Determination of the Efficiency of Controlled Ovarian Hyperstimulation in the Gonadotropin-Releasing Hormone Agonist-Suppression Cycle Using the Initial Follicle Count During Gonadotropin Stimulation

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Purpose: *Our purpose was to evaluate the relationship between the initial follicle count during gonadotropin stimulation after gonadotropin-releasing hormone (GnRH) agonist suppression and the efficiency of controlled ovarian hyperstimulation (COH) in patients receiving treatment with assisted reproductive technologies (ARTs).*
Methods: *A total of 338 COH procedures in 291 couples was performed with cycles that reached the stage of oocyte retrieval. The ovarian antral follicle number was measured using transvaginal ultrasonography at the folliculometry during gonadotropin stimulation by GnRH agonist suppression in patients undergoing ARTs. Controlled ovarian hyperstimulation was accomplished using GnRH agonist downregulation combined with FSH and menotropin stimulation. The characteristics of oocytes after retrieval and embryos after in vitro culture and the pregnancy rates were assessed.*
Results: *The procedures performed included 195 ET cycles, 129 TET cycles, and 14 incomplete cycles. The treatment cycles were divided into four categories according to the antral follicle number (i.e., ≤5, 6–10, 11–15, and ≥16) at the first folliculometry to evaluate the influence of various factors. The antral follicle count correlated significantly with the patient age, dosage of gonadotropins, serum estradiol concentration, number of antral follicles (≥13 mm) while receiving hCG injections, number of oocytes retrieved, and, later, number of embryos transferred. There was a trend toward an increasing number of pregnancies per cycle as the number of antral follicles increased (14.7, 26.5, 44, and 45%, respectively).*
Conclusions: *We were able to predict the efficiency of COH and outcome of ARTs based on the follicle count during the first folliculometry during gonadotropin stimulation after GnRH agonist suppression. The results of the folliculometry significantly predicted the ovarian response to COH and the outcome of ARTs in the current treatment cycle.*

KEY WORDS: Controlled ovarian hyperstimulation; gonadotropin-releasing hormone agonist suppression; initial follicle count; gonadotropin stimulation; folliculometry.

INTRODUCTION

Assisted reproductive technology (ART) is a complex and costly technique with stringent indications. Providing infertile couples with accurate information about their chances of pregnancy is a priority in any ART program. Although many factors can influence the success of ART, there is a general consensus that the key role is played by the ovarian response to stimulation on ART pregnancy rates. The reproductive potential of a woman decreases with age and ovarian reserve (1–3). Assessing a woman's ovarian reserve before undertaking an ART protocol can aid in predicting treatment outcome, provide important information for patients deciding whether to undergo infertility treatment, and allow physicians to individualize the treatment plan.

Several indirect biomarkers (4–8) have been used to assess ovarian reserves including day 3 serum FSH levels and day 3 serum estradiol levels. The clomiphene citrate challenge test (9,10) and dynamic

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hormone patterns (11) following gonadotropin releasing hormone (GnRH) analogue (leuprolide acetate) stimulation have also been used to assess ovarian response. The exogenous follicle-stimulating hormone ovarian reserve test (EFORT) was another functional test of ovarian reserves used in an unselected-group IVF patient (12). However, these tests, which are performed before the treatment cycle, are still not 100% reliable. The most reliable method to monitor and predict the ovarian response to ovulation induction in the current cycle remains unclear. During antral follicle development in the natural menstrual cycle, the recruitment and selection of the dominant follicle occur on days 1 through 7 of the menstrual cycle. Based on this knowledge, the ovarian response to controlled ovarian hyperstimulation (COH) seems to be determined by the initial course of gonadotropin stimulation during the early follicular phase.

So far, there have been no reports in the literature regarding the correlation between the initial follicle count using folliculometry for the assessment of follicular development during gonadotropin stimulation and the ART outcome. Thus, this study examined whether the antral follicle count measured using the first folliculometry during gonadotropin stimulation after GnRH agonist suppression could determine the ovarian response and outcome of pregnancy in patients undergoing current ARTs.

MATERIALS AND METHODS

A total of 338 COH cycles that reached the stage of oocyte retrieval in 291 couples was conducted. The indications for treatment were endometriosis ($n = 48$), tubal factors ($n = 95$), male factors ($n = 111$), uterine factors ($n = 8$), unexplained infertility or ovulatory factors ($n = 46$), and combined factors ($n = 30$). Ovarian stimulation was performed using a long protocol of GnRH agonist (luproid acetate; Takeda, Tokyo) with follicle-stimulating hormone (FSH; Metrodin; Serono Laboratories Inc., Randolph, MA) and human menopausal gonadotropin (hMG; Pergonal; Serono Laboratories Inc.) or, alternatively, FSH only as described previously (13). The initial FSH and hMG dose administered for the first 5 or 6 days of ovarian stimulation was started. Further administration of gonadotropin and human chorionic gonadotropin (hCG) was adjusted according to the standard criteria of follicular maturation as determined by vaginal ultrasound and plasma estradiol concentrations. The monitoring of ovarian response was started on the sixth or seventh day after the beginning of continuous gonadotropin stimulation. The folliculometry to assess follicular development was performed using transvaginal ultrasound (7 MHz; Model VOLUSON 530D; Medison Co., Seoul, Korea) as the mean of two measurements in one two-dimensional plane and recorded. All follicles with diameters $\geq$5 mm were recorded during the first folliculometry following the initial course of gonadotropin stimulation. Only follicles measuring $\geq$13 mm in diameter were included in the antral follicle count on the day of hCG administration during the last folliculometry. Human chorionic gonadotropin (Pregnyl; N.V. Organon, Oss, The Netherlands), usually 10,000 IU, was administered intramuscularly when ultrasound revealed that the two largest follicles had a mean diameter $\geq$16 mm and serum test results revealed adequate E_2 levels. Oocytes were retrieved using transvaginal aspiration under ultrasound guidance 35–37 hr after hCG injection. Sperm concentration and motility were evaluated according to the recommendations of the World Health Organization (14). Kruger's strict criteria were used for the assessment of sperm morphology (15). Semen analysis was done at least once before the treatment cycle to determine whether enough spermatozoa were present in the ejaculate to perform ICSI or in vitro fertilization. The procedure of sperm injection in the intracytoplasmic sperm injection (ICSI) cycle was based on a modified protocol as described previously (16,17). The procedure of sperm insemination in IVF cycles was performed according to our routine protocol (13).

After completion of the IVF procedures, the oocytes were cultured according to our standardized IVF procedure (13). They were assessed for the presence of pronuclei after 16–18 hr of incubation. Fertilization was considered to have occurred when two clear pronuclei were present. If only one pronucleus was observed, a second evaluation was performed 4 hr later to determine whether the pronuclear status had changed. The developmental competence of zygotes with two pronuclei was evaluated after a further 48 hr of in vitro culture. In vitro fertilization with subsequent TET was performed when at least a patent and adhesion-free tube was noted. The luteal phase was supplemented with oral progesterone (Utrogestan; Laboratories Piette International, Brussels, Belgium). All treatment cycles were divided into four groups according to the number of antral follicles (i.e., $\leq$5, 6–10, 11–15, and $\geq$16) during

Table I. Basic Clinical Characteristics, Stimulation Responses, and Pregnancy Results in Patients Who Received ART

Parameter	No. of antral follicles[a]				*P* value
	≦5	6–10	11–15	≧16	
No. of cycles	115	162	50	11	
Mean (±SD) age (yr)	34.2 ± 4.5	32.1 ± 3.7	30.9 ± 3.7	30.7 ± 4.2	0.0001[b]
Mean (±SD) gonadotropin dosage (ampoules)	34.6 ± 9.1	28.2 ± 6.9	27.1 ± 5.6	28.9 ± 7.9	0.0001[b]
Initial course of gonadotropin dosage	20.0 ± 5.3	19.1 ± 4.0	18.3 ± 3.2	20.7 ± 4.0	0.059[b]
Following course of gonadotropin dosage	14.6 ± 7.7	9.1 ± 5.5	9.0 ± 4.6	7.8 ± 6.1	0.0001[b]
Mean (±SD) duration of gondotropin stimulation (days)	9.5 ± 1.9	8.7 ± 1.5	8.5 ± 1.2	8.0 ± 1.1	0.0001[b]
Mean (±SD) estradiol concentration while receiving hCG (pg/ml)	743.3 ± 553.6	1321.47 ± 1022.7	1990.1 ± 1398.7	3657.8 ± 1353.9	0.0001[b]
Mean (±SD) endometrium thickness while receiving hCG (mm)	11.4 ± 2.7	11.7 ± 2.7	11.5 ± 2.6	12.1 ± 1.6	0.83[b]
Mean (±SD) No. of follicles (>1.3 cm) while receiving hCG	4.5 ± 2.2	7.7 ± 2.5	11.3 ± 2.8	16.7 ± 4.2	0.0001[b]
Mean (±SD) No. of oocytes retrieved	3.6 ± 2.0	7.2 ± 2.7	10.6 ± 3.9	20.1 ± 5.0	0.0001[b]
Fertilization rate (2 PN No./total oocyte No.)	73.1%	69.6%	77.4%	70.5%	0.01[c]
Mean (±SD) No. of embryos transferred	2.7 ± 1.3	4.3 ± 1.6	5.1 ± 1.6	4.6 ± 1.5	0.0001[b]
Mean (±SD) cumulative embryo score per transfer	43.5 ± 34.4	75.8 ± 43.4	90.0 ± 44.3	89.2 ± 37.7	0.0001[b]
Pregnancy rate per cycle (%)	17/115 (14.7)	43/162 (26.5)	22/50 (44.0)	5/11 (45.0)	0.001[c,d]
Ongoing pregnancy rate per cycle (%)	15/115 (13.0)	38/162 (23.4)	21/50 (42.0)	5/11 (45.0)	0.001[c,d]
Implantation rate (%)	22/273 (8.0)	69/652 (10.5)	35/251 (13.9)	9/40 (22.5)	0.018,[c] 0.003[d]

[a] Antral follicles detected at the first folliculometry by gonadotropin stimulation.
[b] One-way ANOVA.
[c] Chi-square test.
[d] Mantel–Haenszel monotonic trends test.

the first folliculometry to evaluate the influence of various factors.

Data were analyzed using analysis of variance to test the significance of differences in means among groups, and the chi-square test was used to assess the significance of categorical parameters and pregnancy rates among groups. The Mantel–Haenszel monotonic test for trends was used to analyze factors influencing pregnancy rates among the four groups.

RESULTS

A total of 338 treatment cycles was performed in 291 couples during the study period. The procedures performed were ET ($n = 195$) and TET ($n = 129$). In addition, there were 14 incomplete cycles, 12 without fertilization and 2 in which embryos were frozen due to fluid accumulation in the uterine cavity. Eighty-seven pregnancies were obtained from a total of 324 transfers, resulting in a pregnancy rate (PR) of 26.9% per transfer and 25.7% per cycle. There were 16 spontaneous abortions and two ectopic pregnancies. The overall ongoing PR was 24.4% per transfer and 23.4% per cycle.

Treatment cycles were divided into four groups according to antral follicle count (i.e., ≤5, 6–10, 11–15, and ≥16) during the first folliculometry to examine the association of various treatment results (Table I). Those patients who had an antral follicle count of ≤5 had a significantly higher mean age, gonadotropin dosage, and duration of gonadotropin stimulation and lower number of embryos transferred. The mean initial dose of gonadotropin was similar among the four groups, while the subsequent dose of gonadotropin was reduced depending on the antral follicle count detected during the first folliculometry. A positive correlation was found between the antral follicle count and the treatment cycle (the E_2 level on the day of hCG administration and the mean cumulative embryo score per transfer). There was a trend toward an increasing number of pregnancies per cycle as the number of antral follicles increased (14.7, 26.5, 44, and 45%, respectively).

To rule out the influence of age, we selected patients under the age of 35 years to evaluate the correlation between the antral follicle count during the first folliculometry after gonadotropin stimulation and the number of oocytes retrieved/number of antral follicles (>13 mm) while receiving hCG as shown in Figs. 1 and 2. The correlation between the antral follicle count during the first folliculometry during gonadotropin stimulation after GnRH

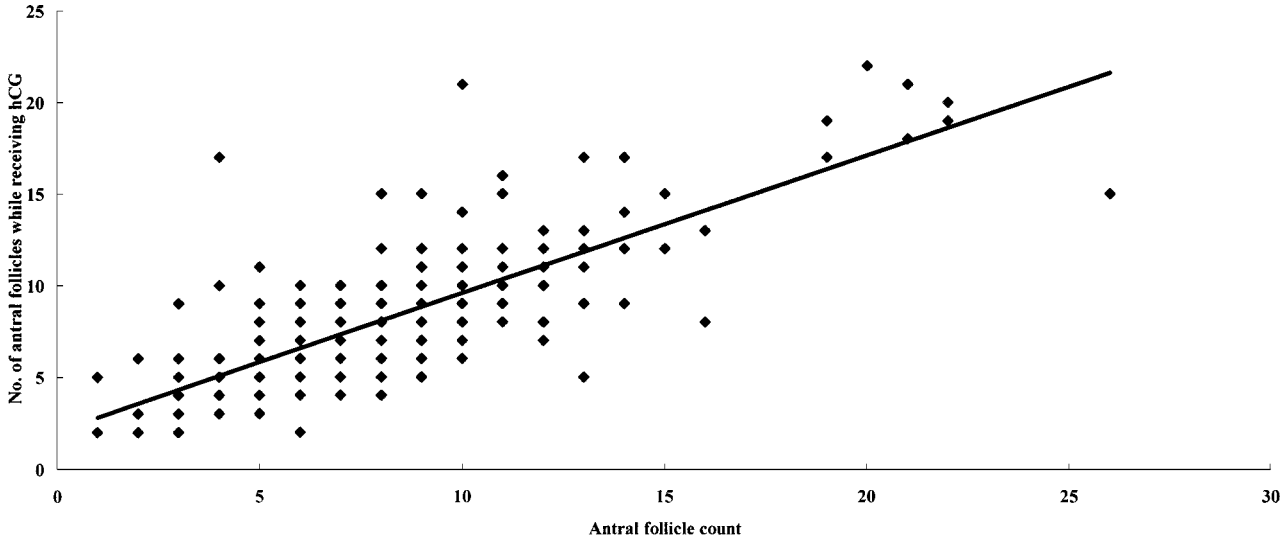

Fig. 1. Scattergram for the correlation analysis between the number of antral follicles in patients while receiving hCG and antral follicle count at the first folliculometry after gonadotropin stimulation in women who underwent ARTs ($R^2 = 0.624$).

agonist suppression and the number of antral follicles (>13 mm) while receiving hCG was statistically significant ($R^2 = 0.624$, $P = 0.0001$) (Fig. 1). The regression line delineates the highly significant correlation found between the antral follicle count during the initial folliculometry by gonadotropin stimulation and the number of oocytes retrieved during the same cycle ($R^2 = 0.53$, $P = 0.0001$) (Fig. 2).

DISCUSSION

In patients undergoing ARTs in this study, their ages (in the ≦5 follicle group) were significantly different from those of patients in other groups according to the ovarian response to exogenous gonadotropin stimulation in the GnRH agonist-suppression cycle and were proportional to the number of oocytes

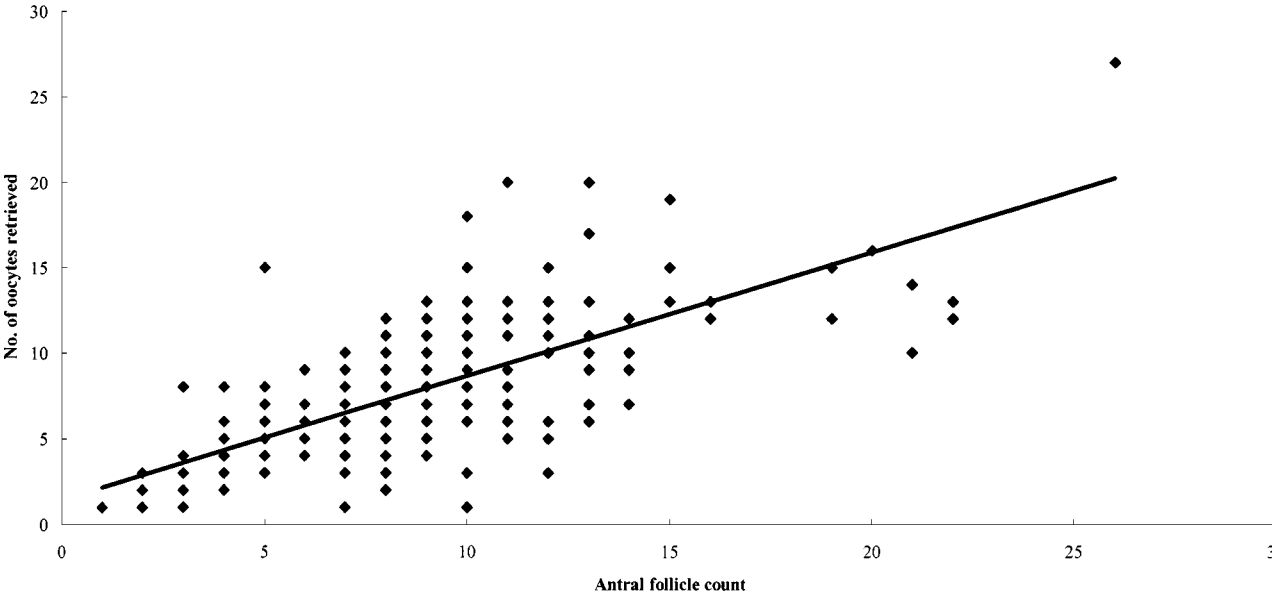

Fig. 2. Scattergram for the correlation analysis between the number of oocyte retrieved and antral follicle count at the first folliculometry after gonadotropin stimulation in women who received ARTs ($R^2 = 0.53$).

retrieved and the number of embryos transferred in the same cycle. In the other three groups, the patients' ages were not significantly different. Regression analysis in these three groups demonstrated that determination of the antral follicle count enabled early prediction of ovarian responses (number of antral follicles on the day of hCG administration and number of oocytes retrieved) during the current ART treatment cycles. This suggests that recruitment and selection at an early follicular phase occurred after the initial course of gonadotropin stimulation and that this phase may be a direct and accurate predictor of ovarian response to COH. To examine further the effects of antral follicle count on pregnancy, we also performed a Mantel–Haenszel monotonic test of the effect of antral follicle count on the success of pregnancy. The results show that the antral follicle count during the first folliculometry had a significant predictive effect on pregnancy rates. However, there have not been any reports in the literature with similar results till now.

There are several explanations for the finding of a relation between initial antral follicle count and ovarian response to COH in this study. From the physiology of the menstrual cycle, we know that the initiation of follicular growth is independent of gonadotropin stimulation until it progresses to the antral stage (18). It has been reported that up to 20 small antral follicles (2 to 5 mm in diameter) are distributed between the two ovaries at the time of luteal regression (19). During the follicular phase of the menstrual cycle, a cohort of follicles is recruited by as early as day 2 of the menstrual cycle. Between day 5 and day 7, the process leading to the selection of a dominant follicle has been completed, and the dominant follicle continues to mature for the next 5 to 7 days, when ovulation occurs (20). By days 5 to 7, this cohort will become visible on ultrasound as small, sonolucent cysts measuring approximately 5 to 8 mm (21). These phenomena show that recruitment and selection of preovulatory follicles occur in the early follicular phase of the menstrual cycle. The important concept regarding the threshold theory for follicular development is that the "threshold" FSH concentration is not a constant value, but rather the requirements of the maturing follicle for FSH change as follicular development proceeds, such that the concentration of FSH required to maintain preovulatory follicular development is lower than the concentration of FSH necessary to initiate follicular growth (22). It has been proposed that multiple follicle development can be induced by elevating FSH concentrations far above the threshold (23). Supraphysiological FSH levels in the early follicular phase are a prerequisite for the induction of multiple follicle development, although the number of preovulatory follicles has been determined by the height of FSH levels during the later stages of the follicular phase (24,25). Hence, most of the synchronized cohort follicles and preovulatory follicles are recruited after gonadotropin stimulation in the early follicular phase of the stimulated cycle. These follicles determine the number of subsequent mature oocytes and embryos produced. This correlation may be due to the finding that the antral follicle number during the initial course of gonadotropin stimulation determines the size of the ovarian pool, which can be recruited and selected during the treatment cycle.

The results of the present study indicate that antral follicle counts provide reliable and direct tools to monitor and predict the ovarian responses and outcomes of ARTs during the current cycle. Thus, it avoids the unnecessary use of expensive and time-consuming treatments (i.e., increase in the stimulation dose and duration after the first folliculometry) in patients destined to respond poorly. Another advantage of this tool is that it not only provides a reliable prediction of response in the current cycle, but also quantifies the anticipated response in terms of the expected number of aspirated oocytes and replaced embryos. Thus, it provides both patients and clinicians with useful and easily applied information that enables them to assess realistically the likelihood of a successful outcome during the current treatment cycle.

In conclusion, the antral follicle count during gonadotropin stimulation seems to be a useful and easily determined predictor of ovarian response using the outcomes of gonadotropin and ART during the current treatment cycle. Although several indirect biomarkers and dynamic tests have provided somewhat reliable predictive values before treatment, the antral follicle count may help to determine the efficacy and, thus, practicality of undergoing gonadotropin and ART procedures during the current cycles, especially in young patients.

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