Original Study

Ovarian Function in Adolescents Conceived Using Assisted Reproductive Technologies

Paulina M. Merino MD [1,*], Patricia Lopez BSc [1,2], Abril Salinas MD, PhD [1], Carolina Pastene MD [3], Alex Muñoz BSc [1,2], Fernando Cassorla MD [1], Ethel Codner MD [1]

[1] Institute of Maternal and Child Research, School of Medicine, University of Chile, Santiago, Chile
[2] Cytogenetics Laboratory, Hospital Clínico San Borja Arriarán, Santiago, Chile
[3] Department of Obstetrics and Gynecology, Clinica Las Condes, Santiago, Chile

A B S T R A C T

Study Objective: To compare ovarian function between adolescents conceived using assisted reproductive technology (AcART) and adolescents who were conceived spontaneously (AcSP).
Design: Multicenter study of ovarian function in AcART because of male or tubal infertility.
Setting: University Hospital.
Participants: We evaluated 22 AcART and 53 AcSP at 1-2 years after menarche. The participants were born at term (≥37 weeks of gestation) with normal birth weights (≥2500 g) from singleton pregnancies.
Interventions: None.
Main Outcome Measures: Differences in ovulation, reproductive hormones, and ovarian morphology.
Results: AcART had an older age of menarche than that of AcSP, even after adjusting for maternal age at menarche, gestational age, and birth weight ($P = .027$). AcART had lower incidence of ovulation ($P = .021$) and higher luteinizing hormone serum levels ($P = .01$) than those of AcSP. The incidence of oligomenorrhea and the cycle length were similar between AcART and AcSP. AcART had levels of anti-Müllerian hormone, inhibin B, follicle-stimulating hormone, estradiol, and androgens similar to those of AcSP. The ovarian morphology, ovarian volume, and follicle counts were similar in both groups.
Conclusion: AcART had later menarche, lower ovulation rates, and higher luteinizing hormone levels than those of AcSP. Future studies should investigate whether these findings are indicative of a risk of ovarian dysfunction later in life for AcART.
Key Words: Ovulation rate, Inhibin B, Anti-Müllerian hormone, Age of menarche, IVF offspring, Assisted reproductive technology

Introduction

Infertility is a condition that affects approximately 10%-15% of couples. This condition leads to significant distress for the affected couple as well as concern regarding achieving pregnancy and the potential effects on offspring as a result of using assisted reproductive technology (ART). After 1978, the most common ART technique was in vitro fertilization (IVF). However, after 2000 in Chile, intracytoplasmic sperm injection (ICSI) increased in popularity because of its indication for causes of infertility other than the male factor (http://redlara.com).

Previous studies have reported an increased risk of perinatal morbidity in children conceived using ART. This risk includes premature birth and low birth weight as well as conditions that might be associated with future comorbidities, especially insulin resistance and altered body fat composition. These conditions can affect the offspring's pubertal development later in life.[1–3]

Three studies with different methodologies and outcomes have reported pubertal development in girls and adolescents conceived using ART (AcART). In 2008, the first report was published of higher levels of luteinizing hormone (LH) and dehydroepiandrosterone sulfate in 19 pubertal premenarcheal girls born as a result of IVF than in girls who were spontaneously conceived.[4] In 2012, Belva et al reported that 14-year-old girls born as a result of ICSI had a similar average age at menarche but less advanced breast development than those of a control group.[5] This group recently published a comparison of the reproductive hormones and antral follicle count in the oldest global cohort of female ICSI offspring and showed that 12 female adults born as a result of ICSI who were not using hormonal contraception had levels of follicle-stimulating hormone (FSH), LH, estradiol (E2), dehydroepiandrosterone sulfate, and anti-Müllerian hormone (AMH) similar to those of a control group. Additionally, no differences were found between the ultrasonographic characteristics of the ovaries of the ICSI and those of the controls.[6] This study had fewer women who were not using contraceptives than did similar studies. Additionally, ultrasound and hormone analyses were performed on a random day of the participants' cycles instead of during the follicular phase.

The aim of this study was to compare ovarian function, reproductive hormones, and ovarian morphology in early postmenarcheal AcART and adolescents who were conceived spontaneously (AcSP) in Chile.

The authors indicate no conflicts of interest.

* *Address correspondence to:* Paulina M. Merino, MD, Institute of Maternal and Child Research, School of Medicine, University of Chile, Santa Rosa 1234, 8360160 Santiago, Chile; Phone: 562-29770855
E-mail address: pmerino@med.uchile.cl (P.M. Merino).

1083-3188/$ - see front matter
https://doi.org/10.1016/j.jpag.2018.11.008

Materials and Methods

Subjects

Postmenarcheal AcSP and AcART were studied. Both groups were at 1-2 years after menarche. Hormonal, ultrasonographic, and ovulatory studies were performed. Ovulation was studied over 2 consecutive menstrual cycles.

The AcART group was conceived in Chile between January 1999 and August 2004. The study included adolescents conceived using IVF or ICSI procedures. The etiologies of infertility in the parents included male factors, tuboperitoneal factors, and unexplained infertility. The 4 mothers with unexplained infertility showed normal tests of ovarian reserve, normal AMH levels, and good response to ovulation stimulation procedures. We excluded adolescents who were born to mothers with polycystic ovarian syndrome (PCOS) or who were poor responders to ovulation stimulation procedures. Other exclusion criteria were multiple gestations, low birth weight, prematurity, chronic diseases, and the use of chronic medications that could alter ovarian function (Gonadotropin-releasing hormone [GnRH] analogues, contraceptives, steroids, antiepileptic drugs, and growth hormone).

The AcSP group was recruited from schools in downtown Santiago, which is a population mostly of European origin, mainly from Spain.[7] The inclusion criteria of the AcSP group were singleton, full-term pregnancies (≥37 weeks of gestation) producing normal birth weight infants (≥2500 g). The exclusion criteria included the use of oral contraceptives, steroids or any other type of medication, or the presence of other concomitant chronic conditions such as genetic syndromes, celiac disease, renal disease, liver disease, cardiac disease, or undernourishment.

Study Protocol

Adolescents were evaluated during the early follicular phase. A complete clinical evaluation and review of participants' perinatal history (birth weight, birth length, and gestational age) and menstrual history was performed. We evaluated the history of infertility and pubertal development of the participants' parents, including maternal age at menarche. The physical examination included anthropometric measures and determination of pubertal status. Standard deviation scores (SDS) were calculated for weight, height, and body mass index using current National Center for Health Statitics (NCHS) standard curves, which have been shown to be applicable to the Chilean population.[8]

A blood sample obtained early during the mornings of days 1 through 7 of the cycle was used to measure gonadotrophins (LH and FSH), E2, androgens (androstenedione and total testosterone), sex hormone-binding globulin (SHBG), AMH, and inhibin B (INHB).

Laboratory Assays

Granulosa cell function was evaluated using serum AMH and INHB levels. AMH was measured using an AMH enzyme linked immunosorbent assay kit (Immunotech-Beckman; sensitivity [S] = 0.7 pmol/L), and INHB was measured using a specific 2-site enzyme linked immunosorbent assay (Diagnostic Systems Laboratories; S = 7 pg/mL).

Theca cell function was evaluated via testosterone and androstenedione levels. Serum testosterone (S = 0.0035 nmol/L) and androstenedione (S = 0.07 nmol/L) levels were measured using a competitive specific binding radioimmunoassay (Diagnostic Systems Laboratories).

Other hormone measurements were as follows: serum LH (S = 0.10 mIU/mL), FSH (S = 0.10 mIU/mL), and SHBG (S = 0.5 nmol/L) levels were measured using immunoradiometric assays (Siemens Healthcare Diagnostics). E2 (S = 18.4 pmol/L) was measured using a competitive specific binding radioimmunoassay (Diagnostic Systems Laboratories). The free androgen index was calculated using the formula ([100 × testosterone (nmol/L)]/SHBG [nmol/L]).

Ultrasonographic Examination

Each participant underwent a gynecological transabdominal ultrasound examination with a 5-MHz probe to evaluate ovarian volume and antral follicles in each ovary (Medison SonoAce 6000C) as previously described.[9] Polycystic ovarian morphology (PCOM) was defined by an ovarian volume of more than 12 mL, according to the definition of PCOM in adolescents by the World Pediatric Consensus for PCOS.[10]

Ovulation

Ovulation was assessed by determining salivary progesterone levels. Salivary samples from 54 adolescents (11 AcART and 43 AcSP) were obtained for hormone assessment on days 13, 18, 23, and 28 of the menstrual cycle for 2 months, resulting in a total of 432 samples. We instructed the adolescents to provide a 0.5-mL sample of morning saliva (fasting, after rinsing the mouth but before toothbrushing) in an Eppendorf tube. A salivary progesterone concentration greater than 0.06 ng/mL is indicative of ovulation, as shown in our published study performed on 20 ovulating young women, which showed that this method provided 90% S and 100% specificity. The area under the receiver operating characteristic curve was 0.93 ($P = .00003$).[11]

The study protocol was approved by the institutional review board of the Central Metropolitan Health Service in Santiago, Chile. The Institutional review board provided ethical agreement to the development of the study. Parents provided informed consent, and volunteers younger than 18 years gave their written consent before entering the study.

Statistical Analyses

Normal distributions were evaluated using the Shapiro–Wilk test. Differences in normal parameters were analyzed using Student t test and are presented as the mean ± SD. Characteristics that did not have a normal distribution were assessed using the Mann–Whitney U test and are presented as medians (Percentile$_{25}$, Percentile$_{75}$) (P_{25}, P_{75}). Categorical variables were compared using a χ^2

test and are presented as absolute numbers (percentage). AMH levels were evaluated as a numerical variable. Two categories were created on the basis of participants' AMH scores: AMH levels lower than P_5 (≤1.3 pmol/L) and AMH levels higher than P_{95} (≥12.7 pmol/L). Furthermore, the adolescents were divided into 2 categories on the basis of their INHB levels: INHB values lower than P_5 (≤15.1 pg/mL) and INHB values higher than P_{95} (≥98.7 pg/mL). These levels were obtained from 102 healthy adolescents as reported previously.[12]

Logistic regression was used to determine whether the AcART group had an older age at menarche, adjusting for gestational age, birth weight-SDS, maternal age at menarche, and maternal age at delivery. Logistic regression was used to determine whether the AcART group was more likely to have differences in ovulation rate, with gestational age and birth weight-SDS and maternal age at delivery included in the model as covariates. Multiple linear regression analysis was used to investigate differences in LH levels between the study groups. This model was adjusted for gestational age, birth weight-SDS, and maternal age at delivery. Statistical significance was defined as a $P < .05$. All statistical calculations were performed using Stata SE 13.0 (StataCorp LLC).

Results

A total of 22 AcART and 53 AcSP were recruited.

Of the 22 AcART, 17 were conceived using IVF, and 5 were conceived using ICSI. The causes of parental infertility were male factors (n = 12), tubal factors (n = 6), and infertility without an apparent cause (n = 4). The adolescents in the IVF and ICSI groups had similar baseline, anthropometric, hormonal, and ultrasonographic characteristics of the ovaries for all parameters evaluated. Therefore, all AcART were analyzed together.

The clinical characteristics of the participants according to the conception method are shown in Table 1. All adolescents were evaluated at a similar chronological and gynecological age. The AcART group showed anthropometric characteristics similar to those of the AcSP group. None of the adolescents received hormonal treatment at the time of evaluation. The AcART group had a lower gestational age and birth weight and higher maternal age at delivery than those of the AcSP group. These adolescents had an older age at menarche than that of the AcSP group, even after adjusting for maternal age at menarche, gestational age, birth weight-SDS, and maternal age at delivery ($P = .027$; Table 1).

The hormonal profiles of adolescents according to type of conception are shown in Table 2. The AcART group showed higher LH levels ($P = .01$) than those of the AcSP group, even after adjusting for gestational age, birth weight-SDS, and maternal age at delivery. No differences were observed in FSH and steroidal hormones between the groups. The AcART group had levels of AMH and INHB similar to those of the AcSP group. Interestingly, we found that a greater proportion of the AcART group than of the AcSP group had an INHB concentration above P_{95} ($P = .049$). Similar proportions of adolescents with AMH levels lower than P_5 and above P_{95} were found.

The ovarian morphology and menstrual characteristics of adolescents according to type of conception are shown in Table 3. The AcART group had a lower incidence of ovulation than that of the AcSP group ($P = .021$), even after adjusting for gestational age, birth weight-SDS, and maternal age at delivery. The incidence of oligomenorrhea and cycle length were similar between the AcART and AcSP groups. The ovarian volume and follicle count were similar in both groups. The AcART group had a proportion of PCOM similar to that of the AcSP group.

Discussion

In this study we evaluated ovarian function, reproductive hormones, and ovarian morphology in early postmenarcheal AcART. AcART were compared with AcSP. Ovulatory function was studied for 2 menstrual cycles. The AcART group showed a later age at menarche, higher LH levels, and lower incidence of ovulation than those of the

Table 1
Clinical Characteristics of Adolescents According to Conception Method (N = 75)

Characteristic	AcART (n = 22)	AcSP (n = 53)	*P*
Age, years	14.3 ± 1.5	13.9 ± 1.3	.26
Age of menarche, years	12.6 (11.9-13.6)	12.0 (11.3-12.6)	.027*
Gynecological age, years	1.7 ± 1.3	1.9 ± 0.9	.45
Weight, kg	55.0 ± 8.0	55.9 ± 7.2	.62
Weight-SDS	0.4 (−0.3 to 1.1)	0.6 (0.3-1.1)	.21
Height, cm	158.8 ± 5.2	157.8 ± 5.5	.47
Height-SDS	−0.1 ± 1.0	−0.2 ± 0.9	.82
BMI	21.8 ± 2.5	22.5 ± 2.7	.29
BMI-SDS	0.6 (0.1-1.0)	0.8 (0.5-1.2)	.13
Waist/hip ratio	0.8 ± 0.1	0.8 ± 0.1	.27
Waist/height ratio	0.45 ± 0.04	0.46 ± 0.05	.88
Gestational age, weeks	38.1 ± 1.6	39.2 ± 1.4	.004*
Maternal age at delivery, years	35.8 ± 4.7	29.1 ± 5.1	.001*
Birth weight, g	2950 (2730-3500)	3450 (3200-3600)	.003*
Birth weight-SDS	−0.7 (−1.1 to 0.5)	0.01 (−0.5 to 0.7)	.027*
Birth length, cm	48.5 ± 1.8	49.7 ± 2.4	.055
Birth length-SDS	−0.6 ± 1.2	−0.4 ± 1.7	.53

AcART, adolescents conceived using assisted reproductive technology; AcSP, adolescents who were conceived spontaneously; BMI, body mass index; SDS, standard deviation score.
Values are presented as the mean ± SD or median (Percentile$_{25}$-Percentile$_{75}$) (P_{25}-P_{75}).
* Significant difference.

Table 2
Hormonal Profile of Adolescents According to Conception Method (N = 75)

Variable	AcART (n = 22)	AcSP (n = 53)	*P*
Estradiol, pg/mL	48.5 (32.4-59.8)	43.0 (32.3-53.7)	.52
LH, mIU/mL	4.0 (2.7-6.0)	2.8 (2.1-4.3)	.01*
FSH, mIU/mL	5.8 ± 2.0	5.6 ± 1.5	.79
SHBG, nmol/L	42.8 (33.3-62.6)	42.5 (36.8-51.3)	.78
Testosterone, ng/dL	32 (25-38)	38 (28-48)	.24
Free androgen index, %	2.2 (1.4-4.3)	3.0 (2.0-4.5)	.24
Androstenedione, nmol/L	1.9 (1.5-2.4)	1.5 (1.2-2.4)	.09
AMH, pmol/L	3.9 (2.8-5.7)	4.8 (3.3-7.7)	.15
Inhibin B, pg/mL	67.7 (30.2-88.3)	44.6 (21.7-75.2)	.12
Participants with AMH < P_5	2 (9)	1 (2)	.17
Participants with AMH > P_{95}	0 (0)	3 (6)	.24
Participants with INHB < P_5	3 (14)	7 (14)	.94
Participants with INHB > P_{95}	3 (14)	1 (2)	.049*

AcART, adolescents conceived using assisted reproductive technology; AcSP, adolescents who were conceived spontaneously; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; INHB, inhibin B; LH, luteinizing hormone; P_5, Percentile$_5$; P_{95}, Percentile$_{95}$; SHBG, sex hormone-binding globulin.
Values are presented as n (%), mean ± SD, or median (Percentile$_{25}$-Percentile$_{75}$) (P_{25}-P_{75}).
* Significant difference.

Table 3
Ovarian Morphology and Menstrual Characteristics of Adolescents According to Conception Method (N = 75)

Characteristic	AcART (n = 22)	AcSP (n = 53)	*P*
Ovulation	7 (64)	31 (72)	.021*
Menstrual cycle duration, days	30 (30-40)	32 (29-36)	.81
Oligomenorrhea	4 (18)	4 (8)	.17
Higher ovarian volume, mL	7.6 (5.2-9.9)	7.6 (5.2-9.9)	.93
Higher follicle number	8 (7-11)	6 (5-9.5)	.06
Ovarian volume, mL	6.2 (4.8-7.6)	6.3 (4.7-7.9)	.92
Follicles	6.8 (6.0-9.5)	5.5 (4-8.5)	.16
FN, follicle 2-5 mm	5.8 (2.5-7)	4.5 (2.5-6.0)	.27
FN, follicle 6-9 mm	1.5 (0.5-3.5)	1.5 (1.0-2.5)	.88
Polycystic ovarian morphology	4 (18)	5 (9)	.3

AcART, adolescents conceived using assisted reproductive technology; AcSP, adolescents who were conceived spontaneously; FN, follicle number.
Values are presented as n (%) or median (Percentile$_{25}$-Percentile$_{75}$) (P_{25}-P_{75}).
* Significant difference.

AcSP group, suggesting a difference in ovulatory function during this period of life.

This study showed that adolescents born as a result of ART had an older age of menarche than that of the control group (by half a year), even after adjusting for maternal age at menarche. The age of menarche is affected by intrinsic and extrinsic factors, ethnic variations, and nutritional or environmental factors. To avoid these confounding factors, statistical analysis of age of menarche was adjusted for maternal age at menarche, gestational age, and birth weight, all of which are known factors that might affect pubertal timing.[13–16] The mechanism explaining this minor delay in age of menarche in AcART is not clear. Previous studies that have evaluated pubertal development in ART offspring have not shown a delay in the age of the first menstrual period.[4,5,17]

To our knowledge, this is the first study to evaluate ovulation in AcART. The incidence of ovulation in the AcART group was significantly lower than that in the AcSP group. This reduction cannot be explained by an association with maternal anovulation because mothers with PCOS or anovulatory infertility were excluded. Despite diminished ovulation rates, a similar menstrual cycle length and prevalence of oligomenorrhea (18% and 8% for the AcART and AcSP groups, respectively) were observed in the 2 studied groups. This discordance of ovulation and prevalence of oligomenorrhea might be explained by the fact that most of the cycles were ovulatory, with a rate of ovulation that might be considered physiologic for adolescents.[11] This result might also be related to the 10% difference in the prevalence of oligomenorrhea between the 2 groups.

In this study we showed higher serum LH levels in ART offspring, similar to findings described by Ceelen et al.[4] High LH levels might be associated with anovulatory states and might indicate a risk of future PCOS development.[18–20] In our study, these higher LH levels were not correlated with androgen levels or PCOM observed in the ultrasonographic evaluation of the ovaries. The finding of higher LH levels associated with late menarche and lower incidence of ovulation might represent a mild hypothalamic dysfunction in this group, which should be investigated in the future.

Our study is the first, to our knowledge, to report of INHB levels in female offspring conceived using ART. A higher proportion of the AcART group than of the control group had INHB levels above P_{95}. The granulosa cells of the preantral follicles and the small antral follicles secrete INHB. An elevated level of this hormone suggests that greater numbers of growing follicles are present in the ovary in these adolescents. AMH serum levels were similar in both groups. Additionally, the proportions of serum AMH levels that were below and above P_5 and P_{95}, respectively, were similar in the AcART and AcSP groups, which is consistent with findings reported by Belva et al.[6]

Ovarian morphology was similar between the AcART and AcSP groups. The prevalence of PCOM was 18% in the AcART group and 9% in the AcSP group, which is similar to the frequency recently reported in a study of 103 healthy postmenarcheal adolescents.[12]

The strength of this study is the assessment of ovulation in AcART. Furthermore, girls born as a result of ART were included only if certain etiologies of infertility were not diagnosed. Preterm delivery and certain pathologic perinatal conditions were excluded. Only postmenarcheal adolescents born with a normal birth weight from a singleton term pregnancy who did not use medications that could alter ovarian function were included. Potential confounders included any factor that could affect age at menarche or ovarian function. To our knowledge, this is the largest cohort of postmenarcheal ART offspring who underwent an exhaustive evaluation of ovarian function that has been reported. However, the limitations of our study are that ovulatory function was only studied for 2 cycles in the first 2 years postmenarche; thus, it is important to maintain surveillance of the menstrual cycles and ovulation in this group of adolescents over a longer period of time.

In conclusion, the data from this study suggest that AcART might exhibit ovarian function characteristics that might be early signs of ovarian dysfunction. Future studies should investigate whether these preliminary findings persist over time and whether they are indicative of a risk of gonadal dysfunction later in life.

Acknowledgments

This work was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico, Chile grant 11130240 to Paulina M. Merino.

References

1. Basatemur E, Sutcliffe A: Follow-up of children born after ART. Placenta 2008; 29(suppl B):135
2. Ludwig AK, Sutcliffe AG, Diedrich K, et al: Post-neonatal health and development of children born after assisted reproduction: a systematic review of controlled studies. Eur J Obstet Gynecol Reprod Biol 2006; 127:3
3. Okun N, Sierra S: Pregnancy outcomes after assisted human reproduction. J Obstet Gynaecol Can 2014; 36:64
4. Ceelen M, van Weissenbruch MM, Vermeiden JP, et al: Pubertal development in children and adolescents born after IVF and spontaneous conception. Hum Reprod 2008; 23:2791
5. Belva F, Roelants M, Painter R, et al: Pubertal development in ICSI children. Hum Reprod 2012; 27:1156
6. Belva F, Roelants M, Vloeberghs V, et al: Serum reproductive hormone levels and ultrasound findings in female offspring after intracytoplasmic sperm injection: first results. Fertil Steril 2017; 107:934
7. Eyheramendy S, Martinez FI, Manevy F, et al: Genetic structure characterization of Chileans reflects historical immigration patterns. Nat Commun 2015; 6:6472
8. Youlton R, Valenzuela C: Growth patterns in height and weight in children aged 0 to 17 years and cranial circumference in children aged 0 to 2 years from medium-high and high socioeconomic status in Santiago. Comparison with

growth in children from medium-low and low status in the Northern area of Santiago. Rev Chil Pediatr 1990;(Spec No):1. [in Spanish].
9. Villarroel C, Merino PM, Lopez P, et al: Polycystic ovarian morphology in adolescents with regular menstrual cycles is associated with elevated anti-Mullerian hormone. Hum Reprod 2011; 26:2861
10. Witchel SF, Oberfield S, Rosenfield RL, et al: The Diagnosis of Polycystic Ovary Syndrome during Adolescence. Horm Res Paediatr 2015; 83:376
11. Codner E, Villarroel C, Eyzaguirre FC, et al: Polycystic ovarian morphology in postmenarchal adolescents. Fertil Steril 2011; 95:702
12. Merino PM, Villarroel C, Jesam C, et al: New diagnostic criteria of polycystic ovarian morphology for adolescents: impact on prevalence and hormonal profile. Horm Res Paediatr 2017; 88:401
13. Cousminer DL, Widen E, Palmert MR: The genetics of pubertal timing in the general population: recent advances and evidence for sex-specificity. Curr Opin Endocrinol Diabetes Obes 2016; 23:57
14. Biro FM, Kiess W: Contemporary trends in onset and completion of puberty, gain in height and adiposity. Endocr Dev 2016; 29:122
15. Witchel SF: Genetics, genome-wide association studies, and menarche. Semin Reprod Med 2016; 34:205
16. Roth CL, DiVall S: Consequences of early life programing by genetic and environmental influences: a synthesis regarding pubertal timing. Endocr Dev 2016; 29:134
17. Beydoun HA, Sicignano N, Beydoun MA, et al: Pubertal development of the first cohort of young adults conceived by in vitro fertilization in the United States. Fertil Steril 2011; 95:528
18. Cortet-Rudelli C, Pigny P, Decanter C, et al: Obesity and serum luteinizing hormone level have an independent and opposite effect on the serum inhibin B level in patients with polycystic ovary syndrome. Fertil Steril 2002; 77:281
19. Hall JE, Taylor AE, Hayes FJ, et al: Insights into hypothalamic-pituitary dysfunction in polycystic ovary syndrome. J Endocrinol Invest 1998; 21: 602
20. McCartney CR, Eagleson CA, Marshall JC: Regulation of gonadotropin secretion: implications for polycystic ovary syndrome. Semin Reprod Med 2002; 20:317