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What is This?
Relationship Between Anti-Müllerian Hormone (AMH) and Insulin Levels During Different Tanner Stages in Daughters of Women With Polycystic Ovary Syndrome

Teresa Sir-Petermann, PhD, MD1, Amanda Ladrón de Guevara, MD1, Ethel Codner, PhD, MD2, Jessica Preisler, MD1, Nicolás Crisostó, PhD, MD1, Bárbara Echiburú, PhD1, Manuel Maliqueo, PhD1, Fernando Sánchez, MD1, Francisco Perez-Bravo, PhD3, and Fernando Cassorla, PhD, MD2

Abstract

Context: We have previously described increased serum levels of anti-Müllerian hormone (AMH) and stimulated insulin in daughters of women with polycystic ovary syndrome (PCOS), suggesting that these girls may have an altered ovarian follicular development which may be modulated by insulin. However, the specific relationship between serum AMH and insulin levels during each Tanner stage of puberty in this cohort has not been established. Objective: The aim of our study was to establish the relationship between AMH and poststimulated insulin serum concentrations during each stage of puberty in daughters of women with PCOS (PCOSd), compared to daughters of control women (Cd). Design: We studied 135 PCOSd and 93 Cd classified according to their Tanner stage. Gonadotrophins, sex steroids, sex hormone-binding globulin (SHBG), and AMH were determined in a fasting sample. Ovarian volume was measured by pelvic ultrasound. In addition, in both groups we performed an oral glucose tolerance test with measurements of glucose and insulin. Results: Anti-Müllerian hormone levels were significantly higher in PCOSd compared to Cd at all Tanner stages. Daughters of women with PCOS having AMH concentrations greater than 2 standard deviation (SD) above the mean AMH value for the Cd group showed decreased serum follicle-stimulating hormone (FSH) concentrations and increased stimulated levels of insulin during Tanner stages I, II, and III. Conclusions: Anti-Müllerian hormone levels are increased in PCOSd during all stages of puberty. We suggest that those PCOSd with the highest AMH levels probably represent a group of girls with more severe ovarian dysfunction and metabolic derangements.

Keywords
hyperandrogenism, puberty, anti-Müllerian hormone

Introduction

Polycystic ovary syndrome (PCOS) is a highly prevalent (5%-10%) endocrine-metabolic disorder in women characterized by ovulatory dysfunction and hyperandrogenism. In addition, PCOS is frequently associated with insulin resistance and its compensatory hyperinsulinemia that enhance the phenotypic expression of PCOS.1-3

Although its pathophysiology is complex and not completely understood, it is now considered that hyperandrogenism is the main feature of PCOS.2 Another important characteristic of this syndrome is an altered folliculogenesis reflected by an excessive number of growing follicles of 2-9 mm.4

Anti-Müllerian hormone (AMH), a dimeric glycoprotein member of the transforming growth factor-β (TGF-β) superfamily,5-6 may constitute a marker of follicular development, and its serum levels seem to correlate with the development of preantral and small antral follicles from puberty to the end of reproductive life.7 In previous studies, we have observed...

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that infant, children, and adolescent daughters of women with PCOS (PCOSd) exhibit higher levels of AMH compared with control girls,8,9 which suggests that the follicular alterations described in adult PCOS women may appear early during development. However, the specific serum AMH levels during each Tanner stage of puberty in a large group of PCOSd compared to controls have not been established. Moreover, according to our study,10 in PCOSd during childhood and adolescence, stimulated insulin levels were significantly higher compared with the control group. However, the possible relationship between insulin levels and AMH during the different Tanner stages of puberty has not been established for these girls.

Therefore, the aim of the present study was to establish the relationship between AMH and stimulated insulin serum concentrations during each stage of puberty in PCOSd, compared to daughters of control women (Cd).

**Material and Methods**

**Participants**

We included 135 PCOSd and 93 Cd between 8 and 16 years of age. From these girls, 28 PCOSd and 33 Cd were included in our previous study.9 The present study was designed to analyze PCOS girls and controls transversally, not including girls evaluated in more than 1 Tanner stage. Both groups of girls were matched using the Tanner stage score for breast development. The girls included in the study were not taking oral contraceptives or any other medications. All girls were born at term from singleton pregnancies.

Mothers with PCOS were recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile, Santiago, Chile. The diagnosis of PCOS was made according to the National Institutes of Health consensus criteria.11 Mothers with PCOS were evaluated before pregnancy and they exhibited chronic oligomenorrhea or amenorrhea, hirsutism, serum testosterone concentration greater than 0.6 ng/mL, free androgen index (FAI) >5.0, and/or androgenorrhea, hirsutism, serum testosterone concentration greater than 3.0 ng/mL. Intra- and interassay coefficients of variation were 5.3% and 8.7%, respectively. Serum LH, FSH, and estradiol were determined by electrochemiluminescence (Roche). Assay sensitivities were 0.1 UI/L, 0.1 UI/L, and 5.0 pg/mL, respectively. Intra- and interassay coefficients of variation were 5.3% and 8.7%, respectively. Serum LH, FSH, and estradiol were determined by electrochemiluminescence (Roche). Assay sensitivities were 0.1 UI/L, 0.1 UI/L, and 5.0 pg/mL, respectively. Intra- and interassay coefficients of variation were 5.3% and 8.7%, respectively.

**Study protocol**

The girls were admitted with their mothers to the Pediatric Unit of our Clinical Research Center at approximately 08:30 hours. We performed a complete physical examination on each girl, including anthropometric measurements (weight, height, waist, hip, body mass index [BMI] and BMI standard deviation [SD] score [SDS] calculated using the Growth Analyser Program, and the US BMI-for-age growth charts).13 These growth curves have been shown to be applicable to contemporary Chilean population.14 Obesity was defined as a body weight >95th percentile. Hirsutism was evaluated by determining the presence of terminal hair using the modified Ferriman-Gallway score.15 The Chilean population is less hirsute than other populations, so a score of 6 or greater was employed to establish the presence of hirsutism.16 The presence of acne and acanthosis nigricans was also determined. Menstrual regularity was not considered in the data analysis, because irregular menses are very frequent during the first years after the onset of menarche.

In both groups of girls, an oral glucose tolerance test (1.75 g/kg, up to a maximum of 75 g glucose in 250 mL water) after a 12-hour overnight fast. Blood samples (5 mL) were drawn before and 30, 60, 90 and 120 minutes after the glucose load was conducted. Serum glucose and insulin were determined in each sample. Glucose tolerance was evaluated using the American Diabetes Association criteria (ADA).17 Insulin resistance was estimated by the Homeostasis Model Assessment for insulin resistance (HOMA-IR), as previously described.18 In addition, we calculated the whole-body insulin sensitivity composite index (ISI).19 Circulating concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone-binding globulin (SHBG), testosterone, androstenedione (Δ4A), 17α-hydroxyprogesterone (17-OHP), estradiol, and AMH were determined in the fasting sample. Basal serum SHBG and testosterone were used to calculate the FAI as the ratio of serum testosterone/SHBG × 100.

Ovarian volume was calculated by transabdominal ultrasonography using the simplified formula for a prolate ellipsoid.20 The larger ovary was used to evaluate the ovarian size.

Postmenarcheal girls were studied during the early follicular phase of the menstrual cycle (days 3-7). In premenarcheal girls, the study was performed whenever feasible.

The protocol was approved by the institutional review boards of the San Juan de Dios and San Borja Arriarán Hospitals and the University of Chile. All parents signed informed consents, and girls gave their assent before entering the study.

**Assays**

Serum AMH was assayed by enzyme immunoassay (Immunotech-Beckman Coulter, Marseille, France).21 Analytical sensitivity was 2.1 pmol/L and intra- and interassay coefficients of variation were 5.3% and 8.7%, respectively. Serum glucose was determined by the glucose oxidase method (Photo metric Instrument 4010; Roche, Basel, Switzerland). The intra-assay coefficient of variation of this method was <2.0%. Serum insulin was assayed by radioimmunometric assay (RIA) Diagnostic Systems Laboratories, Inc, Texas). The intra- and interassay coefficients of variation were 5% and 8%, respectively.

Serum LH, FSH, and estradiol were determined by electrochemiluminescence (Roche). Assay sensitivities were 0.1 UI/L, 0.1 UI/L, and 5.0 pg/mL, respectively. Intra- and interassay coefficients of variation were 5% and 8%, respectively.
coefficients of variation were 1.1% and 2.1% for LH; 1.67% and 3.7% for FSH; and 2.7% and 5.0% for estradiol, respectively.

Serum testosterone was assayed by RIA and the limit of detection was 10 ng/dL. The intra- and interassay coefficients of variation were 7.0% and 11.0%, respectively. This RIA was validated against liquid chromatography, tandem mass spectrometry, as previously described. Serum D4A (Diagnostic Systems Laboratories, Inc, Texas) and 17-OHP (Diagnostic Products Corp. Los Angeles) were assayed by RIA. Sex hormone-binding globulin was determined by radioimmunoassay (Diagnostic Products Corp). Assay sensitivities were 0.1 ng/mL, 0.1 ng/mL, and 0.04 nmol/L, respectively. Intra- and interassay coefficients of variation were 4.3% and 6.0% for D4A; 5.0% and 5.0% for 17-OHP; and 3.8% and 7.9% for SHBG.

Statistical Evaluation

The present study was designed to analyze PCOS girls and controls transversally, not including girls evaluated in more than 1 Tanner stage. Data are expressed as mean and standard error of the mean (SEM). Normal distribution was assessed by the Kolmogorov-Smirnov test. Differences between study groups were assessed with the Student t test when data were normally distributed, or the Mann-Whitney test when not normally distributed. The differences in AMH serum concentrations between each Tanner stages was evaluated by 1-way analysis of variance (ANOVA) test. Moreover, a 2-way ANOVA test was performed, considering the condition (Cd or PCOSd) and the Tanner stage as independent variables, and AMH levels as the dependent variable. The same procedure was used for the analysis of LH, FSH, and 2-hour insulin levels, these data were controlled for Tanner stage and for AMH concentrations greater or lower than 2 SD above the mean AMH values for the Cd group. Multiple regression techniques were performed to assess the effect of BMI SDS on serum AMH concentrations in Cd and PCOSd girls during the different Tanner stages. Categorical data were analyzed using chi-square ($\chi^2$) or Fisher exact test.

Statistical analysis was performed with STATA 7.0 package. A P value of less than .05 was considered statistically significant.

Results

Clinical characteristics and ovarian volume in Cd and PCOSd are shown in Table 1. Both groups were comparable in age, BMI SDS, waist circumference, waist to hip ratio (WHR), and birth weight at each Tanner stage. Ovarian volume was significantly higher in PCOSd compared with Cd at all Tanner stages. Ovarian volume increased during puberty in both groups ($P < .0001$, 1-way ANOVA).

Obesity was present in 10.3% of the PCOSd and in 8.6% of the Cd ($P = .576$). Hirsutism was present in 11.8% of the PCOSd and in 3.2% of the Cd ($P = .387$). The presence of acne was similar in both groups. Menarche occurred at an average age of 11.5 ± 1.0 years in Cd and 11.3 ± 1.4 years in PCOSd. The proportion of postmenarchal girls in each group was similar. Menstrual cycle irregularity was observed in 24% of the postmenarchal Cd and in 53% of the postmenarchal PCOSd. In the PCOSd, a positive correlation between ovarian volume and menstrual cycle irregularity was observed ($P < .05$).

Table 2 shows the main biochemical characteristics of both groups of girls. No differences were observed in serum FSH, LH/FSH ratio, 17-OHP, D4A, or estradiol. In Tanner stage IV, PCOSd showed significantly higher basal LH levels than Cd. Moreover, testosterone was significantly higher in PCOSd compared to Cd during Tanner stages IV and V. Levels of SHBG were lower in Tanner stage V, and FAI was significantly higher in Tanner stages IV and V in the PCOSd group.
<table>
<thead>
<tr>
<th></th>
<th>Tanner I</th>
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<th>Tanner II</th>
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<th>Tanner III</th>
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<th>Tanner IV</th>
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<th>Tanner V</th>
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<tr>
<td></td>
<td>Cd (n = 21)</td>
<td>PCOSd (n = 30)</td>
<td>Cd (n = 17)</td>
<td>PCOSd (n = 26)</td>
<td>Cd (n = 19)</td>
<td>PCOSd (n = 26)</td>
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<td>PCOSd (n = 29)</td>
<td>Cd (n = 19)</td>
<td>PCOSd (n = 24)</td>
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<td>0.4 ± 0.1</td>
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<td>0.6 ± 0.2</td>
<td>3.4 ± 0.7</td>
<td>2.3 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>7.2 ± 1.2b</td>
<td>3.7 ± 0.4</td>
<td>4.7 ± 0.6</td>
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<td>FSH, IU/L</td>
<td>2.6 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td>4.0 ± 0.6</td>
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<td>4.9 ± 0.5</td>
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<td>6.5 ± 0.6</td>
<td>5.8 ± 0.4</td>
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<td>LH/FSH ratio</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
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<tr>
<td>17-OHP, ng/mL</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
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<tr>
<td>Δ4A, ng/mL</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>T, ng/dL</td>
<td>23.3 ± 3.5</td>
<td>26.2 ± 3.3</td>
<td>30.1 ± 4.0</td>
<td>35.3 ± 4.0</td>
<td>40.5 ± 5.0</td>
<td>35.7 ± 4.5</td>
<td>37.8 ± 3.0</td>
<td>68.8 ± 8.6b</td>
<td>44.8 ± 3.2</td>
<td>62.0 ± 4.9b</td>
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<td>Estradiol, pg/mL</td>
<td>9.6 ± 1.7</td>
<td>7.7 ± 1.0</td>
<td>14.9 ± 3.9</td>
<td>14.6 ± 3.0</td>
<td>34.6 ± 5.0</td>
<td>29.4 ± 4.4</td>
<td>50.6 ± 5.5</td>
<td>43.8 ± 9.5</td>
<td>37.9 ± 4.4</td>
<td>41.6 ± 5.9</td>
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<td>SHBG, nmol/L</td>
<td>63.0 ± 7.0</td>
<td>61.6 ± 8.6</td>
<td>60.2 ± 11.1</td>
<td>65.6 ± 8.1</td>
<td>54.9 ± 5.3</td>
<td>58.9 ± 5.4</td>
<td>51.5 ± 4.5</td>
<td>49.2 ± 6.7</td>
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<tr>
<td>FAI</td>
<td>2.1 ± 0.6</td>
<td>2.6 ± 0.9</td>
<td>3.7 ± 1.5</td>
<td>3.0 ± 0.8</td>
<td>3.1 ± 0.5</td>
<td>2.6 ± 0.5</td>
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<td>8.8 ± 2.6b</td>
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<tr>
<td>Glucose, mg/dL</td>
<td>84.1 ± 2.7</td>
<td>87.5 ± 2.1</td>
<td>84.8 ± 3.4</td>
<td>81.7 ± 2.6</td>
<td>89.5 ± 2.1</td>
<td>81.3 ± 2.3</td>
<td>83.9 ± 2.4</td>
<td>85.6 ± 2.4</td>
<td>86.1 ± 2.6</td>
<td>82.9 ± 2.1</td>
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<tr>
<td>Insulin, µIU/mL</td>
<td>9.0 ± 0.8</td>
<td>10.7 ± 1.9</td>
<td>9.3 ± 1.1</td>
<td>11.8 ± 1.7</td>
<td>14.4 ± 2.4</td>
<td>22.8 ± 6.5</td>
<td>15.9 ± 3.1</td>
<td>11.8 ± 1.5</td>
<td>11.6 ± 1.3</td>
<td>14.7 ± 1.7</td>
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<td>2-Hour</td>
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<tr>
<td>Glucose, mg/dL</td>
<td>91.6 ± 4.8</td>
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<td>96.8 ± 6.1</td>
<td>100.3 ± 4.0</td>
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<td>Insulin, µIU/mL</td>
<td>32.1 ± 4.5</td>
<td>66.0 ± 16.7b</td>
<td>35.9 ± 4.3</td>
<td>62.9 ± 12.7b</td>
<td>53.9 ± 5.9</td>
<td>92.9 ± 18.1b</td>
<td>40.9 ± 5.6</td>
<td>65.6 ± 8.4b</td>
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<td>77.2 ± 11.7b</td>
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<td>ISI composite</td>
<td>7.5 ± 0.7</td>
<td>6.7 ± 0.8</td>
<td>6.2 ± 0.6</td>
<td>6.0 ± 0.8</td>
<td>5.6 ± 0.9</td>
<td>5.3 ± 0.8</td>
<td>7.9 ± 1.9</td>
<td>5.4 ± 0.7</td>
<td>5.7 ± 0.5</td>
<td>4.2 ± 0.4b</td>
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</table>

Abbreviations: 17-OHP, 17-OH-progesterone; Δ4A, androstenedione; T, testosterone; FAI, free androgen index; SEM, standard error of the mean; PCOSd, daughters of women with polycystic ovary syndrome; Cd, control daughters; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin; ISI, insulin sensitivity composite index.

Values are mean ± SEM.

*p < .05 between Cd and PCOSd.
compared to controls. Basal glucose and insulin concentrations were similar between both groups. Therefore, HOMA-IR was not different between Cd and PCOSd at any Tanner stage. Two-hour insulin concentrations were significantly higher in PCOSd compared to Cd in all Tanner stages. During early puberty, ISI composite was not different between Cd compared to PCOSd. However, during late puberty, ISI composite was lower in PCOSd compared to Cd.

AMH serum levels are illustrated in Figure 1. AMH levels were significantly higher in PCOSd compared to Cd at all Tanner stages. These differences remained significant after the data were adjusted for BMI SDS. The pattern of AMH fluctuation along different Tanner stages was similar in PCOSd and Cd, nevertheless AMH concentration was always higher in the PCOSd group compared to the Cd group during puberty (P = .498 and P = .596, respectively). The differences regarding AMH concentrations were explained only by the PCOS or control condition and not by the Tanner stage of the girls.

When PCOSd were classified according to their circulating AMH concentrations, we observed that those PCOSd with AMH concentrations greater than 2 SD above the mean AMH values for the Cd group had lower FSH concentrations and higher 2-hour insulin levels during Tanner stages I, II, and III of puberty (Figure 2). In addition, during Tanner stage IV, these girls showed higher LH levels and similar insulin levels compared to the other PCOS daughters (AMH <2 SD; Figure 2). Moreover, we observed that the serum testosterone concentrations approached statistical significance in those PCOSd with the highest AMH levels (76.0 ± 13.0 vs 47.0 ± 8.0 ng/dL; P = .071). During Tanner stage V, there was a trend to higher LH levels in those PCOSd with the highest AMH levels (5.8 ± 0.8 vs 3.8 ± 0.5 IU/L; P = .081). Insulin levels were similar between both groups. No differences in BMI SDS and ovarian volume were observed between PCOSd with the highest AMH levels compared to other PCOSd at any Tanner stage. Moreover, regarding LH, FSH, and 2-hour insulin levels, we observed that all the differences found within the PCOS group were explained by the Tanner stage and the AMH levels.

Discussion

In this study, we evaluated AMH serum concentrations during different Tanner stages in PCOSd, compared to Cd with comparable BMI. We documented that PCOS daughters showed higher AMH serum levels during all Tanner stages, suggesting that the ovarian follicular pool is increased during puberty in these girls. Moreover, the present study revealed that PCOS daughters with the highest AMH concentrations (greater than 2 SD above the mean AMH values for the control group)
exhibited lower FSH and higher stimulated insulin levels during early puberty. This suggests that, despite low FSH concentrations, insulin may promote an increased follicular pool during initial gonadal activation in these girls.

In previous studies, we demonstrated that in PCOSd, AMH serum concentrations were higher during early infancy (2-3 months of age), childhood (4-7 years of age), and in a small group of adolescents (8-18 years of age), suggesting the presence of an increased growing follicular pool, which appears to be established during early development and persists throughout puberty.5,9 However, a careful assessment of circulating AMH concentrations during each Tanner stage of puberty in a large group of adolescents had not been previously performed. The present study shows that AMH serum concentrations are significantly higher in PCOS daughters during all Tanner stages. Since AMH is produced exclusively by the granulosa cells, serum AMH concentrations correlate with the number of growing follicles. Accordingly, we demonstrate in the present study that PCOS daughters show higher AMH levels and higher ovarian volumes than control daughters during all Tanner stages of puberty. This observation is in agreement with a recent study performed in adolescent girls with polycystic ovarian (PCO) morphology,22 which showed that PCO morphology is associated with elevated serum AMH concentrations. It has been documented that ovaries normally have a multifollicular appearance during puberty, so in the present study, the traditional PCO morphology according to the Rotterdam criteria was not employed.23

Recently, we evaluated metabolic and reproductive features before and during puberty in PCOSd, compared to Cd.10 In that study, we observed that at Tanner stage III there is an inflection point for gonadotrophin and androgen levels. Thus, we propose that these girls undergo 2 periods during puberty, one that encompasses Tanner stages I, II, and III (early puberty) where there was no increase in androgens or LH, and the other one, during Tanner stages IV and V (late puberty), where we observed a clear increase in these parameters.

In the present study, we observed that AMH serum concentrations remain high and relatively constant during both periods of puberty in PCOS daughters. Moreover, PCOS daughters showed higher stimulated levels of insulin compared to control girls despite similar BMI. It has been proposed that hyperinsulinemia plays a pivotal role in the pathogenesis of PCOS.24 Hyperinsulinemia stimulates the development of antral follicles, by increasing the sensitivity of granulosa cells to FSH, thus increasing the number of follicles and the ovarian volume.25,26

Interestingly, the present study reveals that during early puberty, PCOS daughters with the highest AMH concentrations (greater than 2 SD above the mean AMH value for the Cd group) exhibited lower FSH and higher poststimulated insulin levels compared to the other PCOS daughters. This suggests a synergistic effect of insulin on FSH action to maintain an increased follicular pool that was probably established during prenatal life.27

In the present study, those girls with the highest AMH levels during late puberty showed higher LH and a trend for higher insulin levels (compared to the control daughters) that may further promote androgen secretion. This observation is in agreement with the concept that insulin acts synergically with LH to stimulate the synthesis of testosterone by ovarian theca cells.28-30 It has been proposed that excess insulin may be responsible for functional ovarian hyperandrogenism in PCOS women, through the overactivation of the CYP17 enzyme pathway.3,31,32 On the other hand, it has been proposed that moderate elevations in testosterone appear to act through the androgen receptor to exert stimulatory effects on LH production at both the hypothalamic and pituitary levels.33,34

The high AMH late puberty PCOSd group probably represents a group of girls with more severe ovarian dysfunction.

The present study confirms the finding of a previous study35 that AMH levels reflect the severity of PCOS. However, as suggested by Rosenfield,36 not all girls present elevated testosterone and increased AMH levels simultaneously. In the present study, 41% of the PCOSd had elevated levels of AMH during late puberty, which was less frequent than the prevalence of the testosterone elevation (nearly 52%) in the same girls during late puberty. This result suggests that the PCOS condition may have high or normal AMH levels, and therefore AMH levels are not a marker of PCOS as previously proposed.22

In conclusion, PCOS daughters show higher AMH levels compared to control daughters during all Tanner stages of puberty. The PCOSd with the highest AMH levels appear to represent a group of girls with more severe ovarian dysfunction, in whom insulin may modulate the size of the growing follicular mass.

Acknowledgments
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Authors’ Note
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