## Original Article

# Endometrial Perfusion on day of hCG Trigger in IVF Cycles Directly Correlates with the Dynamically Changing Endometrial Thickness : A Pilot Study Examining Potential Implications for Endometrial Receptivity and the Development of an Integrated Model to Assess Endometrial Function

**Biswanath Ghosh Dastidar[1], Sudarsan Ghosh Dastidar[2], Jayshree Majumdar[3], Chandan Chakraborty[4], Kakoli Ghosh Dastidar[5]**

Transvaginal Ultrasound (TV-USG) is an indispensable tool for investigation and patient management in infertility and Assisted Reproductive Technology (ART). It provides us with both anatomic and physiologic information about the uterus, endometrium, ovaries and fallopian tubes; apart from facilitating important procedures like Ovum Pick-up (OPU) and Embryo Transfer (ET). Endometrial assessment by sonographic and other means has been advocated as methods to assess endometrial receptivity in IVF cycles to help predict prognosis. TV-USG has been classically used to assess endometrial thickness and pattern. Recent studies have shown the presence of wave like contractions in the sub-endometrial layer. We have previously reported that these contractions result in variations in endometrial thickness and thus a mean value must be used for endometrial assessment in IVF cycles. It is also thought that optimal sub-endometrial spiral artery blood flow might be important to result in a receptive endometrium. We designed this study to evaluate whether sub-endometrial blood flow varies with endometrial thickness. Our results indicate that peak systolic flow in sub-endometrial spiral arteries is positively correlated with endometrial thickness. Future studies should aim to develop an integrated model for endometrial receptivity using these sonographic parameters to facilitate more accurate prediction and management in IVF patients.

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***Key words*** **: Endometrial Thickness (EMT), Sub-endometrial Contractions, Endometrial Perfusion, Color Doppler, Peak Systolic Velocity, Endometrial Receptivity.**

Around 8-12% of couples of reproductive age worldwide are involuntarily infertile[1]. Ultrasonographic (USG) imaging has proven to be an indispensable tool in the field of infertility management and Assisted Reproductive Technology (ART). A trans-vaginal sonography provides us with a vast array of qualitative and quantitative information and guidance in the management of various ART protocols. USG is used to not only obtain detailed anatomic information about the reproductive organs such as the uterus, ovaries and fallopian tubes, but also offers important prognostic information related to the physiologic functioning of ovaries and the uterine endometrium. Color Doppler flow velocimetry studies are particularly useful in assessing endometrial and ovarian physiology.

Despite significant advances in most clinical as well as technical aspects of ART over the last few decades, embryo implantation rates still remain low globally[2]. Thus, there has been much research focus on refining techniques for embryo culture, selection and transfer. Widespread research is also being carried

***Editor's Comment :***

- The Endometrial Thickness (ET) changes cyclically due to wave-like sub-endometrial contractions and while measuring ET it might be good clinical practise to take an average of multiple readings.
- Endometrial perfusion as measured by Peak Systolic Velocity (PSV) of blood flow in the sub-endometrial spiral arteries shows positive correlation with mean endometrial thickness.
- Further studies are needed to establish whether these parameters may be integrated to develop a prognostic marker for embryo implantation and thus IVF outcome.

[1]MBBS (Hons), MSc (Distinction), MS (Obstat & Gynae) (Gold Medallist), Hony Research Director, G D Institute for Fertility Research, Kolkata 700025; At present : Clinical Tutor, Department of Obstetrics and Gynaecology, IPGME&R and SSKM Hospital, Kolkata 700020

[2]MD, Director, G D Institute for Fertility Research, Kolkata 700025

[3]M Tech, Indian Institute of Technology, Kharagpur, West Bengal 721302

[4]PhD, Indian Institute of Technology, Kharagpur, West Bengal 721302

[5]MBBS, PG (Dip), G D Institute for Fertility Research, Kolkata 700025 and Corresponding Author

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out for the assessment of endometrial receptivity in order to predict chance of implantation. Endometrial thickness and the classic triple line appearance of the endometrium on sonography are thought to be markers for endometrial receptivity. Endometrial Thickness (EMT)<7 mm has been correlated with poor implantation, although there is yet to be conclusive evidence of linear correlation between EMT and implantation potential[3]. Molecular and genetic markers of endometrial receptivity have also been studied by various groups[4-8].

### Endometrial Thickness and Pattern :

Multiple studies have tried to correlate endometrial thickness and morphologic pattern with IVF success, with mixed results. Conflicting results have both supported[3,9-12] as well as dismissed[13-15] the role of endometrial thickness to predict prognosis in IVF cycles. Studies on endometrial pattern have also been inconclusive. Three principal endometrial patterns are recognized : Early follicular proliferative, Peri-ovulatory triple-stripe pattern and Luteal secretory pattern (homogeneously hyperechogenic). Studies have shown that patients with triple layer endometrium on day of HCG were more likely to get pregnant, but not across all different endometrial thickness ranges[9,10]. Thus, it appears that more integrated, multi-parameter models are required to more effectively assess the status of endometrial receptivity.

### Sub-endometrial Contractility :

Various studies have reported the presence of wave-like contractions in the endometrial-myometrial Junctional Zone (JZ) which propagate in anterograde or retrograde manner through the uterus[16-18]. We reported that these sub-endometrial contractions resulted in changing endometrial thickness over time and were possibly the first group to recommend that mean value of endometrial thickness measured over multiple contractions be used as a more accurate predictor of IVF prognosis[19]. Some groups have shown that an inverse relationship exists between sub-endometrial wave activity and cycle outcome[20]. However, there is yet to be consensus on how these wave-like contractions may affect embryo implantation and thus IVF results.

### Endometrial Perfusion :

It seems reasonable to assume that good blood flow to the endometrium would be a pre-requisite for optimal endometrial function and receptivity. Many studies have tried to correlate endometrial blood flow with IVF outcome. Although some of the indices showed some advantages towards predicting implantation[21], others have failed to show any beneficial role[22]. The current call in the field appears to be the development of integrative endometrial scores which would include data about endometrial thickness, sub-endometrial contractility, and endometrial blood flow[23], in order to more accurately predict endometrial receptivity and thus, IVF outcome.

Thus, we designed a study to assess if endometrial thickness correlates with the end-organ blood flow reaching the endometrium, as an initial step towards developing a multi-parameter, integrated model to assess endometrial receptivity.

## Materials and Methods

The study was carried out at a tertiary fertility center in India.Institutional ethical committee approval was taken. After informed consent, an initial series of patients undergoing IVF were recruited into the study (n=25). All patients were undergoing Ovarian Stimulation (OS) using long agonist protocol and Human Menopausal Gonadotropin (HMG), with embryo transfer planned in fresh cycle. There were no other inclusion or exclusion criteria. Four patients dropped out of the study at various stages due to cycle cancellations and loss to follow up (Drop-out rate= 16%). Sub-endometrial JZ contractions were observed by transvaginal sonography for each patient on day of hCG (Wipro LogiqPro 500, Wipro-GE, USA) and 3 endometrial thickness values were measured across contractions and the mean value was recorded as the EMT. Simultaneously, endometrial perfusion was measured by color Doppler by measuring the Peak Systolic Velocity (PSV) in spiral artery in the endometrium and JZ upto 10 mm from the endometrial-myometrial interface. Three complete wave forms were recorded over the contractions by Pulsed Doppler with angle correction kept as close to 0 as possible and wall filter at 0.3-0.7. The mean peak systolic flow (cm/sec) over three waveforms of endometrial spiral artery were recorded and their mean value used for data analysis. Data from 5 patients was left out of final data analysis owing to very poor or absent sub-endometrial blood flow and technical difficulty in velocimetry resulting in lack of interpretable PSV data. Sixteen patients completed the study upto data analysis. Data was analysed using Microsoft Excel and SPSS statistical software. Pearson's correlation coefficient (r) was computed to check for possible correlation between sub-endometrial flow and endometrial thickness (Table 1, Figs 1&2).

## Results

Analysis of patient demographic and baseline features shows that 81% of patients had presented

Table 1 — *Summary of Study Data*

| Age | Type of Infertility | Cause of Infertility | Endometrial Thickness (mm) over 3 contractions | Mean Endometrial Thickness (mm) | Number of contractions (over 1 min) | Mean Peak Systolic Flow (cm/sec) |
|---|---|---|---|---|---|---|
| 25 | Primary | Tubal | 11, 9, 17 | 12.3 | 7 | 3.81 |
| 38 | Primary | Tubal | 11, 7, 9 | 9 | 1 | 3.44 |
| 44 | Primary | Tubal | 7, 8, 9 | 8 | 3 | 5.93 |
| 40 | Secondary | Male factor | 11, 6, 10 | 9 | 2 | 1.68 |
| 37 | Primary | Unexplained | 11, 6, 7 | 8 | 3 | 0 |
| 36 | Primary | Unexplained | 8, 6, 7 | 7 | 2 | 2.37 |
| 39 | Primary | Unexplained | 6.6, 6, 9 | 7.2 | 2 | 1.59 |
| 33 | Primary | Male factor | 5.2, 6, 9 | 6.7 | 2 | 2.19 |
| 37 | Primary | Male factor | 13, 11, 13.5 | 12.5 | 2 | 5.39 |
| 28 | Primary | Male factor | 8, 6, 8.3 | 7.4 | 2 | 1.79 |
| 28 | Secondary | Tubal | 8, 6, 8 | 7.3 | 2 | 4.24 |
| 29 | Primary | PCOD | 6.7, 6, 8.7 | 7.1 | 2 | 3.34 |
| 39 | Primary | PCOD | 9.7, 9, 11 | 9.9 | 5 | 2.66 |
| 36 | Secondary | Tubal | 9.7, 9, 11 | 9.9 | 6 | 4.07 |
| 34 | Primary | Male factor | 9, 7, 8 | 8 | 6 | 3.12 |
| 35 | Primary | Male factor | 7, 6, 8 | 7 | 2 | 3.32 |

with primary infertility (n=13) and 19% with secondary infertility (n=3). Distribution of cause of infertility was as follows: 6 with male factor (37%), 5 with tubal factor (31%), 2 with anovulatory/PCOD (13%) and 3 patients with unexplained infertility (19%). Mean age of the patient group was seen to be 34.9 years.

Analysis of sonographic data revealed that there was positive correlation (r=0.401) between mean endometrial thickness (mm) and peak systolic flow in sub-endometrial spiral arteries (cm/sec) as seen on Doppler (Fig 3).

## Discussion

A well-proliferated, well-differentiated endometrium is believed to be more receptive to embryo implantation. Various different studies have tried to characterize the endometrial anatomy and physiology in order to develop markers for endometrial receptivity. These include measurement of endometrial thickness, endometrial perfusion, endometrial microbiome, endometrial genomic expression, as well as proteomic and metabolomic profiles. Although evidence-based objective measures of endometrial receptivity are yet to be established there is general consensus in the field that endometrial thickness of over 7 mm with good morphological echo-pattern is preferable before embryo transfer in IVF cycles.

In this study we show that due to waves of contraction in the sub-endometrial myometrial layer the endometrial thickness changes over time and we suggest that using a mean of 3 different endometrial thickness values over contractions may represent a more accurate measure of endometrial thickness. Moreover, we show that peak systolic flow in the sub-endometrial spiral arteries is positively correlated to the mean endometrial

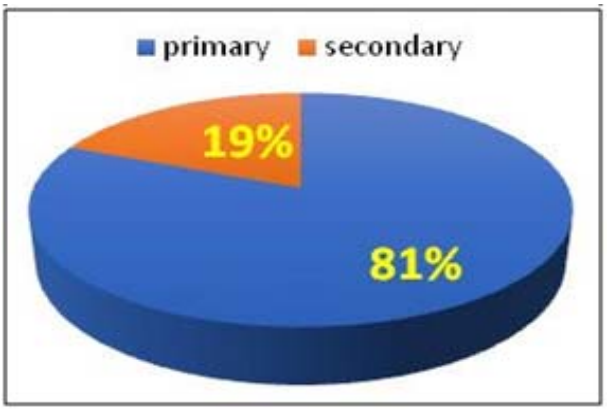


Fig 1 — Distribution of Type of Infertility

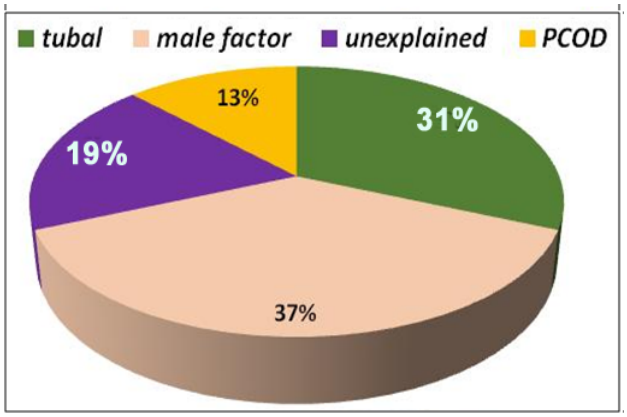


Fig 2 — Distribution of Cause of Infertility

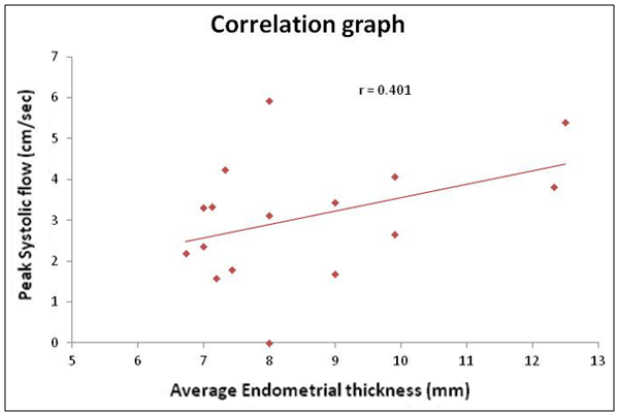


Fig 3 — Positive Correlation (r=0.401) between Endometrial Thickness (mm) and Peak Systolic Flow (cm/sec) in spiral artery

thickness. This finding suggests the very tempting possibility to combine these two markers together to develop a more robust model of endometrial receptivity measurement. Furthermore, we hypothesize that mean endometrial thickness as a function of effective endometrial proliferation and optimal sub-endometrial contractions may be a measure of the innate resistance of the endometrium to blood flow and possibly act as a prognostic marker of endometrial receptivity and IVF outcome.

### Limitations :

Measurement of the changing endometrial thickness over time is labor-intensive, time-consuming and hence practically difficult and cost-prohibitive in a busy IVF practice. Moreover, accurate identification and measurement of the blood flow in small spiral arteries is technically challenging and requires patience and a long learning curve. Very low volume flow in many patients also necessitates careful patient recruitment and pre-screening before enrolment into such a study. Even then, it will be technically difficult to achieve multiple measurements of PSV in the small spiral arteries in some patients. Due to these reasons this pilot study presents the data of a modest number of patients and thus these results need to be interpreted with caution. Furthermore, definite conclusions can only be drawn after these findings are correlated with IVF outcomes in larger comparative studies.

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