Correlation of subendometrial-endometrial blood flow assessment by two-dimensional power Doppler with pregnancy outcome in frozen-thawed embryo transfer cycles

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ABSTRACT

CONTEXT: Various markers have been proposed to evaluate endometrial receptivity, such as molecular markers and sonographic markers. Commonly used sonographic markers include endometrial thickness and pattern. A good endometrial blood flow is considered necessary for improved pregnancy outcome. **AIM:** The aim of the present study is to evaluate the role of subendometrial endometrial blood flow with two-dimensional-power Doppler (2D-PD) in predicting pregnancy outcome in hormone replacement frozen-thawed embryo transfer (FET) cycles. **SETTING AND DESIGN:** Prospective, non-randomized observational study. A total of 165 patients undergoing their first FET cycle were evaluated for subendometrial-endometrial blood flow by 2D-PD once the endometrium was ≥7 mm thick. Group A consisted of 127 women showing the presence of subendometrial-endometrial blood flow. Group B comprised of 38 women in whom subendometrial blood flow was absent. Progesterone supplement was added and transfer of 2-3 cleavage stage good quality embryos was done after 3 days. **STATISTICAL ANALYSIS:** Independent two-tailed *t*-test and Chi-square test. **RESULTS:** There was no significant difference in body mass index, endometrial thickness, follicle stimulating hormone, luteinizing hormone levels, number of mature oocytes, semen parameters and the number of good quality embryos in the two groups ($P > 0.05$). The mean age in Group A was 32.05 years and 33.73 years in Group B, and the difference was statistically significant ($P = 0.04$). Overall pregnancy rate (PR) was 30.90%. PRs were significantly higher in the presence of subendometrial-endometrial blood flow than in its absence (35.43% vs. 15.78%, $P = 0.02$). Furthermore, clinical pregnancy rate and implantation rate were significantly higher in Group A when compared to Group B (31.49% and 14.79% vs. 13.15% and 6.52%, $P = 0.02$ and 0.03, respectively). **CONCLUSION:** The presence of endometrial blood flow significantly improves cycle outcome in hormone replacement therapy-FET cycles.

KEY WORDS: Frozen-thawed embryo transfer, power Doppler, subendometrial-endometrial blood flow

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INTRODUCTION

Ultrasound has emerged as an indispensable modality in the field of assisted reproductive techniques (ART), not only for follicular monitoring, but also for evaluation of the endometrium. Endometrial receptivity still remains an enigma. Various markers have been proposed to evaluate endometrial receptivity, such as molecular markers and sonographic markers. Commonly used sonographic markers include endometrial thickness and pattern. With the advent of Doppler ultrasound, studying endometrial blood flow has emerged as an important means of evaluating endometrial receptivity.

A triple layered endometrial pattern and an endometrial thickness greater than 7 mm have been proposed as markers of endometrial receptivity, but have yielded a high percentage of false-positive results.[1] A good blood supply to endometrium is generally considered necessary for implantation. Thin endometrium is assumed to have suboptimal blood flow and various drugs, e.g., low dose aspirin, vaginal sildenafil have been tried to improve endometrial blood flow and development.[2-4] An elevated pulsatility index (PI) of uterine arteries has been associated with low implantation and pregnancy rates (PRs).[5] However, Doppler velocimetry of uterine arteries is only slightly specific for predicting pregnancy.[1] Blastocyst implants in the endometrium and hence, endometrial blood flow reflects uterine receptivity more appropriately.[6]

Whilst the relationship between endometrial blood flow and PR has been evaluated in many studies in fresh *in vitro*-fertilization-embryo transfer (IVF-ET) cycles,[5,7-11] there is a paucity of data in frozen-thawed embryo transfer (FET) cycles. With the advancement in the field of cryobiology and move towards elective-single embryo transfer, the need to improve PRs in FET cycles has dramatically cropped up. Successful implantation involves good quality embryo, receptive endometrium and a cross-talk between the two. Despite the transfer of good quality embryos (identified by standard embryological techniques), most embryos fail to implant, which makes the evaluation of endometrial receptivity very important.

The purpose of this study was to investigate whether the assessment of endometrial blood flow with two-dimensional-power Doppler (2D-PD) helps to predict pregnancy outcome in hormonal replacement FET cycles.

MATERIALS AND METHOD

It was a prospective non-randomized observational study where 165 women undergoing their first FET in our infertility center from June 2011 to December 2012 were recruited. The study was approved by the Ethical Committee of the hospital, and all patients consented to participate in the study.

Inclusion criteria included patients between 20 and 40 years of age, undergoing their first FET cycle, where vitrification technique was used for freezing and at least two or three good quality embryos were available for transfer after thawing.

Patients with distorted uterine cavity on transvaginal ultrasound or hysteroscopy, as well as with hydrosalpinx or fibroids were excluded from the study.

All patients underwent antagonist IVF cycle and cleavage stage (6-8 cell) good quality embryos were frozen on day 3 of oocyte retrieval by vitrification technique when the embryos were surplus for fresh cycle or when fresh transfer was not possible due to risk of ovarian hyper stimulation syndrome (OHSS).

For the FET cycle (after at least 2 months of oocyte retrieval), the endometrium was prepared using hormone replacement therapy (HRT) with estradiol and progesterone supplements. All patients underwent transvaginal scan to assess uterine and ovarian morphology on day 2 of spontaneous or induced menstrual cycle. Estradiol supplementation (tablet estradiol valerate 2 mg TID) was started on day 2 or day 3 of cycle once endometrial thickness was <6 mm and no ovarian cyst was seen on ultrasound. All patients received folic acid supplements. Ultrasound was repeated after a week of HRT and Doppler study of endometrial blood flow was conducted in all patients once endometrial thickness was >7 mm.

All the ultrasound scans and measurements were performed with the digital platform Voluson E8 system between 9 am and 11 am. A B-Mode exploration of both uterus and ovaries was initially performed. When a longitudinal view of the uterus was obtained endometrium was assessed measuring from outside to outside at its widest point [Figure 1a]. Then 2D-PD assessment was done. The power Doppler characteristics applied in all examinations were as follows: Normal quality of color, color gain-3.4, pulse repetition frequency of 600 Hz and wall motion filter of 50 Hz.

According to Doppler findings, patients were divided into two groups: A and B.

Group A comprised of 127 women in whom blood flow was observed in at least subendometrial region on 2D-PD [Figure 1b]. Group B comprised of 38 women in whom blood flow was absent in the subendometrial-endometrial region.

Progesterone supplement (injection progesterone 100 mg i.m. for 3 days) was added and cleavage stage ET (2 or 3 good quality embryos) was done after 3 days. All patients were

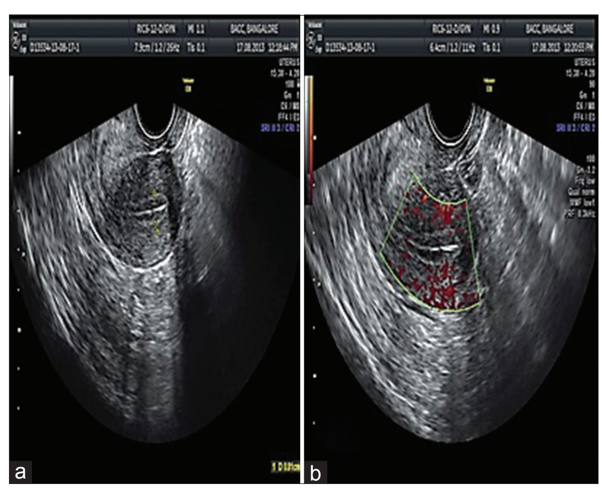


Figure 1: (a) Triple line endometrial pattern with endometrial measurement and, (b) Endometrial blood flow up to zone 4

prescribed vaginal progesterone suppositories and estradiol valerate tablets for luteal phase support after ET along with folic acid supplements for 2 weeks after which blood was tested for β-human chorionic gonadotropin (hCG). β-hCG >50 IU/L was considered as positive for pregnancy. Clinical pregnancy was defined by the appearance of the gestational sac on ultrasound with appropriately rising hCG levels. When no gestational sac could be seen with positive β-hCG by 6 weeks, it was defined as a biochemical pregnancy.

The primary and secondary outcomes of the study were clinical pregnancy rate (CPR) and implantation rate (IR), respectively.

Statistical analysis

In the present study, the data was collected on Microsoft Excel Sheet 2010. Independent two-tailed *t*-test was used to evaluate the significance of continuous variables like age, body mass index (BMI), follicle stimulating hormone (FSH), luteinizing hormone (LH) etc., $P<0.05$ was regarded as significant. Chi-square test was used to evaluate the strength of association between Doppler findings and FET cycles outcome, with $P < 0.05$ taken as statistically significant.

RESULTS

A total of 165 women undergoing their first FET cycle were recruited for the study. 127 women demonstrated blood flow in at least subendometrial region (Group A), whereas subendometrial-endometrial blood flow was absent in 38 women (Group B). There was no significant difference in BMI, FSH and LH levels between the two study groups (Groups A and B). However, women in no subendometrial blood group were of older age with statistically significant difference ($P = 0.04$). The number of mature oocytes obtained and number of good quality embryos formed between the two study groups was not statistically different ($P = 0.44$ and $P = 0.27$, respectively). The endometrial thickness on the day of initiating progesterone was also statistically similar between the two study groups ($P = 0.27$) [Table 1].

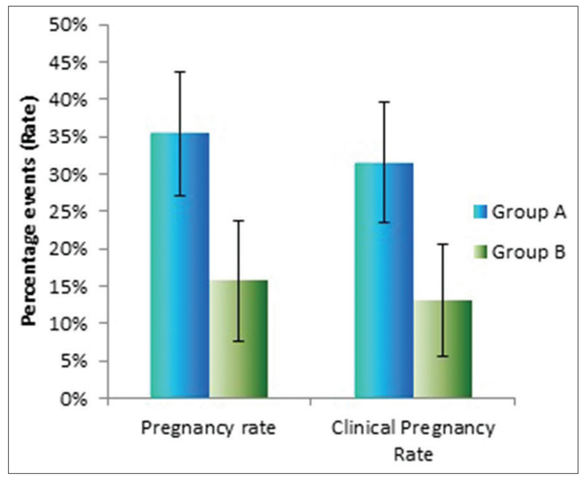
There was no difference in the proportions of abnormal semen parameters across the groups.

In our study, the overall PR was 30.90% (51/165). A total of 45 women conceived in the presence of subendometrial-endometrial blood flow compared with 6 women in the absence of subendometrial blood flow. The PR and the CPR was significantly higher in Group A when compared to Group B ($P = 0.021$ and $P = 0.025$, respectively) [Graph 1]. Furthermore, IR was significantly better in Group A than Group B ($P = 0.03$) [Graph 2].

Table 1: Demographic, oocyte, embryo data, endometrium thickness and hormonal profiles between Group A and Group B

	Mean (SD)		*P* value
	Group A	Group B	
Age (years)	32.05 (3.89)	33.73 (4.5)	0.04
BMI (kg/m^2)	26.31 (2.71)	25.85 (3.17)	0.42
Endometrial thickness (mm)	9.87 (1.17)	9.63 (1.38)	0.27
FSH (IU/L)	5.98 (1.80)	6.08 (2.40)	0.77
LH (IU/L)	5.91 (2.50)	5.76 (2.60)	0.75
Number of M II oocytes	11.70 (4.30)	11.07 (4.93)	0.44
Number of good quality embryos	7.62 (3.13)	6.97 (3.62)	0.27

BMI=Basal metabolic index; FSH=Follicle stimulating hormone; LH=Luteinizing hormone; M II=Metaphase II oocytes; SD=Standard deviation. There was no difference in the proportions of abnormal semen parameters across the groups



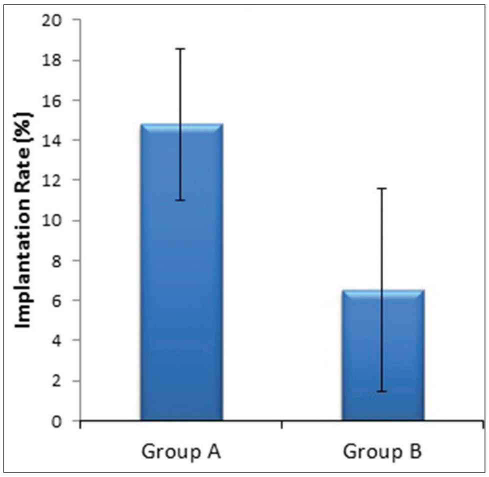
Graph 1: The bar graph represents the percentage of observed events (pregnancy rate 35.43% and 15.78%, respectively and clinical pregnancy rate 31.49% and 13.15%, respectively) for patients in Group A and B, respectively. The error bars represent the 95% confidence intervals for the estimated proportions

Graph 1: Numerical details

	Group A	Group B
Number of patients (*n*)	127	38
Total pregnancies	45	6
Pregnancy rate	35.43	15.78
Clinical pregnancies	40	5
Clinical pregnancy rate %	31.49	13.15

DISCUSSION

Endometrial receptivity is crucial to implantation of an embryo. Ultrasound has emerged as a valuable tool in predicting endometrial preparation before ET both in fresh IVF cycles and FET cycles. Commonly used parameters for sonographic assessment of endometrium include endometrial thickness, endometrial pattern and Doppler indices of blood flow. While uterine artery PI and RI indices are commonly being used, they are not reflective of endometrial blood flow;[12,13] endometrium being the site of blastocyst implantation.



Graph 2: The bar graph represents the percentage of observed events (implantation rate 14.79% and 6.52%) for patients in Group A and B respectively. The error bars represent the 95% confidence intervals for the estimated proportions

Graph 2: Numerical details

	Group A	Group B
Number of sacs	50	6
Number of embryos transferred	338	92
Implantation rate %	14.8	6.5
Standard error	0.019	0.026

Several studies have evaluated the correlation between subendometrial and endometrial blood flow and the PRs in IVF cycles. Some workers have suggested positive correlation of PRs with the subendometrial and endometrial blood flows,[5,7,8,14-18] while others have refuted this.[19-24]

Endometrial-subendometrial blood flow distribution pattern assessed by transvaginal color Doppler before ET was correlated with the implantation and PR of IVF treatment.[7,25] Scoring systems have also been formed including endometrial vascularization to depict the chances of pregnancy. Quantitative assessment of vessel density and perfusion within subendometrial area using three-dimensional-power Doppler (3D-PD) was found to be the strongest predictive factor of IVF success among various sonographic parameters.[14] Vimercati *et al*. stated that power Doppler measurements of intra-endometrial blood flow seem to have a diagnostic value for endometrial receptivity and favorable reproductive outcome.[17]

On the other hand, Yuval *et al*. did not find any correlation between endometrial thickness, blood flow and PR in IVF cycles.[19] de Ziegler *et al*. also stated that Doppler ultrasonography (USG) of endometrial and subendometrial regions does not contribute valid responses as to uterine receptivity and the chances for pregnancy.[20]

Data by Contart *et al*. also revealed that isolated evaluation of endometrial vascularization with power Doppler is not an important factor for the prediction of pregnancy in an intracytoplasmic sperm injection (ICSI) program.[22] Baruffi *et al*.[23] and Aghahoseini[24] *et al*. also could not find endometrial vascularization a good predictor of pregnancy in ICSI cycles.

However, Singh *et al*. stated that endometrial vascularity by PD has a useful predictive value for IR in IVF cycles irrespective of morphological appearance of endometrium.[18]

Though there are many articles correlating endometrial blood flow with IVF success rates, there is a paucity of data in FET cycles. FET cycles contribute to about 25% of all births achieved by ART.[26] However, the CPR associated with FET varies due to differences in the endometrial preparation regimes, quality of embryos that are frozen, the day of development when embryos are frozen and the technique of freezing (slow freezing/vitrification).

Tekay *et al*. found no significant difference in Doppler velocimetry measurements between the conception and non-conception cycles in both the FET and IVF-ET groups. They then concluded that an inadequate uterine blood flow impaired implantation, while optimal uterine perfusion did not necessarily lead to conception.[10] But uterine artery PI on day 14 was found to be significantly higher in non-conceptional HRT-FET cycles. Uterine artery PI did not correlate with endometrial thickness and PI >3.6 predicted 33% of non-conception cycles.[27]

However, Check *et al*. concluded that at least for FET, there does not appear to be any relationship of sonographic endometrial parameters (endometrial and subendometrial blood flow, PI, RI) with PRs.[28] Zácková *et al*. also concluded that measurement of power Doppler indices using 3D ultrasound on the day of the FET does not provide any additional information concerning the outcome of the cycle.[29]

Ng EH, *et al*. demonstrated that the vascularity of the endometrium and subendometrial layers measured by 3D-PD USG was not a good predictor of pregnancy during stimulated IVF and FET cycles if it was measured at 1 time point only.[30]

However, endometrial and subendometrial vascularity was significantly higher in pregnant patients with live birth than in those who suffered a miscarriage.[31]

In contrast, the present study shows that the presence of endometrial blood flow on 2D-PD in HRT-FET cycles is associated with significant improvement in the cycle outcome.

Patients with presence of subendometrial-endometrial blood flow were estimated to be 2.48 times as likely to become pregnant when compared to those with the absence of flow (odds ratio [OR] =2.48; 95% confidence interval = 1.03-5.98; P = 0.06). Though the OR was not statistically significant for patients with subendometrial-endometrial blood flow group but, a clinical trend towards increased pregnancy outcome is seen in this group. A combination of endometrial thickness and Doppler analysis of endometrial blood flow is a simple and effective tool to improve CPR in HRT-FET cycles and should be incorporated into routine clinical practice. Moreover, in line with improvement in IR with increased endometrial blood flow, we can reduce the number of embryos to be transferred and hence, the multiple PRs in FET cycles. Single or double ET can be attempted when good endometrial blood flow (zone 3 or 4) is observed, thereby preventing associated complications of higher order multiple pregnancies. If we can improve PRs in FET cycles, it can also help to reduce the risk of OHSS by using GnRH agonist trigger and/or by elective cryopreservation of embryos in high risk cases. However, this analysis needs to be complemented by research correlating the study protocol with live birth rate.

The differences in observations could be due to differences in the technique of freezing used, developmental stage of embryos at the time of freezing, protocol used for endometrial preparation, endometrial thickness at the time of transfer or the technique of Doppler used for assessing endometrial blood flow.

One limitation of our study could be the small number of patients studied. Another limitation is the observed difference in age in the study groups, suggesting a possible effect on endometrial blood flow. Possibly larger studies are required to reach definite conclusions.

CONCLUSION

Presence of endometrial blood flow on 2D-PD significantly improves pregnancy outcome in HRT-FET cycles.

We suggest that a combination of endometrial thickness and Doppler analysis of endometrial blood flow is a simple and effective tool to improve CPR in HRT-FET cycles and should be incorporated into routine clinical practice.

In addition, this can help us reduce the number of embryos to be transferred and hence, the multiple pregnancies and the associated complications.

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