Effect of Antral Follicle Count on in Vitro Fertilization Outcome

Antral Folikül Sayısının in Vitro Fertilizasyon Sonuçları Üzerine Etkisi

İVF'da Antral Foliküller / Antral Follicles in IVF

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Özet

Amaç: Bu calışmada antral folikül sayısı ve boyutunun over rezervi ile birlikte in vitro fertilizasyon sonuçlari üzerine etkisini incelemek istedik. Gereç ve Yöntem: Zeynep Kamil Egitim ve Araştırma Hastanesi tüp bebek ünitesinde tedaviye başlanan 44 hasta prospektif olarak incelendi. Menstruasyonun 3. Gününde transvaginal ultrason uygulandı. Antral folikül sayıları ve boyutları kaydedildi. Antral folikül boyutuna göre hastalar iki gruba ayrıldı. Grup 1 AF >5mm ve Grup IIAF ≤5mm olarak belirlendi. Kontrollü over stimulasyonu için rec-FSH kullanıldı. Baslangıç dozu hastanın yaşı, over rezervi veya önceki tedaviye olan yanıtına göre belirlendi. Bulgular: Çalışmamızda antral folikül boyutuna göre (AF >5mm ve AF ≤5mm) gruplar arasında total oosit sayısı (11.03± 6.59 vs. 13.71± 5.38), embriyo (5.7± 4.46 vs. 7.29± 5.05) ve gebelik oranları arasında (76.7% vs. 71.4%) istatiksel olarak bir fark saptanmamıştır. Over cevabı açısında receiver operating eğrisi analizi yapıldığında eğri altında kalan alan AF > 5 mm olanlarda 0.675±0.083 (0.517-0.808) olarak tespit edildi. Tartışma: Boyutu 5 mm üzerindeki antral folikul sayısı ile elde edilen matür oosit sayısı arasında ilişki olabilir. Antral folikül sayısının tayini IVF öncesi rutin bir test olması nedeniyle , özellikle 5 mm üzerindeki antral foliküllerinin sayısının önceden belirlenmesi klinisyenin embriyo kalitesi ve gebelik anlamında daha başarılı sonuçlar elde etmesine yardımcı olabilir.

Anahtar Kelimeler

Antral Folikül; IVF; Ultrason

Abstract

Aim: The aim of this study is to evaluate the effects of number and size of antral follicles on ovarian reserve and subsequently in vitro fertilization (IVF) outcome. Material and Method: 44 patients treated with IVF in the Infertility Clinic of Zeynep Kamil Women's and Children Disease Education and Research Hospital (Istanbul, Turkey) were prospectively included in this study. On the third day of menstrual bleeding, transvaginal ultrasound was performed. Antral follicle count (AFC) and antral follicle (AF) size were recorded in these patients. Group I as AF > 5mm and Group II as AF ≤ 5mm were defined. The patients underwent controlled ovarian hyperstimulation with rec-FSH following. Results: Our findings indicated that there was not a statistical difference in number of total oocytes (11.03± 6.59 vs. 13.71± 5.38), embryos (5.7± 4.46 vs. 7.29± 5.05) and pregnancy rates (76.7% vs. 71.4%) between groups according to antral follicle size (AF ≤5mm vs. AF >5 mm) . Under the area of ROC curve estimations for ovarian response revealed that antral follicle (AF) >5 mm had the largest area 0.675±0.083 (0.517-0.808). Discussion: There might be an association between AF that is larger than 5 mm in size and number of retrieved mature oocytes. Since AFC, is a routine screening test in any IVF center, we believe paying closer attention to the measurement of AFC that are larger than 5 mm in size might help clinicians to achieve more successful outcomes in terms of on embryo quality and pregnancy outcome in women undergoing an in vitro fertilization.

Keywords

Antral Follicle; IVF; Ultrasonography

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Introduction

Recent advances in ultrasound technology made it feasible to assess pelvic organs non-invasively [1]. On the other hand, search for highly accurate tools continues to assess ovarian reserve. During this search, a connection between ultrasonographic image of ovaries and their response to induction has been found. As the ovarian volume and antral follicle count increase, ovarian reserve increases as well. Availability of ultrasound nearly in any hospital setting and ease of follicle count made the antral follicle count first line of choice in assessing ovarian reserve [2-5]. There is no consensus, however, on which follicles should be included in total antral follicle count (AFC), and the current evidence base includes studies using follicles measuring 2 to 5 mm, 2 to 6 mm, 2 to 8 mm, or 2 to 10 mm in diameter. Examination of antral follicles grouped into cohorts by their size, particularly the smaller ones, may improve the predictive value of AFCs [6-9].

The aim of this study is to evaluate the effects of number and size of antral follicles on ovarian reserve and subsequently on embryo quality and pregnancy outcome in women undergoing an in vitro fertilization (IVF) .

Material and Method

44 patients treated with IVF in the Infertility Clinic of Zeynep Kamil Women's and Children Disease Education and Research Hospital (Istanbul, Turkey) were prospectively included in this study. Approval of the ethics committee was obtained.

Patients who were included in the study were between the ages of 24-39. They had either tubal, peritoneal, male factor or unexplained reasons for infertility. All the patients' basal follicle stimulating hormone (FSH) value was <15 IU/L, while their tiroid stimulating hormone (TSH) and prolactin (PRL) values were in the normal range. Patients who previously had ovary-related surgeries or who had systematic diseases were excluded from the study.

GnRH antagonist was used in controlled ovarian hyperstimulation (COH). COH was started on the third day of menstrual bleeding, and, on the same day, transvaginal ultrasound was performed. Antral follicle count (AFC) and antral follicle (AF) size were recorded. AF were divided into two groups according to their size: Group I in which AF >5 mm in size and Group II in which AF ≤5 mm in size.Recombinant-FSH were used in COH. Initial dosage was decided according to patient's age, ovarian reserve and response to prior stimulation regime (if applicable). When the dominant follicle size was 13-14 mm, 0.25 mg cetrorelix acetate or 0.25 mg ganirelix acetate were administered and continued until the hCG injection day. When at least 3 dominant follicles that were ≥17 mm in size were found, 10,000 IU hCG was injected to achieve follicular maturation. Follicles were aspirated transvaginally under ultrasound guidance.

All statistical calculations were performed using the NCSS 2007 (NCSS, Kaysville, UT, USA). Data are presented as mean and SD or percentage. Parametric variables were evaluated by chi-square test and Student's t-test, while non-parametric variables were evaluated by the Mann Whitney U-test. Correlations between variables were evaluated with the use of Spearman's correlation coefficient. Subsequently, where individual correlations achieved statistical significance, variables were entered into a linear regression model. Statistical significance was defined as $p<0.05$.

Results

Our findings indicated that there was not a statistical difference between treatment outcome (Table 1) or on pregnancy outcome between antral follicles according to their size (p=0.750) (Table 2). Group II had statistically positive relationship with total oocyte and mature oocyte numbers (Table 3). Weighted linear regression was used to evaluate associations between total oocyte number and Groups I and II; findings were R2=0.163 and p=0.011. Linear regression between total oocyte number, Group I and Group II revealed statistically positive association to Group I (p=0.003), while statistically no significant association to Group II (p=0.750). Weighted linear regression was again used to evaluate associations between mature oocyte number and Groups I and II; findings were R2=0.122 and p=0.028. Linear regression between mature oocyte number, Group I and Group II revealed statistically positive association to Group I (p=0.008), while statistically no significant association to Group II (p=0.621).

After COH, we divided the patients into two sub-groups according to the number of retrieved mature oocytes: >5 and ≤5 mm. We set 5 mature oocytes as a benchmark to achieve a success-

Table 1. Comparison of patients according to the size of antral follicle (AF).

	AF ≤5mm N=30	AF >5mm N=14	t	p
Total oocytes	11.03±6.59	13.71±5.38	-1.33	0.192
Mature ooctyes	8.7±5.24	10.64±5.2	-1.15	0.257
Immature oocytes	1±1.23	1.5±2.1	-0.99	0.326
Germinal vesicle	1.37±2.39	1.57±1.95	-0.28	0.781
O Embryo	5.7±4.46	7.29±5.05	-1.05	0.298
Grade I Embryo	3±2.94	3.64±2.59	-0.70	0.487
Transferred Embryos	1.73±0.87	1.29±0.61	1.73	0.09
Infertility period (years)	6.95±3.13	6.93±1.67	0.03	0.977
Fertilization Rate	0.53±0.30	0.54±0.27	-0.51	0.960

Table 2. Comparison of pregnancy outcome according to the size of antral follicle (AF).

	AF ≤5mm N=30		AF >5mm N=14		
Positive	23	76.7%	10	71.4%	x^2:0.14
Pregnancy Negative	7	23.3%	4	28.6%	p=0.709

Table 3. Correlation of antral follicle size and antral follicle count (AFC) with number of total ooctyes, number of mature ooctyes, fertilization rates and number of grade 1 embryo.

		Total Oocytes	Mature Ooctyes	Fertilization Rate	Grade I Embryo
AFC	r	0.234	0.269	0.079	0.238
	p	0.125	0.078	0.626	0.119
AF >5mm	r	0.444	0.4	0.246	0.247
	p	0.003	0.007	0.126	0.106
AF ≤5mm	r	-0.016	0.018	-0.115	0.122
	p	0.921	0.910	0.486	0.436
AF >5/AFC	r	0.212	0.134	0.081	-0.025
	p	0.168	0.386	0.621	0.871
AF ≤5/AFC	r	-0.21	-0.131	-0.088	0.043
	p	0.170	0.395	0.591	0.782

ful ovarian response.

Under the area of ROC curve estimations revealed that AF >5 mm had the largest area 0.675±0.083 (0.517-0.808). On the other hand, since the area is less than 0.700, predictive value is not very strong.

Discussion

In our study, we tried to find which AF size provides a more successful IVF outcome. Thus, we found that patients who have AF that are equal to or less than 5 mm will respond poorly to gonadotropin treatment

Ovarian reserve is closely related to number and quality of oocytes and indicates a woman's reproductive potential. Negative effect of lessened ovarian support on fertility, provided the 'ovarian reserve' term, which was first used by Navot et al in a study in 1987 [10]. There are several tests to evaluate ovarian reserve of infertile patients. Some of them are simple blood tests and ultrasound scans (such as FSH, estradiol (E2), inhibin-B, anti-müllerian hormone (AMH), FSH, Luteinizing hormone(LH), ovarian volume, AFC), while some are provocative tests (such as clomiphene challenge Test (CCT), exogenous FSH ovarian reserve test (EFFORT), gonadotrophin stimulation test (GAST). On the other hand, studies show that several tests used to evaluate the ovarian response have poor predictive value [3,11]. Basal FSH seems to be convenient to predict low ovarian response in only women with very high values [12]. Basal E2 is also argued to have low predictive value in evaluation of low ovarian reserve in a review consisting of 10 studies. A review by Broekman et al., which included results of 9 studies, argued that 3rd day measure of inhibin-B values may cause exclusion of wrong patients from the treatment due to high rate of false-positive results. Among all tests, AFC and AMH are found to be the most successful in predicting the response to IVF treatment [3,6,9,13,14].

A number of studies that concentrate on AFC argue that AFC declines with age [13,15,16]. A study by Haadsma et al indicated that small AF (2-6mm) declined with age, while large AF (7-10mm) did not change with age [8]. In our study, average age of two AFC groups that were divided according to size, did not have a statistically significant difference (p=0.702). Moreover, we did not see any change in total AFC, AF ≤5 mm in size and AF >5 mm in size with age.

Optimum size of AF that can be included in AFC is still controversial. Jayakprakasan et al. argued that AFC should include AF that are 2-6 mm in size because it represents the ovarian reserve well [9]. In a study done by Pohl et al., a significant increase was found in pregnancy rates in patients that had a high AFC and AF that are 5-10 mm in size[17]. In our study, we classified AF as >5 mm or ≤5 mm. When an association between ovarian reserve, total AFC, AF >5 mm and AF ≤5 mm were evaluated, we found statistically positive association between AF >5 mm, number of retrieved oocytes and number of mature oocytes. Several studies concentrated on a single AF size/range, when investigating the relation between AFC and ovarian reserve. For instance, Chang et al. and Bansci et al. concentrated on AF that are 2-5 mm, Nahum et al. on AF that are 2-6 mm, Frattarelli et al. and Hsieh et al. on AF that are 2-10 mm [7,12,13,18,19]. Since every patient has both small and large AF, it might be important to study different sizes of AF and their effect on the treatment outcome. Study of Jayaprakasan et al. is one of the first to include AF that are in different sizes [9]. We also included different sizes of AF in our study, but our results differed from Jayaprakasan et al. as we found a positive association between AF >5 mm, number of retrieved oocytes and number of mature oocytes. Our findings indicate that AF >5 mm has a stronger association than AF ≤5 mm with both retrieved oocytes and mature oocytes. When studies on ovarian reserve are considered, one other controversial subject is how to define ovarian response. Some studies considered poor response as canceled cycles [20,21]. On the other hand, if patients with canceled cycles are regarded as poor responders, then patients who respond very poorly to gonadotropin treatment, but whose cycles were not canceled, could be wrongly included into the group in which patients respond well to the treatment.

In our study, we tried to find which AF size provides a more successful IVF outcome. For this purpose, we defined poor response as having five or less than five mature oocytes. We compared poor and good responders' AFC, AF ≤5 mm and >5 mm, AFC (>5 in size) /AFC, AFC (≤ 5 mm in size) /AFC, age, FSH and E2 levels. In contrast to Jayaprakasan et al. [9], AFC that is bigger than 5 mm was significantly higher in the good responders than the poor responders in our study (p=0.023). We argue that AF that are bigger than 5 mm has a strong association with ovary's good response to COH. Deb et al. found a significant difference in the measurements of AFC when two-dimensional versus three-dimensional ultrasound is used [22]. This might explain the difference in findings as we used two-dimensional ultrasound, while Jayaprakasan et al. [9] used three-dimensional ultrasound.

Predictive value of AFC for ovarian reserve was measured by the area under the ROC curve of AFC and AF sizes. Area under the ROC curve of AF≤ 5 mm was the largest (AUC:0.675) , yet there was not a statistical difference. A patient, who has AF that is equal to or less than 5 mm in size, is 1.74 more than likely to have less than five mature ooctyes (sensitivity=64%; specificity=63.2%). In light of these findings, we can argue that patients who have AF that are equal to or less than 5 mm will respond poorly to gonadotropin treatment. On the other hand, because of low sensitivity and specificity rates, to simply exclude such patients from the treatment may not be the best mode of action. The major limitation of the study were that we were unable to obtain larger group whose antral follicle size over 5 mm. The research should be further carried out using an enlarged sample size.

Conclusion

There might be an association between AFC that is larger than 5 mm in size and number of retrieved mature oocytes. Since AFC, is a routine screening test in any IVF center, we believe paying closer attention to the measurement of AFC that are larger than 5 mm in size might help clinicians to achieve more successful IVF outcomes in terms of increased number of oocytes, embryos and pregnancy rates.

Competing interests

The authors declare that they have no competing interests.

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