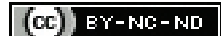


# Does the Pattern of Endometrium Influence the Frozen Embryo Transfer Cycle Outcome?

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## ABSTRACT

**Introduction:** Implantation rates vary between 25-35% despite all advancements in Assisted Reproductive Technology (ART). Successful implantation requires embryo endometrial synchrony. Transvaginal Ultrasonography (TVS) plays an essential role in assessing endometrial receptivity.

**Aim:** To study the influence of different echo patterns of the endometrium in predicting the outcome of Frozen Embryo Transfer (FET) cycle.

**Materials and Methods:** A prospective observational study was performed to determine the effect of endometrial pattern on the outcomes of FET cycles. A total of 348 women who underwent endometrial preparation for FET from January 2012 to December 2017 were included. On the day of starting progesterone, the study participants were divided into three groups based on the endometrial pattern: Type A: Triple line or multilayer pattern; Type B: Isoechoic pattern; and Type C: Hyperechoic pattern.

The association of endometrial pattern with the outcomes of FET cycles- pregnancy rate (primary end point), clinical pregnancy and live birth rate (secondary outcome), were determined. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23.0 software using paired student's t-test, Chi-square test, Fisher's-exact test, Analysis of Variance (ANOVA) and logistic regression. A probability value of <0.05 was considered statistically significant.

**Results:** The baseline characteristics such as Body Mass Index (BMI), duration, type and causes of infertility, were comparable between the study groups. There was no significant difference in the pregnancy, clinical pregnancy and miscarriage rates, and live birth rates between the study groups (Pregnancy rate: Type A: 55.9%; Type B: 51.2%; and Type C: 44.4%; p=0.594).

**Conclusion:** According to the above findings, the endometrial pattern doesn't significantly influence the outcomes of FET cycles.

**Keywords:** Endometrial pattern, Live birth rate, Miscarriage rate, Pregnancy rate, Triple line endometrium, Transvaginal sonography

## INTRODUCTION

The ART is being increasingly used in the management of infertile couple. Over the last 25 years research in this field has led to fine tuning of stimulation protocols to yield better quality oocytes. With the advent of advanced sperm selection techniques and newer methods for embryo assessment there has been considerable improvement in embryo quality and embryo selection. Despite these advances the implantation rates vary between 25-35% [1]. Successful implantation requires embryo endometrial synchrony. TVS plays an essential role in the assessment of endometrial receptivity.

Endometrial pattern constitutes the texture and echogenicity of the endometrium in comparison to the myometrium. The pattern is assessed by transvaginal ultrasound in mid-sagittal plane. The typical pattern of the proliferative endometrium is the trilaminar pattern or pattern A [2]. This consists of a central echogenic line that constitutes the uterine cavity, the outer lines represent the endo-myometrial interface. The functional layer of the endometrium is the region between the central line and the outer two hyperechoic lines. The endometrial pattern has been classified by various criteria by different authors. However commonly used terminologies to describe different sonomorphologic patterns of the endometrium are: A triple line or multilayered pattern, isoechoic pattern and hyperechoic non multilayered pattern [2]. The associations of these endometrial patterns to the various outcomes of FET cycles were poor and inconsistent across various studies [2-4].

Triple line pattern of endometrium was seen in 75% of patients who conceived compared to 42.4% of non pregnant patients (p<0.01) undergoing fresh transfer in ART cycles [5]. A few studies

supported the positive role of favourable endometrial pattern (pattern A and pattern B) on the outcome of ART cycle while many other studies hadn't shown an effect [2-4]. The available evidence on the influence of endometrial pattern on the ART outcome is mutually conflicting and there is lack of uniform consensus till date. The present study was performed to determine the influence of different echogenic patterns of the endometrium on the outcome of FET cycles such as pregnancy rate, clinical pregnancy, miscarriage, and live birth rates.

## MATERIALS AND METHODS

A prospective observational study was conducted in the Department of Reproductive Medicine and Surgery, at a tertiary care university teaching hospital, Chennai, Tamil Nadu, India. Written and informed consent was obtained from all the study participants. The study was approved by the Institutional Ethics Committee (IEC) of our university (Ref: No PhD (RR)/278- P.T./1/ 2012).

**Sample size calculation:** With a power of 80% and type 1 error of 5%, the estimated sample size to produce the desired effect was calculated to be 340 women.

**Inclusion criteria:** The women who gave consent and who underwent endometrial preparation for FET from January 2012 to December 2017 at our centre were eligible to participate in the study.

**Exclusion criteria:** Patients who had history of tuberculosis, intrauterine surgery, endometrial abnormalities like- polyp, fluid, calcification, uterine anomaly on Ultrasonography (USG), cancelled cycles and difficult embryo transfers were excluded.

## Protocol for Endometrial Preparation

The study participants underwent endometrial preparation either by artificial cycle with Gonadotropin Releasing Hormone (GnRH) agonist suppression followed by oestrogen replacement, or by direct Hormone Replacement Therapy (HRT) with estradiol valerate, as per our department protocol. The type of protocol followed was individualised depending on the patient characteristics and the choice of the consultant. In artificial cycles either Leuprolide acetate depot 3.75 mg or Triptorelin depot 3.75 mg was used for down regulation, either on day 21 of periods in regularly cycling women or on 15<sup>th</sup> day of Combined Oral Contraceptive pills (COCs).

Oestrogen replacement was performed using a gradual step-up dosage of estradiol valerate from 4 mg/day for the first five days and 6 mg/day from day 6 of HRT. In direct cycles estradiol valerate was started from day 2 of spontaneous or induced menses at a dose of 6 mg/day. Ultrasound evaluation of endometrium was performed on day 11 of HRT in all the women by sagittal scan using transvaginal probe 9MHz (Ultrasonix- sonixop ultrasound system). Endometrial Thickness (ET) was assessed as maximum distance measured between the outer margins of the outer hyperechoic endometrium (Zone 2) perpendicular to the axis of the uterine cavity, in the mid-sagittal section. In our department, an ET of  $\geq 7$  mm was considered optimal. In all the women with ET  $< 7$  mm on day 11, estradiol valerate was stepped up by 2 mg and reassessed by TVS every 5<sup>th</sup> day till an optimal ET is attained. As per our department protocol the criteria to prepare for transfer was achievement of an ET of at least 7 mm and a minimum duration of HRT of at least 14 days. In the patients planned for transfer progesterone supplementation was performed by parenteral micronised progesterone 100 mg intramuscular for the same number of days equal to the age of the embryo, prior to transfer. Those women who failed to attain an optimal embryo transfer despite a total dose of 18 mg/day of estradiol valerate were counselled for cancellation. However, they were prepared for transfer only if they insist despite being aware of the poorer outcomes.

Endometrial pattern was assessed on the day of progesterone initiation by criteria laid down by Zhao J et al., [2]. Based on these criteria the study participants were divided into three groups as follows: (a) Type A: Trilaminar/multilayered-endometrium with central and outer echogenic lines clearly visualised; (b) Type B: Isoechoic- same reflectivity of the endometrium as the surrounding myometrium and poorly defined central echogenic line; and (c) Type C: hyperechoic- increased reflectivity compared to the myometrium, and the central and the outer echogenic lines not differentiated clearly [2]. Embryo transfer was performed under the guidance of real time transabdominal ultrasound imaging by using a soft catheter (Labotect). Luteal phase was supplemented by oral Dydrogesterone 30 mg/day and vaginal micronised Progesterone 600 mg/day, both in three divided doses. The value of serum Beta Human Chorionic Gonadotropin ( $\beta$ -HCG) of  $> 5$  mIU/mL on the 14<sup>th</sup> day after embryo transfer was considered positive for pregnancy. Clinical pregnancy was confirmed by TVS at six weeks, and these women were further followed-up till delivery.

The following definitions were followed: Clinical pregnancy is a pregnancy diagnosed by ultrasonographic visualisation of one or more viable intrauterine pregnancy (gestational sac containing a foetal pole showing cardiac activity) at 4-5 weeks post-transfer [6]. Miscarriage: Spontaneous loss of a clinical pregnancy before 24 completed weeks of gestational age, in which the embryo(s) or foetus(es) is/are non viable or is/are expelled spontaneously from the uterus [7]. Live birth: The complete expulsion or extraction from a woman of a product of fertilisation, after 24 completed weeks of gestation with signs of life [7,8]. Based on the pregnancy positivity after embryo transfer, the study participants were divided into pregnant and non pregnant groups. The various study parameters were compared to detect the possible effect modifiers.

## STATISTICAL ANALYSIS

The collected data were analysed by SPSS software Version 23.0. Frequency analysis and percentage analysis were used for categorical variables and the mean $\pm$ SD was used for continuous variables. To find the significant difference paired student's t-test, Chi-square test, Fisher's-exact test, ANOVA and logistic regression were used. Probability value (p)  $< 0.05$  was considered statistically significant.

## RESULTS

There was significant difference of age between the study participants, with the mean age being highest in Type C group. The study groups were comparable by BMI, duration [Table/Fig-1]. The type of infertility (primary, secondary) and the various causes of infertility such as male, female, combined, and unexplained factors were comparable between the groups [Table/Fig-2,3]. The study groups had comparable ET on the day of starting progesterone [Table/Fig-4]. There was no significant difference in the FET outcomes in the various sub-groups of ET [Table/Fig-5]. The number of embryos and stage of embryo transfer were comparable between the study groups [Table/Fig-6]. There was no significant difference between the pregnancy and clinical pregnancy rates between the study groups. The miscarriage rates were comparable between the study groups. There was no significant difference in the live birth rates between the study groups [Table/Fig-7]. The intergroup comparisons of the outcomes of FET such as pregnancy rate, clinical pregnancy rate, miscarriage and live birth rates didn't reveal significance between

Parameter	Type A (N=218) (Mean $\pm$ SD)	Type B (N=121) (Mean $\pm$ SD)	Type C (N=9) (Mean $\pm$ SD)	p-value*
Age (years)	30.7 $\pm$ 4.7	32.3 $\pm$ 4.3	34.6 $\pm$ 5.8	0.001
Duration of infertility (years)	5.9 $\pm$ 3.7	6.9 $\pm$ 4.5	7.1 $\pm$ 4.6	0.091
BMI (Kg/m <sup>2</sup> )	26.057 $\pm$ 4.2554	26.983 $\pm$ 4.5654	27.000 $\pm$ 5.0249	0.163

**[Table/Fig-1]:** Comparison of baseline characteristics between the study groups.  
\*Student's t-test; p $< 0.05$  was considered statistically significant

Type of infertility	Type A (%) (N=218) (Mean $\pm$ SD)	Type B (%) (N=121) (Mean $\pm$ SD)	Type C (%) (N=9) (Mean $\pm$ SD)	p-value*
Primary	170 (78)	94 (77.7)	8 (88.9)	0.731, NS
Secondary	48 (22)	27 (22.3)	1 (11.1)	

**[Table/Fig-2]:** Comparison of type of infertility between the study groups.  
\*Fisher's-exact test; p-value  $< 0.05$  was considered statistically significant; NS: Non significant

Causes of infertility (N=348)	Type A (%) N=218	Type B (%) N=121	Type C (%) N=9	p-value*
Female factors (n=144)	82 (37.6)	57 (47.1)	5 (55.6)	0.293, NS
Male factors (n=111)	74 (33.9)	35 (28.9)	2 (22.2)	
Unexplained (n=46)	35 (16.1)	10 (8.3)	1 (11.1)	
Combined (n=47)	27 (12.4)	19 (15.7)	1 (11.1)	

**[Table/Fig-3]:** Comparison of the causes of infertility between the study groups.  
\*Fisher's-exact test; p-value  $< 0.05$  was considered statistically significant; NS: Non significant

Parameter	Type A (%) N=218	Type B (%) N=121	Type C (%) N=9	p-value
Endometrial thickness subgroup (mm)				
$< 7$ (n=4)	2 (1.0)	1 (0.8)	1 (11.1)	0.057, NS <sup>†</sup>
7-8.9 (n=72)	48 (22.0)	22 (18.2)	2 (22.2)	
9-10.9 (n=178)	116 (53.2)	58 (47.9)	4 (44.5)	
11-12.9 (n=83)	48 (22.0)	34 (28.1)	1 (11.1)	
$> 13$ (n=11)	4 (1.8)	6 (5.0)	1 (11.1)	
Endometrial thickness (Mean $\pm$ SD)	9.85 $\pm$ 1.3	10.16 $\pm$ 1.5	9.3 $\pm$ 2.0	0.087, NS <sup>‡</sup>

**[Table/Fig-4]:** Comparison of the endometrial thickness on the day of progesterone between the study groups.

<sup>†</sup>: Fisher's-exact test; <sup>‡</sup>: Student's t-test; p $< 0.05$  was considered statistically significant; NS: Non significant

any of the comparisons [Table/Fig-8]. The baseline characteristics, causes of infertility, endometrial preparation parameters, and number and stage of transferred embryos were similar between the pregnant and non pregnant groups [Table/Fig-9-11]. Except for the day of transfer, there was no significant effect of the potential effect modifiers such as age, BMI, duration of infertility, causes of infertility and ET on the pregnancy rate [Table/Fig-12].

Endometrial thickness subgroup (mm)	Pregnancy rate (%)	Clinical pregnancy rate (%)	Miscarriage rate (%)	Live birth rate (%)
<7	2/4 (50)	2/4 (50)	1/2 (50)	1/4 (25)
7-8.9	38/72 (52.8)	33/72 (45.8)	7/38 (18.4)	25/72 (34.7)
9-10.9	98/178 (55.1)	88/178 (49.4)	22/76 (22.4)	64/178 (36)
11-12.9	44/83 (53)	41/83 (49.4)	15/44 (34.1)	26/83 (31.3)
>13	6/11 (54.5)	6/11 (54.5)	1/6 (16.7)	5/11 (45.5)
p-value*	0.646 NS	0.406 NS	0.396 NS	0.867 NS

**[Table/Fig-5]:** Comparison of the FET outcome with the endometrial thickness. \*Fisher's-exact test; p-value <0.05 was considered statistically significant; NS: Non significant

Parameter	Type A (%) N=218	Type B (%) N=121	Type C (%) N=9	p-value*
Day 3 Transfer (n=168)	103 (47.2)	57 (47.1)	8 (88.9)	0.057, NS <sup>1</sup>
Day 4 Morula (n=100)	59 (27.1)	41 (33.9)	0	
Day 5 Transfer (n=80)	56 (25.7)	23 (19.0)	1 (11.1)	
No. of embryos transferred (mean±SD)	2.6±0.8	2.6±0.6	2.4±0.5	0.648, NS <sup>2</sup>

**[Table/Fig-6]:** Comparison of the embryo transfer characteristics between the study participants. \*1: Fisher's-exact test; \*2: Student's t-test; p<0.05 was considered statistically significant; NS: Non significant

FET cycle outcome parameters	Type A (%) N=218	Type B (%) N=121	Type C (%) N=9	p-value*
Pregnancy rate	122 (55.9%)	62 (51.2%)	4 (44.4%)	0.594, NS <sup>1</sup>
Implantation rate	24.5±28.6	24.1±28.1	14.8±22.7	0.601, NS <sup>2</sup>
Clinical pregnancy	110 (50.4%)	57 (47.1%)	3 (33.3%)	0.538, NS <sup>1</sup>
Ectopic pregnancy	3 (1.3%)	0	0	-
Miscarriage	26 (21.3%)	19 (30.6%)	1 (25%)	0.379, NS <sup>1</sup>
Live birth rate	81 (37.1%)	38 (31.4%)	2 (22.9%)	0.411, NS <sup>1</sup>

**[Table/Fig-7]:** Comparison of ART outcomes between the study groups. \*1: Fisher's-exact test; \*2: Student's t-test; p<0.05 was considered statistically significant

Groups compared	Pregnancy rate *(p-value)	Clinical pregnancy rate *(p-value)	Live birth rate *(p-value)	Miscarriage rate *(p-value)
Type A and C	0.516	0.357	0.605	1.00
Type A and B	0.403	0.695	0.494	0.164
Type B and C	0.742	0.323	0.644	1.00

**[Table/Fig-8]:** Intergroup comparison of ART outcomes between the study groups. \*: ANOVA; p<0.05 was considered statistically significant

Parameter	Pregnant (N=188) (Mean±SD)	Non pregnant (N=160) (Mean±SD)	p-value*
Age (years)	31.4±4.4	31.3±5.0	0.916, NS
Duration of infertility (years)	6.4±4.1	6.2±4.0	0.655, NS
BMI (kg/m <sup>2</sup> )	26.7±4.6	26.1±4.2	0.165, NS

**[Table/Fig-9]:** Comparison of the baseline characteristics with the primary outcome. \*: Student's t-test; p<0.05 was considered statistically significant

Baseline characteristic	Pregnant (%) N=188	Non pregnant (%) N=160	p-value*
Female factor, N=144	80 (42.5)	64 (40.0)	0.762, NS
Male factor, N=111	62 (33.0)	49 (30.6)	
Combined, N=47	24 (12.8)	23 (14.4)	
Unexplained, N=46	22 (11.7)	24 (15.0)	

Type of infertility			
Primary	145 (77.5)	127 (78.9)	0.613, NS
Secondary	43 (22.5)	33 (21.1)	

**[Table/Fig-10]:** Comparison of the causes of infertility with the primary outcome (pregnancy). \*Chi square test; p<0.05 was considered statistically significant

Baseline characteristic	Pregnant N=188	Non pregnant N=160	p-value
Endometrial thickness (mm) (mean±SD)	9.96±1.45	9.95±1.5	0.955 <sup>3</sup>
Stage of embryo transferred			
Cleavage-168 (48.3%)	80 (47.6%)	88 (52.3%)	0.02 <sup>2</sup>
Morula-100 (28.7%)	55 (55.0%)	45 (45.0%)	
Blastocyst-80 (23.0%)	53 (66%)	27 (33.7%)	
No. of embryos transferred (mean±SD)	2.72±0.75	2.63±0.79	0.258 <sup>1</sup>

**[Table/Fig-11]:** Endometrial thickness and embryo quality with the primary outcome (pregnancy). \*1: Fisher's-exact test; \*2: Chi-square test; \*3: Student's t-test; p<0.05 was considered statistically significant

Dependent variables	(*p-value)
Age	0.966
BMI	0.104
Duration of infertility	0.776
Causes of infertility	0.860
Endometrial thickness	0.853
Day of embryo transfer	0.005

**[Table/Fig-12]:** Logistic regression analysis of possible effect modifiers of pregnancy rate. \*Multivariate logistic regression. p<0.05 was considered statistically significant

## DISCUSSION

Overall, various studies have shown inconsistent association of ET and endometrial pattern on the outcome of FET cycles. Shaodi Z et al., performed a retrospective analysis of 10,165 HRT-FET cycles and showed that the optimal live birth rate is achieved when the ET falls in the range of 8.7-14.5 mm on the day of transfer. They comparatively assessed clinical outcomes by grouping women at intervals of 1 mm of ET [9]. This study showed that, the women who had ET <8.7 mm, with each additional 1 mm of ET had 32% higher implantation rates. The pregnancy and clinical pregnancy rates were increased by 36% and 45%, respectively. In a retrospective analysis of 501 FET cycles, Wang Y et al., showed that clinical pregnancy and live birth rates were significantly lower when the ET was <7 mm (18% and 12%; p=0.047 and 0.044) compared to those with ET of 7-12 mm (44.8% and 41.5%) and >12 mm (51% and 44%) [10]. These observations were similar to present study results as authors did not found any association between various subgroups of ET with the clinical pregnancy, miscarriage, and live birth rates.

Endometrial pattern also called echogenicity of endometrium is commonly assessed on the day of starting progesterone prior to embryo transfer in FET cycles. Zhao J et al., reported significantly higher pregnancy rate in women with triple line (Pattern A) and isoechoic endometrium (Pattern B) than in hyperechoic (Pattern C) endometrium (55.2% and 50.9% vs 37.4% p<0.05) [2]. Al Mohammady M et al., showed that trilaminar pattern of endometrium (Type A) showed a higher pregnancy rate compared to other pattern when the ET fell in the range of 10-12.9 mm [11]. In present study a higher pregnancy rate in Type A and Type B groups compared to that of Type C but wasn't statistically significant. This contradictory result could be due to less number of patients with Type C (N=9) endometrium. Abdel Kader M et al., classified the echopattern of endometrium as triple line and non triple line, and observed that triple line endometrium was seen in all the cases who conceived in the study (40 of 100 patients) [6]. Similar findings were observed in the studies performed by Wang L et al., and Singh N et al., that showed



absence of association between echogenicity of endometrium and In Vitro Fertilisation (IVF)-embryo transfer outcome [12,13]. Yang W et al., performed a retrospective observational study on the effect of ET and pattern on ART outcome in 1512 FET cycles [14]. The endometrial pattern was classified into triple line (Pattern A) and non triple line (Pattern B), and ET as group 1 ( $\leq 8$  mm) and group 2 ( $> 8$  mm). There was no influence of endometrial pattern or thickness on live birth rates, but the clinical pregnancy rates were significantly increased with improved ET in pattern A. The clinical pregnancy rates were significantly higher in pattern A compared to pattern B in Group 1, but not in Group 2 [14]. It was believed that premature secretory endometrial pattern (echogenic endometrium or non triple line) was due to premature progesterone rise that could have adverse effect on pregnancy rates [15]. But in a retrospective study by Chen S-L et al., involving 2896 fresh embryo transfer cycles, it was observed that raised serum progesterone was not associated with non triple line endometrium [4]. Another prospective study, showed no significant difference in the echogenic patterns of endometrium between patients with elevated and normal progesterone levels [16]. Endometrial echogenicity was recently attributed to the development of multiple penetrating vessels which produce multiple tissue interfaces and glycogen storage in the endometrial columnar epithelium. It could also be the result of secretions in the endometrial glands and scarce stromal oedema due to increasing oestrogens in the preovulatory phase [17].

The strengths of present study were that it was a prospective observational study with adequate sample size, and the study participants were followed-up till delivery or miscarriage. Further prospective studies evaluating the role of endometrial pattern in specific groups of infertility patients may enhance the understanding of the role of endometrial pattern on the outcome of ART.

### Limitation(s)

The limitations of the study are the study population being heterogenous with various causes of infertility and the non experimental design (observational study). The study wasn't adequately powered to evaluate the role of endometrial pattern in each individual cause of infertility.

### CONCLUSION(S)

There was no significant influence of the endometrial pattern on the outcome of FET cycles. Non significant increase in pregnancy rate was seen in patients with type A and B compared to type C. However, these findings need to be evaluated individually in the participants with each cause of infertility on a larger data.

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