Pituitary and Testicular Function in Sons of Women with Polycystic Ovary Syndrome from Infancy to Adulthood

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Context: An important proportion of male members of polycystic ovary syndrome (PCOS) families exhibit insulin resistance and related metabolic defects. However, the reproductive phenotypes in first-degree male relatives of PCOS women have been described less often.

Objective: The objective of the study was to evaluate the pituitary-testicular function in sons of women with PCOS during different stages of life: early infancy, childhood, and adulthood.

Design: Eighty sons of women with PCOS ($PCOS_S$) and 56 sons of control women without hyperandrogenism (C_S), matched for age, were studied. In all subjects, the pituitary-gonadal axis was evaluated by a GnRH agonist test (leuprolide acetate, 10 μg/kg sc). Serum anti-Müllerian hormone (AMH) and inhibin B were used as Sertoli cell markers. Serum concentrations of gonadotropins, steroid hormones, and SHBG were also determined. A semen analysis was performed.

Results: Basal concentrations of gonadotropins, sex steroids, and inhibin B were comparable between $PCOS_s$ and C_S during early infancy, childhood, and adulthood. Similar results in stimulated gonadotropin and sex steroid concentrations were observed. However, AMH serum concentrations were higher in $PCOS_s$ compared with C_S during early infancy [925.0 (457.3–1401.7) *vs.* 685.6 (417.9–1313.2) pmol/liter, $P = 0.039$] and childhood [616.3 (304.6–1136.9) *vs.* 416.5 (206.7–801.2) pmol/liter, $P = 0.007$). Sperm-count analysis was similar between both groups.

Conclusions: AMH concentrations are increased in prepubertal sons of women with PCOS, suggesting that these boys may show an increased Sertoli cell number or function during infancy and childhood. However, this does not seem to have a major deleterious effect on sperm production.
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Polycystic ovary syndrome (PCOS) is a common familial endocrine-metabolic disorder affecting women of reproductive age, characterized by irregular menses, chronic anovulation, infertility, and hyperandrogenism (1, 2). A genetic cause of the syndrome was suggested many years ago (3). This has been investigated in different populations (4) through phenotypic and family aggregation studies, which have demonstrated that a significant number of female relatives are affected with this condition. However, the reproductive phenotypes in male members of PCOS families have been less documented in the literature. Some of the phenotypes proposed include abnormalities in hair distribution, such as increased hair growth (3) and balding or premature male balding (5). Other studies have described abnormalities in plasma LH levels (6) and dehydroepiandrosterone sulfate

Abbreviations: AMH, Anti-Müllerian hormone; BMI, body mass index; C_s, sons of control women; DHEAS, dehydroepiandrosterone sulfate; 17-OHP, 17α-hydroxyprogesterone; PCOS, polycystic ovary syndrome; $PCOS_s$, sons of women with PCOS; SDS, SD score.

concentrations in male members of PCOS families (7). Recently we reported that brothers of PCOS women show increased 17-hydroxyprogesterone levels in response to leuprolide acetate, resembling those described in women with PCOS (8). However, this latter study was performed in adults, and it is not known whether hormonal dysfunction may be present since early stages of sexual development.

On the other hand, it has been proposed that intrauterine life, as an environmental factor, is implicated in the origin of PCOS (9–11). Therefore, intrauterine life may affect the endocrine/metabolic function of a child born to a PCOS mother, independent of genetic inheritance and sex (12). However, no study has addressed whether the hypothalamic-pituitary-testicular function is affected in sons of women with PCOS from the early stages of sexual development to adulthood. Whereas the function and maturation of the pituitary-testicular axis can be assessed by the GnRH agonist challenge test and a sperm count analysis, testicular function can be more accurately evaluated in prepubertal patients by using direct markers of Sertoli cell function, such as anti-Müllerian hormone (AMH) (13) or inhibin B (14).

The aim of the present study was to evaluate pituitary-testicular function in sons of women with PCOS during three different stages of life: early infancy (2–3 months), childhood (4–7 yr), and adulthood (18–30 yr). Therefore, we determined the basal serum concentrations of AMH and inhibin B as markers of Sertoli cell function, further assessed the pituitary-testicular axis using the GnRH analog leuprolide acetate test, and in addition performed a sperm count analysis in the adults.

Subjects and Methods

Subjects

We studied 84 males (21 infants, 34 children, and 29 adults) born to PCOS mothers [PCOS sons ($PCOS_S$)]. As a control group, we included 60 boys (21 infants, 15 children, and 24 adults) born to mothers with regular menses and without hyperandrogenism [control sons (C_s)]. The $PCOS_S$ and C_s were matched for age. This population has been previously studied and reported (12).

PCOS mothers were recruited from patients attending the Unit of Endocrinology and Reproductive Medicine at the University of Chile. This group of PCOS mothers is part of an unselected group of patients who have attended our clinic since they were diagnosed with PCOS. Diagnosis of PCOS was made according to the National Institutes of Health consensus criteria (15). PCOS women were evaluated before pregnancy and they exhibited chronic oligomenorrhea or amenorrhea, hirsutism, serum testosterone greater than 0.6 ng/ml and/or free androgen index greater than 5.0, and androstenedione greater than 3.0 ng/ml. In addition, PCOS women showed the characteristic ovarian morphology of PCO on ultrasound, based on the criteria described by Adams *et al.* (16). PCOS women were normoglycemic, with varying degrees of hyperinsulinemia, which 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 neoplasms, Cushing's syndrome, and late-onset 21-hydroxylase deficiency as well as thyroid disease. PCOS patients got pregnant after treatment program as previously described (17).

All PCOS sons were born at term after spontaneous conceptions, which led to singleton pregnancies.

As control mothers, we selected 60 women of similar socioeconomic level as the PCOS patients, with a history of singleton pregnancies, regular 28- to 32-d menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism, and no history of infertility or pregnancy complications.

There were no siblings included in the groups studied.

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 and boys older than 8 yr signed an informed consent before entering the study.

Study protocol

Infants and children were admitted with their mothers to the Pediatric Unit of our Clinical Research Center at approximately 0830 h. We performed a complete physical examination on each boy, including anthropometric measurements (weight, height, waist, hip, body mass index (BMI) and BMI SD score (SDS) calculated by the growth analyzer program using the U.S. growth charts BMI for age). Adult males were admitted to our Clinical Research Center at approximately 0830 h. All boys (controls and patients) were evaluated by same blinded andrologist (R.R.). A clinical history and a complete physical examination, including anthropometric measurements, were performed. Hair distribution was assessed clinically and considered pubic and axillary hair, moustache and beard growth, body hair, and head hair. Premature male pattern baldness was assessed by the Hamilton scale and defined as significant frontoparietal hair loss (type IV of Hamilton) (18). Testicular volume was assessed using the Prader orchiometer. In all adult subjects two semen samples were obtained.

All subjects (infants, children, and adults) underwent a leuprolide acetate test. In infants and children, a blood sample (3 ml) was obtained during the fasting state (3–4 h after the last meal) by venipuncture from an antecubital vein. Leuprolide acetate (10 μg/kg, Lupron; Abbott Laboratories, North Chicago, IL) was administered sc, and blood samples were drawn again 3 and 24 h later, as described by Ibañez *et al.* (19) in pubertal children and by us in infants (20). LH and FSH were measured at baseline and 3 and 24 h after leuprolide administration. In adults, baseline blood samples were obtained in the fasting state. Leuprolide acetate (10 μg/kg, Lupron; Abbott Laboratories) was administered sc, and blood samples were drawn 12 and 24 h later, according to the maximal responses for gonadotropins and sex steroids described by Rosenfield *et al.* (21) in adult males and by us (8). LH and FSH were measured at baseline and 12 and 24 h after leuprolide administration. This test was performed after the semen analysis.

In all subjects, testosterone, androstenedione, 17α-hydroxyprogesterone (17-OHP) and estradiol were determined at baseline and 24 h after the leuprolide challenge. Dehydroepiandrosterone sulfate (DHEAS), SHBG, inhibin B, and AMH were measured at baseline.

Assays

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 1.8 and 5.2% for LH; 1.8 and 5.3% for FSH; and 5.7 and 6.2% for estradiol, respectively.

Serum testosterone (Diagnostic System Laboratories, Webster, TX), androstenedione (Diagnostic System Laboratories), 17-OHP (Diagnostic Products Corp., Los Angeles, CA), and DHEAS (Diagnostic Products Corp. LA, USA) were assayed by RIA. SHBG was determined by radioimmunometric assay (Diagnostic Products). Assay sensitivities were 0.1 ng/ml, 0.1 ng/ml, 0.1 ng/ml, 5.0 μg/dl, and 0.04 nmol/liter, respectively. Intra- and interassay coefficients of variation were 9.6 and 8.6% for testosterone; 5.6 and 9.8% for androstenedione; 3.5 and 8.5% for 17-OHP, 4.4 and 6.3% for DHEAS, and 5.3 and 7.9% for SHBG.

Bioavailable testosterone and free testosterone were estimated according to the method proposed by Vermeulen *et al.* (22).

Serum inhibin B was assayed by ELISA (Diagnostic System Laboratories) with a sensitivity of 7.0 pg/ml and intra- and interassay coefficients of variation of 3.5 and 7.6%, respectively.

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

Sperm collection and semen analysis

Semen samples were obtained after 48–72 h of sexual abstinence and were analyzed within 1 h of collection. In all patients, two standard semen analysis were performed within 2 wk. Basic semen parameters, including semen volume, sperm concentration, percentage of sperm motility, and viability and percentage of normal sperm morphology were assessed according to the World Health Organization guidelines (24). Total sperm count was derived by multiplying semen volume times sperm concentration for each sample. Sperm parameters were considered normal when sperm concentration was 20×10^6/ml or greater of semen, motility was 40% or greater, normal sperm forms 30% or greater, and total sperm count 40×10^6 or greater spermatozoa.

Statistical evaluation

Data are expressed as median and range. Normal distribution was assessed by the Kolmogorov-Smirnov test. Differences between study groups were assessed with Student's *t* test when data were normally distributed or Mann-Whitney test when not normally distributed. Comparisons within groups were performed by ANOVA. Maximal values after leuprolide testing were defined as the peak value for gonadotropins at 3 h (infants and children) or 12 h (adults) and for steroids at 24 h. The effect of body weight or BMI on continuous variables was evaluated using multivariate analysis (multiple linear regression techniques). Spearman correlations analysis was used to evaluate the relationship among the variables of interest. Statistical analysis was performed with STATA 7.0 package (STATA Corp., College Station, TX). $P < 0.05$ was considered to be statistically significant.

Results

Table 1 shows the clinical characteristics of C_s and $PCOS_s$ during the three study periods. By design, age was not different between both groups. Birth weights were also comparable between both groups during the three study periods. However, during infancy, childhood, and adulthood, $PCOS_s$ showed a greater weight than C_s. External genitalia were normal in all boys. During adulthood, hair distribution and premature male pattern baldness were not different between C_s and $PCOS_s$. Testicular volume was significantly lower in $PCOS_s$ compared with C_s. However, in all cases testicular volume was in the normal range (>20 ml).

Table 2 shows gonadotropin and sex steroid hormone levels in C_s and $PCOS_s$ before and after leuprolide administration. Basal concentrations of gonadotropins and sex steroids were similar between $PCOS_s$ and C_s during early infancy, childhood, and adulthood. Poststimulated gonadotropin and sex steroid concentrations were also similar in both groups in the three study periods.

During adulthood, SHBG concentrations were significantly lower ($P = 0.021$) and the percentage of bioavailable testosterone was significantly higher in $PCOS_s$, compared with C_s ($P = 0.033$). However, free testosterone concentrations were not different between both groups ($P = 0.209$). DHEAS concentrations were not different between $PCOS_s$ and C_s during infancy [37.9 (21.8–108.5) *vs.* 30.9 (18.1–2.9), $P = 0.260$], childhood [10.3 (5.0–66.8) *vs.* 19.1 (5.0–112.8), $P = 0.160$], or adulthood [243.7 (148.1–569.0) *vs.* 263.5 (148.9–463.2), $P = 0.260$].

Serum concentrations of AMH and inhibin B are shown in Fig. 1. As expected, AMH levels progressively decreased with age in both C_s [early infancy: 685.6 (417.9–1313.2) pmol/liter; childhood: 416.5 (206.7–801.2) pmol/liter; adulthood: 50.9 (22.8–102.5) pmol/liter, $P < 0.007$)] and $PCOS_s$ [early infancy: 925.0 (457.3–1401.7) pmol/liter; childhood: 616.3 (304.6–1136.9)] pmol/liter; adulthood 53.8 (21.7–160.1) pmol/liter, $P < 0.0001$). AMH was significantly higher in $PCOS_s$, compared with C_s, in early infancy ($P = 0.039$) and childhood ($P = 0.007$) but not in adulthood.

In Spearman regression analysis, no correlation between AMH serum concentration and basal and poststimulated testosterone and basal and poststimulated FSH was observed in $PCOS_s$ or C_s.

Basal concentrations of inhibin B were similar between $PCOS_s$ and C_s during early infancy, childhood, and adulthood (Fig. 1).

After adjusting by body weight or BMI, AMH remained significantly different during early infancy and childhood ($P = 0.017$ and 0.034, respectively), but no differences were observed in the other parameters, between $PCOS_s$ and C_s, except for SHBG concentrations in adult group ($P = 0.072$).

As shown in Table 3, semen and sperm parameters was similar in both group. Sperm count/ml slightly below World Health

TABLE 1. Clinical characteristics of C_s and $PCOS_s$

	Infancy		Childhood		Adulthood	
	C_s (n = 21)	$PCOS_s$ (n = 21)	C_s (n = 15)	$PCOS_s$ (n = 34)	C_s (n = 24)	$PCOS_s$ (n = 29)
Age (months/yr)	2.0 (2.0–2.0)	2.0 (2.0–3.0)	5.5 (4.0–7.0)	6.0 (4.0–7.0)	22.5 (18.0–29.0)	22.5 (18.0–29.0)
Weight (kg)	5.6 (5.0–7.5)	6.2 (4.9–8.4)[a]	19.0 (14.5–23.5)	22.5 (14.3–38.7)[a]	72.8 (54.0–99.8)	79.0 (56.2–139.0)[a]
Height (m)	0.6 (0.5–0.6)	0.6 (0.5–0.7)	1.1 (1.0–1.2)	1.2 (1.0–1.3)	1.7 (1.7–1.8)	1.8 (1.6–1.9)
Weight SDS	0.3 (−0.5–2.0)	0.5 (−0.8–3.0)[a]	−0.2 (−1.4–1.9)	1.1 (−1.1–3.1)[a]		
BMI (kg/m^2)			15.1 (13.8–18.8)	17.9 (14.9–24.7)[a]	23.6 (19.4–30.1)	24.9 (20.0–45.4)[a]
BMI SDS			−0.3 (−2.0–2.1)	1.2 (−0.7–3.0)[a]		
Hamilton score					1.8 (1.0–4.0)	1.7 (1.0–4.0)
Testicular volume (cm^3)	2.0 (2.0–2.0)	2.0 (1.0–2.5)	2.0 (2.0–2.0)	2.0 (2.0–2.0)	25.0 (22.5–25.0)	21.0 (20.0–25.0)[a]
Birth weight (kg)	3.5 (2.7–4.0)	3.7 (2.6–4.3)	3.2 (3.0–3.5)	3.5 (3.0–4.4)	3.4 (2.8–4.0)	3.4 (2.9–4.2)

Values are median and range.

[a] $P < 0.05$ between C_s and $PCOS_s$.

TABLE 2. Basal and maximal hormonal responses to leuprolide acetate in C_s and $PCOS_s$

	Infancy		Childhood		Adulthood	
	C_s (n = 21)	$PCOS_s$ (n = 21)	C_s (n = 15)	$PCOS_s$ (n = 34)	C_s (n = 24)	$PCOS_s$ (n = 29)
LH (IU/liter)						
Basal	3.4 (1.8–7.6)	3.6 (0.1–9.8)	0.1 (0.1–0.7)	0.1 (0.1–0.4)	4.2 (2.1–12.0)	3.5 (1.8–17.9)
Maximal	22.6 (13.7–44.8)	21.6 (5.0–56.5)	2.7 (1.0–5.5)	3.2 (1.3–9.1)	30.2 (10.2–90.4)	32.9 (13.7–86.9)
FSH (IU/liter)						
Basal	2.5 (1.2–4.5)	2.6 (0.7–5.1)	0.9 (0.4–1.8)	0.9 (0.4–1.9)	2.2 (1.3–8.0)	3.9 (1.0–8.5)
Maximal	7.3 (3.7–16.0)	8.3 (1.4–22.1)	8.5 (3.5–16.2)	7.6 (2.9–14.8)	5.3 (2.0–15.8)	8.8 (3.0–22.6)
17-OHP (ng/ml)						
Basal	8.0 (3.4–15.3)	7.8 (0.2–13.9)	0.4 (0.2–1.0)	0.5 (0.1–1.7)	2.2 (1.1–3.7)	2.0 (0.7–3.2)
Maximal	11.6 (2.7–22.4)	12.9 (4.2–24.1)	0.4 (0.1–1.2)	0.5 (0.1–1.4)	4.7 (2.5–6.6)	4.5 (1.5–8.7)
Androstenedione (ng/ml)						
Basal	0.4 (0.1–1.2)	0.3 (0.1–1.1)	0.2 (0.1–0.9)	0.3 (0.1–1.6)	2.0 (1.0–4.5)	1.9 (0.9–5.8)
Maximal	0.6 (0.1–1.4)	0.4 (0.1–1.6)	0.2 (0.1–0.6)	0.2 (0.1–0.7)	2.2 (1.0–3.5)	2.5 (1.1–6.8)
Testosterone (ng/ml)						
Basal	0.9 (0.6–2.3)	0.8 (0.1–2.3)	0.1 (0.1–0.3)	0.2 (0.1–0.5)	5.0 (3.3–9.6)	4.4 (3.3–14.8)
Maximal	1.3 (0.7–2.7)	1.2 (0.5–2.9)	0.1 (0.1–0.2)	0.1 (0.1–0.5)	6.7 (3.1–13.2)	6.8 (4.5–13.7)
Estradiol (pg/ml)						
Basal	<5.0	<5.0	<5.0	<5.0	25.3 (14.6–45.2)	32.0 (10.9–42.4)
Maximal	<5.0	<5.0	<5.0	<5.0	70.6 (27.4–106.0)	68.3 (48.8–148.0)
SHBG (nmol/liter)	112.9 (48.3–173.2)	95.6 (30.3–164.3)	97.7 (59.1–128.1)	85.8 (53.9–129.9)	31.4 (13.3–55.0)	22.7 (10.2–44.5)[a]
Bioavailable testosterone (%)	17.5 (12.2–33.5)	20.2 (12.6–45.0)	19.4 (15.5–28.5)	21.6 (15.3–30.5)	51.1 (39.0–73.2)	57.4 (45.0–79.3)[a]
Free testosterone (pg/ml)	7.0 (5.0–24.0)	9.0 (1.0–26.0)	1.0 (1.0–3.0)	1.0 (1.0–5.0)	110.0 (68.0–226.0)	120.0 (40.0–474.0)

Values are median and ranges. For SHBG, free testosterone and bioavailable testosterone were measured only in basal state.

[a] $P < 0.05$ between C_s and $PCOS_s$.

Organization standards was observed in three $PCOS_s$ and one C_s, and low total sperm count was found only in two $PCOS_s$. In three C_s and one $PCOS_s$, a low motility was observed. One C_s and two $PCOS_s$ showed less than 30% of normal sperm forms. Similar results were observed between both semen analysis. Three C_s and one $PCOS_s$ have a paternity history. These low outcome numbers in paternity in both groups, most probably due to the young mean ages of the groups, do not allow to reach any conclusion on fertility rates.

Discussion

In this study, no significant differences in basal and poststimulated gonadotropin or sex steroid levels could be established between C_s and $PCOS_s$. However, AMH levels were increased in prepubertal sons of women with PCOS, suggesting that the number and/or activity of Sertoli cells may be increased in these boys.

AMH, also called Müllerian inhibiting substance, is produced exclusively in the gonads by Sertoli and granulosa cells. AMH is a conspicuous marker of the immature Sertoli cell: high levels are detected in blood throughout childhood, with a progressive decrease during puberty, when it is down-regulated by testosterone and meiotic germ cells (13). The increased serum AMH levels observed in prepubertal $PCOS_s$ suggest that Sertoli cell activity and/or Sertoli cell number is increased from an early age in these boys. Interestingly, pubertal down-regulation of AMH secretion occurred normally in $PCOS_s$, indicating that the effect of androgens and germ cells on Sertoli cell activity was not affected. In experimental mouse models, high AMH expression results in inhibition of Leydig cell differentiation and function (25, 26), although the moderate elevation in serum AMH does not seem to have significantly impaired testosterone secretion in $PCOS_s$. However, we are aware that RIA might not detect minor differences in steroid levels at the low end of assay sensitivity. Therefore, extremely slight differences in testosterone or estradiol levels between $PCOS_s$ and C_s might exist during infancy and childhood. It is difficult to appraise the physiological relevance of those eventual minor differences.

The pathogenesis of the increased AMH concentrations in prepubertal sons of PCOS women could be explained by genetic or environmental factors. It has been suggested that PCOS may have a genetic etiology, and numerous candidate genes have been proposed (27). 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, which may play an important role in the expression of the hyperandrogenic phenotype (27). Interestingly, we have also demonstrated that AMH serum 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 (28). Therefore, elevated concentrations of AMH during the prepubertal period may be a common phenotype in sons and daughters of PCOS women, which reflects the complexity and heterogeneity of this syndrome. Because granulosa and Sertoli cells share many structural and functional characteristics and a common embryologic origin, it is feasible to speculate that genetic and/or environmental factors may affect a specific cell lineage.

FSH plays a central role in regulating Sertoli cell proliferation and AMH secretion (29). In the rat, the AMH expression peak coincides with Sertoli cell mitotic activity, which is under FSH control (30), and AMH mRNA levels are increased in cultured Sertoli cells from human fetal testes after addition of cAMP, the main second messenger involved in FSH signaling (31). In the absence of the androgen inhibitory effect in mice, FSH increases

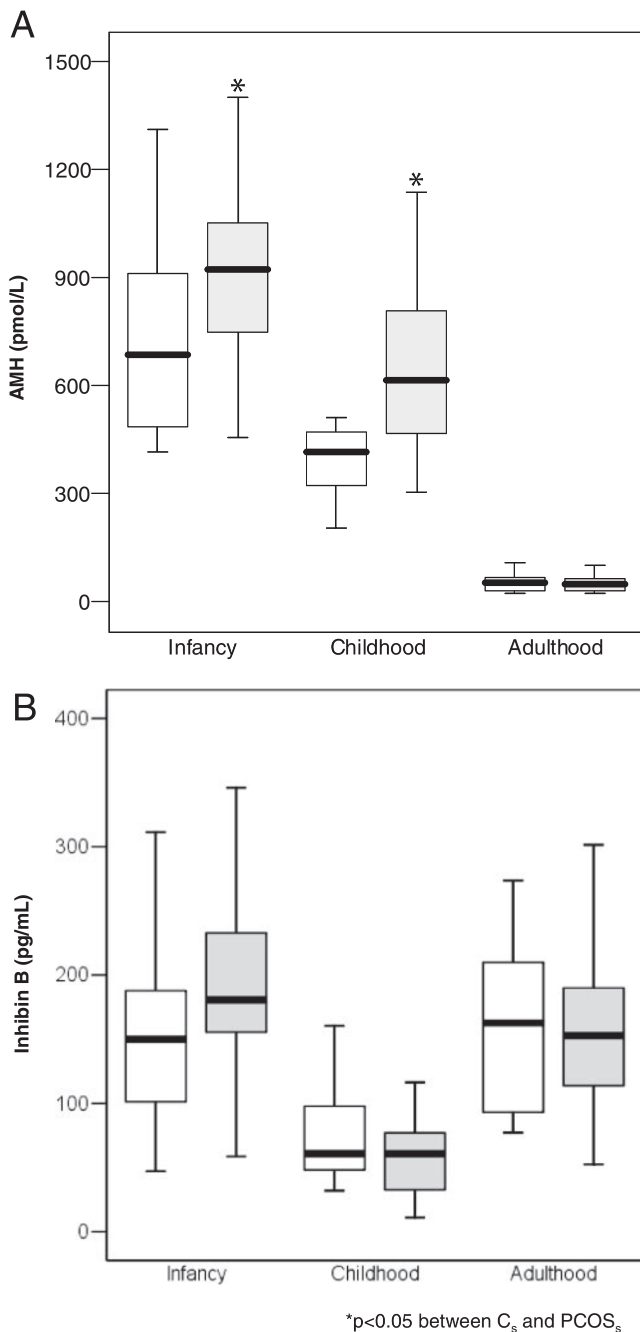

FIG. 1. Comparison of AMH (A) and inhibin B (B) serum concentrations during infancy, childhood, and adulthood in $PCOS_s$ and C_s. $PCOS_s$ are shown in *shaded boxes* and C_s women are shown in *open boxes*.

testicular AMH production (29) through Sertoli cell proliferation and an enhancement of AMH gene transcription mediated by the FSH receptor, protein Gsα, adenylyl cyclase, and protein kinase A (32). Furthermore, the administration of recombinant FSH to patients with hypogonadotropic hypogonadism results in an elevation of serum AMH (33), and an activating mutation in the Gsα protein gene found in one patient with McCune-Albright syndrome resulted in prepubertal macroorchidism and high serum AMH due to Sertoli cell hyperplasia and AMH gene overexpression (34). Altogether these observations indicate that AMH represents a useful marker of FSH action within the testis.

TABLE 3. Semen and sperm parameters in control sons (C_s) and PCOS sons ($PCOS_s$) during adulthood.

	C_s (n = 24)	$PCOS_s$ (n = 29)
Volume (ml)	2.6 (0.9–5.6)	2.9 (0.9–10.6)
Sperm concentration (M/ml)	119.3 (15.0–290.6)	107.8 (15.4–236.0)
Total sperm count (M)	292.0 (55.5–795.0)	258.2 (18.0–1339.8)
Sperm motility (%)	69.5 (27.0–82.0)	73.0 (25.0–85.0)
Sperm morphology (%)	49.0 (21.0–59.0)	50.5 (25.0–63.0)

Values are median and ranges.

In the present study, basal and stimulated FSH concentrations were similar between $PCOS_s$ and C_s. Nevertheless, in a recent experimental study of our group, male sheep born to testosterone-exposed mothers exhibited an increased FSH receptor expression in the testis (35), which could be a novel mechanism to explain the increase in AMH concentrations when FSH levels are not elevated.

$PCOS_s$ had higher AMH in early infancy and childhood, which might reflect FSH stimulation, resulting in both increased Sertoli cell proliferation and AMH gene expression in early infancy. In childhood, higher AMH could indicate the persistence of increased Sertoli cell numbers in $PCOS_s$. In adulthood, testicular volume is represented mainly by germ cells. Because germ cell number is dependent on Sertoli cell number, higher testicular volume would have been expected in $PCOS_s$. However, testicular volume was slightly lower, although within the normal range, in these patients. Sperm production was similar to controls. The interpretation could be that in adult $PCOS_s$, gonads have slightly higher Sertoli cell numbers but produce slightly less germ cells per Sertoli cell. The resulting testicular volume and sperm production would then be compensated.

In a previous work, we demonstrated that women with PCOS exhibit significantly higher androgen concentrations during pregnancy, which could provide a potential source of androgens to the fetus (36). On the other hand, it has been demonstrated that the human fetal testis is a target of estrogen action and that estrogen regulates Sertoli cell proliferation (37); therefore, one possibility in the prenatal androgenized model is that the fetal testis may be exposed to high estrogen levels due to the conversion of androgen to estrogen by the placenta and/or local estrogen production through aromatization of androgen to estrogen by fetal testis aromatase (38). However, according to the data obtained in the experimental model of prenatal androgenization in the sheep (11), the possible effect of androgens cannot be totally ruled out. Further experimental studies in animal models with the nonaromatizable androgen, 5α-dihydrotestosterone, could provide insight in this regard.

Inhibin B, also a member of the TGF family, is produced by granulosa and Sertoli cells. Therefore, like AMH, it is used as a marker of gonadal function; therefore, an increase in inhibin B levels could be expected if an up-regulation of the FSH receptor is present. However, the paracrine mechanisms that regulate AMH and inhibin secretion by Sertoli cells are complex and not necessarily similar. Therefore, a parallelism between AMH and inhibin B secretion is not always observed. For example, in prepubertal boys immature Sertoli cells secrete large amounts of AMH throughout the prepubertal period. However, inhibin B

decreases after the age of 2–4 yr. Moreover, in children the statistical correlation between inhibin B and FSH levels is controversial, and it has been found that inhibin B and FSH levels are either negatively or positively correlated (39).

Regarding different phenotypes proposed in male members of PCOS families, in the present study, there was no increase in the prevalence of premature balding in the sons of PCOS women. This observation is similar to that described in a previous publication (7). We also did not find significant differences in LH or DHEAS concentrations between C_s and $PCOS_s$, as previously described (6, 7). Our results are in agreement with a recent publication aimed at evaluating glucose tolerance status, gonadotropins, and androgens in first-degree relatives of patients with PCOS. In this study, no differences in LH or DHEAS concentrations between control and PCOS male relatives were observed (40).

It was difficult to predict the results that we would find because there are no major antecedents in the literature about hypophyseal and testicular function in sons of PCOS women. In relation to the biological plausibility, it resides in the fact that both daughters and sons of PCOS women develop in a deleterious intrauterine environment, which could have an impact on the metabolic and reproductive functions of the sons and daughters of PCOS women. Up to the moment, we have been able to establish reproductive alterations in daughters, elevated AMH, which reflects an alteration in folliculogenesis, and metabolic alterations in the sons. In this context, it could be expected that the pituitary-testicular function may also be affected. Although our clinical observations cannot provide a direct evidence of the underlying cause for the changes observed, we provide a hypothesis based on observations in an experimental model in which an increase in FSH receptor expression is seen (35).

In conclusion, whereas gonadotropin and sex steroid levels are normal, AMH concentrations are increased in prepubertal sons of women with PCOS, suggesting that these boys may have an increased Sertoli cell number or function during infancy and childhood. Further studies in experimental models may explain the underlying mechanisms. The moderate elevation of AMH levels observed before puberty seems not to have any deleterious long-term effect on the reproductive function in these patients.

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