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Does Peri-Follicular Blood Flow on the day of Ovum Pick-up Predict the Likelihood of Retrieving an Oocyte?

Yumurta Toplama Günü Perifoliküler Kan Akımı Oosit Toplama Şansını Tahmin Edebilir mi?

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Abstract

Objective: To determine whether peri-follicular blood flow predicts pick up of an oocyte at egg retrieval.

Patients and Methods: A prospective cohort study was made on forty-seven women undergoing in vitro fertilization treatments in a university hospital. Peri-follicular blood flow was analyzed on the day of egg retrieval. Two groups were formed regarding the presence or absence of oocyte during retrieval. Oocyte grades in different peri-follicular blood flow groups were compared.

Results: There was no significant difference between the groups regarding the perivascular blood flow per follicle. The oocyte grade did not differ between follicles with different peri-follicular vascularity.

Conclusions: It appears that high peri-follicular vascularity on the day of egg retrieval does not predict the likelihood of obtaining an oocyte. High peri-follicular vascularity does not confirm good egg quality even if an egg could be collected.*(Marmara Medical Journal 2012;25:118-22)*

Key Words: Blood flow, Peri-follicular vascularity, Egg quality, Egg retrieval

Özet

Amaç: Yumurta toplama günü folikül etrafı kan akımının oosit çıkma olasılığını öngörebilebilmesini araştırmak.

Hastalar ve Yöntem: Bir üniversite hastanesinde in vitro fertilizasyon tedavisi alan 47 kadın prospektif kohort çalışmaya dahil edildi. Folikül etrafı kan akımı yumurta toplama günü değerlendirildi. Toplama sırasında yumurta çıkma veya çıkmamasına göre iki grup belirlendi. Farklı perifoliküler kan akım gruplarına göre oosit sınıflamaları karşılaştırıldı.

Bulgular: Gruplar arasında folikül başına perifoliküler kan akımında fark görülmedi. Farklı perifoliküler kanlanma gruplarında oosit sınıflaması değişmedi.

Sonuç: Yumurta toplama günü yüksek perifoliküler kanlanma yumurta elde etme şansını öngöremez. Yüksek perifoliküler kanlanma yumurta toplanabilse dahi kalitesini belirleyemez. *(Marmara Üniversitesi Tıp Fakültesi Dergisi 2012;25:118-22)*

Anahtar Kelimeler: Kan akımı, Perifoliküler kanlanma, Yumurta kalitesi, Yumurta toplama

Introduction

There is no single factor(s) secreted into the circulation or present in the follicular fluid predicting the developmental competence of the oocyte-embryo. Analysis of follicular fluid biochemistry (content of dissolved O2, growth factors, and pH), in vitro granulosa cell behavior (presence of metabolic products and regulatory proteins), and peri-follicular blood flow and correlation of the results with various oocyte-embryo developmental capacities under in vitro conditions and after uterine embryo transfer (ET), have been reported[1-5].

Blood flow increases around developing follicles during the follicular phase in ovarian stimulation[6]. Colour Doppler indices (CDI) of follicular blood flow are correlated with oocyte recovery[4,7], fertilization rate[4], developmental potential of the oocyte[1,8] and pregnancy rate[9] of in vitro fertilization (IVF) treatment. Power Doppler is more sensitive than the conventional CDI, enables flows with lower volumes and velocities to be displayed and can display perfused regions where the mean velocity is zero[10].

Patients who have follicles with good vascularity shown by power Doppler scanning are associated with better pregnancy rates following IVF[5,11].

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Oocyte competence[2,4,12] and consequent embryo quality[4,5,11] are the crucial factors in determining the outcome of the IVF cycles. Peri-follicular blood flow is correlated with oocyte recovery[4,12], implantation potential[13], and pregnancy[5,9,11]. The objective of our study was to evaluate whether peri-follicular blood flow predicts retrieving oocyte at egg retrieval in intracytoplasmic sperm injection (ICSI) cycles.

Patients and Methods

Forty-seven women undergoing consecutive IVF treatments at the Assisted Reproductive Techniques (ART) Unit at Marmara University Hospital in Istanbul, Turkey, were recruited in a prospective cohort study. The study was approved by the Medical School Local Research Ethics Committee. All practices and protocols conformed to the ethical requirements for assisted reproductive technology programs of the Ethics Committee of Marmara Medical School and it conforms to the provisions of the Declaration of Helsinki. A detailed explanation, both verbal and written, was given to the patients prior to recruitment. Consent to participate in the study was obtained from each patient.

The standard protocol used was a gonadotropin-releasing hormone (GnRH), agonist down-regulated, long luteal protocol. Buserelin acetate 0.2 mL (Lucrin daily, Abbott, Turkey) was administered by subcutenous injection starting on the 21st day of the previous cycle. Ovarian stimulation was started after low levels of oestradiol (E2) and luteinizing hormone (LH) had been confirmed, usually after at least 10 days of buserelin. Ovarian stimulation was accomplished with recombinant follicle stimulating hormone (FSH), preparations, either Gonal F (Merck-Serono,Turkey) or Puregon (Schering-Plough,Turkey) at starting doses between 150 and 300 IU per day. Dose adjustments were individualized after 5 days of ovarian stimulation. Oocyte maturation was induced by human chorionic gonadotropin (HCG, 10,000 IU Pregnyl, Schering-Plough, Turkey) when the two leading follicles were 18 mm in mean diameter. The GnRH agonist was continued till the day of HCG injection.

All patients underwent a transvaginal ultrasound documenting antral follicle count (AFC) on day 3 of the cycle (CD3) before their stimulation. All measurements were performed on one ultrasound machine (General Electric Loqic 200, 8-4 MHz, Istanbul, Turkey). The ultrasonographic intensity which was <50 mW/cm^2 was within the safety limits approved by the American Medical Ultrasound Institute.

AFCs measured all follicles between 2 and 8 mm on each ovary. On that day, serum samples were collected in the morning after a 12 h fast. Serum FSH and LH levels were measured by an electrochemiluminescent immunoassay (ECLIA) kit (Elecsys systems 1010/2010/modular Analytics E170 (Elecsys module), Roche Diagnostic Gmbh, D-68298, Mannheim/Germany). E2 concentration was determined by an immulite chemiluminescent competitive immunoassay kit (DPC, Los Angeles).

Follicles were aspirated 34 hours after HCG administration under guided vaginal sonography, using a single lumen 17-gauge needle (Cook, Tekservis Ltd, Turkey) with the patient in a sedated but conversant state. The follicles were not flushed; aspiration was done once per follicle. Approximately 2-3 h before oocyte collection, each patient underwent a transvaginal ultrasound scan. The vascularity of various follicles of different diameters were examined depending on the patient's ovarian response. The vascularity of each follicle studied was subjectively graded by an experienced operator at the time of the scan using power Doppler image. The grading system consisted of assessing the percentage of the follicular circumference in which most flow was identified from a single cross-sectional slice. The grading system was as follows: F1 = <25% of circumference in which flow was identified; F2=26-50% of follicular circumference in which flow was identified; F3 = 51-75% of follicular circumference in which flow was identified ; F4 = 76-100% of follicular circumference in which flow was identified. In addition, the mean follicular diameter and the position within the ovary of each studied follicle were recorded. The follicles were mapped in longitudinal and transverse planes by the surgeon who was to perform the aspiration, and subjective cartoons were made of the location of the follicles in each ovary. Because of possible changes in ovarian architecture with each aspiration, at most four follicles were studied per ovary to ensure accuracy of localization and consistency of data. The intraobserver coefficient of variation has been shown to be 5% for peri-follicular vascularity and zero for peri-follicular grading.

At oocyte collection, all study follicles were identified and each one was aspirated separately into one test tube and was individually processed thereafter. Embryological processing was carried out without prior knowledge of the vascularity grading. Follicles were flushed with MOPS buffer (G-MOPS, Vitrolife, SISMED, Istanbul, Turkey). Cumulus-oocyte complexes were isolated from the follicular aspirates, washed with fertilization medium (G-IVF, Vitrolife, SISMED, Istanbul, Turkey), each placed individually in a 100 µl well of fertilization medium under parafin oil (Ovoil, Vitrolife, SISMED, Istanbul, Turkey) and incubated at 37 ^{0}C in a humidified atmosphere of 6% CO2, 5% O2. Human oocyte grading before intracytoplasmic sperm injection was done as follows: Grade 1, fragmented first polar body and large perivitelline space; grade 2, intact first polar body and large perivitelline space; grade 3, fragmented first polar body and normal perivitelline space; grade 4, intact first polar body and normal perivitelline space[14].

Patients were placed into two groups; women with no oocytes retrieved (NOR) from follicles and women with oocyte retrieved (OR) from follicle. Furthermore, follicles with F3 and F4 grades formed a high vascularity (HV) group and follicles with F1 and F2 made the low vascularity (LV) group. Oocyte retrieval rates between groups NOR and OR were calculated. Oocyte grades were compared between the LV and HV groups.

All analyses used StataSE 10.0 (Statacorp, Texas, USA). Statistical analysis was by Student's t-test on mean values and chi-square test on outcome rates related to vascularity and oocyte grades. P<0.05 was considered to be significant. The distribution of oocytes among F1-F4 groups was analyzed by using the Mann-Whitney U test. The Bonferroni adjusted level of significance needed to obtain statistical significance was calculated as p<0.0083 (0.05/6 = 0.0083).

Multivariable logistic regression modeling was used to compute the odds ratios (ORs) of variables predictive of oocyte retrieval from the follicle. The independent variables were age, body mass index, total dose of FSH administrated, CD3 FSH levels, CD3 E2 levels, serum E2 levels on the day of HCG, F1 vasculature and F4 vasculature.

Table I. The patient demographics

	N=47
Mean age (years)	28.97±3.61
BMI (kg/m^2)	23.00±2.55
Cycle day 3 FSH (IU/L)	5.14±1.83
Cycle day 3 estradiol (pg/ml)	44.98±17.23
Antral follicular count (AFC)	10.53±2.54
Duration of stimulation (days)	9.22±1.38
Total dose of gonadotropin (IU)	2044.27±666.01
Mean E2 level on HCG day (pg/ml)	2256.70±810.76
Male factor infertility	57.4%
Unexplained infertility	17%
Tubal infertility	25.6%

All values are shown as mean ±standard deviation
Infertility factors are given as percentages

Results

The patient demographics are shown in table I. A total of 350 follicles were prospectively analysed. Two groups were formed in terms of whether oocytes were retrieved (OR) or not (NOR). The percentage of follicles with HV was similar in both groups. So was the percentage of follicles with LV. When peri-follicular vasculature gradings were analysed separately, the percentage of follicles with F1 and F2 were similar between the OR and NOR groups. However, the OR group had higher percentage of follicles with F3 vasculature than the NOR group (Table II).

When oocyte grades were compared beween the HV and LV groups, there was no significant difference in percentage of distribution. There were 4 germinal vesicles (GVs) in the LV and 7 GVs in the HV groups (Table III). The same was true when oocytes were analysed separately regarding the follicular gradings. There were 2 GVs in the F4, F2 and F1 groups, and 5 GVs in the F3 group (Table IV).

Table II. The distribution of peri-follicular blood flow between the groups regarding whether an oocyte was retrieved or not

	Follicle from which no oocyte was retrieved (NOR) (n=242)	Follicle from which an oocyte was retrieved (OR) (n=108)	P value[a]
F4	75	29	0.42
F3	59	38	0.04
F2	60	24	0.60
F1	48	17	0.36
High peri-follicular blood flow	134	67	0.26
Low peri-follicular blood flow	108	41	0.24

All values are shown as numbers; p<0.05 is statistically significant; a χ^2 test

Table III. Distribution of oocytes in high and low peri-follicular vasculature groups

Oocyte grade	Peri-follicular vasculature		P value[a]
	Low vasculature	High vasculature	
Grade1	19 (46.34±0.50)	35 (52.24±0.50)	0.55
Grade2	8 (19.51±0.40)	14 (20.90±0.41)	0.86
Grade3	7 (17.07±0.38)	6 (8.96±0.29)	0.21
Grade4	3 (7.46±0.26)	5 (7.32±0.26)	0.98

Values are given as numbers and percentage ± standard deviation in parantheses a χ^2 test ; p<0.05 is statistically significant

Table IV. Distribution of oocytes in different peri-follicular vasculature groups

Oocyte grade	Peri-follicular vasculature			
	F4	F3	F2	F1
Grade1	19 (65.52±0.48)	16 (42.10±0.50)	11 (45.83±0.51)	8 (47.06±0.51)
Grade2	5 (17.24±0.38)	9 (23.68±0.43)	5 (20.83±0.41)	3 (17.65±0.39)
Grade3	1 (3.45±0.18)	5 (13.16±0.34)	3 (12.50±0.34)	4 (23.53±0.44)
Grade4	2 (6.89±0.26)	3 (7.89±0.27)	3 (12.5±0.34)	0

Values are given as numbers; percentage±standard deviation in parentheses
P value <0.0083 is statistically significant
For all comparisons p >0.0083

Table V. Multivariate analysis

Chance of retrieving an oocyte from the follicle	Odds Ratio	P value	95% confidence interval
Age	0.97	0.48	0.89-1.05
Body mass index	1.06	0.32	0.94-1.20
Total dose of gonadotropin used	1.00	0.55	0.99-1.00
Serum estradiol on HCG day	0.99	0.73	0.99-1.00
Day 3 FSH	0.91	0.37	0.74-1.12
Day 3 Estradiol	1.01	0.15	0.99-1.02
F4 vasculature	0.58	0.10	0.30-1.11
F1 vasculature	0.37	0.02	0.16-0.84

The multivariate analysis showed that F1 vasculature decreased the chance of retrieving an oocyte from the follicle (odds ratio (OR), 0.37; confidence interval (CI), 0.16-0.84; p=0.02), while F4 vasculature (OR, 0.58; CI, 0.30-1.11; p=0.10) had no effect (Table V).

Discussion

The uterine and ovarian blood flow changes during the menstrual cycle, and colour Doppler ultrasound is used to assess changes in ovarian and follicular vascularity. Transvaginal pulsed Doppler has been used to assess ovarian blood flow patterns in stimulated cycles[15]. The changes in blood flow of maturing follicles have been demonstrated by transvaginal colour Doppler studies[6,16]. The results have shown a general increase in intra-follicular blood flow over the peri-ovulatory period[16] and increasing peak systolic velocity with increasing follicular size[6].

Lower Doppler indices of the peri-follicular vasculature are indicative of poor blood flow, which provides oxygen and protein-rich blood to the developing oocyte and cumulus-corona complex[17]. Oxygenation appears to be a significant factor in adequate oocyte spindle formation, chromosomal aggregation, oocyte maturity and fertilization, and, ultimately, implantation of the subsequent embryo[1,18]. The association between follicular fluid hypoxia and preimplantation embryo quality has also been reported[1-3]. The cumulus cells maintain high adenosine triphosphate and oxygen requirements for completion of meiosis[19]. As the follicle grows, an insufficient increase in blood flow during follicular maturation is demonstrated by the fall in the partial pressure of oxygen and pH[20]. Severely hypoxic follicles having a reduced vascularity may lead to oocytes with cytoplasmic defects, disorganized chromosomes, impaired organization and stability of the meiotic metaphase spindle[1] and cleavage stage embryos with multinucleated[1-3].

An increase in intrafollicular blood flow in the periovulatory period[16,21] coinciding with the oocyte maturity prior to ovulation has been suggested. Following the administration of HCG, the peri-follicular peak systolic velocity increases together with the increase in follicular size[6]. It has been postulated that changes in the periovulatory follicle and its vascularity may initiate important biochemical events within the follicular environment[12]. The low grade follicle vascularity before HCG might affect the uptake of HCG and result in impaired maturation of the cumulus-oocyte-complex. Furthermore, higher perfusion may lead to the increased access of FSH to those follicles, promoting better maturation of oocytes.

Our results contradicted with those of Bhal et al.[11]. They studied peri-follicular vascularity of normal responders, following different stimulation protocols (ultrashort, short and long). They had shown that follicles with high grade vascularity were associated with higher oocyte retrieval, fertilization and pregnancy rates in IVF treatment. Unlike Bhal, we used long protocol for all our patients. The HV and LV groups in our study had similar oocyte retrieval rates. Just as our results disputed those of Nargund et al.[4,12] they were in accordance with the findings of what Chui et al.[5] had reported. They did not indicate any difference in oocyte retrieval rates related with follicular vascularity. Follicular growth commences as follicles become recognizable as class 1 during the early luteal phase (EL) and develop as class 5 (selectable stage) 70 days later, in the late luteal phase (LL), and constitute a population from which the follicle destined to ovulate during the subsequent cycle will be selected[22]. Hence along this developmental path many intrinsic factors could setle preliminaries for further oocyte development dependent on FSH.

Complete maturation of the oocyte determines oocyte quality. Complete maturation of oocytes includes both nuclear maturation and cytoplasmic maturation. For oocyte nuclear maturation, resumption and progression of meiosis to MII cannot be used as the sole determinant of an oocyte's developmental competence[23]. Extensive changes in protein synthesis and post-translational modifications in the cytoplasm take place simultaneously with nuclear maturation[24]. Both nuclear and cytoplasmic maturation play important roles in achieving successful fertilization and subsequent development[25]. The grading for each oocyte was done thoroughly in our study rather than just analysing the mature oocytes. Accordingly the distribution of oocytes with each grade was compared among the HV and LV groups. The difference of percentage was insignificant among the groups. As the fate of the embryo is largely dependent on the oocyte from which it originates, studies have been conducted to investigate a possible association between oocyte morphology and developmental potential[26]. The extent to which the follicular microenvironment affects oocyte competence and as

a consequence, embryo viability, has been investigated[27,28]. Biochemical analysis of human follicular fluid obtained in stimulated cycles has demonstrated significant differences in concentrations of steroids, growth factors, enzymes and cytokines among follicles at oocyte recovery time[29,30].

This study has some limitations. First, the sample size was not large enough to make definite conclusions. Second, we analyzed only young normal-responders who were treated at Marmara University Hospital ART Clinic, a tertiary center. The study was not designed to include the low-responder patients. It is possible that the predictive value of power Doppler analysis could be different in these patients. Third, endpoints like fertilization rate, embryo quality and pregnancy rate were not examined since our main focus was on egg quality.

Conclusion

It seems that high peri-follicular vascular flow on the day of ovum pick-up does not validate the likelihood of oocyte retrieval. Furthermore, the chance for obtaining grade 1 oocytes from follicles with HV is not verified since follicles with F1 vascular flow would yield grade 1 oocytes.

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The authors declare that they have no conflict of interest

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