GEOGRAPHICAL VARIATIONS IN POLYCYSTIC OVARIAN MORPHOLOGY IN INDIA- AND UNITED STATES-BASED WOMEN WITH POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Background. Polycystic ovary syndrome (PCOS) is the most common reproductive disorder in women affecting 1 and 8 reproductive age women worldwide. PCOS is based on the presence of two or more cardinal features including ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology. Ethnic and racial variations in the clinical presentation of PCOS have been reported suggesting that screening and diagnosis of PCOS may require consideration of regional differences in reproductive symptomology. Little to no data exist on the potential for geographic variations in polycystic ovarian morphology on ultrasonography. As such, the utility of the current international standard for defining polycystic ovaries is in question.

Study Question. Are there differences in ovarian morphology between India- and United States-based women with PCOS?

Objective and Rationale. To determine whether ovarian morphology differs between women with PCOS from two different geographic regions of the globe in order to support the appropriateness of a singular international standard to define polycystic ovarian morphology.

What is Known Already. The International Evidence-Based Guideline for the Assessment and Management of PCOS supports phenotypic differences in the presentation of PCOS, including variations in the reproductive and cardiometabolic profiles among women of different races and ethnicities. However, few studies have directly contrasted clinical, endocrine and morphological variables across races, ethnicities and/or geography.

Study design, Size, Duration. A retrospective, cross-sectional analysis of de-identified research and medical records from women with PCOS collected between 2010 to 2022 from two geographic regions (India and the United States) was performed. The study population comprised a total of 331 reproductive age (18-38 years) women with PCOS for which both an ultrasound scan of the ovaries and clinical data to corroborate a PCOS diagnosis were available.

Participants, Setting, Methods. In India, data for inclusion were available for 119 women with PCOS attending a gynecological practice for evaluation and treatment of reproductive disorders. In the United States, data for inclusion were available from 212 women with PCOS that engaged in a research study across three clinical research centers in New York State. Two sample t-tests and chi-square or Fisher's exact tests were used to assess differences in continuous and categorical variables across groups, respectively. Bivariate Pearson correlation analysis was used to determine any correlations between ovarian morphology markers with reproductive and metabolic features for the two geographical groups.

Main Results. Ovulatory dysfunction as judged by menstrual irregularity was more prevalent in India-based women compared to U.S.-based women, with a greater majority reporting cycles greater than 35 days (99 vs. 87%, $p<0.0001$). Likewise, a higher prevalence of hyperandrogenism was apparent in India- versus U.S.-based women with PCOS (99% vs. 52%, $P<0.0001$). Both the proportion of those with elevated testosterone (45% vs. 17%) and Ferriman-Gallwey scores greater than 6 (100% vs. 43%) were higher in the India-based group compared to U.S.-based participants, with a difference also noted in the severity of hirsutism scores (all $P<0.001$). Nearly all participants in both groups met the criteria for polycystic ovarian

morphology (99%) with the India-based group demonstrating a higher prevalence of follicle excess compared to the U.S.-based group (94% vs. 86%, p = 0.039). Prevalence of ovarian enlargement was similar across groups (India 71% vs. United States 70%, p=0.900). Further, follicle number per ovary (35.3 ±13.4 vs. 39.1 ±20.6), follicle number per single cross-section (8.8 ±2.8 vs. 9.6 ±4.0), and ovarian volume (9.0 ± 3.9 vs. 8.8 ± 4.4) did not differ between India- and -U.S.-based groups (all p>0.05). Of the non-conventional ovarian features assessed, stromal area (4.3 ± 1.4 vs. 4.2 ±1.4), and stromal to ovarian area ratio (0.8 ± 0.1 vs. 0.8 ± 0.1), did not differ between groups – albeit ovarian area was slightly larger in women with PCOS from India (6.3 ±1.7 vs. 6.0 ±1.8, p=0.030). Collectively, a vast majority of participants from India met the criteria for Frank PCOS whereas U.S.-based participants had a more heterogeneous phenotypic presentation, with a majority having a Mild variant of PCOS. Subgroup analyses of women meeting the criteria for Frank PCOS confirmed little to no variations in ovarian size and stromal characteristics across the India- and U.S.-based groups. However, follicle number per ovary (48.6±23.2 vs. 35.9± 13.2, P<0.0001) and follicle number per single cross-section (10.9±3.9 vs. 8.9±2.7 p=0.0003) were higher in the U.S.- versus India-based group, respectively. Associations between ovarian morphology and reproductive markers [menstrual cycle length (ρ= 0.16 – 0.25), Ferriman-Gallwey hirsutism scores (ρ= 0.16 – 0.26), and/or total testosterone (ρ= 0.24 – 0.34)] were noted in both the U.S.- and India-based group on both the full and subgroup analysis (all, p<0.05). By contrast, associations detected between ovarian morphology and metabolic status markers (fasting blood glucose (ρ= -0.29 – 0.31), diastolic blood pressure (ρ= -0.24 – -0.26), body mass index (ρ= 0.12 – 0.32), triglycerides (ρ= 0.18 – 0.34), and HDL (ρ= -0.12 – -0.33), were inconsistent across both groups with additional associations emerging in the subgroup analysis (2-hour glucose (ρ= -0.29 – 0.31), and waist to hip ratio (ρ= -0.31 – 0.43).

Limitations, Reasons for Caution. Ultrasonographic scans from India were conducted either post-natural menses or after a progesterone-induced bleed whereas, scans conducted in the U.S. never occurred following progesterone stimulation. While all scans were conducted in the follicular phase, we cannot exclude the possibility that progesterone withdrawal in a subset of participants in India impacted morphological findings across groups. Different biochemical assays were used across geographic regions. Since metabolic marker assays used for both groups were not comparable, analyses were run separately for each region, thereby restricting the analyses to subgroup regression analyses (India and U.S.). Participants were evaluated in different settings (i.e., research center versus clinical practice). Therefore, differences in PCOS symptomology between groups may relate to a higher likelihood of severe clinical manifestations presenting primarily to a clinical practice rather than a research setting. Lastly, data from healthy women with regular cycles were not available for inclusion across both sites. Therefore, the generation of regional-specific criteria for polycystic ovarian morphology and their performance could not be directly evaluated.

Conclusion and wider implications of the findings. Geographic differences exist in the clinical presentation of PCOS. However, variations in ovarian morphology may not be sufficient to warrant regional definitions of polycystic ovarian morphology. Ovarian dysmorphology served as a biomarker of the severity of reproductive symptomology in both regions consistent with the ovary being a central component of the pathophysiology of PCOS. However, more research is needed to fully elaborate its utility as a marker of metabolic status across races and ethnicities.

BIOGRAPHICAL SKETCH

Hilary Huimin Zhang was born on February 3rd, 1999 in Manhattan, NY to Jenny Zhang and Shao Bin Zhang. Being a lover of magical things and possessing a thirst for scientific knowledge, Hilary grew up wondering which was superior to the other. Only to find out, both can exist in a vacuum.

As a young girl, Hilary always had her head buried in a book. Every time she read; she was able to time travel to different worlds. It started with the Magic School Bus Series and then graduated to non-fiction research books as she got older. It was then she discovered her passion for nutritional sciences. Coupled with her personal growth journey at the time, she sought to learn about holistic health as an outlet for her budding passion for all things nutrition and health. From then, she changed gears from being a biology major at Stonybrook University to a nutrition and food studies major at New York University (NYU) with the hopes of becoming a dietitian. Her undergraduate career was bustling with opportunities, just like the city. She was part of Dr. Kathleen Woolf's lab during her time at NYU, working with the elderly and chronic kidney disease. Dr. Woolf encouraged her to seek research as a future option, but Hilary was ultimately unsure despite knowing that she always had a curious spirit to pursue this route. This was also the time that she was working at a pediatric office to feed her passion for working with the maternal and pediatric populations. At the end of her undergraduate degree, she was accepted into the Cornell Dietetic Internship (DI) Program where she went on to complete and obtain her Registered Dietitian Credential.

After partaking in Cornell's research-heavy DI program, Hilary's desire to one day publish her own paper pushed her to seek avenues to make this dream come true. She decided to seek more research opportunities and ended up continuing her higher education at Cornell

University in the College of Human Ecology. It was here that she found a home in Dr. Marla Lujan's Lab. For the next year, she spent her time learning about the ovary and investigating ovarian morphology in relation to reproductive and metabolic dysfunctions in two different geographical locations.

This thesis is dedicated to my family who I cherish dearly.

To my grandparents, my mother Jenny Zhang, my father Shao Bin Zhang and my two younger siblings Emily and Steven Zhang.

Thank you for your undying love and support that has allowed me to blossom into the woman I am today.

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My academic journey here at Cornell was short but sweet. Everything that I have accomplished would not have been possible without the help of the many brilliant individuals that I have crossed paths with within this past year.

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

TABLES

APPENDIX

FIGURES

APPENDIX

GLOSSARY

Abbreviation	Term
PCO	Polycystic Ovaries
PCOS	Polycystic Ovary Syndrome
PCOM	Polycystic Ovarian Morphology
AEPCOS	Androgen Excess and Polycystic Ovary Syndrome Society
NIH	National Institute of Health
ESHRE	European Society of Human Reproduction and Embryology
ASRM	American Society for Reproductive Medicine
FNPO	Follicle Number Per Ovary
FNPS	Follicle Number Per Cross-section
OV	Ovarian Volume
OA	Ovarian Area
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
BMI	Body Mass Index
SDXCA	Siemens Dimension Expand Chemistry Analyzer
GOD-POD	Glucose Oxidase and Peroxidase

GPO-TRINDER	Glycerol Phosphate Oxidase
CHOD-PAP	Cholesterol Oxidase Phenol 4-aminoantipyrine Peroxidase
SA	Stromal Area
S/A	Stromal Area/Ovarian Area Ratio
ICC	Intraclass Correlation Coefficient
WC	Waist Circumference
WHR	Waist-to-hip Ratio
BP	Blood Pressure
TG	Triglycerides
TC	Total Cholesterol
CYP11A	Cholesterol Side Chain Cleavage Enzyme
CYP17	17a-hydroxylase/17,20-desmolase

I. INTRODUCTION

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age.[1] The global burden of PCOS is significant, affecting nearly 20%[2] of reproductive age women worldwide.[3-6] The etiology of PCOS remains unclear, but both environmental factors and genetics are posited to contribute to its development.[3,4] PCOS is characterized by a combination of three cardinal features: ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology (PCOM). The condition is also associated with a variety of metabolic complications, such as, but not limited to, obesity, insulin resistance, dyslipidemia, hypertension and other risk factors for cardiovascular disease and diabetes.[7] PCOS also contributes to an increased risk for psychological complications such as anxiety and depression and contributes to a reduction in quality of life.[8-11] Currently, there is no cure for PCOS. Treatment is focused on preventative measures and mitigation of symptoms as per the individual unique clinical presentation and personal goals.

Cardinal Features of PCOS

There are 3 distinct cardinal features of PCOS that are used in the diagnostic algorithm for this condition.[2] Ovulatory dysfunction is judged by the degree of menstrual cycle irregularity, Hyperandrogenism can manifest by either clinical or biochemical indicators of androgen excess including male-patterned hair growth (hirsutism) and increased serum total or free testosterone levels, respectively. Last, polycystic ovaries on ultrasonography are defined by either follicle excess and/or ovarian enlargement. Several attempts have been made to generate a consensus on a diagnostic algorithm for the condition of PCOS.[2,12-14] Each of these attempts has debated the

significance or relevance of one or more of the cardinal features. At present, no one cardinal feature is considered absolutely required for the diagnosis of PCOS.

PCOS Phenotypes

The first diagnostic algorithm for PCOS was developed by the National Institutes of Health (NIH) in 1990 wherein PCOS was based on the combined presence of oligo-anovulation and either clinical or biochemical evidence of hyperandrogenism.[12] Although this was a transformative moment in establishing a universally accepted diagnostic algorithm for PCOS, it failed to recognize the importance of morphological variations in ovarian morphology that were common in women with PCOS[15]. In 2003, a subsequent set of criteria established by the Rotterdam Working Group (a joint venture by the American Society for Reproductive Medicine and European Society of Human Reproduction and Embryology) included polycystic ovaries on ultrasonography as a diagnostic marker for diagnosis.[13] The "Rotterdam criteria" required individuals to present at least 2 out of the 3 cardinal features in order to be diagnosed with PCOS. However, these updated criteria remained controversial in that PCOS could manifest in the absence of hyperandrogenism. As a response, the Androgen Excess and PCOS Society (AE-PCOS) proposed a third diagnostic algorithm in 2006 that required both androgen excess (clinical or biochemical) and ovarian dysfunction (oligo-anovulation or PCOM) in order to define PCOS.[14]

Most recently, the 2018 International Guideline supports the centrality of polycystic ovaries as a diagnostic marker of PCOS, as well as the broad clinical spectrum of PCOS represented by the Rotterdam criteria. Accordingly, depending on the cluster of cardinal features present in the individual, PCOS can exist across four clinical phenotypes that vary in severity.

The phenotypic variations of PCOS are relatively well studied.[6,16-20] The PCOS phenotypes that coincide with the 3 different diagnostic algorithms for PCOS are depicted in **Table 1**. Frank PCOS manifests as the presence of all 3 cardinal features and is considered the most severe, whereas those involving the presence of only two cardinal features such as non-PCO, ovulatory and non-androgenic PCOS would be considered less severe. By defining PCOS phenotypes, it provides a more comprehensive and distinct categorization of women with PCOS and their actual health risks. For example, hyperandrogenic phenotypes have a higher risk for cardiometabolic complications whereas, anovulatory phenotypes have a greater risk for infertility and endometrial complications. Ultimately, variable etiological mechanisms may underlie phenotypic differences in the presentation of PCOS.

Table 1. Diagnostic Criteria and Phenotyping

Phenotype	Frank	Non-PCO (Classic)	Ovulatory	Non-Androgenic (Mild)
Features				
Hyperandrogenism	Present	Present	Present	Absent
Menstrual irregularity	Present	Present	Absent	Present
Polycystic ovarian morphology	Present	Absent	Present	Present
Criteria				
NIH 1990	✓	✓		
AE-PCOS 2006	✓	✓	✓	
Rotterdam 2003	✓	✓	✓	✓
International Guideline 2018	✓	✓	✓	✓

Adapted from Azziz et al 2016[21] and Carmina and Azziz 2006[22].
Abbreviations: NIH = National Institutes of Health; AE-PCOS = Androgen Excess and PCOS.

Racial and Ethnic Influences on PCOS

The International PCOS Guideline supports the presence of ethnic and racial differences in the clinical presentation of PCOS and the need for research to understand the implications of regional differences in PCOS for diagnosis, risk assessment and treatment.[2] Studies conducted in specific regions of the globe, suggest that East Asian women exhibit reproductive dysfunction, but hirsutism and high BMI are less common.[23, 24] European (i.e., Italy and Greek) and U.S.-based studies, show lower rates of obesity and higher fasting insulin levels in Italy- and Greek-based groups versus U.S.-based groups. [25-27] By contrast, the prevalence and severity of hyperandrogenic and metabolic dysfunction are significant in South Asian women with PCOS[28-32] versus U.S.-based. In a systematic review and meta-analysis, we confirmed that U.S.-based Hispanic women with PCOS exhibited greater impairments in glucoregulatory status than U.S.-based Non-Hispanic White women albeit disparities in reproductive risks could not be concluded as data were unable to be harmonized across studies.[31] Likewise, we showed in another systematic and meta-analysis that U.S.-based Black women with PCOS had a more adverse cardiometabolic risk profile compared to their White counterparts. [32] In terms of regional influences, there are few studies that have directly compared symptoms across women with PCOS from various countries. In the most comprehensive cross-sectional analysis of PCOS symptomology across the globe, Chan et al.[28] showed differences in the presentation of PCOS from women living in the U.S., India, Brazil, Finland and Norway. Namely, Norwegian and Indian women showed a higher prevalence of metabolic syndrome (independent of obesity), whereas Finnish and Norwegian women had a lower prevalence of clinical and/or biochemical hyperandrogenism compared to the other regional groups. Further, women living in Finland and Norway had the highest documentation of polycystic-appearing ovaries (90 – 100%), whereas

the prevalence ranged from 67 – 79% in Brazil, India and the U.S. Unlike the prevalence of oligo-amenorrhea which was consistent across the various countries, markers of androgen status and polycystic ovarian morphology were sufficiently variable to suggest that regional considerations might be warranted to detect manifestations of these cardinal features across races and ethnicities.

Rationale and Study Aims

Differences in the prevalence and severity of the cardinal features of PCOS have been documented, with few studies directly comparing features across geographical regions. In the case of polycystic ovaries on ultrasonography, the degree to which individual morphological features contribute to these regional differences is unknown. An understanding of which ovarian markers best capture ovarian dysmorphology in women with PCOS from various races and ethnicities would clarify the suitability of one international standard to define PCOM versus the need for regional definitions. To that end, the purpose of this cross-sectional analysis was to contrast ovarian morphology in women with PCOS residing in the U.S. and India. Further, the degree to which ovarian morphology can serve as a biomarker of reproductive and metabolic status across regions was also explored.

II. METHODS

This was a retrospective cross-sectional analysis of de-identified research and clinical records designed to compare PCOS symptoms across two geographic regions with differing cultural and ethnic backgrounds. Ethics approval for primary data collection and analysis for the U.S.-based studies was granted by the Cornell University, Weill Cornell Medicine and the University of Rochester's Institutional Review Boards on the use of Human Subjects in Research (IRB approval #:1108002383, 1303003665, 1202002774, 0908000633, 1207003154, 00000436, 00007519, 1410015577). The retrospective reviews of de-identified clinical records were deemed exempt from full review by Cornell's Institutional Review Board (IRB exemption #: 1712007665).

Population

The two populations of interest were women of reproductive age with PCOS residing in India and the United States (U.S.). In India, the population varied in ethnicities within Odisha, India. Data were amassed from medical records of patients attending a gynecological practice for evaluation and treatment of reproductive disorders. All patients of record who satisfied the criteria for PCOS (described below) between 2018 to 2021 were considered for inclusion in this study. In the U.S., data were garnered from participants with PCOS that engaged in a clinical research study across three research centers in New York State. The U.S.-based population also varied in race and ethnicity.

Participant Selection

Data from 567 participants that had completed a research study at Cornell University or an affiliated site in New York State from 2010 to 2022 were available for inclusion in the following analysis. Participants were excluded if they were: (1) not of reproductive age(<18 or >38) (n=22); (2) using contraceptives or medication known to interfere with glucose or lipid metabolism within two months of study participation (n=20); (3) did not have sufficient clinical and ultrasonographic data to assess endpoints of interest (n=185); (4) were evaluated with transabdominal, instead of transvaginal, ultrasonography (n=1); (5) both ultrasonographic images and serum levels were not conducted during the follicular phase (n=6); (6) poor image quality (n=1); (7) did not have PCOS based on international standards (n=102); or (8) were duplicate participants in the dataset (n=17). In addition, a participant was excluded due to the presence of a dominant follicle in both ovaries (n=1). Ultimately, 212 U.S.-based women were qualified for analysis.

Medical charts were obtained from 178 consecutive patients seen at a primary care center (Kar Clinic and Hospital Pvt. Ltd.) in India for concerns over PCOS from 2018 to 2021. Patients were excluded if they were: (1) not of reproductive age (<18 or >38 years old) (n=1); (2) lacking sufficient clinical and ultrasonographic data to assess endpoints of interest (n=21); (3) were evaluated with transabdominal, instead of transvaginal, ultrasonography (n=33); (4) poor image quality of ovarian ultrasounds (n=2); or (5) not diagnosed with PCOS using international standards (n=2). The remaining 119 India-based women were qualified for inclusion in the analysis.

The final sample included 331 women from both the United States and India.

Data and Measures

(i) Clinical Features of PCOS

Women were categorized as having PCOS according to the 2018 International Guidelines[2] of two or more cardinal features: 1) ovulatory dysfunction, 2) hyperandrogenism and 3) polycystic ovarian morphology in the absence of other medical conditions known to interfere with reproductive function, including congenital adrenal hyperplasia, hyperprolactinemia, untreated thyroid dysfunction, and premature ovarian failure. Ovulatory dysfunction was based on evidence of menstrual cycle irregularity. Menstrual cycle length was categorized into ranges that reflected their self-reported typical cycle length over the reproductive years including: <35, 35-44, 45-89, 90-179 and >180 days. Women with a cycle length of ≥ 35 days were defined as having irregular cycles. Both clinical and biochemical androgen status were assessed. Clinical measures were assessed using the Ferriman-Gallwey hirsutism scale of male-patterned hair growth on nine regions of the body[33]. The threshold used to define clinical hyperandrogenism was a Ferriman-Gallwey score >6. Biochemical hyperandrogenism was assessed using serum concentrations of total testosterone. Two different thresholds of elevated total testosterone were used to account for variations in assay technology and performance across sites (U.S. threshold: ≥ 61.5 mg/dL; India threshold: > 45 mg/dL). Polycystic ovarian morphology (PCOM) was based on ultrasonographic assessments of follicle number per ovary (FNPO) and ovarian volume (OV). Thresholds to define PCOM were an average FNPO of ≥20 and/or an average OV ≥ 10cm^3 across both ovaries. In instances in which only one ovary was visualized, or a dominant follicle was present (N=47), measurements made in a single ovary were deemed sufficient. Further, a sensitivity analysis was conducted with participants with mild elevations of thyroid

stimulating hormone (N=4) and prolactin (N=2) levels with no impact on the results; therefore, these participants were included in the analysis.

(ii) Metabolic Markers

Metabolic status markers included anthropometric measurements (body mass index, waist circumference, waist-to-hip ratio), vitals (blood pressure) and blood glucose levels (fasting followed by a 75-gram 2-hour oral glucose tolerance test). Height, weight, waist-to-hip circumference, and blood pressure were assessed across sites using standard clinical approaches. Venous blood glucose was measured using the glucose-oxidase peroxidase method at the India site and standard glucometer at the U.S. sites. If a participant had a fasting blood glucose greater than 126 mg/dL, data at 0- and 2-hour post-glucose ingestion was censored to 127mg/dL and 200mg/dL, respectively.[34] Fasting lipid measures included total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides. All lipids were measured using a Siemens Dimension Xpand Chemistry Analyzer at the U.S. site. Methods used to measure lipids in India are summarized in **Table 2**.

Features of the metabolic syndrome[35-38] were defined as: (1) central or abdominal obesity (>88cm. for women), (2) high triglycerides (≥150 mg/dL), (3) low HDL cholesterol (<50 mg/dL for women), (4) high blood pressure (≥130/85 mmHg), and (5) high fasting glucose (≥100 mg/dL). Thresholds to define metabolic syndrome are presented in **Table 2**.

Table 2. Clinical and Biochemical Reference Ranges for the United States and India Cohort

Variable	Methodology	Normal range	Abnormal range
United States			
Menstrual cycle length (d)	Self-report	21 – 35	≥ 36
Modified hirsutism score	Ferriman-Gallwey Scale[40]	0 – 6	≥ 7
Total testosterone (ng/dL)	LC/MS/MS	< 61.5	≥ 61.5
BMI (kg/m^2)	Standard approaches	18.5–24.9	≥ 25.0
Waist circumference (cm)	Standard approaches	< 80	≥ 80
Waist-to-hip ratio	Standard approaches	< 0.85	≥ 0.85
Blood pressure (mmHg)	Standard approaches	< 140/90	≥ 140/90
Glucose, 0-hour (mg/dL)	Glucometer	< 100	≥ 100
Glucose, 2-hour (mg/dL)	Glucometer	< 140	≥ 140
Triglycerides (mg/dL)	SDXCA	< 150	≥ 150
Total Cholesterol (mg/dL)	SDXCA	< 200	≥ 200
HDL (mg/dL)	SDXCA	≥ 50	< 50
LDL (mg/dL)	SDXCA	< 100	≥ 100
India			
Menstrual cycle length (d)	Self-report	21 – 35	≥ 36
Modified hirsutism score	Ferriman-Gallwey Scale[40]	0 – 6	≥ 7
Total testosterone (ng/dL)	Chemiluminescence immunoassay	< 76.0	≥ 76.0
BMI (kg/m^2)	Standard approaches	18.5–24.9	≥ 25.0
Waist circumference (cm)	Standard approaches	< 80	≥ 80
Waist-to-hip ratio	Standard approaches	< 0.85	≥ 0.85
Blood pressure (mmHg)	Standard approaches	< 140/90	≥ 140/90
Glucose, 0-hour (mg/dL)	GOD-POD	< 100	≥ 100
Glucose, 2-hour (mg/dL)	GOD-POD	< 140	≥ 140
Triglycerides (mg/dL)	GPO-TRINDER	< 170	≥ 170
Total Cholesterol (mg/dL)	CHOD-PAP	< 200	≥ 200
HDL (mg/dL)	Direct HDL-C assay	≥ 45	< 45
LDL (mg/dL)	Direct LDL-C assay	< 130	≥ 130

Abbreviations: LC/MS/MS = liquid chromatography tandem mass spectrometry, SDXCA = Siemens Dimension Xpand Chemistry Analyzer, GOD-POD = glucose oxidase and peroxidase, GPO-TRINDER = glycerol phosphate oxidase, CHOD-PAP = cholesterol oxidase phenol 4-aminoantipyrine peroxidase, BMI = body mass index, HDL = high density lipoprotein, LDL = low density lipoprotein

(iii) Ovarian Image Analysis and Image Processing

Ultrasounds were conducted on both women with PCOS in NYS and India using 3D ultrasonography on a GE Voluson ultrasound machine (i.e., E8 Expert, S6, or S10 Series) and 5–9 or 6–12 MHz endovaginal transducer (GE Healthcare, Milwaukee, WI) and either coincided with the early follicular phase (U.S.-based participants), post-natural menses (India-based participants) or after a progesterone-induced bleed (India-based participants). At both sites, images of both left and right ovaries were saved and exported for off-line analysis at the Cornell Ovary Lab (Ithaca, NY).

For 2D ultrasound methods, volume files were partitioned from their native file format into two 2D cineloop videos (.dcm file format) representing each of the orthogonal planes. A single cineloop was selected as the reference plane and analyzed using a generic DICOM reader software (DICOM Viewer Mac, v, 2.0.10). Follicle populations were counted and measured using an offline approach with a programmable grid overlay (2D-Grid) which has been shown to improve the reliability of follicle counts of polycystic ovaries.[39,40] The largest cross-sectional plane of the ovary was used to determine OV, ovarian area (OA), stromal area (SA), stromal-to-total area (S/A), and follicle number per single cross-sectional image (FNPS). OV was calculated using the largest cross-sectional measurements in two orthogonal planes and the prolate ellipsoid formula which is considered the conventional clinical method.[41] OA was calculated as the average of the perpendicular measurements at the largest ovarian cross-section across both transverse and sagittal planes. SA was calculated indirectly by subtracting the total follicular area from the ovarian area from a fixed frame and S/A was subsequently determined by dividing SA with OA. Measurements reported represent average values across the left and right ovaries. In instances where a participant had a follicle greater than 10mm, the contralateral ovary alone was

used to determine ovarian (OA and OV) and stromal (SA and S/A) measures. Ultrasound images were analyzed by one of fourteen raters with an intraclass correlation coefficient (ICC) of >0.874 across all ovarian measurements as judged by a standard internal training protocol.

Statistical analysis

Data analysis was carried out using JMP Pro 16 Statistical Software (SAS Institute, Cary, NC). Significance was determined at the 5% level. Continuous variables were summarized using mean and standard deviations. Categorical variables were summarized using counts and percentages. Two sample t-tests were used to assess differences in a continuous variable across the two groups. Chi-square tests were used to assess differences in categorical variables across groups. Fisher's exact tests were used for comparisons in which there was a frequency <5 samples. Bivariate correlations between ovarian morphology markers and reproductive and metabolic features were estimated. Normality and homogenous variances of residuals were determined using graphical methods and when needed response and predictor variables were log-transformed.

III. RESULTS

Demographic and PCOS Diagnostic Features

The demographic and diagnostic features of the participants in both groups are reported in **Table 3**. The age of the women in both groups was similar (p = 0.34), as was body mass index (BMI) (p = 0.84). Ethnicity was not assessed in India and therefore data were not available. In the U.S.-based group, a majority of the participants were non-Hispanic or non-Latino (87%) and racially identified as White (67%). The proportions of participants identifying as Black or Asian were relatively similar at 12% and 16%, respectively. The "Other" category (4%) was composed of a combination of the participants that identified either as mixed, Pacific Islander or Native American. Data were pooled due to the small number of participants in each category.

Menstrual irregularity was more prominent (p = <0.001) in India-based women compared to U.S.-based women, with a majority of them having oligomenorrhea (defined as typically having a menstrual cycle length between 36 and 89 days) (proportion of oligomenorrhea in India vs. U.S.-based group: p = 0.019). Hyperandrogenism was present in a majority of both U.S. and India-based participants. Notably, a higher prevalence of hyperandrogenism was apparent in India- versus U.S.-based women with PCOS (99% vs. 52%, P<0.0001). However, the proportion of those with elevated total testosterone and Ferriman-Gallwey score greater than 6 was higher in the India-based group compared to U.S.-based participants (100% vs. 43%, p=<.0001). Lastly, nearly all participants in both groups met the criteria for PCOM with the India-based group demonstrating a higher prevalence of follicle excess compared to the U.S.-based group (94% vs. 86%, p = 0.039). Collectively, a vast majority of participants in India met the criteria for Frank PCOS (97%) whereas U.S.-based participants had a more heterogeneous presentation, with a

majority having a Mild variant of PCOS (50%). A comparison of demographic and reproductive features between those meeting the criteria for Frank PCOS across India and the U.S. also showed a higher prevalence of hirsutism in the India-based participants (**Supplemental Table 1**).

Table 3. Demographic and Reproductive Characteristics of Study Participants

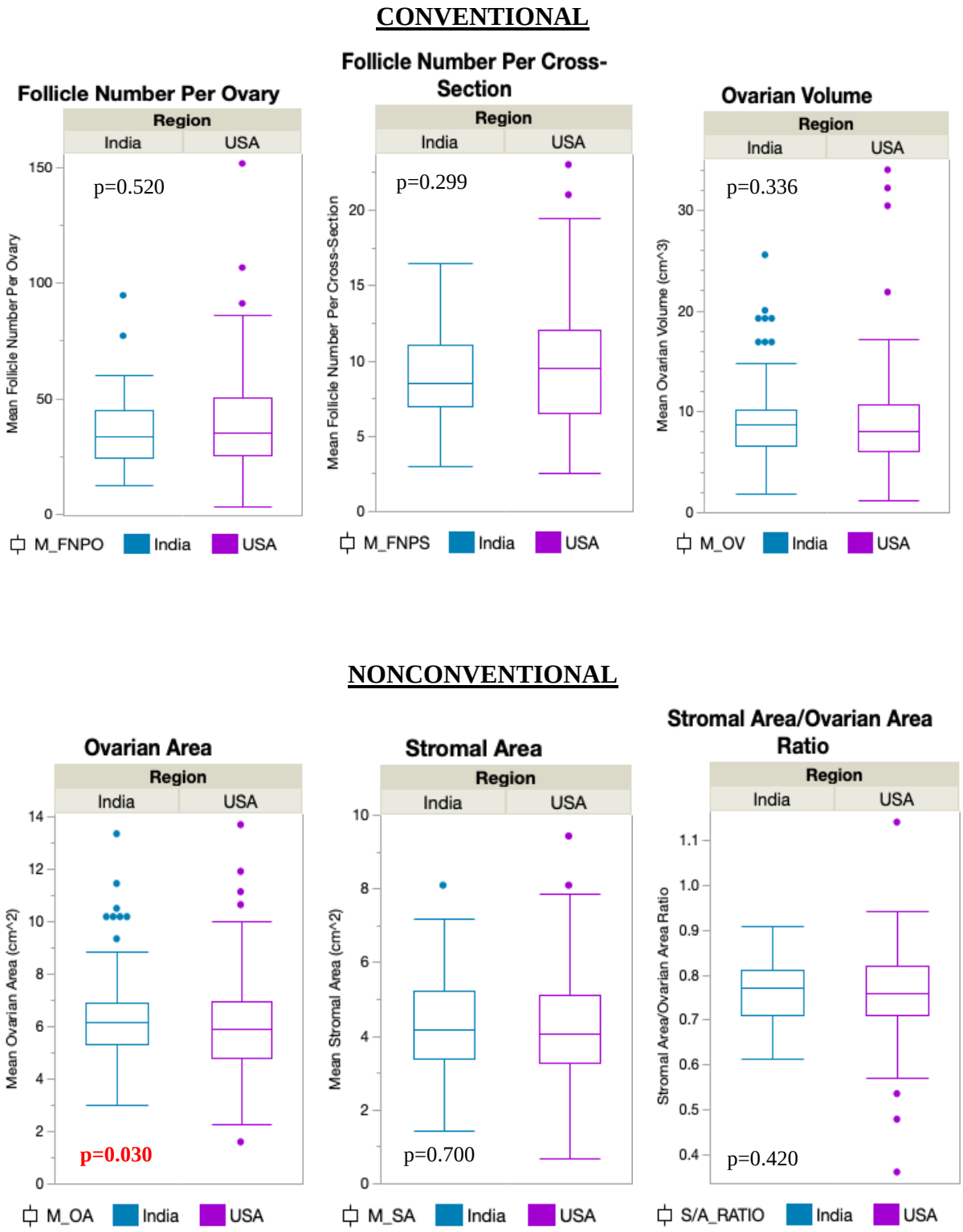
Variable	**India** N = 119		**U.S.** N = 212		**P-value**
Demographics	n	Mean (SD)	n	Mean (SD)	
Age (years)	119	26.5 (3.6)	212	26.0 (5.4)	0.338[3]
BMI (kg/m^2)	119	28.1 (5.1)	205	29.0 (8.7)	0.843[3]
Ethnicity	n	Count (%)	n	Count (%)	
Hispanic or Latino	-	-	203	17 (1)	
Not Hispanic or Latino	-	-	203	177 (87)	
Other	-	-	203	9 (4)	
Race					
White	-	-	205	138 (67)	
Black	-	-	205	25 (12)	
Asian	-	-	205	33 (16)	
Other	-	-	205	9 (4)	
Reproductive Features	n	Count (%)	n	Count (%)	
Menstrual Irregularity	119	118 (99)	206	179 (87)	**<0.0001[1]**
Oligomenorrhea (36-89 days)	119	81 (68)	206	112 (54)	**0.019[2]**
Amenorrhea (≥90 days)	119	132 (31)	206	215 (29)	0.705[2]
Hyperandrogenism	116	115 (99)	207	107 (52)	**<0.0001[1]**
Hyperandrogenemia	38	17 (45)	194	33 (17)	**0.0004[1]**
Hirsute (>6)	114	114 (100)	205	88 (43)	**<0.0001[1]**
Polycystic Ovarian Morphology	119	118 (99)	211	210 (99)	1[1]
Excess FNPO (≥20)	119	112 (94)	206	178 (86)	**0.039[2]**
Enlarged Ovarian Volume (>10cm^3)	119	85 (71)	212	149 (70)	0.900[2]
Phenotypes (if had **all** three criteria above)					
Frank	116	113 (97)	200	73 (37)	**<0.0001[2]**
Non-PCO	116	1 (1)	200	1 (1)	1[1]
Ovulatory	116	1 (1)	200	26 (13)	**<0.001[1]**
Mild	116	1 (1)	200	99 (50)	**<0.001[1]**

Abbreviations: N = total number in group, n = # of non-missing for this variable, SD = standard deviation; FNPO = follicle number per ovary, BMI = body mass index; WC = waist circumference, HC = hip circumference, WHR = waist-to-hip ratio, 1 = p-value from Fisher's exact test, 2 = p-value from Pearson chi-squared test, 3 = p-value from two sample t-test

Ovarian Morphology Markers

Figure 1 depicts a comparison of conventional and nonconventional markers of PCOM across India and U.S.-based participants. No differences in FNPO, FNPS and OV were noted between groups. By contrast, ovarian area was higher in the India group compared to the U.S. group ($p=0.030$). On subgroup analysis involving only those meeting the criteria for Frank PCOS, FNPO ($p<0.0001$) and FNPS ($p=0.0003$) were higher in the U.S.-based group compared to the India-based group (**Supplemental Figure 1**).

Figure 1. Visual Depiction of Conventional and Nonconventional Markers of PCOM



Metabolic Markers

Metabolic characteristics and the prevalence of those meeting the criteria for metabolic syndrome features defined by the International Diabetes Federation[39], National Heart, Lung and Blood Institute[37], American Heart Association[38], and Androgen Excess and Polycystic Ovary Syndrome Society statement[36] are presented in **Table 4**. Participants in the India group had a higher prevalence of elevated waist-to-hip ratio (WHR) ($p<0.0001$) compared to the U.S. group, who had a higher prevalence of waist circumference ($p=0.032$). Likewise, the prevalence of elevated 2-hour glucose ($p<0.0001$), increased triglycerides ($p<0.0001$) and LDL levels ($p=0.005$), and lower HDL levels ($p<0.0001$) were higher in the India group compared to the U.S. group. On subgroup analysis involving only those meeting the criteria for Frank PCOS (**Supplemental Table 2**), increased prevalence of abnormalities in WHR, 2hr glucose, triglycerides and HDL persisted in the India-based group (all, $p<0.0001$).

Table 4. Metabolic Markers and Prevalence within India- and U.S.-based Groups

Variable	India		U.S.		P-value
	N = 119		N = 212		
	n	Mean (SD) or count (%)	n	Mean (SD) or count (%)	
Metabolic Measures					
Waist circumference (cm)	115	91.0 (11.1)	192	89.8 (21.3)	0.506[1]
Prevalence of elevated WC (>89cm)		63 (55)		81 (42)	**0.032**[2]
Waist-to-hip ratio	115	0.9 (0.04)	192	0.8 (0.1)	**<0.0001**[1]
Prevalence of elevated WHR (>0.85)		107 (93)		70 (36)	**<0.0001**[2]
FBS (mg/dL)	93	98.2 (22.6)	187	93.8 (12.6)	0.059[1]
Prevalence of elevated 0-hour glucose (≥100mg/dL)		28 (30)		46 (25)	0.325[2]
2-hour glucose (mg/dL)	77	143.5 (42.1)	166	97.1 (26.4)	**<0.0001**[1]
Prevalence of elevated 2-hour glucose (>139mg/dL)		41 (53)		8 (5)	**<0.0001**[2]
BP Systolic (mmHg)	117	118.8 (9.7)	181	112.7 (15.7)	**<0.0001**[1]
Prevalence of elevated systolic BP (>130mmHg)		23 (20)		25 (14)	0.180[2]
BP Diastolic (mmHg)	117	72.2 (10.9)	181	72.0 (11.8)	0.782[1]
Prevalence of elevated diastolic BP (>85mmHg)		11 (9)		22 (12)	0.460[2]
TC (mg/dL)	58	178.5 (37.0)	185	174.2 (31.4)	0.579[1]
Prevalence of elevated TC (>199mg/dL)		15 (26)		31(17)	0.123[2]
TG (mg/dL)	59	150.2 (71.3)	185	85.5 (56.5)	**<0.0001**[1]
Prevalence of elevated TG (>150mg/dL)		28 (47)		18 (10)	**<0.0001**[2]
HDL (mg/dL)	59	40.4 (7.9)	185	58.0 (16.4)	**<0.0001**[1]
Prevalence of low HDL (<50mg/dL)		50 (85)		63 (34)	**<0.0001**[2]
LDL (mg/dL)	59	110.6 (28.6)	185	96.9 (26.2)	**0.002**[1]
Prevalence of elevated LDL (>99mg/dL)		38 (64)		80 (43)	**0.005**[2]

p-values come from two-sample t-tests and Fisher's exact tests; two-sample t-tests used log-transformed data.
Abbreviations: N = total number in group, n = # of non-missing for this variable, SD = standard deviation, FBS = fasting blood sugar, BP = blood pressure, TC = total cholesterol, TG = triglycerides, HDL = high density lipoprotein, LDL= low density lipoprotein; p-values come from two-sample t-tests

Associations Between Reproductive Markers and PCOM.

The relationships between reproductive markers and ovarian morphology are depicted in **Table 5**. FNPO, FNPS and OV were positively associated with menstrual cycle length, Ferriman Gallwey hirsutism scores and total testosterone concentrations (all $p<0.02$). SA and S/A were positively associated with menstrual cycle length in U.S.-based participants ($P<0.05$) whereas only a trend was noted between SA and total testosterone concentrations ($p=0.08$). No correlations were noted between FNPO, FNPS and OV with reproductive markers in the India cohort. However, positive associations were apparent between SA and S/A with either menstrual cycle length or Ferriman Gallwey hirsutism scores ($p<0.05$). On subgroup analysis involving only those meeting the criteria for Frank PCOS (**Supplemental Table 3**), FNPO ($p=0.034$), SA ($p=0.007$) and S/A ($p=0.016$) remained positively associated with total testosterone concentrations and/or Ferriman Gallwey scores in either U.S. or India-based participants.

Table 5. Regional Correlations Between Ovarian Morphology and Reproductive Markers

	FNPO		FNPS		OV		SA		S/A	
Reproductive Markers	India	U.S.	India	U.S.	India	U.S.	India	U.S.	India	U.S.
Menstrual cycle length (d)	+0.01	**+0.23**	+0.01	**+0.19**	+0.08	**+0.25**	**+0.18**	**+0.17**	-0.09	**+0.16**
Ferriman-Gallwey hirsutism score	+0.08	**+0.21**	+0.04	**+0.16**	-0.08	**+0.17**	**+0.26**	+0.07	**+0.21**	+0.00
Total testosterone (ng/dL)	+0.06	**+0.34**	+0.14	**+0.26**	+0.02	**+0.24**	-0.12	+0.12	-0.18	-0.06

Represented as Pearson's correlation coefficients of the non-log transformed data; shading reflects a significant or trending correlation: $P < 0.01$ (black) and $P < 0.05$ (dark gray)

Abbreviations: FNPO = follicle number per ovary, FNPS = follicle number per cross-section, OV = ovarian volume, SA = stromal area, S/A = stromal area/ovarian area ratio

Associations Between Ovarian Morphology and Metabolic Markers.

Table 6 depicts relationships between ovarian morphology and metabolic features in both groups. Positive associations were noted between OV and BMI ($p=0.03$) in addition to S/A with fasting blood glucose ($p=0.007$) and triglycerides ($p=0.020$) in the U.S. cohort. By contrast, both FNPO ($p=0.049$) and SA ($p=0.020$) negatively correlated with HDL levels. In the India group, only FNPS negatively correlated with diastolic pressure ($p=0.006$). On subgroup analysis involving only those meeting the criteria for Frank PCOS (**Supplemental Table 4**), positive associations were noted between SA and S/A with 2-hour BMI, WHR and/or triglycerides ($p<0.05$) in the U.S.-based group. Further, FNPS and S/A were negatively associated with 2-hour glucose, WHR and HDL in the U.S.-based group ($P<0.05$). By contrast, only FNPS negatively correlated with diastolic blood pressure in the India-based group ($p=0.010$).

Table 6. Regional Correlations Between Ovarian Morphology and Metabolic Markers

	FNP0		FNPS		OV		SA		S/A	
Metabolic Markers	India	U.S.	India	U.S.	India	U.S.	India	U.S.	India	U.S.
2-hr glucose	-0.09	-0.00	+0.04	-0.15	+0.02	+0.08	+0.01	+0.06	-0.05	+0.10
FBS	-0.07	-0.07	+0.03	-0.16	-0.01	+0.06	-0.05	+0.09	-0.05	**+0.19**
BPSYS	-0.07	+0.08	-0.13	-0.05	-0.01	+0.11	-0.03	+0.08	-0.01	+0.05
BPDYS	-0.14	+0.02	**-0.26**	-0.08	+0.01	+0.08	-0.02	+0.07	+0.11	+0.02
BMI	-0.01	+0.03	-0.01	-0.13	+0.03	**+0.12**	-0.04	+0.10	-0.08	+0.05
WHR	+0.15	-0.01	+0.13	-0.13	+0.02	+0.06	+0.07	+0.04	+0.03	+0.07
TC	-0.00	+0.06	+0.16	+0.03	-0.08	+0.04	-0.18	-0.06	-0.17	-0.05
TG	+0.17	+0.04	+0.29	-0.09	+0.05	-0.03	+0.07	+0.05	-0.10	**+0.18**
HDL	+0.07	**-0.12**	+0.09	+0.03	-0.01	-0.12	-0.17	**-0.18**	-0.18	-0.10
LDL	-0.08	+0.09	+0.02	+0.02	-0.11	+0.10	-0.15	+0.02	-0.15	-0.03

Represented as Pearson's correlation coefficients of the non-log transformed data; shading reflects a significant or trending correlation: $P < 0.01$ (black) and $P < 0.05$ (dark gray); p-values from using log-transformed data.

Abbreviations: N = total number in group, n = # of non-missing for this variable, FNPO = follicle number per ovary, FNPS = follicle number per cross-section, OV = ovarian volume, SA = stromal area, S/A = stromal area/ovarian area ratio, FBS = fasting blood sugar, BPSYS = systolic blood pressure, BPDYS = diastolic blood pressure, BMI = body mass index, WHR = waist-to-hip ratio, TC = total cholesterol, TG = triglycerides, HDL = high density lipoprotein, LDL= low density lipoprotein

IV. DISCUSSION

The current study aimed to determine differences in ovarian morphology between two different geographic regions of India and the United States within a PCOS group. We also sought to explore relationships between ovarian morphology and the severity of reproductive and metabolic disturbances in both groups. We hypothesized that features of ovarian morphology might differ between geographical groups owing to previous reports of relatively lower follicle counts and ovarian size in studies involving women with PCOS in India.[42-44] In addition, we also posited that the strength of the relationships between ovarian morphology and PCOM symptomology might differ owing to reports of relatively worse reproductive and metabolic profiles in Indian women with PCOS compared to that of U.S.-based groups.[28] Ultimately, we showed that no significant differences exist in either conventional or non-conventional markers of polycystic ovarian morphology between India and U.S.-based women with PCOS, broadly defined. However, in subgroup analyses, follicle populations may be higher in women with severe manifestations of PCOS residing in the U.S. – albeit this difference may not be clinically relevant. Further, we confirmed that the ovary reflected reproductive symptomology in both India and U.S.-based participants consistent with the role of the ovary as a biomarker. However, the relationship between ovarian morphology and metabolic features was inconsistent across regions.

Potential for regional differences in markers of PCOM.

Ultrasonography is an important tool in PCOS diagnosis in that it allows for real-time and non-invasive assessments of ovarian morphology.[45] According to the International PCOS Guideline, polycystic ovaries are defined as containing 20 or more follicles ranging in 2-9mm throughout

the entire ovary and/or increased ovarian volume of >10cm^3.[2] Unlike previous definitions, subjective assessments of stromal size, stromal echogenicity and/or follicle distribution pattern are not considered as we and others have shown them to have less diagnostic accuracy for the condition of PCOS. That said, non-conventional markers of PCOM have been shown to have high specificity and reflect the degree of PCOS symptomology. However, as far as we are aware these markers have not been evaluated in Indian women with PCOS and therefore their utility in global settings is unknown. To our knowledge, there has not been any study contrasting variations in specific markers of ovarian morphology across different geographical regions. However, differences in the prevalence of PCOM across women from different geographic regions would suggest potential for variations in ovarian morphology. In the case of India-based populations, one study showed that a FNPO threshold of 12 and an OV threshold of 6.15cm^3 has the best diagnostic accuracy for PCOS in women from this region.[42] These thresholds are substantially lower than those reported in U.S.-based studies[40,46] and do not align with current international standards. Contrary to our hypothesis, we noted no significant differences in the conventional (FNPO, FNPS, OV) and a majority of the nonconventional markers (SA, S/A ratio) of PCOM across groups. While a statistically significant difference was detected for ovarian area (p=0.030) across groups, the difference in ovarian size was clinically insignificant (~0.3cm^2) with the U.S., and not the India, group having the "smaller" ovaries. Reasons for differences across studies might relate to differences in methodology across studies. In this study, we used standardized approaches with high reproducibility to measure conventional markers of PCOM for both geographic groups. Standardization of approaches and direct access to stored imaging enabled us to perform a reliable and direct comparison across groups. In the

case of stromal markers, our data represent the first report of these measures in an India-based population and support similarities across geographic groups.

Phenotypes across India and US.

More participants in the India group possessed all the three main cardinal features of PCOS consistent with a significantly higher prevalence of Frank PCOS compared to the U.S.-based group. Namely, there was more oligomenorrhea, severe clinical hyperandrogenism and a higher prevalence of follicle excess albeit actual follicle counts per ovary were comparable across groups. Our finding of the great prevalence of Frank PCOS is consistent with previous reports that have shown South Asian women with PCOS to present with more severe reproductive symptoms (i.e., higher degrees of infertility, acne and hirsutism) compared to other ethnic groups.[29,48] In contrast, the U.S. participants consisted of a majority of Mild PCOS followed by Frank PCOS consistent with representation on both sides of the PCOS severity spectrum. Differences in the prevalence of PCOS phenotype mostly likely relate to the study design. The data from the India-based group were garnered from patients presenting to a reproductive health clinic seeking evaluation and/or care for PCOS and/or infertility. Whereas data from the U.S.-based group captured individuals within the community that had presented for participation in a research study on reproductive health and/or PCOS pathophysiology. Therefore, it is likely that clinic-based populations in India would have a higher likelihood of exhibiting a more severe PCOS phenotype compared to those volunteering from the general population wherein Mild and Ovulatory PCOS were relatively more common. Nevertheless, we did note that non-PCO PCOS was exceptionally rare across both regional groups – consistent with PCOM being a significant marker in the condition of PCOS.

On subgroup analysis that considered only those meeting the criteria for Frank PCOS, the prevalence of clinical hyperandrogenism remained higher in those from India consistent with South Asian populations having higher degrees of androgen excess compared to other geographic regions. Theca cells from polycystic ovaries produce excess androgens owing to the overexpression of two genes, cholesterol side chain cleavage enzyme (CYP11A) and 17a-hydroxylase/17,20-desmolase (CYP17), which are key regulators of androgen biosynthesis.[49] With this in mind, the genetic etiology of why South Asian women may have a greater propensity for hyperandrogenism may be due to the activity of these two genes within South Asian women with PCOS. A retrospective analysis by Pusalkar et al. in a South Asian population confirmed polymorphisms in the promoter regions of androgen-regulating genes (CYP11A and CYP17) that had significant associations with testosterone levels, both individually and cumulatively within this population.[50] In the case of ovarian morphology, we noted that markers of follicle excess were increased in U.S.-based populations compared to the India group. This increase is estimated on average 13 follicles for FNPO and 2 follicles for FNPS. The clinical relevance of this difference is uncertain. Participants were matched for age and body mass index, and it is unlikely that these factors would have contributed to differences between groups. We did not have access to biological specimens across both groups and were unable to directly access and pool data for biochemical markers of ovarian function (i.e., total testosterone and anti-Mullerian hormone) that could have provided functional evidence for follicle excess to corroborate the relevance of this morphological difference. However, a possible reason for this finding may be attributed to follicle reserve differences in South Asian-based compared to other races and ethnicities. In a cross-sectional study by Iglesias et al. looking at ovarian reserve markers (i.e., AMH, FSH, E2 and AFC) between Spanish and Indian women, Indian women had

a greater decline in AMH relative to age suggesting ethnic differences in ovarian aging.[51] Accordingly, Indian women have been shown to exhibit higher rates of premature menopause (5.5%) before the age of 40 years[52], in contrast to the 1-2% reported internationally.[53]

Region-specific correlations of ovarian morphology with reproductive characteristics. Ultrasonographic metrics of polycystic ovarian morphology have been shown to be a biomarker for reproductive features in PCOS.[54-57] When looking at reproductive features in relation to ovarian morphology, the U.S. group showed consistent positive associations in conventional markers of PCOS (FNPO, FNPS, OV) with the degree of menstrual cycle irregularity, Ferriman-Gallwey hirsutism score and total serum testosterone levels as well as with some non-conventional markers (SA and S/A). This was in contrast to the India group in which only non-conventional ovarian markers were associated with menstrual cycle length and Ferriman-Gallwey scores. The lack of association between conventional ovarian markers and the cardinal features of PCOS within the India group may be related to the homogenous nature of the India group wherein the range of values for menstrual cycle length, hirsutism scores and total testosterone were narrower compared to the variation noted in the U.S.-based group (data not shown). Accordingly, when a subgroup analysis was performed on only those meeting criteria for Frank PCOS, positive associations between menstrual cycle length and hirsutism scores were lost in the U.S. based group likely owing to the homogeneous nature of the study samples. Nonetheless, the presence of association with stromal assessments (SA and S/A) does suggest that the stroma has greater relevance in driving clinical manifestations of PCOS in different races and ethnicities.

Region-specific correlations of ovarian morphology with metabolic characteristics.

Consistent with previous reports[28-30], the India-based group showed a higher prevalence of metabolic aberrations compared to the U.S.-based group that persisted even on subgroup analysis limited to Frank manifestations of PCOS. It is well-accepted that androgen excess underlies many of the metabolic aberrations seen in PCOS.[58-61] As discussed above, Indian women with PCOS demonstrate a more hyperandrogenic variant of PCOS which is consistent with the presence of more metabolic dysfunction in this population. We noted few associations between ovarian morphology and metabolic features in women with PCOS from India – with the negative association between diastolic blood pressure and follicle excess (i.e., FNPS) being difficult to explain. In contrast, we noted several associations between metabolic features and ovarian morphology in the U.S.-based group likely owing again to a greater range of metabolic dysfunction in this population. Associations between follicle excess, ovarian enlargement and stromal features with markers of obesity, insulin resistance and/or dyslipidemia suggest that ovarian dysmorphology could serve as an indicator of a higher likelihood of concurrent metabolic dysfunction. That said, we did note some associations that were counter-productive in the subgroup analysis such as negative associations between FNPS and fasting blood glucose and waist to hips ratio which suggest that some markers may not have the same level of performance for metabolic dysfunction compared to other sonographic markers. Together, our data support the use of FNPO, OV and stromal markers over FNPS when considering any potential for the ovary to serve as a biomarker of concurrent metabolic aberrations.

Strengths and Limitations.

To the best of our knowledge, this is the first study to directly explore phenotypic variations in ovarian morphology across geographical locations. In addition, we assessed the severity of reproductive and metabolic disturbances in both cohorts in relation to ovarian morphology which is a novel approach. Our study consisted of relatively large sample sizes across both geographic regions to enable the detection of significant differences among our primary endpoints of ovarian morphology. Further, we had access to raw images for both populations and used standardized image analysis approaches with low levels of inter-observer variability to ensure a high degree of rigor and reproducibility. However, our study is not without limitations. First, differences in the timing of the ultrasound scans across regions may have impacted findings. In India, scans were conducted either post-natural menses or after a progesterone-induced bleed whereas, scans conducted in the U.S. never occurred following progesterone stimulation. While all scans were conducted in the follicular phase, we cannot exclude the possibility that progesterone withdrawal in a subset of participants in India impacted morphological findings across groups. Secondly, clinical and biochemical endpoints were evaluated with different rubrics and assays across sites. Consequently, since metabolic marker assays used for both groups were not comparable, analyses were run separately for each region, thereby restricting the analyses to subgroup regression analyses (India and U.S.). In addition, hormone levels were not consistently available for some individuals, making the sample sizes for certain comparisons smaller. This was primarily the case in India, where the lack of serum levels was largely due to a lack of coverage by the health insurance system for that particular analyte. We did not have access to stored biological samples for the India cohort, so we were unable to re-assay for any biochemical variable of interest. The associations identified within this study may have been impacted by the

use of different assays and variability in sample sizes and should be considered exploratory. Thirdly, differences in approaches for participant recruitment may have impacted findings. Participants were evaluated in different settings in that U.S.-based participants were garnered from a research center versus clinical practice which was the case in India. Therefore, differences in PCOS symptomology between groups may relate to a higher likelihood of severe clinical manifestations presenting primarily to a clinical practice rather than a research setting. In an attempt to account for this discrepancy, we performed subgroup analyses limited to frank manifestations of PCOS.

V. CONCLUSION

Geographic differences exist in the clinical presentation of PCOS. However, variations in ovarian morphology may not be sufficient to warrant regional definitions of polycystic ovarian morphology. Ovarian dysmorphology served as a biomarker of the severity of reproductive symptomology in both regions consistent with the ovary being a central component of the pathophysiology of PCOS. However, more research is needed to fully elaborate its utility as a marker of metabolic status across races and ethnicities albeit our exploratory analysis is promising.

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APPENDIX

Supplemental Table 1

Demographic and Reproductive Characteristics of Study Participants (Frank PCOS)

Variable	**India** N = 113		**U.S.** N = 73		**P-value**
Demographics	n	Mean (SD)	n	Mean (SD)	
Age (years)	113	26.5 (3.5)	73	25.8 (5.0)	0.282[3]
BMI (kg/m^2)	113	28.0 (5.1)	72	30.0 (8.7)	0.106[3]
Reproductive Features	n	Count (%)	n	Count (%)	
Menstrual Irregularity	113	113 (100)	73	73 (100)	
Oligomenorrhea (35-89 days)	113	76 (67)	73	39 (53)	0.058[2]
Amenorrhea (≥90 days)	113	37 (33)	73	33 (45)	0.087[2]
Hyperandrogenism	113	113 (100)	73	73 (100)	
Hyperandrogenemia	37	17 (46)	67	25 (37)	0.390[2]
Hirsute (>6)	112	112 (100)	72	58 (81)	**<0.0001[1]**
Polycystic Ovarian Morphology	113	113 (100)	73	73 (100)	
Excess FNPO (≥20)	113	109 (96)	70	66 (94)	0.484[2]
Enlarged Ovarian Volume (>10cm^3)	113	79 (70)	73	41 (56)	0.056[2]

Abbreviations: N = total number in group, n = # of non-missing for this variable, SD = standard deviation; FNPO = follicle number per ovary, BMI = body mass index; WC = waist circumference, HC = hip circumference, WHR = waist-to-hip ratio

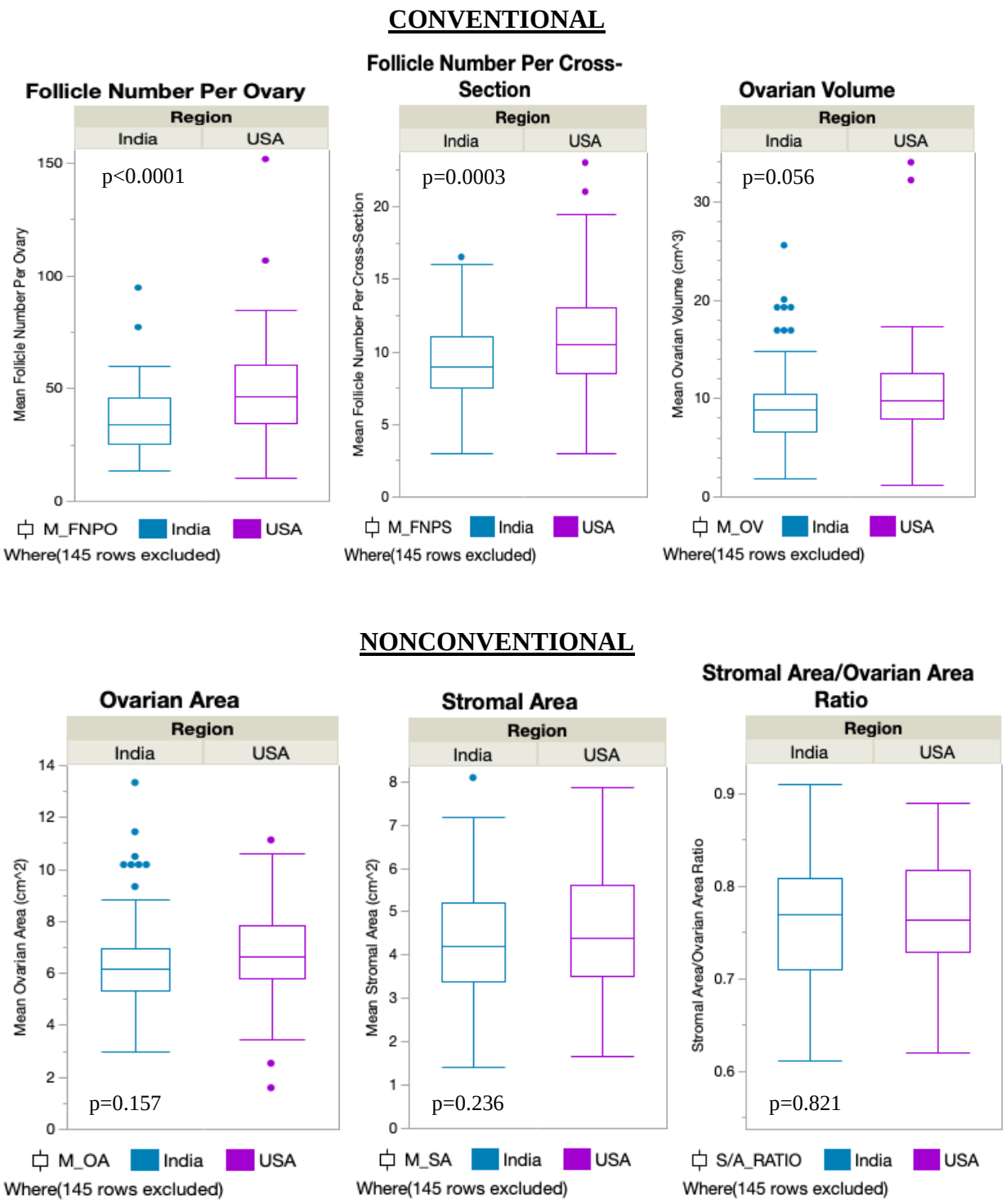
1 p-value from Fisher's exact test

2 p-value from Pearson chi-squared test

3 p-value from two sample t-test

Supplemental Figure 1

Visual Depiction of Conventional and Nonconventional Markers of PCOM (Frank PCOS)



Supplemental Table 2

Metabolic Markers and Prevalence within India- and U.S.-based Groups (Frank PCOS)

Variable	India N = 113		US N = 73		P-value
	n	Mean (SD) or count (%)	n	Mean (SD) or count (%)	
Metabolic Measures					
Waist circumference (cm)	111	91.0 (11.0)	67	92.2 (23.5)	0.664[1]
Prevalence of elevated WC (>89cm)		62 (56)		33 (49)	0.392[2]
Waist-to-hip ratio	111	0.9 (0.04)	67	0.8 (0.1)	**<0.0001**[1]
Prevalence of elevated WHR (>0.85)		104 (94)		32 (48)	**<0.0001**[2]
FBS (mg/dL)	92	98.3 (22.7)	62	94.0 (12.3)	0.134[1]
Prevalence of elevated 0-hour glucose (≥100mg/dL)		28 (30)		15 (24)	0.397[2]
2-hour glucose (mg/dL)	75	144.8 (41.7)	56	99.2 (27.7)	**<0.0001**[1]
Prevalence of elevated 2-hour glucose (>139mg/dL)		41 (55)		3 (5)	**<0.0001**[2]
BP Systolic (mmHg)	113	118.6 (9.7)	61	112.6 (15.1)	**0.0057**[1]
Prevalence of elevated systolic BP (>130mmHg)		21 (19)		8 (13)	0.356[2]
BP Diastolic (mmHg)	113	72.0 (10.9)	61	71.5 (11.4)	0.785[1]
Prevalence of elevated diastolic BP (>85mmHg)		9 (8)		9 (15)	0.161[2]
TC (mg/dL)	57	178.5 (37.3)	63	179.0 (37.4)	0.951[1]
Prevalence of elevated TC (>199mg/dL)		15 (26)		17 (27)	0.934[2]
TG (mg/dL)	58	149.9 (71.9)	63	87.3 (64.6)	**<0.0001**[1]
Prevalence of elevated TG (>150mg/dL)		27 (47)		7 (11)	**<0.0001**[2]
HDL (mg/dL)	58	40.5 (8.0)	63	55.5 (16.3)	**<0.0001**[1]
Prevalence of low HDL (<50mg/dL)		49 (84)		24 (38)	**<0.0001**[2]
LDL (mg/dL)	58	110.5 (28.8)	63	102.12 (30.0)	0.118[1]
Prevalence of elevated LDL (>99mg/dL)		37 (64)		31 (49)	0.106[2]

p-values come from two-sample t-tests and Fisher's exact tests; two-sample t-tests used log-transformed data.

Abbreviations: N = total number in group, n = # of non-missing for this variable, SD = standard deviation, FBS = fasting blood sugar, BP = blood pressure, TC = total cholesterol, TG = triglycerides, HDL = high density lipoprotein, LDL= low density lipoprotein

Supplemental Table 3

Regional correlations of associations between ovarian morphology and reproductive markers (Frank PCOS)

	FNPO		FNPS		OV		SA		S/A	
Reproductive Markers	**India**	**U.S.**	**India**	**U.S.**	**India**	**U.S.**	**India**	**U.S.**	**India**	**U.S.**
Menstrual cycle length (d)	-0.07	+0.18	-0.07	+0.16	+0.05	+0.18	+0.19	+0.03	-0.07	+0.04
Ferriman-Gallwey hirsutism score	+0.04	-0.04	+0.01	-0.02	-0.10	-0.05	**+0.25**	-0.08	**+0.23**	-0.13
Total testosterone (ng/dL)	+0.08	**+0.26**	+0.12	+0.10	+0.02	+0.12	-0.13	+0.08	-0.17	+0.11

Represented as Pearson's correlation coefficients of the non-log transformed data; shading reflects a significant or trending correlation: $P < 0.01$ (black) and $P < 0.05$ (dark gray); p-values from using log-transformed data.

Abbreviations: FNPO = follicle number per ovary, FNPS = follicle number per cross-section, OV = ovarian volume, SA = stromal area, S/A = stromal area/ovarian area ratio

Supplemental Table 4

Regional correlations of associations between ovarian morphology and metabolic markers (Frank PCOS)

	FNP0		FNPS		OV		SA		S/A	
Metabolic Markers	**India**	**U.S.**	**India**	**U.S.**	**India**	**U.S.**	**India**	**U.S.**	**India**	**U.S.**
2-hr glucose	-0.10	-0.04	+0.04	**-0.29**	+0.02	+0.11	+0.01	**+0.25**	-0.06	**+0.31**
FBS	-0.06	-0.08	+0.03	-0.26	-0.01	+0.01	-0.05	+0.12	-0.06	+0.19
BPSYS	-0.08	+0.28	-0.13	+0.04	+0.00	+0.20	-0.01	+0.20	-0.01	+0.24
BPDYS	-0.13	+0.16	**-0.24**	-0.03	+0.03	+0.12	+0.01	+0.17	+0.10	+0.10
BMI	-0.01	+0.04	-0.00	-0.17	+0.02	+0.04	-0.06	+0.15	-0.10	**+0.32**
WHR	+0.18	-0.03	+0.16	**-0.31**	+0.03	-0.06	+0.06	+0.07	+0.01	**+0.43**
TC	-0.005	+0.03	+0.16	+0.07	-0.08	-0.01	-0.18	+0.01	-0.17	+0.05
TG	+0.18	-0.02	+0.30	-0.16	+0.05	-0.17	+0.06	+0.01	-0.10	**+0.34**
HDL	+0.05	-0.08	+0.07	+0.07	-0.01	-0.06	-0.16	-0.21	-0.18	**-0.33**
LDL	-0.08	+0.03	+0.02	+0.06	-0.11	+0.01	-0.15	+0.06	-0.16	+0.15

Represented as Pearson's correlation coefficients of the non-log transformed data; shading reflects a significant or trending correlation: $P < 0.01$ (black) and $P < 0.05$ (dark gray); p-values from using log-transformed data.

Abbreviations: N = total number in group, n = # of non-missing for this variable, FNPO = follicle number per ovary, FNPS = follicle number per cross-section, OV = ovarian volume, SA = stromal area, S/A = stromal area/ovarian area ratio, FBS = fasting blood sugar, BPSYS = systolic blood pressure, BPDYS = diastolic blood pressure, BMI = body mass index, WHR = waist-to-hip ratio, TC = total cholesterol, TG = triglycerides, HDL = high density lipoprotein, LDL= low density lipoprotein