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Endometrial thickness is a good predictor of clinical pregnancy and live birth rates in fresh and frozen-thawed embryo transfer cycles

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Abstract. – OBJECTIVE: The aim of this study was to investigate the effect of the endometrial thickness (EMT) measured on embryo transfer day on clinical pregnancy (CPR), live birth (LBR), and miscarriage rates (MR) in fresh and frozen-thawed embryo transfer cycles.

PATIENTS AND METHODS: This prospective cohort study consisted of 160 patients, 80 frozen-thawed and 80 fresh cycles. Endometrial thickness was measured on the day of embryo transfer for fresh and frozen cycles. In addition to the endometrial thickness, the endometrial appearances of the patients in both groups were also recorded. Those without trilaminar appearance were excluded from the study. Both groups were classified according to the EMT values measured on the day of the transfer. The number of groups was calculated considering 1 mm intervals of EMT, and a total of 8 groups were formed. The initial group started with <6 mm, while the final group was >12 mm. The relationship between endometrial thickness, clinical pregnancy, live birth and miscarriage rates was analyzed using multivariable regression analysis.

RESULTS: A significant correlation was observed between endometrial thickness values, clinical pregnancy rates, live birth rates in the analyses performed after adjusting for age, infertility duration, body mass index, number of MII oocytes, number and quality of embryos transferred. Based on univariate analysis, each 1 mm increase in EMT resulted in a significant increase in CPR (OR=1.08, 95% CI: 1.07-1.09, *p*<0.01). Similarly, the increase in EMT led to a significant increase in LBR (OR=1.12, 95% CI: 1.10-1.14, *p*<0.01). Although the relationship between miscarriage rates and EMT is not as clear as LBR and CPR, the increase in EMT led to a significant reduction in MR (OR=1.05, 95% CI: 1.03-1.05, *p*=0.03). The lowest CPR was detected at EMT <6 mm, while the EMT value with the highest CPR was 11-12 mm in both groups. Likewise, in both groups, the lowest LBR was detected at EMT <6 mm, while the EMT value with the highest LBR was 11-12 mm. Although MR showed a fluctuating course according to EMT values, it reached its highest rate at EMT <6 mm (100%). In EMT 11-12 mm, MR reached its lowest level (12.5%). If EMT >12 mm, an increase in MR rates was observed again (33.3%).

CONCLUSIONS: Clinical pregnancy and live birth rates remain optimal if the endometrial thickness is between 11-12 mm in both fresh and frozen-thawed cycles. A fluctuating course is observed between EMT values and miscarriage rates.

Key Words:
Fresh ET, Frozen ET, Endometrial thickness, CPR, LBR, MR.

Introduction

The endometrium is a privileged tissue with selectivity and receptivity functions. Under the influence of the ovarian sex steroids estrogen and progesterone, the endometrium, which first acquires proliferative and then secretory features, provides suitable decidual conditions and allows successful implantation to take place. However, changes in the endometrium are often insufficient to provide suitable conditions for implantation in natural or artificial cycles. For this reason, implantation does not occur despite good-quality embryo transfer. Although many clinicians[1,2] prefer knowing the receptive status of the endometrium before the embryo transfer, there is no easy, repeatable, inexpensive, noninvasive, and reliable test to evaluate receptivity.

Ultrasonographic evaluation of changes in the endometrium has long been used to detect receptive endometrium. Endometrial thickness (EMT) is the most widely used sonographic predictive marker[1-3]. In addition, endometrial volume and pattern changes are also used to predict receptivity[4,5]. However, data

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regarding EMT predicting receptivity are inconsistent. Pregnancy rates are very low in the presence of weak and not enough thick EMT. However, pregnancy does not always occur in endometriums with sufficient thickness[2,6]. These findings suggest that endometrial thickness is not only a sonographically measured parameter but also has other components. There are new studies[1,2] on which is reported that the thickness of the endometrium is most receptive on the day of embryo transfer. It has been reported that optimal pregnancy is achieved if EMT >12 mm in fresh cycles, while pregnancy rates above EMT >15 mm have been reported to decrease[1,2]. In another study[3], it was emphasized that the required EMT for optimal live birth in frozen-thawed cycles (FET) is 8-14 mm.

Considering all these data, no clear value has been determined for the optimal EMT value in fresh or frozen-thawed ET cycles. Since receptivity is not only proportional to endometrial thickness, it is obvious that other parameters are also involved[1,2,5]. There are studies[1,7] that separately evaluated the effects of EMT values on fertility outcomes in fresh and FET cycles. However, there are no comprehensive studies comparing changes in fertility outcomes according to EMT values in fresh and FET cycles. This study was planned to test the effect of EMT, measured on the day of embryo transfer, on clinical pregnancy, live birth, and miscarriage rates in fresh and FET cycles.

Patients and Methods

This prospective cohort study consisted of 160 patients, 80 frozen-thawed and 80 fresh cycles. Endometrial thickness was measured on the day of embryo transfer. In addition to the endometrial thickness, the endometrial appearances of the patients in both groups were also recorded. Both groups were classified according to the EMT values measured on the day of transfer. 8 groups were formed by considering 1 mm intervals of EMT between the groups. The initial group started with <6 mm, while the final group was >12 mm. The fertility outcome of each group, consisting of clinical pregnancy, live birth and miscarriage, was determined separately according to the groups. Inclusion criteria were the presence of at least one good-quality embryo and the absence of a compressive lesion in or around the endometrium. Those with subendometrial contraction during embryo transfer were excluded from the study. Participants with thin and non-homogeneous endometrium were not included in the study. The trilaminar appearance of the endometrium before embryo transfer was another inclusion criterion. Patients with endometrioma, hydrosalpinx, adenomyosis, endometrial adhesion and the presence of polyps were excluded from the study. Patients with intramural or submucous myoma pressing on the cavity and those with a history of mechanical endometrial injury were also excluded from the study. Ethical approval was obtained from the Diyarbakır Memorial Hospital (Ethic approval number: 2022/64).

All patients in the frozen transfer group received hormone replacement therapy for artificial endometrial preparation. The endometrium was prepared artificially using the estrogen and progesterone protocol. On the 3rd day of the cycle, a total of 4 mg estradiol treatment (Estrofem 2 mg tab, Novo Nordisk Saglik Urunleri Tic, Ltd, Şti, Istanbul, Turkey) was started, twice a day. Dose adjustment was made by measuring EMT every four days. Estradiol treatment was continued for at least 10 days. The dose was increased up to 8 mg daily in those with EMT thin. When the endometrial thickness reached 8 mm at the end of ten days, progesterone treatment (Progestan 50 mg/ml 1x2 IM, Koçak Farma İlaç ve Kimya Sanayi A.Ş., Istanbul, Turkey) was started. ET time was determined according to the freezing day of the egg. Embryos frozen on the third day were transferred on the 4th day of progesterone treatment, and the blastocyst-stage embryos were transferred on the 6th day. Embryos with 7-9 blastomeres with uniform cytoplasm and normal morphology are considered high quality. In addition, embryo fragmentation was required to be below 10%. Gardner scoring system[8] was used for blastocyst scoring. Thus, it was possible to transfer a good-quality embryo or blastocyst to each patient. Each patient was given luteal phase support until at least the human chorionic gonadotropin (hCG) day with two different progesterone applications (Progestan 50 mg/ml 1x1 IM and Progestan 200 mg capsule, 3x200 mg, Koçak Farma İlaç ve Kimya Sanayi A.Ş., Istanbul, Turkey). Clinical pregnancy was defined as evidence of an intrauterine gestational sac, confirmed by ultrasound examination. Live birth was defined as the delivery of a live fetus after 20 completed weeks of gestational age. The loss of a fetus before 12 weeks of gestation was defined as an early miscarriage. Clinical pregnancy, miscarriage, and live birth rates per cycle of frozen-thawed or fresh ET were calculated using the formulas below. Clinical pregnancy rate=total number of clinical pregnancies/total number of ET cycles x100%; live birth rate=total number of live births/total number of ET

cycles x100%; Abortion rate=total number of abortions/total number of clinical pregnancies x100%. The relationship between endometrial thickness, clinical pregnancy, live birth and miscarriage rates was analyzed using multivariate regression and threshold effect analysis.

Statistical Analysis

SPSS v. 21 (IBM Corp., Armonk, NY, USA) was used for statistical analysis of all data. The distribution pattern of collected data was analyzed by Kolmogorov-Smirnov test. Continuous variables were presented as mean±SD, and categorical variables as n (%). One-way ANOVA or Mann-Whitney U test was used for comparison between groups. Chi-square test was used for categorical variables. One-way ANOVA test was performed without distinguishing between fresh and frozen-thawed cycles. Multivariate regression analysis was performed on each fresh or frozen-thawed cycle. Parameters with a 10% change effect on clinical pregnancy, live birth and miscarriage rates were selected as confounders. The adjusted and non-adjusted values of the confounders were presented as OR, 95% CI. $p<0.05$ was considered statistically significant.

Results

Demographic data of both groups were similar in terms of age, BMI, infertility duration, serum ollicle-stimulating hormone (FSH), the luteinizing hormone (LH), estradiol (E2) levels and antral follicle count (AFC) (Table I). The number of embryos transferred was similar. Antagonist protocol was applied to both groups. The groups were similar in terms of CPR and LBR. Miscarriage rates were higher in the FET group [11/35 (31.4%) *vs.* 6/35 (17.1%), $p<.03$]. There was no difference between the groups in terms of EMT values on the day of ET (10.9±1.22 mm *vs.* 11.3±2.05, $p<0.78$). On the day of embryo transfer, all patients had a trilaminar appearance.

Univariate Analysis of Factors Affecting CPR, LBR and MR

Based on univariate analysis, each 1 mm increase in EMT resulted in a significant increase in CPR (OR=1.08, 95% CI: 1.07-1.09, $p<0.01$). Similarly, the increase in EMT led to a significant increase in LBR (OR=1.12, 95% CI: 1.10-1.14, $p<0.01$). Although the relationship between miscarriage rates and EMT is not as clear as LBR and CPR, the increase in EMT led to a significant reduction in MR (OR=1.05, 95% CI: 1.03-1.05, $p=0.03$). As the patient's age and infertility duration increased, their negative effects on CPR and LBR increased. The increase in progesterone also had a negative effect on the fertility outcome. The increase in anti-Mullerian hormone (AMH) and AFC levels positively affected fertility outcomes. The increase in the number of MII oocytes and the number of transferred embryos were among the parameters that contributed positively to the fertility outcome.

Table I. Baseline characteristics of fresh ET and FET groups.

	Fresh ET (n=80)	Frozen ET (n=80)	*p*-value
Age (y)	25.9±3.40	27.6±2.56	0.13
BMI (kg/m^2)	23.9±3.48	24.5±4.01	0.40
Infertility duration (yrs)	3.12±1.06	3.29±1.45	0.56
LH (mIU/ml)	5.80±2.09	5.11±2.06	0.49
FSH (mIU/ml)	4.90 ±1.03	5.27±1.90	0.77
Basal E2 (pg/mL)	41.2±4.30	39.6±3.12	0.60
AFC	10.7±2.75	11.9±2.07	0.29
COS protocol	Antagonist	Antagonist	NA
Endometrial thickness (mm)	10.9±1.22	11.3±2.05	0.78
ET	Single ET	Single ET	NA
Clinical pregnancy, n (%)	35 (43.7%)	35 (43.7%)	0.22
Miscarriage, n (%)	6/35 (17.1%)	11/35 (31.4%)	0.03
Live birth, n (%)	23 (28.7%)	21 (26.2%)	0.43

Body mass index (BMI), serum ollicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), antral follicle count (AFC), controlled ovarian stimulation (COS).

Comparison of CPR, LBR, and Miscarriage Rates According to EMT Values of the Frozen-Thawed ET Group

The CPR and LBR of the patients, who were divided into 8 groups according to their EMT values, increased in proportion to the increasing EMT values (Tables II and III). The lowest CPR was detected at EMT <6 mm, while the EMT value with the highest CPR was 11-12 mm. Similarly, the lowest LBR was detected at EMT <6 mm, while the EMT value with the highest LBR was 11-12 mm. There was a significant decrease in both CPR and LBR rates at EMT >12 mm. CPR, which was determined as 57.1% when EMT value was 11-12 mm, decreased to 46.1% when EMT was >12 mm. Likewise, LBR, which was detected as 35.7% when EMT was 11-12 mm, decreased to 30.7% when EMT was >12 mm. Although MR showed a fluctuating course according to EMT values, it reached its highest rate at EMT <6 mm (100%). In EMT 11-12 mm, MR reached its lowest level (12.5%). If EMT >12 mm, an increase in MR rates was observed again (33.3%).

Comparison of CPR, LBR, and Miscarriage Rates According to EMT Values of the Fresh ET Group

In parallel with the increase in EMT values, a significant increase was found in the CPR and LBR of fresh ET patients. If EMT >12 mm, both CPR and LBR rates started to decrease again. Based on EMT values, the change in CPR, LBR, and MR was similar to the frozen-thawed ET group. The lowest CPR was detected at EMT <6 mm, while the EMT value with the highest CPR was 11-12 mm. Similarly, the lowest LBR was detected at EMT <6 mm, while the EMT value with the highest LBR was 11-12 mm. There was a significant decrease in both CPR and LBR rates at EMT >12 mm. CPR, which was determined as 53.8% when EMT value was 11-12 mm, decreased to 45.4% when EMT was >12 mm. Likewise, LBR, which was detected as 38.4% when EMT was 11-12 mm, decreased to 36.6% when EMT was >12 mm. Although MR showed a fluctuating course according to EMT values, it reached its highest rate at EMT <6 mm (100%). In EMT 10-11 mm, MR reached its lowest level (12.5%). If EMT >12 mm, an increase in MR rates was observed again (20.0%). However, the fact that the MR is zero when the EMT is 6-7 mm and 7-8 mm prevents us from talking about the exponential decrease in MR. The small number of patients in these two groups may have led to a non-exponential decrease in MR (Tables III-V).

Discussion

Although embryos transferred in the implantation window have a higher chance of attachment and invasion to the endometrium, successful im-

Table II. Clinical pregnancy, live birth and miscarriage rates according to EMT values of patients who underwent frozen-thawed ET.

EMT*	<6 mm	6-7 mm	7-8 mm	8-9 mm	10-11 mm	11-12 mm	>12 mm	9-10 mm	*p***
N	*4*	*5*	*7*	*10*	*12*	*15*	*14*	*13*	
Clinical pregnancy	1 (25%)	2 (40%)	3 (42.8%)	4 (40%)	5 (41.6%)	7 (46.6%)	8 (57.1%)	6 (46.1%)	0.001
Live birth	0 (0%)	1 (20%)	1 (14.2%)	2 (20%)	3 (25%)	5 (33.3%)	5 (35.7%)	4 (30.7%)	0.001
Miscarriage	1 (100%)	1 (50%)	1 (33.3%)	2 (50%)	1 (20%)	2 (28.5%)	1 (12.5%)	2 (33.3%)	0.03

*: EMT was measured during embryo transfer and recorded in mm. **: $p<0.05$ indicates statistical significance. Endometrial thickness (EMT).

Table III. Multivariate logistic regression analysis of the relationship between EMT and fertility outcomes in frozen-thawed ET cycles.

Fertility outcome	Non-adjusted		Adjusted	
	OR, 95% CI	*p*-value	OR, 95% CI	*p*-value
Clinical pregnancy	1.04 (1.03, 1.05)	0.001	1.03 (1.02, 1.05)	0.001
Live birth	1.05 (1.04, 1.08)	0.001	1.05 (1.04, 1.07)	0.001
Miscarriage	0.97 (0.96, 0.98)	0.002	0.95 (0.94, 0.96)	0.001

Adjusted for age, infertility duration, BMI, number of MII oocytes, and number and quality of embryos transferred.

Table IV. Clinical pregnancy, live birth and miscarriage rates according to EMT values of patients who underwent fresh ET cycle.

EMT*	<6 mm	6-7 mm	7-8 mm	8-9 mm	10-11 mm	11-12 mm	>12 mm	9-10 mm	p**
N	*3*	*5*	*6*	*12*	*14*	*16*	*13*	*11*	
Clinical pregnancy	1 (33.3%)	2 (40.0%)	2 (33.3%)	4 (33.3%)	6 (42.8%)	8 (50.0%)	7 (53.8%)	5 (45.4%)	0.001
Live birth	0 (0%)	1 (20.0%)	1 (16.6%)	2 (16.6%)	4 (28.5%)	6 (37.5%)	5 (38.4%)	4 (36.6%)	0.002
Miscarriage	1 (100%)	0 (0%)	0 (0%)	1 (25%)	1 (16.6%)	1 (12.5%)	1 (14.2%)	1 (20.0%)	0.02

*: EMT was measured during embryo transfer and recorded in mm. **: $p<0.05$ indicates statistical significance. Endometrial thickness (EMT).

Table V. Multivariate logistic regression analysis of the relationship between EMT and fertility outcomes in fresh ET cycles.

	Non-adjusted		Adjusted	
Fertility outcome	**OR, 95% CI**	***p*-value**	**OR, 95% CI**	***p*-value**
Clinical pregnancy	1.02 (1.04, 1.05)	0.001	1.03 (1.02, 1.04)	0.001
Live birth	1.05 (1.04, 1.06)	0.001	1.05 (1.03, 1.07)	0.001
Miscarriage	0.98 (0.97, 0.99)	0.001	0.96 (0.95, 0.98)	0.001

Adjusted for age, infertility duration, BMI, number of MII oocytes, number, and quality of embryos transferred.

plantation is not achieved in approximately two-thirds of cases despite high-quality embryo transfer[9,10]. In order to capture the receptive endometrial period, most clinicians[9] have tried to clearly define the implantation window and increase the rate of attachment per transferred embryo. Embryo transfer was performed by accepting the implantation window between the 20th and 23rd days of the mid-luteal phase (LH+7-9), but no significant increase in implantation rates was achieved[9]. Subsequently, endometrial receptivity array (ERA) tests have come to the fore to increase implantation. Due to the invasive nature of the biopsy, different expressions of receptivity gene sequences even in healthy endometrium, and failure to take into account the simultaneous progesterone levels, receptivity tests have not found widespread use. On the other hand, difficulties in interpreting possible changes in endometrial genes due to hormone replacement applied in FET cycles further limited the use of receptivity tests[11,12].

Endometrial thickness, measured transvaginally, has long been used as a non-invasive predictor test that can measure the receptive phase of the endometrium more sensitively than invasive biopsy techniques[1,2]. However, it is not always possible to determine the fertility outcome based on EMT values. Some authors[1] argue that EMT values between 12-15 mm are necessary for optimum fertility outcomes in fresh cycles. Others accept this range as 8-14 mm in FET cycles[2]. It has been reported[2,3] that EMT being between these values also reduces abortion rates. It has been reported[1] that an EMT increase above 15 mm causes a decrease in pregnancy rates and an increase in abortion rates. However, there are also studies[13] reporting that there is no correlation between EMT values and pregnancy and abortion rates. In the current study, we compared the relationship between EMT measured on ET day and fertility outcome in fresh and FET cycles. We found an increase in CPR and LBR values, and a decrease in miscarriage rates with an increase in EMT in both groups. In both FET and fresh cycles, the EMT value with the highest CPR and LBR and the lowest miscarriage was 11-12 mm. CPR and LBR were found to be the lowest in cases with an EMT value of less than 6 mm. The increase in the EMT value above 12 mm caused a decrease in the fertility outcome again. In FET cycles, CPR decreased from 57% when EMT was 11-12 mm, to 46% when EMT was >12mm. Similarly, LBR decreased from 35% when EMT was 11-12 mm, to 30% when EMT was >12 mm. Similarly, an increase in EMT above 12 mm in fresh cycles led to a significant decrease in CPR (53% *vs.* 45%) and LBR (38% *vs.* 36%). In FET cycles, MR reached its lowest level (12.5%) while EMT was in the range of 11-12 mm. If EMT >12 mm, an increase in MR rates was observed again (33.3%).

Similarly, in fresh cycles, MR reached its lowest level (12.5%) while EMT was in the 10-11 mm range. If EMT >12 mm, an increase in MR rates was observed again (20.0%).

This is the first comprehensive study comparing embryo transfer day EMT values with fertility outcomes in fresh and frozen-thawed cycles. In previous studies[14,15], the EMT measurement days varied considerably. EMT was measured on the hCG trigger day in some studies[15,16], on the oocyte retrieval day[17], on the day of progesterone supplementation[14], and on the day of ET in others[14-16]. Some studies[18,19] have measured EMT twice on the progesterone administration day and transfer day. EMT measurement twice in the same cycle provided important information in terms of determining the effect of changes in EMT on fertility outcome. In FET cycles, 10% increases in EMT between the day of progesterone initiation and the day of transfer did not lead to a significant increase in CPR and LBR rates[14]. However, there are also authors[20,21] reporting that the increase in EMT after progesterone treatment positively affects the fertility outcome. These differences between studies[20,21] may be related to study design, EMT measurement days, and measurement patterns. In most studies[15,22], the relationship between EMT and fertility outcome has been analyzed retrospectively. Most of the EMT measurement was done transabdominally, which often does not provide a precise measurement. The fact that the results of studies[14,16] by different authors using the same methods are different is a piece of evidence supporting the importance of the difference between EMT measurement and interpretation criteria. Some authors[21,23] have considered endometrial compaction together with endometrial thickness. A decrease of >10% in endometrial thickness after progesterone treatment was accepted as compaction. Unlike these studies, we made a prospective design study. We included cases with both FET and fresh cycles. We measured EMT vaginally and did not evaluate compaction. As a result, we found a significant relationship between EMT increase and fertility outcome in both fresh and FET cycles. The most optimal EMT value of this relationship was 11-12 mm for both groups. Fertility outcome was adversely affected in EMT values above 12 mm.

We found a clear relationship between increased EMT and fertility outcome in both FET and fresh cycles. However, after EMT >12 mm, we determined that this relationship changed, and it caused a negative fertility outcome. The relationship between EMT increase and miscarriage rates was not as clear as LBR and CPR. However, multiple logistic regression analyses of factors related to CPR, LBR, and MR indicated that endometrial thickness on ET day was associated with improved fertility outcomes in both fresh and frozen cycles. Factors such as the age of the expectant mother, infertility duration, BMI, number of MII oocytes, and number and quality of embryos transferred were included in the regression analysis, thus excluding confounders that affect fertility outcomes other than EMT. Unlike our results, in a study[15] comparing fresh and FET EMT values of the same patients, a significant relationship was found between fresh cycle EMT values and fertility outcomes, but this relationship was not found in FET cycles. However, the authors reporting 84% CPR over EM 16 mm is inconsistent with other studies[16-18]. In most studies[1,2], the maximum EMT value found for an optimal fertility outcome has been reported as 14-15 mm. We also showed that the fertility outcome of EMT >12 mm is adversely affected.

Conclusions

Despite some limitations, our study provides important clinical data in terms of comparing the relationship between transfer day EMT values and fertility outcomes in fresh and frozen ET cycles. Although the unequal number of participants in EMT groups is an important limitation, the number of participants was statistically significant in discussing the results. Although endometrial thickness increase is a good predictor of receptivity, the relationship between receptivity genes and thickness is unknown. When we say a sufficient thickness, we combine morphological, glandular, vascular, and decidual components. Considering the individual changes in these parameters, not every increase in thickness will be a good predictor of receptivity, and every decrease in thickness will not show a bad fertility outcome.

Ethics Approval

Ethical approval was obtained from Diyarbakır Memorial Hospital Ethical Committee (Date: 27/07/2022 and record number: 64).

Informed Consent

All patients were informed about the study. All patients signed and approved the “informed patients consent”.

Availability of Data and Materials

All generated materials and data were presented in the study.

Authors' Contributions

UD, ZK, and FÇ read and approved the final version of the manuscript. All authors equally contributed to the manuscript writing, editing, drafting, and conceptualizing stage.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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