Follicle number, not assessments of the ovarian stroma, represents the best ultrasonographic marker of polycystic ovary syndrome

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Objective: To compare the diagnostic potential of ultrasonographic markers of ovarian morphology, used alone or in combination, to predict polycystic ovary syndrome (PCOS).
Design: A diagnostic test study using cross-sectional data collected from 2006–2011.
Setting: Academic hospital and clinical research unit.
Patient(s): Eighty-two women with PCOS and 60 healthy female volunteers.
Intervention(s): None.
Main Outcome Measure(s): Follicle number per ovary (FNPO), ovarian volume (OV), follicle number per single cross-section (FNPS), follicle distribution pattern, stromal area, ovarian area, stromal-to-ovarian area ratio (S:A), and stromal index (SI).
Result(s): Follicle number per ovary best predicted PCOS ($R^2 = 67\%$) with 85% sensitivity and 98% specificity, followed by OV ($R^2 = 44\%$), and FNPS ($R^2 = 36\%$). Neither S:A nor SI had predictive power for PCOS. In combination, FNPO+S:A and FNPO+SI most significantly predicted PCOS ($R^2 = 74\%$ vs. 73%, respectively). The diagnostic potentials of OV and FNPS were substantially improved when used in combination (OV+FNPO, $R^2 = 55\%$).
Conclusion(s): As a single metric, FNPO best predicted PCOS. Although the addition of S:A or SI improved the predictive power of FNPO, gains were marginal, suggesting limited use in clinical practice. When image quality precludes a reliable estimation of FNPO, measurements of OV+FNPS provide the next closest level of diagnostic potential. (Fertil Steril® 2014;101:280–7. ©2014 by American Society for Reproductive Medicine.)
Key Words: Polycystic ovary syndrome, ultrasound, follicle, stroma, hyperandrogenism

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Polycystic ovary syndrome (PCOS) is a heterogeneous disorder, originally characterized by Stein and Leventhal (1) in 1935, given the consistent presence of abnormal ovarian morphology among patients. The significance of ovarian morphology to PCOS has since been debated. The 1990 National Institutes of Health (NIH) consensus statement on diagnostic criteria for PCOS did not include ovarian morphology, indicating that it was only suggestive—and not definitive—evidence of a diagnosis (2). However, more recent diagnostic criteria proposed by the American Society of Reproductive Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE) in 2003 (3, 4), as well as by the Androgen Excess and PCOS Society in 2006 (5), have reasserted the importance of ovarian morphology to the diagnosis of PCOS.

Since the 1980s, ultrasonography has allowed for the noninvasive

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assessment of polycystic ovarian morphology (6). The earliest studies assessing polycystic ovaries (PCO) commented on three seemingly unique characteristics of the ovarian stroma: hypertrophy, increased echogenicity, and a tendency for the peripheral distribution of small ovarian follicles much like a "string of pearls" (6–9). Although this subjective assessment of the ovarian stroma was once common in the diagnosis of PCO (7), it has since been replaced by more quantitative measures of follicle number per ovary (FNPO) and ovarian volume (OV). Increased FNPO and OV continue to be favored vs. other morphological characteristics of PCO, despite criticism that these metrics are not purely specific to PCOS. In healthy women, OV has been found to naturally vary with age (10, 11), stage of puberty (12, 13), body mass index (BMI) (14), and insulin levels (15). Multiple reports have indicated that the threshold for FNPO supported by the ASRM/ESHRE Rotterdam consensus ($\geq$12 follicles) has contributed to an increased prevalence of PCO among healthy women of reproductive age (16–18). In addition, significant intraobserver and interobserver variability exists when counting follicles throughout the entire ovary (19, 20), suggesting that assessments of FNPO might be too subjective to serve as part of the diagnosis of PCO. There is also no uniform consensus on whether follicles should be counted throughout the entire ovary or in a single cross-sectional view of the ovary.

Although there appears to be inherent limitations to assessments of ultrasound features of PCO numerous investigative groups have recently reported improvements in reliability of these measures given advancements in imaging technology. We developed a systematic approach for counting ovarian follicles that showed exceptional reproducibility in follicle counts made in PCO (21). As a result, we and other investigators have recently revised diagnostic thresholds for FNPO and OV in PCO, which are obviating the artificial increase in polycystic ovarian morphology in the general population (22–24). Objective assessments of the ovarian stroma are now possible as a result of improved ultrasonographic imaging software. Fulghesu and colleagues (25) have developed a metric for assessing the ovarian stroma—called the stromal-to-total area ratio (S:A)—that appears highly specific to PCOS. Digital technology now also enables an unbiased method of quantifying stromal echogenicity by evaluating the mean pixel intensity of selected regions in the ovary (26). Finally, quantitative assessments of follicle distribution pattern by multiple observers were shown to be associated with fair levels of agreement, suggesting that distribution pattern evaluations may not be as subjective as once considered (20).

Although ultrasound metrics appear to have diagnostic utility on their own, few have quantified the predictive power gained when using these factors in combination to detect PCOS (9). Doing so may allow for an even more sensitive and specific diagnosis based on ultrasound. Thus, the primary objective of this study was to identify the diagnostic potential of each sonographic criterion to predict PCOS. The secondary objective was to determine the diagnostic capabilities of these morphological characteristics in combination to predict PCOS.

MATERIALS AND METHODS

Subjects

Eighty-two women diagnosed with PCOS by the NIH criteria as having oligoamenorrhea and hyperandrogenism were recruited to the study. Oligoamenorrhea was defined as a history of unpredictable menstrual cycles shorter than 21 days or longer than 36 days. Hyperandrogenism was defined as a modified hirsutism score $\geq$7 (internally validated value having 83% sensitivity and 96% specificity to distinguish between PCOS and controls) and/or an elevated total T concentration $\geq$114.12 ng/dL (internally validated value having 87% sensitivity and 100% specificity to distinguish between PCOS and controls). Sixty women from the general population with no hyperandrogenism and regular menstrual cycles served as controls. Participants were recruited from the general population using ads seeking healthy women of reproductive age or women with concerns of outward features of PCOS such as irregular periods, obesity, excess hair growth, and/or infertility. Volunteers ranged in age from 18–38 years and could not have used hormonal contraception, fertility medications, or insulin sensitizers in the 3 months before enrollment. Subjects were ineligible if they had a previous history of ovarian surgery or current abnormalities in cortisol (F), PRL, thyroid hormone, DHEAS, or 17-hydroxyprogesterone (17-OHP) secretion. Of the 62 women presenting for evaluation as controls, 2 were excluded because of hirsutism. Of the 134 women presenting for evaluation of PCOS features 98 met the NIH criteria for PCOS. Of the 60 controls that proceeded for an ultrasound scan (described later), all were judged to have sufficient image quality to be included in the study. Of the 98 participants with PCOS, 16 were excluded because of borderline image quality, thereby generating a PCOS cohort of 82 participants.

Ultrasound Procedures and Measurements

Participants were evaluated by transvaginal ultrasonography by one of two experienced ultrasonographers. Control subjects were scanned on days 2–5 of the menstrual cycle and women with PCOS were scanned at an unspecified time. Ovaries were scanned from their inner to outer margins in the longitudinal plane using a 5- to 9-MHz transducer on an Ultrasonix RP System or a 6- to 12-MHz transducer on a GE Voluson E8 System. The age of the equipment varied by 3.5 years. A post-hoc comparison of ultrasound measures noted only a 4.5% difference in FNPO (absolute difference $\pm$ 1.27 follicles) and 7.7% difference in OV (absolute difference $\pm$ 0.88 cm^3) for the pooled data set. Because this difference in measurements among machines was considered within the error for these parameters, data obtained using both machines were pooled. Digital cine loops throughout each ovary (DICOM file format) and static images of the largest cross-sectional view of each ovary (JPEG file format) were archived for off-line analysis using Santesoft DICOM Editor (©Emmanouil Kanellopoulus, Athens, Greece).

Largest cross-sectional views of the ovary were evaluated by a single investigator for follicle distribution pattern, stromal area (SA), ovarian area (OA), S:A, mean stromal echogenicity, mean total echogenicity, and the stromal index (SI).