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Polycystic ovarian morphology in adolescents with regular menstrual cycles is associated with elevated anti-Müllerian hormone

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BACKGROUND: The significance of polycystic ovarian morphology (PCOM) during adolescence is not clear. The aim of this study was to determine the relationship between PCOM and anti-Müllerian hormone (AMH), inhibin B, testosterone and insulin levels in healthy girls during the second decade of life. We also determined whether AMH could be used as a surrogate marker of PCOM during adolescence.

METHODS: Seventy-four non-obese adolescents (age range: 13.5−19.75 years old) with regular menstrual cycles participated in this study. Transabdominal ultrasound and blood samples were obtained during the follicular phase.

RESULTS: PCOM was present in 33.8% of the subjects. Girls with PCOM had higher AMH levels than girls without PCOM (72.5 ± 6.1 versus 33.4 ± 2.6 pmol/l; $P < 0.0001$) and lower FSH levels (5.4 ± 0.3 versus 6.2 ± 0.2 mUI/ml; $P < 0.036$). Similar levels of inhibin B, androgens and LH were observed in girls with and without PCOM. PCOM prevalence and AMH levels were not associated with age ($P = 0.745$ and 0.2, respectively) or BMI-SDS ($P = 0.951$ and 0.096, respectively). AMH levels positively correlated with the of 2–5 mm follicle number. AMH levels ≥60.15 pmol/l had a sensitivity and specificity of 64.0 and 89.8%, respectively, to diagnose PCOM (area under the curve = 0.873).

CONCLUSIONS: These data confirm that PCOM in healthy non-hyperandrogenic girls with regular menstrual cycles is prevalent and is not associated with hyperandrogenism. The elevated AMH and lower FSH levels observed in healthy girls with regular menses and PCOM suggest that this ovarian pattern is secondary to a larger number of 2–5 mm follicles. An elevated AMH level is suggestive of the presence of PCOM during adolescence.

Key words: polycystic ovarian morphology / hyperandrogenism / polycystic ovarian syndrome diagnosis / adolescence / anti-Müllerian hormone

Introduction

Polycystic ovarian morphology (PCOM) is one of the diagnostic criteria of polycystic ovarian syndrome (PCOS) in adult women according to the Rotterdam and the Androgen and PCOS Society criteria (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004; Azziz *et al.*, 2006, 2009). The significance of this ultrasonographic finding during adolescence is controversial, and the International Consensus published by Balen *et al.* (2003) suggested that this diagnostic element should not be employed during adolescence. Even though several recent publications have included PCOM as a diagnostic criterion for PCOS in adolescents, the presence of this finding has not been validated in this population (Hickey *et al.*, 2009; Nur *et al.*, 2009; Hart *et al.*, 2010a). This lack of consensus regarding the significance of PCOM during this stage of life is explained by the frequent presence of multifollicular ovaries (Adams *et al.*, 1985) that may be confused with polycystic ovaries, and concerns about the diagnostic accuracy of transabdominal ultrasonography (TA-US), the test usually used in adolescents (Balen *et al.*, 2003; Carmina *et al.*, 2010).

Previously, we studied the prevalence of PCOM in a group of 20 normal or overweight girls followed from 2 to 4 years past menarche

and observed that PCOM was observed in one-third of the girls and was not associated with ovulatory dysfunction (Codner et al., 2011). A similar prevalence was reported by Hart et al. (2010a) in girls who were 2 years past menarche. Studies evaluating the prevalence of PCOM in hyperandrogenic or PCOS girls have also shown that one-third of these girls exhibit PCOM (Ibanez et al., 2008; Fruzzetti et al., 2009). However, there is little information available regarding PCOM prevalence in a larger group of girls, during the second decade of life, or the relationship of the ultrasonographic pattern with anti-Müllerian hormone (AMH), inhibin B, androgen and insulin levels.

AMH is a glycoprotein secreted by the granulosa cells of small, growing follicles, and AMH serum levels correlate with the number of small antral follicles (2–5 mm) observed by transvaginal ultrasound in adult women (Pigny et al., 2003, 2006). Whether AMH levels correlate with the number of 2–5 mm follicles observed during adolescence is unknown. AMH levels are slightly elevated in girls with oligomenorrhoea, whereas they have normal or elevated androgen levels (Park et al., 2010a).

Inhibin B is a glycoprotein secreted by larger antral follicles (8–10 mm) (Andersen et al., 2010), and some publications have reported that inhibin B is elevated in adult women with PCOS (Chu et al., 2005; Bayrak et al., 2007). Recently, a positive correlation between inhibin B and antral follicle number (FN) has been described in adolescents (Hart et al., 2010b), but there is no information about the association of this hormone with PCOM during the second decade of life, as well as its use as a diagnostic tool for this condition.

In the current study, we evaluated healthy girls between 1 year after menarche and 19 years old. We postulated that similar to the situation in adults, AMH levels are increased in girls with PCOM, and a relationship between AMH levels and the number of small antral follicles exists, even when measured by TA-US. We also determined whether AMH can be used as a surrogate marker of PCOM during adolescence. In addition, we studied the prevalence of PCOM and its relationship with age, AMH, inhibin B, androgens, insulin and gonadotrophin levels during the second decade of life, performed a cross-sectional study in healthy adolescents who were between 1 year past menarche and 19 years old and had regular menstrual cycles.

Materials and Methods

Subjects

We studied 74 healthy post-menarchal girls between 1 year past menarche and 19 years old (mean 16.3 years old, range: 13.5–19.75 years old) in a cross-sectional study. The subjects were recruited from schools in downtown Santiago, which is a middle-class area. Fifteen girls who had participated in our previous prospective research were included in the present study (Codner et al., 2011); five subjects were excluded due to obesity. Each girl participated in the study only once. The inclusion criteria were as follows: the absence of significant clinical signs of hyperandrogenism, such as moderate-to-severe acne or hirsutism [Ferriman–Gallwey (FG) score $\geq$7], and the presence of regular menstrual cycles, defined as a menstrual cycle length between 21 and 45 days, according to the recent consensus for adolescents developed by the American College of Obstetrics and Gynecology (ACOG) and the American Association of Pediatrics (AAP) (American Academy of Pediatrics et al., 2006). The exclusion criteria included the following: use of oral contraceptives, steroids or any other type of medication; the presence of other concomitant chronic conditions, such as genetic syndromes, coeliac disease, renal disease, liver disease, cardiac disease or undernourishment; premature pubarche (defined as the appearance of pubic hair in girls younger than 8 years of age); intrauterine growth retardation (neonatal birth/length $<$2 SDs for gestational age, according to Chilean standards); and obesity (BMI greater than the 95th percentile). All subjects had normal fasting blood glucose and thyroid hormone levels.

A total of 102 girls from three schools located near our hospital were invited, and 88 girls agreed to participate in this study. In addition, 14 girls were excluded from the study for the following reasons: hirsutism ($n = 8$), hormonal contraception ($n = 2$), obesity ($n = 2$) and cycles longer than 45 days ($n = 2$).

The protocol was approved by the Institutional Review Board of the San Borja Arriarán Hospital. Parents provided informed consent, and volunteers gave their written assent before entering the study. Adolescent girls older than 18 years old signed the consent form.

Study protocol

Girls were evaluated during the follicular phase (Days 1–7 of the cycle). A complete clinical and physical exam was performed. The age at menarche was obtained retrospectively. Cycle length was obtained from the mean length of the last three menstrual cycles. Gynaecological age was defined as the number of years past menarche at the moment of enrolment in the study. The BMI, FG score and waist-to-hip ratio (WHR) were determined. Standard deviation scores (BMI-SDS) were calculated for BMI, using the current NCHS standard curves; these growth curves have been shown to be applicable to contemporary Chilean populations (Youlton and Valenzuela, 1990). Overweight was defined as a BMI between the 85th and 94th percentiles.

An early-morning blood sample was obtained during the follicular phase (Days 1–7 of the cycle) for the measurement of LH, FSH, estradiol, 17-hydroxyprogesterone (17-OHP), dehydroepiandrosterone sulphate (DHEAS), androstenedione, total testosterone, sex hormone-binding globulin (SHBG), AMH and inhibin B. To measure insulin sensitivity, homeostatic model assessment of insulin resistance (HOMA-IR) values were calculated based on the fasting insulin and glucose levels of each subject (Pacini and Mari, 2003).

Ultrasonographic study

PCOM was identified according to the Rotterdam consensus as the presence of either 12 or more follicles measuring 2–9 mm in diameter and/or an ovarian volume (OV) $>$10 ml in one or both ovaries (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004).

TA-US was performed by a single observer (C.V.) on the same day that the blood sample was obtained. The exam was performed with a 5-MHz transabdominal probe using a Medison SonoAce 6000C (Medison, Seoul, Korea). Measurements were performed in real time, using the highest possible magnification to view the ovaries. The longest medial axis (length) and its corresponding thickness and width were measured to calculate OV. OV was estimated according to the formula for a prolate ellipsoid: OV = $\pi/6 \times$ length $\times$ width $\times$ thickness (Porter, 2008). The FN was established by scanning each ovary from the inner to the outer margins in a longitudinal cross-section. Follicle diameter was obtained from the mean of the maximum and its corresponding perpendicular diameter. All follicles between 2.0 and 9.0 mm were counted. Then, the numbers of follicles measuring between 2–5 mm and 6–9 mm were determined. The intra-observer variation coefficients of the ultrasonographic study were 3.2 and 4.1% for OV and FN, respectively (Codner et al., 2011). In cases where a dominant cyst/

follicle larger than 10 mm was observed, the ultrasonographic exam was repeated in the following menstrual cycle. The ovary with the larger OV and the number of follicles of the ovary with the larger FN was reported.

Laboratory assays

Serum testosterone (sensitivity [S] = 0.0035 nmol/l), androstenedione ([S] = 0.07 nmol/l), 17-OHP ([S] = 0.03 nmol/l), DHEAS ([S] = 0.07 nmol/l) and estradiol ([S] = 18.4 pmol/l) were measured by competitive specific binding radioimmunoassays (Diagnostic Systems Laboratories, Webster, TX, USA), inter-assay coefficients of variation (CVs) were 8.1, 8.9, 7.3, 7.7 and 6.1%, respectively, and intra-assay CVs were 5.3, 4.2, 7.7, 5.3 and 4.1%, respectively.

Serum LH ([S] = 0.10 mIU/ml), FSH ([S] = 0.10 mIU/ml) and SHBG ([S] = 0.5 nmol/l) levels were measured by immunoradiometric assays from Siemens Healthcare Diagnostics (USA). Intra-assay CVs were 6.5% for LH, 3.6% for FSH and 3.9% for SHBG. Inter-assay CVs were 7.6% for LH, 6.2% for FSH and 6.9% for SHBG (Codner *et al.*, 2005). The free androgen index (FAI) was calculated by the formula [100 × testosterone (nmol/l)]/[SHBG (nmol/l)] (Villarroel *et al.*, 2010). The LH/FSH ratio was calculated. Serum AMH was assayed using the AMH/MIS ELISA kit (Immunotech-Beckman, Marseilles, France) as described previously (Codner *et al.*, 2007). The AMH assay had a sensitivity of 0.7 pmol/l and intra- and inter-assay CVs of 5.3 and 8.7%, respectively. Serum inhibin B was measured using a specific two-site ELISA assay (Diagnostic Systems Laboratories). The assay sensitivity was 7 pg/ml, and the intra- and inter-assay CVs were 4.8 and 7.1%, respectively (Rey *et al.*, 2005; Soto *et al.*, 2009).

Statistical analysis

Normal distributions were evaluated using the Kolmogorov–Smirnov test. 17-OHP did not pass the normality test and was transformed to its logarithm; after being logarithmically transformed 17-OHP levels showed a normal distribution. The other hormones measured passed the normality test.

Continuous variables observed in girls who did or did not fulfil PCOM criteria were compared using the Student's *t*-test. Logistic regression analysis was performed to determine the effects of chronological age, age at menarche, gynaecological age and BMI-SDS on the presence of PCOM. Pearson's correlation analysis was performed to evaluate the relationships between continuous variables.

Receiver-operating characteristic (ROC) analysis was employed to determine the diagnostic accuracy of AMH, inhibin B, testosterone, FSH serum levels and FAI for the diagnosis of PCOM. A significance level of 5% was employed. All statistical calculations were performed using SPSS for Windows, version 18.0 and GraphPad Prism version 5.0. The data are shown as arithmetic means ± SEM, except for the FG score and the data presented in Fig. 1, which are shown as the median, 5th and 95th percentile.

Results

The clinical characteristics of all participants according to the presence of PCOM are shown in Table I. PCOM was observed in 33.8% of the girls. An OV > 10 ml in at least one ovary was observed in 19 girls (25.7%). The presence of 12 follicles or more between 2 and 9 mm was observed in 13 girls (17.6%). Girls with and without PCOM

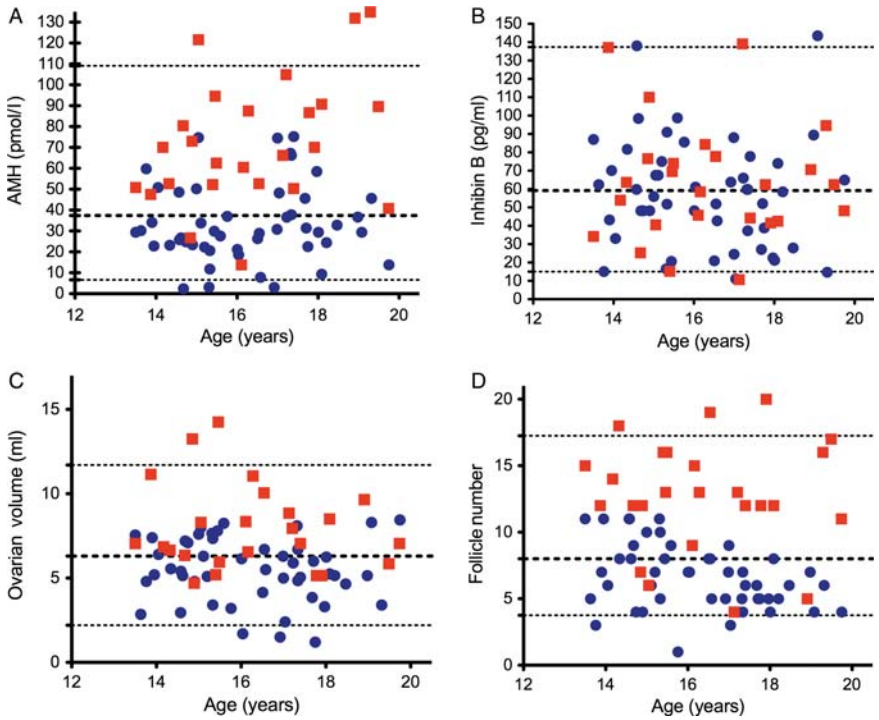

Figure 1 Serum AMH levels, inhibin B levels and ultrasonographic findings according to age and the presence of PCOM in 74 healthy girls during the second decade of life. Black dotted lines represent the median, 5th percentile and 95th percentile. Red squares represent girls with PCOM. Blue circles represent girls without PCOM.

Table I Clinical characteristics according to ovarian morphology.

	PCOM (−)	PCOM (+)
n (%)	49 (66.2)	25 (33.8)
Age (years)	16.2 ± 0.2	16.4 ± 0.4
Age of menarche (years)	12.2 ± 0.1	12.0 ± 0.2
Gynecological age (years)	3.6 ± 0.3	4.0 ± 0.5
BMI (kg/m^2)	22.7 ± 0.4	22.5 ± 0.5
BMI-SDS	0.5 ± 0.1	0.5 ± 0.1
Menstrual cycle (days)	30.0 ± 0.4	31.4 ± 0.8
Overweight (%)	15 (30.6)	4 (16.0)
Height (cm)	158.6 ± 0.8	160.3 ± 1.0
Waist-to-hip ratio	0.8 ± 0.01	0.8 ± 0.01
Ferriman–Gallwey	2.0 (0.0–6.0)	3.0 (0.0–7.7)

Data are shown as means ± SEM. FG scores are shown as medians (5th to 95th percentile). BMI, body mass index; BMI-SDS, body mass index-standard deviation score.

had a similar chronological age ($P = 0.723$), age at menarche ($P = 0.623$), gynaecological age ($P = 0.391$), BMI-SDS ($P = 0.938$), length of the menstrual cycle ($P = 0.098$), overweight prevalence ($P = 0.174$), WHR ($P = 0.344$) and FG scores ($P = 0.671$). Logistic binary regression showed that gynaecological age ($P = 0.397$), chronological age ($P = 0.745$), age at menarche ($P = 0.617$), length of menstrual cycle ($P = 0.095$) and BMI-SDS ($P = 0.951$) were not associated with PCOM.

The hormonal profiles and ultrasonographic characteristics of girls without PCOM and with PCOM are shown in Table II. Higher AMH levels and lower FSH levels were observed in girls with PCOM ($P < 0.0001$ and 0.036, respectively). AMH levels were higher in girls who fulfilled the OV criteria of PCOM (>10 ml) compared with those with OV < 10 ml (76.2 ± 11.1 and 40.3 ± 2.9, respectively; $P = 0.007$), and the same results were observed in girls who met the FN criteria of PCOM compared with those who did not meet the FN criteria (74.3 ± 5.3 and 37.0 ± 3.2, respectively; $P < 0.0001$). Inhibin B levels ($P = 0.432$), testosterone ($P = 0.674$), androstenedione ($P = 0.696$), LH ($P = 0.651$), the LH/FSH ratio ($P = 0.159$), SHBG ($P = 0.939$), insulin levels ($P = 0.163$), HOMA-IR ($P = 0.127$) and FAI ($P = 0.440$) were similar in girls with and without PCOM and in girls who fulfilled the criteria of PCOM defined by either OV or FN. As expected, the presence of PCOM was associated with a larger OV and FN than in the absence of PCOM. A larger number of follicles with a 2–5-mm diameter ($P < 0.0001$) were observed in girls with PCOM compared with those without PCOM, but there was no difference in the number of follicles with a 6–9-mm diameter ($P = 0.295$).

Figure 1 shows individual AMH and inhibin B levels and ultrasonographic findings according to age and the presence of PCOM. AMH (median: 37.4 pmol/l; p5–p95: 6.6–109.1 pmol/l, Fig. 1A) and inhibin B levels (median: 59.2 pg/ml; p5–p95: 15.0–137.3 pmol/l; Fig. 1B) did not correlate with age or BMI-SDS (Table III). Similarly, no correlation was observed between OV (median: 6.9 ml; p5–p95: 2.3–15.1 ml; Fig. 1C) and FN (median: 8.0; p5–p95: 3.7–17.3;

Table II Hormonal profiles and ultrasonographic characteristics according to ovarian morphology.

	PCOM (−)	PCOM (+)
n (%)	49	25
AMH (pmol/l)	33.4 ± 2.6**	72.5 ± 6.1**
Inhibin B (pg/ml)	57.4 ± 4.2	63.3 ± 6.5
LH (mIU/ml)	3.9 ± 0.3	4.2 ± 0.5
FSH (mIU/ml)	6.2 ± 0.2*	5.4 ± 0.3*
LH/FSH	0.6 ± 0.1	0.9 ± 0.1
Estradiol (pmol/l)	169.2 ± 12.5	167.3 ± 13.6
Testosterone (nmol/l)	1.6 ± 0.1	1.6 ± 0.1
SHBG (nmol/l)	53.3 ± 3.3	52.9 ± 3.4
Free androgen index (%)	3.8 ± 0.4	3.4 ± 0.3
DHEAS (nmol/l)	4485 ± 254	4482 ± 362
Androstenedione (nmol/l)	5.6 ± 0.3	5.9 ± 0.3
17OH-Progesterone (nmol/l)	3.3 ± 0.3	4.5 ± 0.9
Glucose (mmol/l)	4.5 ± 0.1	4.3 ± 0.1
Insulin (mIU/ml)	6.5 ± 0.4	5.7 ± 0.5
HOMA-IR	1.3 ± 0.1	1.1 ± 0.1
Ovarian volume (ml)	6.3 ± 0.3**	9.9 ± 0.7**
Follicle number (*n*)	6.6 ± 0.4**	12.8 ± 0.8**
FN, follicle 2–5 mm (*n*)	5.2 ± 0.4**	11.2 ± 0.9**
FN, follicle 6–9 mm (*n*)	1.9 ± 0.3	2.4 ± 0.5

Results are reported as means ± SEM. The analysis was performed using Student's *t*-test P. Conversion to metric units: testosterone, nmol/l × 0.2882 = ng/ml; androstenedione, nmol/l × 0.2865 = ng/ml; DHEAS, nmol/l × 370.37 = ng/ml; estradiol, pmol/l × 0.2725 = pg/ml; 17OH progesterone, nmol/l × 0.3300 = ng/ml; SHBG, nmol/l/ 34.67 = μg/dl; AMH, pmol/l × 0.1400 = μg/l; glucosem mmol/l/0.0555 = mg/dl.
*PCOM$^+$ versus PCOM$^-$: $P < 0.05$.
**PCOM$^+$ versus PCOM$^-$: $P < 0.0001$.

Table III Relationships between AMH, inhibin B, FSH levels and insulin, androgens and ovarian parameters.

		AMH	Inhibin B	FSH
Age	r	0.195	−0.068	−0.131
BMI-SDS	r	−0.151	0.119	−0.100
Ovarian volume	r	0.396**	0.339**	−0.281*
Follicle number	r	0.376**	0.183	−0.141
2–5 mm follicles number	r	0.299**	0.205	−0.170
6–9 mm follicle number	r	0.132	0.004	−0.079
FSH	r	−0.193	0.164	–
Testosterone	r	0.124	0.150	−0.179
FAI	r	0.081	0.074	−0.145
Insulin	r	−0.311**	0.040	−0.035
HOMA-IR	r	−0.306**	0.095	−0.041

The results are shown as the Pearson *r* correlation.
*$P < 0.05$.
**$P < 0.01$.

Table IV Comparison of ROC plot analysis of different ovarian criteria for PCOM.

Hormone	Area under the curve	Cut-off value (pmol/l)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*P*-value
AMH levels	0.873	50.25	84.0	83.7	83.7	84.0	<0.0001
		60.15	64.0	89.8	86.3	71.4	<0.0001
		70.10	48	99.9	99.8	65.8	<0.0001
Inhibin B	0.542	61.75	52	59.2	56.0	55.2	0.560
Free androgen index	0.517	3.15	52	55.2	53.7	53.5	0.815
Testosterone	0.506	0.5	44	61.2	53.1	52.2	0.936

PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

Fig. 1D) with age ($r = -0.113$, $P = 0.341$ and $r = 0.007$, $P = 0.956$, respectively).

The correlations between AMH, inhibin B and FSH levels with the clinical characteristics, ultrasonographic findings and measured hormone levels are shown in Table III. AMH levels exhibited positive correlations with OV, FN and the size of the 2–5-mm follicle pool, but not with the size of the 6–9-mm follicle pool. A negative correlation between AMH with insulin levels and the HOMA-IR index was observed. No correlation was observed between AMH and FSH with androgen levels. Inhibin B and FSH levels showed a positive and negative correlation, respectively, with OV, but showed no correlation with FN. Testosterone and FAI did not exhibit a significant correlation with inhibin B, FSH, OV and FN.

The ROC analysis is shown in Table IV. The area under the curve of the ROC for AMH reached a value of 0.873 [0.782–0.963, 95% confidence interval (CI)]. Several threshold values of serum AMH levels were analysed in terms of specificity and sensitivity from the ROC curve data. The best compromise between specificity (89.2%) and sensitivity (64%) was obtained with a cut-off value of 60.15 pmol/l with a good positive predictive value (86.3%) and negative predictive value (71.4%). The diagnostic accuracy of AMH levels for PCOM defined by OV > 10 ml [area under the curve (AUC) = 0.767, 95% CI = 0.607–0.927] was lower than PCOM defined by FN > 12 (AUC = 0.894 95% CI = 0.824–0.965). ROC analyses showed that testosterone, FAI, FSH and inhibin B had no significant diagnostic utility for detecting PCOM, as shown by a small area under the ROC curve.

Discussion

We report a study of ovarian function in 74 non-obese adolescent girls with regular menstrual cycles and confirmed that PCOM is a prevalent condition in these girls that is not associated with hyperandrogenism or insulin resistance; therefore, this ultrasonographic pattern may correspond to a physiological finding in these girls. In addition, we showed that PCOM is associated with elevated AMH and lower FSH levels, which suggest that this ovarian pattern is secondary to a higher number of 2–5 mm follicles present in these adolescents. Similar elevations in AMH levels were observed in girls who fulfilled PCOM criteria by OV or FN.

We observed that 33.8% of the adolescents in our study exhibited PCOM defined by the Rotterdam criteria, similar to the 35.4% prevalence of PCOM recently reported in healthy girls who were 2 years post-menarche (Hickey *et al.*, 2009; Hart *et al.*, 2010a) and the 38 and 37% observed in adolescents with hyperandrogenism or PCOS, respectively (Ibanez *et al.*, 2008; Fruzzetti *et al.*, 2009). In contrast, adult women with hyperandrogenism or PCOS exhibit a large difference in the prevalence of PCOM compared with healthy women (80–95 versus 15–25%, respectively) (Polson *et al.*, 1988; Azziz *et al.*, 2006; Codner *et al.*, 2006; Barber *et al.*, 2009; March *et al.*, 2010), suggesting a stronger association of PCOM with hyperandrogenism/PCOS in adult women than in adolescents.

It has been reported that the prevalence of PCOM varies with age (Murphy *et al.*, 2006; Duijkers and Klipping, 2010; Johnstone *et al.*, 2010) and is associated with a physiological decline in FN and OV (Alsamarai *et al.*, 2009). Recent research has shown that PCOM is more prevalent in adult women younger than 30 years old than in older women (Murphy *et al.*, 2006; Duijkers and Klipping, 2010; Johnstone *et al.*, 2010). We did not find an association between OV, follicle count or PCOM prevalence and age, suggesting that the prevalence of PCOM does not change during the second decade of life in girls.

Another important finding was that AMH levels were relatively constant during the second decade of life. These findings are similar to the ones reported by Hagen *et al.* (2010), which recently reported a wide range of normal levels of AMH in healthy girls during adolescence. It is possible that a third of these girls with elevated AMH levels described by Hagen *et al.* (2010) could have PCOM, which would explain the wide range of normal values found in this study.

The negative association between insulin, HOMA-IR and AMH levels has been previously reported in healthy adult women (Chen *et al.*, 2008; Park *et al.*, 2010b). It has been postulated that in women without PCOS, insulin resistance might exert its negative effect on the inhibitory action of AMH on follicular development (Durlinger *et al.*, 2001, 2002a,b; Jonard and Dewailly, 2004) and, therefore, increase the sensitivity of granulosa cells to FSH.

The lower levels of FSH observed in girls with PCOM, in addition to the negative correlation between FSH and OV, suggest that PCOM is not mediated by gonadotrophin action, but rather that this diminished FSH level is associated with an increased follicular mass (Durlinger *et al.*, 2002a). The levels of FSH are regulated by inhibin B serum levels, which depend on follicular mass (Knight and Glister, 2001; Rosencrantz *et al.*, 2010). We did not find an association between these two hormones that may be explained by the fact that the serum sample was obtained in early follicular phase, a moment of the menstrual cycle that is not the appropriate for the measurement of inhibin B levels (Andersen *et al.*, 2010) or by

the high variability that this hormone shows during the follicular phase (Roberts, 2010).

Previous studies in adolescent girls and adult women with polycystic ovaries and menstrual irregularities have shown higher androgen, insulin and LH levels and lower SHBG levels compared with individuals without polycystic ovaries (Norman *et al*., 1995; Venturoli *et al*., 1995; Carmina *et al*., 1997; van Hooff *et al*., 2000; Adams *et al*., 2004; Johnstone *et al*., 2010). In contrast, our data show that the presence of PCOM in a girl with normal menstrual cycles, defined by a length of 21–45 days (American Academy of Pediatrics *et al*., 2006), is not associated with hyperandrogenism or elevated insulin levels.

In the current study, we determined through ROC analysis that an AMH cut-off level of 60.15 pmol/l is the best diagnostic accuracy for detecting PCOM, with a sensitivity and specificity for the diagnosis of PCOM of 64.0 and 89.8%, respectively. This cut-off level and sensitivity are similar to the results described by Pigny *et al*. of 60 pmol/l, using the same assay, in adult women with PCOS (Pigny *et al*., 2006). In addition, the diagnostic accuracy of AMH levels for the diagnosis of PCOM was somewhat lower when the diagnosis was based on the OV criteria compared with the FN criteria. However, we demonstrated a better diagnostic accuracy of AMH for the detection of PCOM in adolescents compared with the accuracy described by Hart *et al*. (2010a). The differences in these results may be explained by the fact that in the latter study, the authors evaluated girls with menstrual irregularities, a confounding factor that also leads to elevated AMH levels and, hence, may have decreased the discriminating value of the AMH assay (Park *et al*., 2010a). The AMH levels found in asymptomatic girls with PCOM are similar to the levels we have recently shown in hyperandrogenic adult women with PCOS using the same assay (Codner *et al*., 2007). However, the AMH levels we found in healthy adolescents with PCOM in our study are higher than in girls with oligomenorrhea, as previously reported by Park *et al*. (2010a) (72.5 ± 6.1 versus 37.8 pmol/l, respectively). This finding suggests that though AMH levels may be mildly elevated in girls with oligomenorrhea, the degree of this elevation is not comparable to girls with PCOM.

Previously, we prospectively followed 20 girls from 2 years past menarche and performed annual ultrasonographic studies and assessment of ovulation. We observed a normal ovulatory rate in girls with PCOM and concluded that '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 physiological condition during early adolescence' (Codner *et al*., 2011). In agreement with our previous publication, the current research, in which a larger number of girls were studied, did not find hyperandrogenism or insulin resistance in girls with PCOM. Because PCOM was an inconstant finding throughout the study period of our previous paper, it is possible that elevated AMH levels may also vary with time. A limitation of the current study is the lack of long-term follow-up study of the girls with PCOM and elevated AMH levels.

In summary, we show that PCOM in healthy adolescents is associated with elevated AMH and lower FSH levels, without biochemical hyperandrogenism. These data suggest that PCOM in a normal weight adolescent with regular menstrual cycles is a physiological ovarian pattern secondary to a larger number of 2–5 mm follicles. In addition, the prevalence of PCOM observed in our study is similar to previous studies performed in hyperandrogenic adolescents, suggesting that this ultrasonographic finding is not clearly associated with this disorder. These data confirm that PCOM in girls with normal menstrual cycles is a physiological condition that may be linked to a larger follicle mass. An elevated AMH level is suggestive of the presence of PCOM, and this hormone could be used as a surrogate marker of PCOM in adolescents with regular menstrual cycles. Future studies should evaluate the long-term outcome of high AMH levels and PCOM in healthy girls.

Authors' roles

C.V. contributed with the study design, execution, analysis, manuscript drafting and its critical discussion. P.M.M. participated in the execution, manuscript drafting and critical discussion. P.L., F.C.E. and A.V. participated in the execution of the study protocol. G.I. contributed with the execution and manuscript drafting. E.C. contributed to the conception, design and analysis and interpretation of data, manuscript drafting and critical discussion.

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