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Original Article

The significance of antral follicle size prior to stimulation in predicting ovarian response in a multiple dose GnRH antagonist protocol

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Abstract: Prediction of ovarian responses prior to stimulation is not only useful for patient counseling, but also important in tailoring the optimal dosage of gonadotrophin for individual patients. By prospectively study of 214 women undergoing in vitro fertilization and embryo transfer (IVF-ET) treatment, we obtained data supporting that antral follicle size could be an additional valuable predictive marker other than the antral follicle count (AFC) in predicting ovarian response. Our studies revealed that AFC achieved the best predictive value in relation to the number of oocyte obtained, followed by antral follicle size, basal follicle stimulating hormone (FSH) and body mass index (BMI). Unlike AFC, antral follicle size was noted to be negatively correlated with the dosage (R = -0.493) and duration (R = -0.465) of rFSH stimulation. Antral follicle size was also found with higher negative regression coefficient (B = -0.661) as compared with that of basal FSH concentration (B = -0.326) and BMI (b = -0.281). More importantly, women with antral follicle size 6-7mm showed significantly higher AFC, oocytes retrieved, fertilized oocytes and grade I/II embryos along with much lower transfer cycle cancellation rate (7.5% vs. 16-17%). Together, our data suggest that basal antral follicle size could be a valued predictive marker in women with IVF-ET treatment, in which women with antral follicle size 6-7mm are likely predisposed to better IVF-ET outcomes.

Keywords: IVF-ET, antral follicle count, antral follicle size, ovarian response, oocytes

Introduction

The recruitment and development of multiple follicles in response to gonadotrophin stimulation are essential for the successful treatment of infertility by the assisted reproductive techniques. Poor ovarian response has been suggested to be associated with poor pregnancy rates and high cycle cancellation rates [1-4], while exaggerated ovarian response is also found to be a risk factor for both ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy [5-7]. Therefore, prediction of ovarian responses prior to stimulation is a critical point necessary to be addressed during patient counseling, which can also provide help to tailor the optimal dosage of gonadotrophin administration for a particular patient.

Traditionally, the patient age and early phase serum follicle stimulating hormone (FSH) levels are considered to be the most useful parameters for the prediction of ovarian reserve [8-11]. Particularly, early FSH concentration is thought to be the most powerful predictive marker for assessing ovarian response as compared with that of patient age [12, 13]. However, follow up studies have then consistently demonstrated the significant predictive value for antral follicle count (AFC) in ovarian response and pregnancy rate of patients with in vitro fertilization (IVF) treatment, in which AFC achieved the best predictive value, followed by basal FSH, body mass index (BMI) and age of the patient [14]. Other than these markers, additional hormonal and ultrasound markers such as serum inhibin B [15-18], serum anti-MÜllerian hormone [19-23],

ovarian volume [24-27] and ovarian stromal blood flow [28-33], have also been assessed, but the results are not conclusive.

Despite the extensive studies indicated above, there is no report in the literature to assess the predictive value of antral follicle size in ovarian responsiveness. Haadsma and colleagues reported that the number of small antral follicles (2 – 6 mm) is declined with age, while the number of larger follicles (7 - 10mm) remains constant [34]. Interestingly, after a 19-day of pituitary consumption of endogenous FSH and luteinizing hormone (LH) by gonadotropin-releasing hormone (GnRH) agonist, higher pregnancy rate was only noted in those patients showing higher number of antral follicles with size 5-10mm, and patients with antral follicle size predominantly > 11mm displayed higher cancellation rate due to low ovarian response [35]. Given that small antral follicles are significantly related to age, AFC and various endocrine parameters, the number of small antral follicles could represent the capacity of functional ovarian reserve. To systematically address this question, we thus conducted the current prospective study to assess whether antral follicle size is a valuable predictive marker for ovarian response and successful pregnancy rate in women undergoing IVF treatment.

Materials and methods

Subjects

A total of 214 patients and 214 cycles between January 2008 and July 2012 were included for the study. All patients had regular menstrual cycles (25-32 days), and were undergoing treatment for infertility due to tubal, endometriosis, male, unexplained or mixed factors. In addition, all patients did not process ovarian stimulation 3 months prior to this cycle and did not receive oral contraceptive pill (OCP) pretreatment before this cycle. Consent forms were obtained from all subjects and the studies were approved by the Tongji Hospital Human Assurance Committee.

Procedures for controlled ovarian stimulation

On day 3 of menstrual period, a basic evaluation was conducted by ultrasound examination. Medication was then initiated with recombinant FSH (rFSH) (Gonal-F, Serono) at the day of ultrasound examination, in which younger patients (< 35 years old) took two ampoules (150 IU) of Gonal-F daily, and older patients (≥ 35 years old) took three ampoules (225 IU) of Gonal-F daily. The dose was fixed for the first 5 days of stimulation. After a 5-consecutive days of medication, transvaginal B ultrasound examination was then performed to monitor the induction of follicles, and the dose of rFSH was optimally adjusted based on the number and size of developing follicles. The GnRH antagonist, cetrorelix, was next administered daily by s.c. injection (0.25mg/d) in the morning (8:00-12:00AM) from day 6 of the stimulation cycle to the day of human chorionic gonadotropin (HCG) administration. Additional transvaginal B ultrasound examinations were also performed post days 8, 10 and 12 of medication.

Table 1. Demographic and clinical information for the studying subjects

Parameters	Average
No. of patients	214
Age (years)	34.5±5.0
BMI(kg/m^2)	21.6±2.59
Basal FSH concentration (IU/l)	7.2±2.9
Duration of infertility (years)	7.74±5.30
Infertility (%)	
Primary	87(40.7)
Secondary	127(59.3)
Size of antral follicle (mm)	6.9±1.5
No. of antral follicle	8.3±4.5
Days of rFSH	7.9±1.6
Ampoules of rFSH	25.8±10.7
Oestradiol on HCG day (pmol/l)	1420.8±975.1
No. of oocytes retrieved	7.5±6.5

BMI: body mass index; FSH: follicle stimulating hormone; HCG: human chorionic gonadotropin.

Oocyte retrieval procedures

Gonal-F and cetrorelix were administered continuously until three follicles reached ≥ 17mm. HCG (10,000 IU, Serono) was then administrated, and serum concentrations for estradiol (E2), LH, and progestone (P) were tested on the day of HCG administration. The hormones were determined by an Immulite Automated Analyser System (ECL2012, Siemens, Germany) as instructed. Oocytes were retrieved 34-38h after injection of HCG and were fertilized in vitro according to the standard procedures.

Table 2. Comparison of antral follicle size between infertile subjects with different causes

Causes of infertility	No. of Women	Antral Follicle Size (average)
Tubal factor	108	6.8±1.6
Endometriosis	10	6.9±0.8
Male factor	33	7.2±1.5
Unexplained	19	7.3±1.2
Mixed	44	6.9±1.4
Total	214	6.9±1.5

Embryo transfer and data collection

Embryo transfer (ET) was carried out 72h after oocyte retrieval. A maximum of three embryos were transferred into each patient. Progesterone (in oil) was i.m. administered daily (80mg/day, from day 1 post oocyte retrieval) to maintain luteal functionality. Clinical pregnancy was defined as elevated serum β-hCG 14 days after ET and the presence of gestational sac(s) by ultrasonography.

Data on age, body mass index (BMI), basal FSH concentration, the number and size of antral follicles, the duration/dosage of rFSH used, serum concentrations for oestradiol, LH and progesterone, the number of follicles with size ≥ 14mm, the endometrial thickness on the day of HCG administration, and the number of oocytes aspirated/fertilized were recorded. The patients were also followed for the number of good quality embryos and the rates of fertilization, implantation and the presence of clinical pregnancy.

Statistical analysis

The SPSS 17.0 for windows was used for statistical analysis. Basal antral follicular size and count were measured by vaginal ultrasonography on menstrual day 3 prior to gonadotropin (Gonal-F) administration. Antral follicle was defined as diameter 2-10mm follicle. The follicular size was given as the mean of the two largest diameters in the same plane and perpendicular to each other [36]. Normally, we measured 5-8 antral follicles for each patient.

All values were expressed as mean ± SD or number (percentage). Continuous data were log transformed to correct for skewness prior to further statistical analysis. Analysis of variance (ANOVA) with multiple comparisons (Tukey HSD) was employed for continuous data, and χ^2 or Fisher's exact tests were used for analysis of categorical data. Correlation was assessed by the Pearson method to evaluate the predictive values of antral follicle size on the IVF outcome. Multiple logistic regression analysis was used to assess the ability of each variable on the prediction of ovarian response. In all cases, a *p*-value (two-tailed) < 0.05 was considered with statistical significance.

Results

Demographic information and clinical characteristics

All demographic information and clinical characteristics for the studying subjects are summarized in **Table 1**. The age for the patients ranged from 22 to 42yr old with an average age of 34.5 ± 5.0yr. Among all 214 subjects recruited, 87 of which (40.7%) were present with primary infertility, and the rest 127 women (59.3%) were associated with secondary infertility. The mean duration of infertility was 7.74 ± 5.3yr, ranging from 3 to 11yr. The lowest BMI was noted only 17.02, while the highest one reached 27.02 (average 21.6± 2.59). In average, the number for antral follicles was 8.3 ± 4.5, and the size for antral follicles was 6.9 ± 1.5mm. The average rFSH stimulation days were 7.9 ± 1.6day, and the average rFSH ampoules used for each subject were 25.8 ± 10.7. In general, oestradiol levels on the day of HCG administration were 1420.8 ± 975.1pmol/l, and 7.5 ± 6.5 oocytes were retrieved from each subject.

The infertility causative factors do not affect antral follicle size

Among all subjects included in the current study, 108 women were diagnosed with tubal problems, 10 with endometriosis, 33 with male factors, and 44 with mixed causes. However, 19 of which failed to reach a confirmative diagnosis, and therefore, they were defined to the category with unexplained factors. Surprisingly, we failed to observe a significant difference for the average size of antral follicles between women with different causes of infertility (**Table 2**), indicating that the causative factors for infertility do not have a perceptible impact on antral follicle size.

Table 3. Correlation coefficients of antral follicle count and size with parameters relevant to ovarian response (log transformed data)

Parameters	Antral Follicle Size	Antral Follicle Count
Age (years)	-0.013	-0.304[b]
BMI	-0.114	-0.012
Basal FSH concentration (IU/l)	-0.119	-0.228[b]
No. of total antral follicle	0.007	-
Antral follicle size (mm)	-	0.007
rFSH duration (days)	-0.493[b]	0.059
rFSH dosage (ampoules)	-0.465[b]	-0.280[b]
Day of HCG		
Follicles ≥ 14mm	-0.099	0.646[b]
Oestradiol (pmol/l)	-0.128	0.519[b]
Progesterone (ng/ml)	-0.095	0.213[b]
Endometrial thickness (mm)	0.049	0.152[a]
No. of oocytes retrieved	-0.119	0.599[b]

[a]$p < 0.05$; [b]$p < 0.01$.

Correlation analysis of factors relevant to ovarian response

Given that antral follicle count (AFC) has been suggested to be a pivotal marker in predicting ovarian response, we thus first conducted analyses to evaluate its correlation with other factors related to ovarian response. In consistent with previous report, our data revealed that AFC was significantly correlated with patient age, basal FSH concentration, rFSH dosage, serum oestadiol and progesterone concentration, follicles size ≥ 14mm, endometrial thickness on the day of HCG administration, and the potential number of oocytes to be obtained. Together, these results provided strong evidence supporting that AFC is a valuable marker in predicting ovarian response (**Table 3**).

We next sought to address the correlation between antral follicle size and the above indicated factors related to ovarian response. It was interestingly noted that unlike AFC, antral follicle size was only negatively correlated with the dosage (R = -0.493) and duration (R = -0.465) of rFSH (**Table 3**). This is a very exciting discovery, demonstrating that patients with smaller basal size for antral follicles on menstrual day 3 would need higher rFSH dosage and longer duration of stimulation during the course of IVF treatment. Indeed, a simulation analysis of our retrospective data further confirmed this trend (data not shown). Collectively, our data suggest that antral follicle size could be an additional predictive marker in ovarian response other than AFC.

Multiple regression analysis of factors in predicting the number of oocytes to be obtained

To characterize the factors with predictive values for the number of oocytes to be obtained, AFC, antral follicle size, basal FSH concentration, BMI, and the age of patients were entered in a stepwise fashion in the multiple regression analysis, in which the number of oocytes obtained was defined as a dependent variable. It was noted that AFC yielded the highest coefficient determination (R^2 = 0.358) and positive regression coefficient (B = 0.823) (**Table 4**), indicating that AFC provides the best predictive value among all of these parameters examined. Indeed, in our clinical practice basal AFC was found with high potency to predict the exact range for the number of oocytes to be obtained for a particular patient. Importantly, our data also provided a logical explanation why poor responders always have high cycle cancellation rate, and why patients with polycystic ovary syndrome (PCOS) are always more susceptible to the development of ovarian hyperstimulation syndrome (OHSS).

Excitingly, antral follicle size once again was found with higher negative regression coefficient (B=-0.661) as compared with that of basal FSH concentration (B = -0.326) and BMI (B = -0.281) (**Table 4**). These results provided additional evidence supporting that antral follicle size could be a better predictive marker in the setting of IVF treatment as compared with that of basal FSH concentration and BMI. Surprisingly, we failed to detect a discernable impact for the patient age on the number of oocytes to be obtained.

The impact of antral follicle size on IVF-ET outcomes

In view of the above findings, we next performed analysis to assess the impact of antral follicle size on IVF-ET outcomes. For this pur-

Table 4. Multiple regression analysis of parameters in predicting the number of oocytes to be obtained (log transformed data)

	B (95%CI)	β	R^2 change	*p*-value
Constant	13.692 (6.159, 21.226)	-	-	-
Antral follicle count	0.823 (0.668, 0.978)	0.565	0.358	0.000
Antral follicle size	-0.661 (-1.118, -0.205)	-0.152	0.015	0.005
Basal FSH concentration	-0.326 (-0.567, -0.084)	-0.145	0.017	0.008
BMI	-0.281 (-0.544, -0.018)	-0.112	0.012	0.036

Subject age was excluded in the equation: Log (total egg no.)= 13.692 + 0.823×log (antral follicle no.) – 0.661(antral follicle size) – 0.326(D_3FSH) – 0.281(BMI). B: Regression coefficient; R (multiple correlation coefficient) = 0.635 ; Adjusted R^2 = 0.392; β: Partial regression coefficient, CI : Confidence interval.

pose, the subjects were recategorized into four groups according to their antral follicle size on menstrual cycle day 3, which include patients with antral follicle sizes 2-6mm, 6-7mm, 7-8mm and 8-10mm, respectively. We failed to detect a significant difference between patients in different groups for the age, BMI, basal FSH concentration, HCG day progesterone levels and endometrial thickness. Remarkably, patients with antral follicle size 6-7mm showed significantly higher AFC, oocytes retrieved, fertilized oocytes, and grade I/II embryos as compared with that of patients in the other 3 groups. Although we did not detect an obvious difference for the implantation rate and clinical pregnancy rate among groups, the transfer cycle cancellation rate in patients with antral follicle size 6-7mm (7.5%) was significantly lower than that in the other three groups (16-17%) (**Table 5**).

Analysis of the outcomes in patients with antral follicle size 8-10mm revealed a significant reduction for the rFSH duration and dosage. However, numbers for the oocytes obtained, fertilized oocytes, and grade I/II embryos were significantly reduced as well. Furthermore, the implantation rate and clinical pregnancy rate were also found lower than the patients in other groups. In contrast, we failed to observe a significant difference for the oocytes obtained, fertilized oocytes, grade I/II embryos, implantation rate and clinical pregnancy rate between patients with antral follicle size 2-6mm and patients with antral follicle size >7mm (the 7-8mm and 8-10mm group), but patients with antral follicle size 2-6mm showed significantly higher rFSH dosage and longer rFSH duration (**Table 5**). Taken together, our data indicate that antral follicle size impacts the outcomes for IVF-ET treatment, and patients with antral follicle size 6-7mm are likely predisposed to better clinical outcomes.

Discussion

Prediction of ovarian responses prior to stimulation is useful in patient counseling, which may also be helpful in tailoring the dosage for gonadotrophin in each individual patient. Therefore, many clinical, hormonal and ultrasound parameters have been extensively evaluated for their feasibility to predict ovarian responses during ovarian stimulation. It has now generally accepted that AFC is thus far the most powerful marker for predicting ovarian response [37-39]. Indeed, Chang and colleges found that patients with antral follicle number ≤ 3 had a significantly higher rate of cycle cancellation and higher human menopausal gonadotropin (HMG) dosage as compared with those patients with antral follicle number 4-10 or ≥ 10 [37]. Similarly, Tomas and co-workers demonstrated that AFC is strongly correlated with the number of oocytes to be retrieved before ovarian stimulation. They concluded that AFC is a better predictor for ovarian response than that of ovarian volume or age alone [38]. Of note, our prospective studies in the current report also found that AFC on menstrual cycle day 3 is significantly correlated with patient age, basal FSH concentration, rFSH dosage, serum oestadiol and progesterone concentration, antral follicle size ≥ 14mm, endometrial thickness on the day of HCG administration and the number of oocytes to be obtained. These data provided additional evidence supporting that AFC is a valuable marker in ovarian response prediction.

Other than AFC, antral follicle size may also impact the capacity of ovarian response. Indeed, Haadsma and colleagues found that the endocrine function of an antral follicle is related to its size [34]. A prospective study

Table 5. Comparison of IVF-ET outcomes of subjects with different antral follicle size

Antral Follicle Size	2-6mm	6-7mm	7-8mm	8-10mm
Cycles	52	53	61	48
Age (years)	34.6±5.5	34.7±4.7	34.4±4.6	34.5±5.2
BMI	21.6±2.4	22.1±2.7	21.7±2.6	21.2±2.5
Basal FSH (IU/l)	7.6±2.2	7.7±3.6	6.7±3.0	7.0±2.5
Antral follicle count	6.8±3.7[b]	10.2±5.2[b]	8.9±4.5	7.1±3.3[b]
rFSH duration (days)	8.8±1.6	8.5±1.6	7.5±1.2	6.9±1.2
rFSH dosage(amp)	31.7±12.1[b]	27.8±11.1	23.7±8.7[b]	19.9±6.3[b]
HCG day				
Oestradiol (pmol/l)	1513.4±1247.9	1700.2±1154.6[a]	1323.0±705.8	1149.9±590.2[a]
Progesterone(ng//ml)	1.3±0.5	1.4±0.8	1.2±0.5	1.2±0.6
Endometrial thickness(mm)	9.9±2.2	10.7±2.2	11.1±2.7	9.9±2.1
≥14mm follicles	5.7±3.8	6.6±3.8[a]	5.3±2.8	4.4±3.1[a]
Oocytes retrieved	7.2±5.8	10.3±9.4[b]	7.1±4.7	5.3±4.1[b]
Fertilized oocytes	4.5±4.1[a]	6.0±5.7[b]	3.9±2.8	2.6±2.4[a, b]
Fertilization rate (%)	245/391 (62.7)	320/543 (58.9)	249/455 (54.7)	132/266 (49.6)
Embryos transferred no.	1.9±1.0	2.3±0.9[b]	1.8±1.0	1.7±1.0[b]
Grade I/II embryos	2.4±2.6	3.1±3.1[b]	1.8±2.0	1.4±1.6[b]
Transfer cycles cancelled (%)	9(17.3)	4(7.5)	10(16.4)	8(16.7)
Implantation rate (%)	18/101(17.8)	24/121(19.8)	27/117(23.1)	11/83(13.3)
Clinical pregnancy (%)	14/45(33.3)	16/49(32.7)	21/54(38.9)	8/42(19.0)

[a]$p < 0.05$; [b]$p < 0.01$.

including 474 women further revealed that the number of small follicles (2–6 mm) in a particular woman may represent her functional ovarian reserve [34]. Pohl and co-workers studied 113 patients, in which they categorized the patients based on antral follicle size into < 5mm, 5-10mm, 11-20mm and > 20mm four groups to assess the impact of antral follicle size on IVF outcomes [35]. They have interestingly noted that patients with antral follicle size 5-10mm displayed a significantly higher pregnancy rate, whereas those patients with antral follicles size > 11mm presented a higher cancellation rate due to low ovarian response [35]. Together, these studies provided suggestive evidence supporting a role for antral follicle size in ovarian response prediction, while the exact impact of antral follicle size on ovarian response and IVF outcome is yet to be fully assessed.

In the current report, we have systematically assessed the feasibility and potency of antral follicle size in ovarian response prediction and IVF outcome. By prospectively study of 214 women undergoing IVF-ET treatment, we noted that antral follicle size is significantly correlated with the dosage and duration of rFSH stimulation. Particularly, higher dosage and longer duration of rFSH stimulation were required for those women with antral follicle size 2-6mm. However, the dosage and duration of rFSH stimulation were significantly reduced for those women with antral follicle size 8-10mm, but the implantation rate and clinical pregnancy rate were reduced, and lower estradiol levels were noticed in these women as well. In sharp contrast, women with antral follicle size 6-7mm showed much more AFC, higher potency for oocytes to be retrieved along with higher fertilized oocytes and grade I/II embryos. Importantly, although we failed to characterize an obvious difference for the implantation rate and clinical pregnancy rate between these women and those with antral follicle size < 6mm or > 8mm, the transfer cycle cancellation rate was significantly lower in women with antral follicle size 6-7mm (7.5% vs. 16-17%). All together, our data support that the basal antral follicle size 6-7mm can produce the best predictive value on clinical and laboratory results during IVF-ET processes.

It has been suggested that subject age is also one of the important predictive parameters in ovarian response. Indeed, woman fertility is remarkably reduced along with the increase of

age in both spontaneous conceptions [40, 41] and assisted reproductive methods [42, 43]. The decrease of fertility with aging is apparently due to the decrease for the number of primordial follicles [44] and antral follicles with size ≥ 2mm [45]. In our data, subject age was found to be correlated with AFC. Unexpectedly, subject age was excluded from our multiple regression analysis, suggesting that age alone has quite limited value in predicting ovarian response for women undergoing assisted reproduction methods.

Basal FSH concentration has been shown to be a better predictor of ovarian response to stimulation than the age of women [12, 13]. It is generally suggested that higher basal FSH concentration would impair ovarian response. In line with this notion, our data also revealed that basal FSH concentration was negatively correlated with AFC and the number of oocytes to be obtained. Similarly, BMI is considered to be another predictive marker for ovarian response. Our studies demonstrate that higher BMI impairs the number of oocytes to be obtained. In consistent with our results, obese patients are generally thought to require significantly higher dose of gonadotropin in a longer duration of stimulation, and therefore, they should be advised to reduce weight before attempting IVF treatment [46]. Of importantly note, our data suggest that antral follicle size possesses higher predictive power than that of basal FSH concentration and BMI, as manifested by that antral follicle size has significantly higher negative regression coefficient. Given the fact that very few studies have been conducted relevant to this topic, the predictive importance of antral follicle size in clinical IVF-ET treatment is generally neglected.

In summary, we have demonstrated evidence supporting that antral follicle size is probably a new and valuable predictive marker for ovarian response during IVF-ET treatment. Antral follicle size 6-7mm can achieve the best predictive value. However, additional prospective studies with larger sample size would be necessary to further validate the conclusion.

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