Number and Size of Antral Follicles as Predictive Factors In Vitro Fertilization and Embryo Transfer

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Submitted: November 24, 1999

Accepted: January 29, 2000

Purpose: The purpose of the study was to evaluate whether number and size of antral follicles can predict the outcome of in vitro fertilization–embryo transfer.

Methods: A total of 113 patients were prospectively included into this study. After 19 days of down-regulation, number and size of follicles were determined by using recent three-dimensional transvaginal ultrasound technology. Before application of gonadotropin, all follicles had been defined as antral follicles. According to size, antral follicles were categorized into four different groups: group I included antral follicles < 5 mm, group II follicles 5–10 mm; group III 11–20 mm; and group IV >20 mm. Pregnant and non-pregnant patients were compared in terms of their number of antral follicles of group I–IV. These four groups were then compared regarding implantation rate, number of retrieved oocytes, endometrium thickness, and age.

Results: Pregnant patients showed a significant higher number of follicles with the size between 5 and 10 mm ($P = 0.04$). A significant correlation was found between number of retrieved oocytes and antral follicle size of 5–10 mm ($P = 0.0001$). Antral follicles with a diameter between 5 and 10 mm decreased significantly with age ($P = 0.008$). In groups III and IV, a significant correlation was found between antral follicle size ($P = 0.016$) and serum estradiol level after gonadotropin-releasing hormone-agonist down-regulation ($P = 0.011$).

Conclusions: We demonstrated that patients with a higher number of follicles between 5 and 10 mm showed a significantly higher pregnancy rate, whereas patients with a dominant number of antral follicles > 11 mm have a higher cancellation rate due to ovarian low response.

KEY WORDS: Antral follicle; three-dimensional ultrasound.

INTRODUCTION

Recent advances in ultrasound technology have made accurate noninvasive assessment of the pelvic organs feasible (1). The advent of three-dimensional ultrasound scanning, currently mainly a research tool, offers potential new applications in the evaluation of the infertile woman. Three-dimensional (3D) scanning offers several options extending conventional two-dimensional scanning. Digital documentation of whole volumes enables full evaluation without loss of information at a later point (2, 3). In this study 3D scanning enabled us to store digitally the volume of ovaries on an external hard disk and to accurately analyze and work up data later.

Poor ovarian response is associated with a higher risk of cancellation of treatment cycle and poor outcome of treatment. On the other hand, an exaggerated ovarian response is associated with an increased risk of ovarian hyperstimulation syndrome (OHSS) with its attendant complications (4). Determination of the appropriate starting dose for an ideal controlled ovarian stimulation with gonadotropin therefore is generally an aim in assisted reproduction.

Tomas *et al.* (5) could predict patient responsiveness to exogenous gonadotropins and the number oocytes retrieved by antral follicle count before application of gonadotropins. Chang *et al.* (6) demonstrated that age-related changes in antral follicle count significantly predicted the dominant follicle count and the pregnancy outcome. The aim of our study was to evaluate not only the antral follicle count but also antral follicle size as a predictive factor in in vitro fertilization (IVF) treatment by using the 3D transvaginal ultrasound.

MATERIALS AND METHODS

A total of 113 patients were prospectively included in this study. All patients were stimulated with gonado-

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tropin 150 IE follicle-stimulating hormone (rFSH) (Gonal F, Serono) after routine pituitary suppression with nasal administration of gonadotropin-releasing hormone-agonist (GnRH-a) (3×4 sniffs per day, Suprefact nasal spray; Hoechst) for 3 weeks. After 19 days of down-regulation, 3D ultrasound (US) was performed before the application of gonadotropin. The US equipment used was a commercially available US machine (Kretztechnik-Medison, Zipf, Austria) with an integrated 3D VIEW (Volume Imaging Evaluation Workplace; Kretztechnik-Medison) software package. Technical details and the procedure of 3D scanning with this system have been published in detail previously (7, 8). To carry out a volume scan, the ovary as the region of interest in this study has been visualized in B-mode. Within a few seconds, the machine scans the total volume consisting of sequential B-mode images. Arbitrary sectional images within the stored volume are displayed simultaneously on the monitor. The scanned ovary then can be rotated visually around the x, y, or z axis, and the displayed sectional perpendicular planes (A, B, and C planes) (Fig. 1) can be scrolled separately through the entire truncated cone.

In working up the ovaries, follicles were counted and measured initially. We defined them as antral follicle independent of follicle size. Evaluation of hormone

serum levels [FSH, luteinizing hormone (LH), estradiol (E_2)] was performed on the day of 3D US. According to size, antral follicles were categorized into four different groups: group I included antral follicles less than 5 mm; group II follicle size between 5 and 10 mm; group III size between 11 and 20 mm; and group IV size greater than 20 mm. Furthermore, endometrium thickness was measured. Pregnant and nonpregnant patients were compared in terms of their number of antral follicles of groups I–IV. These four groups were then compared regarding implantation rate, number of retrieved oocytes, endometrium thickness, and age. Stimulation cycles of patients with $E_2 > 50$ pg/dl and/or FSH/LH > 15 mg/ml were canceled. Otherwise, patients continued 2 days after blood sample with 150 IU rFSH (Gonal F, Serono) daily until at least two follicles reached a mean diameter ≥ 18 mm. Ovulation was induced with 10,000 IU human chorionic gonadotropin (hCG) (Pregnyl; Organon), and 36 hr later transvaginal oocyte retrieval was performed.

Oocytes were collected, cultured, and inseminated in vitro as described previously (9, 10). Two to five days after oocyte retrieval, two or three embryos were transferred; 14 days later an urine pregnancy test was performed.

Fig. 1. Scanned ovary demonstrated in perpendicular planes (A, B, and C planes) with measured antral follicles.

Fig. 2. Number of antral follicles in all four groups are demonstrated with significance (*) in pregnant and nonpregnant women.

Analysis of variance were used for the deviation of follicle size in pregnant or nonpregnant patients. All other variables were calculated by simple regression analysis.

RESULTS

Pregnant patients showed a significantly higher number of follicles, with the size between 5 and 10 mm ($P = 0.04$) (Fig. 2). A significant correlation was found between number of retrieved oocytes and antral follicle group II ($P = 0.0001$). Antral follicles with a mean diameter between 5 and 10 mm decreased significantly with age ($P = 0.008$) (Table I).

A significant correlation could be found between follicles with a mean diameter <5 mm and endometrium thickness. Furthermore, endometrium thickness correlated significantly with follicles with a mean diameter > 20 mm.

Out of 113 IVF cycles 30 had to be canceled after down-regulation because of an E_2 level > 50 pg/ml. Upon US, 8 patients (26%) of the 30 showed at least one antral follicle with a mean diameter > 20 mm and 5 patients (16%) of the 30 had at least one follicle with a mean diameter between 11 and 20 mm. A significant correlation was found between antral folli-

cle size and serum E_2 level after GnRH-a down-regulation in group III ($P = 0.016$) and IV ($P = 0.011$) (Table I). There was no significant correlation between follicle size and pregnancy rate, number of retrieved oocytes, and age in groups III and IV (Table I). There was no significant correlation between FSH and LH serum levels after GnRH-a down-regulation and antral follicle size or count.

DISCUSSION

Assessing a woman's ovarian reserve before undertaking a therapeutic protocol can aid in individualizing treatment plans and in predicting treatment outcome. In a recent investigation, determination of the ovarian antral follicle count with the use of transvaginal US correlated well with the patient's age and previously obtained autopsy data of ovary (11, 12). Determination of the number of antral follicles before the administration of gonadotropins might depend on the size of the ovarian follicle pool. Tomas *et al.* (5) could predict patient responsiveness to exogenous gonadotropins and the number oocytes retrieved. Therefore, the number of antral follicles were grouped as follows: <5 follicle, 5–15 follicles, and >15 follicles. Our data show that not only the antral follicle count but also the follicle size correlates with ovarian response to exogenous gonadotropin stimulation and proportionately with the number oocytes retrieved and pregnancy rate. In contrast to Tomas *et al.* (5), we defined the follicles as previously described and established four groups. Patients with a dominant number of antral follicles according to group II show a significantly higher pregnancy rate, a significantly higher number of retrieved oocytes, and are significantly younger.

The influence of age on oocyte quality and the pregnancy rate has been widely accepted. Chang *et al.* (6) demonstrated that age-related changes in antral follicle count significantly predicted the dominant follicle count and the pregnancy outcome. An age-related curve of ovarian follicles (less than 0.1 mm) was established by Faddy *et al.* (13) by pooling the combined data from autopsy and surgery in premenopausal women. Their model demonstrated that the follicle number declined biexponentially and an accelerated disappearance of ovarian follicles occurs in midlife which can forecast menopause. Chang *et al.* (6) found that decreased total antral follicle count are associated with a reduction in the number of dominant follicles.

Twenty-seven percent of 113 IVF cycles had to be canceled in our study because of serum E_2 > 50 pg

Table I. Correlations Between Number of Retrieved Oocytes (n), Age, Estradiol Serum Concentration (pg/ml), and Endometrial Thickness (mm) and Number and Size of Antral Follicles (mm)

	Retrieved oocytes (P value)	Age (P value)	Estradiol (P value)	Endometrial thickness (P value)
>5	0.34	0.446	0.206	0.047
5–10	0.0001	0.008	0.717	0.925
11–20	0.419	0.887	0.016	0.073
>20	0.496	0.408	0.011	0.001

after 21 days of luteal down-regulation with GnRH-a. We could observe in 26% out of the canceled cycles at least one cyst formation with a mean diameter more than 20 mm (group IV) and 16% out of them had a mean diameter with size between 11 and 20 mm. The cancellation rate in our study could be explained on the one hand by poor ovarian response and on the other hand by incorrect application of GnRH-a nasal spray. Jenkins *et al.* (14) found that large cysts >30 mm after luteal phase GnRH-a administration were associated with a poor IVF outcome in combination with an elevated baseline E₂ level. Cycles in which cysts were formed were associated with a significant higher age. Keltz *et al.* (15) demonstrated that baseline cyst formation after luteal-phase GnRH-a administration is both a marker for poor responders and a reliable predictor of poor stimulation and low pregnancy rates in IVF-ET.

In conclusion, it is easy to count the number and measure the size of antral follicles just before the administration of exogenous gonadotropins by transvaginal ultrasound. Three-dimensional ultrasound offers several options extending conventional two-dimensional scanning. Digital documentation of whole volumes enables full evaluation without loss of information anytime after scanning. In this study, 3D scanning enabled us to store the digital volume of ovaries on an external hard disk and to accurately analyze and work up data later. One result suggests that expensive blood samples are not needed to define ovarian reserve. With the technology of transvaginal ultrasound ovarian response and pregnancy success may be predicted for patients undergoing assisted reproduction technique.

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