial vellus hair is wrongly perceived as terminal hair. Terminal hair is distinctive as they can grow beyond 5 mm. It is also important to bear in mind that different ethnic groups can have denser vellus hair and hence hirsutism can be overestimated. Confirming hirsutism can also be challenging as many women have often treated excess hair growth before presenting to the clinics.

One of the methods that is most widely used to assess hirsutism and grade its severity is the modified Ferriman–Gallwey (mFG) score. It includes assessment of nine body areas, and each area is visually scored from 0 to 4. A pictorial representation is then made.

The biggest controversy is defining a ‘cut-off’ value for the mFG score to diagnose hirsutism. There is a strong difference in the prevalence and severity of hirsutism in different ethnic groups. Although this is known, unfortunately, there appears to be little difference in the cut-off values for determining excess terminal facial and body hair as abnormal (i.e. defining ‘hirsutism’). The mFG cut-off score can be based on percentile with a score >6–8 consistent with the 95th percentile of unselected women.29–31 It can also be defined by a lower percentile (85th–90th percentile) or by cluster analysis where the score is analysed in relation to other features of PCOS. Many studies have concluded that a lower mFG score32 for black and white women compared with Asian women33 represents true abnormality. Thus, generalising a cut-off at the 95th percentile is not appropriate and hence the ESHRE PCOS guideline 2018 recommended the cut-off of ≥4–6 on mFG score. Overall, more than half of women with mFG scores of 3–534 and almost three fourths (>70–90%) of women with scores >529,35 have elevated androgens or PCOS.

**Acne**

Acne can be associated with biochemical hyperandrogenism. Unlike hirsutism which offers a good predictive value for hyperandrogenism, the predictive value of acne is still unclear. There are not many studies looking at predictive value of acne, most of them being retrospective.36,37 Unlike hirsutism, we lack an accepted scoring system to clinically evaluate and measure acne. Overall, while acne in women might indicate androgen excess, the predictive value of acne alone for hyperandrogenism remains unclear.

**Male pattern hair loss**

Diffuse sagittal alopecia can be seen in women with PCOS. The Ludwig scale,38 with a range from grade I to grade III, indicates increasing severity and can be used to visually assess scalp hair loss. On its own, its predictive value as a marker of androgen excess is unclear.

**Ultrasound and polycystic ovarian morphology**

Ovarian follicles undergo a continuous process of recruitment and apoptosis through reproductive life. This starts in foetal life, continues throughout childhood and adulthood and stops at menopause. OV changes over time with changes in antral follicles and stromal development. Most women with clinical and endocrinological features of PCOS demonstrated ovaries which are polycystic on ultrasound; hence this feature was added as a third inclusion criterion in 2003.39 Various ultrasound features have been identified as a feature in women with PCOS which include antral follicle count (AFC), follicular number per ovary (FNPO), OV, ovarian area (OA), ovarian blood flow and ratio of stroma to total ovarian size.

**Antral follicle count**

AFC is considered to be a good measure to identify the severity of reproductive dysfunction in PCOS. Increased AFC was most significantly associated with increased androgens and LH:FSH ratio.40 The polycystic ovaries in PCOS can be confused with other causes of multifollicular ovaries which could be both physiological (puberty) and pathological (hypothalamic anovulation, hyperprolactinaemia, central precocious puberty). As multifollicular ovaries are considered physiological in the early years of reproductive life (within 8 years of menarche), diagnosing PCOS based on ultrasound criteria in adolescents is not appropriate.
Earlier studies showed greatest sensitivity for defining polycystic ovaries from a count of 10 or more follicles arranged peripherally around dense core stoma (Adams criteria)\(^41\) while a definition of 12 or more follicles\(^42\) offered a greater specificity. Most of authors had initially set this threshold at 10\(^43,44\) but some authors recommended 15.\(^45\) Following a consensus opinion in 2003, the count was then changed over to >12 follicles measuring 2–9 mm in diameter.

With advances in ultrasound and better transducer frequencies, significant increase in FNPO was reported with transducer frequency of >8 MHz. The previous threshold for FNPO of 12 or more resulted in a significantly greater prevalence of polycystic ovarian morphology (PCOM) in women especially below 30 years of age in a general population.\(^46–49\) Eleven studies which included 2961 participants looked at FNPO and concluded optimal sensitivity and specificity with >19 per ovary. The grid system method to count antral follicle, which was devised by Lujan and colleagues in 2010, is the most reproducible technique. This technique showed a sensitivity of 85% and specificity of 94% when 26 follicles per ovary was taken as a cut-off. This cut-off may miss mild form of PCOS. Others\(^50\) suggested lower threshold of 19 follicles.

Counting antral follicles and setting a threshold is controversial, especially, as different populations and counting techniques may account for differences. There are also differences in the method of counting the follicles, observer variability in assessing follicle number and variable ultrasound technology.

When diagnosing PCOM on transvaginal scan, ESHRE PCOS guideline group 2018 have suggested to use a cut-off of FNPO of 20 or more in one or both the ovaries or OV >10 mL without inclusion of dominant follicle or corpus luteum or any cysts. This cut-off is to be used when using a transvaginal scan with a frequency band of >8 MHz. The cut-off of FNPO of 12 or more or OV of >10 mL should be used when the ultrasound machine of older technology is used.

Few studies show that using 3D scan to provide automatic volume calculations of antral follicles (e.g. VOCAL\(^\text{TM}\) and SonoAVC\(^\text{TM}\)) showed better accuracy\(^51–53\) as well as reduced interobserver variation in follicle counts\(^54,55\) compared with manual 2D measurements. Not many studies have attempted to look into the reliability of 3D ultrasound to estimate the follicular population in polycystic ovaries.\(^56–62\) More studies are necessary to confirm its importance before its recommendation for routine practice.

**Ovarian size.** The size of the ovary changes through the reproductive life of a woman with slow decline during adulthood and rapid shrink-age during menopause. Ovary achieves its maximal size during adolescence. As only small changes in OV occur between the age of 20 and 39 years, an age-specific OV cut-off is not warranted.

Histopathological studies show that stromal hypertrophy as well as increased follicular count which reflects PCOM correlates to OV and OA. Rotterdam criteria have set a threshold of OV >10 mL for diagnosis of PCOM.\(^63\) Women with PCOS have a higher ovarian size compared with normal women who are matched for age and body weight.\(^63–65\) There has been several interests in setting a lower cut-off volume for OV including 6.4,\(^66\) 6.7,\(^67\) 7.0,\(^68,69\) and 7.5 mL.\(^70\) These different cut-offs could be because of variation in population characteristics.

OV cut-off of >10 mL was solely based on the results from various studies, where the upper limit was defined as either being maximum value of controls or 95th percentile of control range. The currently accepted cut-off of >10 cm\(^3\) has a sensitivity of 98.2% and a lower sensitivity of 45%, in diagnosing PCOM.\(^42\) It should be noted that OV measurement still holds its place when the image resolution does not allow an accurate AFC.

**Other Ultrasound measurements**

**Ovarian stroma.** Only few studies have looked at ovarian stroma as a diagnostic tool for polycystic ovaries. Fulghesu and colleagues\(^70\) proposed a cut-off of 0.32 for ratio of stroma to total ovarian size. They suggested that this cut-off value is associated with hyperandrogen-aemia. It appears that stromal volume and ovarian size correlate well and hence adding stromal volume to clinical practice does not provide much value.
Ovarian blood flow. To date, there are no homogeneous data confirming the importance of measuring ovarian blood flow to diagnose PCOM. There is also no cut-off values that have been proposed to differentiate PCOM from normal ovaries.

Increased serum AMH concentrations as a marker of PCOM
AMH is a glycoprotein which is produced by granulosa cells of pre-antral and antral follicles of the ovary. As serum AMH reflects the antral follicles, a significantly higher level of AMH is seen in women with PCOS compared with normal women. There is also a direct correlation between AMH levels and ultrasound parameters of FNPO and OV. Dewailly and colleagues proposed a new adaptation to diagnose PCOS based on FNPO and serum AMH levels. They concluded that measuring serum AMH and its elevated levels in PCOS and PCOM could be much more reproducible than FNPO which can show interobserver variation as well as variation from unit to unit. This is if a universally available assay is used. They proposed a classification which takes into consideration previous classification for the diagnosis of PCOS. They used FNPO > 19 and AMH > 35 pmol/L as surrogates when either oligo/anovulation or androgen excess was missing. This classification is shown in Table 2.

| Oligo/ anovulation | Androgen excess (clinical or biochemical) | FNPO > 19 or AMH > 35 pmol/L [5 ng/mL] | Diagnosis          |
|-------------------|------------------------------------------|---------------------------------------|-------------------|
| +                 | +                                        | +/-                                   | PCOS              |
| +                 | -                                        | +                                     | PCOS              |
| -                 | +                                        | +                                     | PCOS              |
| -                 | -                                        | +                                     | PCOM              |
| +                 | -                                        | -                                     | Idiopathic anovulation |
| -                 | +                                        | -                                     | Idiopathic hyperandrogenism |

AMH, anti-Mullerian hormone; FNPO, follicular number per ovary; PCOM, polycystic ovarian morphology; PCOS, polycystic ovary syndrome.

Many studies that looked at correlation between AMH levels and diagnosis of PCOS used Diagnostic Systems Laboratories (DSL) or Immunotech (IOT) assays. These assays are not available anymore. Assays which used Gen II kit which have been used more recently also need cautious interpretation. The recent new automated assays that are used have very little data on them. It is also worth noting that International Federation of Clinical Chemistry does not provide a standard regarding assay methods. In view of all these caveats, serum AMH value as a surrogate to diagnose PCOM is still not accepted. AMH can be a potential surrogate marker for diagnosing PCOM in the future, provided further research confirms its validation in vast population of different backgrounds.

Conclusion
PCOS still remains a controversial topic due to its varied etiology and undetermined phenotypic spectrum. The existing diagnostic criteria
are those suggested by the NIH in 1990, Rotterdam criteria 2003 and AES criteria 2005. Expanding the diagnostic criteria in 2003 was aimed at targeting the different phenotypes that exist. In 2005, the AES task force accepted the original 1990 NIH criteria along with modifications, considering the 2003 Rotterdam criteria. Despite being in widespread global use, each diagnostic criterion stems an unresolved controversy as much of the evidence is only based on consensus opinion rather than robust evidence. Although there is a clear cut-off for ‘irregular cycles’ in adults, defining ‘irregular cycles’ in adolescents poses a great controversy. Further longitudinal studies are needed to look at natural history of PCOS and also early predictors in adolescents. Assessing hyperandrogenism clinically is highly subjective and further studies are needed to determine the cut-off values for mFG scoring system. There is insufficient evidence regarding the best method to use for measurement of androgens. Also, the methods used to measure are of insufficient precision. FNPO, which forms one of the diagnostic criteria, has been well researched. Eleven studies including 2961 participants concluded that an optimal sensitivity and specificity was achieved when a cut-off of >19 follicles was used. With OV, 12 studies with 2096 participants did not provide a clear cut-off for the optimal OV with both 5–8 cm³ and 9–10 cm³. There is inadequate evidence for the use of other ultrasound parameters to diagnose PCOS. The use of AMH as a substitute for diagnosis of PCOS is hindered by the fact that current assays need improved standardisation. The evidence also does not adequately support the role of AMH currently. In conclusion, large scientific and clinical research is needed in this field.

Conflict of interest statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

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References
1. Wolfe WM, Wattick RA, Kinkade ON, et al. Geographical prevalence of polycystic ovary syndrome as determined by region and race/ethnicity. Int J Environ Res Public Health 2018; 15: E2589.
2. Stein IF and Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935; 29: 181–191.
3. Azziz R and Adashi EY. Stein and Leventhal: 80 years on. Am J Obstet Gynecol 2016; 214: 247.e1–247.e11.
4. Zawadzki JK and Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens J, Haseltine F, et al. (eds) Polycystic ovary syndrome. 1st ed. Oxford: Blackwell Scientific, 1992, pp. 377–384.
5. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81: 19–25.
6. Azziz R, Carmina E, Dewailly D, et al. Criteria for defining polycystic ovarian syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab 2006; 91: 4237–4245.
7. Apter D and Viiko R. Serum pregnenolone, progesterone, 17-hydroxyprogesterone, testosterone and 5 alpha-dihydrotestosterone during female puberty. J Clin Endocrinol Metab 1977; 45: 1039–1048.
8. Lemarchand-Béraud T, Zufferey MM, Reymond M, et al. Maturation of the hypothalamo-pituitary-ovarian axis in adolescent girls. J Clin Endocrinol Metab 1982; 54: 241–246.
9. NICE Clinical Guideline. Fertility: assessment and treatment for people with fertility problems, February 2013, p. 102, https://www.nice.org.uk/guidance/cg156/evidence/full-guideline-pdf-188539453
10. The ESHRE Capri Workshop. Guidelines to the prevalence, diagnosis, treatment and management of infertility, 1996. Hum Reprod 1996; 11: 1775–1807.
11. Hull MG, Savage PE, Bromham DR, et al. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle (‘ovulation’) derived from treated and untreated conception cycles. Fertil Steril 1982; 37: 355–360.
12. Abdulla U, Diver MJ, Hipkin LJ, et al. Plasma progesterone levels as an index of ovulation. Br J Obstet Gynaecol 1983; 90: 543–548.
13. Wathen NC, Perry L, Lilford RJ, et al. Interpretation of single progesterone measurement in diagnosis of anovulation and
22. International evidence-based guideline for the assessment and management of polycystic ovary syndrome. Melbourne, VIC, Australia: Monash University, 2018.

23. Emans SJ, Grace E and Goldstein DP. Oligomenorrhea in adolescent girls. J Pediatr 1980; 97: 815–819.

24. Vermeulen A, Verdonck L and Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999; 84: 3666–3672.

25. Rosner W, Auchus RJ, Azziz R, et al. Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. J Clin Endocrinol Metab 2007; 92: 405–413.

26. Hahn S, Kuehnel W, Tan S, et al. Diagnostic value of calculated testosterone indices in the assessment of polycystic ovary syndrome. Clin Chem Lab Med 2007; 45: 202–207.

27. Escobar-Morreale HF, Asunción M, Calvo RM, et al. Receiver operating characteristic analysis of the performance of basal serum hormone profiles for the diagnosis of polycystic ovary syndrome in epidemiological studies. Eur J Endocrinol 2001; 145: 619–624.

28. Stanczyk FZ. Androgen measurements: methods, interpretation and limitations. In: Azziz R, Nestler JE and Dewailly D (eds) Androgen excess disorders in women. 2nd ed. Totowa, NJ: Humana Press, 2006, pp. 63–72.

29. Knochenhauer ES, Key TJ, Kahsar Miller M, et al. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab 1998; 83: 3078–3082.

30. Ferriman D and Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 1961; 21: 1440–1447.

31. Hatch R, Rosenfield RL, Kim MH, et al. Hirsutism: implications, etiology, and management. Am J Obstet Gynecol 1981; 140: 815–830.

32. deUgarte CM, Bartolucci AA, Woods KS, et al. Degree of facial and body terminal hair growth in unselected black and white women: toward a populational definition of hirsutism. J Clin Endocrinol Metab 2006; 91: 1345–1350.

33. Zhao X, Ni R, Li L, et al. Defining hirsutism in Chinese women: a cross-sectional study. Fertil Steril 2011; 96: 792–796.

34. Souter I, Sanchez A, Perez M, et al. The prevalence of androgen excess among patients with minimal unwanted hair growth. Am J Obstet Gynecol 2004; 191: 1914–1920.

35. Yüldiz BO, Bolour S, Woods K, et al. Visually scoring hirsutism. Hum Reprod Update 2010; 16: 51–64.

36. Lizneva D, Gavriloja-Jordan L, Walker W, et al. Androgen excess: investigations and management. Best Pract Res Clin Obstet Gynaecol 2016; 37: 98–118.

37. Uysal G, Sahin Y, Unluhizarci K, et al. Is acne a sign of androgen excess disorder or not? Eur J Obstet Gynecol Reprod Biol 2017; 211: 21–25.

38. Ludwig E. Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. Br J Dermatol 1977; 97: 247–254.

39. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS): the Rotterdam ESHRE/
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ASRM-sponsored PCOS consensus workshop group. *Hum Reprod* 2004; 19: 41–47.

40. Christ JP, Vanden Brink H, Brooks ED, et al. Ultrasound features of polycystic ovaries relate to degree of reproductive and metabolic disturbance in polycystic ovary syndrome. *Fertil Steril* 2015; 103: 787–794.

41. Alsamarai S, Adams JM, Murphy MK, et al. Criteria for polycystic ovarian morphology in polycystic ovary syndrome as a function of age. *J Clin Endocrinol Metab* 2009; 94: 4961–4970.

42. Jonard S, Robert Y, Cortet-Rudelli C, et al. Ultrasound examination of polycystic ovaries: is it worth counting the follicles? *Hum Reprod* 2003; 18: 598–603.

43. Adams J, Polson DW, Abdulwahid N, et al. Multifollicular ovaries: clinical and endocrine features and responses to pulsatile gonadotrophin releasing hormone. *Lancet* 1985; 326: 1375–1379.

44. Takahashi K, Eda Y, Abu-Musa A, et al. Transvaginal ultrasound imaging, histopathology and endocrinopathy in patients with polycystic ovarian syndrome. *Hum Reprod* 1994; 9: 1231–1236.

45. Fox R, Corrigan E, Thomas PA, et al. The diagnosis of polycystic ovaries in women with oligo-amenorrhea: predictive power of endocrine tests. *Clin Endocrinol* 1991; 34: 127–131.

46. Duijkers IJ and Klipping C. Polycystic ovaries, as defined by the 2003 Rotterdam consensus criteria, are found to be very common in young healthy women. *Gynecol Endocrinol* 2010; 26: 152–160.

47. Johnstone EB, Rosen MP, Neril R, et al. The polycystic ovary post-Rotterdam: a common, age-dependent finding in ovulatory women without metabolic significance. *J Clin Endocrinol Metab* 2010; 95: 4965–4972.

48. Kristensen SL, Ramlav-Hansen CH, Ernst E, et al. A very large proportion of young Danish women have polycystic ovaries: is a revision of the Rotterdam criteria needed? *Hum Reprod* 2010; 25: 3117–3122.

49. Jokubkiene L, Sladkevicius P and Valentin L. Number of antral follicles, ovarian volume, and vascular indices in asymptomatic women 20 to 39 years old as assessed by 3-dimensional sonography: a prospective cross-sectional study. *J Ultrasound Med* 2012; 31: 1635–1649.

50. Dewailly D, Gronier H, Poncelet E, 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: 3123–3129.

51. Raine-Fenning N, Jayaprakasan K, Clewes J, et al. SonoAVC: a novel method of automatic volume calculation. *Ultrasound Obstet Gynecol* 2008; 31: 691–696.

52. Lamazou F, Arbo E, Salama S, et al. Reliability of automated volumetric measurement of multiple growing follicles in controlled ovarian hyperstimulation. *Fertil Steril* 2010; 94: 2172–2176.

53. Salama S, Arbo E, Lamazou F, et al. Reproducibility and reliability of automated volumetric measurement of single preovulatory follicles using SonoAVC. *Fertil Steril* 2010; 93: 2069–2073.

54. Jayaprakasan K, Walker KF, Clewes JS, et al. The interobserver reliability of off-line antral follicle counts made from stored three-dimensional ultrasound data: a comparative study of different measurement techniques. *Ultrasound Obstet Gynecol* 2007; 29: 335–341.

55. Deb S, Jayaprakasan K, Campbell BK, et al. Intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC. *Ultrasound Obstet Gynecol* 2009; 33: 477–483.

56. Allemand MC, Tummon IS, Phy JL, et al. Diagnosis of polycystic ovaries by three-dimensional transvaginal ultrasound. *Fertil Steril* 2006; 85: 214–219.

57. Ng EH, Chan CC and Ho PC. Are there differences in ultrasound parameters between Chinese women with polycystic ovaries only and with polycystic ovary syndrome? *Eur J Obstet Gynecol Reprod Biol* 2006; 125: 9292–9288.

58. Lam PM, Johnson IR and Raine-Fenning NJ. Three-dimensional ultrasound features of the polycystic ovary and the effect of different phenotypic expressions on these parameters. *Hum Reprod* 2007; 22: 3116–3123.

59. Lam P, Raine-Fenning N, Cheung L, et al. Three-dimensional ultrasound features of the polycystic ovary in Chinese women. *Ultrasound Obstet Gynecol* 2009; 34: 196–200.

60. Sun L and Fu Q. Three-dimensional transrectal ultrasonography in adolescent patients with polycystic ovarian syndrome. *Int J Gynecol Obstet* 2007; 98: 35–38.

61. Pascual MA, Graupera B, Hereter I, et al. Assessment of ovarian vascularization in the polycystic ovary by three-dimensional power
Doppler ultrasonography. *Gynecol Endocrinol* 2008; 24: 631–666.

62. Battaglia C, Battaglia B, Morotti E, *et al.* Two- and three-dimensional sonographic and color Doppler techniques for diagnosis of polycystic ovary syndrome. The stromal/ovarian volume ratio as a new diagnostic criterion. *J Ultrasound Med* 2012; 31: 1015–1024.

63. Balen AH, Laven JS, Tan SL, *et al.* Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Update* 2003; 9: 505–514.

64. Carmina E, Orio F, Palomba S, *et al.* Ovarian size and blood flow in women with polycystic ovary syndrome and their correlations with endocrine parameters. *Fertil Steril* 2005; 84: 413–419.

65. Alsamarai S, Adams JM, Murphy MK, *et al.* Criteria for polycystic ovarian morphology in polycystic ovary syndrome as a function of age. *J Clin Endocrinol Metab* 2009; 94: 4961–4970.

66. Köşüş N, Köşüş A, Turhan NÖ, *et al.* Do threshold values of ovarian volume and follicle number for diagnosing polycystic ovarian syndrome in Turkish women differ from western countries? *Eur J Obstet Gynecol Reprod Biol* 2011; 154: 177–181.

67. Chen Y, Li L, Chen X, *et al.* Ovarian volume and follicle number in the diagnosis of polycystic ovary syndrome in Chinese women. *Ultrasound Obstet Gynecol* 2008; 32: 700–703.

68. Jonard S, Robert Y and Dewailly D. Revisiting the ovarian volume as a diagnostic criterion for polycystic ovaries. *Hum Reprod* 2005; 20: 2893–2898.

69. Dewailly D, Gronier H, Poncet E, *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: 3123–3129.

70. Fulghesu AM, Angioni S, Frau E, *et al.* Ultrasound in polycystic ovary syndrome the measuring of ovarian stroma and relationship with circulating androgens: results of a multicentric study. *Hum Reprod* 2007; 22: 2501–2508.

71. Cook CL, Siow Y, Brenner AG, *et al.* Relationship between serum Mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *Fertil Steril* 2002; 77: 141–146.

72. Seifer DB and MacLaughlin DT. Mullerian Inhibiting Substance is an ovarian growth factor of emerging clinical significance. *Fertil Steril* 2007;88: 539–546.

73. Pigny P, Merlen E, Robert Y, *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: 5957–5962.

74. Laven JS, Mulders AG, Visser JA, *et al.* Anti-Müllerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. *J Clin Endocrinol Metab* 2004; 89: 318–323.

75. Piltonen T, Morin-Papunen L, Koivunen R, *et al.* 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: 1820–1826.

76. Villarroel C, Merino PM, López P, *et al.* Polycystic ovarian morphology in adolescents with regular menstrual cycles is associated with elevated anti-Müllerian hormone. *Hum Reprod* 2011; 26: 2861–2868.

77. Eilertsen TB, Vanky E and Carlsen SM. Anti-Müllerian hormone in the diagnosis of polycystic ovary syndrome: can morphologic description be replaced? *Hum Reprod* 2012; 27: 2494–2502.

78. Robin G, Gallo C, Catteau-Jonard S, *et al.* Polycystic Ovary-Like Abnormalities (PCO-L) in women with functional hypothalamic amenorrhea. *J Clin Endocrinol Metab* 2012; 97: 4236–4243.

79. ESHRE/ASRM. *International evidence-based guideline for the assessment and management of polycystic ovary syndrome* (Technical repost), 2018.

80. Pigny P, Gorisse E, Ghulam A, *et al.* Comparative assessment of five serum antimullerian hormone assays for the diagnosis of polycystic ovary syndrome. *Fertil Steril* 2016; 105: 1063–1069.e3.

81. Villarroel C, Lopez P, Merino PM, *et al.* Hirsutism and oligomenorrhea are appropriate screening criteria for polycystic ovary syndrome in adolescents. *Gynecol Endocrinol* 2015; 31: 625–629.