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## **BASAL ANTRAL FOLLICLE COUNTS BY TRANSVAGINAL ULTRASONOGRAPHY FOR THE PREDICTION OF OVARIAN RESPONSIVENESS AND PREGNANCY RATES IN IVF/ICSI TREATMENT CYCLES**

### **BROJ BAZALNIH FOLIKULA TRANSVAGINALNOM ULTRASONOGRAFIJOM ZA PRETKAZIVANJE OVARIJSKOG ODGOVORA I STOPE TRUDNOĆE U IVF/ICSI CIKLUSIMA**

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*Original paper*

*Key words:* basal antral follicle count, ovarian response, pregnancy rates, ultrasound

**SUMMARY.** *The aim* of this study is to determine the predictive capacity of BAFC measurements in the prediction of IVF outcome with regard to both ovarian response and pregnancy rates. *Methods.* Transvaginal ultrasound was performed on day 2–3 of the stimulation cycle, after ovarian down-regulation and before beginning treatment with gonadotropins. All ovarian follicles measuring 2 mm to 10 mm on both ovaries were counted by a single investigator. Patients were examined vaginally with an ATL transvaginal transducer (HDI 5000 diagnostic ultrasound system; Bothell, WA; USA). In total, 85 patients aged 24–42 years who underwent IVF/ICSI cycles were included in this prospective study. The patients were divided into two groups: group 1 patients who had basal antral follicle count  $\leq 10$  and group 2 patients who had basal antral follicles  $>10$ . *Results.* Basal antral follicle count correlated directly with number of follicles, number of mature oocytes, number of embryos and peak  $E_2$  levels. We failed to show any significant effect of basal antral follicle count on pregnancy rates. Furthermore, basal antral follicle count correlated inversely with ampoules of gonadotropin used, days of stimulation, patient age, and day 3 FSH levels. Nevertheless, transvaginal ultrasound ovarian measurements can predict poor response, thereby allowing physicians to inform patients that they are at an increased risk for poor stimulation and cycle cancellation. Physicians can then potentially optimize stimulation protocols. *Conclusion.* Based on the results of this study, we suggest using a higher starting dose of gonadotropin in women with an antral follicle count of  $\leq 10$  and a lower starting dose of gonadotropin in women with an antral follicle count of  $>10$ .

*Izvorni članak*

*Ključne riječi:* bazalni antralni folikuli, ovarijalni odgovor, stopa trudnoća, ultrazvuk

**SAŽETAK.** *Cilj* ovog istraživanja je odrediti valjanost mjerenja bazalnih antralnih folikula u pretkazivanju ishoda IVF glede odgovora ovarija i stopa trudnoća. *Metode.* Transvaginalni ultrazvuk je rađen 2.–3. dana stimuliranoga ciklusa, nakon ovarijalne »down-regulation« i prije početka terapije gonadotropinima. Isti pretraživač je u oba jajnika izbrojio sve folikule veličine 2–10 mm. Pacijentice su pregledavane vaginalno pomoću ATL aparata (HDI 5000 diagnostic ultrasound system; Bothell, WA; USA). Ukupno je 85 pacijentica dobi 24–42 godine bilo podvrgnuto IVF/ICSI postupku. Pacijentice su bile podijeljene u dvije skupine: 1. skupina s  $\leq 10$  antralnih bazalnih folikula; i 2. skupina s  $>10$  antralnih folikula. *Rezultati.* Broj bazalnih antralnih folikula je direktno korelirao s brojem folikula, brojem zrelih oocita, brojem postignutih embrija i vrškom  $E_2$ . Nismo mogli pokazati znakovit efekt broja bazalnih antralnih folikula na stopu trudnoća. Nadalje, broj bazalnih antralnih folikula je bio obrnuto proporcionalan s brojem uporabljenih ampula gonadotropina, danima trajanja stimulacije, dobi pacijentica i razinom FSH-a trećeg dana. Štoviše, transvaginalno ultrazvučno mjerenje jajnika može pretkazati loš odgovor, što omogućuje liječniku da informira pacijenticu o mogućnosti nedovoljne stimulacije te da odgodi taj ciklus. Liječnik tada može optimalno primijeniti protokol stimulacije. *Zaključak.* Na temelju rezultata ovog istraživanja, preporučujemo uporabu više početne doze gonadotropina u žena s brojem antralnih folikula  $\leq 10$  i nižu početnu dozu gonadotropina u žena s brojem antralnih folikula  $>10$ .

## Introduction

Evaluation of ovarian reserve has been the focus of much clinical research over the past several years.<sup>1–12</sup> Knowing that there is a high risk of a poor outcome of the IVF treatment may help physicians as well as patients decide to withdraw from IVF treatment and to search for alternatives, such as adoption or oocyte donation. To prevent patients from being wrongly assigned to poor prognosis group, the markers that will be used for the prediction of a poor prognosis need to be highly specific. It would be clinically as well as economically helpful if there were accurate methods to predict poor prospects of pregnancy, before entering IVF treatment. Assessment of ovarian reserve is valuable to determine stimulation protocols and predicting ART outcome.

Many tests have been evaluated to predict cycle outcome. Although age is associated with ovarian reserve and responsiveness, age alone is a weak predictor of IVF success.<sup>2–5</sup> A variety of hormonal markers has been used to predict poor ovarian reserve in infertile women. Cycle day 3 FSH, E<sub>2</sub>, and inhibin B, as well as challenge tests, have been attitude for their predictive value.<sup>6–12</sup> However, despite its increasing acceptance in clinical practice, these tests are far from perfect in predicting ovarian response, cancellation rates, and ultimately, who will or will not get pregnant. Several studies have demonstrated the predictive value of the hormonal tests described above.<sup>5–13</sup> Despite its increasing acceptance in clinical practice, the tests have a variety of shortcomings, including a lack of predictive value of a normal result. Investigators therefore continue the search for other markers to identify patients whose ovarian reserve is insufficient for conception. It would be clinically as well as economically helpful if there were accurate methods to predict poor prospects of pregnancy, before entering IVF treatment. Measuring the volume or maximal diameter of both ovaries or counting the number of small antral follicles by ultrasound has been studied as a noninvasive and more accurate test in comparison to the endocrine ovarian reserve tests.<sup>13–16</sup> A low number of small antral follicles is associated with decreased ovarian response during controlled ovarian hyperstimulation for IVF, supporting the concept of reduced numbers of primordial follicles delivering a small antral follicle cohort. Moreover, Chang et al.<sup>15</sup> reported a trend toward lower pregnancy rates in women with few antral follicles. However, this trend lacked statistical significance. The predictive capacity of AFC for the outcome in IVF patients has not been assessed systematically. There are a limited number of studies that assessed the performance of AFC in predicting the occurrence of non pregnancy and/or poor response. Unfortunately, most of these studies report on a small number of patients. The aim of this study is to determine the predictive capacity of BAFC measurements in the prediction of IVF outcome with regard to both ovarian response and pregnancy rates.

## Materials and methods

Between June 2004 and June 2007, a total of 85 patients at the IVF Unit, The Sistina Medical Center were found to be eligible for this prospective study. All couples previously had been evaluated by day 3 hormones levels, preovulatory US evaluation, hysterosalpingography, semen analysis, and hysteroscopy and laparoscopy, if indicated. The age range of the women was 22–42 years. The predominant diagnoses were male factor, tubal factor or unexplained infertility.

The inclusion criteria were that the participant had a normal basal FSH level per our laboratory (<14 mIU/mL), presence of both ovaries (ability to visualize both ovaries on transvaginal ultrasonography) and absence of ovarian abnormalities or ovarian cysts >10 mm. All patients were included in this study only once to avoid selection bias.

Exclusion criteria were history of ovarian or uterine surgery, distortions of the uterine cavity or apparent endometrial pathologies (submucous myoma, synechia, polyps, Sy PCO) etc. Women with a or who had had three more failed attempts at IVF and embryo transfer or who received frozen–thawed embryos were not included.

### Basal antral follicle measurement

The main outcome measure was the number of oocytes recovered. Transvaginal ultrasound was performed on day 2–3 of the stimulation cycle, after ovarian down-regulation and before beginning treatment with gonadotropins. All ovarian follicles measuring 2 mm to 10 mm on both ovaries were counted by a single investigator. The total number of follicles per patient was used for calculations.

All patients were examined after spontaneous emptying of the urinary bladder, lying supine with the knees slightly bent and with a small pillow under the buttocks. Patients were examined vaginally with an ATL (HDI 5000 diagnostic Ultrasound system; Bothell, WA; USA) transvaginal transducer. Wall filter was set to 25–50 Hz, and the limit of aliasing 1 cm/sec. One sonographer performed all sonographic measurements.

*Women undergoing ovulation induction were routinely down-regulated with triptorelin acetate (Decapeptyl, 0.1 mg; ER-KIM, Ilac San.; 0.1 mg/d) or buserelina acetate SC (Suprefact 7ml; Aventis Pharma GmbH; 0.5 mg/d) starting from the 21st day of the preceding cycle in long down-regulation protocol and from the 2nd day of the cycle in the short down-regulation protocol. The analogue was continued until the day of hCG. After the down-regulation, ovulation induction was performed by daily injections of 150–300 IU of recombinant FSH (Follitropin alfa; Gonal-f; Serono).*

The ovulation was triggered by 10,000 IU of hCG (Profasi, 5,000 IU ampule, Serono; or Pregnyl, 5,000 IU amp, Organon Ilac San.), when there were at least two leading follicles with a diameter of >18 m. After 34–36 hours, egg collection was performed by transvaginal ul-

trasound. In vitro fertilization or intra-cytoplasmic sperm injection and embryo transfer was performed for all the patients. Luteal phase support was performed by progesterone (Utrogestan, 100 mg capsules, two vaginal capsules three times per day; Besins-I'Scovesco Lab., Paris, France). Embryo transfer was performed 2-5 days after the oocyte aspiration. Embryos were classified as previously reported<sup>17,18</sup> as follows: grade 1: perfectly symmetrical with no fragmentation; grade 2: perfectly symmetrical with slight fragmentation (<20% fragmentation of the total embryonic volume); grade 3: uneven blastomeres with gross fragmentation (>20% fragments). Embryos of Veeck grades 1 or 2 were considered high quality.

Two to five days after oocyte retrieval, usually three, but occasionally two or four embryos per patient were replaced depending on the age of the patient, the indication for IVF, the number of previous attempts, and the number and quality of embryos available for replacement.

Clinical pregnancy was defined as the presence of gestational sac by ultrasound with appropriately rising  $\beta$ -hCG levels. Miscarriage was defined as pregnancy loss before 12 weeks of gestation.

### Statistical analysis

The collected data were input into a computer database, pre-defined according to a specially prepared form and software for the needs of the study. The data processing as well as their analysis was done with the statistical software »Statistica for Windows/Release 7.1«.

Data were analyzed by analysis of variance to test the significance of means among groups, and  $\chi^2$  test was used to assess the significance of categories parameters and pregnancy among groups. A multiple regression analysis was performed for numerically dependent variables and a multiple logistic regression analysis was used for a model with binomial outcomes.

Table – Tablica 1. Age of patients / Dob pacijentica

N	Mean	Confidence – 95.0%	Confidence +95.0%	Min	Max	SD
85	33.45	32.49	34.41	24.0	42.0	4.45

Univariate analysis included regression and correlation coefficients examining the association of antral follicle count with measures of ovarian reserve and response. Logistic regression was used to compare binomial outcome rates with antral follicle count. Data are reported as means ( $\pm$  SD).  $P < 0.05$  was considered significant.

### Results

In total, 85 patients aged 24-42 years were included in the study (Table 1).

The patients were divided into two groups according to the total number of basal antral follicles; group 1 patients who had antral follicles  $\leq 10$  (N=65) and group 2 patients who had antral follicles  $> 10$  (N=20).

There were no significant differences between either group in patients' age, basal levels of LH and E2 and down-regulation days (Table 2). No cycle was canceled after initiation of gonadotropin stimulation.

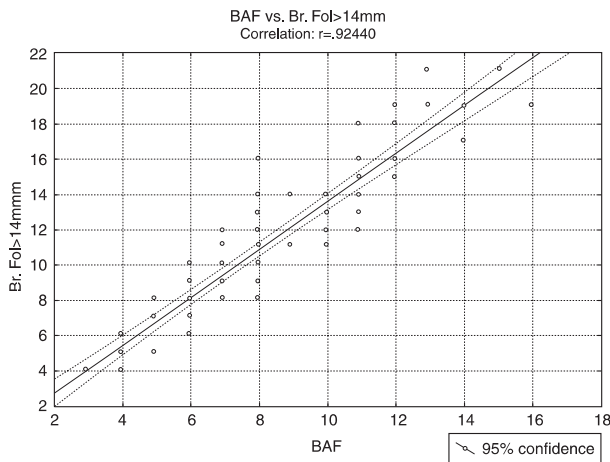
A total of 29 pregnancies resulted; a crude pregnancy rate was 29/85 (34.12%). Of these 6/29 (20.69%) were spontaneous abortions.

There was a significantly higher basal level of FSH in group 1 versus group 2 ( $8.16 \pm 2.25$  mIU/mL vs.  $6.97 \pm 1.58$  mIU/mL,  $P < 0.05$ ,  $t$  2.19). There were significantly more stimulation days and total gonadotropin ampoules needed in group 1 versus group 2 ( $12.31 \pm 2.48$  days vs.  $9.75$  days  $\pm 1.25$ ,  $p < 0.05$ , and  $36.17 \pm 8.97$  vs.  $31.45 \pm 7.34$ ,  $P < 0.05$ ). There was a significantly higher number of growing follicles of  $\geq 14$  mm at the time of hCG injection in group 2 versus group 1 and the number of oocytes retrieved at follicular aspiration ( $9.00 \pm 3.03$  vs.  $16.65 \pm$

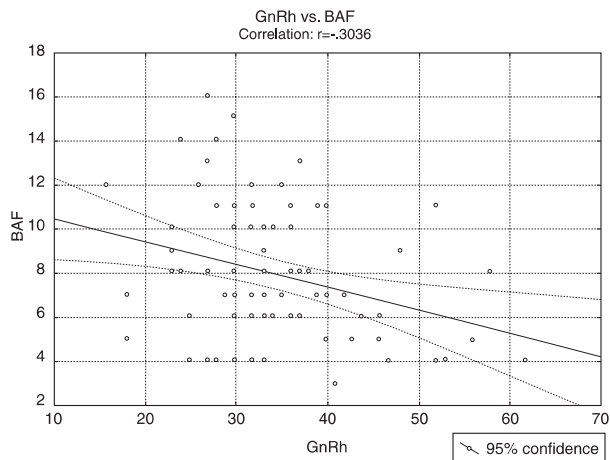
Table 2. The number of BAF. Differences between groups  
Tablica 2. Broj bazalnih antralnih folikula. Razlika među grupama

Parameter	Number of BAF		t	Z	p
	Gr. 1 $3-10$ (n=65)	Gr. 2 $>10$ (n=20)			
Age	$33.66 \pm 4.56$	$32.75 \pm 4.10$	0.79		0.43
FSH 3 day mIU/mL	$8.16 \pm 2.25$	$6.97 \pm 1.58$	2.19		0.03*
LH 3 day mIU/mL	$5.93 \pm 2.77$	$5.91 \pm 1.95$		-0.43	0.67
E <sub>2</sub> pmol/L	$35.26 \pm 13.21$	$30.69 \pm 7.31$		1.45	0.15
Gonadotropin ampoule / N <sup>o</sup>	$36.17 \pm 8.97$	$31.45 \pm 7.34$		-1.91	0.05*
Days of stimulation	$12.31 \pm 2.48$	$9.75 \pm 1.25$		-2.43	0.03*
N <sup>o</sup> of follicles $>14$ mm	$9.00 \pm 3.03$	$16.65 \pm 2.70$		-6.26	0.000***
N <sup>o</sup> of oocytes	$6.55 \pm 2.21$	$12.80 \pm 2.39$		-6.50	0.000***
E <sub>2</sub> pik pmol/L	$1535.86 \pm 661.62$	$2883.50 \pm 1090.07$	-6.75		0.000***
N <sup>o</sup> ET	$2.71 \pm 0.74$	$3.15 \pm 0.67$		-2.09	0.04*
Pregnancies %	22 (33.85%)	7 (35.00%)			0.92

\*  $p < 0.05$ ; \*\*\*  $p < 0.001$



Graf 1. BAF vs N° oocytes  $\geq 14$   
Grafikon 1. BAF prema broju oocita  $\geq 14$



Graf 2. Gonadotropin ampoules vs BAF  
Grafikon 2. Ampule gonadotropina prema BAF

2.70,  $P < 0.01$ ;  $6.55 \pm 2.21$  vs.  $12.80 \pm 2.39$ ,  $P < 0.001$ ). However, women with antral follicles of  $\leq 10$  have a lower  $E_2$  peak level on day of administration of hCG ( $1535.86$  vs  $2883$ ,  $P < 0.01$ ).

There was a significant difference on the number of embryos transferred between either group ( $2.71 \pm 0.74$  vs.  $3.15 \pm 0.67$ ,  $P < 0.04$ ). There was not significant difference in pregnancy rates (33.85% vs. 35%,  $P = 0.92$ ) in group 1 versus group 2.

The multiple regression analysis indicated that the total antral follicle count was negatively correlated with total gonadotropin dose (ampoules) ( $r = -0.30$ ;  $p < 0.05$ ) meaning that for each number of antral follicles  $\leq 10$  women will need a 30% higher total gonadotropin dose (Graf 2). Also multiple regression analysis indicated that number of basal antral follicles was negatively correlated with the days of stimulations ( $r = -2.43$ ;  $p < 0.05$ ) and number of oocytes  $\geq 14$  ( $r = 0.92$ ;  $p < 0.05$ ) (Graf 1).

In the present study, our data showed that on day 2–3 of stimulated cycle the number of antral follicles did not

Table 3. Day of ET and pregnancy rates  
Tablica 3. Dan embriotransfera i stopa trudnoća

Day of ET	$\beta$ HCG +	$\beta$ HCG –	Total
3+4	13	47	60
	15.29	55.29	70.59
5	16	9	25
	18.82	10.59	29.41
Total	29	56	85
	34.12	65.88	100

predict pregnancy rates in IVF/ICSI-ET cycles. No cycle was canceled due to poor response despite the fact that 76.4% of our patients had an antral follicle count of  $\leq 10$  (range 3–10).

Unexpectedly, the quality of embryos and day of ET were not affected by the low antral follicle count. Obviously, the pregnancy rate is influenced by many factors other than antral follicles count alone. We found in this study that cycle outcome was strongly correlated with good-quality embryos. Grade 1 embryos and day of ET ( $\chi^2 = 14.48$ ;  $p < 0.001$ ) were the strongest factors have an impact on pregnancy rates (Table 3). Significantly higher pregnancy rate was achieved after transfer of blastocysts compared to cleavage-stage embryos

## Discussion

The number of human oocytes in both ovaries decreases irreversibly after its formation during fetal life.<sup>19</sup> The total number of oocytes is approximately 6–7 million at 16–20 weeks of gestation, 1–2 million at birth, and 300,000 at the onset of puberty. The store of follicles decreases steadily throughout a woman's reproductive years at an assumed rate of attrition of 1,000 per menstrual cycle. This rate is believed to be even higher between 37.5 years of age and menopause.<sup>20</sup> The number of follicles leaving the pool of resting follicles to enter the growth phase toward the antral stages of development decreases with increasing age.<sup>21</sup>

It would be helpful to have a reliable test that would tell us how many eggs a woman has remaining at a point in time – as well as telling us about the quality of those eggs. The term »ovarian reserve« is useful in the field of reproductive medicine. In other words, a woman's ovarian reserve is her remaining fertility potential.

A variety of hormonal markers has been used to predict poor ovarian reserve in infertile women. Cycle day 3 FSH,  $E_2$ , and inhibin B, as well as challenge tests, have been studied for their predictive value. However, despite their increasing acceptance in clinical practice, these tests are far from perfect in predicting ovarian response, cancellation rates, and ultimately, who will or will not get pregnant.<sup>1–12</sup>

Transvaginal ultrasonography is an accurate tool for evaluating the ovary. Measuring the volume or maximal diameter of both ovaries or counting the number of small antral follicles by ultrasound has been studied as a noninvasive and more accurate test in comparison to the



endocrine ovarian reserve tests.<sup>13–16</sup> Frattarelli<sup>16</sup> and Saxton<sup>22</sup> in his studies demonstrated that variations in ovarian measurements between examiners are very small. Therefore, results of basal transvaginal ultrasonography are valuable in counseling women before starting ART cycle.

Similar to previous reports on ovarian reserve, we found that basal antral follicle count (BAFC) is specific but not sensitive. This characteristic makes BAFC a good tool for counseling patients, but it cannot be used to predict success or failure with 100% accuracy. We also found that basal antral follicle count correlated with the measures currently used to assess ovarian reserve but not correlated with pregnancy outcome. These findings correspond to those reported by Hendriks<sup>23</sup> and Klinkert<sup>24</sup> and don't correspond to those reported by Nahum<sup>25</sup> and Saleh.<sup>26</sup>

We used multiple methods to attempt to limit bias. All data were collected prospectively after obtaining patient consent. Ultrasonography was performed by one investigator. Similar to other studies,<sup>16,23</sup> we found that antral follicle number is a better prognostic indicator than age or endocrine markers. In patients undergoing IVF-ET program, our data also show that the antral follicle count correlates well with ovarian response to exogenous gonadotropin stimulation and proportionately with the number of oocytes and the number of embryos transferred in the same cycle. As expected, the number of antral follicles was found to be significantly correlated with the total number of follicles  $\geq 14$  mm at hCG injection and this led to a significant correlation with the number of oocytes. These findings were in agreement with those previously reported.<sup>13–16,23–26</sup> To examine further the effect of antral follicle count on pregnancy rates, we also performed a multivariate logistic regression analysis on the success of pregnancy, while controlling for the number of oocytes. We failed to show any significant effect of basal antral follicle count on pregnancy rates. The antral follicle count significantly predicted the number of oocytes and embryos, but not the quality of the oocytes and embryos or the success of pregnancy.

Obviously, the pregnancy rate is influenced by many factors other than antral follicles alone. We found in this study that cycle outcome was strongly correlated with good-quality embryos and the day of embryo transfer. Pregnancy, however, is largely dependent on oocyte quality, although other factors also probably will play a role.<sup>23,24</sup>

In summary basal antral follicle count correlated directly with number of follicles, number of mature oocytes, number of embryos and peak  $E_2$  levels. Furthermore, basal antral follicle count correlated inversely with ampoules of gonadotropin used, days of stimulation, patient's age, and day 3 FSH levels.

## Conclusion

It is easy to count the number of antral follicles that measure 2–10 mm just before the administration of ex-

ogenous gonadotropins. Nevertheless, transvaginal ultrasound ovarian measurements can predict poor response, thereby allowing physicians to inform patients that they are at an increased risk for poor stimulation and cycle cancellation. Physicians can then potentially optimize stimulation protocols.

Based on the results of this study, we suggest using a higher starting dose of gonadotropin in women with an antral follicle count of  $\leq 10$  and a lower starting dose of gonadotropin in women with an antral follicle count of  $> 10$ .

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**1<sup>st</sup> Session. Epidemiology and new data:** A. Baldredeen, Qatar • C. Derom, Belgium • F. Goffinet, France • T. K. Lau, Hong Kong • B. Luke, USA • M. Merialdi, Switzerland • N. Sebire, United Kingdom;

**2<sup>nd</sup> Session. Risk factors, PMA contributions, diagnosis:** P. Barri, Spain • V. Englert, Belgium • I. Ghi, Italy • N. Manning, United Kingdom • G. Monni, Italy • R. Romero, USA • M. Walker, Canada;

**3<sup>rd</sup> Session. Clinical issues, Management:** A. Antsaklis, Greece • G. Clerici, Italy • I. Deprest, Belgium • G. C. di Renzo, Italy • T. Frusca, Italy • E. Gratacos, Spain • K. Hecher, Germany • K. Nicolaidis, United Kingdom • R. Quintero, USA • Y. Ville, France;

**4<sup>th</sup> Session. Follow up. Psycho correlates. Ethics:** B. Arabin, The Netherlands • M. I. Bastian, USA • V. Carnielli, Italy • T. Tuan, China • F. Fachinelli, Italy • M. Kilby, United Kingdom • A. Kurjak, Croatia • U. Simeoni, France

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