

## Polycystic ovarian morphology in postmenarchal adolescents

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**Objective:** To evaluate the association of polycystic ovary morphology (PCOM) with ovarian function in adolescents and to determine its time course during two years of follow-up.

**Design:** Prospective study.

**Setting:** Academic center.

**Patient(s):** Twenty healthy adolescents were followed from 2–4 years after menarche.

**Intervention(s):** We performed annual ultrasonographic and hormonal studies. Ovulation was assessed during 6 consecutive months by measuring salivary progesterone levels.

**Main Outcome Measure(s):** Persistence of PCOM during the years following menarche; ovulation in girls with PCOM.

**Result(s):** PCOM was observed in 40%, 35%, and 33.3% of the ultrasonographic studies performed at 2, 3, and 4 years after menarche, respectively. The concordance between ultrasonographic diagnosis at 2 and 4 years postmenarche (50%) was nonsignificant ( $\kappa = 0.08$ ). PCOM was not associated with abnormalities in ovulatory rate, menstrual cycle duration, lipid levels, or homeostatic model assessment of insulin resistance. However, lower FSH ( $4.8 \pm 1.3$  vs.  $6.1 \pm 1.9$  mIU/ml) were observed in girls with PCOM compared with those without PCOM. Similar T and stimulated 17-hydroxyprogesterone on the leuprolide test were observed in girls with and without PCOM.

**Conclusion(s):** PCOM is an inconstant finding in healthy adolescents and does not appear to be associated with decreased ovulatory rate or metabolic abnormalities in healthy adolescents. This finding suggests that PCOM may correspond to a physiologic condition during early adolescence. (Fertil Steril® 2011;95:702–6. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Polycystic ovary morphology, polycystic ovary syndrome, adolescents, adolescence, menarche, ovulation, salivary progesterone

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The presence of polycystic ovarian morphology (PCOM) has recently been included as a key element for the diagnosis of polycystic ovary syndrome (PCOS) in adults, leading to two different sets of diagnostic criteria (1, 2). The definitive criteria for diagnosing PCOS in adult women remain controversial, and few publications have reported on this issue in adolescents. Recently, some authors have postulated that the same criteria described for the adult population should be used for diagnosing PCOS in adolescents (3). De Zegher and Ibañez (4) have suggested, however, that PCOS should be diagnosed when the patient has both clinical and biochemical hyperandrogenism (4).

Whether polycystic ovaries in the absence of hyperandrogenism in adults should be considered pathologic for the purpose of PCOS diagnosis remains controversial (2, 5, 6). This controversy extends to adolescents, because few studies have evaluated the significance of polycystic ovaries during this stage of life. Recently, Mortensen et al. (7) studied a group of adolescent girls between the ages of 11 and 18 years and observed that almost half of them showed PCOM. In addition, they observed that girls who exhibited PCOM

in the ultrasonographic study had some mild elements of ovarian functional hyperandrogenism. These authors postulated that a significant proportion of adolescents with PCOM exhibit a “subclinical PCOS type of ovarian dysfunction”, as suggested by higher peak 17-hydroxyprogesterone (17OHP) in response to GnRH agonist test, and they propose that PCOM may be subsequently associated with the development of anovulatory cycles.

In this study, we investigated the possible relationship between PCOM and ovulation and ovarian function. In addition, we followed these adolescents longitudinally for 2 years by performing serial ovulation studies, ovarian ultrasound examinations, and metabolic studies.

## MATERIALS AND METHODS

### Subjects

We studied 20 healthy, postmenarchal girls who had reached menarche during the previous 24–30 months. They were recruited from schools in downtown Santiago, which is a middle-class area in Chile. Adolescents with any clinical signs of hyperandrogenism, such as moderate to severe acne or hirsutism, or significant menstrual irregularities were excluded from the study. Additional exclusion criteria included premature pubarche or intrauterine growth retardation—defined as the appearance of pubic hair in girls younger than 8 years or neonatal birth and length lower than  $-2$  SD for gestational age according to Chilean standards, respectively—first-degree relatives with diabetes mellitus, BMI below or above the 5th and 95th percentiles, use of oral contraceptives, steroids or any other type of medication, and the presence of other concomitant chronic conditions, such as genetic syndromes, celiac disease, renal disease, liver disease, cardiac disease, or undernourishment. All subjects had normal fasting blood glucose and thyroid hormone levels. The protocol was approved by the institutional review board of the San Borja Arriarán Hospital. Parents provided informed consent, and patients gave their assent before entering the study.

### Study Protocol

Girls were evaluated at baseline, which corresponds to a period approximately 2 years postmenarche (2YPM), and were followed prospectively with clinical, ultrasonographic, and hormonal studies at 1-year intervals, at approximately 3 years postmenarche (3YPM) and 4 years postmenarche (4YPM). A study of ovulation was performed during each period.

PCOM was defined by either 12 or more follicles measuring 2–9 mm in diameter and/or an ovarian volume greater than 10 ml in one or both ovaries (1, 8). In case a dominant cyst or follicle larger than 10 mm was observed, the ultrasonographic examination was repeated in the following cycle. Annual transabdominal ultrasonographic studies were performed and analyzed by a single observer (C.V.) who was blinded to the results of the previous sonograms for each girl. The examination was performed with a 5-MHz abdominal probe using Medison Sonoace 6000C equipment (Medison, Seoul, South Korea). Ovarian volume was calculated using the simplified formula for a prolate ellipsoid (9). The follicle number was obtained by counting the number of 2- to 9-mm follicles in the longitudinal cross-section of the ovary. The intraobserver variation coefficients of the ultrasonographic study were 3.2% for ovarian volume and 4.1% for follicle number.

An early-morning sample of blood was obtained during the follicular phase (days 1–7) for the measurement of LH, FSH,  $E_2$ , 17OHP, dehydroepiandrosterone sulfate (DHEAS), androstenedione, total T and sex hormone-binding globulin (SHBG). In addition, the girls underwent a leuprolide test with 500  $\mu$ g of leuprolide acetate injected subcutaneously at 2YPM and 4YPM for determination of stimulated 17OHP, as described previously (10, 11). An 1-24ACTH (Synacthen Novartis-Pharma, Hünigues, France) test was performed to exclude congenital adrenal hyperplasia by determining 17OHP levels 60 minutes after intravenous injection of 250  $\mu$ g ACTH (12). As a measure of insulin sensitivity, homeostatic model assessment of insulin resistance (HOMA-IR) values were calculated based on the fasting insulin and glucose levels of each subject.

### Assessment of the Ovulation Rate

Evaluation of ovulation was based on the measurement of salivary P (13). Salivary samples were collected from 19 and 17 girls during the first and second years of the study, respectively. A fasting salivary sample for the measurement of P was obtained after a mouth rinse with clear water on the 13th, 18th, 23rd, and 28th days of each menstrual cycle. The girls were requested to collect 1.5 ml of saliva in Eppendorf tubes. Regular telephone calls were made by one of the investigators (F.E.C. or P.L.) on the night before each scheduled saliva collection. Samples were stored in a case, frozen at home, and collected every 3 months. The duration of each menstrual cycle was determined through telephone calls and from calendars that were prospectively filled out by the study subjects. An ovulatory cycle was diagnosed if at least one of the samples had levels of salivary P greater than the threshold.

For determination of the salivary P threshold level, we studied 20 healthy young women with regular menstrual cycles and five women taking oral contraceptive pills, who served as negative controls. In addition, we performed simultaneous measurements salivary P on the 13th, 18th, 23rd, and 28th days of the menstrual cycle. The presence of an ovulatory cycle was confirmed by a serum P level of at least 4 ng/ml on days 20–24 of the menstrual cycle (14). (The maximum salivary P level attained in each cycle compared to the corresponding serum level is shown in Supplemental Figure 1A (available online)). A significant correlation between the salivary and serum P levels was observed (Pearson's  $r = 0.7178$ ; 95% confidence interval (CI), 0.4504–0.8671;  $P < 0.0001$ ). Receiver operating characteristic (ROC; Supplemental Figure 1B, available online) analysis was used to determine the salivary P levels with the best diagnostic accuracy. According to ROC analysis, a salivary P level  $\geq 0.06$  ng/mL had a diagnostic sensitivity and specificity of 80% and 100%, respectively, to detect a serum P level suggestive of the presence of ovulation. For this analysis, the ROC area under the curve was 0.93 ( $P = 0.00003$ ). This threshold level is similar to that reported by Gandara et al. (13).

### Definitions

The rate of ovulation was defined as the number of ovulatory cycles per 100 days of follow-up for each girl. The proportion of ovulatory cycles was defined as the fraction of studied cycles that were ovulatory, and it was calculated as the number of ovulatory cycles divided by the number of cycles studied in T1D or C and multiplied by 100. Oligomenorrhea was diagnosed if at least one menstrual cycle was longer than 45 days, as recently suggested by the American Academy of Pediatrics (15).

### Laboratory Assays

The salivary P level was determined by a commercial competitive radioimmunoassay kit obtained from Diagnostic System Laboratories (Webster, TX). The salivary samples were concentrated 20 times using an Eppendorf concentrator (Hamburg, Germany). In this condition, the assay had a sensitivity of 0.01 ng/mL. The intraassay and interassay coefficients of variation were 4.8% and 7.2%, respectively. T, 17OHP,  $E_2$ , DHEAS, SHBG, LH, and FSH were measured as previously described (10).

### Statistical Analysis

Clinical and laboratory data are shown as mean  $\pm$  SD. The comparison of continuous variables in girls at 2YPM, 3YPM, and 4YPM (Table 1) were compared in trend analyses with the generalized equation estimation methodology using an exchangeable covariance matrix (Table 1). The comparison of menstrual cycle duration and ovulatory rate during the two observed periods (2–3YPM and 3–4YPM) was analyzed with the Wilcoxon signed-rank test. The differences in the prevalence of oligomenorrhea during these two periods were compared with McNemar statistics.

The differences in the prevalence of PCOM during the three different periods were evaluated with Pearson's chi-square test (Table 2). Follicle number and ovarian volume at 2YPM, 3YPM, and 4YPM were compared in trend analyses with generalized equation estimation using an exchangeable covariance matrix. Agreement between the ultrasonographic diagnosis at baseline and during the following evaluations was determined using kappa statistics (Fig. 1). Comparison of the rates of ovulation in each period

**TABLE 1****Clinical characteristics, hormonal profile, and ovulatory and menstrual function of the girls at baseline and at each follow-up.**

	2YPM (baseline)	3YPM	4YPM
N	20	20	18
Age (y)	13.8 ± 0.8	14.9 ± 0.8	16.1 ± 1.0 <sup>a</sup>
Age of menarche (y)	11.8 ± 0.8		
Gynecologic age (mo)	24.9 ± 2.7	37.4 ± 2.0	49.8 ± 3.5 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	23.4 ± 3.5	23.8 ± 3.6	23.3 ± 4.3
BMI SDS	0.9 ± 1.0	0.8 ± 1.0	0.5 ± 1.2 <sup>a</sup>
Height (cm)	156.8 ± 5.9	157.6 ± 5.5	157.9 ± 5.8 <sup>a</sup>
Waist/hip ratio	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
LH (uU/mL)	2.4 ± 1.1	4.6 ± 3.6	5.2 ± 7.7 <sup>b</sup>
FSH (uU/mL)	5.0 ± 1.1	5.5 ± 1.2	6.3 ± 2.6 <sup>c</sup>
E <sub>2</sub> (pg/mL)	49 ± 13	57 ± 20	61 ± 44
17OH P (ng/mL)	1.2 ± 0.4	1.8 ± 1.9	1.1 ± 0.5
Stimulated 17OHP (ng/mL)	2.2 ± 0.8		2.6 ± 1.0
T (ng/dL)	40.4 ± 10.7	41.8 ± 18.6	40.1 ± 11.0
SHBG (nmol/L)	44.1 ± 14.2	44.2 ± 15.6	52.5 ± 27.5
Free androgen index	4.0 ± 2.2	4.1 ± 3.1	3.5 ± 2.0
DHEAS (mg/L)	1241 ± 511	1408 ± 558	1608 ± 606 <sup>a</sup>
Androstenedione (ng/mL)	1.3 ± 0.3	1.7 ± 0.8	1.6 ± 0.4 <sup>d</sup>
Insulin (mUI/mL)	7.4 ± 3.4	8.5 ± 4.1	7.0 ± 2.6
HOMA-IR	1.3 ± 0.7	1.5 ± 0.8	1.2 ± 0.5
Ovulation, menstrual cycle			
Cycles studied by girl (N)		4.8 ± 1.3	5.2 ± 2.1
Ovulation rate (n per 100 d)		1.3 ± 5.4	1.5 ± 1.2
Menstrual cycle duration (d)		32.8 ± 5.4	32.2 ± 4.9
Oligomenorrhea in ≥ 1 cycle (%)		35	11.8 <sup>b</sup>

Note: Results are shown as mean ± SD. Comparison of the data at 2YPM, 3YPM, and 4YPM was performed by generalized equation estimation. Comparison of the prevalence of oligomenorrhea was evaluated with McNemar statistics. SDS = standard deviation score; 2YPM = 2 years postmenarche; 3YPM = 3 years postmenarche; 4YPM = 4 years postmenarche.

<sup>a</sup>  $P < 0.001$ .

<sup>b</sup>  $P = 0.05$ .

<sup>c</sup>  $P < 0.01$ .

<sup>d</sup>  $P < 0.05$ .

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(Online-Only Appendix Table 1) by ultrasonographic status was performed with the Mann–Whitney test.

To evaluate the association of PCOM with clinical, metabolic, and hormonal abnormalities, all the data associated with a PCOM<sup>+</sup> test were compared with data associated with a PCOM<sup>-</sup> test. Adjusted analysis was performed with a multilevel model that included gynecologic age and ultrasonographic findings, which were nested for each girl. All statistical calculations were run on Stata 10.5 (College Station, TX), and  $P < 0.05$  was considered to indicate statistical significance. Data are shown as mean ± standard deviation (SD).

## RESULTS

The clinical characteristics, hormonal profile, and menstrual characteristics of the patients at baseline and at follow-up are shown in Table 1. All the girls completed the 3YPM follow-up, but two girls withdrew from the study between the 3YPM and 4YPM periods. Increasing LH, FSH, DHEAS and androstenedione levels were observed with increasing gynecologic age. A similar menstrual cycle duration and ovulatory rate were observed at 3YPM and 4YPM. The proportion of girls who had at least one menstrual cycle with oligomenorrhea decreased from 3YPM to 4 YPM (35.8% to 11.8%, respectively;  $P < 0.05$ ).

Ultrasonographic data are shown in Table 2. The prevalence of PCOM observed at the three different observation times was similar

(40%, 35%, and 33.3% of the girls at 2YPM, 3YPM, and 4YPM, respectively). Follicle number and ovarian volume were similar for the three observation periods.

The time course of the three ultrasonographic studies at 2YPM, 3YPM, and 4YPM is shown in Figure 1. Seven girls had a normal ovarian appearance in the three time points, whereas one had a PCOM morphology during the three time points. The remaining nine girls had a PCOM in some gonadal ultrasonograms and not in others. Agreement between the first and final ultrasonographic diagnoses was 50% ( $\kappa = 0.08$ ;  $P = 0.7$ ). Agreement for 2YPM and 3YPM and for 3YPM and 4YPM was nonsignificant.

PCOM<sup>+</sup> was associated with lower basal FSH compared with PCOM<sup>-</sup> ( $4.8 \pm 1.3$  and  $6.1 \pm 1.9$  uU/ml, respectively;  $P = 0.02$ , mixed multi level model). Similar BMI, menstrual cycle duration, ovulatory function, HOMA-IR, insulin, androgen, and cholesterol level were observed in girls with and without PCOM (data not shown). The ovulatory rate and menstrual cycle characteristics according to gynecologic age and ultrasonographic findings are shown in the Supplemental Table 1, available online.

## DISCUSSION

The results of our study suggest that PCOM is frequent in adolescents and is not associated with decreased ovulatory function,

**TABLE 2**

**Ultrasonographic findings and prevalence of polycystic ovarian morphology in healthy young adolescents. 2YPM: 2 years postmenarche; 3YPM: 3 years postmenarche; 4YPM: 4 years postmenarche.**

	2YPM	3YPM	4YPM
N	20	20	18
PCOM (N/%)	8 (40)	7 (35)	6 (33)
PCOM by volume only (N)	3	2	4
PCOM by follicle only (N)	3	1	1
PCOM by both criteria (N)	2	4	1
Ovarian follicles (N)	6.7 ± 3.1	6.8 ± 2.4	6.6 ± 2.6
Follicle number in the ovary with more follicles (N)	8.6 ± 4.4	8.6 ± 3.7	7.6 ± 3.5
Ovarian volumen (mL)	6.8 ± 3.8	7.2 ± 4.9	6.4 ± 2.2
Ovarian volume in the larger ovary (mL)	8.4 ± 5.8	8.7 ± 5.9	7.7 ± 3.1

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hyperandrogenism, or metabolic abnormalities. In addition, we observed that PCOM is an inconstant finding in this young postmenarcheal group of healthy girls, with 50% concordance at the 2-year follow-up.

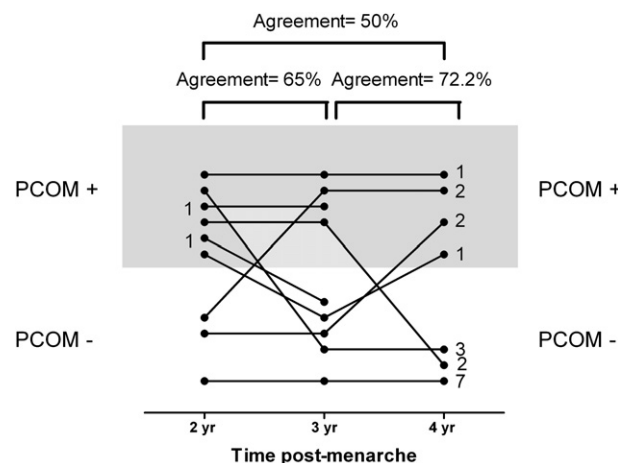
We observed that 36.2% of the ultrasonographic studies performed in young adolescents showed evidence of PCOM. This finding is similar to the recently reported prevalence of 35.4%, using Rotterdam criteria, by Hickey et al. in healthy adolescents (16), but it is lower than the 54% prevalence observed by Mortensen et al. (7), who used an ovarian volume greater than 10.5 mL as the diagnostic criterion for polycystic ovaries. Previously, PCOM prevalence rates of 26% and 31% were described in English and obese American adolescents, respectively. Importantly, these authors used different diagnostic criteria (17). These data suggest that the prevalence of PCOM in adolescents is far higher compared with that among healthy adult women with regular menstrual cycles, as the latter population shows evidence of this ultrasonographic finding in only 13%–25% of cases (18–20).

The prevalence of PCOM in adolescents observed in our study was similar to that recently reported by Fruzzetti et al. in adolescents with PCOS (21). This group studied 120 adolescents who fulfilled the National Institutes of Health criteria for PCOS and observed that 35% of the population had a PCOM. The similar prevalence of PCOM in adolescents with and without PCOS suggests a poor association of this ovarian pattern with the hyperandrogenic condition. The similar prevalence of PCOM in healthy adolescents and girls with PCOS differs from the data reported in adults, given that 93%–100% of the adult patients with a diagnosis of PCOS according to the National Institutes of Health criteria also present with PCOM (22).

Our study shows a low degree of agreement between initial and final diagnoses of PCOM, because approximately half the girls did not maintain their initial findings. Similarly, Murphy et al. (23) observed a low concordance for PCOM in adult women, who showed disappearance of PCOM after 8 years in 50% of the women who initially presented with PCOM. Similarly, van Disseldorp et al. (24) showed a 29% intercycle variability of antral follicle count in healthy adult women who were followed for several cycles. In contrast to healthy adolescents, polycystic ovaries appear to persist over time in girls with menstrual irregularities (25).

**FIGURE 1**

Longitudinal study of PCOM in individual girls at baseline and follow-up. Concordance of the ultrasonographic study was evaluated with kappa statistics. The concordance between ultrasonographic diagnosis at 2YPM and 4YPM was non-significant ( $\kappa=0.08$ ,  $P=0.7$ ). Agreement was also non-significant for 2YPM and 3YPM ( $\kappa=0.25$ ,  $P=0.1$ ) and for 3YPM and 4YPM ( $\kappa=0.35$ ,  $P=0.07$ ). The ultrasonographic status of the 18 girls who participated in the three evaluations is shown at the right; the numbers for the two girls who withdrew from the study are shown on the left.



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Our study showed that PCOM in adolescents was not associated with decreased ovulation, menstrual irregularities, or metabolic disturbances. These data suggest that detection of this ultrasonographic pattern in a nonhyperandrogenic adolescent should be considered a normal finding. The high prevalence of a polycystic ovarian pattern, which may be associated with physiologic presence of longer menstrual cycles during this stage of life (15, 26), can lead to the overdiagnosis of PCOS according to the Rotterdam criteria in this group of adolescents.

Previous studies performed in adults have shown that PCOM is associated with functional ovarian hyperandrogenism, as demonstrated by higher stimulated 17OHP levels during the GnRH agonist test (27). Similarly, Mortensen et al. (7) studied 12 adolescents with PCOM and observed that half of them had either PCOS diagnosed by the Rotterdam criteria or by an abnormal response to GnRH. We did not observe higher stimulated 17OHP levels in the leuprolide test in girls with PCOM. Our data do not support the notion that PCOM in healthy adolescents is associated with mild ovarian dysfunction.

The main strength of this study is the careful follow-up of healthy adolescents, which included hormonal and ovulation assessment. It also included an ultrasonographic profile performed by the same observer. However, some limitations of the study should be acknowledged: the number of girls studied was small, and future studies evaluating a larger number of girls should be performed in order to confirm these observations; and the evaluation of ovarian morphology was performed by transabdominal imaging, which is the accepted technique for studying gynecologic tract in adolescents, but is not the recommended technique for evaluating the presence of PCOM (8).

Our results suggest that PCOM diagnosed in otherwise healthy girls during the first few years following menarche is not necessarily

associated with anovulation or hyperandrogenism. This study should alert physicians caring for adolescents that the presence of PCOM in nonhyperandrogenic girls without significant menstrual irregularities may correspond to a physiologic event. Additional studies should evaluate the long-term prognosis for young girls with PCOM to confirm these findings.

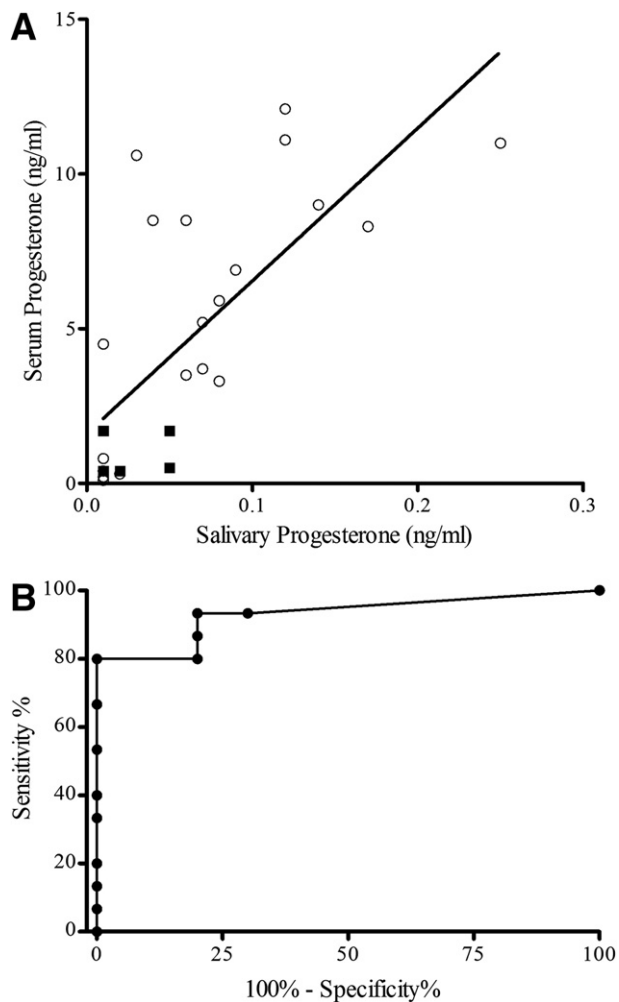
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## SUPPLEMENTAL FIGURE 1

**(A)** Correlation between maximum salivary P attained during days 13, 18, 23, and 28 of the menstrual cycle and the corresponding serum P levels at days 20–24 of the menstrual cycle (Pearson's  $r = 0.7178$ ; 95% CI, 0.4504–0.8671;  $P < 0.0001$ ). Data pertaining to women taking contraceptive pills are presented as *filled squares*. **(B)** Receiver operating characteristic (ROC) curve analysis was used to determine the salivary P levels with the best diagnostic accuracy. According to ROC analysis, a salivary P level  $\geq 0.06$  ng/mL had a sensitivity and a specificity of 80% and 100%, respectively (ROC area under the curve, 0.93;  $P = 0.00003$ ).



Codner. Polycystic ovaries in adolescents. *Fertil Steril* 2011.

**SUPPLEMENTAL TABLE 1**

Menstrual cycle characteristics and ovulatory rates in girls with and without PCOM: the data are shown for the 2YPM–3YPM and 3YPM–4YPM follow-ups.

	2YPM to 3YPM		3YPM to 4YPM	
	PCOM+	PCOM–	PCOM+	PCOM–
N	8	11	5	12
Cycles evaluated by girl (N)	4.6 ± 1.4	5.0 ± 1.3	7.2 ± 2.2	4.4 ± 1.6 <sup>a</sup>
Ovulation rate (n per 100 d)	1.2 ± 0.9	1.3 ± 0.8	1.2 ± 1.1	1.4 ± 1.1
Menstrual cycle duration (d)	32 ± 4	33.3 ± 6.3	31.9 ± 2.0	32.2 ± 5.6

<sup>a</sup>  $P < 0.05$ , girls at 3YPM–4YPM compared with 2YPM–3YPM.

Codner. Polycystic ovaries in adolescents. *Fertil Steril* 2011.