ORIGINAL ARTICLE: REPRODUCTIVE ENDOCRINOLOGY

Higher luteinizing hormone levels associated with antimüllerian hormone in postmenarchal daughters of women with polycystic ovary syndrome

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Objective: To study the reproductive and metabolic differences between daughters of women with polycystic ovary syndrome (PCOSd) and control women (Cd) after menarche.
Design: Case-control study.
Setting: Clinical endocrinology unit.
Patient(s): We studied 43 PCOSd and 28 Cd 1.5–6 years after menarche.
Intervention(s): Determination of anthropometry, pubertal development, hirsutism, oral glucose tolerance test, and GnRH analogue test.
Main Outcome Measure(s): Ferriman score, sex steroids, gonadotropins, antimüllerian hormone (AMH), ovarian volumes, and glucose and insulin levels.
Result(s): The groups were similar in chronologic, gynecologic, and menarchal ages and anthropometric variables. Ferriman score, ovarian volumes, and AMH were higher in PCOSd. Propensity score analysis showed that there were significant differences in LH, LH-FSH ratio, T and free androgen index, post-stimulated LH and LH-FSH ratio, and 2-hour insulin that could be attributed only to the fact of being a PCOS daughter. The generalized linear model showed that higher LH levels were positively associated with AMH and T levels.
Conclusion(s): We found that higher LH, androgen, and insulin levels are present in PCOSd during the postmenarchal period, which may establish the basis for the development of PCOS during adulthood. Moreover, LH levels were associated with AMH levels, which supports that the neuroendocrine feedback proposed for AMH and LH is present in humans and that this feature is probably programed in utero, as recently shown in mice. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: PCOS daughters, LH, AMH

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Polycystic ovary syndrome (PCOS) is a highly prevalent (5%–10%) endocrine-metabolic dysfunction in adult women, characterized by chronic anovulation and hyperandrogenism. In addition, most women with PCOS also have neuroendocrine dysfunction, reflected by increased LH pulsatility and peripheral insulin resistance, which plays a key role in the pathogenesis of this syndrome (1, 2) and its long-term health

consequences, such as type 2 diabetes and cardiovascular disease (3). A genetic cause of the syndrome was suggested many years ago (4), and phenotypic and family aggregation studies have demonstrated that a significant number of female relatives of PCOS patients are affected with the condition (5–9). On the other hand, alterations during intrauterine life have been implicated in the origin of PCOS (9–13) and may modify the endocrine and metabolic function of a child born to a PCOS mother independently from the genetic inheritance and sex (14–16). Recently antimüllerian hormone (AMH) has been implicated in the pathogenesis of PCOS, inducing high LH secretion during pregnancy in mice and PCOS-like features in the offspring of treated mothers (13).

In an attempt to understand the sequence of appearance of the pathophysiologic components of PCOS, we designed a strategy that involved the study of daughters born to PCOS mothers at different stages of development. We have described several early metabolic and reproductive markers of the syndrome that may be modified through interventions that improve the adverse pregnancy environment present in PCOS women (15, 17). During early infancy (2–3 months old) and childhood (4–7 years old) PCOS daughters (PCOSd) show increased AMH levels, which is a marker of ovarian follicle number (18). Peripubertal PCOSd show an increased ovarian volume and significantly higher levels of 2-hour poststimulated insulin compared with control daughters at all Tanner stages. In Tanner stages IV and V, basal and post–leuprolide-stimulated LH, T, and 17-OHP concentrations are higher in PCOSd compared with daughters of control women (Cd). Along all these stages, AMH levels and ovarian volumes are higher in the PCOSd group (17). Other researchers have also found this same sequence of metabolic abnormalities preceding the onset of hyperandrogenism, as shown by Ibañez et al. in girls with premature pubarche (19).

On the other hand, as mentioned before, a new pathophysiologic mechanism has been reported in mice, showing a positive feedback between AMH and LH (20). This feature is programmed in utero and has not been demonstrated in humans (13).

Therefore, the aim of the present study was to identify which of all these features are present in PCOS daughters during the postmenarchal period.

MATERIALS AND METHODS

Subjects

Forty-three postmenarchal girls born to PCOS mothers (PCOSd) and 28 postmenarchal girls born to mothers with regular menstrual cycles and without hyperandrogenism (Cd) were studied. Girls were 11–17 years old with a gynecologic age (years after menarche) of 1.5–6 years. All were born from singleton pregnancies and were not taking oral contraceptives or any other medication during the 6 months before the study. Girls with any chronic diseases were excluded. Most of these girls participated in previous studies by our group (17, 21). Each girl participated in the present study only once. None of these girls have been previously diagnosed with PCOS.

PCOS mothers were recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile, Santiago, Chile. The diagnosis of PCOS of the mother was made during early reproductive age according to the National Institutes of Health consensus criteria (22). Mothers with PCOS exhibited chronic oligomenorrhea or amenorrhea and hirsutism during reproductive age. In addition, PCOS women showed the characteristic ovarian morphology of polycystic ovaries on ultrasound, based on the criteria described by Adams et al. (23). These inclusion criteria for PCOS mothers have been previously reported (17).

As control mothers, we selected women of similar socioeconomic level with a history of singleton pregnancies and regular 28–32-day menstrual cycles, without hirsutism or other clinical manifestations of hyperandrogenism, infertility, or pregnancy complications during their early reproductive age.

Study Protocol

The girls were admitted with their mothers to our Clinical Research Center. We performed a complete physical examination, including anthropometric measurements (weight, height, waist, hip, body mass index [BMI], and BMI and height for age *z*-score on the basis of World Health Organization 2007 growth references (24)). These growth curves have been shown to be applicable to the contemporary Chilean population (25). The age at menarche was registered. The mean cycle length of the past 12 months according to what was reported by each study subject was recorded (cycles per year). Gynecologic age was defined as the number of years since menarche at the time of the study.

A single observer (A.L.d.G) assessed the pubertal development according to the Tanner criteria. Hirsutism was evaluated by determining the presence of terminal hair with the use of the modified Ferriman-Gallwey score. Girls with a history of early puberty and precocious pubarche were excluded.

In both groups, we performed 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 for the measurement of glucose and insulin concentrations before and 30, 60, and 120 minutes after the glucose load. Glucose tolerance was evaluated with the use of the American Diabetes Association criteria. Impaired glucose tolerance was defined as 2-hour postload glucose of $\geq$140 mg/dL and $<$200 mg/dL (26). The homeostasis-model assessment of insulin resistance (HOMA-IR) and the whole-body insulin sensitivity composite index (ISI) were calculated as previously described (27, 28). Circulating concentrations of SHBG and serum lipids (total cholesterol, triglycerides, low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]) were determined in the fasting sample.

On the next day, transabdominal ultrasound was performed and analyzed by an observer blinded to the condition of the subject. The examination was performed with the use of a 5-MHz abdominal probe and Medison Sonoace X8

equipment. Ovarian volume was calculated using the simplified formula for a prolate ellipsoid (29). The larger ovary was used to evaluate ovarian size.

Subsequently, all subjects underwent a GnRH agonist test with 10 μg/kg subcutaneous leuprolide acetate (Lupron; Abbott Laboratories). The test was started between 8:00 and 9:00 a.m.; serum LH and FSH levels were measured before and 3 hours after leuprolide injection. Serum T, A, 17α-hydroxyprogesterone (17-OHP), and E_2 concentrations were determined at baseline and 24 hours after leuprolide administration. Basal serum SHBG and T were used to calculate the free androgen index (FAI) as the ratio of serum T to SHBG × 100. Maximal values after leuprolide testing were defined as the peak value for gonadotropins at 3 hours and for steroids at 24 hours.

The girls were studied during the early follicular phase of the menstrual cycle (day 3–7) or whenever feasible in those with amenorrhea.

Ethical Approval

The protocol was approved by the Institutional Review Board of the University of Chile. All parents signed informed consents, and the girls gave their assents before entering the study.

Assays

Serum AMH was assayed by means of enzyme immunoassay (Immunotech; Beckman Coulter). Analytical sensitivity was 2.1 pmol/L, and intra- and interassay coefficients of variation were 5.3% and 8.7%, respectively. Serum glucose and lipid profile were determined with the use of the glucose oxidase method (Biosystems). The intraassay and interassay coefficients of variation of this method were <1.2% and <2.7%. LDL-C was calculated according to the Friedewald formula.

Serum insulin was assayed by means of immunoradiometric assay (IRMA; Diasource Immunoassay) with a sensitivity of 1.0 μIU/mL and intra- and interassay coefficients of variation of 3% and 7%. Serum LH, FSH, and SHBG were determined by means of IRMA (Izotop). Assay sensitivities were 0.05 mIU/mL, 0.02 mIU/mL, and 0.22 nmol/L, respectively. Intra- and interassay coefficients of variation were 1.0% and 3.1% for LH, 2.5% and 2.7% for FSH, and 4.9% and 3.8% for SHBG.

Serum T, A, and 17-OHP were assayed by means of radioimmunoassay (RIA; Diasource Immunoassay), and the limits of detection were 0.1 ng/mL, 0.1 ng/mL, and 0.03 ng/mL. The intra- and interassay coefficients of variation were 3.5 and 5.5%, 3.8% and 7.5%, and 4.6 and 7.7%, respectively.

Serum E_2 was assayed by means of RIA (Pantex) with a sensitivity of 10 pg/mL and intra- and inter-assay coefficients of variation of 4.7% and 5.4%.

The T RIA was validated against liquid chromatography–mass spectrometry (Supplemental Fig. 1, available online at www.fertstert.org) at Barnafi-Krause Laboratories, Santiago, Chile, with the use of the methods described by Maliqueo et al. (30).

Statistical Evaluation

Data are expressed as median and interquartile range. Data distribution was assessed by means of the Kolmogorov-Smirnov test; most of the variables showed a nonnormal distribution, for which differences between study groups were assessed with the use of the Mann-Whitney test. In the few normally distributed variables a Student *t* test was performed as indicated on each table. Statistical analysis was performed with the use of the Graph Pad Prism 6.0 package and the SPSS 22 package. A propensity score analysis, performed with the use of the Stata 14.0 package, using the variables chronologic age, gynecologic age, Tanner stage, age of menarche, BMI, waist circumference, hip circumference, and birth weight, was applied to establish if the differences observed were attributable only to the PCOSd condition. A Pearson correlation was made for T validation analysis and a Spearman correlation to assess the association between LH and AMH. A generalized linear model was used to analyze the relationship between LH and AMH, age, BMI, FSH, A, and T to explore which factors explain LH levels. A P value of $<.05$ was considered to be statistically significant.

RESULTS

Clinical and Anthropometric Characteristics

The groups were similar in chronologic, gynecologic, and menarchal ages. Birth weight and anthropometric variables, such as weight, *z*-score of height and BMI, waist and hip diameter, waist-to-hip ratio, and systolic and diastolic blood pressures were also similar between groups. Ferriman-Gallwey score, ovarian volumes, and AMH levels were significantly higher in PCOSd compared with Cd. No differences were observed in the number of cycles per year (Table 1). Eleven Cd girls had a Ferriman score >6 and of these, six had oligoamenorrhea and only one had an ovary with polycystic morphology, but none of them had the three features simultaneously (0% PCOS). In the PCOSd group, 28 girls had a Ferriman score >6 and of these, 13 had oligoamenorrhea and 13 had an ovary with polycystic morphology. Seven PCOSd had the three features of PCOS simultaneously (16.2% PCOS).

Hormonal Characteristics

Basal T and FAI were significantly higher in PCOSd compared with Cd, whereas SHBG was lower. After leuprolide stimulation, LH, LH-FSH ratio, and A were significantly higher in PCOSd (Supplemental Table 1, available online at www.fertstert.org).

Metabolic Characteristics

Fasting glucose and insulin and 2-hour glucose levels were similar between groups. On the other hand, 2-hour insulin levels were higher in PCOSd compared with Cd. HOMA-IR and ISI composite did not differ between the groups. Six PCOSd (13.95%) presented glucose intolerance. No differences were observed in lipid profile (Supplemental Table 2, available online at www.fertstert.org).

TABLE 1

Clinical characteristics of postmenarchal control (Cd) and polycystic ovary syndrome (PCOSd) daughters.

Characteristic	Cd (n = 28)	PCOSd (n = 43)	*P* value
Age (y)	14.96 (14.08–15.67)	15.42 (14.17–16.17)	.410[a]
Gynecologic age (y)	2.55 (2.30–3.25)	3.20 (2.30–3.80)	.099[b]
Menarchal age (y)	12.00 (11.00–13.25)	12.00 (11.00–13)	.789[b]
Weight (kg)	57.15 (50.90–66.25)	60.00 (52.00–72.50)	.312[b]
Height (m)	1.58 (1.55–1.62)	1.57 (1.53–1.61)	.454[a]
Z-score height	−0.42 (−0.87 to 0.06)	−0.62 (−1.16 to 0.02)	.089[a]
BMI (kg/m^2)	22.86 (20.35–26.06)	25.28 (21.70–28.72)	.134[b]
Z-score BMI	0.81 (0.32–1.61)	1.24 (0.48–2.07)	.397[a]
Waist (cm)	71.00 (67.00–75.00)	73.50 (68.00–83.00)	.124[b]
WHR	0.83 (0.80–0.87)	0.84 (0.81–0.86)	.839[b]
Birth weight (kg)	3.30 (2.98–3.81)	3.20 (2.95–3.35)	.123[b]
SBP (mm Hg)	100.0 (90.0–110.0)	100.0 (90.0–110.0)	.291[b]
DBP (mm Hg)	60.0 (60.0–70.0)	60.0 (60.0–70.0)	.689[b]
Ferriman-Gallwey score	6.5 (2.0–8.0)	9.5 (5.0–15.0)	.006[b]
Cycles per year	12.0 (9.0–12.0)	11.0 (8.0–12.0)	.108[b]
Ovarian volume (cm^3)	6.52 (4.30–9.00)	9.18 (6.40–12.45)	.013[b]
AMH (pmol/L)	24.31 (13.39–47.31)	40.05 (17.49–52.12)	.049[a]

Note: Data are presented as median (interquartile range). Cd missing values: 1 for waist and birth weight, 2 for WHR, cycles per year, and ovarian volume, 3 for SBP and DBP, and 6 for Ferriman-Gallwey score. PCOSd missing values: 1 for waist, WHR, and Ferriman-Gallwey score, 2 for birth weight, and 4 for SBP and DBP. AMH = antimüllerian hormone; BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure; WHR = waist-to-hip ratio.

[a] Student *t* test.

[b] Mann-Whitney *U* test.

Crisosto. High LH related to AMH in PCOS daughters. Fertil Steril 2018.

Propensity Score Analysis

A propensity score analysis was performed with the use of age, gynecologic age, menarchal age, BMI, waist, WHR, and birth weight as matching variables. The analysis showed that once cases were perfectly paired with controls (Supplemental Table 3, available at www.fertstert.org), there were significant differences in Ferriman-Gallwey score, basal LH, LH-FSH ratio, T and FAI, peak LH and peak LH-FSH ratio, and 2-hour insulin that could be attributed only to the PCOSd condition (Table 2).

LH and AMH Relationship

In the total group, there was a positive correlation between AMH and LH levels. This correlation was significant in the PCOSd group but not in the Cd group (Fig. 1). The generalized linear model showed that LH levels were negatively associated with BMI and positively associated with testosterone and AMH (Table 3).

DISCUSSION

In the present study we have identified, during the postmenarchal period, three main pathophysiologic components that are strongly associated with the condition of being a daughter of a patient with PCOS: increased LH secretion (neuroendocrine component), higher androgen levels, and a hyperinsulinemic response in the oral glucose tolerance test.

Regarding the neuroendocrine component, in adolescents with PCOS, increased LH has been described as an early

TABLE 2

Clinical, hormonal and metabolic parameters that were significantly different between groups after propensity score matching.

Parameter	Cd (n = 18)	PCOSd (n = 40)	*P* values
Clinical parameter			
Ferriman-Gallwey score	7.0 (0.0–7.0)	9.0 (5.0–15.0)	.008
Hormonal parameters			
Basal			
LH (UI/mL)	1.74 (1.21–3.23)	4.24 (2.15–9.23)	.005
LH-FSH ratio	0.28 (0.13–0.55)	0.63 (0.37–1.47)	.001
T (ng/mL)	0.54 (0.49–0.67)	0.70 (0.59–1.02)	.003
FAI	3.55 (2.97–5.63)	7.30 (3.32–18.52)	<.001
Post-leuprolide			
LH (UI/mL)	39.9 (32.2–79.8)	85.4 (54.2–108.8)	.002
LH/FSH	1.87 (1.26–2.38)	2.98 (1.89–2.98)	.009
Metabolic parameter			
2-hour insulin (uUI/mL)	53.6 (39.6–62.1)	63.9 (41.0–108.8)	.036

Note: Data are presented as median (interquartile range). FAI = free androgen index. *P* values were calculated by Student *t* test.

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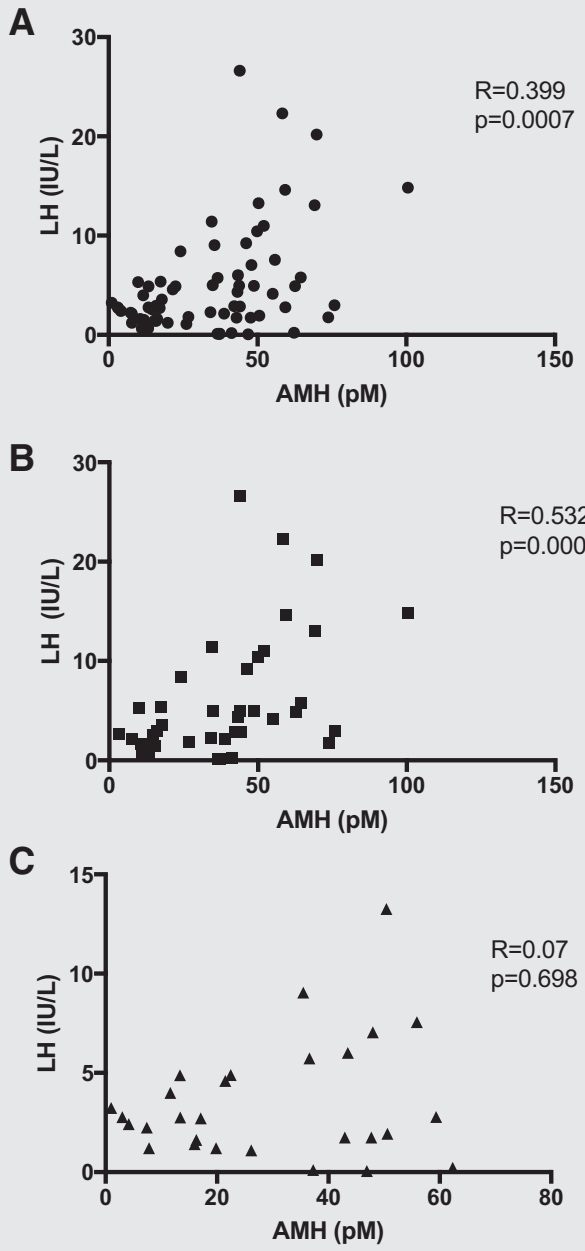
FIGURE 1



Spearman correlations between LH and antimüllerian hormone (AMH) levels in the (**A**) total group, (**B**) polycystic ovary syndrome (PCOS) daughters group, and (**C**) control group.

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TABLE 3

Generalized lineal model for the different factors that may influence LH levels.

Factor	Coefficient (95% CI)	*P* value
Age	0.17 (−0.24 to 0.60)	.410
BMI	−0.11 (−0.22 to 0.015)	.025
FSH	0.17 (−0.09 to 0.44)	.210
Insulin	0.02 (−0.04 to 0.09)	.456
AMH	0.06 (0.02 to 0.10)	.002
Testosterone	3.41 (1.06 to 5.77)	.004
Androstenedione	−0.02 (−0.41 to 0.35)	.883

Note: CI = confidence interval; other abbreviations as in Table 1.

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feature in the establishment of the syndrome (31). Accordingly, in our studies, PCOS daughters exhibited elevated peak LH levels after leuprolide administration during early postnatal life and late puberty (15, 17). Similarly, postmenarchal PCOS daughters exhibited elevated LH levels and LH-FSH ratios, indicating that the neuroendocrine defects are present at this age. Although these alterations are not consistently evidenced in adult women with PCOS, probably owing to the effect of increased BMI, it seems to be a very strong marker during postmenarche. In this regard, it has been observed that LH levels are negatively correlated with BMI and that obesity reduces the amplitude of the LH pulses (32–34). In our generalized linear model BMI was negatively associated with LH levels and positively correlated with AMH. Because this is only an association,we can not be sure if AMH is increasing LH or if LH is increasing AMH. In this regard, in human follicles from PCOS women, LH has been shown to increase AMH production, but not significantly (35). On the other hand, Cimino et al. demonstrated in a rodent model that AMH is able to directly increase GnRH-dependent LH pulsatility (20). In the present study, PCOSd show a strong correlation between AMH and LH, which is confirmed in the generalized linear model. This correlation was not significant in the control group, probably because they do not have a broad enough spectrum of AMH levels to find a statistically significant correlation, which does not mean that this correlation is not present in normal subjects.

Our study is the first to show an association between AMH and LH levels in humans after controlling for other factors, such as age and BMI. On the other hand, it was recently demonstrated that the female offspring of mice treated with AMH during pregnancy showed this same neuroendocrine feature (13). We have also previously shown that infant girls born to PCOS mothers have higher LH levels after leuprolide stimulation (15). The present data support this finding, showing that in the postmenarchal period daughters of women with PCOS have increased LH levels, probably driven by high AMH levels. Nevertheless, this is still a speculation, because there may be many other environmental and genetic factors influencing this relationship.

The second component is higher androgen levels. Our T measurements were performed with the use of RIA and validated by means of mass spectrometry, which is very important to generate valid data. We previously reported that PCOSd exhibit higher basal and peak T levels (15) in Tanner stages IV and V compared with control girls (17). In the present study, basal T levels and FAI were significantly higher in PCOSd after carefully matching them to a control group, which is clinically reflected by an increased Ferriman-Gallwey score. T levels were associated with LH levels in the generalized linear model, which is physiologically expected

because LH drives androgen production in the ovary. These findings also support recent data from Tata et al. (13) showing that the offspring of PCOS patients have higher T levels along with higher LH levels, as demonstrated in the mouse model.

The genetic background of our population (50% Spanish) might explain the seemingly high Ferriman-Gallwey scores observed in the control group. It is known that Mediterranean, Hispanic, and Middle Eastern women have higher Ferriman-Gallwey scores, so the scores that determine hirsutism in this populations should be >9–10 (36, 37). We also have to point out that in the present study, by design, we selected the control mother, not the daughter; thus, we did not exclude girls in terms of their Ferriman-Gallwey scores or PCOS features, so some of our control girls may develop PCOS in time. In the current international recommendations for the assessment and management of PCOS (38), ultrasound features should not be used before 8 years after menarche, so it is possible that in our control group we have girls at risk of PCOS that show oligoamenorrhea and hyperandrogenism. Only follow-up of these girls will clarify their final clinical status.

The third component is a higher insulin response in the oral glucose tolerance test, which may reflect metabolic disruption, which is fundamental to the severity of PCOS expression. In this regard, insulin resistance, exacerbated by obesity, plays a major role in the metabolic abnormalities of this syndrome. Nevertheless, the relationship between androgens and insulin is reciprocal and there are emerging evidences that androgens can induce insulin resistance (39). We previously observed that before the onset of puberty also, it is possible to find biochemical and clinical signs of hyperinsulinemia in daughters of PCOS women (17, 40). Although the assessment of insulin resistance is not part of the diagnosis of PCOS, it has been demonstrated that this metabolic component appears early in life and persists over time. Moreover, it is exacerbated during puberty (41). We found that the most relevant metabolic feature in prepubertal and peripubertal PCOS daughters is higher poststimulated insulin levels, which is present even before the onset of hyperandrogenism, suggesting that insulin may play an early and pivotal role in the pathogenesis of this syndrome (17, 40). In the present study we found that this feature remains during the postmenarchal period, suggesting the presence of an intrinsic insulin resistance that is maintained throughout development. This can be aggravated by the addition of external components, such as obesity, poor eating habits, and lack of exercise, adding an extrinsic component to this preexisting intrinsic insulin resistance (42). In the present study we carefully matched our subjects according to BMI. Therefore, the higher 2-hour insulin levels in the PCOSd group are most probably due to intrinsic insulin resistance not yet amplified by a higher BMI.

Although not significantly different in the propensity score analysis, AMH and ovarian volumes were higher in the PCOSd group (Table 1), as shown previously at earlier stages (17, 21). Very importantly, AMH levels seem to be driving LH levels up, as recently shown in the mouse model (20). Our data support the presence of this feedback in humans and, moreover, in the offspring of PCOS patients during the postmenarchal period, which is in line with the recent model proposed by Tata et al. (13), where the offspring of mice treated with AMH showed higher LH levels. Nevertheless, as stated before, this is still theoretical because other factors may also be involved, apart from a disrupted intrauterine environment. On the other hand, in the propensity score analysis, AMH levels were similar between the two groups; this could be due to the known normal increase in ovarian size during adolescence, which makes the normal ovary morphologically similar to a polycystic ovary (43) and thus making AMH levels similar between the two groups when we use a more strict method of analysis, such as propensity score.

In summary, we have found three main pathophysiologic components (higher LH levels, higher androgen levels, and higher insulin levels) present in PCOSd during the postmenarchal period that may establish the basis for the development of PCOS during adulthood. Higher LH levels were associated with AMH support in the human model, in accord with the proposed hypothesis of increased GnRH-dependent LH pulsatility driven by AMH, as shown in rodents (20), and the possible in utero programming of these features in the offspring of PCOS patients (13, 15). Further studies are needed to confirm this hypothesis.

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