CLINICAL ASSISTED REPRODUCTION

The Antral Follicle Count Predicts the Outcome of Pregnancy in a Controlled Ovarian Hyperstimulation/Intrauterine Insemination Program

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Submitted: August 4, 1997
Accepted: September 29, 1997

Purpose: *Our purpose was to test whether age-related changes in antral follicle counts can predict the pregnancy outcome in the early follicular phase of a controlled ovarian hyperstimulation/intrauterine insemination (COH/IUI) program.*
Methods: *A selected group of 107 women (36 healthy women requesting child sex preselection, 52 women with unexplained infertility, and 19 with minimal endometriosis) who underwent controlled ovarian hyperstimulation with clomiphene citrate (CC) plus human menopausal gonadotrophin (hMG) and subsequent intrauterine insemination were enrolled in the study. Transvaginal ultrasonography (7.0 MHz) was used to determine the total number of antral follicles (2–8 mm) in the right and left ovaries. The association among the antral follicle count, age, dominant follicle, and estradiol (E_2) level on the day of human chorionic gonadotropin (hCG) was analyzed. The association of the pregnancy rate and OHSS with the antral follicle count, dominant follicle count, and age was also examined.*
Results: *The total antral follicle number decreased with age ($P < 0.0001$). Dominant follicle number increased with total antral follicle number in women who received CC plus hMG/IUI ($P < 0.0001$). The pregnant group had a higher number of antral follicle and dominant follicles in comparison with the nonpregnant group ($P < 0.01$ and $P < 0.02$, respectively). The E_2 level on the day of hCG injection increased positively with the total number of antral follicles ($P < 0.0001$) and the total number of dominant follicles ($P < 0.0001$). In women aged younger than 35 years, the pregnancy rate and dominant follicle number rose as the number of antral follicles increased ($P < 0.03$ and $P < 0.0001$, respectively). The pregnancy rate was low (2/39) in women aged older than 35 years regardless of the number of antral follicles ($P < 0.05$) and the extent of hMG administration ($P < 0.02$). Women aged older than 35 also produced fewer dominant follicles ($P < 0.001$). No pregnancy was achieved in a patient with an antral follicle number of less than five (17 cases).*
Conclusions: *Age-related changes in antral follicle count significantly predicted the dominant follicle count and the pregnancy outcome. In women with antral follicle counts of less than five or who are older than 35 years, the application of COH/IUI may not be indicated.*

KEY WORDS: antral follicle count; dominant follicle count; controlled ovarian hyperstimulation/intrauterine insemination; pregnancy rate.

INTRODUCTION

A reduced pregnancy rate has been shown to be associated with poor oocyte quality and endometrial receptivity (1). Although the influence of advanced age on the endometrial receptivity has been disputed, the influence of age on oocyte quality and the pregnancy rate has been widely accepted. Elevated levels of follicle stimulating hormone with or without small decreases in the level of estradiol in the early follicular phase are common findings in older women. However, these events do not generally occur in women with underlying abnormal ovarian function, especially, in young

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1058-0468/98/0100-0012$15.00/0

women who receive ovarian surgery and whose day 3 (D3) FSH levels are still within normal limits. These findings were suggested to have profound effects on the timing of menopause. However, whether fecundity was affected remains unclear. There are now many methods and tests to determine the ovarian reserve, with various success rates (2–14).

The observation of decreasing antral follicle counts (0.1 cm or greater) with age has been shown on the basis of autopsy (15,16). This age-related decrease in antral follicles (2 mm or greater) has been confirmed by a study using high-frequency transvaginal transducers in the follicular and luteal phase in different age groups (17). However, clinical variation in therapy based on these age-related changes of antral follicle number in patients desiring pregnancy have yet to be reported in either intrauterine insemination (IUI) or in vitro fertilization and embryo transfer (IVF-ET). In this study, we tested whether age-related changes in antral follicle counts detected by transvaginal ultrasonography could predict the dominant follicle count and the pregnancy rate in the early follicular phase of a controlled ovarian hyperstimulation/IUI program.

MATERIALS AND METHODS

A total of 107 patients was included in the study. Patients were selected for study if clomiphene citrate (CC) plus human menopausal gonadotropin (hMG; Pergonal; Serono, Switzerland)/IUI was indicated for the following reasons: unexplained infertility ($n = 52$), patients seeking child sex preselection ($n = 36$), and patients who had minimal endometriosis after laparoscopy (rAFS less than 4) ($n = 19$). All of the patients had a history of regular menstrual cycles of 23 days to 35 days, with no missed periods, pregnancies, or hormonal medications. All of the patients were nonsmokers. None of the patients had a history of ovarian surgery, ovarian cyst, or polycystic ovarian syndrome.

An Acuson computed 120 XP/10 ultrasonography (Acuson Inc., Mountain View, CA) with high-frequency transvaginal probe (7.0 MHz) was used to scan the ovaries horizontally at about 1-mm intervals by one operator (C.H.C.). Space frames of each interval were printed and yielded 10 to 36 prints. Each antral follicle (sonolucent cystic area of 2 mm or greater) was counted through several frames to determine its maximum diameter. The total numbers of follicles in both right and left ovaries were summed to count the total number of antral follicles (2–8 mm) for each patient and were randomly evaluated by a second author (M.Y.C.). A blood sample was taken from each patient for analysis of baseline follicle-stimulating hormone (FSH) level and baseline estradiol (E_2) level following ultrasonography.

For controlled ovarian hyperstimulation, 100 mg clomiphene citrate (CC) was given orally from day 3 to day 7 and 2 ampoules of hMG were given subcutaneously from day 4 to day 8 and boosted if needed due to poor response of the patients. The follicles and E_2 levels were routinely checked every other day from day 5 until the day of human chorionic gonadotropin (hCG; Pregnyl; Organon) injection. hCG (5000 IU) was given when at least two leading follicles reached a diameter of 16 mm. The number of dominant follicles (greater than 14 mm) and E_2 level were also determined on the day of hCG injection. IUI was performed within 36 hr of hCG administration. Pregnancy was defined as detection of either intrauterine or extrauterine gestational sac by sonography. Ovarian hyperstimulation syndrome (OHSS) was recognized according to World Health Organization criteria (1973) when the patient had enlarged ovaries (diameter, greater than 12×12 cm) with abdominal complaints (mild form); or abdominal swelling with nausea, vomiting, and diarrhea (moderate form); or ascites and/or pleural effusion with hematological changes (severe form) 5 to 10 days after the first hCG injection.

We evaluated the relationship among the total antral follicle number, age, number of dominant follicles, and E_2 levels on the day of hCG injection with ordinary least-squares linear regression after stimulation with CC plus hMG. Then patients were further classified into pregnant and nonpregnant groups to compare differences in age, baseline FSH, baseline E_2, ampoules of hMG given, endometrial thickening, antral follicle count, dominant follicle count, and E_2 level on the day of hCG injection. The significance of pregnancy rate (PR) and OHSS association with age, antral follicle count, dominant follicle count, and E_2 level on the day of hCG injection was also studied. Statistical comparisons were made using paired Student's t test or ANOVA. Data are presented as mean ± SD. A P value less than 0.05 was considered statistically significant.

RESULTS

The results showed that the number of follicles decreased with age in the linear model (Fig. 1). The total counts decreased by 0.47 follicle per year of age ($P < 0.0001$, $\chi^2 = 0.08$). The number of dominant follicles increased with increases in the number of

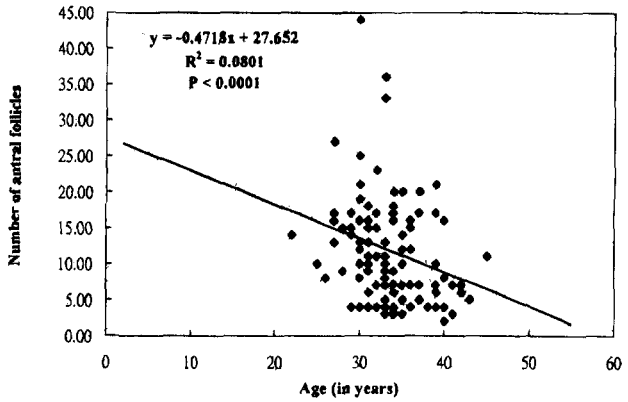

Fig. 1. A linear model of the total number of follicles in the early follicular phase by age. Number of follicles = 27.7 − 0.47 × (age).

antral follicles (Fig. 2). The estimated dominant follicles counts increased by 1.4 follicles per 5 antral follicles ($P < 0.0001$, $\chi^2 = 0.34$). E_2 levels on the day of hCG injection increased positively with the number of antral follicles ($P < 0.0001$, $\chi^2 = 0.2081$) and number of dominant follicles ($P < 0.0001$, $\chi^2 = -0.0542$), respectively (Figs. 3 and 4). The associations among age, baseline FSH level, baseline E_2 level, ampoules of hMG given, and endometrial thickness were not statistically significant between the pregnant and the nonpregnant groups (Table I). The pregnant group had higher antral follicle counts ($P < 0.01$), more dominant follicles ($P < 0.02$), and higher E_2 levels on the day of hCG injection ($P < 0.02$).

Of the 107 patients who received CC plus hMG and IUI, 15 became pregnant. The impact of antral follicle number and age on pregnancy rate and OHSS was also

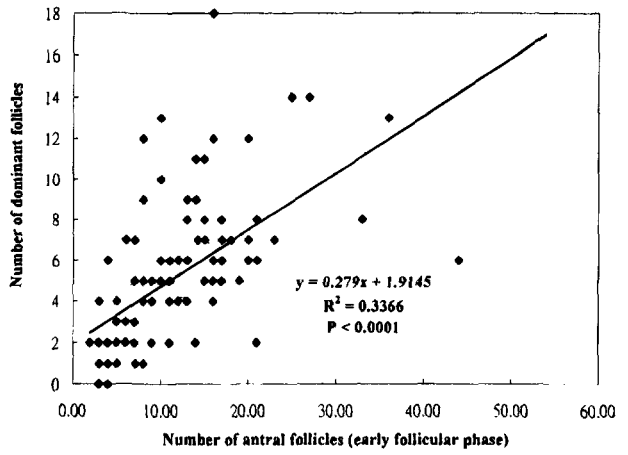

Fig. 2. Total number of dominant follicles versus number of antral follicles in the early follicular phase. Number of dominant follicles = 1.91 + 0.28 × (number of antral follicles).

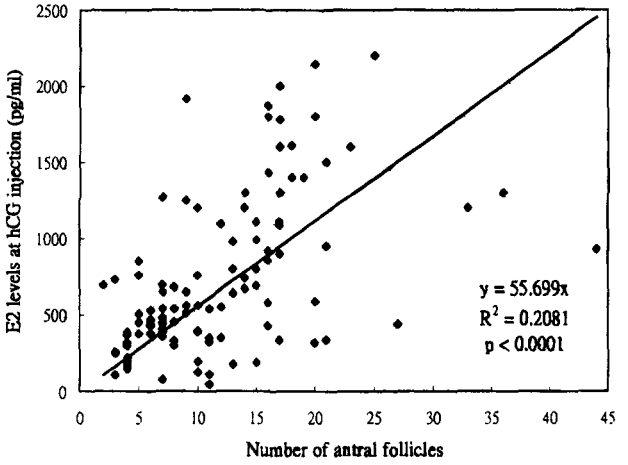

Fig. 3. Total number of antral follicles versus E_2 levels at hCG injection. Estimated E_2 levels at hCG-injection = 55.699 × (number of antral follicles).

studied (Table II). In patients with fewer than five antral follicles (17 cases), no pregnancy was achieved by IUI procedures regardless of age. The pregnancy rate was lower for patients older than 35 years of age than for patients younger than 35 years (2/39; 5.1%; $P < 0.05$), and more ampoules of hMG were required to induce ovulation ($P < 0.02$) and fewer dominant follicles were produced ($P < 0.001$). The number of dominant follicles and E_2 levels on the day of hCG injection increased with increasing number of antral follicles in both age groups of patients ($P < 0.0001$ and $P < 0.002$ in those younger than 35 years and $P < 0.0001$ and $P < 0.0001$ in those aged 35 and older, respectively). In patients aged younger than 35 with more than 15 antral follicles, the pregnancy rate and dominant follicle counts were significantly higher

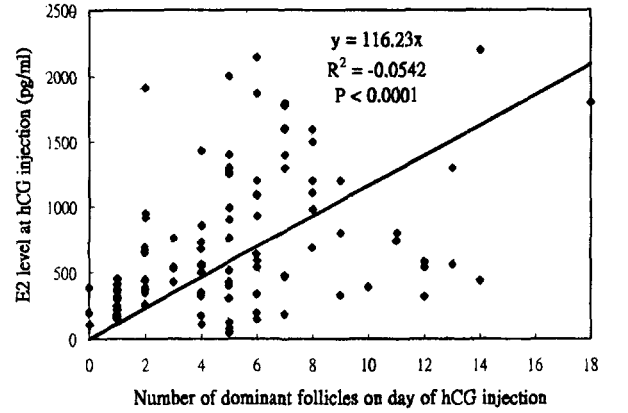

Fig. 4. A linear model of the total number of dominant follicles by E_2 level at hCG injection. Estimated E_2 levels at hCG injection = 116.23 × (dominant follicles).

Table I. Variables Correlating with Treatment Outcome[a]

	Conception cycles (n = 15)	Nonconception cycles (n = 92)	P^b
Age (yr)	32.7 ± 2.4	33.8 ± 4.5	NS
Baseline FSH (IU/L)	5.7 ± 1.1	6.6 ± 3.2	NS
Baseline E_2 (pg/ml)	90.9 ± 79.9	49.1 ± 21.9	NS
Total hMG dose (ampoules)	12.5 ± 3.8	13.4 ± 3.2	NS
Endometrial thickeness (mm)	7.9 ± 3.3	8.6 ± 2.8	NS
Number of antral follicles	18.2 ± 10.8	10.8 ± 5.8	0.01
Number of dominant follicles	7.2 ± 3.3	5.0 ± 3.4	0.02
E_2 on day of hCG (pg/ml)	996.1 ± 422.0	689.6 ± 518.5	0.02

[a] Values are means ± SD.
[b] Chi-square test.

than in those with antral follicle counts from 5 to 15 and less than 5 ($P < 0.03$ and $P < 0.0001$, respectively). The dose of hMG administered was also lower in patients with antral follicle counts greater than 15 compared to the other two groups ($P < 0.05$). The incidence of OHSS was high as the number of antral follicles increased in the group aged younger than 35 ($P < 0.02$).

DISCUSSION

Many methods and tests used in the evaluation of the ovarian reserve have pitfalls (1). The effect of age on ovarian reserve is a useful parameter in predicting the reproductive outcome in IVF-ET (2–4). However, this relationship cannot be generally applied to women who have undergone ovarian surgery or oophorectomy due to changes in the basis of the ovarian volume which result from these procedures. Previous reports on basal day 3 FSH screening have shown that women with elevated serum FSH levels might have lower pregnancy rates in IVF-ET (5–7). However, this criterion cannot indicate a poor ovarian reserve with a normal basal FSH level. Despite the presence of a normal D3 FSH, some patients still respond poorly to gonadotroprin treatment and have a poor reproductive success rate. Also, some investigators have reported that elevated D3 estradiol levels that are independent of D3 FSH levels and elevated day 10 progesterone levels and elevated FSH/LH ratios are associated with lower pregnancy rates and higher cancellation rates in IVF-ET (8–10). The CC challenge test was designed to uncover a poor ovarian reserve women with normal basal FSH levels (11). In the study, basal FSH was measured on D3 and then again on D10 after stimulation with CC, 100 mg orally, from D5 to D9. An abnormal test was defined as either a D3 or a D10

Table II. Variables Correlating with the Number of Antral Follicles[a]

			Number of antral follicles			
Age (yr)	Variable	Mean	<5	5–15	>15	P^b
<35	Age (yr)	31.0 ± 2.5***	32.2 ± 1.7	30.8 ± 2.7	30.8 ± 2.3	NS
	Baseline FSH (IU/L)	6.4 ± 3.5	6.6 ± 0.6	6.8 ± 4.6	5.6 ± 1.0	NS
	Baseline E_2 (pg/ml)	53.4 ± 41.9	62.2 ± 13.7	58.1 ± 43.3	59.9 ± 20.4	NS
	Total hMG dose (ampoules)	12.7 ± 3.7**	14.2 ± 1.7	13.1 ± 3.9	12.5 ± 1.0	0.05
	Number of dominant follicles	6.1 ± 3.7***	1.7 ± 1.0	5.8 ± 2.8	8.6 ± 3.7	0.0001
	E_2 on day of hCG (pg/ml)	755.4 ± 514.7	196.3 ± 58.5	644.5 ± 391.7	1150.1 ± 511.8	0.0001
	Conception rate	13/68*	0/9	5/37	8/22	0.03
	OHSS	12/68	0/9	4/37	8/22	0.01
≥35	Age (yr)	38.2 ± 2.6***	38.1 ± 2.3	38.5 ± 3.0	37.4 ± 1.8	NS
	Baseline FSH (IU/L)	6.5 ± 1.8	7.3 ± 1.3	6.3 ± 2.0	6.3 ± 1.6	NS
	Baseline E_2 (pg/ml)	54.4 ± 41.9	77.2 ± 22.8	62.9 ± 19.1	66.1 ± 22.4	NS
	Total hMG dose (ampoules)	14.3 ± 3.4**	14.8 ± 1.4	14.6 ± 4.5	13.3 ± 1.4	NS
	Number of dominant follicles	3.8 ± 2.4***	1.6 ± 1.2	4.0 ± 2.5	5.2 ± 1.4	0.002
	E_2 on day of hCG (pg/ml)	692.9 ± 518.9	392.1 ± 199.1	521.0 ± 239.9	1380.3 ± 604.8	0.0001
	Conception rate	2/39*	0/8	1/22	1/9	NS
	OHSS	3/39	0/8	1/22	2/9	NS

[a] Values are means ± SD.
[b] ANOVA.
* $P < 0.05$.
** $P < 0.02$.
*** $P < 0.001$.

FSH concentration exceeding 10 IU/L. However, two blood samples and 5 days were needed to obtain a result and the test carried a small risk of OHSS development in women with underlying polycystic ovarian syndrome (PCOS). Although the exogenous FSH ovarian reserve test showed that an age-related decline occurs in the ovarian reserve (12), this test is expensive and is also a risk factor for OHSS for patients with underlying PCOS. In addition, this test cannot prospectively predict the ovarian reserve in the early follicular phase. Recently, the GnRH analogue stimulation test was used to predict the reproductive outcome based on changes in the E_2 level from cycle D2 to D3. The pregnancy rate was found to increase when the E_2 level was elevated (13,14). Nevertheless, elevated E_2 levels on D3 do not accurately predict the number of follicles. Basically, all of the aforementioned tests (single or dynamic tests) are based on the detection of abnormal levels of specific hormones in the blood and have the disadvantages of being tedious, expensive, and inconsistent between laboratories. As a result, they have achieved various levels of success in evaluating the ovarian reserve (5–14).

Faddy *et al.* established the age-related curve of ovarian follicles (less than 0.1 mm) by pooling the combined data from autopsy and surgery in premenopausal women. Their model showed that the follicle number declined biexponentially and an accelerated disappearance of ovarian follicles occurs in midlife which can forecast menopause (15). Using high-frequency transvaginal sonography in the follicular and luteal phase, Reuss *et al.* found that an age-related curve of antral follicles (2 mm or greater) existed among three age groups. They found that the numbers of antral follicles of 2 mm or greater decreased by approximately 60% between 22 and 42 years of age, in concordance with the autopsy findings of Block (16). They suggested that ultrasonographically derived counts of antral follicles provide a relatively noninvasive tool to study changes in the ovarian antral follicle pool and to relate these changes to reproductive function (17). Tomas *et al.* used the antral follicle count detected by transvaginal ultrasound (6.5 MHz) to predict ovarian responsiveness in IVF/ET (18). They found that women with antral follicle numbers greater than 15 (PCO-like ovaries) had larger ovaries and higher numbers of recovered oocytes than patients with normal ovaries (5–15 antral follicles) or inactive ovaries (less than 5 antral follicles), but they did not compare the reproductive outcomes. Our study found that decreased total antral follicle counts are associated with a reduction in the number of dominant follicles. Thus, the numbers of oocytes and embryos may subsequently decrease, leading to a decrease in the clinical pregnancy rate (PR). In this study, no pregnancy occurred in patients with antral follicle counts lower than five. In these patients, COH/IUI therapy is probably not indicated, and more potent protocols for ovulation induction in ART are recommended. On the other hand, an increased antral follicle count was associated with a higher dominant follicle count and increased chances of selection of more oocytes and embryos of a good quality. Thus, a higher PR could be achieved in these patients.

The positive correlation of D3 antral follicle number with dominant follicle number of controlled ovarian hyperstimulation implied that the D3 antral follicle number could predict the number of dominant follicles and the effectiveness of the ovulation-induced protocol prior to the use of hyperstimulation regimens. Our data showed that women aged younger than 35 with more D3 antral follicles had a higher dominant follicle number and a resulting higher PR. If the D3 antral follicle number was low in the early follicular phase, the dominant follicle number might be subsequently decreased and a reduced PR could be anticipated. On the other hand, if the number of dominant follicles was less than expected in the early follicular phase after stimulation with exogenous gonadotropins, it could be a sign of a poor response to these agents.

The results of this study demonstrate that the antral follicle count as determined by transvaginal sonography is a novel predictor of ovarian responsiveness to ovulation induction and clinical PR. By using more sensitive markers for ovarian reserve, the outcome of IUI theoretically could be improved by the precise selection of patients. In women with antral follicle counts of less than five or aged over 35, the application of COH/IUI should be considered. We speculate that, in the future, the combination of age, antral follicle count, basal FSH, and other newly developed factors might be able to predict more accurately the outcome of IUI and other forms of ART.

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