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RESEARCH ARTICLE

A prospective study on the colour doppler indices of follicular and endometrial blood flow as a predictor of pregnancy in intrauterine insemination (IUI) cycles

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ABSTRACT

Objectives: Success rates of intrauterine insemination (IUI) as first-line treatment for subfertile couples depend on various factors including follicular and endometrial blood flow and thickness. This study aimed to evaluate the color doppler indices of endometrial and follicular blood flow to predict pregnancy in IUI cycles. **Methods**: A prospective observational study was done on 100 infertile women (21-35 years age) who underwent IUI cycles. On the day when trigger was planned, patients underwent color doppler study of follicular and endometrial flow by 2D ultrasound. Parameters such as endometrial thickness and endometrial blood flow, follicular vascularity grading, pulsatility index (PI) and the resistance index (RI) of uterine and ovarian arteries were calculated. The association of these factors with pregnancy outcomes was evaluated. **Results**: Out of 100 women, 84% of women were nonpregnant, 14% got pregnant with live intrauterine pregnancy (LIUP), and 2% had poor outcome. Among the clinical characteristics, number of follicles was significantly associated with pregnancy outcomes ($p<0.0001$). Mean values of uterine artery PI, uterine artery RI, intraovarian artery (IOA) PI, and IOA RI were 3.27±1.63, 0.88±0.09, 1.92±1.94, and 0.74±0.69, respectively. Follicular vascularity grading was grade 1 and 2 in 50% of the total patients (mention in pregnant women). Uterine artery pulsatility index ($p=0.021$), follicular vascularity grading ($P<.0001$), and IOA PI ($P<.0001$) showed significant association with pregnancy outcomes. ROC curves showed that IOA PI was the best predictor of clinical pregnancy. **Conclusion**: Color doppler flow study of uterus and ovary at the time of trigger in IUI cycles are good predictors of successful IUI outcome.

Keywords: Color doppler, endometrial thickness, endometrial blood flow, follicular vascularity grading, pulsatility index, resistance index.

Intrauterine insemination (IUI) is a recognized alternative in the list of techniques of assisted reproduction (ART). The procedure of IUI continues to be used routinely due to its low cost, less invasive technique and good success rates[1]. To increase the chances of clinical pregnancy in IUI, a better harmonization between characteristics of ovarian follicles and endometrium is required. It will be prudent if the utero-ovarian vascularity and endometrial thickness is gauged together at the time of ovarian stimulation [1, 2].

Color doppler (CD) is emerging as a valuable diagnostic modality during IUI to assess the uterine, endometrial, and follicular blood flows which are useful markers in predicting the uterine receptiveness, follicular maturity, and pregnancy outcomes [2]. However this link has been well established in IVF cycles and less researched in IUI cycles [3, 4].

Pulsatility index (PI) and resistive index (RI) are popular indexes to assess the flow characteristics of the vascular system in ultrasound. Pulsatility is one of the intrinsic property of cardiac and vascular system that is overseen by the "resistance differential across the arteriolar bed" [4, 5].

The present study was conducted to determine the association of follicular and endometrial blood flow with the

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clinical pregnancy rates and to have predictive cutoffs of doppler variables (RI and PI) of uterine and intra ovarian artery for the pregnancy outcome in IUI cycles.

Materials and methods

This was a prospective observational study that included infertile women between 21-35 years age who were planned to undergo IUI cycles and had basal FSH level <10 mIU/mL. Women with intrauterine pathology, acute vaginal/cervical infection, pelvic inflammatory disease, disease of ovary (dermoid, endometrioma), tubal factor (hydrosalpinx), male factor infertility (sperm count <15 million and/or progressive motility <32%) and those who took any adjuvant drug were excluded from study.

After applying inclusion/exclusion criteria and taking informed consent, 100 women were included. The demographic and clinical details like age, duration of infertility, type of infertility, cause of infertility were recorded in a predesigned proforma. For ovulation induction, either clomiphene citrate (50-100 mg) or letrozole (2.5mg) was used. On the day, when trigger was planned, infertile women underwent colour doppler study of follicular and endometrial flow by ultrasound.

The primary outcome was clinical pregnancy (as determined by ultrasound done between 6-8 weeks), and secondary outcomes included miscarriages and ectopic pregnancy. Ethical committee permission was taken for the study (19/14/17IEC-2012/PGIMSR). The trial is registered in CTRI (REF/2019/03/024562).

The sample size calculation was based on the study of Chui DK et al[5], who observed pregnancy rate of 26.3% per embryo transfer based on follicular vascularity grade. Taking this value as reference, the minimum required sample size with 10% margin of error and 5% level of significance was 75 patients. The total sample size taken in our study was 100.

On the day when trigger was planned, infertile woman underwent color doppler study of follicular and endometrial flow and thickness by ultrasound (2D ultrasound transvaginal probe 7.5MHZ). During ultrasound scanning of each ovary, the power doppler color box was positioned over dominant follicle and the cross-sectional image of the follicle with maximum color indication in the follicular circumference was frozen and then perifollicular blood flow (PFBF) was graded based on study of Chui et al.[5]

Grade 0: Follicles do not have any detectable blood flow around the follicular circumference.

Grade 1: Follicles have blood flow visible in 1-25% of the follicular circumference.

Grade 2: Follicles have blood flow visible in 26-50% of the follicular circumference.

Grade 3: Follicles have blood flow visible in 51-75%of the follicular circumference.

Grade 4: Follicles have blood flow visible in 76-100% of the follicular circumference.

On the basis of vascularity of follicle blood flow, grade 1 and grade 2 were categorized low grade; grade 3 and 4 were in high grade and mixed grade (if more than one dominant follicles with perifollicular flow of different grades).

The flow velocity waveform from ovarian stromal or intraovarian arteries of both ovaries was obtained. The arteries within ovarian stroma were visualized with power doppler technique. The doppler gate was then positioned over stromal vessels and flow velocity waveforms were traced until at least three waveforms with similar amplitude occurred. Computer-generated autocalculation box was positioned over two cardiac cycles, and the PI and RI were calculated. Blood vessels in the subendometrial region were located using the color doppler technique. Endometrial blood flow was detected by intra-endometrial or the adjacent subendometrial regions within 2 mm of the echogenic endometrial borders.

The patients were divided into three groups according to the condition of the endometrial blood flow as adapted from the study by Singh N et al [6].

Zone A: No endometrial blood flow detected

Zone B: Subendometrial blood flow detected

Zone C: Both endometrial and subendometrial blood flow detected

Endometrial morphology pattern was classified as “pattern A (a triple-line pattern consisting of a central hyperechoic line surrounded by two hypoechoic layers), pattern B (an intermediate isoechogenic pattern with the same reflectivity as the surrounding myometrium and a poorly defined central echogenic line), or pattern C (homogenous, hyperechogenic endometrium) as per the classification suggested in Zhao J et al [7].Both uterine arteries flow waveform were obtained from ascending branch of the uterine artery on right and left side of cervix before it entered the uterus in longitudinal plane in order to calculate the pulsatility index (PI) and the resistance index (RI). The doppler gate was then positioned over uterine artery and flow velocity waveforms were traced until at least three waveform with similar amplitude occurred. Computer generated autocalculation box was positioned over two cardiac cycle and the PI and RI were calculated. The mean of both uterine

artery PI and RI was taken. The cases were tested for pregnancy after 2 weeks by urine pregnancy test (UPT). If UPT was positive, ultrasound was done for the confirmation of intrauterine pregnancy between 6 and 8 weeks.

Statistical analysis: Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Quantitative variables were associated using ANOVA/Kruskal Wallis test (when the data sets were not normally distributed) between the three groups. Qualitative variables were associated using chi-square test. A p-value <0.05 was considered statistically significant.

Results

Table 1: Association of clinical characteristics with pregnancy outcome

Clinical characteristics	Non pregnant (n=84)	LIUP (n=14)	Poor outcome (n=2)
Age (years)	28.5(26-31.25)	28.5(26.5-31.75)	34.5(33.25-35.75)
Duration of infertility (years)	6(4-8)	6(4-7.75)	5.5(4.25-6.75)
Type of infertility			
Primary	54 (64.29%)	11 (78.57%)	2 (100%)
Secondary	30 (35.71%)	3 (21.43%)	0 (0%)
Cause of infertility			
Anovulation	40(47.62%)	6 (42.85%)	1 (50%)
Unexplained	44 (52.38%)	8 (57.14%)	1 (50%)
Number of IUI cycles			
1	8(9.52%)	4(28.57%)	0(0%)
2	22(26.19%)	1(7.14%)	1(50%)
3 or more	54(64.29%)	9(64.29%)	1(50%)
Ovulation induction drugs			
Clomiphene	18 (21.43%)	5 (35.71%)	1 (50%)
Letrozole	66 (78.57%)	9 (64.29%)	1 (50%)
Diameter of follicles (mm)	20(19-21)	20(19-20)	19.25(18.875-19.625)
Number of follicles*	1(1-1)	2(2-2)	2(2-2)
Number of days from starting stimulation to hCG administration	15(14-16)	14(13-16)	15(14-16)

*p value <0.05, Median (IQR 25-75% interquartile), n(%)

In the present study on 100 women, 84% of women remained nonpregnant, 14% of women got pregnant with live intrauterine pregnancy (LIUP), and 2% had poor outcome, among which 1 patient had abortion (miscarriage) and 1 patient had ectopic pregnancy. The demographic and clinical characteristics of the study patients are shown in table 1. The mean (SD) age of the study patients were 29.15 (3.88) years with a median duration of infertility of 6 years. The type of infertility was primary in 67% and secondary in 33%. The cause of infertility was unexplained in 53% and anovulation in 47%. Number of IUI cycles were ≥3 in 64%, two in 24%, and one in 12% patients. Letrozole and clomiphene were the drugs used for ovulation induction in 76% and 24% patients, respectively. Pregnancy outcomes showed no significant association with the type of drug used for ovulation induction (p=0.271). The mean diameter of the follicles was 20.12 ± 1.57 mm and the mean number of follicles was 1.28 ± 0.49. Mean (SD) number of days from starting ovarian stimulation to hCG administration were 14.65+-1.57 days. Among the various demographic and clinical characteristics, only the number of follicles was significantly associated with pregnancy outcomes (1.17 ± 0.41 in non-pregnant vs 1.86 ± 0.53 in LUIP vs 2 ± 0 in poor outcomes, p<0.0001) as shown in table 1.

Table 2: Association of endometrial characteristics with pregnancy outcomes

Parameters	Non pregnant (n=84)	LIUP (n=14)	Poor outcome (n=2)
Morphology of endometrium			
Type A (Triple line)	54 (64.29%)	10 (71.43%)	0 (0%)
Type B (Hyperechoic)	16 (19.05%)	3 (21.43%)	2 (100%)
Type C (Diffuse)	14 (16.67%)	1 (7.14%)	0 (0%)
Endometrial thickness (mm)	8.5(7.975-9.225)	8.5(8-9.275)	8.55(8.375-8.725)
Endometrial blood flow*			
Zone A	30 (35.71%)	0 (0%)	0 (0%)
Zone B	33 (39.29%)	9 (64.29%)	1 (50%)
Zone C	21 (25%)	5 (35.71%)	1 (50%)

* p value < 0.05, Median (IQR 25-75% interquartile), n(%)

Morphology of endometrium was type A (triple line) in 64%, type B (hyperechoic) in 21%, and type C (diffuse) in 15% patients. Mean (SD) endometrial thickness was 8.63 (1.32) mm. Endometrial blood flow was zone A, B, and C in 30%, 43%, and 27% patients, respectively. Among the endometrial characteristics of type, thickness and blood flow type, higher endometrial blood flow in zone B and C was found to be significantly more in LIUP as compared to non-pregnant women, p=0.016 (table 2).

Mean values of uterine artery pulsatility index, uterine artery resistance index, intraovarian artery (IOA) pulsatility

index, and IOA resistance index were 3.27 ± 1.63, 0.88 ± 0.09, 1.92 ± 1.94, and 0.74 ± 0.69, respectively. Follicular vascularity grading was low in 50% cases, mixed in 44%, and high in 6% cases.

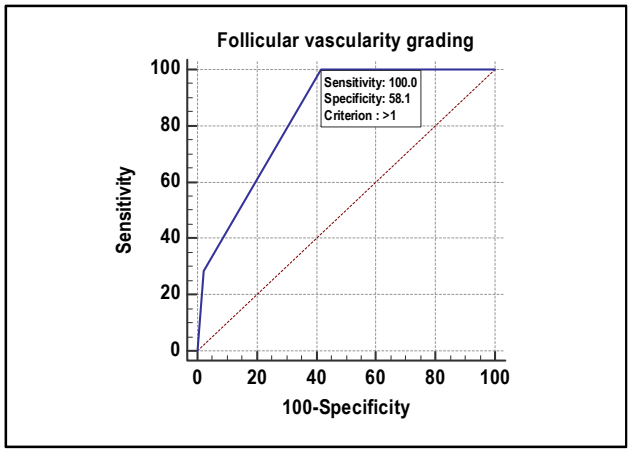

Figure 1.1: Receiver operating characteristic curve of follicular vascularity grading for predicting clinical pregnancy.

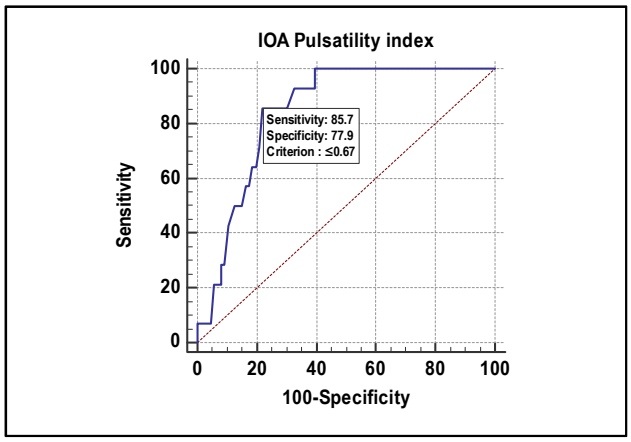

Figure 1.2: Receiver operating characteristic curve of IOA pulsatility index for predicting clinical pregnancy

For predicting the outcomes of pregnancy, the follicular vascularity grading and endometrial blood flow had sensitivity of 100% while other parameters such as IOA pulsatility index, uterine artery pulsatility index, uterine artery resistance index and IOA resistance index carried a lower sensitivity of 85.71%, 85.71%, 64.29% and 78.57% respectively. On the other hand, IOA pulsatility index had specificity of 77.91% followed by IOA resistance index (65.12%), uterine artery resistance index (60.47%), follicular vascularity grading (58.14%). In prediction of clinical pregnancy, endometrial blood flow had lowest specificity of 34.88% followed by uterine artery pulsatility index (52.33%). Highest positive predictive value was found in IOA pulsatility index (38.70%) and highest negative predictive value was found in follicular vascularity grading (100%) and endometrial blood flow (100%). There is always a trade-off between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity) so we choose that variable as best in which combination of sensitivity and specificity gives the maximum predictive value i.e. maximum diagnostic accuracy so overall IOA pulsatility index was best predictor of clinical pregnancy.

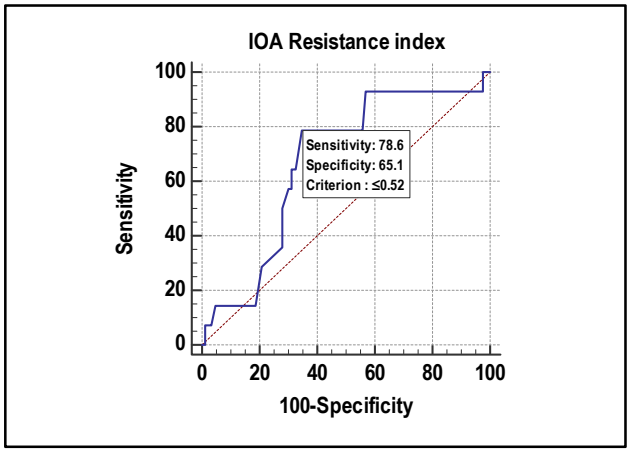

Figure 1.3: Receiver operating characteristic curve of IOA resistance index for predicting clinical pregnancy

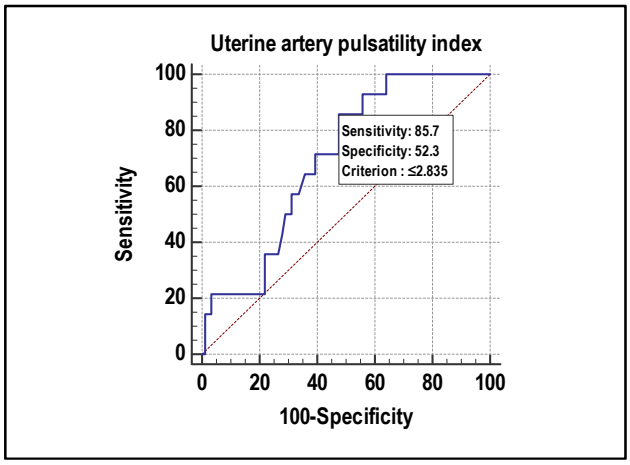

Figure 1.4: Receiver operating characteristic curve of uterine artery pulsatility index for predicting clinical pregnancy

Further, as per the ROC curves, we found that the discriminatory power of IOA pulsatility index (AUC 0.844;

95% CI: 0.758 to 0.909) and follicular vascularity grading (AUC 0.839; 95% CI: 0.752 to 0.905) was excellent and discriminatory power of IOA resistance index (AUC 0.669; 95% CI: 0.568 to 0.760), endometrial blood flow (AUC 0.663; 95% CI: 0.561 to 0.754) and uterine artery pulsatility index (AUC 0.695; 95% CI: 0.595 to 0.783) was acceptable. On the other hand, discriminatory power of uterine artery resistance index (AUC 0.603; 95% CI: 0.500 to 0.699) was non-significant (figure 1.1-1.6). Among all the parameters, IOA pulsatility index was the best predictor of clinical pregnancy at cut off point of ≤0.67 with 84.40% chances of correctly predicting clinical pregnancy.

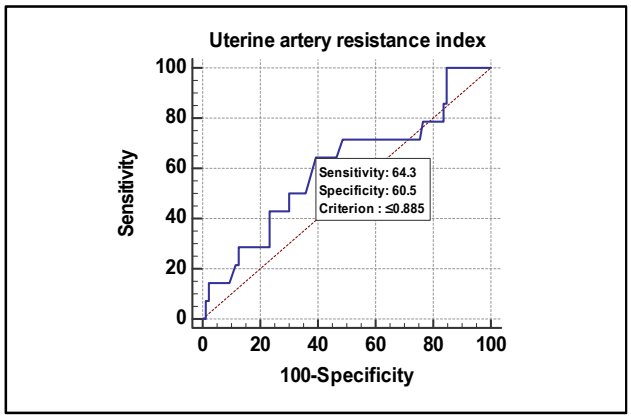

Figure 1.5: Receiver operating characteristic curve of uterine artery resistance index for predicting clinical pregnancy

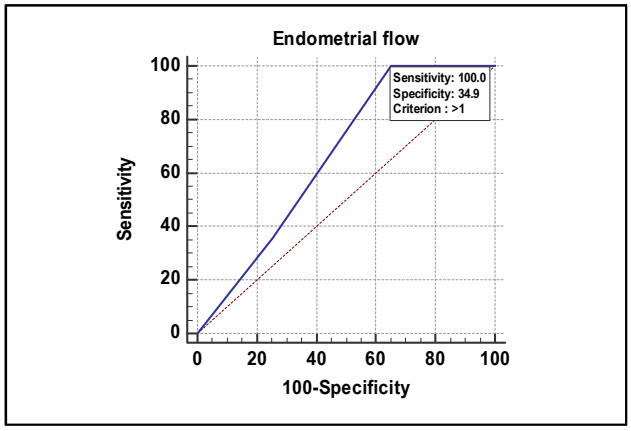

Figure 1.6: Receiver operating characteristic curve of endometrial flow for predicting clinical pregnancy

Discussion

Though the mechanisms underlying the variation in perifollicular and endometrial blood flow are poorly understood, it has certainly proved to be of significance in achieving a live intrauterine pregnancy following IUI. In our study, 14% LIUP was observed against literature reports ranging from 8.75% - 20%[8, 9]. Parameters such as age, duration of infertility, type of infertility, cause of infertility, number of IUI cycles and endometrial morphology and thickness showed no significant association with the pregnancy outcomes.

Doppler indices of uterine arteries and ovarian arteries were found to be significant predictors of clinical pregnancy. There was a decrease in PI and RI of both uterine arteries and ovarian arteries in LIUP group. IOA PI ≤ 0.67 showed the best prediction of achieving a clinical pregnancy. It can be suggested that better prediction of ovarian artery doppler parameters in comparison to uterine artery parameters stress upon the role of ovarian vascularity in providing a better milieu for generating mature quality follicles. Madkour NM et al, reported that mean ovarian artery PI in non-pregnant and pregnant women were 0.91 ± 0.08 and 0.847 ± 0.03 (p=0.016), and mean ovarian RI were 0.495 ± 0.05 and 0.46 ± 0.05, (0.047) respectively; with a statistically significant difference in ovarian PI and RI between pregnant and nonpregnant cases (p <0.05) [1]. However few studies failed to observe statistical difference in these parameters. Wang et al [10] found that the mean PI and RI in group 1 (non-pregnant), group 2 (intrauterine pregnancy with live fetus), and group 3 (poor pregnancy outcomes) were 1.0 ± 0.3, 1.1 ± 0.4, 0.9 ± 0.2, and 0.6 ± 0.4, 0.6 ± 0.1, 0.6 ± 0.1, respectively; with no significant difference between three groups (p>0.05). Even Guzel AI et al [11], found that there was no statistically significant differences in PI and RI between the pregnant and nonpregnant groups (p>0.05).

In the index study, follicular vascularity >75% significantly predicted the pregnancy with 64% accuracy (p < 0.0001), LIUP was observed to be 0% in low grade, 10(71.43%) in mixed grade and 4(28.57%) in high grade perifollicular flows. The findings corroborate with study by Madkour NM et al [1], where 6 out of 8 pregnancies were from high grade vascularity group; 2 cases in mixed grades group and no cases got pregnant in low grade group.

Another study by Bhal et al (2001)[12] done in IUI cycles to assess perifollicular vascularity reported that pregnancy rate was more in highly vascularized follicles (n = 101, 31%) as opposed to 18% and 0% in mixed pattern (n=60) and poorly vascularized follicles (n = 21) (p<0.05). The pregnancy rate achieved was higher in Bhal et al [12] study as gonadotropin was used for ovulation induction and mean

number of follicles might have independently affected the outcomes to an extent.

In present study oral ovulogens only was used and the median number of follicles were two in LIUP group as compared to one in non-pregnant group ($p<0.0001$).

The endometrial aspect and its effects on the pregnancy outcomes showed a significant association with the endometrial blood flow but not with the endometrial thickness. A median endometrial thickness of 8.5 mm was found in both non-pregnant and LIUP group ($p=0.971$). The findings are in line with the other studies which showed the clinical pregnancy rates following IUI to be independent of endometrial thickness [13 -15]. The findings of high endometrial blood flow resulting in significantly more live clinical pregnancies as seen in our study corroborates with the study of Masrour MJ et al [16] who in their study found higher proportion of women with a meaningful blood flow in the pregnant women compared to non-pregnant women group (58.3% vs 17.2%, $p<0.0001$).

The main strength of present study is that it studied color doppler flow of uterus and ovary at the time of trigger in predicting pregnancy outcomes in IUI cycles whereas most of the studies till date focus on color Doppler indices in predicting pregnancy outcome in IVF cycles.Further, our study can act as a stepping zone for further larger studies to find out usefulness of color doppler indices in Indian women undergoing IUI.One of the limitations is the small sample size of the women included in this study. Another limitation is that the accuracy of measurement of blood flow velocity in ovarian artery may at times be difficult as the arteries within the ovary are not only small but tortuous.

Conclusion

Perifollicular and uterine blood flow assessment by doppler ultrasonography are good predictors for the pregnancy outcome in stimulated IUI cycles with IOA pulsatility index presenting as the best predictor.

Conflict of interest: None. **Disclaimer:** Nil.

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