Assessment of Endometrial Receptivity using Doppler Ultrasonography in Infertile Women in Controlled Ovarian Stimulation: An Observational Study

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Abstract

Objective: To evaluate endometrial and subendometrial blood flow parameters, follicular vascularity, assess the relationship between blood flows and pregnancy outcome.

Material and method: A prospective study done in tertiary care center. 204 women with infertility undergoing controlled ovarian stimulation and intrauterine insemination were enrolled. Transvaginal ultrasound with the Doppler flows was performed, on the day of hCG trigger. Endometrial blood flow, endometrial morphology, follicular vascularity and pulsatility index of uterine artery was studied. These findings were correlated with the pregnancy outcomes. The primary outcome was clinical pregnancy rate and secondary outcomes were miscarriages and ectopic pregnancy.

Results: The maximum clinical pregnancy rate (11.18%) was observed with triple line endometrium and endometrial thickness more than 8 mm on the day of trigger. 11 cases who conceived and continued the pregnancy had endometrial blood flow till Zone 3. 15.59% clinical pregnancy rate when follicular vascularity was between 51% and 75%. The clinical pregnancy in 16 cases (*p*-value = 0.323) was observed when the pulsatility index of uterine artery was between 2 and 3.2 on the day of trigger.

Conclusion: The pregnancy outcome is poor if the endometrial flows are absent, PI of uterine artery is less than 2 or more than 3.2 and follicular vascularity is less than 50% on the day of trigger. The endometrial blood flow Doppler studies and follicular vascularity on the day of trigger in COS cycles with intrauterine insemination can be useful in predicating pregnancy outcomes. Interventions can be planned in COS cycles to improve pregnancy outcomes.

Keywords: Infertility; Color Doppler ultrasound; Uterine and ovarian blood flows; Controlled ovarian stimulation.

Introduction

The success of an artificial reproduction technique procedure depends on the uterine endometrial receptivity. The window period when endometrium becomes receptive to embryo implantation is the crucial period. The uterine, endometrial and ovarian blood flow during this window period effects the implantation. The blood flow pattern can be assessed by using ultrasonography with

color Doppler. Color Doppler of uterine and ovarian blood flows has been used as a measure to predict the endometrial receptance to embryo, hence predicting the pregnancy outcome in IVF/ICSI procedures.

Ultrasound with color Doppler has emerged as a non-invasive technique to study the anatomy and physiological changes of uterus and ovary during the artificial reproduction techniques. The measurement of uterine, endometrial and ovarian blood flow by the ultrasound has played an important role in the predicting the pregnancy outcome in IVF/ICSI cycles.[1]

This study is an attempt to assess the same parameters in infertile women undergoing controlled ovarian stimulation and intrauterine insemination.

Materials and Methods

This was a longitudinal prospective observational clinical study conducted at IVF centre, ESIC PGIMSR, Delhi, India. The study was approved by the IEC. All the couple attending infertility clinic with either primary or secondary infertility, with women age between 20 and 38 years were enrolled in the study. Informed written consent prior to enrollment was taken. The baseline infertility work-up was done. The women with intrauterine pathology, acute vaginal and cervical infection, endometriosis and hydrosalpinx were excluded.

The 204 infertile women were recruited in the study. They underwent baseline ultrasound transvaginal on Day 2/Day 3 of menstrual cycle.

The measurement of endometrial thickness in the antero-posterior dimension were noted. The transducer was then angled to the right and left of midline in the sagittal plane to examine the number and size of follicles in the ovaries (antral follicle count).

Subjects with endometrial thickness <5 mm and follicle size less than 10 mm on ultrasound were planned for controlled ovarian stimulation with intrauterine insemination according to standard protocol followed in the institution.

Ultrasound follicular monitoring of all patients was performed by the investigator starting from Day 7 of stimulation cycle on 2D transvaginal sonography with 7.5–9 MHz. When the dominant follicle was 18 mm in gonadotropin cycle or 22–24 mm in clomiphene cycle and the trigger was planned. The endometrial pattern and blood flows, follicular flows and uterine arteries blood flows with color Doppler ultrasound were assessed.

Ascending branch of uterine artery was identified using color Doppler in the two-dimensional mode. A gate was placed over the vessel, the width of the sample gate (2–3 mm) is adjusted according to the vessel diameter and a blood velocity wave-form was obtained. When the signals passing the gate returned, the Doppler frequency shift echoes were converted electronically by a mathematical technique and displayed as a Doppler shift versus time waveform. The PI was calculated using three consecutive uniform heart beats and averaged. The mean value of uterine artery PI and RI/PSV from both ascending uterine artery branches were calculated (Fig. 1).

Fig. 1: Uterine artery Doppler.

Endometrial blood flow was detected by intra-endometrial or the adjacent sub-endometrial regions within 10 mm of the echogenic endometrial borders.

Double thickness of the endometrium was measured (maximum distance between each myometrial/endometrial interface through the longitudinal axis of the uterus).

The morphology of the endometrium on ultrasound was categorized into Type A: triple-line; Type B: slight-triple-line; Type C: hyperechoic type.

The subjects were categorized into three groups according to the endometrial blood flow: In Group A, no endometrial blood flow detected; Group B, sudendometrial blood flow detected; and Group C, both endometrial and subendometrial blood flow detected.

The color Doppler was placed over the ovarian follicle and took the cross-sectional image of follicle with the maximum color intensity representing the greatest Doppler frequency shifts. The follicular circumference was frozen and perifollicular blood flow was graded.

The subjects were divided into three groups according to the pregnancy outcomes. Group 1: non-pregnant group; Group 2: intrauterine pregnancy with living fetus (displaying a gestational sac and primary embryonic pulse on Day 30 by transvaginal ultrasonography); and Group 3: poor pregnancy outcome, including the patients with biochemical pregnancy, ectopic pregnancy, or miscarriage.

Outcomes

The primary outcome of the study was clinical pregnancy rate which was defined as ultrasound confirmation of gestational sac. Clinical pregnancy rate was defined as number of patients with clinical pregnancy divided by number of patients who underwent COS cycle.

The secondary outcome of the study was endometrial thickness, endometrial blood flows and follicular blood flow pattern. These were correlated with the pregnancy outcome.

Statistical Analysis

The statistical analysis was entered in the MS Excel spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Qualitative variables were correlated using chi-square test/ Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Results

Out of total 204 subjects, there were 20 pregnancies (9.80%) out of which 16 ongoing pregnancy (7.8%) and 1.96% had poor reproductive outcome.

There was no statistically significant difference in the demographic characteristics of the pregnant and the non-pregnant subjects (Table 1). The 54.9% subjects were age above 25 years. 92.6% subjects had primary infertility with 57.84% were unexplained.

The triple-line endometrium pattern had 11.18% pregnancy rate (Table 2). The endometrial flow was detected in 174 subjects. The detectable flow

Table 1: Demographic comparison between pregnant and non-pregnant group.

		Negative (*n* = 184) (90.20%)	Pregnancy Positive (*n* = 20) (9.80%)	Total	*p*-value
Age	1)< = 25	79 (85.87%)	13 (14.13%)	92 (100.00%)	0.060
	2) > 25	105 (93.75%)	7 (6.25%)	112 (100.00%)	
Type of infertility	Primary	168 (89.36%)	20 (10.64%)	188 (100.00%)	0.376
	Secondary	16 (100.00%)	0 (0.00%)	16 (100.00%)	
Cause	Male	34 (79.06%)	9 (20.93%)	43 (100.00%)	
	Anovulation	16 (94.11%)	1 (5.88%)	17 (100.00%)	
	Tubal	8 (88.88%)	1 (11.11%)	9 (100.00%)	
	Premature ovarian failure	6 (100.00%)	0 (0.00%)	6 (100.00%)	0.009
	Septal resection	8 (80%%)	2 (20%)	10 (100.00%)	
	Thin endometrium	1 (100.00%)	0 (0.00%)	1 (100.00%)	
	Unexplained	111 (94.07%)	7 (5.93%)	118 (100.00%)	

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till Zone 3 observed pregnancy rate 12.5% (Table 3). The follicular flow between 26 and 50% was detected in 57.84%. The clinical pregnancy rate of 15.69% was observed in subjects with follicular blood flow of 51%–75% (Tables 4,5).

Table 2: Relationship of endometrial morphology with pregnancy outcome

Endometrial morphology Group 1 n = 184 (90.20%)	Group 2 n = 16 (7.84%)	Group 3 n = 4 (1.96%)		Total	p-value
			n = 204		
Diffuse	12 (92.31%)	1 (7.69%)	0 (0%)	13 (100.00%)	
Hyperechoic	45 (93.75%)	2 (4.16%)	1 (2.08%) abortion	48 (100.00%)	0.751
Triple layer	127(88.81%)	13 (9.09%)	3 (2.09%) 2 ectopic 1 abortion	143 (100.00%)	

Table 3: The relationship of endometrial blood flow with pregnancy outcomes

Endometrial blood flow	Group 1 n = 184 (90.20%)	Group 2 n = 16 (7.84%)	Group 3 n = 4 (1.96%)	p-value
Zone 1	27 (90.00%)	0	3 (10.0%)	0.0008
Zone 2	64 (92.75%)	5 (7.25%)	0	0.338
Zone 3	70 (87.50%)	10 (12.50%)	0	0.42
Zone 4	23 (92.00%)	1 (4.00%)	1 (4.00%)	0.562

Table 4: The relationship between parameters used in color Doppler imaging of blood flow and pregnancy outcomes

Group	Endometrium thickness on the day of trigger	Uterine artery blood flow on the day of trigger			Follicular blood flow on the day of trigger.			
		S/D	Pulsatility Index (PI)	Resistive Index (RI)	F1 (0–25%)	F2 (26%–50%)	F3 (51%–75%)	F4 (76%–100%)
Group 1 non-pregnant n = 184 (90.20%)	8.21 ± 1.6	10.81 ± 11.87	2.4 ± 1.43	1.24 ± 1.18	26 (89.66%)	109 (92.37%)	43 (84.31%)	6 (100.00%)
Group 2 pregnant n = 16 (7.84%)	8.7 ± 1.71	8.58 ± 4.83	3.32 ± 4.19	0.91 ± 0.3	0	8 (6.783%)	8 (15.69%)	0
Group 3 poor pregnancy outcome n = 4 (1.96%)	8.4 ± 1.85	7.24 ± 4.98	2.6 ± 0.37	1.24 ± 0.32	3 (10.34%)	1 (0.85%)	0	0
p-value 1 vs 2; z value	0.304;1244	0.971;1464	0.323;1252.500	0.398;1284.500	p-value 0.0006	p-value 0.313	p-value 0.031	p-value 0.715
p-value 1 vs 3; z value	0.911;356	0.636;317	0.145;211	0.061;166.500				
p-value 2 vs 3; z value	0.635;.225	0.571;.321	0.508;.438	0.023;5.166				

Table 5: Correlation between endometrial blood flow parameters and pregnancy outcomes

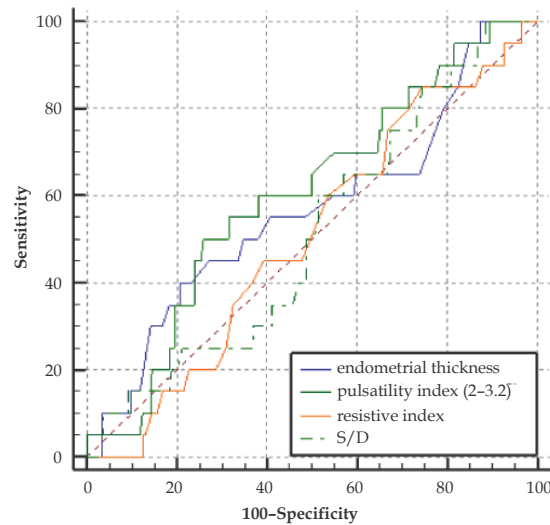|	p-value	Sensitivity	95% CI	Specificity	95% CI	+PV	95% CI	–PV	95% CI
Endometrial thickness	0.3742	40	19.1–63.9	79.35	72.8–85.0	17.4	7.8–31.4	92.4	87.1–96.0
Pulsatility index	0.1132	50	27.2–72.8	73.91	66.9–80.1	17.2	8.6–29.4	93.2	87.8–96.7
Resistive index	0.9519	0	0.0–16.8	87.5	81.8–91.9	0	0.0–14.8	89	83.5–93.1
S/D	0.856	25	8.7–49.1	63.04	55.6–70.0	6.8	2.3–15.3	88.5	81.8–93.4

Fig. 2: Area under the ROC curve (AUC)

ROC curve for comparison of specificity, sensitivity, positive predictive value and negative predictive value.

Area under the ROC curve (AUC)

endometrial thickness	0.565
pulsatility index	0.602
resistive index	0.504
S/D	0.512

Discussion

The ultrasound on the day of trigger to study endometrial parameters with Doppler blood flow and follicular vascularity can help to study endometrial receptivity in artificial reproduction techniques. The ultrasound is non-invasive means to predicate pregnancy outcomes.

In the present study, the transvaginal ultrasound was done on the day of trigger prior to the injection hCG. The hCG injection causes increased impedance of uterine arteries and thus influence the predictive value of ultrasonography.[2]

In this study, the endometrial thickness on the day of trigger with the range of 7–9 mm had pregnancy rate of 7.84%. No pregnancy was documented below 7 mm endometrium thickness. The finding was in consistent with Khan MS et al. study which reported that no pregnancy occurred when endometrial thickness was below 7 mm.[3] The study by Kader et al.[4] (2016) and Singh et al.[1] (2011) found that endometrial thickness between 8 and 10 mm (50% of them achieved pregnancy)

was strongly correlated with successful pregnancy in IVF/ICSI cycles. No pregnancies were achieved when endometrial thickness was less than 8 mm.

The endometrial pattern independently affects the pregnancy outcomes. In the present study the pregnancy rate was 9.09% when the triple-layer endometrium was present on the day of trigger. The pregnancy rate decreased to 7.6% with diffuse endometrium and 4.13% with hyperechoic endometrium on the day of trigger (*p*-value-0.751). Khan MS et al. reported 68.5% pregnancy rate in triple-line endometrium.[3] The Zhao et al. concluded that a triple-line pattern with a moderate endometrial thickness (>7 mm) appeared to be associated with a 55.2% clinical pregnancy rate.[5] Various studies concluded that pregnancy is achieved when a triple-line pattern of the endometrium is present on day of hCG. (Kader et al.,[4] El-Zenneni et al.[6] (2014), Singh et al.[1] (2011).

The endometrial and periendometrial areas are divided into the following four zones[7]:

- Zone I: A 2-mm thick area surrounding the hyperechoic outer layer of the endometrium.

- Zone II: The hyperechoic outer layer of the endometrium.

- Zone III: The hypoechoic inner layer of the endometrium.

- Zone IV: The endometrial cavity.

In the present study,12.5% pregnancy rate was seen when the endometrial blood flow was till Zone III followed by 7.25% in Zone II. In the series by Khan MS et al.,[3] the pregnancy rate was significantly higher in patients with multifocal

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vascularity in Zone III. Absent endometrial vascularization was associated with no conception which was supported by study.

In the study by Kader et al.[4], 57.5% of the pregnant women had Zone III of endometrial blood flow, 37.5% had Zone II of blood flow while only 5% of the pregnant women had Zone I of blood flow.

The 12.87% pregnancy were seen with PI value 2–3.2 (p-value 0.312). 4.35% conception reported when PI value above 3.2. The study by Wang et al.,[8] found no association between uterine arterial blood flow and pregnancy outcome.

Tekay et al. reported no pregnancy when the PI was more than 3.[9]

Grading system for the follicles vascularity, expressed in percentage of follicular circumference in which flow was identified. (Chui et al., 1997)[10] F1 <25% of the follicular circumference, F2 25–50% of the follicular circumference, F3 51–75% of the follicular circumference, F4 > 75% of the follicular circumference.

In the present study, 15.59% had follicular vascularity of F3 (p = 0.003) which had pregnancy. Follicular vascularity Grade F1 had significant relation with negative pregnancy outcome (p-value 0.0006). Thus, poor perifollicular vascularity on the day of trigger was associated with no pregnancy outcomes.

The study done by Ragni et al.[11] stated that perifollicular vascularity does not have predictive value with the pregnancy outcome in IUI cycles.

Huyghe et al.[12] investigated the predictive value of the perifollicular flow on the outcome of assisted reproduction. The study showed an important prognostic value of perifollicular blood flow and Doppler on the pregnancy rate after IVF. But the prognostic value was contradictory in IUI.

The study done by Naredi N et al.[13] in IVF-ET cycles, they had 733 follicles aspirated and retrieved 627 oocytes. The 75.8% oocytes from Grade: III and IV. The percentage of oocytes retrieved with their maturity and fertilization rates were of statistical significance (p = 0.0001) when high and low perifollicular vascularity grades were compared.

Conclusion

The endometrial thickness 7 mm or more with triple -line pattern and good endometrial flow on the day of trigger can be useful in predicting pregnancy outcomes in IUI cycles.

The pulsatility index (PI) of uterine artery on the day trigger between 2 and 3.2 correlates with good pregnancy outcomes in IUI cycles.

The F3 and F4 follicular vascularity on the day of trigger is associated with good follicular perfusion.

Thus, Doppler ultrasonography to study endometrial sensitivity is a promising prognostic tool in predicating pregnancy outcomes in artificial reproductive outcome.

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