

Increased Anti-Müllerian Hormone Serum Concentrations in Prepubertal Daughters of Women with Polycystic Ovary Syndrome

Teresa Sir-Petermann, Ethel Codner, Manuel Maliqueo, Bárbara Echiburú, Catalina Hitschfeld, Nicolás Crisosto, Francisco Pérez-Bravo, Sergio E. Recabarren, and Fernando Cassorla

Endocrinology and Metabolism Laboratory (T.S.-P., M.M., B.E., C.H., N.C.), West Division, School of Medicine; Institute of Maternal and Child Research (E.C., F.C.), School of Medicine; and Institute of Nutrition and Food Technology (F.P.-B.), University of Chile, Casilla 052 Correo 33, Santiago, Chile; and Laboratory of Animal Physiology and Endocrinology (S.E.R.), School of Veterinary Medicine, University of Concepción, 380-1061 Chillán, Chile

Context: Anti-Müllerian hormone (AMH) is produced by the granulosa cells and reflects follicular development. Adult women with polycystic ovary syndrome (PCOS) have increased levels of AMH associated with an excessive number of growing follicles. However, it is not known whether these abnormalities are present before the clinical onset of PCOS.

Objective: Our objective was to investigate whether prepubertal daughters of women with PCOS have increased AMH levels.

Design: Fourteen female infants (2–3 months old) and 25 prepubertal girls (4–7 yr old) born to PCOS mothers were studied. As a control group, we studied 21 female infants and 24 prepubertal girls born to mothers with regular menses and without hyperandrogenism. The group with PCOS mothers and the control group had normal birth weight and were born from spontaneous singleton pregnancies. Circulating concentrations of gonadotropins, testosterone, androstenedi-

one, estradiol, 17-OH-progesterone, SHBG, inhibin B, and AMH were determined by specific assays.

Results: Serum concentrations of AMH were significantly higher in the PCOS group compared with the control group during early infancy (20.4 ± 15.6 vs. 9.16 ± 8.6 pmol/liter; $P = 0.024$) and during childhood (14.8 ± 7.7 vs. 9.61 ± 4.4 pmol/liter; $P = 0.007$). Gonadotropin and serum sex steroid concentrations were similar in both groups during the two study periods, except for FSH, which was lower during childhood in girls born to PCOS mothers.

Conclusions: We conclude that serum AMH concentrations are increased in prepubertal daughters of PCOS women, suggesting that these girls appear to show evidence of an altered follicular development during infancy and childhood.

POLYCYSTIC OVARY SYNDROME (PCOS) is a highly prevalent (5–10%) endocrine-metabolic dysfunction in premenopausal women and is the most frequent cause of anovulatory infertility and hyperandrogenism in women. Ovarian morphology, assessed by ultrasound and by histological examination, has shown that polycystic ovaries are characterized by an excessive number of growing follicles compared with normal ovaries (1–3), suggesting an altered folliculogenesis in this syndrome.

Anti-Müllerian hormone (AMH), a dimeric glycoprotein member of the TGF β superfamily (4), may constitute a marker of follicular development. AMH is produced exclusively in the gonads (5), and its dimorphic expression in ovary and testis is crucial for the normal differentiation of reproductive structures (6, 7). During early fetal development, Sertoli cell expression of AMH results in Müllerian duct regression in the male (5). In contrast, in the female fetus, granulosa cell expression of AMH occurs during late gestation, after the Müllerian ducts have lost

sensitivity to AMH (8). Serum AMH levels seem to be correlated with the development of preantral and small antral follicles, from puberty to the end of reproductive life (9). During this period, AMH expression in the human ovary is similar to that observed in the mouse and rat (10), suggesting an important role for AMH in the regulation of early follicle growth.

It has been established that AMH levels are 2- to 3-fold higher during reproductive life in women with PCOS compared with healthy women (11, 12). Increased serum AMH concentrations in PCOS women are related to the increased number of growing follicles that secrete AMH (13). However, it is not known whether AMH increases before the clinical onset of PCOS, as defined by the Rotterdam ESHRE/ASRM-sponsored PCOS consensus (14). Phenotypic and family aggregation studies in different populations have demonstrated that hyperandrogenic symptoms and polycystic ovaries assessed by ultrasound are more frequent in first-degree relatives of PCOS patients compared with controls (15–19). Therefore, we investigated whether daughters of women with PCOS have increased AMH levels during early infancy or during childhood.

Subjects and Methods

Subjects

As the PCOS group (PCOSG), we studied 14 female infants (2–3 months old) and 25 prepubertal girls (4–7 yr old) born to PCOS mothers. As a control group, we studied 21 female infants and 24 prepubertal girls

Abbreviations: AMH, Anti-Müllerian hormone; BMI, body mass index; CG, girls born to control mothers; 17-OHP, 17-OH-progesterone; PCOS, polycystic ovary syndrome; PCOSG, girls born to PCOS mothers; SDS, SD score.

(CG) born to mothers with regular menses and without hyperandrogenism. PCOSG and CG had normal birth weight and were born from spontaneous singleton pregnancies. Both groups of girls were matched for age and body mass index (BMI) at the beginning of the study.

PCOS mothers were recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile. Diagnosis of PCOS was made according to the National Institutes of Health consensus criteria (20). PCOS mothers were evaluated before pregnancy, and all of them exhibited chronic oligomenorrhea or amenorrhea, hirsutism, serum testosterone concentration more than 0.6 ng/ml and/or free androgen index more than 5.0, and androstenedione concentration more than 3.0 ng/ml. In addition, PCOS women showed the characteristic ovarian morphology of PCOS on ultrasound, based on the criteria described by Adams *et al.* (21). PCOS mothers were normoglycemic, with different degrees of hyperinsulinemia that were evaluated by an oral glucose tolerance test. All patients had an elevated waist-to-hip ratio greater than 0.85. We excluded patients with hyperprolactinemia, androgen-secreting neoplasm, Cushing's syndrome, and late-onset 21-hydroxylase deficiency as well as thyroid disease.

PCOS patients included in this study were selected from a group of 39 PCOS patients who were seeking treatment for infertility and were placed on a 6-month diet and an exercise program consisting of a low-fat diet and a daily 30-min walk. During this program, 12 patients ovulated and became pregnant. Another 25 patients became pregnant with the addition of metformin at a dose of 1500 mg, which was immediately discontinued when they became pregnant. Only two patients became pregnant after pharmacological induction of ovulation with clomiphene citrate, 100 mg/d from cycle d 5–9.

As control mothers, we selected 45 women of similar age and socioeconomic level with singleton pregnancies. Control mothers had a history of regular 28- to 32-d menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism, and no history of infertility or pregnancy complications.

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 before entering the study.

Study protocol

All girls were admitted with their mothers to the pediatric unit of the Clinical Research Center at approximately 0830 h. We performed a complete physical examination on each girl, including anthropometric measurements. Girls were excluded if we observed signs of a genetic disorder or pubertal development. In the female infants of both groups, weight, length, and head circumference were recorded at birth. Weight and length were transformed into SD scores (SDS) using Chilean normative data for newborns (22), adjusting for differences in gestational age and gender. SDS were calculated using the formula $SDS = (x - \text{mean}) / \text{SD}$ percentile. For the prepubertal girls (4–7 yr) of both groups, anthropometric birth data were obtained retrospectively from the clinical history.

A fasting sample (3 ml) was obtained by venipuncture of an antecubital vein. Circulating concentrations of gonadotropins, testosterone, androstenedione, estradiol, 17-OH-progesterone (17-OHP), SHBG, inhibin B, and AMH were determined by specific assays.

Assays

Serum AMH was assayed by enzyme immunoassay (Immunotech-Beckman Coulter, Marseille, France) (23). Analytical sensitivity was 2.1 pmol/liter, and intra- and interassay coefficients of variation were 5.3 and 8.7%, respectively.

Serum LH, FSH, and estradiol were determined by electrochemiluminescence (Roche, Basel, Switzerland). Assay sensitivities were 0.1 IU/liter, 0.1 IU/liter, and 5.0 pg/ml, respectively. Intra- and interassay 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 (Diagnostic Systems Laboratories, Inc., Webster, TX), and androstenedione, 17-OHP, and SHBG were determined by radioimmunoassay (DPC, Los Angeles, CA). Assay sensitivities were 0.1 ng/ml, 0.1 ng/ml, 0.1 ng/ml, and 0.04 nmol/liter, respectively. Intra- and interassay coefficients of variation were 7.0 and 11.0% for testosterone, 3.7 and 4.9% for androstenedione,

3.5 and 5.0% for 17-OHP, and 3.8 and 7.9% for SHBG. Serum inhibin B was assayed by ELISA (Diagnostic Systems Laboratories) with an assay sensitivity of 7.0 pg/ml and intra- and interassay coefficients of variation of 6.3 and 4.6%, respectively.

Statistical evaluation

Data were normally distributed and were expressed as means and SD. Differences between groups were assessed by Student's *t* test. The correlation between continuous variables was assessed by Spearman regression analysis. Statistical analysis was performed using the Statistical Package for Social Science software (SPSS 10.0). A *P* value of <0.05 was considered statistically significant.

Results

As expected, PCOS mothers had increased serum testosterone and insulin concentrations and free androgen index. In addition, they had decreased levels of SHBG. Table 1 shows the clinical and endocrine characteristics of the PCOS mothers, compared with a group of 71 normal women studied by us (24).

Both groups of girls were comparable in gestational age (control, 38.9 ± 1.7 , vs. PCOS, 38.6 ± 1.2 wk; *P* = 0.327) and birth weight (control, 3370 ± 550 , vs. PCOS, 3334 ± 481 g; *P* = 0.756). Table 2 shows the clinical and biochemical characteristics of CG and PCOSG during early infancy (2–3 months of age). No difference was observed in age, weight, height, or weight SDS between both groups. In addition, no significant differences were observed in serum concentrations of sex steroids, SHBG, or inhibin B.

Table 3 shows the endocrine and biochemical characteristics of CG and PCOSG during childhood (4–7 yr of age). We did not observe any significant differences in age, weight, or height. Waist circumference and waist-to-hip ratio, as well as serum concentrations of sex steroids, inhibin B, and SHBG, were similar in both groups. However, during this period, FSH concentrations were significantly lower in the PCOSG compared with the CG.

Serum AMH concentrations in CG and PCOSG during the two study periods are shown in Fig. 1. Serum concentrations of AMH were significantly higher in the PCOSG compared with the CG during early infancy (PCOSG, 20.4 ± 15.6 , vs. CG, 9.16 ± 8.6 pmol/liter; *P* = 0.024) and during childhood (PCOSG, 14.8 ± 7.7 , vs. CG, 9.61 ± 4.4 pmol/liter; *P* = 0.007). AMH levels were not different between early infancy and childhood in the PCOSG (*P* = 0.140) or in the CG (*P* = 0.830). No correlation between AMH and FSH or estradiol in the PCOSG or CG was observed during infancy or childhood by Spearman analysis. Moreover, there was no association be-

TABLE 1. Clinical and endocrine characteristics of PCOS mothers

	PCOS mothers (n = 39)	Reference values (24) (n = 71)
Age (yr)	29.8 ± 7.0	24.4 (16.0–36.0)
BMI (kg/m ²)	27.3 ± 6.3	27.1 (19.0–41.9)
SHBG (nmol/liter)	24.4 ± 4.2	61.2 (20.5–138.6)
Testosterone (ng/ml)	0.80 ± 0.04	0.35 (0.15–0.57)
FAI	13.2 ± 1.7	1.98 (0.8–4.8)
Fasting glucose (mg/dl)	85.2 ± 2.5	78.0 (51.0–107.0)
Fasting insulin (μIU/ml)	22.3 ± 2.0	8.5 (3.0–19.7)

Values are means ± SD. FAI, Free androgen index.

TABLE 2. Clinical and biochemical characteristics of CG and PCOSG during infancy

	CG (n = 21)	PCOSG (n = 14)
Age (months)	2.4 ± 0.6	2.5 ± 0.5
Weight (kg)	5.9 ± 0.8	5.7 ± 0.8
Height (cm)	58.3 ± 2.0	58.0 ± 2.9
Weight SDS	1.2 ± 1.3	0.8 ± 1.0
LH (IU/liter)	0.3 ± 0.5	0.4 ± 0.9
FSH (IU/liter)	5.4 ± 5.0	3.6 ± 2.1
Testosterone (ng/ml)	0.3 ± 0.2	0.3 ± 0.1
Androstenedione (ng/ml)	0.7 ± 0.5	1.0 ± 0.9
17-OHP (ng/ml)	9.2 ± 2.2	9.6 ± 3.6
Estradiol (pg/ml)	10.2 ± 5.2	16.4 ± 17.4
SHBG (nmol/liter)	95.8 ± 42.3	102.0 ± 30.5
Inhibin B (pg/ml)	38.5 ± 25.8	42.1 ± 40.4

Values are means ± SD.

tween the infertility treatment of the PCOS mothers and the AMH levels in their daughters.

Discussion

The present study shows that AMH levels are increased in prepubertal daughters of women with PCOS, suggesting that the ovarian preantral follicular pool may be increased in these girls. AMH is synthesized by granulosa cells of preantral and small antral follicles (25, 26). Therefore, the increased serum AMH levels observed in prepubertal daughters of women with PCOS suggest that the ovarian follicle pool is increased from an early age in these girls and persists elevated during childhood (4–7 yr), at a time when the gonadal axis is relatively quiescent.

In this study, we selected only appropriate-for-gestational-age infants born to PCOS and control mothers, to avoid the possible effect of fetal size on gonadal function. A relationship between reduced fetal size and PCOS has been suggested (27, 28). In addition, we have recently demonstrated that PCOS mothers show a significantly higher prevalence of small-for-gestational-age newborns (29).

During early infancy, daughters of PCOS women exhibited a normal pituitary gonadal profile (30, 31), except for the fact that serum AMH levels were elevated. It is likely that the

TABLE 3. Clinical and biochemical characteristics of CG and PCOSG during childhood

	CG (n = 24)	PCOSG (n = 25)
Age (yr)	5.7 ± 0.9	5.6 ± 1.0
Weight (kg)	23.7 ± 5.9	23.4 ± 5.5
Height (cm)	114.2 ± 7.8	114.1 ± 7.7
BMI (kg/m ²)	17.8 ± 2.3	17.8 ± 2.4
Weight SDS	0.9 ± 0.9	1.0 ± 0.8
Waist circumference (cm)	57.3 ± 7.3	55.6 ± 6.2
Waist-to-hip ratio	0.9 ± 0.1	0.9 ± 0.1
LH (IU/liter)	<0.1	<0.1
FSH (IU/liter)	2.4 ± 1.5	1.6 ± 0.6 ^a
Testosterone (ng/ml)	0.2 ± 0.1	0.2 ± 0.1
Androstenedione (ng/ml)	0.5 ± 0.3	0.4 ± 0.3
17-OHP (ng/ml)	0.8 ± 0.8	0.7 ± 0.5
Estradiol (pg/ml)	6.5 ± 2.9	5.7 ± 2.0
SHBG (nmol/liter)	70.4 ± 18.4	84.2 ± 28.0
Inhibin B (pg/ml)	9.6 ± 4.3	10.2 ± 6.4

Values are means ± SD.

^a *P* < 0.05.

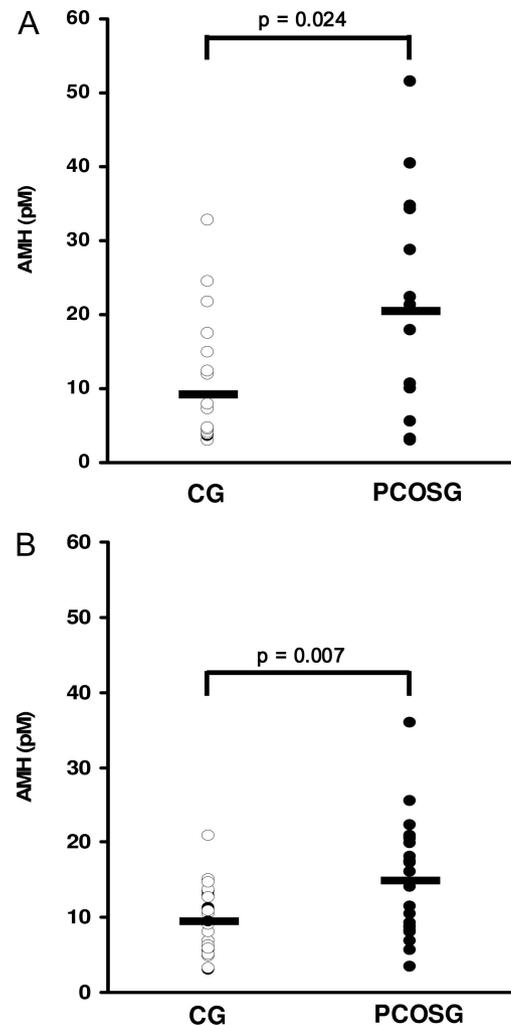


FIG. 1. Comparison of AMH serum concentrations in infant girls 2–3 months of age (A) and prepubertal girls 4–7 yr of age (B). ●, Daughters of PCOS women; ○, daughters of control women.

detection of an elevated AMH concentration in female infants of PCOS mothers at 2–3 months of age reflects an increase in the ovarian follicular pool (32) that is already present during intrauterine life. This phenomenon could be explained by genetic or environmental factors. It has been suggested that PCOS may have a genetic etiology (33), and numerous candidate genes have been proposed (34). However, given the large number of genetic variants found in association with this syndrome, it has been recently suggested that PCOS is a complex multigenic trait, subject to environmental influences that may play an important role in the expression of the hyperandrogenic phenotype (35). In addition, it has been proposed that the endocrine, nutritional, and metabolic milieu of the fetus may have a programming effect, which may persist into adult life (36–39). Recently, it has been established that prenatal androgen exposure in females born to androgenized sheep or monkey mothers is associated with growth retardation, infertility, obesity, and insulin resistance during adulthood, even though some of these characteristics could have been already expressed early during extrauterine life (40–44). Moreover, prenatal expo-

sure to androgens in rhesus monkeys and sheep produces endocrine changes that are similar to PCOS (45, 46).

We have recently demonstrated that women with PCOS exhibit a significant increase in androgen concentrations during pregnancy, which could provide a potential source of androgens to the fetus (47). Therefore, it is possible that the high androgen levels observed in pregnant PCOS women may influence fetal physiology, leading to changes in ovarian morphology and function as described in experimental models. Data from rhesus monkeys strongly suggest that intraovarian androgens promote granulosa cell proliferation and inhibit apoptosis, especially in small follicles whose granulosa cells are rich in androgen receptors (48). Accordingly, polycystic ovaries are characterized by an excessive number of growing follicles of up to 2–5 mm in size (preantral and small antral follicles) (1). Based on these observations, Jonard and Dewailly (49) postulate that the follicular dysfunction in PCOS is 2-fold, with two abnormalities that are linked. First, intraovarian hyperandrogenism may promote early follicular growth, leading to an excess of 2- to 5-mm follicles. Second, the ensuing excessive number of growing follicles may inhibit the selection process, presumably through follicle-follicle interaction involving granulosa cell products such as AMH. Recently, Stubbs *et al.* (50) showed that AMH is expressed in primordial and transitional follicles. However, in PCOS patients, a lower AMH expression was observed. These authors suggest that the relative deficiency in AMH results in an enhanced primordial follicle recruitment, which is in agreement with the notion that AMH inhibits the recruitment of primordial follicles into the pool of growing follicles (51). As a consequence, more growing follicles will be present, resulting in increased serum AMH levels. According to our data, these abnormalities may be present during the early stages of postnatal life.

The presence of elevated serum AMH concentrations in daughters of PCOS women during childhood, at a time when the gonadal axis is relatively quiescent and other hormonal markers of ovarian function such as estradiol and inhibin B are very low, suggests that AMH may be used as an early marker of ovarian follicular development. This assumption is in agreement with previous studies in adult women with PCOS. According to the study by Laven *et al.* (52), inhibin B was elevated in only 20% of the PCOS cases, and a recent study by Fanchin *et al.* (53) demonstrated that serum AMH levels were better correlated with follicular number than inhibin B, estradiol, FSH, and LH on cycle d 3.

FSH levels were lower in our PCOS girls during childhood compared with controls, but inhibin B levels were similar in both groups. Assuming that AMH levels reflect the follicular mass, lower FSH levels in the PCOS group could indirectly reflect the effect of other FSH-regulatory peptides of follicular origin on FSH levels (54). We did not find lower FSH levels in the PCOS group compared with controls during infancy. However, the present study was not designed to find differences in FSH concentrations, so the statistical power of this observation is low.

In children, AMH determination has been used to investigate gonadal development and differentiation (23, 55). To our knowledge, this is the first study of AMH serum concentrations in a cohort of prepubertal daughters of PCOS

women. Our study shows that serum AMH concentrations are increased in these girls, suggesting that they may show evidence of altered follicular development during infancy and childhood. Prospective studies are needed to establish the clinical significance of elevated AMH levels during the prepubertal period in these girls and its possible relationship with the subsequent development of PCOS.

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Address all correspondence and requests for reprints to: Prof. T. Sir-Petermann, Laboratory of Endocrinology, Department of Medicine West Division, School of Medicine, Las Palmeras 299, Interior Quinta Normal, Casilla 33052, Correo 33, Santiago, Chile. E-mail: tsir@med.uchile.cl.

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