ANALYSIS

24(103), May - June, 2020

ISSN
2321–7359
EISSN
2321–7367

Medical Science

The role of endometrial thickness and echo pattern on the day of embryo transfer in Frozen Embryo Transfer (FET) cycle outcome

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Article History
Received: 23 February 2020
Reviewed: 24/February/2020 *to* 30/March/2020
Accepted: 31 March 2020
E-publication: 10 April 2020
P-Publication: May - June 2020

Citation
Elham Ghorbani, Somaye Hajiahmadi. The role of endometrial thickness and echo pattern on the day of embryo transfer in frozen embryo transfer (FET) cycle outcome. *Medical Science*, 2020, 24(103), 1578-1585

ABSTRACT

Introduction: Nowadays infertility is an important worldwide issue. Frozen embryo transfer (FET) is currently used widely but the influential factors in the success of implantation are still a question. So, the aim of this study was the role of endometrial thickness and echo pattern on the day of embryo transfer in frozen embryo transfer (FET) cycle outcome. *Material and Methods:* This is a census cross-sectional study conducted on 140 patients in Isfahan fertility and infertility center. The patients were divided into two groups using random even/odds numbers. Groups were divided based on endometrial preparation for endogenous and exogenous

hormone cycles. Demographics including; age, duration of infertility, BMI, number of transfer cycles, endometrial thickness, endometrial echo pattern, method of endometrial preparation and cycle outcome were collected and recorded in a checklist and variables were analyzed via SPSS 20 version. *Results:* In this study, 140 females were studied. The mean age of the patients was 32.9 ± 5.1 years. Patients with successful implantation were statistically different from those with unsuccessful cycles in terms of age, infertility duration, number of previous unsuccessful cycles and endometrium thickness prior to HCG therapy and on the day of implantation (P-value < 0.05). Considering 9.15 millimeter of endometrial thickness prior to HCG therapy had 79.1% sensitivity and 78.9% specificity while cut-off of 9.45-millimeter endometrial thickness had 79.1% sensitivity and 80% specificity for successful implantation. *Conclusion*: Based on findings of current study, endometrium thickness of 9.45 millimeter can be accompanied with high rate of FET success with sensitivity of 79.1% and specificity of 80%. In addition, other factors such as patients' age, infertility duration, number of previous unsuccessful cycles and endometrium thickness prior to HCG therapy can affect results of FET.

Keywords: Endometrial thickness, frozen embryo transfer, Infertility.

1. INTRODUCTION

Nowadays infertility is an important worldwide issue. It is defined as inability to get pregnant in spite of having frequent unprotected sexual intercourse for minimal duration of a year and even six months in particular conditions (Vander Borght and Wyns, 2018). Fortunately, there are numerous safe and effective therapies for overcoming infertility such as hormone treatments and medications in men, stimulating ovulation with fertility drugs in women or assisted reproductive technology (ART) in men and women (Lunenfeld et al., 2019). Assisted reproductive technology (ART) is used to treat infertility. It includes fertility treatments that handle both a woman's egg and a man's sperm. It works by removing eggs from a woman's body. The most common ART technique is IVF. By improvement of this technique, in order of uncontrolled multiple gestations prevention, fewer number of embryos have been recommended to be transferred (Lunenfeld et al., 2019). Another less expensive ART method is embryo cryopreservation that has been improved to a superior method called frozen embryo transfer (FET). FET method has been proved to be accompanied with higher rates of pregnancy (Kolibianakis et al., 2009). Numerous variables have been mentioned for successful IVF. Achieving success is associated with two main factors including appropriate blastocyst supposed to be transferred and receptive endometrium supposed embryo to be grown in (Singh et al., 2011).

In this term different variables including age, duration of infertility, endometrium thickness, endometrium echo-pattern and primary/secondary infertility status were estimated as influential factors related to endometrium receptivity for successful FET. Previous studies have utilized various methods in order of finding receptivity of endometrium including histologic dating of endometrium using biopsy (Noyes et al., 1950) and evaluation of cytokines produced by endometrium (Ledee-Bataille et al., 2002). Another common non-invasive method for endometrial receptivity assessment is ultra-sonography, as appropriate blood supply can be considered as an important factor for successful fertilization (Singh et al., 2011). In order of frozen-thawed embryo replacement (FER), estrogen therapy has been used at initiation until reaching acceptable endometrial thickness, but unsolved problem in this term is lacking of appropriate threshold as target endometrial thickness (Check et al., 2004). Although some authors have recommended that embryo implantation in < 7 mm or > 14 mm endometrial thickness may be accompanied with high rate of failure, this cut-off is still under the debate (El-Toukhy et al., 2008).

The aim of the current study is to assess the effect of endometrial thickness and echo pattern on the day of transfer of frozen embryo transfer (FET) on pregnancy outcome and also to identify endometrial thickness cut-off on the day of embryo transfer for an acceptable chance of transfer success.

2. MATERIAL AND METHODS

This is a census cross-sectional study conducted on 140 patients in Isfahan fertility and infertility center. 20-39 years old infertile females who had optimal quality embryos confirmed by an embryologist were included and those with uterine abnormality, immune system disorders and body mass index > 30 were excluded. Consent forms for participating and all needed information about the study were given to the patients. This study was approved by Research Council and Ethics Committee of School of Medicine of Isfahan University of Medical Sciences.

The patients were divided to two groups using random even/odds numbers. Groups were divided based on endometrial preparation to endogenous and exogenous hormone cycles. Endometrial thickness in mid-sagittal plane on the day of embryo transfer using transvaginal ultrasound (mind-ray) and also from the outer edge of the endometrial-myometrial interface to the outer edge in the widest part of the endometrium was measured. Endometrial echo pattern was described as A (hypo echoic and triple

layer), B (heterogenous echogenic), and C (uniform echogenic). Demographics including; age, duration of infertility, BMI, number of transfer cycles, endometrial thickness, endometrial echo pattern, method of endometrial preparation and cycle outcome were collected and recorded in a checklist.

If the patients had missed period 16 – I8 days after embryo transfer, a quantitative beta HCG assay was performed. When the beta HCG was positive, it repeated 2 to 4 days after and later a suitable rise, an ultrasound was carried out 3 weeks after the positive pregnancy test. Pregnancies were classified as pathological, ongoing and normal. Number of successful embryos during a pregnancy (singleton, twins, etc.) was assessed by heartbeat identified on ultrasound. All pregnancy that resulted in damage (ectopic pregnancy, missed abortion, spontaneous abortion, etc.) was considered as an abnormal pregnancy. All sonograms were obtained from the same machine (G40™, Siemens Healthcare, Mountain View, CA, USA) with a 6-9 MHz transducer.

Then data were analyzed with IBM SPSS20 - United States software. Descriptive data were reported in mean ± standard deviation. For analytic data, independent T-test, paired T-test, Chi-square and ANOVA were used. P-value of less than 0.05 considered significant.

Ethical committee approval number

This article was approved by ethical committee of Isfahan University of medical science with ethic code IR.MUI.REC.395875.

3. RESULT

In this cross-sectional study, number of 140 females was studied. Based on the Table 1, mean age of the patients was 32.9 ± 5.1 years (range of 24-47 years). These patients had history of 6.4 ± 5 years infertility (range of 1-24 years). Mean body mass index (BMI) measured in our patients was 26.1 ± 2.5 (range of 19-29 years).

Table 1 Mean of age, duration of infertility and BMI

Max	Min	Standard deviation	Mean	Variable
47	24	5.1	32.9	Age (year)
24	1	5	6.4	duration of infertility (yaer)
29	19	2.5	26.1	BMI

Table 2 is showing mean number of previous unsuccessful cycles of each patient was 2.1 ± 0.9 (range: 1-4), endometrial thickness prior to Human chorionic gonadotropin (HCG) therapy was 9.1 ± 1.8 (range: 6-16) and endometrial thickness on the day of embryo transfer was 9.2 ± 1.8 (range: 7-15).

Table 2 Mean of cycles, endometrial thickness before HCG and transfer day

Max	Min	Standard deviation	Mean	Variable
4	1	0.9	2.1	Number of cycles
16	6	1.8	9.1	Endometrial thickness before hCG
15	7	1.8	9.2	Endometrial thickness per day transfer

Patients' distribution based on endometrial echo-pattern prior to HCG therapy, echo-pattern on the day of embryo transfer, endometrial preparation method and fertility status of patients have been shown in Table 3.

Table 3 Frequency distribution of echo-pattern before HCG, echo-pattern transmission, endometrium preparation method and fertility status in studied subjects

%	Number		Variable
59.3	83	A	Echo-pattern before hCG
35	49	B	
5.7	8	C	
60.7	85	A	Echo-pattern transmission
39.3	55	B	

62.1	87	Exogenous	Endometrium preparation method
37.9	53	Endogenous	
67.9	95	Lack of fertility	Fertility status
31.1	45	fertility	

Table 4 is presenting endometrial thickness comparison prior to HCG therapy and on the day of transfer based on fertility status.

Table 4 Mean endometrial thickness before HCG and day of transfer compared to normal fertility

P-value	Fertility		Lack of fertility		Variable
	Standard deviation	Mean	Standard deviation	Mean	
<0.001	1.9	10.4	1.4	8.5	Endometrial thickness Before hCG
<0.001	1.9	10.7	1.4	8.6	Endometrial thickness per day transfer

Based on Figure 1, the area under ROC curve was 0.819. Thus endometrial thickness prior to HCG therapy can be considered a useful index for fertility prediction (Figure 1). Using the ROC Curve, the endometrial thickness cut-off point (COP) before HCG was 15.9 for predicting infertility was estimated to be 9.15 with 79.1% sensitivity and 78.9% specificity (Table 5).

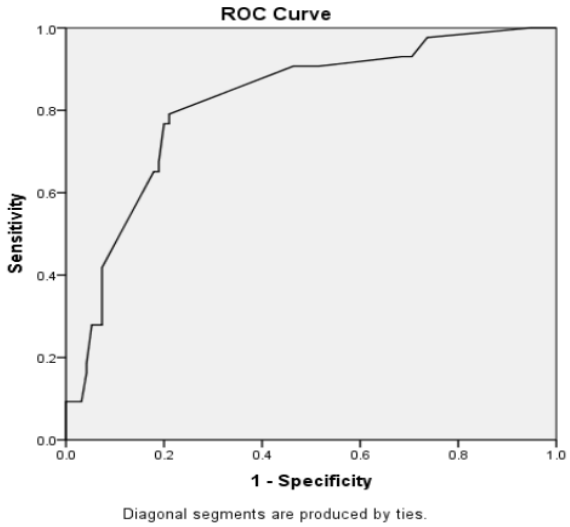

Figure 1 ROC curve for diagnostic evaluation of endometrial thickness before HCG for predicting fertility

Table 5 Indicators to evaluate the diagnostic value of endometrial thickness before HCG for predicting infertility

Amount	Index
0.819	area under the curve(AUC)
9.15	Cutt off point (COP)
79.1%	sensitivity (sen)
78.9 %	Specificity (sp)
63.4%	Positive predictive value(PPV)
89.3%	Negative predictive value (NPV)

Based on Figure 2, the area under ROC curve was 0.847. Thus endometrial thickness on the day of implantation can be considered a useful index for fertility prediction. Using the ROC Curve, endometrial thickness cut-off point (COP) at the day of transfer for predicting infertility was estimated to be 9.25% with 49.1% sensitivity and 80% specificity (Table 6).

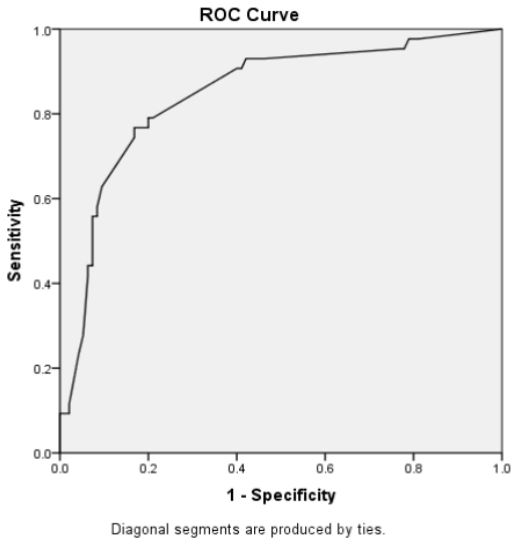

Figure 2 ROC curve for diagnostic evaluation of endometrial thickness on transfer day to predict fertility

Table 6 Indicators for the evaluation of the diagnostic value of endometrial thickness on the day of transfer to predict fertility

Amount	Index
0.847	area under the curve(AUC)
9.45	Cutt off point (COP)
79.1%	sensitivity (sen)
80 %	Specificity (sp)
64.2%	Positive predictive value(PPV)
89.4%	Negative predictive value (NPV)

Next assessment is comparison of endometrial echopattern prior to HCG therapy and on the day of implantation based on fertility state (Table 7). Fertility pattern did not have any association with endometrial preparation technique (P-value = 0.35).

Table 7 Frequency distribution of echo-pattern prior to HCG and on transfer day by fertility status

P-value	C		B		A		Fertility status	Time
	%	N	%	N	%	N		
0.03	100	8	76.6	36	61.4	51	Lack of fertility	Before HCG
	0	0	23.4	11	38.6	32	fertility	
<0.001	0	0	94.3	50	52.9	45	Lack of fertility	Transfer day
	0	0	5.7	3	47.1	40	fertility	

The next assessment was comparison of unsuccessful and successful implantation in patients in terms of evaluations performed. Number of 95 (67.9%) of patients had unsuccessful implantation, 45 (32.1%) patients underwent successful embryo implantation that 2 (1.4%) of implantations led to ectopic pregnancy. Independent t-test showed that the mean of age (P=0.04) duration of infertility (P<0.001), and number of cycles (P=0.01) in those who had fertility were significantly lower than those who did not. However, there was no significant difference in mean of BMI between the two groups (P=0.62) (Table 8).

Table 8 is demonstrating fertility status due to endometrial thickness prior to HCG therapy, age, infertility duration, number of unsuccessful cycles, endometrial echo-pattern prior to HCG therapy and BMI. Based on this table, mentioned variables can predict fertility success in 85.7% of cases both prior to HCG therapy and on the day of implantation (Table 9). Considering Wald score, endometrial thickness prior to HCG therapy and duration of infertility has the most ability of fertility success prediction (P-value < 0.001) (Table 9). About the day of implantation, endometrial thickness prior to HCG therapy (P-value < 0.001), duration of infertility (P-value = 0.001) and echo-pattern on the day of implantation (P-value = 0.002) have the most ability for fertility status prediction, respectively (Table 10).

Table 8 Mean age, duration of infertility, body mass index and number of cycles by fertility status

P-value	fertility		Lack of fertility		Variable
	Standard deviation	Mean	Standard deviation	Mean	
0.04	5.9	31.6	4.7	33.5	Age (year)
<0.001	1.9	3.2	5.4	7.7	duration of infertility (year)
0.62	2.9	26.3	2.3	26.03	BMI
0.01	0.9	1.8	0.8	2.2	Number of cycles

Table 9 Logistic Regression Analysis to predict fertility status according to endometrial thickness before hCG, Age, duration of infertility, number of Cycles, Echo-pattern before hCG and BMI

95% C.I for OR		OR	P-value	Amount of Wald	Beta	Variable
upper limit	Lower limit					
3.37	1.51	2.26	<0.001	15.98	0.81	Endometrial thickness Before hCG
1.13	0.94	1.03	0.50	0.45	0.03	Age
0.84	0.57	0.69	<0.001	13.30	0.36	duration of infertility
1.98	0.63	1.12	0.71	0.14	0.11	Number of cycles
1.27	0.21	0.51	0.15	2.10	0.67	Echopattern before hCG
1.11	0.76	0.92	0.37	0.79	0.09	BMI

Table 10 Logistic Regression Analysis to predict fertility status according to Endometrial thickness per day transfer, Age, duration of infertility, BMI, number of Cycles, Echo-pattern transmission

95% C.I for OR		OR	P-value	Amount of Wald	Beta	Variable
upper limit	Lower limit					
2.68	1.34	1.89	<0.001	13	0.64	Endometrial thickness per day transfer
1.15	0.94	1.04	0.42	0.64	0.04	Age
0.88	0.60	0.72	0.001	10.91	0.32	duration of infertility
1.15	0.78	0.95	0.58	0.30	0.05	BMI
1.83	0.55	1.009	0.98	0.001	0.009	Number of cycles
0.50	0.04	0.15	0.002	9.23	1.92	Echo-pattern transmission

4. DISCUSSION

In present census study, efforts have been made to provide a broader view of endometrial factors that may affect success rate of frozen embryo transfer. According to findings of current study, patients who experienced successful embryo implantation were significantly different in terms of age, duration of infertility, number of previous unsuccessful cycles, endometrial thickness prior to HCG therapy and endometrial thickness on the day of embryo transfer. In our study, we have found that age, duration of infertility, BMI, endometrial echo-pattern and endometrial thickness whether prior to HCG therapy or on the day of implantation can be useful

in prediction of FET success. These findings are similar to some studies while in contrast with others. These results are controversial to what was reported by other studies. Also these results may be attributed to low number of successful cycles in comparison to unsuccessful ones.

In one study performed in 2014, the only influential factors were age and FSH level (Eftekhar et al., 2014). Another study presented similar results about the age as they mentioned older ages above 36 years were significantly accompanied with less rate of success (Check et al., 2007) but they presented opposite results about FSH level as they found lower rate of successful embryo transfer in higher levels of FSH (Eftekhar et al., 2014). The other study reported that females' age did not affect FET outcomes statistically (Ashrafi et al., 2011). One of the limitations of our study is that we did not evaluate FSH level. Another statistical significant difference was endometrial thickness that in group with successful implantation was thicker. This result is in accordance with findings of several other reports about this issue that presented endometrial thickness as identifying factor for supplementation of progesterone and therefore FET success (El-Toukhy et al., 2008 and Eftekhar et al., 2014 and Check et al., 2007 and Ashrafi et al., 20118). In these studies endometrial thickness of 9mm on HCG therapy or on the day of embryo transfer has been reported as appropriate thickness for implantation success prediction (Ashrafi et al., 20118) while there are studies that found no difference in the success rate of FET in females with endometrial thickness of 9 mm and above in comparison to those with thickness of less than 8 mm (Zhang et al., 2005 and Noyes et al., 1995). It is obvious that endometrium receptivity plays an important role in success of in vitro fertilization (IVF)/frozen embryo transfer (FET). In fact, blood flow of endometrium is steeply in association with implantation process.

Most of the patients with successful implantation had hypo echoic and triple layer endometrial echo pattern in our presentation. This type of echo pattern is consistent with previous studies that presented triple-line endometrium as the echo pattern with more successful rate of FET in comparison to heterogenous echogenic pattern in absence of central line or homogenous hyperechoic pattern (Check et al., 1993). Another study revealed similar results that triple layer endometrial pattern at the time of HCG injection and also on the day of embryo transfer may be associated with higher rate of successful implantation which can be followed by successful gestation (Zhao et al., 2014 and Gingold et al., 2015). One of the questions answered in this study was cut-off of endometrium thickness for embryo transfer. We found that thickness of 9.45 millimeters had sensitivity of 79.1% and specificity of 80% for fertility success. It seems that this thickness can be associated with high rate of implantation success.

Although various experts have assessed thickness of endometrium in order of finding proper thickness for implantation performance, there is no unanimous measurement accepted by specialists. In spite of studies that reported failure of pregnancy occurrence in endometrial thickness of less than 7 millimeter (El-Toukhy et al., 2008), one study has presented 6 millimeter of thickness (Coulam et al., 1994) and the other even reported 4 millimeter (Sundström, 1998) as endometrial thickness that was accompanied with successful results. In addition, Weissman et al. have mentioned that endometrial thickness above 14 millimeter has been associated with higher rate of miscarriage (Weissman et al., 1999). Singh et al. have presented that the least thickness with successful gestation was 5.8 but the most rate was occurred in endometrial thickness of 8-10 millimeter (Singh et al., 2011). This was similar to findings of our study. Also other studies had wider range of thickness of 9 to 14 millimeters (Tang et al., 2006). In the current study we found that cut-off thickness of 9.15 millimeter of endometrium prior to hCG therapy is accompanied with 79.1% of sensitivity and 78.9% of specificity for starting hormone therapy. Rinaldi et al. presented that outcomes of IVF were considerably better as the endometrial thickness was above 10 millimeter at the time of hCG injection (Rinaldi et al., 1996). Zhang et al. presented 9-14 millimeter of thickness for more success in comparison to less than 9 millimeter for hCG injection (Zhang et al., 2005).

5. CONCLUSION

In conclusion, our study revealed that endometrium thickness of 9.45 millimeter can be accompanied with high rate of FET success with sensitivity of 79.1% and specificity of 80%. In addition, the study showed that other factors such as patients' age, infertility duration, number of previous unsuccessful cycles prior to HCG therapy can affect results of FET.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

List of abbreviations

FET: Frozen embryo transfer
BMI: Body mass index
HCG: Human chorionic gonadotropin

ART: Assisted reproductive technology
IVF: In vitro fertilization
FER: Frozen–thawed embryo replacement
COP: Cut-off point
FSH: Follicle-stimulating hormone

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