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Individual 17-Hydroxyprogesterone Responses to hCG Are Not Correlated With Follicle Size in Polycystic Ovary Syndrome

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Context: In women with polycystic ovary syndrome (PCOS), 17-hydroxyprogesterone (17-OHP) responses to gonadotropin stimulation vary from increased to indistinguishable compared with normal controls.

Objective: To determine whether 17-OHP responses to recombinant-human chorionic gonadotropin (r-hCG) are individually correlated to the size of antral follicles among women with PCOS.

Design, Setting, and Participants: A prospective study conducted in 19 women with PCOS and 20 normal controls at an academic medical center.

Interventions: Blood samples were obtained before and 24 hours after administration of 25 μg of r-hCG. Ovarian imaging was conducted with three-dimensional pelvic ultrasonography. Each subject underwent a 2-hour oral glucose tolerance test.

Main Outcome Measures: Basal and stimulated levels of 17-OHP, androgens, estradiol, progesterone, anti-Mullerian hormone (AMH), insulin, glucose, follicle number, and size.

Results: In women with PCOS, mean antral follicle count (AFC) was greater than that of controls, although the size of cohort follicles within individual subjects was not correlated to 17-OHP responses. The numbers of 2- to 3-mm and 3- to 4-mm follicles in PCOS were significantly greater than in controls, whereas differences between larger follicles were not observed. Increased AMH in PCOS was correlated to AFC, but not 17-OHP responses. Insulin sensitivity did not correlate to r-hCG–stimulated 17-OHP after adjustment for body mass index.

Conclusions: 17-OHP responses to hCG in individuals with PCOS were not correlated to the distribution of antral follicles. Greater numbers of small antral follicles in women with PCOS than in controls suggest an extension of accelerated growth from the preantral stage.

Freeform/Key Words: 17-OHP, follicle, polycystic ovary syndrome

In women with polycystic ovary syndrome (PCOS), excessive androgen production is derived from ovarian theca cells (TCs), resulting in elevated circulating levels of testosterone (T) and androstenedione (A4). Mechanisms contributing to androgen overproduction include

Abbreviations: 17-OHP, 17-hydroxyprogesterone; A4, androstenedione; AMH, anti-Mullerian hormone; AFC, antral follicle count; BMI, body mass index; BMP, bone morphogenetic protein; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; GC, granulosa cell; GDF-9, growth differentiation factor 9; hCG, human chorionic gonadotropin; P_4, progesterone; PCOS, polycystic ovary syndrome; r-hCG, recombinant-human chorionic gonadotropin; T, testosterone; TC, theca cell.

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increased LH secretion, hyperinsulinemia, and increased TC responsiveness to gonadotropin stimulation. With respect to the latter, we and other investigators have demonstrated exaggerated 17-hydroxyprogesterone (17-OHP) responses to gonadotropin administration in women with PCOS compared with responses in normal controls [1–5]. However, despite a significant mean increment of 17-OHP following gonadotropin stimulation among women with PCOS, individual responses ranged widely and included some that were indistinguishable from those of normal women. These results may reflect varying TC sensitivity to gonadotropin stimulation among women with PCOS. Alternatively, the spectrum of 17-OHP responses to human chorionic gonadotropin (hCG) may be a direct result of corresponding TC hyperplasia within individual follicle populations.

Regarding the latter, we have raised the possibility that heterogeneous 17-OHP responses in women with PCOS may reflect differences in maturational growth of the follicle cohort among individuals with this disorder [5]. Previous studies showed that the increased follicle population in PCOS is predominantly composed of small (2 to 5 mm) antral follicles [6, 7]. Although larger follicles (6 to 9 mm) comprise a small percentage of the follicle population in PCOS, the number of these advanced follicles is similar to that observed in women with normal ovaries [6]. During normal follicular growth, increasing size is accompanied by greater TC mass. Correspondingly, in women with PCOS, advanced follicle growth might be expected to increase TC hyperplasia, which, at least in part, would contribute to greater ovarian androgen excess. A detailed analysis of the proportion of follicles according to size among individual women with PCOS has not been performed and may shed light on disparate 17-OHP responses to hCG.

To determine whether the number and size distribution of follicles in women with PCOS account for varied 17-OHP responsiveness to hCG, we performed a detailed analysis of ovarian follicle number and corresponding size using three-dimensional ultrasonography among normal controls and women with PCOS undergoing hCG stimulation.

1. Subjects and Methods

A. Subjects

Nineteen women with PCOS and 20 normal women with regular menstrual cycles were enrolled in this study. Women with PCOS and normal subjects were recruited through University of California, San Diego outpatient clinics and responses to advertisements in the San Diego area. All women with PCOS were oligomenorrheic or amenorrheic and demonstrated either biochemical (serum T >55 ng/mL) or clinical evidence of androgen excess or clinical evidence of androgen excess (Ferriman-Gallwey score >8). Women with PCOS demonstrated evidence of bilaterally enlarged ovaries with more than 12 antral follicles per ovary. Circulating TSH and prolactin levels were normal and were not significantly different between the two groups. Serum 17-OHP was greater than 2 ng/mL in three women with PCOS. Nonclassical congenital adrenal hyperplasia was excluded by an ACTH stimulation test. No participant received any hormone medication or metformin within 2 months of study enrollment. The study was approved by the Human Research Protection Program at the University of California, San Diego (IRB#140260), and written informed consent was obtained from each individual prior to participation.

B. Procedures

Subjects were admitted to the Clinical and Translational Research Institute at the University of California, San Diego on the days of testing. Normal subjects were studied during the midfollicular phase (cycle days 5 to 8), whereas women with PCOS were studied on a random day. Each subject received IV administration of recombinant hCG (r-hCG), 25 μg. Blood samples were collected prior to and 24 hours following r-hCG injection. Each subject also underwent a 75-g oral glucose tolerance test. Blood was obtained prior to (t = 0) and at t = 15, 30, 60, and 120 minutes following glucose ingestion. Insulin sensitivity was derived from the

formula described by Matsuda and DeFronzo [8]. For the imaging portion of the study, each participant underwent transvaginal ultrasonography (Voluson E8 Expert; GE Healthcare, Chicago, IL) using a 4- to 9-MHz probe (RIC5-9-D Endocavity transducer; GE Healthcare, Chicago, IL) to obtain three-dimensional imaging of each ovary using the SonoAVC™ software. Assessment of ovarian morphology included the total antral follicle count (AFC) of both ovaries in each subject as well as the size of each follicle as determined by its diameter in 1-mm increments between 2 and 9 mm. None of the subjects with PCOS experienced recent ovulation, as evidenced by the absence of recent menstrual bleeding for 2 months before the study and serum progesterone (P_4) level less than 1.0 ng/mL at a baseline sample.

C. Hormone Measurements

Blood was assayed by the Ligand Assay and Analysis Core Laboratory of the University of Virginia Center for Research in Reproduction. All assays were validated using the following indices: accuracy, linearity, functional sensitivity (*i.e.,* the lowest concentration with accuracy to a known standard within 20% and intra-assay coefficient of variation [CV] [1] <20%), precision, and correlation to a previous or established method. LH, FSH, insulin, total T, and P_4 levels were measured by chemiluminescence (Immulite 2000; Siemens, Los Angeles, CA); sensitivities = 0.1 IU/L, 0.1 IU/L, 2.0 uIU/mL, 10 ng/dL, and 0.1 ng/mL; intra-assay CVs = 3.9%, 3.0%, 2.5%, 4.9%, and 4.2%; and interassay CVs = 5.2%, 5.5%, 7.7%, 7.1%, and 5.8%, respectively [9–13]. 17-OHP, A4, and dehydroepiandrosterone (DHEA) were measured by ELISA (ALPCO, Salem, NH); sensitivities = 0.15 ng/mL, 0.1 ng/mL, and 0.4 ng/mL; intra-assay CVs = 6.1%, 4.4%, and 5.7%; and interassay CVs = 7.1%, 8.9%, and 9.7%, respectively [14–16]. Estradiol (E_2) was measured by ELISA (CalBiotech, El Cajon, CA); sensitivity = 10 pg/mL; intra-assay CV = 6.7%; and interassay CV = 9.8% [17]. Anti-Mullerian hormone (AMH) was measured by ELISA (ANSH, Webster, TX); sensitivity = 0.16 ng/mL; intra-assay CV = 1.6%; and interassay CV = 6.1% [18, 19]. Glucose was measured by the glucose oxidase method using the Analox Instrument (Stourbridge, UK); sensitivity = 1.0 mg/dL; intra-assay CV = 0.6%; and interassay CV = 1.2%.

D. Statistical Analysis

Statistical analysis was performed using JMP program version 13 (SAS Institute, Cary, NC). Results are presented as means ± SEM (SE). A value of $P < 0.05$ was considered statistically significant. Normality of distribution was assessed by the Shapiro-Wilk W test. In the absence of normality, data were appropriately transformed or nonparametric testing (Wilcoxon/Kruskal Wallis test, Wilcoxon signed rank test) was carried out when appropriate. To analyze distribution of follicles by percentage of total, follicle counts for each size range were converted to proportion of overall counts for each individual. Pooled data were transformed by the method of Box and Cox and subjected to ANOVA followed by *post hoc* testing between specific pairs using the Student *t* test for specific differences between groups based on diagnosis.

2. Results

A. Clinical Data

Clinical data for individual women with PCOS and normal women are listed in Table 1. The mean (±SE) ages for the PCOS and normal groups were 26.3 ± 1.1 and 26.9 ± 1.3 years, respectively. The mean body mass index (BMI) of subjects with PCOS was 30.9 ± 1.5 kg/m^2, compared with 26.0 ± 2.2 kg/m^2 in control participants (P = 0.02). The total number of follicles as well as the number of follicles according to 1-mm increments from 2 to 9 mm in individual normal women and women with PCOS are also shown in Table 1. In the normal group, total follicle numbers ranged from 11 to 70, compared with women with PCOS, in whom the range of follicle numbers was 25 to 132.

Table 1. Clinical Data for Normal Controls and Women With PCOS

Subject	Age (y)	Wt (kg)	BMI (kg/m^2)	Foll[a] Total	Foll[a] 2–3 mm	Foll[a] 3–4 mm	Foll[a] 4–5 mm	Foll[a] 5–6 mm	Foll[a] 6–7 mm	Foll[a] 7–8 mm	Foll[a] 8–9 mm
Control											
1	27	50.6	21.3	22	2	2	5	8	5	0	0
2	19	66.8	25.8	28	6	3	10	5	1	0	1
3	26	51.2	19.2	23	2	3	7	3	4	3	1
4	32	55.1	21.0	24	6	1	2	3	5	4	1
5	25	64.9	23.0	11	1	3	3	3	0	0	0
6	28	85.0	28.2	60	12	14	12	8	5	2	3
7	34	51.8	20.5	21	5	1	4	2	2	2	2
8	29	55.6	23.1	21	3	3	6	2	3	1	1
9	27	54.4	19.7	16	1	3	5	2	0	2	0
10	27	78.9	29.7	49	7	3	10	7	15	2	2
11	30	59.3	22.2	53	8	10	11	10	8	2	0
12	21	59.9	21.5	39	10	8	8	2	7	1	1
13	31	69.8	29.1	43	10	10	9	6	1	0	1
14	25	52.7	21.1	26	6	2	3	3	4	2	3
15	20	72.6	29.1	70	22	11	22	6	4	2	0
16	20	71.0	24.0	26	4	8	5	2	1	0	1
17	20	50.7	19.9	50	13	5	9	6	4	6	1
18	19	58.8	24.8	33	6	6	8	4	3	2	0
19	28	78.6	27.2	13	2	2	4	1	0	1	0
20	26	71.4	25.0	36	7	5	11	3	1	2	2
PCOS											
1	34	49.9	18.7	109	35	34	27	8	0	0	0
2	33	65.7	23.3	90	27	29	19	6	0	0	0
3	29	85.8	32.5	111	10	9	18	22	35	12	2
4	34	84.2	31.0	51	14	10	12	12	0	0	1
5	33	82.4	30.1	25	5	3	2	3	5	2	2
6	33	68.7	26.8	119	34	32	20	15	1	0	0
7	25	76.7	28.6	62	6	6	10	8	22	6	4
8	20	77.3	25.4	92	28	32	21	5	0	0	0
9	35	72.7	26.1	84	17	16	18	26	5	0	0
10	37	83.9	29.7	97	53	23	8	1	0	1	0
11	27	70.6	29.8	80	31	31	13	1	0	0	0
12	23	99.5	36.8	103	27	27	21	17	3	0	0
13	26	69.6	27.9	38	16	10	5	1	0	0	0
14	26	69.2	26.7	132	28	29	46	17	6	0	0
15	29	97.8	32.7	40	7	10	9	8	2	0	0
16	19	99.2	35.0	65	16	17	12	3	3	0	0
17	26	52.2	19.6	58	12	8	16	14	6	0	2
18	28	97.5	35.8	60	17	17	12	9	3	0	1
19	19	61.6	24.4	31	9	11	5	4	2	0	0

[a]Number of follicles.

B. Basal Hormone Levels

Basal levels of serum LH, 17-OHP, A4, T, E_2, and AMH were significantly higher in women with PCOS than in the group of normal controls (Table 2). In women with PCOS, FSH was significantly lower than in controls, whereas circulating levels of DHEA and DHEAS were similar.

C. Steroid Hormone Responses to r-hCG

Individual 17-OHP responses following r-hCG in both groups are illustrated in Fig. 1. To account for differences in basal hormone levels, the percentage change from baseline following r-hCG was used to calculate hormone responsiveness. Following administration of

Table 2. Steroid Hormone Levels Prior to and 24 H After r-hCG, 25 μg, in Normal Controls and Women With PCOS

Hormone	Basal	24 H	%Δ Max
17-OHP, ng/mL			
Control	0.9 ± 0.1	1.6 ± 0.1	67%
PCOS	1.4 ± 0.1	3.2 ± 0.5	140%[a]
A4, ng/mL			
Control	2.2 ± 0.1	2.5 ± 0.1	17%
PCOS	2.8 ± 0.2	3.5 ± 0.2	27%
T, ng/mL			
Control	14.8 ± 1.5	17.3 ± 2.2	20%
PCOS	29.7 ± 3.8	42.6 ± 4.3	97%[b]
DHEA, ng/mL			
Control	9.8 ± 1	9.6 ± 0.7	8%
PCOS	7.8 ± 0.8	10 ± 1.7	32%

Data are expressed as mean adjusted ± SE. To convert to SI units, multiply by the following conversion factor: 17-OHP (3.03); A4 (3.49); T (3.47); DHEA (3.47). %Δ Max, percent change from basal values.
[a] $P < 0.01$.
[b] $P < 0.05$.

r-hCG, significant percentage increases in 17-OHP and T were observed in women with PCOS compared with normal controls, whereas A4 and DHEA responses between groups were not different. As noted in Fig. 1, among women with PCOS, individual 17-OHP responses to r-hCG were highly variable, with many individual responses appearing similar to those of controls. These findings are consistent with our previously published results as well as those of other investigators. There was no significant relationship between serum AMH and 17-OHP responses to r-hCG in contrast to our earlier reported results.

D. Oral Glucose Tolerance Test

During the oral glucose tolerance test, integrated insulin responses (area under the curve) were higher in women with PCOS than in controls. Correspondingly, insulin sensitivity was significantly lower in women with PCOS, 6.9 ± 0.9, than in normal women, 12.5 ± 1.5. In women with PCOS, 17-OHP responses to hCG were correlated with insulin sensitivity. However, after adjusting for BMI differences in insulin responses, insulin sensitivity between groups was no longer observed.

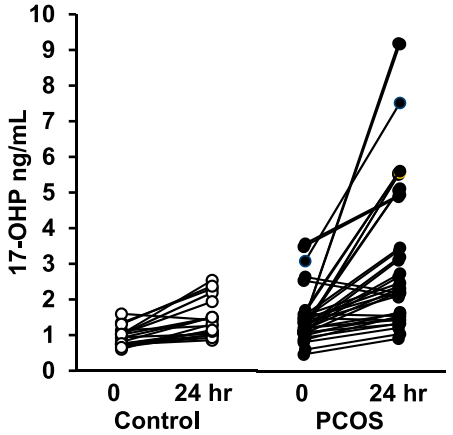

Figure 1. Individual serum 17-OHP levels prior to and 24 h following administration of r-hCG, 25 μg IV, in normal controls and women with PCOS.

E. Ovarian Follicle Morphology

As expected, in women with PCOS the mean total AFC was markedly greater than that of normal controls (75 ± 6 vs 32 ± 6; $P = 0.001$). In addition, the AFC was positively correlated with serum AMH ($P < 0.0001$). Analysis of follicles by 1-mm increments revealed significantly increased absolute mean numbers of follicles in individual groups of 2 to 3 mm through 5 to 6 mm in women with PCOS compared with normal controls (Fig. 2). At larger sizes, mean follicle numbers between groups were not statistically different. Based on the percentage of total follicles within an individual subject, only 2- to 3-mm and 3- to 4-mm follicles in PCOS ovaries were significantly greater than those in normal ovaries (Fig. 2). Beyond 4 mm, differences in mean percentage of follicle number between PCOS and normal ovaries were not noted. Among individual women with PCOS, the distribution of follicles by absolute number and by percentage of total was not correlated to 17-OHP responses to hCG. In addition, associations were not found between androgen responses to hCG and follicle number or serum AMH.

3. Discussion

The results of this study have confirmed our previous findings that in women with PCOS mean 17-OHP responses to hCG were significantly greater than those of normal controls, although individual responses were wide ranging. As expected, the AFC was higher in women

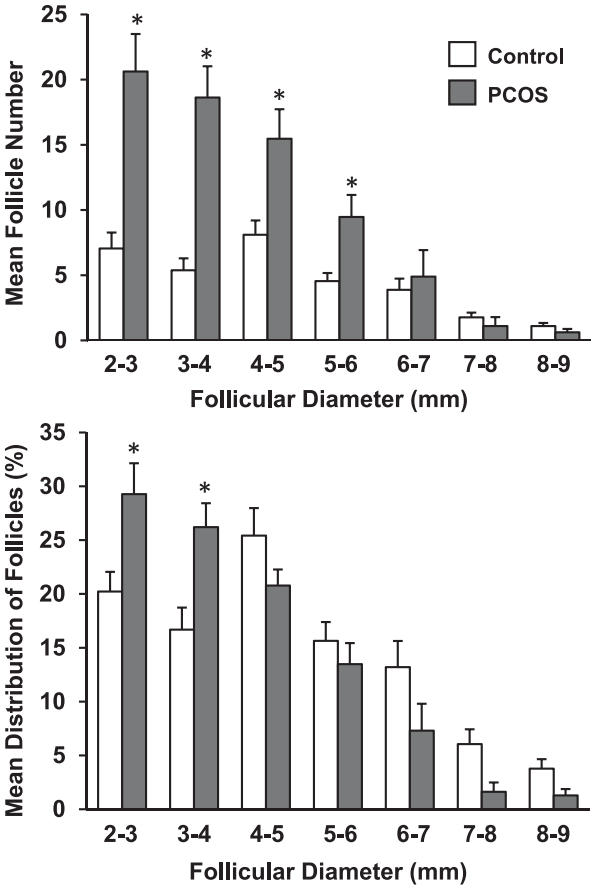

Figure 2. Mean (±SE) follicle number and mean (±SE) distribution of follicles (percentage of total follicle number within an individual) according to size in normal controls and women with PCOS. *, $P < 0.05$.

with PCOS than in normal women. However, analysis of follicle distribution within individual women with PCOS revealed that 17-OHP responses to hCG were not related to the distribution of follicles by size. There were greater numbers of small antral follicles in ovaries of women with PCOS than in ovaries of normal controls. However, beyond 4 mm this distinction was not evident between groups.

Increased 17-OHP responsiveness to hCG in women with PCOS compared with that of normal women is consistent with findings reported by others [1–5]. Notably, individual responses in women with PCOS were highly variable, as some were exaggerated whereas others were equivalent to those observed in normal women. It was previously reported that increased 17-OHP responses to gonadotropin stimulation were associated with reduced insulin sensitivity compared with observations in normal women [4]. It was proposed that increasing insulin resistance and greater hyperinsulinemia provided a basis for enhanced TC responsiveness. In the current study, 17-OHP responses were not correlated to basal insulin levels or insulin sensitivity after adjustment for BMI. The disparity of BMI between groups as well as limited numbers of subjects studied precluded comparison of our data with that of Pasquali *et al.* [4].

There are data suggesting an inherent defect of TC steroidogenesis in women with PCOS [20–22]. In particular, Gilling-Smith *et al.* [21] demonstrated that in human TC cultures, LH-stimulated A4 production per cell was significantly greater in cells obtained from women with PCOS than in those from normal controls. In a subsequent clinical study, the same authors examined 17-OHP and A4 responses to hCG stimulation in women with PCOS and normal women before and 1 month after an injection of a long-acting GnRH agonist [22]. After 1 month, reduced gonadotropin secretion and ovarian suppression were evident, as basal 17-OHP and A4 levels were decreased in both groups. In normal women, there was a corresponding reduction of 17-OHP and A4 following hCG stimulation. However, in women with PCOS, the heightened magnitude of response following hCG was unchanged from pretreatment values. Whether the high dose of intramuscular hCG—10,000 IU—obscured subtle differences among individual subjects is unclear. Subsequently, Rosencrantz *et al.* [23] performed a dose-response study revealing that maximal A4 and T responses to hCG in women with PCOS were achieved at low doses of hCG, whereas minimal responses at comparable doses of hCG were observed in normal control subjects. Although these data support a primary abnormality of androgen steroid production in women with PCOS, this concept does not account for the heterogeneous 17-OHP responsiveness exhibited by our subjects.

Alternatively, it has been reported that intraovarian factors may influence TC steroidogenesis. Among members of the tumor growth factor β superfamily, growth differentiation factor 9 (GDF-9) appears to have a vital role in TC development [24]. In GDF-9 knockout mice, the TC compartment failed to develop [25]. Moreover, in this model, GDF-9 was responsible for induction of CYP17 expression and androgen production in TCs of follicles at the preantral stage [26, 27]. In ovaries from women with PCOS, we showed that GDF-9 mRNA expression was significantly reduced in oocytes of growing and small antral follicles compared with that observed in normal oocytes [28]. Because lowered GDF-9 mRNA was evident in nearly all PCOS oocytes, it is unlikely that GDF-9 contributed to variable 17-OHP responses to hCG.

It was previously reported that certain bone morphogenetic proteins (BMPs) may exert inhibitory effects on TC androgen production [26, 29]. In bovine TCs, BMP-4, BMP-6, and BMP-7 decreased basal and LH-stimulated androgen accumulation primarily through suppression of CYP17 mRNA expression [14]. The role of these BMPs was underscored by the ability of chordin and gremlin to antagonize the stimulation of androgen production [20]. Notably, within the ovary, gremlin and chordin are derived from granulosa cells as is BMP-6, whereas BMP-4 and BMP-7 are from TCs. These findings suggest that interactions between these BMPs and their antagonists involve complex paracrine or autocrine mechanisms yet to be precisely defined. Consequently, at this time the role of BMPs in TC androgen production in PCOS cannot be determined.

There is little question that other granulosa cell factors may impact TC androgen production. Both *in vitro* and *in vivo* studies performed in animals and humans have indicated

that FSH may stimulate ovarian androgen production. Early studies showed that the addition of inhibin to TC culture resulted in increased basal as well LH-stimulated androgen responses [30–34]. In women with PCOS, we reported significant increases in ovarian androgen production following FSH administration compared with that in normal women [35]. The incremental androgen responses exhibited by women with PCOS accompanied by similar significant increments in FSH-stimulated inhibin-B levels. To determine whether inhibin mediates TC CYP17 mRNA expression, ovarian TCs were examined in the presence or absence of granulosa cells treated with FSH, inhibin, inhibin antibody, or β-glycan antibody [36]. CYP17 mRNA expression was dose-dependently increased by FSH, which suggests that paracrine factors from granulosa cells (GCs) mediated CYP17 mRNA expression. Antibodies against inhibin and β-glycan prevented FSH stimulation of CYP17 mRNA expression. However, inhibin alone did not increase CYP17 mRNA level to the same extent. Although these findings suggest that inhibin may participate in regulating TC CYP17 mRNA expression, other paracrine factors produced by FSH-stimulated granulosa cells appear to be involved.

Regarding women with PCOS in whom 17-OHP responses were indistinguishable from those of normal women, an obvious explanation is not apparent. In the report by Pasquali *et al.* [4] exaggerated 17-OHP responses to GnRH agonists in women with PCOS were attributed to hyperinsulinemia as reflected by significant elevations of plasma insulin during an oral glucose tolerance test. However, in women with PCOS with normal 17-OHP responses, insulin levels during glucose loading were also significantly greater than in controls. Ibañez *et al.* [3] examined hCG-stimulated 17-OHP levels in 23 women with PCOS and found that in 15 individuals responses were above the 95th percentile of 28 control subjects. Normal 17-OHP responsiveness observed in the remaining eight women with PCOS was not addressed. In an early study by Barnes *et al.,* women with PCOS exhibited significantly increased 17-OHP responses to a GnRH agonist [20]. Maximal responses did not overlap with those of normal controls, although the number of women with PCOS studied was limited to eight. In a previous publication of disparate 17-OHP responses to hCG in women with PCOS, we considered whether differences in follicle cohort populations among individuals might account for our findings [5].

Previous histological studies of ovaries from women with PCOS have demonstrated predominant numbers of preantral and early antral follicles that reflect maturational arrest at the midstage of follicle development [37–39]. Our ultrasonographic findings are consistent with these observations. However, follicle arrest does not appear to be complete cessation of progressive growth, as advanced antral follicles exist and spontaneous ovulation is known to occur in these women. Despite these considerations, results of the current study revealed that in individual women with PCOS, 17-OHP and androgen responses to hCG were not correlated to the size distribution of antral follicles. Thus, greater 17-OHP responses do not appear to be attributable to a cohort of larger antral follicles with corresponding greater TC mass. In addition, the distribution pattern of follicles in PCOS ovaries suggests that accelerated follicle growth is a characteristic feature of small antral follicle development in this disorder.

This increased rate of growth may represent an extension of preantral follicle advancement as previously reported [38, 39]. In an earlier study by Webber *et al.* [38], the accumulation of growing follicles in PCOS was greater than that in normal controls. Of note, the proportionate increase of growing follicles in PCOS was accompanied by a reciprocal decrease in the proportion of primordial follicles. In a subsequent study by Maciel *et al.* [39], there was a stockpiling of primary and secondary follicles in ovaries from women with PCOS that was significantly greater than those in normal ovaries. Although there was no difference in the number of primordial follicles between groups, the percentage of growing follicles relative to the number of primordial follicles was nearly twofold higher in PCOS than in normal ovaries. Thus, both studies suggested an accelerated transition of primordial to growing preantral follicle development in PCOS. Interestingly, initiation of primordial follicle growth has been observed in 2-day-old mouse ovaries treated with androgen. It was shown that both T and DHT stimulated an increase of phosphatidylinositol 3-kinase/protein kinase B (AKT), which resulted in activation of Forkhead box (Foxo)-3α [40]. This led to translocation of Foxo protein from the nucleus to the cytoplasm, allowing for transitional primordial follicle

growth. These findings are consistent with studies conducted in nonhuman primates and sheep in which T treatment during gestation resulted in enhanced preantral follicle growth [41, 42]. In particular, prenatal exposure to T in sheep resulted in a proportionate increase of primary follicles and a reduction of primordial follicles [43].

In our study, we noted that beyond 4 mm a difference in the number of follicles between groups was no longer present, implying a slowing of follicle growth in PCOS. Whether this relative decline in the rate of growth by PCOS follicles is due to an inhibitory process or a lack of continued stimulation is unknown. Nevertheless, at this early stage of antral growth, PCOS follicles appear to be quite vital, as shown by Das *et al.* [44]. Examination of follicles (4 to 5 mm) obtained from unstimulated follicles revealed lower rates of apoptosis and increased proliferation in GCs from women with PCOS compared with those from normal women. Moreover, GCs from PCOS follicles exhibited lower expression of apoptotic effector caspase-3 and increased amounts of inhibitor of apoptosis protein-2 than those of normal GCs.

In follicles larger than 4 to 5 mm, the impact of androgen on development and health is less clear. Using an encapsulated *in vitro* ovarian follicle growth system, the effect of androgen on multilayered secondary follicles was examined during progressive growth in mice [45]. Treatment with DHT resulted in advanced growth and accelerated preantral to antral follicle development. Prolonged exposure to DHT had no effect on steroidogenesis on days 2 and 4 of culture, but it decreased endogenous androgen synthesis on day 6. In addition, low-dose DHT enhanced follicle growth and survival, whereas these effects were reversed or not apparent with increasing doses.

In women with PCOS, it has been well regarded that arrest of follicle growth occurs at the midantral stage of development. According to the results of previously cited studies, together with our imaging data, it appears that this process may be initiated at or just beyond 4 to 5 mm. Of note, with increasing size, the follicle number as percentage of total follicle count in PCOS actually was less than that of controls, although the number of advanced-size follicles was low in both groups.

There was considerable overlap in total follicle numbers among individual normal controls and women with PCOS. This is not necessarily surprising because polycystic ovary morphology has been reported to be at least as high as 50% in women with normal ovulation [46, 47]. Moreover, it seems unlikely that similar follicle morphology as determined by ultrasonography reflects comparable follicle function between normal women and women with PCOS. To date, assessment of polycystic ovary morphology on a functional basis in normal women and those with PCOS has not been explored in detail. As a result, this consideration represents a limitation of our study. Studies attempting to address this issue are currently under way.

Similar to our previously published results, serum AMH levels were increased in women with PCOS compared with normal controls [5, 48]. However, unlike our earlier findings, in the current study AMH levels were not inversely correlated with 17-OHP responses to hCG. These results differ from those recently published by Rosenfield *et al.* [49] in that AMH levels in women with PCOS were positively correlated to 17-OHP responses to a GnRH agonist. Interestingly, among 48 women with PCOS and ovarian hyperandrogenism, 77% exhibited high responses, whereas in a much larger study of 148 women with PCOS, excessive 17-OHP responses to a GnRH agonist were found in 50% [4]. However, AMH was not measured in the latter study. By comparison, the limited numbers of subjects in both our early study and the current study preclude any interpretation of the relationship between AMH levels and 17-OHP responses. Currently, another study involving far greater numbers of normal women and subjects with PCOS is being conducted and includes evaluation of follicle populations. Another shortcoming of our study was that subjects were not matched for weight or BMI. As a result, the positive correlation between 17-OHP response to hCG and reduced insulin sensitivity was lost after adjustment for BMI.

In summary, the results of this study have shown that the spectrum of 17-OHP responses to hCG in women with PCOS was not correlated to the size of antral follicles among individual subjects. Notably, the distribution of follicles by size in women with PCOS suggests an

extension of accelerated growth from preantral stage to early antral stage development. Thereafter, follicle growth is slowed, most likely reflecting the initiation of follicle arrest in this disorder.

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