INTRODUCTION

A number of ovarian reserve markers that influence the ovarian response to stimulation and determine the success of in vitro fertilization (IVF) are being used to predict pregnancy. Advanced age of the woman is arguably the most important predictor, but even young women respond poorly to controlled ovarian hyperstimulation perhaps due to environmental factors such as smoking, surgical procedures on the ovaries or pathological conditions such as ovarian endometriosis and pelvic infections. With a diminished ovarian reserve the recruitable follicle population responds unfavorably to gonadotropin stimulation. This recruitable follicle cohort is represented by the antral follicle count which is influenced by the degree of ovarian blood flow and the dose of gonadotropins used during stimulation in an IVF cycle. As a woman's age advances there is a decline in the ovarian vascularity. Thus, a measure of the ovarian blood flow may reflect the ovarian response to controlled ovarian hyperstimulation in an IVF cycle.

The growing oocyte is provided with a developmental milieu like the intrafollicular level of oxygen and vascular endothelial growth factor (VEGF) that play a crucial role in the health of the developing oocyte. The vascularity of the parent follicle from which the oocyte is retrieved during IVF is known to influence the viability of the embryo.
Selecting an optimally viable embryo for a fresh transfer has always remained a challenge and there is evidence that assessment of perifollicular vascularity may assist in selecting the embryo with better potential for implantation. With the development of power Doppler imaging the pattern of perifollicular perfusion can be determined accurately. This ovarian perifollicular vascularity when assessed by power Doppler ultrasound (PDU) on the day of oocyte retrieval can be used as an in vivo ovarian marker for oocyte competence, embryo viability and subsequently implantation potential.[6] Authors have demonstrated increased clinical pregnancy rates in IVF cycles when embryos from highly vascularized follicles were transferred suggesting angiogenesis as a determinant of outcome in these cycles.[7-9]

Our prior results on infertile women with female genital tuberculosis (FGTB) which was proven on convention/molecular and endoscopy showed that in these infertile women, the ovarian reserve was compromised when compared with the fertile controls without tuberculosis (TB).[10] In a developing country like India the burden of genital TB among the infertile women has incidence as high as 19%.[11,12] Women suffering from TB have higher basal follicle-stimulating hormone (FSH) levels, lower peak estradiol (E2) levels and yield fewer oocytes during IVF cycles compared with women without the disease.[13] Their requirement for exogenous gonadotropins during ovulation induction is higher however, fertilization results in fewer embryos. The question of whether the perifollicular blood flow (PFBF) is decreased in women with genital TB has not been investigated previously. The objective of this study was to assess perifollicular Doppler blood flow in normoresponders with or without genital TB undergoing IVF/intracytoplasmic sperm injection (ICSI) and correlate them with the cycle outcome. Our hypothesis was that women demonstrating genital TB would have significantly lower ovarian blood flow in response to ovarian stimulation and cycle outcome when compared with women without genital TB.

**MATERIALS AND METHODS**

This prospective observational study was performed in the Assisted Reproductive Unit in the Department of Obstetrics and Gynecology from January 2009 to December 2011. A total of 254 women undergoing IVF for tubal factor infertility were recruited in this study. An Ethical clearance was obtained from the Institute’s Ethics Committee and an informed written consent to participate in the study was obtained from each patient after explaining the objective of the study to them. Normogonadotropic women aged 20-38 years; with normal ovarian reserves and with no previous surgeries on the ovary/ovaries with tubal factor or unexplained infertility were included in the study. We excluded women with endometriosis, polycystic ovarian disease, prior surgery, single ovary, male factor infertility and poor ovarian reserves.

All infertile women underwent an endometrial aspirate in the premenstrual phase of their cycle. The sample was tested for histopathology, acid fast bacilli (AFB) stain, polymerase chain reaction (PCR) to detect *Mycobacterium tuberculosis* deoxyribonucleic acid. In these infertile, a diagnosis of FGTB was made based on AFB smear and culture, histopathological studies and PCR in conjunction with laparoscopy and hysteroscopy findings. Sixty nine women who had evidence of genital TB were started on antitubercular therapy (ATT) drugs as per the World Health Organization’s directly observed treatment strategy guidelines for category 1 extra pulmonary severe disease as per the revised national TB control program of India (RNTCP). After completing a course of ATT they underwent IVF cycle.

Ovarian stimulation was done using the long agonist protocol (n = 170) or antagonist protocol (n = 84), in women undergoing IVF (n = 201) or ICSI (n = 53) cycles.

All patients underwent serial transvaginal ultrasound scans starting from day 6 to 7 of the cycle and continued every alternate day until human chorionic gonadotropin (HCG) was administered using Siemens Acuson Antares (Siemens Medical Solution, USA, Inc.) with power and color Doppler facilities. The size of the follicle was calculated by using the mean of two maximum diameters. Perifollicular Doppler blood flows were assessed in dominant follicles >16 mm before oocyte recovery. We placed the power Doppler color over each ovarian follicle and then took a cross-sectional image of the follicle with the maximum color intensity representing the greatest Doppler frequency shifts. The follicular circumference was frozen and the PFBF was graded based on Chui *et al.*[9] According to this modified grading system, ovarian PFBF was represented as: Grade 0: 0%, Grade 1: 1-25%, Grade 2: 26-50%, Grade 3: 51-75%, Grade 4: 76-100%.

The flow velocity waveforms from the ovarian stromal or intra-ovarian arteries of both the ovaries were obtained in order to calculate the resistive index, pulsatility index (PI), peak systolic velocity (PSV) and systolic/diastolic ratio. A recording was considered satisfactory for measurement when there were three intense waveforms in a row and an average of each index in both ovaries was calculated. During oocyte recovery, each follicle was aspirated individually into individually numbered tubes, to allow the Embryologist to follow the performance of each follicle separately and correlate this with IVF outcomes including E2 levels on the
day of HCG, number and quality of oocytes, fertilization rate, cleavage rate, number of good quality embryos and pregnancy rate. ICSI was done in women who had unexplained infertility or in those women in whom less number of oocytes was retrieved on the day of ovum pick up.

Statistical analysis was performed using STATA 11.0 (College Station, Texas, USA). Data were presented as a number (%) or mean ± standard deviation (SD)/median (range). The differences in Mean/Median values were compared between patients with TB and those without the disease using Student’s t-test (normal data distribution)/Wilcoxon Ranksum Test (for categorical variables). The correlation between outcome measures and ovarian blood flow were calculated using Spearman rank correlation. $P < 0.05$ was considered to be statistically significant.

RESULTS

The average age ($±$ SD) of these 254 infertile women was 31.3 ($±$4.0) years with a mean duration of infertility of 8.4 ($±$4.1) years. The average day 2 FSH, inhibin B and anti-Mullerian hormone were 6.4 ($±$2.1) mIU/ml, 47.7 ($±$34.6) pg/ml and 4.4 ($±$3.0) ng/ml respectively [Table 1]. 69 women had TB in the study. The average oocytes retrieved after controlled ovarian hyperstimulation was 10.4 ($±$6.6), being higher in agonist cycles (12.6 ($±$4.2)) when compared with antagonist (8.2 ($±$5.2)) cycles which was not statistically significant ($P = 0.3$). The fertilization rate was 69.4% ($±$25.8) and cleavage rate was 95.2% ($±$14.5). The result of demographic outcome variables and ovarian blood flow between women with TB and those without the disease is given in Table 2. The median (range) inhibin B levels in patients with TB were 36.2 (5.2-103.8) and those without the disease was 43.03 (2.5-193.3) respectively, which was statistically significant ($P = 0.04$). The mean cleavage rate was 9 and 5 times higher respectively in tubercular patients compared to those without FGTB. Grade 4 blood flow was observed in 35.7% of patients without the disease compared to only 8.7% in those with the disease with a likelihood ratio of 4.3 ($P = 0.01$) and blood flow in patients without FGTB. A positive correlation was also been seen between ovarian reserve and PSV ($r = 0.17$), $P < 0.05$ which was statistically significant.

We observed a trend of having poor ovarian blood flow in patients with genital TB when compared to those without the disease with a $P < 0.001$. The likelihood of having Grade 0 blood flow was 28 times higher in tubercular patients compared to controls. Similarly, the likelihood of having Grade 1 and Grade 2 blood flow was 9 and 5 times higher respectively in tubercular patients compared to those without FGTB. Grade 4 blood flow was observed in 35.7% of patients without the disease compared to only 8.7% in those with the disease with a likelihood ratio of 1 [Figure 1].

DISCUSSION

Until date, there are no reported studies describing the PFBF in women with and without genital TB undergoing IVF treatment. Our study being the first clearly showed that the PFBF indices differed between infertile patients without genital TB when compared to those with FGTB.

Genital TB has preponderance toward women in the reproductive age group of 20-40 years. Being hematogenous in its spread, it commonly affects the fallopian tubes (90%), followed by the endometrium (50-60%), ovary (10-30%) and rarely cervix, vagina and vulva (5% all combined) eventually causing tubal blockage, pelvic, abdominal and perihepatic adhesions (Fitz-Hugh-Curtis syndrome). [15-16]

Being paucibacillary and asymptomatic, it is difficult to detect and treat. [17,18]

Table 1: Baseline characteristics ($n=254$)

| Demographic and hormonal profile | Ovarian blood flow | Outcome | Mean±SD |
|----------------------------------|--------------------|---------|---------|
| Age in years                     | Blood flow in %    | ET on day of HCG in mm | 8.6±1.8 |
| BMI in kg/m²                      | PSV                | E2 on day of HCG in mm  | 3876.2±2982.9 |
| Duration of infertility in years | PI                 | Oocytes retrieved      | 10.4±6.6 |
| AMH in ng/ml                     | RI                 | M II                 | 7.7±5.8 |
| Inhibin B in pg/ml               | S/D                | Day 1 2PN            | 7.2±5.8 |
| Day 2 FSH in mIU/ml              | 1.9±0.9            | Cleavage rate         | 95.2±14.5 |
| Day 2 LH in mIU/ml               | Fertilization rate  |                     | 69.4±25.8 |
| Total dose of gonadotropins in IU | Number of embryos  |                     | 6.9±5.4 |
| Total days of stimulation        |                    |                     | 9.7±1.6 |

PSV=Peak systolic velocity, PI=Pulsatility index, RI=Resistive index, S/D=Systolic/diastolic ratio, BMI=Body mass index, ET=Embryo transfer, HCG=Human chorionic gonadotropin, AMH=Anti-mullerian hormone, LH=Luteinizing hormone, FSH=Follicle stimulating hormone, SD=Standard deviation
Table 2: Demographic and hormonal profile of women with and without genital tuberculosis

| Demographic and hormonal profile | Women without FGTB n=185 | Women with FGTB n=69 | P value |
|----------------------------------|--------------------------|----------------------|---------|
| Age in years (mean±SD)           | 31.3±3.9                 | 31.5±4.8             | 0.91    |
| BMI in kg/m² (mean±SD)           | 24.8±3.5                 | 25.1±3.5             | 0.85    |
| Duration of infertility (median±range) | 7.5 (2-20)           | 7.5 (2-22)           | 0.90    |
| AMH in ng/ml (median±range)      | 3.1 (0.63-12.2)          | 4.8 (2.6-7.7)        | 0.21    |
| Inhibin B in pg/ml (median±range) | 43.03 (2.5-193.3)       | 36.2 (5.2-103.8)     | 0.04    |
| Day 2 FSH in mIU/ml (mean±SD)    | 6.4±2.1                  | 6.3±1.9              | 0.62    |
| Day 2 LH in mIU/ml (median±range) | 4.5 (0.74-20.3)         | 4.9 (1.6-19.5)       | 0.31    |
| AFC (median±range)               | 6.5 (1-24)               | 6.5 (3-24)           | 0.85    |
| Ovarian blood flow in % (median±range) | 57.5 (0-100)         | 60 (0-90)            | 0.20    |
| PSV (median±range)               | 4.8 (0-14.9)             | 4.7 (0-10.2)         | 0.37    |
| PI (median±range)                | 0.71 (0-4.7)             | 0.68 (0-1.5)         | 0.67    |
| RI (median±range)                | 0.47 (0-8.8)             | 0.47 (0-5.6)         | 0.66    |
| S/D (mean±SD)                    | 1.9±0.9                  | 1.8±0.9              | 0.91    |
| Total dose of gonadotropins in IU (mean±SD) | 2900.5±910.5           | 2900.7±897.6         | 0.99    |
| ET on day of HCG in mm (mean±SD) | 9.7±1.6                 | 9.8±1.7              | 0.77    |
| E2 on day of HCG in (median±range) | 2704.7 (495.2-15090)    | 2740.5 (372-13240)   | 0.90    |
| Oocytes retrieved (median±range) | 9 (0-37)                | 9 (0-35)             | 0.75    |
| M II (median±range)              | 7 (0-35)                 | 7 (0-32)             | 0.96    |
| Day 1 2PN (median±range)         | 6 (0-30)                 | 6 (0-29)             | 0.90    |
| Cleavage rate (mean±SD)          | 98.4±7.4                | 93.8±16.2            | 0.04*   |
| Fertilization rate (mean±SD)     | 69.9±25.8               | 67.6±26.3            | 0.56    |
| Number of embryos (median±range) | 6 (0-30)                | 5 (0-30)             | 0.97    |

*P<0.05 significant. FGTB=Female genital tuberculosis, PSV=Peak systolic velocity, PI=Pulsatility index, RI=Resistive index, S/D=Systolic/diastolic ratio, BMI=Body mass index, AMH=Anti-mullerian hormone, FSH=Follicle stimulating hormone, LH=Luteinizing hormone, AFC=Antral follicle count, ET=Embryo transfer, HCG=Human chorionic gonadotropin, SD=Standard deviation

Table 3: Correlation between outcome measures and ovarian blood flow indices

| Outcome measures | Blood flow | PSV | PI | RI | S/D |
|------------------|------------|-----|----|----|-----|
|                  | r   | P   |    |    |     |
|                  | r   | P   |    |    |     |
| Endometrial thickness on day of HCG |       |     |    |    |     |
| Women without FGTB | 0.14 | 0.08 | 0.13 | 0.09 | 0.16 | 0.04* |
| Women with FGTB   | −0.13 | 0.34 | −0.02 | 0.88 | −0.09 | 0.48 |
| E2 on day of HCG  |       |     |    |    |     |
| Women without FGTB | 0.15 | 0.05* | 0.09 | 0.24 | 0.01 | 0.88 |
| Women with FGTB   | 0.18 | 0.17 | 0.06 | 0.62 | 0.06 | 0.67 |
| Oocytes retrieved |       |     |    |    |     |
| Women without FGTB | 0.25 | 0.001* | 0.17 | 0.03* | 0.07 | 0.40 |
| Women with FGTB   | 0.07 | 0.58 | 0.01 | 0.91 | 0.01 | 0.93 |
| M II              |       |     |    |    |     |
| Women without FGTB | 0.24 | 0.001* | 0.14 | 0.08 | 0.05 | 0.56 |
| Women with FGTB   | 0.09 | 0.48 | −0.04 | 0.73 | 0.06 | 0.64 |
| Day 1 2PN         |       |     |    |    |     |
| Women without FGTB | 0.16 | 0.04* | 0.11 | 0.16 | 0.08 | 0.28 |
| Women with FGTB   | 0.02 | 0.89 | −0.02 | 0.90 | 0.11 | 0.39 |
| Cleavage rate     |       |     |    |    |     |
| Women without FGTB | 0.009 | 0.91 | −0.05 | 0.51 | −0.06 | 0.43 |
| Women with FGTB   | 0.03 | 0.84 | −0.11 | 0.38 | 0.06 | 0.65 |
| Number of embryos |       |     |    |    |     |
| Women without FGTB | 0.16 | 0.04* | 0.08 | 0.31 | 0.09 | 0.25 |
| Women with FGTB   | 0.02 | 0.89 | −0.02 | 0.85 | 0.12 | 0.38 |
| Fertilization rate |       |     |    |    |     |
| Women without FGTB | −0.13 | 0.11 | −0.08 | 0.34 | 0.09 | 0.26 |
| Women with FGTB   | −0.07 | 0.62 | −0.12 | 0.34 | 0.15 | 0.26 |

*P<0.05 significant. PSV=Peak systolic velocity, PI=Pulsatility index, RI=Resistive index, S/D=Systolic/diastolic ratio, HCG=Human chorionic gonadotropin, FGTB=Female genital tuberculosis
Women suffering from TB have higher basal FSH levels, lower peak E2 levels and yield fewer oocytes on stimulation than women who do not harbor this infectious disease. Despite requirements of higher doses of exogenous gonadotropins for ovulation induction fewer embryos are formed following fertilization in an IVF cycle.[13] The tubal and endometrial involvement of TB is evident during laparoscopy and hysteroscopy, however, knowledge about the ovarian involvement in women with genital TB is limited. The quality of an oocyte determines the embryo quality. It is the follicular microenvironment of the human oocyte that plays a crucial factor in its developmental competence.[19] The quality and maturity of the oocyte depends on the intrafollicular levels of oxygen which in turn is directly proportional to the degree of follicular vascularity.[20] In our study, the demonstration of a difference in ovarian PFBF during IVF stimulation between women with or without FGTB suggests that there may be a difference in the egg quality obtained or competence between these two groups based on the measurement of the surrogate end point of the ovarian follicle vascularity. Our study did not show a significant correlation between the outcome variables like fertilization rate and cleavage rate with perifollicular Doppler blood flow. On comparing the response to COH the present study showed that the total number of gonadotropins administered, duration of stimulation, endometrial thickness and plasma E2 levels (on the day of HCG), number of oocytes retrieved and embryos transferred did not differ between women with or without FGTB.

In the present study, we observed a trend of having poor ovarian blood flow in patients with genital TB when compared to those without the disease, which was statistically significant. Previous studies have assessed the ovarian PFBF semi quantitatively using the grading system (grades 1-4) in IVF treatment with the help of PDU or color Doppler ultrasound (CDU).[6-8] In these patients, the ovarian PFBF was assessed on the day of HCG trigger in the CDU study or on the day of oocyte retrieval in the PDU studies. All these studies demonstrated a significant improvement in the pregnancy rate when embryos resulting from the fertilization of eggs with better perfused follicles were used. In their study 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.[6] Similarly, 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.[7] The compromised perifollicular microcirculation leads to hypoxia which probably causes an increased incidence of aneuploidal oocytes. However, the question remains unanswered whether the HCG trigger injection used to induce ovulation alters the pattern of PFBF by the time oocyte recovery is performed. It has been demonstrated that the exogenous HCG or endogenous luteinizing hormone surge increases angiogenesis of human ovarian follicles by increasing the local angiogenic factor VEGF production.[21] An adequate blood supply remains fundamental in the regulation of intrafollicular oxygen levels and the determination of oocyte quality.

We performed this study using 2D power Doppler which has its own limitations. The information regarding the vascularization and blood flow is derived from a single artery, which may not be the true representative of the surrounding vasculature or total ovarian blood flow.[22] 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.\textsuperscript{[2,24]} Our study was designed prospectively using 2D color and power Doppler technology and included women with genital TB proven on histopathology and conventional methods. The door is open to larger studies using 3D Doppler technology to be performed on women with genital Koch's proven histopathologically in order to substantiate our results. Three dimensional power Doppler is essentially more sensitive, angle independent and evaluates the total vascularization and blood flow of the ovary.\textsuperscript{[25]}

**CONCLUSION**

In our study, the PFBF provided a favorable prediction on the quantity of oocytes retrieved MII, day 1 2PN and number of embryos but did not show any significant statistical difference in other outcome variables including fertilization or cleavage rate in women with or without genital TB undergoing IVF/ICSI cycles. Hence, PFBF may be used as a valid indirect marker of oocyte quality. Women with genital TB require early diagnosis with timely and complete short-course ATT before initiating them into IVF - embryo transfer so as to optimize their chance of achieving a successful live birth.

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