A comparison of ovarian morphology and reproductive and metabolic features between South Asian and White women with polycystic ovary syndrome (PCOS)

Honors Thesis
Presented to the College of Arts and Sciences
Cornell University
in Partial Fulfillment of the Requirements for the
Biological Sciences Honors Program

by
Ashley Tam
May 2019
Supervisor: Dr. Marla Lujan

ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine condition, with known variability in the severity of its cardinal features and long-term health consequences across races and ethnicities. Diagnosis of PCOS involves the ultrasonographic identification of polycystic ovarian morphology (PCOM). However, current diagnostic thresholds for PCOM do not account for any impact of race or reflect the potential for race-specific differences in the presentation of PCOS. I aimed to evaluate differences in ovarian morphology between South Asian and White women with PCOS and assess relationships between reproductive / metabolic features and PCOM in both cohorts. **Methods:** White (n = 30) and South Asian (n = 31) women with PCOS underwent clinical evaluation, venipuncture, and transvaginal ultrasonography. Markers of PCOM (i.e., follicle number per ovary [FNPO] and ovarian volume [OV]) and reproductive / metabolic status were compared between groups. **Results:** South Asian women with PCOS had lower OV (9.0 vs. 12.5 mL, $P < 0.01$) and slightly lower FNPO (31 vs. 44 follicles, $P = 0.06$) than White women with PCOS. South Asian women also had more metabolic disturbances than White women, as indicated by higher waist circumference, waist-to-hip ratio, and 2-hour glucose levels (All: $P < 0.05$). Correlations between ovarian morphology and reproductive / metabolic features were variable across cohorts and challenging to interpret due to limited sample sizes. **Conclusions:** My findings support the need for race-specific diagnostic criteria for PCOM. Future studies would benefit from the inclusion of larger cohorts of South Asian and White women to clarify the importance of race in the manifestation and treatment of PCOS.

INTRODUCTION

Polycystic Ovary Syndrome. Polycystic ovary syndrome (PCOS) is a complex endocrine condition that affects 8–13% of premenopausal women worldwide.[1] PCOS is heterogeneous in nature and characterized by a combination of three cardinal features: (1) hyperandrogenism, (2) menstrual irregularities, and (3) polycystic ovarian morphology (PCOM) on ultrasonography.[2] The condition is not well understood, because the etiology is unclear and the phenotypic presentation varies with age, race, ethnicity, and physiologic factors (i.e., adiposity and androgen status).[3] PCOS is linked to metabolic comorbidities, including obesity, insulin resistance, inflammation, hypertension, and dyslipidemia,[4] which can increase the risk for infertility, adverse pregnancy outcomes, cardiovascular disease, diabetes mellitus, stroke, and certain gynecological cancers.[3] Many patients also experience an array of psychological issues including poor quality of life, low self-esteem, anxiety, and depression.[5] Because the reproductive, metabolic, and psychological comorbidities have serious implications for women and their families, it is critical to establish diagnostic criteria that accurately identify those with PCOS and allow for early prescription of preventative therapies.

Diagnosis of PCOS. Due to its complex and likely multi-factorial etiology, the diagnostic criteria for PCOS have been changed and heavily debated over time. The diagnosis not only involves an identification of the cardinal features, but exclusion of other disorders that manifest like PCOS (e.g., thyroid disorders, congenital adrenal hyperplasia, and androgen-secreting tumors).[6,7] Three sets of diagnostic criteria for PCOS have been published to date and are summarized in **Table 1**. In 1990, the National Institute of Child Health and Human Development (NICHD) proposed that

the definition of PCOS include evidence of hyperandrogenism and menstrual irregularities, but not PCOM.[8] Many experts believed that PCOM was not necessary for the diagnosis of PCOS, because anecdotal reports estimated that 30% of patients did not have the ovarian phenotype.[8] However, it soon became clear that the condition was much broader than what the NICHD definition allowed and that women could exhibit signs of ovarian dysfunction despite normal androgen status.[9] In 2003, the Rotterdam PCOS Consensus Workshop Group (hosted by the American Society for Reproductive Medicine and the European Society for Human Reproduction and Embryology) proposed that the presence of any two of the three cardinal features (i.e., hyperandrogenism, menstrual irregularities, or PCOM) was sufficient for the diagnosis of PCOS.[9] The "Rotterdam" criteria expanded the range of possible clinical phenotypes and increased the number of women that could be classified as having PCOS. Then, in 2006, the Androgen Excess and PCOS (AE-PCOS) Society re-assessed whether the broadened definition captured the true population of women with PCOS or instead overestimated the prevalence.[10] The AE-PCOS Society ultimately emphasized that PCOS is a disorder of androgen excess and cannot be identified in its absence.[11] Together, recognition of different diagnostic criteria for PCOS has resulted in variable global estimates of the prevalence of PCOS and obscured our understanding of the pathogenesis of the condition.[12] In an effort to standardize methods for diagnosing and treating PCOS, Teede *et al.* recently published the first International Evidence-based Guideline for the Assessment and Management of PCOS in 2018. Based on expert opinion and the benefits of studying all existing variants of PCOS, the authors' supported the sole use of the Rotterdam criteria in both clinical practice and research.[1]

Table 1. Diagnostic Criteria for PCOS

	NICHD (1990)[8]	**Rotterdam (2003)**[9]	**AE-PCOS (2006)**[10]
Hyperandrogenism	Required	Optional	Required
Menstrual irregularities	Required	Optional	Optional
PCOM		Optional	Optional
Notes	Ignores PCOM	Requires 2 of 3 features	Requires hyperandrogenism

Adapted from Lujan et al. 2008.[7] *Abbreviations:* PCOM, polycystic ovarian morphology; NICHD, National Institute of Child Health and Human Development; AE-PCOS, Androgen Excess and PCOS.

Definition of PCOM. Inclusion of the ovary in the diagnostic criteria for PCOS requires an accurate definition for PCOM. PCOM is characterized by follicular excess and increased ovarian size compared to normal ovaries.[13] Although recent technological advances have facilitated the detection of discrete aspects of ovarian morphology, differences in equipment and operator skills across healthcare centers have prevented diagnostic thresholds for ultrasonographic markers from being universally agreed upon.[13] Debates have centered on the thresholds beyond which follicle number and ovarian size should be regarded as "abnormal." The Rotterdam criteria included the first-ever ultrasonographic definition of PCOM, i.e., the presence of ≥ 12 follicles (2 – 9 mm in diameter) in the entire ovary (FNPO) and/or ovarian volume (OV) ≥ 10 mL.[13] FNPO ≥ 12 was initially accepted and widely used in clinical practice and research, but is now no longer seen as valid.[14] Its use led to an over-diagnosis of PCOM in healthy women due to the increased visualization of smaller follicles with newer imaging technology (e.g., 2 – 4 mm).[15] Higher thresholds have been proposed for FNPO in the past five years (**Table 2**), with Dewailly *et al.* and Lujan *et al.* publishing the best-designed diagnostic test studies in the field to date. Using high frequency ultrasound transducers and receiver operating characteristic curve analysis, the two groups observed that FNPO ≥ 19 and ≥ 26, respectively, offered the best diagnostic potential for PCOS.[15,16] In 2014, the AE-PCOS Society Task Force reaffirmed a threshold for FNPO of ≥ 25,

by considering the upper limit of follicle counts in a healthy control population.[14] The AE-PCOS Task Force also concluded that FNPO is the most reliable metric of PCOM and PCOS, and suggested that OV only be used in cases of reduced image quality.[14] Then, in 2018, Teede and colleagues recommended a threshold for FNPO of ≥ 20[1] based on expert opinion (Table 2). Notably, although higher thresholds for FNPO aim to reduce the misdiagnosis of PCOM, they remain limited by the lack of attention to the demographic and physiologic factors that modify the presentation of the syndrome.

Table 2. Summary of Proposed Thresholds to Define PCOM by FNPO and OV

Group	Year	Country of Origin	N	FNPO	OV (mL)
Rotterdam[9]	2003	France	C: 112 P: 214	≥ 12	≥ 10
Dewailly et al.[16]	2011	France	C: 105 P: 62	≥ 19	N/A
Lujan et al.[15]	2013	North America	C: 70 P: 98	≥ 26	≥ 10
AE-PCOS Task Force[14]	2014	International	C: >1000 P: N/A	≥ 25	≥ 10
International Guideline[1]	2018	International	C: NR P: N/A	≥ 20	≥ 10

Across publications, the thresholds for FNPO and OV were not determined with the same ultrasound technology or methodology. Efforts to standardize technology and methodology are ongoing in the field of PCOS. *Abbreviations:* N, sample size; FNPO, follicle number per ovary; OV, ovarian volume; C, Control; P, PCOS; N/A, not assessed; NR, not reported.

Race, Ethnicity, and PCOM. Race and ethnicity are believed to play a role in the presentation of PCOM.[1,3,14] Previous diagnostic test studies have derived thresholds for FNPO and OV from cohorts of predominantly White women in Europe and North America (Table 2). Some authors have argued that such approaches are not adequate to detect PCOM in other populations and have pointed to the need for race- or ethnicity-specific thresholds.[17,18,19,20] Four groups have performed diagnostic test studies in Asian cohorts and the results are listed in **Table 3**. Proposed thresholds have ranged from FNPO ≥ 8 in Turkish women[18] to FNPO ≥ 12 in Chinese[17] and Indian[20] women.

Thresholds for OV have ranged from ≥ 6 to ≥ 8 mL.[17,18,19,20] Of the two studies that have considered PCOM in Indian women, each one has identified a different threshold for FNPO and OV. Ahmed *et al.* proposed an FNPO ≥ 9 and OV ≥ 8 mL,[19] while Kar *et al.* proposed an FNPO ≥ 12 and OV ≥ 6 mL[20] (Table 3). Together, existing data support the notion that ovarian morphology may differ sufficiently across races and ethnicities, wherein application of current international consensus thresholds for PCOM may not be reliable for the ultrasound diagnosis of PCOS in all women. However, to the best of my knowledge, no studies have directly compared PCOM across women of different races. Given the variability in proposed thresholds for Indian cohorts, such studies are needed to quantify the actual degree to which ovarian morphology differs in White versus South Asian women with PCOS.

Table 3. Summary of Proposed Thresholds to Define PCOM in Asian Countries

Group	Year	Race	Country of Origin	N	FNPO	OV (mL)
Chen[17]	2008	Asian	China	C: 153 P: 432	≥ 12	≥ 8
Köşüş[18]	2011	Asian	Turkey	C: 65 P: 251	≥ 8	≥ 6
Ahmed[19]	2014	Asian	India	C: 77 P: 119	≥ 9	≥ 8
Kar[20]	2018	Asian	India	C: 45 P: 86	≥ 12	≥ 6

Abbreviations: N, sample size; FNPO, follicle number per ovary; OV, ovarian volume; C, control; P, PCOS.

Impact of Race and Ethnicity on the Presentation of PCOS. Previous studies have reported racial and ethnic differences in the presentation of PCOS, including in the severity of hirsutism and the prevalence of co-morbid metabolic disorders.[21–24] A large international cross-sectional study found that Asian women with PCOS from India have higher modified hirsutism scores than White women with PCOS from Europe and North America.[23] Indian women with PCOS also have increased fasting insulin,[24] greater insulin responses to a glucose challenge,[24] and a heightened

prevalence of metabolic syndrome compared to White women with PCOS.[23] Different features appear to contribute to the heightened prevalence of metabolic syndrome in Indian women – particularly a higher prevalence of obesity, elevated fasting glucose levels, and low HDL.[23] Similarly, a recent systematic review concluded that Asian women with PCOS exhibit the highest risk of impaired glucose tolerance compared to White women with PCOS, even when cohorts are matched for body mass index (BMI).[22,23] Interestingly, genetic factors may not explain racial or ethnic differences in the severity of PCOS. Previous genome-wide associations studies have shown that the genetic determinants of metabolic syndrome are similar in Indian and European men.[25] In addition, susceptibility loci for PCOS have not differed between Han Chinese and European women, suggesting that the genetic risk for the condition is similar across races and ethnicities.[26–28] Such findings support the notion that other factors, including differences in lifestyle or environment, could be involved in the mechanism behind racial and ethnic differences in the severity of PCOS. Studies that compare the severity of PCOS by country of origin and country of residence might help to clarify the environmental versus genetic contributions to racial and ethnic differences. Nevertheless, if metabolic disorders are more prevalent in South Asian than White women, then there is an even greater need to refine the ultrasound criteria for PCOS to ensure reliable early evaluation and treatment.[23]

Utility of Ultrasound Beyond the Diagnosis of PCOS. It should be acknowledged that ovarian morphology may be able to provide information beyond the diagnosis of PCOS. In particular, PCOM may also reflect the severity of a patient's reproductive and metabolic disturbances. We previously showed that the number of total follicles (2 – 9 mm diameter) and small antral follicles (3 – 4 mm) in the ovaries were positively associated with androgen concentrations in women with

PCOS, while the number of medium-sized follicles (5 – 6 mm) was positively associated with both androgens and menstrual cycle length. Smaller follicles (≤ 4 mm) were also negatively linked to BMI, waist-to-hip ratio, and markers of insulin resistance.[29] Further, the number of medium and large follicles (5 – 11 mm) were positively associated with features of metabolic health, but negatively associated with aspects of reproductive disturbance in PCOS.[29] In a second report, we showed that women with PCOS who developed dominant follicles (≥ 10 mm) had lower BMIs and improved glucoregulation compared to women that did not develop dominant follicles.[30,31] Such information may be useful in clinics that can afford the one-time purchase of an ultrasound machine, but have limited financial resources to purchase multiple endocrine and metabolic assays. Some blood tests may not be covered by patients' insurance companies or may not be available in rural areas. If both the diagnosis of PCOM and an indication of metabolic status can be garnered from a single ultrasound scan, then there may be an opportunity to improve the accessibility of care and identify patients that would not otherwise be treated for metabolic issues (e.g., younger or normal-weight women with PCOS). The role of ultrasonography for triaging medical care in women may be especially important in lower-income countries like India.

AIMS AND HYPOTHESES

Overall, there is little information on the differences in ovarian morphology between White and South Asian women with PCOS and whether or how these differences might reflect reproductive or metabolic status. My objectives were to: (1) evaluate differences in ovarian morphology between South Asian and White women with PCOS, and (2) assess any relationships between reproductive / metabolic features and PCOM in both cohorts. Based on the studies referenced in Table 3, I hypothesized that estimates of FNPO and OV would be lower in South Asian women with PCOS compared to White women with PCOS. Likewise, based on our previous observations in White women,[29] I hypothesized that correlations of similar direction, but different magnitude would be found between reproductive / metabolic features and PCOM in the South Asian and White cohorts. The present work is especially timely, given the recent calls to action to consider race and ethnicity in the diagnosis and treatment of PCOS.[1,3,14]

METHODS

Study Population. The present study involved the secondary analysis of data from (a) clinical research studies conducted in New York State, United States *(White women with PCOS)* and (b) patient medical charts and ultrasound images shared by a gynecologic primary care center in Odisha, India *(South Asian women with PCOS)*. Data collection for the clinical research studies occurred at Cornell University and the University of Rochester from 2010 to 2018. The original study protocols were approved by the Institutional Review Boards at both universities (ClinicalTrials.gov Identifiers: NCT01927471, NCT01859663, NCT01927432, NCT01785719). In all cases, written, informed consent was obtained before the study procedures were initiated. White women were selected for inclusion in the present analysis from a database of past participants (n = 220). Women were excluded if they: (1) were not of reproductive age (i.e., <18 or >38 years) (n = 11); (2) did not self-identify as both Non-Hispanic and White (n = 96); (3) were using hormonal contraception or medications known to interfere with glucose or lipid metabolism (n = 7); (4) did not provide sufficient clinical and ultrasonographic data to assess the endpoints of interest (n = 14); (5) were evaluated in the pre-ovulatory or luteal phase of the menstrual cycle (n = 3); (6) were evaluated with transabdominal, instead of transvaginal, ultrasonography (n = 0); (7) had abnormal thyroid-stimulating hormone or prolactin levels (n = 0); or (8) did not have PCOS based on the NICHD criteria (as described below; n = 59).[8] The remaining cohort included 30 White women with PCOS. Medical charts were obtained from consecutive patients seen at the primary care center in India from January to May 2018 (n = 67). The retrospective chart reviews were confirmed to be an exempt research activity by Cornell's Institutional Review Board. As with the White cohort, South Asian women were excluded from the present analysis if they: (1) did not

provide sufficient clinical and ultrasonographic data to assess the endpoints of interest (n = 16); (2) were evaluated in the pre-ovulatory or luteal phase of the menstrual cycle (n = 1); (3) were evaluated with transabdominal, instead of transvaginal, ultrasonography (n = 18); or (4) did not have PCOS based on the NICHD criteria (as described below; n = 1).[8] The remaining cohort included 31 South Asian women with PCOS. For both cohorts, I elected to rely on self-reported race, due to evidence that an individual's identification with a particular racial category is highly reliable.[32]

Clinical Definition of PCOS. Women in both cohorts were retrospectively categorized as having PCOS according to the NICHD criteria.[8] The NICHD criteria were used to allow for unbiased comparisons of ovarian morphology between groups. Menstrual cycle length was assessed categorically (i.e., as being 21 – 35 or ≥ 36 days apart). Women that reported menstrual cycles ≥ 36 days apart were considered to have menstrual irregularities. Clinical androgen status was evaluated with modified hirsutism score, or male-patterned hair growth on nine regions of the body, according to the Ferriman-Gallwey Scale.[33] Biochemical androgen status was judged with serum total testosterone concentration. Women with elevated modified hirsutism scores (≥ 7) or serum total testosterone concentrations (i.e., ≥ 61.5 ng/dL in the White cohort or ≥ 76.0 ng/dL in the South Asian cohort) were considered to have hyperandrogenism. The same thresholds were used to assess menstrual cycle length and hirsutism in both cohorts due to limited data detailing differences in the features between White and South Asian women. However, different thresholds were employed for total testosterone in each cohort due to the use of separate, non-comparable assays between sites. Descriptions of the assays used to measure total testosterone are listed in **Table 4**.

Measurement of Reproductive and Metabolic Features. Reproductive and metabolic endpoints were evaluated by anthropometry (for height, weight, and waist and hips circumference), vitals assessment (for systolic and diastolic blood pressure), and 75-gram 2-hour oral glucose tolerance tests (OGTT). Clinical procedures were conducted using internationally standardized approaches at both sites.[34] Waist-to-hip ratio was calculated as the ratio of waist circumference (cm) to hips circumference (cm), and BMI was calculated by the ratio of weight (kg) to height squared (m2). Blood samples were obtained before (i.e., at fasting or 0 hours) and after the OGTT (i.e., at 2 hours). Fasting samples were assayed for circulating glucose, total testosterone, and anti-Müllerian hormone (AMH) concentrations, and 2-hour samples were assayed for glucose (Table 4). Given the retrospective nature of the present study's design, the use of different biochemical assays at each site could not be avoided. I also acknowledge the potential for inter-site differences in the assessment of menstrual cycle length, hirsutism, anthropometry, and vitals. Therefore, to be as conservative as possible in group comparisons, all reproductive and metabolic endpoints were judged categorically between cohorts (i.e., "normal" or "abnormal" values), based on variable- and assay-specific reference ranges (Table 4).

Table 4. Clinical and biochemical methodologies and normal reference ranges for each cohort

Endpoint	Methodology	Normal Range	Abnormal Range	Intra-Assay CV (%)	Inter-Assay CV (%)
White women with PCOS (United States)					
Menstrual cycle length (d)[1]	Self-report	21 – 35	≥ 36	N/A	N/A
Modified hirsutism score	Ferriman-Gallwey Scale[33]	0 – 6	≥ 7	N/A	N/A
Total testosterone (ng/dL)	LC/MS/MS	< 61.5	≥ 61.5	< 8	< 5
AMH (ng/dL)	ELISA	0.2–16.0	< 0.2 or > 16.0	< 7	< 7
BMI (kg/m^2)[35]	Standard approaches	18.5–24.9	≥ 25.0	N/A	N/A
Waist circumference (cm)[35]	Standard approaches	< 80	≥ 80	N/A	N/A
Waist-to-hip ratio[35]	Standard approaches	< 0.85	≥ 0.85	N/A	N/A
Blood pressure (mmHg)[35]	Standard approaches	< 140/90	≥ 140/90	N/A	N/A
Glucose, 0-hour (mg/dL)[36]	Glucometer	< 100	≥ 100	N/A	N/A
Glucose, 2-hour (mg/dL)[36]	Glucometer	< 140	≥ 140	N/A	N/A
South Asian women with PCOS (India)					
Menstrual cycle length (d)[1]	Self-report	21 – 35	≥ 36	N/A	N/A
Modified hirsutism score	Ferriman-Gallwey Scale[33]	0 – 6	≥ 7	N/A	N/A
Total testosterone (ng/dL)	CLIA	< 76.0	≥ 76.0	< 5	< 5
AMH (ng/dL)	ELFA	1 – 10	> 10	< 9	< 9
BMI (kg/m^2)[35]	Standard approaches	18.5–24.9	≥ 25.0	N/A	N/A
Waist circumference (cm)[35]	Standard approaches	< 80	≥ 80	N/A	N/A
Waist-to-hip ratio[35]	Standard approaches	< 0.85	≥ 0.85	N/A	N/A
Blood pressure (mmHg)[35]	Standard approaches	< 140/90	≥ 140/90	N/A	N/A
Glucose, 0-hour (mg/dL)[36]	GOD-POD	< 100	≥ 100	< 3	< 3
Glucose, 2-hour (mg/dL)[36]	GOD-POD	< 140	≥ 140	< 3	< 3

Abbreviations: BMI, body mass index; AMH, anti-Müllerian hormone; LC/MS/MS, liquid-chromatography tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence immunoassay; ELFA, enzyme-linked immunofluorescent assay; GOD-POD, glucose oxidase and peroxidase; CV, coefficient of variation; N/A, not applicable.

Transvaginal Ultrasonography and Ovarian Image Analysis. Ultrasound examinations were conducted in the United States for White women with PCOS and in India for South Asian women with PCOS. Across clinical research centers and countries, ovaries were assessed using a GE Voluson ultrasound machine (i.e., E8 Expert, S6, or S10 Series) and 5 – 9 (n = 6) or 6 – 12 MHz endovaginal transducer (n = 55) (GE Healthcare, Milwaukee, WI). To the best of our knowledge, ultrasound machine brand and series are unlikely to influence estimates of follicle number or ovarian size. Examinations were conducted during the early follicular phase (i.e., in the absence of follicles > 13 mm) and on the same day as all other endpoints were collected. Three-dimensional volumes of each ovary were obtained using customized settings and the automated volume modality, in order to simultaneously collect data in the sagittal and transverse anatomical planes. Two-dimensional cineloops were then extracted by individually partitioning each plane (Slice Thickness: 0.5 mm) and then archived for offline analysis with Santesoft DICOM Editor Medical Imaging Software (Sante DICOM Editor, Santesoft LTD, Athens, Greece). Image analyses were conducted by investigators at Cornell University, regardless of the site of data collection, to minimize variability in estimates of ovarian morphology. Endpoints of interest included: FNPO, follicle number per cross-section (FNPS), and OV. Reliable estimates of FNPO (i.e., $FNPO_{2–9mm}$) were obtained using the grid system method, as previously described by Lujan *et al*.[37] Follicles were measured and categorized as either small (i.e., $FNPO_{2–5mm}$) or medium-sized (i.e., $FNPO_{6–9mm}$). FNPS and OV were assessed in the largest cross-sectional view of each ovary, as previously described by Christ *et al.*[29] OV was estimated using the simplified formula for a prolate ellipsoid: 0.5 × (transverse diameter) × (longitudinal diameter) × (anteroposterior diameter).[29] Mean values between ovaries were tabulated for FNPO, FNPS, and OV. Women were then categorized as having PCOM according to current international consensus or proposed diagnostic thresholds (i.e.,

$FNPO_{2–9mm}$ ≥ 20 follicles[1]; FNPS ≥ 9 follicles[15]; and OV ≥ 10 mL^{1}). Notably, five investigators assessed the cineloops for the White cohort, and I assessed all of the cineloops for the South Asian cohort. I recognize that it would have been ideal for a single observer to assess all images, while blinded to race. However, use of the grid system method[37] likely minimized potential variability and bias, as we have previously shown that the method yields excellent intra- and inter-rater agreement in follicle counts. Moreover, all six investigators were required to participate in a standardized training program and demonstrate strong inter-rater agreement (i.e., intra-class correlation coefficient >0.08) for an internal reliability assessment, prior to working with study data. Due to unforeseen differences in ultrasound scanning protocols between sites, I could not reliably estimate OV in 14 of the 31 South Asian women with PCOS. Therefore, data relating to ovarian size are presented for only 17 South Asian women with PCOS.

Statistical Analysis. Statistical analyses were performed using JMP Pro 13 Statistical Software (SAS Institute, Cary, NC). The threshold for statistical significance was set a $P < 0.05$. Normality was determined using the Shapiro-Wilk W test. Most variables were not normally distributed; therefore, all data are reported as median (10th – 90th percentile). Differences in demographic, diagnostic, ovarian, and metabolic characteristics between the two cohorts were determined by two-sample t-tests (continuous, parametric variables), Mann-Whitney U tests (continuous, non-parametric variables), or chi-squared tests (categorical variables), as appropriate. Within each cohort, relationships between reproductive / metabolic features and ovarian morphology were assessed by Spearman's rank correlation coefficients.

RESULTS

Demographic and Diagnostic Characteristics of Study Subjects. Demographic and diagnostic characteristics of each cohort are reported in **Table 5**. South Asian and White women with PCOS were similar in age ($P = 0.95$). There were no differences between groups in BMI ($P = 0.16$) or the proportion of women with overweight or obesity ($P = 0.84$). Both cohorts showed evidence of PCOS, as judged by menstrual cycle irregularity and clinical or biochemical hyperandrogenism (Table 4). Most women were diagnosed with hyperandrogenism based on elevated modified hirsutism scores. However, South Asian women with PCOS exhibited higher modified hirsutism scores ($P = 0.04$) and an increased prevalence of hirsutism compared to White women with PCOS (100% vs. 80%, respectively; $P < 0.01$). There were no differences between groups in the proportion of women with elevated total testosterone ($P = 0.22$) or AMH concentrations ($P = 0.19$).

Table 5. Demographic and diagnostic characteristics of study subjects with PCOS

	South Asian Cohort ($n = 31$)	White Cohort ($n = 30$)	*P*
Age (y)	27 (21–30)	25 (20–34)	0.95
BMI (kg/ m^2)	27.2 (22.3–31.9)	29.2 (20.8–44.9)	0.16
Prevalence of overweight/obesity	22/31 (71%)	22/30 (73%)	0.84
Prevalence of menstrual irregularities	31/31 (100%)	30/30 (100%)	-
Modified hirsutism score	10 (7–18)	9 (2–15)	0.04
Prevalence of clinical HA	31/31 (100%)	24/30 (80%)	<0.01
Total testosterone (ng/dL)	56.6 (38.6–94.7)[a]	52.1 (21.6–102.6)	N/A
Prevalence of biochemical HA	4/25 (16%)[a]	9/30 (30%)	0.22
AMH (ng/dL)	7.2 (2.1–12.2)[a]	9.7 (5.3–29.0)[a]	N/A
Prevalence of abnormal AMH	1/15 (7%)[a]	6/27 (22%)[a]	0.19

Data are presented as median (10th – 90th percentile) or n (%), where appropriate. Differences in raw data for total testosterone and AMH were not assessed. [a]Data were not available for all study subjects. *Abbreviations:* BMI, body mass index; HA, hyperandrogenism; AMH, anti-Müllerian hormone; N/A not assessed.

Comparisons of Ovarian Morphology. Ultrasonographic markers of ovarian morphology are shown for each cohort in **Table 6**. South Asian women with PCOS exhibited lower OV compared to White women with PCOS (Median: 9.0 mL vs. 12.5 mL, respectively; $P < 0.01$), wherein the median value in the South Asian cohort fell below the current international diagnostic threshold (i.e., OV ≥ 10 mL). Consequently, the prevalence of PCOM, as indicated by OV, was lower in South Asian versus White women with PCOS (18% vs. 73%, respectively; $P < 0.01$). $FNPO_{2–9\ mm}$ also tended to be lower in the South Asian cohort ($P = 0.06$). However, there were no differences between groups in the prevalence of PCOM by $FNPO_{2–9\ mm}$ ($P = 0.26$), and median follicle counts in both groups exceeded the current international diagnostic threshold (i.e., FNPO ≥ 20).[1] There were no differences between groups in FNPS, $FNPO_{2–5\ mm}$, or $FNPO_{6–9\ mm}$ (All: $P > 0.05$).

Table 6. Comparison of ovarian morphology between South Asian and White women with PCOS

	South Asian Cohort ($n = 31$)	White Cohort ($n = 30$)	*P*
OV (mL)	9.0 (4.8–10.6)[a]	12.5 (6.5–17.9)	<0.01
Prevalence of PCOM by OV	3/17 (18%)[a]	22/30 (73%)	<0.01
FNPS	9 (5–13)	10 (5–17)	0.12
Prevalence of PCOM by FNPS	15/31 (48%)	18/30 (60%)	0.36
$FNPO_{2–9mm}$	31 (22–53)[b]	44 (16–84)[c]	0.06
Prevalence of PCOM by $FNPO_{2–9mm}$	30/31 (96%)[b]	26/29 (90%)[c]	0.26
$FNPO_{2–5mm}$	29 (16–51)[b]	37 (14–77)[c]	0.12
$FNPO_{6–9mm}$	3 (0–8)[b]	5 (0–11)[c]	0.13

Data are presented as median (10th – 90th percentile) or n (%), where appropriate. [a]Reliable estimates of OV were only available for 17 of the 31 South Asian women with PCOS. [b]FNPO was likely underestimated due to compromised image quality. [c]Data were missing for one subject due to technological malfunction. *Abbreviations:* OV, ovarian volume; FNPS, follicle number per section; FNPO, follicle number per ovary.

***Comparisons of Metabolic Features*.** Metabolic features of each cohort are described in **Table 7**. South Asian women with PCOS exhibited higher waist-to-hip ratios compared to White women with PCOS (Median: 0.92 vs. 0.87, respectively; $P < 0.01$). Although waist circumference did not differ between groups ($P = 0.72$), more women had elevated waist circumference (90% vs. 63%

respectively; $P = 0.01$) and elevated waist-to-hip ratio (100% vs. 57%, respectively; $P < 0.01$) in the South Asian cohort than in the White cohort. In addition, more women had elevated 2-hour glucose concentrations in the South Asian cohort than in the White cohort (56% vs. 10%, respectively; $P < 0.01$). Diastolic blood pressure tended to be lower in South Asian women with PCOS ($P = 0.09$), but there were no differences between groups in the prevalence of elevated diastolic blood pressure ($P = 0.37$). There were no differences between groups in systolic blood pressure or fasting glucose (All: $P > 0.05$).

Table 7. Comparison of metabolic features between South Asian and White women with PCOS

	South Asian Cohort ($n = 31$)	White Cohort ($n = 30$)	*P*
Waist circumference (cm)	93 (78–100)	93 (68–132)	0.72
Prevalence of elevated WC	28/31 (90%)	19/30 (63%)	0.01
Waist-to-hip ratio	0.92 (0.87–0.96)	0.87 (0.72–0.98)	<0.01
Prevalence of elevated WHR	31/31 (100%)	17/30 (57%)	<0.01
Systolic BP (mmHg)	110 (110–130)	121 (98–138)	0.44
Prevalence of elevated systolic BP	1/31 (3%)	2/30 (7%)	0.53
Diastolic BP (mmHg)	70 (60–80)	76 (57–93)	0.09
Prevalence of elevated diastolic BP	2/31 (6%)	4/30 (13%)	0.37
Glucose, 0-hour (mg/dl)	98 (84–110)[a]	96 (85–116)	N/A
Prevalence of elevated 0-hour glucose	7/19 (37%)[a]	10/30 (33%)	0.80
Glucose, 2-hour (mg/dl)	144 (92–219)[a]	93 (69–154)	N/A
Prevalence of elevated 2-hour glucose	15/27 (56%)[a]	3/30 (10%)	<0.01

Data are presented as median (10th – 90th percentile) or n (%), where appropriate. Differences in raw data for glucose were not assessed. [a]Data were not available for all study subjects. *Abbreviations:* BP, blood pressure; N/A, not assessed.

Correlations of Reproductive / Metabolic Features and Ovarian Morphology. Race-specific correlations between ovarian morphology and reproductive and metabolic features are presented in **Table 8**. Both South Asian and White women with PCOS showed significant correlations between OV and AMH, but the direction of the relationships were opposite in each cohort

(Spearman's rho: –0.84 vs. 0.41, respectively; $P < 0.05$). $FNPO_{2–9mm}$ and $FNPO_{2–5mm}$ were positively correlated with AMH in the White cohort and with modified hirsutism score in the South Asian cohort (All: $P < 0.05$). FNPS was only positively correlated with AMH in the White cohort ($P < 0.01$). $FNPO_{2–9mm}$ and $FNPO_{2–5mm}$ had negative correlations with systolic and diastolic blood pressure in South Asian, but not White, women with PCOS (All: $P < 0.05$). Likewise, FNPS was also negatively correlated with systolic blood pressure in the South Asian cohort ($P = 0.02$). In White women with PCOS alone, OV was positively correlated with 2-hour glucose ($P = 0.05$) and $FNPO_{6–9mm}$ was negatively correlated with WHR ($P = 0.04$) as well as diastolic blood pressure ($P = 0.03$).

Table 8. Race-specific correlations between reproductive / metabolic features and ovarian morphology

	OV[a]		FNPS		$FNPO_{2–9mm}$		$FNPO_{2–5mm}$		$FNPO_{6–9mm}$	
	S. Asian	*White*	*S. Asian*	*White*	*S. Asian*	*White*	*S. Asian*	*White*	*S. Asian*	*White*
Modified hirsutism score	-0.08	0.12	0.09	0.00	0.37	0.01	0.36	-0.02	0.03	0.18
Total testosterone	-0.51	0.21	0.14	-0.03	0.19	0.07	0.11	0.04	0.13	-0.01
AMH	-0.84	0.41	-0.03	0.58	-0.13	0.83	-0.08	0.84	-0.26	-0.07
BMI	-0.02	0.23	0.06	-0.18	-0.02	0.03	0.00	0.07	-0.20	-0.21
Waist circumference	-0.14	0.04	0.02	-0.25	0.01	-0.09	0.06	-0.04	-0.30	-0.35
Waist-to-hip ratio	-0.35	-0.01	0.06	-0.29	0.09	-0.05	0.17	0.01	-0.42	-0.38
Systolic blood pressure	-0.11	0.33	-0.43	0.03	-0.47	0.32	-0.43	0.36	0.07	-0.31
Diastolic blood pressure	0.06	0.13	-0.33	-0.13	-0.51	0.14	-0.43	0.20	-0.18	-0.40
Glucose, 0-hour	-0.41	0.28	0.28	-0.16	-0.12	0.10	0.04	0.14	-0.28	-0.28
Glucose, 2-hour	-0.45	0.36	0.18	-0.01	-0.15	0.21	-0.14	0.24	-0.01	-0.17

Data are presented as Spearman's rho. The shading reflects a significant or trending correlation: $P < 0.01$ (black), $P < 0.05$ (dark gray), $P < 0.10$ (light gray).
[a]Reliable estimates of OV were only available for 17 of the 31 South Asian women with PCOS. *Abbreviations:* BMI, body mass index; AMH, anti-Müllerian hormone.

DISCUSSION

Summary. The present study aimed to determine differences in ovarian morphology between South Asian and White women with PCOS and whether or how these differences reflected reproductive or metabolic status. Such efforts have implications for understanding the impact of race and ethnicity on diagnostic thresholds for PCOM and for elucidating novel opportunities for ultrasound in the evaluation of PCOS globally. Data in the present study are unique in that I performed direct comparisons of ultrasonographic markers of ovarian morphology between South Asian and White cohorts using newer ultrasound technology and reliable measurement techniques. Overall, I detected lower OV in South Asian versus White women with PCOS, as well as unexpected, opposite correlations between PCOM and reproductive / metabolic features across cohorts.

Race-Specific Differences in OV. To the best of my knowledge, the present study is the first to describe lower OV in South Asian versus White women with PCOS. My findings align with my hypothesis that estimates of OV would be lower in South Asian women. I reported a median OV of 9.0 mL in South Asian women with PCOS compared to 12.5 mL in White women with PCOS. Moreover, 82% of South Asian women with PCOS had OV < 10 mL, wherein a large proportion of the cohort did not meet the current international criteria for PCOM by OV. Only 27% of White women with PCOS had OV <10 mL. My findings are not surprising, given that previous diagnostic test studies have proposed thresholds of ≥ 6 – 8 mL to adequately distinguish South Asian women with PCOS from South Asian women without PCOS.[19,20] The proposed thresholds for South Asian women are substantially lower than the diagnostic threshold for OV (i.e., ≥ 10 mL),[1] which was

established in predominantly White cohorts.[14] In clinical practice, false negative diagnoses could lead to missed opportunities for timely identification and treatment of comorbidities related to PCOS. Therefore, my findings support the need for a race-specific threshold for OV. Although FNPO is heralded as the best ultrasonographic marker of PCOS, there is still value in considering diagnostic thresholds for OV. Poor image quality and/or use of older imaging technology can compromise visualization of follicles within the ovary, and therefore, the reliability of FNPO to detect PCOM. In these cases, OV can be prioritized to diagnose PCOM.[1,14,38] Ultimately, to confirm whether a lower diagnostic threshold is appropriate for South Asian women, future studies should include South Asian and White control groups. Both control cohorts are needed to identify thresholds that balance sensitivity and specificity for PCOS. The present study was not able to include controls, because the gynecologic primary care center in India, from which we acquired patient charts, does not see healthy women without PCOS.

Race-Specific Differences in FNPO. The present study also appears to be the first to report a potential difference in FNPO between South Asian and White women with PCOS. I detected a lower median $FNPO_{2–9mm}$ of 31 follicles in South Asian women, compared to 44 follicles in White women, but the difference did not reach significance. The trend aligns with my hypothesis that estimates of FNPO would be lower in South Asian women. Previous diagnostic test studies have proposed thresholds of ≥ 9–12 follicles throughout the entire ovary[19,20] to adequately distinguish South Asian women with PCOS from those without PCOS. The proposed thresholds for FNPO in the South Asian population are substantially lower than the current diagnostic threshold (i.e., ≥ 20 follicles).[1] It is important to acknowledge that FNPO was substantially higher among the South Asian women in the present study than in the studies by Ahmed *et al.* (*Median:* 13 follicles) and

Kar *et al*. (*Mean:* 20 follicles).[19,20] I found that only 4% of South Asian women with PCOS had FNPO < 20 and could not detect differences between the two cohorts in the prevalence of PCOM by FNPO. I appreciate that utilization of the grid system method[37] may have allowed me to conduct a more thorough analyses of the ovaries of South Asian women with PCOS, thereby leading to an increased follicle count compared to previous studies. Both Ahmed *et al.* and Kar *et al.* reported counting follicles by 'scanning throughout the entire ovary,' presumably in real time.[19,20] Emerging evidence from our group suggests that FNPO derived from real-time approaches is lower than FNPO derived from offline counts. Real-time counts are conducted while the patient is undergoing the ultrasound scan, likely leading to a need for faster-paced and less meticulous assessments. Without the ability to focus counts to a particular region of the ovary and flag the follicles that have already been counted, real-time counts could lead to under-estimates of FNPO. Therefore, OV may serve as a more reliable marker of PCOM, if offline follicle counts cannot be conducted in clinical settings.[38] Nevertheless, my findings suggest that the current threshold for FNPO may be sufficient to detect PCOM in South Asian women. As with OV, future studies should include both South Asian and White control groups to corroborate the adequacy of the current thresholds in South Asian populations.

Ovarian Morphology and AMH. AMH showed significant and positive correlations with FNPS ($\rho = 0.58$, $P < 0.01$), $FNPO_{2\text{-}9mm}$ ($\rho = 0.83$, $P < 0.01$), and $FNPO_{2\text{-}5mm}$ ($\rho = 0.84$, $P < 0.01$) in White women with PCOS. My findings were expected in light of existing evidence on AMH in PCOS. Previous studies have reported positive correlations between AMH and antral follicle count (i.e., FNPO × 2) in women with and without PCOS.[39–42] AMH is produced by pre-antral and antral follicles (≤ 8 mm)[39,43] and consequently believed to be an indirect biomarker of the ovarian

reserve.[44] Women with PCOS have increased circulating concentrations of AMH, likely due to the increased number of small follicles in polycystic ovaries (i.e., 2 – 5 mm)[31,40] and heightened per-follicle production of AMH.[45] Lack of a significant correlation with $FNPO_{6–9mm}$ ($\rho = –0.07$, $P > 0.10$) is consistent with evidence of reduced numbers of medium-sized follicles (i.e., 6 – 9 mm) in PCOS.[31] Surprisingly, AMH did not relate to follicle number or follicle size in South Asian women (All: $\rho < 0.30$, $P > 0.10$). Racial and ethnic differences in ovarian reserve, as reflected by AMH, have been reported across other populations and are thought to arise from a combination of genetic and environmental factors.[46] The precise mechanisms remain unclear but could provide a physiologic basis for the presence of different relationships between AMH and FNPO in White versus South Asian women with PCOS. AMH also showed unexpected relationships with OV. I noted a moderate, positive correlation between AMH and OV in White women ($\rho = 0.41$, $P < 0.05$), and a strong, negative correlation between AMH and OV in South Asian women ($\rho = –0.84$, $P < 0.05$). A consistent link between AMH and OV would add to the hormone's clinical utility as a surrogate marker of PCOM.[44] Race could mediate the relationship between AMH and ovarian size, but I suspect that the negative correlation in South Asian women with PCOS had more to do with the assay that our collaborator used to measure AMH. AMH is susceptible to substantial intra-individual and inter-assay variation and most commercially available methods are considered unreliable.[47] As a result, the International Evidence-Based Guideline for the Assessment and Management of PCOS recently concluded that AMH may have the potential to indicate PCOM, but not until the assays are standardized.[1]

Ovarian Morphology and Blood Pressure. Systolic ($\rho = –0.47$, $P < 0.01$) and diastolic blood pressure ($\rho = –0.51$, $P < 0.01$) showed significant and negative correlations with $FNPO_{2-9mm}$ in

South Asian women with PCOS. My findings were unexpected, given that we did not find significant associations between blood pressure and follicle number in our previous study.[29] I also noted a trending, positive association between systolic blood pressure and $FNPO_{2\text{-}9mm}$ in White women with PCOS ($\rho = 0.32$, $P < 0.05$). The opposing relationships with blood pressure across cohorts could be interpreted to mean that metabolic abnormalities have differential negative effects on follicle development across populations. However, it is important to mention that very few women in the present study had elevated systolic (*South Asian:* 3%; *White:* 7%) or diastolic blood pressure (*South Asian:* 6%; *White:* 13%) based on international diagnostic thresholds for hypertension.[35] The narrow range of blood pressure measurements could have led to spurious findings and limits my ability to draw conclusions about any mediating effect of race on the relationship between blood pressure and ovarian morphology.

Other Relationships. The remaining reproductive and metabolic features showed relatively weak correlations with follicle number, follicle size, and ovarian size (All: $\rho < 0.50$, $P > 0.10$). The direction of the relationships also differed across cohorts – to an extent that was difficult to explain. Of the reproductive features, modified hirsutism score was positively correlated with $FNPO_{2\text{-}9mm}$ and $FNPO_{2\text{-}5mm}$ in South Asian but not White women with PCOS. Androgens are produced by small follicles and believed to play an intra-ovarian role in follicular excess and anovulation in PCOS.[48] As a result, previous studies have reported positive relationships between serum testosterone, $FNPO_{2\text{-}9mm}$, and $FNPO_{2\text{-}5mm}$.[29,49] However, modified hirsutism score has not been consistently linked to follicle number or size. One reason could be that male-patterned hair growth reflects long-term exposure to elevated circulating androgens from any source (e.g., the ovaries or adrenal gland), while serum testosterone reflects current androgen status, and by extension, current

follicle populations.[29,49] In the present study, South Asian women with PCOS had significantly higher modified hirsutism scores compared with White women, and all women in the South Asian cohort were diagnosed with PCOS based on clinical evidence of hyperandrogenism. Such characteristics may have contributed to my findings.

Severity of Metabolic Disturbances. Of the metabolic features, poorer metabolic health (e.g., elevated waist-to-hip ratio and elevated diastolic blood pressure) were negatively correlated with $FNPO_{6\text{-}9mm}$ in White women with PCOS. My findings reaffirm those of our previous studies,[29,30] which showed that metabolic status may be involved in the regulation of antral follicle growth to dominance and ovulation. The absence of similar relationships in South Asian women with PCOS could reflect the relatively small numbers of 6 – 9 mm follicles (*Mean:* 3 follicles), which may have reduced my ability to detect correlations with metabolic features. The conflicting correlations across groups could also be explained by the overall good health of the two cohorts. Despite evidence of PCOS and abdominal obesity, most women in the present study had normal blood pressure and normal glucose concentrations. I might have been able to detect more consistent relationships had the women shown greater severity of reproductive and metabolic comorbidities. Nevertheless, a heightened prevalence of metabolic disturbances in the South Asian cohort supports current recommendations for lifestyle intervention as the first-line therapy in overweight / obese women with PCOS.[1] I appreciate that future studies in broader populations are ultimately needed to understand the role of ultrasonography in facilitating healthcare versus standard clinical metrics (e.g., BMI or waist circumference).

Strengths and Limitations. The present study had two major strengths. First, to the best of my

knowledge, no other studies have directly compared ultrasonographic markers of PCOM across races. Evaluating the prevalence of PCOM based on current international diagnostic thresholds also allowed me to consider whether the current criteria are appropriate in South Asian women. My honors thesis can, therefore, be thought of as providing proof-of-concept for future studies to establish race-specific diagnostic thresholds for PCOM. Second, I used the grid system method to reliably determine FNPO in both cohorts. Such an approach allowed for meticulous analysis of the ovaries and reduced the impact of inter-method and inter-observer variability when comparing follicle data across races. However, the present study also had three main limitations. First, due to the retrospective design of the study, I was able to compare ovarian, reproductive, and metabolic features between 31 South Asian women with PCOS and 30 White women with PCOS. *Post hoc* sample size calculations revealed that I only had power (i.e., 0.80) to detect significant differences between groups in OV ($\alpha = 0.05$) and correlations with effect sizes ≥ 0.5 ($\alpha = 0.05$). It is possible that the unexpected findings across cohorts was influenced by the small sample size. An ideal study design would have included 64 women per group, because that number would have enabled me to confirm significant differences in FNPO and FNPS and detect correlations with weaker effect sizes (i.e., ~0.35). The inclusion of healthy controls of both races would also have strengthened the study. Efforts are ongoing in our group to acquire more patient records and confirm the relationships presented herein. Second, biochemical endpoints were evaluated with different assays across sites. It would have been challenging to use the same assays, because we were only able to receive medical charts and ultrasound scans (not sera) from our collaborator. I attempted to control for the impact of assay characteristics by calculating the prevalence of women with abnormal endocrine or metabolic values and by not combining reproductive / metabolic data for the correlation analysis. However, I cannot exclude the possibility that use of different assays

contributed to the unexpected, opposing relationships detected across cohorts. Finally, both study populations consisted of women with a narrow range of metabolic disturbances. Larger cohorts with more diverse metabolic statuses may have revealed greater differences in ovarian morphology between White and South Asian women with PCOS, as well as more striking correlations with reproductive / metabolic features. Because metabolic disturbances could manifest for a variety of reasons, future studies should explore any impacts of country of origin, country of residence, and lifestyle behaviors on correlations with ovarian morphology.

CONCLUSION

In summary, ovarian morphology differs between White and South Asian women with PCOS. My findings are consistent with recent international recommendations that urge for race and ethnicity to be considered in the diagnosis of PCOS.[1,14] My results also indicate that South Asian women with PCOS have greater metabolic disturbances compared to White women with PCOS. Future studies in larger cohorts of South Asian and White women are ultimately needed to understand the utility of the ovary for reflecting systemic health across populations. Broadly, my findings highlight the importance of considering race in both the diagnosis and treatment of PCOS.

ACKNOWLEDGEMENTS

I would like to thank Dr. Marla Lujan, my primary research mentor, and Dr. Brittany Jarrett, my project supervisor, for their unwavering support throughout my honors thesis. I would also like to thank Dr. Sujata Kar for sharing her patients' clinical and ultrasound data and Dr. Kathleen Hoeger of the University of Rochester for her support of our studies in New York State. I would like to thank our study participants for their dedication to our research. Finally, I am grateful to Lydia

Anderson, Brittany Jarrett, Andres Ortiz, Adrienne Pisch, and Heidi Vanden Brink for their previous efforts in completing the image analyses for the White cohort. The present study was funded by the Division of Nutritional Sciences at Cornell University, as well as the President's Council of Cornell Women, the United States Department of Agriculture (Grant 2010-34324-20769), and National Institutes of Health (Grant R56-HD089962).

REFERENCES

1. Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome†‡. *Hum Reprod*. 2018;33(9):1602-1618. doi:10.1093/humrep/dey256
2. Escobar-Morreale HF. Polycystic ovary syndrome: Definition, aetiology, diagnosis and treatment. *Nat Rev Endocrinol*. 2018;14(5):270-284. doi:10.1038/nrendo.2018.24
3. Fauser BCJM, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril*. 2012;97(1):28-38.e25. doi:10.1016/j.fertnstert.2011.09.024
4. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: A systematic review and meta-analysis. *Obes Rev*. 2013;14(2):95-109. doi:10.1111/j.1467-789X.2012.01053.x
5. Dokras A, Stener-Victorin E, Yildiz BO, et al. Androgen Excess- Polycystic Ovary Syndrome Society: position statement on depression, anxiety, quality of life, and eating disorders in polycystic ovary syndrome. *Fertil Steril*. 2018;109(5). doi:10.1016/j.fertnstert.2018.01.038
6. Mohammad MB, Seghinsara AM. Polycystic Ovary Syndrome (PCOS), Diagnostic Criteria, and AMH. *Asian Pac J Cancer Prev*. 2017;18(1):17-21. doi:10.22034/APJCP.2017.18.1.17
7. Lujan ME. Diagnostic Criteria for Polycystic Ovary Syndrome: Pitfalls and Controversies. *J Obstet Gynaecol Canada*. 2008;30(8):671-679. doi:10.1016/S1701-2163(16)32915-2.Diagnostic

8. Zawadzki J, Dunaif A. Diagnostic Criteria for Polycystic Ovary Syndrome: Towards a Rational Approach. *Polycystic Ovary Syndr*. 1992;1:377-384.

9. Fauser BCJM. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81(1):19-25. doi:10.1016/j.fertnstert.2003.10.004

10. Azziz R, Carmina E, Dewailly D, et al. *The Androgen Excess and PCOS Society Criteria for the Polycystic Ovary Syndrome: The Complete Task Force Report*. Vol 91.; 2009. doi:10.1016/j.fertnstert.2008.06.035

11. Azziz R, Carmina E, Dewailly D, et al. Position statement: Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: An androgen excess society guideline. *J Clin Endocrinol Metab*. 2006;91(11):4237-4245. doi:10.1210/jc.2006-0178

12. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: A systematic review and meta-Analysis. *Hum Reprod*. 2016;31(12):2841-2855. doi:10.1093/humrep/dew218

13. Balen AH, Laven JSE, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: International consensus definitions. *Hum Reprod Update*. 2003;9(6):505-514. doi:10.1093/humupd/dmg044

14. Dewailly D, Lujan ME, Carmina E, et al. Definition and significance of polycystic ovarian morphology: A task force report from the androgen excess and polycystic ovary syndrome society. *Hum Reprod Update*. 2014;20(3):334-352. doi:10.1093/humupd/dmt061

15. Lujan ME, Jarrett BY, Brooks ED, et al. Updated ultrasound criteria for polycystic ovary syndrome: Reliable thresholds for elevated follicle population and ovarian volume. *Hum*

Reprod. 2013;28(5):1361-1368. doi:10.1093/humrep/det062

16. 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(11):3123-3129. doi:10.1093/humrep/der297
17. 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(5):700-703. doi:10.1002/uog.5393
18. Köşüş N, Köşüş A, Turhan NÖ, Kamalak Z. 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(2):177-181. doi:10.1016/j.ejogrb.2010.10.007
19. Ahmed S, Pahwa S, Das C, et al. Comparative evaluation of sonographic ovarian morphology of Indian women with polycystic ovary syndrome versus those of normal women. *Indian J Endocrinol Metab*. 2014;18(2):180-184. doi:10.4103/2230
20. Kar S, Samparna S. 2D and 3D Transvaginal Sonography to Determine Cut-offs for Ovarian Volime and Follicle Number Per Ovary for Diagnosis of Polycystic Ovary Syndrome in Indian Women. *J Repro*. 2018;19(3):146-151.
21. Williamson K, Gunn AJ, Johnson N, Milsom SR. The impact of ethnicity on the presentation of polycystic ovarian syndrome. *Aust New Zeal J Obstet Gynaecol*. 2001;41(2):202-206. doi:10.1111/j.1479-828X.2001.tb01210.x
22. Kakoly NS, Khomami MB, Joham AE, et al. Ethnicity, obesity and the prevalence of impaired glucose tolerance and type 2 diabetes in PCOS: A systematic review and meta-

regression. *Hum Reprod Update*. 2018;24(4):455-467. doi:10.1093/humupd/dmy007

23. Chan JL, Kar S, Vanky E, et al. Racial and ethnic differences in the prevalence of metabolic syndrome and its components of metabolic syndrome in women with polycystic ovary syndrome: a regional cross-sectional study. *Am J Obstet Gynecol*. 2017;217(2):189.e1-189.e8. doi:10.1016/j.ajog.2017.04.007

24. Norman RJ, Mahabeer S, Masters S. Ethnic differences in insulin and glucose response to glucose between white and Indian women with polycystic ovary syndrome. *Fertil Steril*. 1995;63(1):58-62. doi:10.1016/S0015-0282(16)57297-5

25. Zabaneh D, Balding DJ. A genome-wide association study of the metabolic syndrome in Indian Asian men. *PLoS One*. 2010;5(8):1-6. doi:10.1371/journal.pone.0011961

26. Tian Y, Zhao H, Chen H, et al. Variants in FSHB are associated with polycystic ovary syndrome and luteinizing hormone level in Han Chinese Women. *J Clin Endocrinol Metab*. 2016;101(5):2178-2184. doi:10.1210/jc.2015-3776

27. Louwers Y V., Stolk L, Uitterlinden AG, Laven JSE. Cross-Ethnic meta-Analysis of genetic variants for polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2013;98(12):2006-2012. doi:10.1210/jc.2013-2495

28. Brower MA, Jones MR, Rotter JI, et al. Further investigation in europeans of susceptibility variants for polycystic ovary syndrome discovered in genomewide association studies of chinese individuals. *J Clin Endocrinol Metab*. 2015;100(1):E182-E186. doi:10.1210/jc.2014-2689

29. Christ JP, Vanden Brink H, Brooks ED, Pierson RA, Chizen DR, Lujan ME. Ultrasound features of polycystic ovaries relate to degree of reproductive and metabolic disturbance in polycystic ovary syndrome. *Fertil Steril*. 2015;103(3):787-794.

doi:10.1016/j.fertnstert.2014.12.094

30. Lujan ME, Kepley AL, Chizen DR, Lehotay DC, Pierson RA. Development of morphologically dominant follicles is associated with fewer metabolic disturbances in amenorrheic women with polycystic ovary syndrome: A pilot study. *Ultrasound Obstet Gynecol*. 2010;36(6):759-766. doi:10.1002/uog.7751

31. Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C, Dewailly D. Ultrasound examination of polycystic ovaries: Is it worth counting the follicles? *Hum Reprod*. 2003;18(3):598-603. doi:10.1093/humrep/deg115

32. McAlpine DD, Beebe TJ, Davern M, Call KT. Agreement between self-reported and administrative race and ethnicity data among Medicaid enrollees in Minnesota. *Health Serv Res*. 2007;42(6 P2):2373-2388. doi:10.1111/j.1475-6773.2007.00771.x

33. Ferriman D, Gallwey JD. Clinical assessment of body hair in women. *J Clin Endocrinol Meta*. 1961;21(3):1440–7. doi:10.1210/jcem-21-11-1440

34. CDC. *Anthropometry Procedures Manual*.; 2011. https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/Anthropometry_Procedures_Manual.pdf.

35. Alberti KGM, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetes Med*. 2006;23:469-480. doi:10.1155/2010/307409

36. CDC. Getting Tested: Type 1 Diabetes, Type 2 Diabetes, and Prediabetes. https://www.cdc.gov/diabetes/basics/getting-tested.html. Published 2019.

37. Lujan ME, Brooks ED, Kepley AL, Chizen DR, Pierson RA, Peppin AK. Grid analysis improves reliability in follicle counts made by ultrasonography in women with polycystic

ovary syndrome. *Ultrasound Med Biol*. 2010;36(5):712-718. doi:10.1016/j.ultrasmedbio.2010.02.008

38. Christ JP, Willis AD, Brooks ED, et al. Follicle number, not assessments of the ovarian stroma, represents the best ultrasonographic marker of polycystic ovary syndrome. *Fertil Steril*. 2014;101(1):280-287. doi:10.1016/j.fertnstert.2013.10.001

39. Christiansen SC, Eilertsen TB, Vanky E, Carlsen SM. Does AMH reflect follicle number similarly in women with and without PCOS? *PLoS One*. 2016;11(1):1-13. doi:10.1371/journal.pone.0146739

40. 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(12):5957-5962. doi:10.1210/jc.2003-030727

41. Leonhardt H, Hellström M, Gull B, et al. Ovarian morphology assessed by magnetic resonance imaging in women with and without polycystic ovary syndrome and associations with antimüllerian hormone, free testosterone, and glucose disposal rate. *Fertil Steril*. 2014;101(6):1747-1756. doi:10.1016/j.fertnstert.2014.02.022

42. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB. A validated model of serum Anti-Müllerian hormone from conception to menopause. *PLoS One*. 2011;6(7):1-7. doi:10.1371/journal.pone.0022024

43. Jeppesen J V., Anderson RA, Kelsey TW, et al. Which follicles make the most anti-Müllerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. *Mol Hum Reprod*. 2013;19(8):519-527. doi:10.1093/molehr/gat024

44. Dewailly D, Andersen CY, Balen A, et al. The physiology and clinical utility of anti-Müllerian hormone in women. *Hum Reprod Update*. 2014;20(3):370-385. doi:10.1093/humupd/dmt062

45. Bhide P, Dilgil M, Homburg R, Akwaa C, Shah A, Gudi A. Each small antral follicle in ovaries of women with polycystic ovary syndrome produces more antimüllerian hormone than its counterpart in a normal ovary: an observational cross-sectional study. *Fertil Steril*. 2015;103(2):537-541. doi:10.1016/j.fertnstert.2014.10.033

46. Tal R, Seifer DB. Potential Mechanisms for Racial and Ethnic Differences in Antimüllerian Hormone and Ovarian Reserve. *Int J Endocrinol*. 2013;2013:1-7. doi:10.1155/2013/818912

47. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Müllerian hormone and antral follicle count as biomarkers of ovarian response. *Hum Reprod Update*. 2015;21(6):698-710. doi:10.1093/humupd/dmu062

48. Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Hum Reprod Update*. 2004;10(2):107-117. doi:10.1093/humupd/dmh010

49. Brink H Vanden, Willis AD, Jarrett BY, et al. Sonographic markers of ovarian morphology, but not hirsutism indices, predict serum total testosterone in women with regular menstrual cycles. 2016;105(5):1322-1329. doi:10.1016/j.fertnstert.2015.12.136.Sonographic