Perifollicular Vascularity in Poor Ovarian Responders in In vitro Fertilization Cycles

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ABSTRACT

Context: Poor response is reported in 9%–24% of stimulated cycles. Color Doppler indices of follicular blood flow are correlated with oocyte recovery, fertilization rate, developmental potential of oocyte, and pregnancy rate in in vitro fertilization (IVF) treatment. Aim: The aim of this study is to find out the correlation between perifollicular vascularity with clinical outcomes in poor ovarian responders during IVF cycles. Settings and Design: A total of 49 poor ovarian responder women undergoing conventional IVF-embryo transfer procedure at a tertiary care hospital between September 2014 and 2015 were included in the study. It was a prospective observational study. Subjects and Methods: Patients were recruited on the day of trigger following a transvaginal ultrasound if they developed ≤4 dominant follicles of ≥16 mm diameter. After ovarian stimulation patients who had all follicles with low-grade vascularity were classified as Group A, those with follicles with high-grade vascularity were Group C, and Group B included patients with follicles of both good and poor vascularity. Statistical Analysis Used: Analysis of variance and Chi-square/Fisher’s exact test and software, namely SAS 9.2 and SPSS 15, has been used. Results: A total 49 patients were recruited for the study. 10 patients were allocated in Group A, 26 patients in Group B and 13 in Group C. Both groups were comparable in terms of age, period of infertility, follicle-stimulating hormone, luteinizing hormone, and gonadotrophin requirement. The number of metaphase II oocytes and good quality oocytes was significantly higher in Group C. Implantation rate, clinical pregnancy rate, multiple pregnancy rate, miscarriage rate, and live birth rate were comparable among all groups. Conclusions: Perifollicular vascularity has an important role to play in clinical outcomes in poor ovarian responders in IVF cycles.

KEYWORDS: Perifollicular vascularity, poor ovarian responders, colour doppler indices

INTRODUCTION

Poor responders were reported in 9%–24% of stimulated cycles.[1] The management of poor ovarian responders has been extensively reviewed[1‑3] but remains a great challenge in assisted reproduction. Cycles with poor ovarian response are usually canceled because they are associated with low pregnancy rates.[1] Color Doppler indices of follicular blood flow are correlated with oocyte recovery, fertilization rate, developmental potential of oocyte, and pregnancy rate in in vitro fertilization (IVF) treatment.[4,5] The assessment of perifollicular vascularity in poor responders may be useful in selecting a subgroup of patients with better outcomes.[6] In the recent years, follicular blood flow has been evaluated by bidimensional pulsed Doppler[4,5] and the perifollicular blood flow by color mapping.[5,7,8] The perifollicular peak systolic velocity (PSV) before the human chorionic gonadotropin (HCG) administration was significantly related to the possibility of retrieving oocytes and developing embryos of high preimplantation potential.

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potential.[4] By evaluating the percentage of perifollicular color mapping circumference by power Doppler, it was concluded that follicles showing a high degree of vascularity produce significantly higher number of mature oocytes and have high fertilization rate.[7,9] Oocytes obtained from follicles with good perifollicular blood flow show a lower frequency of abnormalities in the chromosomal arrangement within the metaphase II (MII) spindle, greater dissolved oxygen content, and a high concentration of vascular endothelial growth factor.[5] Moreover, oocytes from those follicles with a lower degree of vascularity develop embryos with a high rate of triploids.[7] All these explain why the pregnancy rate is higher when the transferred embryos come from oocytes with highly vascularized follicles.[7,9] Therefore, assessment of perifollicular vascularity in poor responders may be a useful tool to predict clinical outcomes in poor ovarian responders in IVF cycles. Those patients with poorer outcomes could perhaps be advised against transvaginal ultrasound-guided oocyte retrieval. We aimed to study the correlation between perifollicular vascularity with clinical outcomes in poor responders in IVF cycles.

**Subjects and Methods**

It was a prospective observational study. The study included couples undergoing conventional IVF-embryo transfer (ET) procedures conducted at Milann Hospital, Bangalore. The sample size was 49 women undergoing IVF for 12 months from September 2014 to September 2015. Protocols for ovarian stimulation included mainly antagonist and microflare. Ovarian stimulation was carried out using gonadotrophins in the form of recombinant follicle-stimulating hormone (FSH)/luteinizing hormone (LH)/human menopausal gonadotropin. The dose of gonadotrophins was adjusted based on ovarian follicular response and blood estradiol levels. Patients were recruited if they developed ≤4 dominant follicles of ≥16 mm in diameter after ovarian stimulation. HCG trigger was given when the two-lead follicle size was ≥18 mm. On the day of trigger, transvaginal Doppler assessment was done. All Doppler ultrasound examinations were carried out by an experienced operator using a 6.5 MHz vaginal probe (GE Voluson E8). Patients were segregated into three groups:

- **Group A**: Perifollicular blood flow <50%/PSV <10 mm/RI >0.5/pulsatility index (PI)>1.
- **Group B**: Includes patients with follicles of both good and poor vascularity.
- **Group C**: Perifollicular blood flow >50%/PSV >10 mm/RI<0.5/PI <1.

Transvaginal oocyte retrieval was scheduled 36 h after the HCG injection and was performed using a double-lumen needle under ultrasound guidance. The double-channel needle allowed aspiration and flushing of follicles >10 mm on both sides. The follicle with the highest grade perifollicular vascularity was first aspirated. Maturity and the quality of oocytes were assessed following which intracytoplasmic sperm injection was performed. Cell-stage embryos were graded based on consensus scoring system.[10] ET was performed through an ultrasound-guided technique 48 or 72 h after oocyte retrieval and one to three embryos were replaced. Excess good quality embryos were frozen. Luteal phase was supported by three doses of HCG and micronized progesterone. Serum estradiol, progesterone, and beta-HCG was done 14 days after fresh ET. If it was positive, ultrasound examination was performed 10–14 days later to confirm intrauterine pregnancy and to determine the number of gestational sacs present.

**Exclusion criteria**

- Age <18 years or >45 years of age
- Hepatic and cardiovascular disorders
- Concurrent medical illness (including asthma)
- Patients with >4 dominant follicles and size of >16 mm after ovarian stimulation.

**Inclusion criteria**

- Age >18 years or <45 years
- Tubal factor
- Endometriosis
- Male factor infertility
- Unexplained infertility
- Patients with <3 dominant follicles and size of >16 mm after ovarian stimulation.

**Statistical analysis**

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented as mean ± standard deviation and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Analysis of variance has been used to find the significance of the study parameters between three or more groups of patients. Chi-square/Fisher exact test has been used to find the significance of the study parameters on categorical scale between two or more groups. Statistical software: The Statistical software, SAS 9.2, SPSS Inc., version 15.0, Chicago, IL, USA, were used for the analysis of the data.

**Results**

A total of 49 patients were included in the study; 10 patients in Group A, 26 patients in Group B,
and 13 patients in Group C. All the groups were comparable in terms of age, period of infertility, FSH, LH, and gonadotrophin requirement. The mean serum anti-Müllerian hormone was significantly lower in Group A \( (P = 0.003) \) [Table 1]. The most common cause of subfertility was poor ovarian reserve –55.5% endometriosis and male factor –16.3% each, and unexplained infertility –8.2%.

The duration of ovarian stimulation was significantly higher in Group A \( (P = −0.033) \). All the groups had similar peak estradiol, LH, progesterone, and number of follicles on the day of HCG trigger. The pulsatility index \( (P = < 0.009) \) and PSV index \( (P = < 0.011) \) in Group C was significantly higher. The resistance index was suggestive of significance in Group C. No significant difference was seen in oocyte retrieval rate, fertilization rate among the three groups. However, significantly higher number of MII oocytes \( (P = < 0.001) \) and good-quality oocytes were seen in Group C \( (P = < 0.005) \). Failed fertilization was encountered in three cycles in Group A and two cycles in Group B [Table 2].

**DISCUSSION**

The management of poor ovarian responders remains a great challenge in assisted reproduction. Cycles with

![Figure 1: Comparison of clinical outcomes. IR: Implantation rate, CPR: Clinical pregnancy rate, MPR: Multiple pregnancy rate, MC: Miscarriage rate, LBR: Live birth rate](image_url)

**Table 1: Comparison of demographic variables**

| Variables               | Group A \( (n=10) \) | Group B \( (n=26) \) | Group C \( (n=13) \) | \( P \)  \\
|-------------------------|-----------------------|-----------------------|-----------------------|--------
| Age (years)             | 35±3.68               | 32.42±3.89            | 34.23±4.27            | 0.161  
| Duration of infertility (years) | 6.90±4.20          | 6.02±2.96             | 6.38±3.31             | 0.773  
| Basal FSH (IU/l)        | 8.96±3.54             | 9.08±4.66             | 9.64±4.28             | 0.913  
| Basal LH (IU/l)         | 4.72±1.93             | 5.10±1.84             | 5.10±2.42             | 0.868  
| AMH (ng/ml)             | 0.67±0.24             | 1.95±1.67             | 1.25±0.97             | 0.033* 

*Moderately significant \( (P \text{ value: } 0.01 < P \leq 0.05) \), FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, AMH: Anti-Müllerian hormone

**Table 2: Comparison of ovarian response**

| Variables               | Group A \( (n=10) \) | Group B \( (n=26) \) | Group C \( (n=13) \) | \( P \)-value   \\
|-------------------------|-----------------------|-----------------------|-----------------------|----------------
| Dose of gonadotrophins (IU) | 1749±191              | 1737±96               | 1664±81               | 0.166          
| Duration of stimulation (days) | 11.20±1.99           | 10.04±1.28            | 9.54±1.45             | 0.033*         
| Peak estradiol (pg/ml)    | 113.48±924.54         | 1531.27±747.9         | 1223.15±788.8         | 0.314          
| Peak LH                  | 5.15±5.27             | 5.45±8.42             | 3.03±1.87             | 0.559          
| Peak progesterone         | 0.62±0.27             | 0.84±0.42             | 0.82±0.57             | 0.416          
| Number of follicles      | 1.70±0.91             | 2.1±1.13              | 2.05±2.05             | 0.176          
| PI                      | 0.49±0.51             | 0.68±0.43             | 0.81±0.29             | 0.009**        
| RI                      | 0.55±0.45             | 0.62±0.37             | 0.74±0.30             | 0.054*         
| Peak systolic velocity (cm/s) | 8.37±5.53           | 8.66±3.75             | 10.82±3.92            | 0.011*         
| Oocyte retrieval rate (%) | 9 (90)                | 24 (92.3)             | 13 (100)              | 0.637          
| Number of metaphase II oocytes (%) | 19 (70.4)          | 65 (54.6)             | 39 (83)               | <0.001**       
| Number of good-quality oocytes (%) | 7 (25.9)          | 47 (39.5)             | 30 (63.8)             | 0.005**        
| Fertilization rate (%)    | 7 (70)                | 24 (92.3)             | 13 (100)              | 0.232          
| Good-quality embryos (1×8 cell grade A)% | 12 (44.4)         | 52 (46.7)             | 34 (72.3)             | 0.070*         

*Moderately significant \( (P \text{ value: } 0.01 < P \leq 0.05) \), **Strongly significant \( (P \text{ value: } P < 0.01) \), Suggestive significance \( (P \text{ value: } 0.05 < P < 0.10) \), L.H: Luteinizing hormone, PI: Pulsatility index, RI: Resistance index

There were 2 (20%) clinical pregnancies, and consequently 2 (20%) live births among which one was preterm twin pregnancy in Group A. In Group B, there were 8 (30.8%) clinical pregnancies, among which 2 resulted in tubal ectopic pregnancy, 1 missed miscarriage, and 5 (19.2%) live births. In Group C, there was 1 clinical pregnancy (7.7%) resulting in 1 (7.7%) live birth [Figure 1]. There were 2 biochemical pregnancy in Group B, 3 in Group C, and none in Group A. No significant difference was seen in implantation rate, clinical pregnancy rate, multiple pregnancy rate, miscarriage rate, and live birth rate among the three groups [Table 3]. No congenital anomalies were seen in the babies of any of the groups.
Table 3: Comparison of clinical outcomes

| Variables                  | Group A | Group B | Group C | P   |
|----------------------------|---------|---------|---------|-----|
| Implantation rate (%)      | 2 (20)  | 10 (38.5) | 4 (30.8) | 0.560 |
| Clinical pregnancy rate (%)| 2 (20)  | 8 (30.8) | 1 (7.7) | 0.316 |
| Multiple pregnancy rate (%)| 1 (10) | 0 (0)   | 0 (0)   | 0.264 |
| Miscarriage rate (%)       | 0 (0)   | 3 (11.5) | 0 (0)   | 0.411 |
| Live birth rate (%)        | 2 (20)  | 5 (19.2) | 1 (7.7) | 0.675 |

Poor ovarian response are usually cancelled because they are associated with low pregnancy rates. Different stimulation regimens have been tried in poor responders with an attempt to improve the ovarian response or pregnancy rate, but their efficacy remains unproven or appears very limited. The concerns of the continuation of treatment are the known risk of retrieving no oocytes with the associated economic and psychological consequences and poor pregnancy rate. Perifollicular vascularity has an important role to play in clinical outcomes in poor ovarian responders in IVF cycles and can be used to refine the management of assisted reproduction cycles. Color Doppler indices of follicular blood flow are correlated with oocyte recovery, fertilization rate, developmental potential of the oocyte, and pregnancy rate of IVF treatment. In our study, all the groups had similar number of follicles on the day of HCG trigger, oocyte retrieval rate, and fertilization rate. The pulsatility index ($P < 0.009$) and PSV index ($P < 0.011$) in Group C was significantly higher. The resistance index was suggestive of significance in Group C. Results from our study indicate that number of MII oocytes and good-quality oocytes was significantly higher in Group C containing follicles with high-grade perifollicular vascularity. However, implantation rate, clinical pregnancy rate, multiple pregnancy rate, miscarriage rate, and live birth rate were comparable among the three groups. Our result was similar to the study conducted by Kan et al., where no significant difference was found in the clinical pregnancy rate among poor responders with and without high-grade perifollicular vascularity. Implantation and live birth rates were lower in patients without high-grade perifollicular vascularity than those with high-grade vascularity, but the difference did not reach statistical significance. Multiple pregnancies and live birth rates appeared higher and miscarriage rate lower in group with high perifollicular vascularity, but the difference did not reach statistical significance. Our results are different from a study by Oyesanya et al., who found significantly lower oocyte retrieval and fertilization rates in follicles without high-grade vascularity. In a study by Borini et al., embryos originating from oocytes developed from well-vascularized follicles had a statistically higher pregnancy rate compared to poor vascularized oocytes (34% vs. 13.7%). In a study by Ozturk, perifollicular vascular impedance was found to be lower in the pregnant group at both day 10 and the last stimulation day before HCG injection. No correlation was detected between the degree of perifollicular vascularity and the vascular impedance expressed as pulsatility index. Furthermore, there were no differences in the perifollicular vascularity values between pregnant and nonpregnant women. Our result contradicted with that of Bhal et al., in which mean follicular diameter, oocyte retrieval rate, number of mature oocytes recovered, and fertilization rates were all significantly higher ($P < 0.05$) and triploidy rate significantly lower ($P < 0.05$) from the cohort of follicles with high-grade vascularity. Cycles with follicles of uniformly high grade vascularity were associated with significantly higher pregnancy rate (34%) than cycles with mixed grade vascularity follicles (18%), with no pregnancies occurring in the group with low grade vascularity. However, they studied perifollicular vascularity of normal responders following different stimulation protocols (ultrashort, short, and long). In study by Chui et al., there was no significant difference in fertilization rates with different degrees of vascularity, although there was a trend toward higher fertilization rates with higher grade vascularity. Pregnancies were confined to those women whose embryos were derived from follicles with Grade 3 and 4 vascularity (pregnancy rates per ET of 12.5 and 61.5%, respectively), with only those from Grade 4 follicles resulting in live births. This study suggested that high-grade follicular vascularity is associated with increased pregnancy rate and implantation. In a study by Coulam et al., all pregnancies occurred in women with high follicular vascularity and 91% of pregnancies occurred with follicular PSV ≥10 cm/s. Even in a population at high risk for IVF failure, women with follicular flow of PSV ≥10 cm/s and Grade 3–4 had a 13% pregnancy rate that cycle. This finding is consistent with previous studies that reported a significant relationship between follicular PSV within a given follicle and the recovery of an oocyte and the subsequent production of an embryo. These findings are consistent with our study where higher number of MII oocytes ($P < 0.001$), good-quality oocytes ($P < 0.005$), and higher number of good-quality embryos were seen in Group C. However, there was no significant difference in the clinical pregnancy rate, multiple pregnancy rate, miscarriage rate, and live birth rate. Although perifollicular vascularity may provide another means to select embryos for transfer with highest implantation potential, the moderate predictive power (sensitivity and specificity of 60%–70%) may limit its clinical applicability. A potential limitation
of our study is the data taken for the study was from a single center, so the sample was small which may lead to nonsignificant findings and the generalizability of the findings may be limited. Thus, more studies are required to assess the clinical outcomes and perifollicular vascularity which can prove to be an effective tool in the management of poor responders.

**Conclusions**

These data suggest that perifollicular vascularity has an important role to play in clinical outcomes in poor ovarian responders in IVF cycles.

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**Conflicts of interest**

There are no conflicts of interest.

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