Section: Obstetrics and Gynaecology

Original Research Article

ROLE OF ENDOMETRIAL RECEPTIVITY AND PERIFOLLICULAR BLOOD FLOW BY 2D POWER COLOUR DOPPLER AS A PREDICTIVE FACTOR FOR PREGNANCY OUTCOME

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Abstract

Background: The use of ultrasound to examine the endometrium is a non-invasive method used during in vitro fertilization. Studies have reported a correlation between endometrial receptivity and perifollicular blood flow where a good endometrial blood flow is considered necessary for an improved pregnancy outcome. **Materials and Methods:** The prospective, observational, cohort study was conducted at the Institute of Obstetrics and Gynecology, Egmore for a duration of 8 months among 200 women attending the infertility clinic. Patients age between 20 to 35 years of age were included with unexplained primary or secondary infertility and normal levels of basal FSH and LH levels. After the ethical approval patients were prescribed with T.Letrozole (2.5mg) BD for 5 days and ultrasonography (USG) was performed to assess the endometrial receptivity and perifollicular blood flow on Day 9 of the cycle. Data were analyzed based on variables and quantitative assessment. **Result:** A total of 200 patients were enrolled with equal distribution for a history of timed intercourse and intrauterine insemination (IUI). A majority of the patients were in the age group of 25-30 years (40.50%), including 15.0% of patients with a history of IUI. TIC reported with higher pregnancy rates (22%) and IUI (10%). Endometrial blood flow revealed that zone 3 patients had a better rate of pregnancy (79.41%) along with perifollicular blood flow >75% (61.7%). A good myometrial blood flow also reported higher pregnancy outcome (55.56%), followed by urinary artery doppler rate <3 (57.14%), and endometrial thickness of >8 mm (93.75%) with a significant difference (P <0.001). **Conclusion:** The endometrial, perifollicular, and myometrial blood flow is strongly associated with positive pregnancy outcomes along with an endometrial thickness >8 mm and urinary artery doppler rate <3.

INTRODUCTION

Infertility is one of the major complications experienced by at least one in seven couples in the western world and one in every four couples in developing countries.[1] In certain regions such as South Asia, Middle East, and Central Asia the infertility rates may reach 30%.[2]

The idiopathic type of infertility has been defined as the lack of an obvious cause for a couple's infertility and the inability of a female to get pregnant after at least 12 cycles of unprotected intercourse or at least six cycles in women above the age of 35 years.[1] During the menstrual cycle, the endometrium has no adhesive capabilities until the implantation window phase, when the endometrial facilitates the implantation of gestational sacs for a relatively brief time.[2] This characteristic is known as endometrial receptivity. Endometrial receptivity has long been the primary focus in the field of assisted reproduction because the synchronization of endometrial alterations with embryonic development is the foundation for embryonic implantation.[3]

Although a triple-layered endometrial pattern and an endometrial thickness higher than 7 mm have been proposed as measures of endometrial receptivity, they have produced a significant percentage of false-positive outcomes.[4] A strong blood supply to the endometrium is usually thought to be required for implantation. The thin endometrium is thought to have poor blood flow, and several medications,

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including low-dose aspirin and vaginal sildenafil, have been attempted to promote endometrial blood flow and development.[5] A high uterine artery pulsatility index (PI) has been linked to reduced implantation and pregnancy rates (PRs).[6] Uterine blood flow is a significant element in uterine receptivity and can be measured using two-dimensional (2D)-power color doppler (PCD) ultrasound.[7] Previous literature has reported the use of this technique to differentiate between the conception and non-conception cycles of women. [8]
On the basis of previously conducted studies, it has been reported that doppler ultrasonography is one of the potential methods to predict the outcome of stimulated cycles in women. The current study aims to evaluate the role of endometrial receptivity by assessing the vascularity of endometrium and identifying any potential relationship between perifollicular blood flow and fertility outcome.

MATERIALS AND METHODS

The prospective, observational, cohort study was conducted at the Institute of Obstetrics and Gynecology, Egmore, for a duration of 8 months after taking ethical approval from the ethics committee and patient consent was obtained. Women attending the infertility clinic at the Institute of Obstetrics and Gynecology were enrolled in the study.

Inclusion Criteria

- Women attending OPD at the infertility clinic
- Women age between 20 to 35 years
- Women with primary or secondary infertility
- Patients with unexplained infertility
- Patients with at least one patent fallopian tube
- Patients with normal basal FSH and LH levels

Exclusion Criteria

- Patients with a previous history of ovarian surgery
- Patients with poor ovarian reserve
- Patients with chronic systemic diseases such as hypertension, diabetes, coronary artery disease, cerebrovascular accident, or connective tissue diseases
- Patients with pulmonary/hepatic disease/ prolactinemia
- Patients who were SERO Positive
- Spouse with oligospermia, asthenospermia, azoospermia

Data Collection

Patients were enrolled in the study after ethical approval and obtaining informed consent. Demographic details such as medical history, age, and other past history were collected. Patients prescribed T. Letrozole (2.5 mg) twice a day (BD) for 5 days who were on day 2 of the cycle and serial ultrasonography was conducted to assess the endometrial receptivity and perifollicular blood flow by endometrial and perifollicular doppler which was performed on the day 9 of the cycle.

Endometrial receptivity assessment

Combination of ―triple-layered endometrium‖ (ET>8mm) with low vascular impedance in uterine arteries (i.e) PI <3.0, and endometrial vascularity (Zone III vascularity) are considered good indicators.

Zones of vascular penetration

- ZONE I- Subendometrial zone
- ZONE II- Outer hyperechogenic zone
- ZONE III- Inner hyoechogenic zone

Perifollicular Blood Flow (PFBF)

Vascular perfusion of the maturing follicle (intra-ovarian stromal blood flow) was graded based on a percentage of follicular circumference seen to be vascularised.

Good PFBF-Vascularity of >3/4 (i.e)>75% circumference.

Following induction with T. Letrozole, participants were monitored for the follicular size from Day 9 of the cycle. When the size of the follicle of 15mm is achieved participants were advised for TIC (Timed intercourse).

Participants with previously failed induction and participants with a long period of infertility were advised for IUI (intrauterine insemination) when the follicle size reaches 15mm.

The pregnancy outcome correlated with these factors and the participants are followed up.

Data Analysis

The collected data was analyzed using SPSS 21.0 software, continuous variables were derived by mean ± SD. Qualitative variables were derived using percentages and the test of significance (P <0.05) was considered significant. Unpaired T-test, Chi-square test, and relative risk (RR) were used.

Primary and Secondary Outcomes

Pregnancy outcome was considered the primary outcome variable, followed by endometrial blood follow, endometrial thickness, perifollicular blood flow, myometrial blood flow, and uterine artery doppler was considered as an explanatory variable. Data were also represented using appropriate bar diagrams and pie charts.

RESULTS

From a total of 200 patients, 100 patients were treated with timed intercourse (50.0%) and 100 patients had a history of the intra-uterine insemination (IUI) method [Table 1].

The majority of the patients were in the age group of 25-30 years of age accounting for 81 patients (40.50%), followed by the age group of 31-35 years of age with 61 patients (30.50%), and 21-25 years of age comprising of 58 patients (29.0%) [Table 2]. The mean duration of marriage was 4.87±1.66 years.

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Table 1: Descriptive analysis of the study population

Study Group	Frequency	Percentages
TIC	100	50.00%
IUI	100	50.00%

Table 2: Age distribution in patients

Age	Frequency	Percentages
21-25yrs	58	29.00%
25 - 30yrs	81	40.50%
31 - 35yrs	61	30.50%

Patient's BMI was calculated which revealed that 54.0% of the study population were in the range of 23 to 24.99, followed by 24.0% of patients with BMI > 25, and 22.0% between the BMI of 18.5 to 22.9 respectively [Figure 1]. Further to this polycystic ovary syndrome was reported in 55 patients (27.50%) and 58 patients (29.00%) had a history of hypothyroidism.

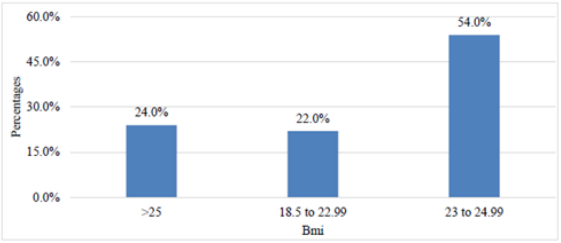

Figure 1: BMI distribution in the study population

Based on the history of ovulation, 28.00% of patients had 4 cycles, followed by 41.0% with 3 cycles, and 17.00% had 2 cycles. 1 cycle was reported in one patient. The urinary positive test was positive in 32 patients (16.00%) whereas, 168 patients were negative.

Table 3: History of ovulation induction in the patients

H/O Ovulation Induction	Frequency	Percentages
1cycle	1	0.50%
2cycles	34	17.00%
3cycles	82	41.00%
4cycles	56	28.00%
Nil	27	13.50%

From a total of 200 patients, 30 patients had a history of IUI accounting for 15.00% of the study population [Figure 2].

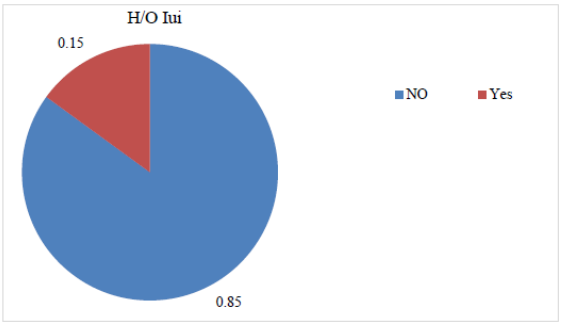

Figure 2: History of IUI in patients

Endometrial blood flow assessment in patients revealed that 45.50% of patients were in zone 2, 37.50% of patients in zone 1, and 17.00% were in zone 3 [Table 4]. In addition, endometrial thickness revealed that 153 patients (76.50%) had a thickness <8 mm, and 47 patients (23.50%) were reported with a thickness >8 mm. A total of 146 patients had poor endometrial blood flow (73.00%) and 54 patients (27.00%) were seen with good endometrial blood flow.

Table 4: Endometrial blood flow assessment in patients

Endometrial Blood Flow	Frequency	Percentages
Zone1	75	37.50%
Zone2	91	45.50%
Zone3	34	17.00%

Perifollicular blood flow assessment revealed that 77 patients (38.50%) reported with blood flow between 50-75%, followed by 76 patients (38.00%) with blood flow <25% and 47 patients (23.50%) reported with blood flow >75% [Table 5].

Table 5: Perifollicular blood flow in the study population

Perifollicular Blood Flow	Frequency	Percentages
<25%	76	38.00%
>75%	47	23.50%
50 - 75%	77	38.50%

Myometrial blood flow assessment revealed that 73.00% of patients had low/poor myometrial blood flow whereas, 27.00% of patients had good myometrial blood flow [Figure 3].

The viability test revealed that 28 patients (14.00%) were positive for viability whereas, 172 patients were not viable.

A positive outcome of pregnancy was reported in 22 patients who had timed intercourse and 10 patients were positive with the use of the IUI method. A significant difference was reported between the pregnancy outcome and study group (P value <0.021) [Table 6].

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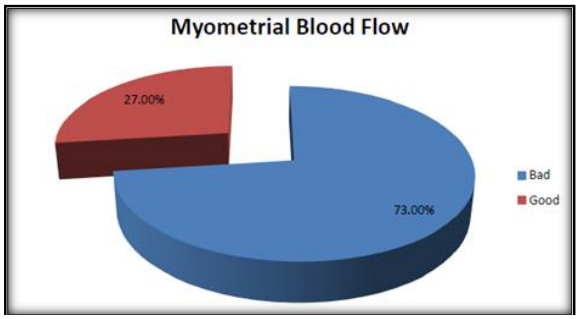

Figure 3: Myometrial blood flow assessment in the study population

Table 6: Comparison of pregnancy outcome based on the used methods

Pregnancy Outcome	Study Group		Chi square	P value
	Tic (N=100)	Iui (N=100)		
Negative	78 (78%)	90 (90%)	5.357	0.021
Upt Positive	22 (22%)	10 (10%)		

The comparison of endometrial blood flow and pregnancy outcome reported a significant difference with p-value <0.001. A positive pregnancy outcome was reported in 5 patients in zone 2 of blood flow and 27 patients in zone 3 of blood flow [Table 7].

Table 7: Pregnancy outcome and endometrial blood flow

Endometrial Blood Flow	Pregnancy Outcome		P value
	Negative	Upt Positive	
Zone1 (N=75)	75 (100%)	0 (0%)	<0.001
Zone2 (N=91)	86 (94.51%)	5 (5.49%)	
Zone3 (N=34)	7 (20.59%)	27 (79.41%)	

Among the study population patients with perifollicular blood >75% reported positive outcomes comprising 29 patients (61.7%) and 3 patients (3.9%) with perifollicular blood between 50-75%. A significant difference was reported between the perifollicular blood flow and pregnancy outcomes [Table 8].

Table 8: Comparison of perifollicular blood flow and pregnancy outcome

Perifollicular Blood Flow	Pregnancy Outcome		P value
	Negative	Upt Positive	
<25% (N=76)	76 (100%)	0 (0%)	<0.001
>75% (N=47)	18 (38.3%)	29 (61.7%)	
50 - 75% (N=77)	74 (96.1%)	3 (3.9%)	

In addition, 30 patients with good myometrial blood flow reported positive pregnancy outcomes whereas only 2 patients with bad flow reported positive outcomes. A significant difference (p-value <0.001) was reported between the myometrial blood flow and pregnancy outcomes [Table 9]. Uterine artery doppler <3 in 32 patients were reported with positive pregnancy outcomes with a significant difference (p-value <0.001) respectively [Table 9].

Table 9: Comparison of endometrial and myometrial parameters with pregnancy outcomes

Myometrial Blood Flow	Pregnancy Outcome		Chi-square	P value
	Negative	Upt Positive		
Bad (N=146)	144 (98.63%)	2 (1.37%)	86.117	<0.001
Good (N=54)	24 (44.44%)	30 (55.56%)		
Urinary Artery Doppler				
<3 (N = 56)	24 (42.86%)	32 (57.14%)		<0.001
>3 (N = 144)	144 (100%)	0 (0%)		
Endometrial Thickness				
<8 mm	151 (89.88%)	2 (6.25%)	104.576	<0.001
>8 mm	17 (10.12%)	30 (93.75%)		

Further to this, patients with endometrial thickness >8 mm had a significant difference with positive pregnancy outcomes in 30 patients when compared with patients with endometrial thickness <8 mm.

DISCUSSION

The study evaluated the role of endometrial blood flow in pregnancy outcomes measured by using 2D power color doppler. A significant correlation between endometrial parameters and positive pregnancy outcomes. Among the study population, an equal distribution of patients was seen with timed intercourse and IUI. Endometrial receptivity is critical for embryo implantation. Endometrial thickness and pattern are both agreed to be useful as predictive factors for successful pregnancy in in-vitro fertilization/intracytoplasmic sperm injection and embryo transfer (IVF/ICSI-ET). Implantation requires adequate perfusion of the endometrium.

In our study, we find that the majority of infertility was prevalent in the age group of 25 to 30 years of age at 30.50%, followed by 31 to 35 years of age with 29% of the patients. A study conducted by Singh et al, reported a higher prevalence of infertility in the age group of 31-35 years of age at 30.7% which was similar to our study findings. [9] Approximately 87% of patients in our study were reported with primary infertility and 13% had secondary infertility which was also similar to the findings of Singh et al, where primary infertility was 74.25% and secondary infertility was 25.74% respectively.[9]

The current study findings report positive pregnancy outcomes in patients with endometrial blood flow of

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zone 3 blood flow comprising 29 patients (79.41%) and in zone 2 blood flow with 5 patients. A high endometrial blood flow has been postulated with improved pregnancy outcomes as the current study also observed a significant difference between pregnancy outcome and endometrial blood flow (P value <0.001). Singh et al, also observed similar observation where zone 3 patients (51.8%) had higher pregnancy rates when compared with zone 1 patients (14.8%).[9] Consistency in data was also reported in the study conducted by Zaidi et al, where zone 3 endometrial blood flow patients were seen with a 37.9% of pregnancy rate and zone 2 patients with 37.6% respectively.[10]

A significant difference was also reported between endometrial thickness >8 mm with 30 patients (93.75%) which signifies that thicker endometrium correlates with higher pregnancy rates. Gonen et al reported with similar findings where higher endometrial thickness >7 mm was acceptable for implantation and revealed positive pregnancy outcomes.[11] Oliveria et al, also reported that endometrial thickness <7 reported low pregnancy outcomes in patients with infertility.[12] Coulam et al, reported that patients with endometrial thickness of more than 6 mm can be accepted for implantation with positive pregnancy outcomes.[13] In contrast, Weissman et al reported that endometrial thickness >14 mm reveals a higher miscarriage rate which was not observed in the current study.[14] In a prospective study by Wang et al, 182 women reported that improved endometrial thickness, echo pattern, and blood flow eight hours prior to human chorionic gonadotropin (hCG) injection reported higher pregnancy rate and implantation rate in women with detectable blood flow.

The current study finding also reports a significant difference in positive pregnancy outcomes with perifollicular flow >75%. Amanda et al, demonstrate a similar observation where higher perifollicular blood flow is associated with better pregnancy outcomes.[16] Bhal et al also reported similar results where higher perifollicular blood flow was associated with improved pregnancy outcomes. Thus, follicular assessment can be used as one of the prognostic markers to improve the rate of in-vitro fertilization treatment cycles.[17]

The current study reports that endometrial thickness and blood flow can be used as prognostic markers to assess the current condition of infertility and improve the rate of pregnancy in patients with infertility. In addition, improved perifollicular blood flow, and high vascularity are associated with positive pregnancy outcomes.

CONCLUSION

An improved perifollicular blood flow and endometrial receptivity of zone III had more positive pregnancy outcomes. Patients with endometrial thickness >8 mm, low pulsatility index in uterine artery doppler, and good myometrial blood flow are more prevalent with higher pregnancy rates.

Hence, Endometrial receptivity and Perifollicular vascularity assessment could be employed to improve the success of artificial reproductive procedures in the future. There is a need to conduct further studies with adjuvants such as HMG and a large study population may be required.

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