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Perifollicular Blood Flow in Controlled Ovarian Hyperstimulation

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ABSTRACT

Background: The aim of this study was to investigate the association of perifollicular blood flow with oocyte quality , metaphase 2 conversion and embryo quality after controlled ovarian hyperstimulation in antagonist cycles .

Objective: The primary aim was to measure the perifollicular Doppler blood flow indices in graffian follicle and correlate it with number of mature oocytes retrieved in women undergoing intracytoplasmic sperm injection (ICSI) in controlled ovarian hyperstimulation with gonadotropins in antagonist cycles .The secondary aim was to assess the blastocysts obtained from these oocytes.

Results: Antagonist protocol was used for controlled ovarian stimulation with sequential gonadotropin protocol . Peripheral Vascularity, pulsatility Index (PI) and resistive index (RI) of perifollicular perfusion were assessed on day 10 for 65 follicles. After oocyte retrieval the count of metaphase stage 2 oocytes, and day 5 embryos obtained were evaluated.

RI and PI indices had a positive correlation with age (p value nonsignificant). Follicles with ≥18 mm diameter and >75% of circumferential perfusion (p value= 0.04, significant), Follicle with > 75% circumferential vascularity with low PI and RI were associated with high number of metaphse 2 oocytes (p value significant). Follicles with >75% of vasculaity with low PI and RI were associated high number of Day 5 blastocyst (p value significant).

Conclusion: In our study, the Doppler indices of perifollicular blood flow provided a reliable prediction of the number and quality of metaphase 2 oocytes and day 5 embryos. Hence perifollicular blood flow is a simple, non-invasive and economical marker to predict response to gonadotropins in controlled ovarian hyperstimulation.

Key words: Doppler sonography–Graffian Follicle–Metaphase–oocytes–Blastocyst

1 INTRODUCTION

Perifollicular perfusion and ovarian stromal blood flow are useful markers for optimal evaluation of follicles, with the former having a direct relationship with follicular oxygena-

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tion and oocyte maturation [1] .The chance of obtaining a high-quality oocyte, and thus a good embryo, increases when perifollicular blood flow which is preferably between 50% and 75% [2,3] . In the ovary, primordial and preantral follicles have no independent blood supply of their own and derive their vascularity from the stromal blood vessels. However, subsequently, the growth of the primary follicles leads to the development of a vascular network in the theca layer with increased follicular vascularity.[4,5]

The inner granulosa cells aromatize androgen to produce oestrogen. They also produce other protein hormones and secrete proteoglycan to produce an osmotic gradient and fluid-filled cavity [6]. The resulting capillary network mediates the transport of oxygen, nutrients, and precursor substances [7,8]. Vascularization is the primary essential step in follicular growth, and the follicular microenvironment is an essential factor in oocyte growth .

It was observed that embryos from oocytes resulting from well vascularized follicles had a higher implantation rate than those from oocytes developed in poorly vascularized follicles.[6]The introduction of transvaginal power Doppler ultrasound has facilitated non-invasive study of the vascularity of ovarian follicles in detail.[9] Ovarian perifollicular blood flow assessment using Doppler Ultrasound has been postulated to be a good marker of oocyte competence, embryo viability, and implantation potential too.[10] Thus, this study aims to find out the role of perifollicular vascularity in patients undergoing Controlled Ovarian Hyerstimulation (COH) cycles in predicting oocyte maturity and embryo development . The perifollicular perfusion by colour Doppler ultrasonography (USG) was done and their correlation with the quality of oocytes and thus the embryo quality were evaluated.

2 MATERIALS AND METHODS

This prospective study was conducted on 65 Graffian follicles for a period of 4 months in the ARC International fertility hospital and research centre, affiliated to Saveetha University Chennai India. Informed, written consent will be taken from all participants

All participants were 20 - <40 years old women who will be in the first ART cycle. All participants and regular menstruation cycles, were non-smokers, did not use any drug and had basal Follicle Stimulating Hormone level (FSH) <10 mIU/m. All patients underwent serial transvaginal ultrasound scans starting from day 8 of the cycle and continued every day until trigger was administered using Mindray DC 70 Ultrasound with power and Doppler facilities. The size of the follicle was calculated by using the mean of two maximum diameters. Perifollicular Doppler blood flow was assessed in follicles > 18 mm before oocyte recovery. We placed the power Doppler colour over each ovarian follicle and then took a cross-sectional image of the follicle with the maximum colour intensity representing the greatest Doppler frequency shifts. The follicular circumference was frozen and the perifollicular vascularity was graded based on Chui*et al* . According to this modified grading system, perifollicular vascularity was represented as: Grade 0: 0%, Grade 1: 1-25%, Grade 2: 26-50%, Grade 3: 51-75%, Grade 4: 76-100%.

Women with congenital uterine anomaly, endometriosis confirmed by laparoscopy, history of partial or complete surgical resection of the ovary, myoma (intramural <5mm away from endometrium and features of Ovarian hyperstimulation syndrome (OHSS) were excluded from the study.

Doppler ultrasound was performed by transvaginal USG machine having a 8 MHz transvaginal probe from 10^{th} day of stimulation till trigger injection or lead follicle size >18mm on each participant. For all the scans, the velocity range, wall filter, and colour gain were standardized in the USG machine. The flow velocity waveforms from the Graffian folliular perpheral vascular sheath was used to calculate Resistive index and Pulsatility index (Figure 1). A recording was considered satisfactory for measurement when there were three consecutive waveforms with gentle peak. During oocyte recovery, each follicle was aspirated individually into individually numbered tubes, quality of oocytes, fertilization rate, cleavage rate, number of good quality embryos was evaluated .

A total of 65 patients were included in the study. The patient characteristics like age, follicular diameter, resistive index ,pulsatility index, maturity of oocyte, blastocyst conversion were recorded. Descriptive and inferential statistics were used to analyze the data. Comparisons of age, AMH, AFC, resistivity index, pulsatility index and oocyte maturity and Blastocyst conversion rate were performed with the use of Pearson Correlation. Correlation Coefficient (r) was used for measuring the linear dependence of two variables. Correlation coefficient, -1 <= r <= 1, 1 represents strongly positively correlated, -1 represents strongly negatively correlated, 0 represents no correlation. A scatter plot was plotted using Cartesian coordinates to show values for two variables. Chi square test with Yate's correction was used for categorical variables. One tailed p value< 0.05 was considered significant .Statistical analysis was done using MEDCALC (Belgium).

3 RESULTS

Satisfactory waveforms were obtained in 63 follicles. The mean age of patients with follicle diameter more than 18 and peripheral vascularity > 75% was 29.34+3.15 (mean+SD). The mean Body Mass Index (BMI) of women with recruited follicles were recrited in the study was 26.19+2.25 (mean+SD).

The description of various parameters of study population such as age, Antimullerian Hormone(AMH) , Antral follicular count , Pulsatility Index and Resistiity Index is given in Table 1 **.** There was a significant association between grade of peripheral vascularity and maturity of oocyte till Metaphase 2 stage (pvalue=0.0076, Table 2).There was also a significant corellation between grade of peripheral vascularity and number of embryos till blastocyst stage(p value=0.0473, Table 3)

Table 1. The corellation between various parameters of study population(Age, Antimullerian Hormone, Antral follicular count, Pulsatility Index and Resistiity Index)

Serial no.	Characteristic (n=63)	Coefficient of correlation (Confidence interval)	p value	Inference
1	Age v/s Pulsatility Index	0.013509 (-0.024-0.26)	0.9136	Positive Non significant
2	Age v/s Resistivity Index	0.024854 (-0.22-0.27)	0.8470	Positive Non significant
3	Age v/s Anti Mullerian Hormone	-0.094332(-0.33-0.16)	0.462257	Negative Non significant
5	Age v/s Antral Follicular Count	-0.076526(-0.32-0.17)	0.5538	Negative Non significant
6	Anti Mullerian Hormone v/s Antral Follicle Count	0.835291(0.74-0.90)	<0.0001	Positive Significant

Table 2. Grade of peripheral vascularity and maturity of oocyte till Metaphase 2 stage (pvalue=0.0076, significant)

Peripheral Vascularity	Germinal Vesicle and metaphase 1 oocyte	Metaphase 2 oocyte	Total
Grade 1 and 2	11	8	19
Grade 3 and 4	10	34	44
Total	21	42	63

Table 3. Grade of peripheral vascularity and number of embryos till blastocyst stage(pvalue=0.0473, significant)

Peripheral Vascularity	Arrested Embryos	Blastocyst Day 5 Embryos	Total
Grade 1 and 2	5	3	8
Grade 3 and 4	10	30	40
Total	17	33	48

4 DISCUSSION

A good quality oocyte often results in a good quality embryo [11]. The follicular microenvironment of human oocyte plays a crucial factor in its developmental competence of resulting embryo[11]. The quality and maturity of the oocyte depends on the intrafollicular levels of oxygen which in turn is directly proportional to the amount of follicular vascularity[12].

Figure 1. Measurement of follicular peripheral vascularity Doppler Indiices (PS=Peak systolic, ED=EndDiastolic, RI=Resistivity Index, PI=Pulsatility Index) in 2D colour Doppler Sonography.

The study by Chui *et al.* demonstrated that oocytes derived from follicles with low grade vascularity resulted in a significantly higher proportion of triploid embryos when compared to those derived from follicles with high grade vascularity[13].

It is evident in this study that with advancing age there is an increase in PI and RI (Figure 1 and Figure 2). This study also demonstrates that Resistivity Index and Pulsatility index have negative correlation with number of Metaphase 2 oocytes retrieved (Figure 3 and Figure 4) . This study also shows that Resistivity Index and Pulsatility index have negative correlation with Blastocyst and Blastocyst conversion (Figure 5 and 6)

Similarly, a study by Bhal *et al.* showed a significantly higher oocyte retrieval rate, maturity and fertilization rate with significantly lower triploidy rate in the group with high grade vascularized follicles.[14] The compromised perifollicular microcirculation leads to hypoxia which probably causes an increased incidence of aneuploid oocytes[15].

However our study has some limitations. Pulsatility index and resistivity index used for assessing vascularization and blood flow are derived from a single artery, which may not be the true representative of the surrounding vasculature or total follicular blood flow.[16,17] Furthermore, the accuracy of measurement of blood flow velocity is dependent on the angle of insonation to the blood vessels. This may at times be difficult to measure correctly as the arteries within the ovary are not only small but tortuous also.

The recent advent of high resolution and 2 D and 3 D Doppler ultrasound scanning has led to the formulation of several uterine and ovarian sonographic markers which can predict ooutcome in assisted reproductive treatment cycles[18]. Of these markers, the assessment of peri follicular blood flow using colour Doppler imaging is a useful predictor of ovarian responsiveness to gonadotropins.

5 CONCLUSION

In our study, the perifollicular blood flow p rovided a favourable predictionon the quality of oocytes retrieved at Metaphase 2 stage an number of embryos. Hence, perifollicular blood flow may be used as a valid indirect marker of oocyte quality The association between perifollicular vascular perfusion and follicular oxygenation and oocyte maturation does exist which ultimately gets translated to quality of embryos . However how the effects of vascularization in perifollicular regions are mediated in controlled ovarian hyperstimulation in ART, is still unclear. The addition Doppler ultrasonography with gray scale can be used to predict out-come.

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