Impact of Intraendometrial Vascularity on Implantation Rates in Frozen Blastocyst Transfer

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ABSTRACT

Introduction: Implantation remains the main limiting factor in the success of *in vitro* fertilization treatment, despite major advances made in this field. This study was done to evaluate whether intraendometrial vascularity affects the outcome in cases of Frozen embryo transfer (FET).

Materials and methods: In total, 226 patients undergoing frozen embryo transfer out of the total 233 patients were recruited for the study. Endometrial preparation was done by artificial cycle protocol in all the patients with estradiol valerate. Endometrium is evaluated for its thickness and its morphology as seen on B-mode ultrasound. Endometrial vascularity is evaluated by power Doppler (PD) and pulse-wave Doppler. Post-embryo transfer of two Grade 1 frozen-thawed blastocyst, judicious luteal support was given. Beta human chorionic gonadotropin (β -hCG) was checked after 14 days for confirmation of pregnancy in all the patients. In all β -hCG positive cases, ultrasound was done after 10 days. All the patients with pregnancy were followed up by ultrasound for the entire first trimester and miscarriages were documented.

Results: Patients showed higher positive pregnancy rate and lesser miscarriage rate with vascular penetration in zone 3 and zone 4 of the endometrium. Conception rate and miscarriage rate did not vary significantly with change in endometrial thickness beyond 7 mm in Frozen embryo transfer cycles.

Keywords: Blastocyst, Conception rates, Endometrial vascularity, Frozen embryo transfer, Miscarriage rates.

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INTRODUCTION

Successful implantation is a result of a positive dialogue between the endometrium and embryo.¹ For a very short time during the menstrual cycle, the endometrium allows for the implantation of gestational sac and this is referred to as endometrial receptivity.² There are many variables of successful implantation and those related to embryo are difficult to study in spite of all advances in technology. Molecular changes play a major role in endometrial receptivity and are also controlled by hormonal changes. These reflect as changes in endometrial morphology and vascularity.

Transvaginal ultrasonography is universally accepted and most extensively used noninvasive tool to assess the endometrial receptivity during assisted reproductive treatment.³ Ultrasound is easily repeatable, yet highly reproducible, patient friendly and yet accurate modality for endometrial receptivity.⁴ Assessment of blood flow as a functional dimension of endometrial receptivity along with endometrial thickness, has important role in the prediction of pregnancy outcomes FET (frozen embryo transfer) cycles.⁵⁻⁷ This study is aimed to assess the impact of endometrial morphology, thickness and endometrial vascularity in pregnancy outcomes in FET cycles.

MATERIALS AND METHODS

Study Design

This is retrospective cross-sectional study. Total 233 patients undergoing frozen embryo transfers (FET) in our centre from December 2019 to December 2020 were recruited for the study. The study was approved by the institutional ethical committee.

Inclusion Criteria

- Females between 20 and 40 years of age.

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- Females with BMI-22–29.9.
- Patients with normal uterus (no Mullerian anomalies).
- Patients with mild adenomyosis, that allows endometrium to grow to at least 6 mm.
- Patients with no endometrial pathology like polyp, subendometrial fibroid, acute or chronic endometritis.
- Patients with mild/moderate endometriosis.

Exclusion Criteria

- Females under 20 years and above 40 years of age.
- Females with BMI more than 22 and less than 30.
- Patients with severe endometriosis.
- Patients with Mullerian anomalies.
- Patients with endometrial polyps, submucous fibroids or endometriosis.
- Patients with cervical pathologies that restrict the passage of embryo transfer cannula.
- Patients with uterine artery PI >3.2.¹²

Method

For all the patients, a pilot transvaginal ultrasonography was performed with transvaginal volume probe of 5–9 MHz, on Voluson E10 BT20 (GE Medical Systems Kretztechnik GmbH, Zipf, Austria) during the preovulatory phase of the previous cycle. Systematic and detailed scan of the pelvic organs-uterus, ovaries, and fallopian tubes was done to exclude any abnormalities for each patient.

Out of total of 233 patients who underwent FET during the study period, seven patients were excluded from the study. Of these one patient had endometrial polyp, two patients had intramural fibroid, larger than 4 cm in diameter, one patient had subendometrial fibroid, one patient was at 42 years of age and two patients had B.M.I. of more than 30. Total 226 patients were then taken forward for the study.

Estradiol valerate 2 mg was given thrice a day orally to these patients, from day 2 of the treatment cycle for endometrial preparation along with aspirin 75 mg given orally once daily. The endometrium was assessed for its morphology and thickness on day 8–9 of the cycle.

B-mode ultrasound was used to assess the endometrial thickness and morphology. Endometrium was assessed in the mid-sagittal plane of the uterus. B-mode image is optimized

by bringing the uterus in the centre of the image so that it covers 2/3rd of the screen, adjusting the angle, depth, and focal zone. Endometrial thickness was measured from the outer margin of the anterior hyperechoic line of the endometrium to the outer margin of posterior hyperechoic line of the endometrium at the broadest part of the endometrium, excluding the junctional zone; perpendicular to the central endometrial line of the endometrium (Fig. 1). Depending on the thickness of the endometrium, a repeat visit is planned when the endometrium is likely to grow till the thickness of at least 6 mm.⁸ This can be decided on the basis of the fact that endometrium grows at a rate of 1–2 mm per day.⁸

Morphologically the multi-layered endometrium⁹ is graded as (Fig. 2):

Grade A: Triple line endometrium with echogenicities in intervening area not more than that of anterior myometrial echogenicity.

Grade B: Multilayered or triple line endometrium with hypoechoic intervening area.

Grade C: Homogeneous isoechoic endometrium.

Endometrial vascularity was assessed with HD flow power Doppler. For HD flow power Doppler, PRF is adjusted at 0.3, wall filter at low 1 and volume gate size of 2 mm. The Doppler box is placed on the mid-sagittal plane of the endometrium, large enough to include the entire endometrium.

Vascularity is graded according to Applebaum¹⁰ as (Fig. 3):

Zone 1: When blood vessels reach the hypoechoic endometrial-myometrial junction.

Zone 2: When blood vessels reach the outer hyperechoic line of endometrium.

Zone 3: When blood vessels reach the intervening hypoechoic area between the two hyperechoic lines.

Zone 4: When blood vessels reached the central echogenic line.

At least 5 mm² area of a particular zone must show vascularity to designate that zone is adequately vascularized¹¹ (Fig. 4). Pulse-wave Doppler is used to assess the flow in these spiral vessels, supplying the endometrium. At least 4–5 good waveforms are obtained; each demonstrating the maximum Doppler shift.

The resistance index (RI) was calculated on three consecutive uniform waveforms. The pulse Doppler of spiral vessels with RI 0.49–0.59 was considered a favorable prognostic factor for conception.¹⁴

Fig. 1: B-mode image of the mid-sagittal plane of the uterus showing endometrial thickness measurement

Figs 2A to C: (A) B-mode ultrasound image of mid-sagittal plane of the uterus showing grade A morphology with thick multilayered endometrium and smooth central line. The intervening area between the central line and outer lines show echogenicities similar to that of anterior myometrium; (B) B-mode ultrasound image of mid-sagittal plane of the uterus showing grade B morphology with thick multilayered endometrium and smooth central line. The intervening area between the central line and outer lines is hypoechoic; (C) B-mode ultrasound image of mid-sagittal plane of the uterus showing grade C morphology with thick but homogeneous endometrium, isoechoic to the myometrium

Figs 3A to D: (A) Mid-sagittal plane of the uterus with high definition power Doppler showing blood vessels only up to zone 1 (till endometrio-myometrial junction); (B) Mid-sagittal plane of the uterus with HD power Doppler showing blood vessels up to zone 2 (till outer hyperechoic lines of the endometrium); (C) Mid-sagittal plane of the uterus with HD power Doppler showing blood vessels up to zone 3 (till the intervening area between the outer hyperechoic lines of endometrium); (D) Mid-sagittal plane of the uterus with HD power Doppler showing blood vessels up to zone 4 (touching the central line)

Fig. 4: 5 mm² area in zone 3 showing vascularity on HD power Doppler

Endometrial thickness of >6 mm, was taken as a cut-off to start progesterone and plan embryo transfer. If, when the scan is done on day 8–9 of the cycle and the endometrial growth is not documented as compared to the first scan, the dose of estradiol valerate was increased to 8 mg. Estrogen priming of the endometrium for a minimum of 12 days and maximum up to 28 days is done before progesterone was started as a final preparation for embryo transfer. Preparation of the endometrium for embryo transfer is done with natural micronized progesterone gel 8% twice a day and subcutaneous administration of aqueous progesterone 25 mg; for all the patients undergoing frozen embryo transfer. Estrogen is continued in the dose; that was given before starting progesterone.

Day 5 embryo transfer was done in all the cases. The cryopreserved embryos were thawed on the day of the embryo transfer. Two Grade 1; blastocyst were transferred in all patients. Luteal phase was supported with progesterone, estradiol valerate and aspirin in the same dose as before the transfer to support implantation after the embryo transfer.

Serum β -hCG level estimation was done after 14 days of the embryo transfer. Serum β -hCG level more than 10 mIU/ml was indicative of a positive pregnancy test which was the primary desired result. If the pregnancy test was positive, same luteal support was continued till 12 weeks of the pregnancy. Transvaginal ultrasound was performed 10 days after positive β -hCG to diagnose a clinical pregnancy. Presence of gestational sac in the endometrial cavity was considered as ultrasound evidence of pregnancy.

Pregnancy was considered only biochemical when no evidence of an intrauterine or extrauterine gestational sac

on transvaginal ultrasonography with beta human chorionic gonadotropi (β -hCG) levels >10 miu/ml but not increasing to more than double in 72 hours. Failure of the appearance of the yolk sac at 20 mm gestational sac or no foetal pole at 25 mm of gestational sac is considered a failed pregnancy.¹⁵ The gestational sac does not grow at the normal rate of 1–1.3 mm/day¹⁶ or 4 mm/week was also considered as failure of pregnancy. Weekly follow-up was done up to eight weeks to confirm ongoing pregnancy. The miscarriage group included patients with biochemical pregnancy and clinical miscarriage.

RESULTS

The current study included 226 patients who underwent Frozen-embryo transfer. The pre-procedure assessment included assessment of endometrial thickness, morphology and endometrial vascularity on the day progesterone was started as a final endometrial preparation for frozen embryo transfer. Progesterone was started when the endometrial thickness was at least 6 mm when Estradiol valerate was given for minimum 12 days to maximum 28 days.

The study showed overall pregnancy rate of 42.92% (97/226) based on β -hCG levels and included 14 miscarriages (14.43%) out of the 97 positive pregnancies. The average age of the patients in the pregnant group was 32.3 years and that in the nonpregnant group was 31.9 years and BMI in both the groups was between 22 and 30.

For evaluation of the results and the aim to find the most favorable endometrial thickness and vascularity for conception and to minimize miscarriage rates, the patients were divided primarily based on the result of the frozen transfer cycle-positive or negative β -hCG results. The results for both the groups were analyzed. The results were compared for both the groups for different endometrial thickness range and for different zones of vascularity.

Assessing the endometrial thickness distribution (Table 1), 17/226 (7.52%) patients had endometrial thickness of ≤ 7 mm, 103/226 (45.57%) had endometrial thickness between 7 and 9 mm, 70/226 (30.97%) patients had endometrial thickness between 9 and 11 mm, 28/226 (12.38%) patients has endometrial thickness of between 11 and 13 mm and 8/226 (3.53%) patients had endometrial thickness of between 13 and 15 mm.

Of all the patients (Fig. 5); 4/17 (23.25%) patients with endometrial thickness <7 mm, 49/103 (47.57%) patients with endometrial thickness of 7–9 mm, 27/70 (38.57%) patients with endometrial thickness of 9–11 mm, 13/28 (46.42%) patients between endometrial thickness of 11–13 mm and 5/8 (50%) patients with endometrial thickness of 13–15 mm got positive

Fig. 5: Endometrial thickness and the pregnancy outcome

Fig. 6: Endometrial vascularity zone and pregnancy outcomes

Table 1: Endometrial thickness (binned) and pregnancy result– cross tabulation

		Positive pregnancy	Negative pregnancy	Total patients	Pregnancy rate
Endometrial thickness (Binned)	≤7.00	4	13	17	23.52%
	7.01–9.00	49	54	103	47.57%
	9.01–11.00	27	43	70	38.57%
	11.01–13.00	13	15	28	46.42%
	13.01–15.00	4	4	8	50%
Total		97	129	226	

Table 2: Endometrial vascularity score and pregnancy result– cross tabulation

		Positive pregnancy	Negative pregnancy	Total patients	Pregnancy rate
Endometrial vascularity score	Zone 1	1	5	6	16.66%
	Zone 2	11	25	36	30.55%
	Zone 3	75	91	166	45.18%
	Zone 4	10	8	18	55.55%
Total		97	129	226	

Table 3: Endometrial thickness and abortion

		No abortion	Abortion	Total pregnancies	Abortion rate
Endometrial thickness (Binned)	≤7.00	5	0	5	0
	7.01–9.00	42	9	51	17.64%
	9.01–11.00	20	4	24	16.66%
	11.01–13.00	12	1	13	7.69%
	13.01–15.00	4	0	4	0
Total		83	14	97	

pregnancy test. Though the conception rates were significantly lower when endometrial thickness was ≤7 mm, for the rest of the endometrial thicknesses the conception rates were not significantly different. Although the conception rates were significantly higher when endometrial thickness was 13–15 mm.

Assessing patient distribution for endometrial vascularity, 6/226 (2.65%) patients had vascularity in zone 1, 36/226 (15.92%) had vascularity in zone 2, 166/226 (73.45%) patients vascularity in zone 3, 18/226 (7.96%) patients had vascularity in zone 4 (Table 2).

Of all the patients (Fig. 6) 1/6 (16.66%) patients with endometrial vascularity in zone 1, 11/36 (30.55%) patients with vascularity in

zone 2, 77/166 (45.18%) patients with endometrial vascularity in zone 3 and 10/18 (55.55%) patients with endometrial vascularity in zone 4 got positive pregnancy test.

The findings suggest that the conception rate consistently increased as the vascularity penetrated deeper in the endometrium.

Evaluating the secondary aim of the study, the abortion rate in relation to the endometrial thickness. For endometrial thickness of <7 mm, abortion rate were calculated as 0%, for endometrial thickness 7–9 mm, abortion rate was 17.64%, for 9–11 mm, it was 16.66%, 7.69% for endometrial thickness of 11–13 mm and again zero for endometrial thickness of 13–15 mm (Table 3). No definite pattern

Table 4: Endometrial vascularity zone and abortion rates

		No abortion	Abortion	Total pregnancy	Abortion rate
Endometrial vascularity zone	Zone 1	3	0	3	0
	Zone 2	13	6	19	31.57%
	Zone 3	59	8	67	11.94%
	Zone 4	8	0	8	0
Total		83	14	97

Fig. 7: Endometrial thickness and miscarriage rates

of abortion rates were documented with increasing endometrial thickness (Fig. 7). This indicates that the abortion rate cannot be related to the endometrial thickness in frozen embryo transfer cycles.

Evaluating the abortion rates for vascularity in different endometrial zones (Table 4). The abortion rates were (8/67)14.28% and no abortions were noted when the endometrial vascularity was in zone 3 and zone 4 respectively. Whereas when the vascularity was in zone 2, miscarriage rate was (6/19) 31.57%. Though (0/3) abortions were documented when the vascularity was seen in zone 1 only, this was probably too small a number to endorse low abortion rate in this case (Fig. 8).

DISCUSSION

Implantation is a complex process involving several biochemical and molecular processes. Angiogenesis plays an important role in female reproductive processes such as development of dominant follicle, formation of a corpus luteum, growth of endometrium and implantation.^{17–19} Endometrial angiogenesis is thought to be crucial to endometrial receptivity, but the studies have shown controversial results. The only parameter that has been fairly consistently been shown to be a reliable marker of endometrial receptivity is endometrial thickness.

We preferred to do the endometrial thickness and vascularity studies, on the day when we planned to start progesterone after assessing endometrial thickness, morphology, and vascularity by power Doppler, to maintain the consistency about the time of assessment. There is a decrease in endometrial thickness and vascularity immediately after starting progesterone due to initial compacting effect of progesterone on the endometrium and slow gradual building up of progesterone level to improve the endometrial circulation by decreasing uterine artery resistance

Fig. 8: Endometrial vascularity and miscarriage rate

due to smooth muscle relaxation.²⁰ This is the need of physiology also because relatively low oxygen tension is present around the blastocyst during the time of implantation.²¹

Yaman et al. demonstrated in their study that there was no significant difference in the conception and abortion rates in FET cycles, between endometrial thickness of 7–13 mm.²² This can be related to our findings too. Several other studies have also shown that endometrial thickness and pattern have low positive predictive value and specificity in the prediction of IVF outcome.^{3,23–25} When the thickness measured by ultrasound is <7 mm, the functional layer is thin or absent, and the implanting embryo would be much closer to the spiral arteries and the higher vascularity and oxygen concentrations of the basal endometrium. The high oxygen concentration near the basal layer could be detrimental compared with the low oxygen tension of the surface endometrium.^{26,27,38} Singh et al.⁵ reported lower cut-off of 5.8 mm of endometrial thickness for implantation, with the most favorable thickness of 8–10 mm. Sundström²⁵ also reported a successful pregnancy with an endometrial thickness as little as 4 mm. According to these and many other studies therefore it is difficult to establish a definite relationship and the conception rates.^{28–30} These results are in agreement with the results of our present study. Also, lower conception rate was documented with endometrial thickness more than 14 mm, but up to 14 mm of thickness no significant change in conception rate was found.³¹

When relating the conception rates to endometrial vascularity before starting progesterone as final endometrial preparation in FET cycles in our studies, we have found significantly better conception rates for better endometrial vascularity in terms of vascular penetration (zone of vascularity). This also correlates with our previous study relating endometrial vascularity to conception rates in ovum donation, fresh embryo transfer cycles, when endometrial vascularity was assessed on the day of trigger.³²

Considering and comparing our results with other workers, Mojgan et al.³³ found that women who had a more favorable cycle showed a higher endometrial vascular flow rate as against the non-pregnant ones in IUI cycles. Riad et al.³⁴ also reported that the presence of subendometrial flow is associated with successful IUI in women under stimulated cycles undergoing IUI. Wu et al.³⁵ also demonstrated in 54 patients that subendometrial VFI on the day of hCG was significantly higher in the pregnant group and was superior to endometrial volume, subendometrial VI and FI in predicting the outcome. This indicates that not just the subjective assessment of more vascular penetration, even quantitative assessment of the endometrial vascularity proved that better conception rates can be achieved when endometrium was well vascularized on the day of trigger in IVF–fresh transfer cycles.

Wang et al.⁴ found in prospective study of 182 patients undergoing IVF-ET, that patients with detected endometrial blood flow had a higher pregnancy and implantation rate and suggested that blood supply and distribution of the endometrium had a strong association with the possibility for embryo implantation and development, indicating the value of detecting the existence of endometrial blood flow in predicting IVF-ET outcomes. They also found a significant increasing multiple pregnancy rate in those who had better endometrial blood flow. We in our study also found that deeper the penetration of vascularity in endometrium, better is the positive pregnancy outcome. Nygren et al.³⁶ have also suggested positive correlation of the endometrial and subendometrial blood flows to the outcome of IVF/ET.

In a recent paper, Ng et al.³⁷ discuss the relationship of endometrial blood flow between those with a thin (less than or equal to 8 mm) endometrium and in those with a low volume (less than or equal to 2.5 ml) endometrium. Endometrial and subendometrial vascularity measured by 3D power Doppler ultrasound was significantly lower (p value less than or equal to 0.003) in patients with a low volume endometrium, but not in those with a thin endometrium. Therefore, according to this study, considering the vascular parameters along with endometrial thickness provides better positive pregnancy outcomes than only when the endometrial morphological parameters and its thickness are taken into account.

Dechaud et al.³⁹ considered that, the endometrial pattern, thickness and end-diastolic blood flow were shown to be the most effective combination for the evaluation of uterine receptivity. Divya sardana et al.⁴⁰ also suggested that a combination of endometrial thickness and Doppler analysis of the endometrial blood flow was a simple and effective tool to improve the outcome of IVF/ET. But we have not found any correlation between the endometrial thickness and conception rates when the endometrial thickness was between 7 and 13 mm.

Though Ng et al.⁴¹ did not demonstrate a significant difference in vascularity parameters of the endometrium and positive pregnancy outcome, when endometrial vascularity assessment was done a day after the LH surge started in natural cycle or clomiphene citrate stimulated FET cycles. This may correlate with the known decreased vascularity of the endometrium soon after the start of LH surge. Raine-Fenning et al.⁴² proposed that the degree of change in endometrial perfusion from the late follicular phase through to the early luteal phase was a more important determinant of endometrial receptivity. Thus indicating that the dynamic changes in the endometrial vascularity are more relevant to the possibility of conception and serial assessment

of the endometrial vascularity may better predict and relate to conception rates.

About 10–15% of clinically recognised pregnancies end in spontaneous miscarriage. Though 50% of the first trimester spontaneous miscarriage may have an abnormal karyotype,⁴³ in the rest it may also be due to abnormal uterine or endometrial blood flow indices.⁴⁴ Measuring the endometrial blood flow indices in IVF and FET treatment, even before the time of implantation, may open up a possibility to start therapeutic measures to reduce the miscarriage in these patients to improve endometrial flow. In one of our previous studies, we have also found significantly lower abortion rates in IUI cycles when the conception had occurred with vascularity in zone 3 and zone 4 of the endometrium on the day of ovulation trigger.⁴⁵ Chien et al. stated that, when pregnancy is achieved in absence of endometrial and subendometrial flow on the day of embryo transfer, more than half of these pregnancies will finish as spontaneous miscarriage.⁴⁶

In one of our other studies; patients with endometrial vascularity in zones 3 and 4, RI <0.6, had a high conception rate and low miscarriage rate but with endometrial volume of <1.5 ml, not a single pregnancy was noted. If the endometrial FI before the trigger for ovulation is between 37 and 41 and endometrial VFI was between 25 and 27, not only there are significantly higher chances of leading to conception, but these values also carry a high chance of ongoing pregnancy.⁴⁷ In another study by Ng et al., a multiple logistic regression analysis, only endometrial VI was significantly associated with the chance of live birth with an odds ratio (OR) of 1.384 [95% confidence interval (CI) 1.025–1.869, $p = 0.034$]. In the prediction of live birth, in FET cycles, the area under the receiver operating characteristic (ROC) curve for endometrial VI was 0.64 and the odds ratio (OR) was 3.2 (95% CI: 1.5–6.8) with a cut-off of >0.306.¹³ This study indicates better endometrial perfusion indicates higher chances of live birth and is consistent with the results of our study.

CONCLUSION

This study indicates that zone 3 (intra-endometrial) vascularity is an important parameter when evaluating endometrial receptivity and can be positively correlated to the conception rates and negatively correlated with the miscarriage rates. We recommend; therefore, to evaluate the endometrium before FET by Doppler and decide on starting progesterone for frozen embryo transfer only if intra-endometrial vascularity is seen.

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