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Does Endometrial Compaction Predict Clinical Pregnancy Rate after Cleavage Stage Frozen Embryo Transfer?

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Abstract

Objectivess: This study aimed to determine the relationships between endometrial compaction and pregnancy outcome in patients receiving artificial endometrial preparation for frozen embryo transfer (FET) cycles.

Materials and Methods: This prospective cohort study was performed in a university-affiliated fertility clinic from March 2020 to March 2021. The eligible women undergoing their first or second FET cycle and having the top grading cleavage stage embryos were enrolled. All patients received the same endometrial preparation regime. The alteration in endometrial thickness (EMT) between the day of progesterone initiation and the day of embryo transfer (ET) was measured using consecutive transvaginal sonography. The patients were divided into three groups based on the percentage of endometrial compaction (i.e., the difference of EMT at end of the estrogen-only phase and after three days of progesterone administration (ET day) divided by the EMT on the terminal day of the estrogen-only exposure).

Results: Overall, 300 eligible women were evaluated and only 27.3% (82/300) of the studied cycles showed ≥5% compaction, whereas 72.6% (218/300) either expanded or showed minimal compaction. The clinical and ongoing pregnancy rates in group 2 (any expansion) were significantly higher than those in groups 1 and 3 ($P=0.002$ and $P=0.01$, respectively). Multivariable logistic regression test indicated that the cycles with any expansion in ET were independently associated with 3.1 times improvement in clinical pregnancy rate in comparison to those with any compaction ($P=0.002$).

Conclusion: Gross endometrial compaction occurred in one-third of FET cycles with no significant positive effect on pregnancy outcomes after cleavage-stage ET.

Keywords: Endometrium/diagnostic imaging, Frozen-warmed embryo transfer, Pregnancy outcome

Introduction

To date, most fertility clinics have preferred the "freeze-all" strategy resulting from new progressions in laboratory methods for cryopreservation and thawing of embryos as well as prohibition of ovarian hyper-stimulation syndrome risk (1). The embryo morphology and the type of endometrial preparation are two main parameters to achieve success in the frozen/thawed embryo transfer (FET) cycles (1). The specific changes in the surface epithelium, infrastructure vascular network, as well as expression of glycoproteins, receptors, integrins, and chemokines are necessary to develop a receptive environment for implantation of embryo, which are existed for a short interval of time (approximately 5 days) and named as "the window of implantation" (2,3). Different modalities and assays have been investigated to evaluate and optimize endometrial receptivity, including transvaginal ultrasound (TVU), the endometrial receptivity array, and traditional histopathologic tests (3). Among these technological tools, TVU has received more attention due to its easier access, low cost, and non-invasiveness. It has been confirmed that imaging modalities present details about endometrial receptivity up to a specified time, as pregnancies occur even in thin endometrium <5 mm and in hyperechogenic endometria; therefore, molecular techniques can greatly assist us in discovering the receptivity stage of the endometrium (4). These recent molecular techniques still require to be accredited in prospective studies, and modern generations of the early assays that are used presently may be developed and applied across centers (4). However, clinicians still judge the endometrium from the image obtained from TVU.

Several studies have examined the effect of the endometrial thickness (EMT) and pattern prior to or on final oocyte triggering day as well as at the time of embryo transfer (ET) on pregnancy outcome, and some of them have reported a positive correlation while some others failed to find any relationship. Liu et al (5) analyzed over 40 000 ET cycles and reported a lower limit of EMT <8 mm in fresh and <7 mm in FET cycles that induced lower clinical pregnancy rates. On the other hand, El-Toukhy et al found that an upper limit >13 mm was associated with a lower pregnancy rate, and suggested that there was

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Key Messages

- Progesterone administration makes endometrial compaction in 1/3d of FET cycles with no significant positive effect on pregnancy outcomes after cleavage-stage ET.

an optimal range for EMT to achieve a better pregnancy rate (6). In a meta-analysis study, Gao et al assessed nine prospective and 21 retrospectives published researches including a total of 88 056 cycles, and concluded that the lower EMT in comparison to the higher EMT was associated with lower pregnancy rates in the studied women (7). There may be a relationship between EMT and implantation rate, although this process is too complex and a single ultrasound measurement may not confirm the existence of this relationship (8). For the first time, Haas et al suggested that the alteration in the EMT between the terminal day of the estrogen phase and the time of ET, named as "endometrial compaction", may have been a greater predictive value for pregnancy outcome than the simple measure of EMT at the time of ET (9). Afterwards four cohort studies examined the association between endometrial compaction and pregnancy outcome in FET cycles and reported conflicting findings. In two studies by the same group, a positive relationship was detected (9,10); in two other studies, however, endometrial compaction did not improve pregnancy and live birth rates (3,11). Reviewing the existing studies suggests that further studies are required to clarify and draw a better conclusion in this area. This prospective study, therefore, was conducted to investigate any possible relationships between endometrial compaction and pregnancy outcome in patients receiving artificial endometrial preparation for FET cycles.

Materials and Methods

This prospective observational cohort study was performed to evaluate infertile women who underwent FET in a university hospital (Imam Khomeini hospital) affiliated with Tehran University of Medical Sciences between March 2020 and March 2021. On the first day of endometrial preparation, eligible patients were screened and, after checking all the inclusion and exclusion criteria, informed consent was taken from all participants. The women aged under 40 years, with a body mass index (BMI) of <30 kg/m^2, and undergoing their first FET cycle with two top-quality cleavage stage embryos were included in the study. The patients with recurrent miscarriages, more than two implantation failures, donated oocyte or embryo, severe male factor and uterine diseases or presence of hydrosalpinx, and poor progression of thickness during the serial ultrasonic evaluation of endometrium were excluded from the study.

The endometrial preparation was carried out for all the patients with hormone replacement therapy (HRT) including administration of oral micronized estradiol on days 2-3 of the cycle after pituitary down-regulation with oral contraceptive pills accompanying GnRH agonist. The ultrasound scan and serum estradiol (E_2) assessment were conducted at of the beginning of the menstrual cycle to confirm adequate pituitary suppression. The administration of estradiol valerate (6 mg) was performed on a daily basis if the EMT <5 mm and serum estradiol level <50 pg/mL were observed. After 12-14 days of performing estradiol exposure, if triple-layer endometrium ≥7 mm was confirmed by ultrasound, estradiol valerate was proceeded with a similar dose and then vaginal progesterone suppository (Cyclogest® 400 mg, Actoverco, Iran) was performed twice a day on the subsequent morning. If not, the dosage of estradiol was enhanced to 8 mg/day until the adequate EMT was achieved. The administration of estradiol valerate and progesterone was continued until the day of the pregnancy test and continued until the 10th week of gestation in the cases with the positive pregnancy tests.

The results of EMT measurements by TVU at the day of progesterone initiation (P+0) and the third day after progesterone administration or ET day (P+3) were recorded. All the ultrasound assessments were carried out using a Philips Affiniti 70 ultrasound machine with a C10-3v Pure-Wave endo-vaginal probe by an experienced infertility fellowship. The patients were classified in three groups based on the endometrial compaction percentage (i.e., the difference of EMT at end of the estrogen-only phase and after three days of progesterone administration (ET day) divided by the EMT on the terminal day of the estrogen-only exposure). Group 1 was defined as a decrease in EMT of ≥5% (endometrial compaction), and group 2 was described as an increase in EMT. Otherwise, if the percentage of compaction was less than 5%, the cycles were assigned to group 3.

Embryo Morphology Assessment and Transfer

The cleavage-stage embryos' assessment was performed based on the criteria reported by the ESHRE consensus workshop on embryo assessment (12). The vitrification of transferable embryos with grades 1–3 was conducted three days after the insemination using a standard procedure which was described in detail by Fahy and Wowk (13). The patients with top grading (A and AB) embryos for transfer were included in the present study. All ETs were done using the same type of transfer catheter (Cook catheter, Queensland, Australia). Laser-assisted hatching was performed on the transferred embryos immediately before the ET. Based on the women's age, up to two frozen embryos with top-quality were thawed and transferred at the cleavage stage on the 4th day of progesterone administration.

Outcome Measures

The primary outcome was the association between

endometrial compaction and clinical pregnancy rate (the observation of a gestational sac with heartbeat on vaginal ultrasound). The secondary outcomes included the ongoing pregnancy rate (continuing pregnancy beyond week 20) and miscarriage rates (the spontaneous abortion of a clinical pregnancy prior to pregnancy week 20).

Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 21.0. The categorical variables of the groups were compared using chi-square test. The Kolmogorov–Smirnov test showed that the distribution of all continuous variables in this study was non-normal; therefore, the Kruskal-Wallis test was used instead of the one-way analysis of variance (ANOVA) test for comparing the groups. The multivariable logistic regression was performed to detect the independent significant predictive factors responsible for clinical pregnancy in the study population. The significance level was set at $P<0.05$.

Results

Overall, 300 eligible women with HRT-FET cycles were evaluated. According to the results from comparing EMT on days P+0 and P+3, only 27.3% (82/300) of the studied cycles showed ≥5% (gross) compaction, whereas 72.6% (218/300) cycles showed either lower than 5% compaction or even expansion in EMT. Of the cycles with no gross compaction, 32.5% (71/218) had minimal compaction or showed no change, and the rate for 67.5% (147/218) expanded, Clinical pregnancy was 24.6% (74/300), with a miscarriage rate of 14.8% (11/74) and ongoing pregnancy rate of 85.2% (63/74) in total evaluated cycles.

Table 1 summarizes the results from comparing study groups regarding the baseline and cycle characteristics. There were no significant differences among the groups in terms of women's age and BMI, type and the etiology of infertility, duration of infertility, and quality of transferred embryos. The mean of anti-Müllerian hormone level in minimal compaction no-change group (group 3) was significantly lower than those of other two groups (P=0.001). No significant difference was found among groups regarding the mean of EMT on the terminal day of the E_2-only exposure.

Table 2 indicates the pregnancy outcomes in the study groups. The chi-square test demonstrated that the clinical and ongoing pregnancy rates in any expansion group were significantly higher than those of groups 1 and 3 (P=0.002 and $P=0.01$).

All possible confounders were inserted in the multivariable logistic regression model in order to determine any significant factors related to the clinical pregnancy rate independently. The analysis revealed that while transferring cleavage state frozen embryos in artificially prepared endometrium, the cycles with any expansion in EMT was independently associated with 3.1 times improvement in clinical pregnancy rate in comparison to those with any compaction (odds ratio: 3.1; CI: 1.50-6.55, $P=0.002$) (Table 3).

Discussion

This study indicated that the endometrial compaction evaluated by sequential trans-vaginal sonography occurred approximately in a quarter of the FET cycles,

Table 1. Comparing the Patients' Basic Characteristics in Three Groups

	Gross Compaction (n=82)	Any Expansion (n=147)	Minor Compaction or No Change (n= 71)	*P* Value
Age (y) (Mean±SD)	36.7±5.71	35.91±7.00	35.40 ± 6.33	0.13[a]
Body mass index (Mean ± SD)	22.04±2.75	22.23±2.28	21.88±2.21	0.58[a]
Anti-Müllerian hormone (Mean ± SD)	3.51±2.71	3.12±3.77	2.62±3.34	**0.001**[a]
Infertility duration (y) (Mean ± SD)	6.05±3.81	5.13±3.79	4.50±3.29	0.07[a]
Type of infertility, No. (%)				
Primary	48 (58)	103 (70)	41 (58)	0.10[b]
Secondary	34 (42)	44 (30)	30 (42)	
Cause of infertility, No. (%)				
Male factor	7 (8.5)	25 (17)	7 (10)	
Female factor	45 (55)	73 (50)	27 (38)	0.07[b]
Both factors	12 (14.5)	22 (18)	18 (25)	
Unexplained factor	18 (22)	22 (15)	19 (27)	
Endometrial thickness on the end of E_2 only period (mm) (Mean ± SD)	8.77±1.34	8.76±1.55	9.01±1.50	0.70[a]
Grade of transferred embryos, No. (%)				
A	74 (56)	209 (66)	68 (59)	0.50[b]
AB	58 (44)	106 (34)	46 (41)	

[a] This analysis performed by Kruskal-Wallis test. [b] This comparison performed by chi-square test.

Table 2. Comparing Pregnancy Outcomes Between Groups According to Endometrial Thickness Changes

	Gross Compaction (n=82)	Any Expansion (n=147)	Minor Compaction or No Change (n= 71)	*P* Value
Clinical pregnancy rate	11 (13.5)	49 (33.3)	14 (20)	0.002
Miscarriage rate	2 (2.4)	7 (4.7)	2 (2.8)	0.90
Ongoing pregnancy rate	9 (11)	42 (28.5)	12 (17)	0.01

Note: This analysis was performed by chi-square test.

Table 3. Determining the Prognostic Factors for Clinical Pregnancy Rate by Multivariable Logistic Regression Analysis

	B	OR	(95% CI)	*P* Value
Woman's age	-0.04	0.96	(0.91- 1.007)	0.09
Women's BMI	0.03	1.03	(0.92-1.16)	0.54
Serum level of anti-Müllerian hormone	0.08	1.08	(0.99- 1.17)	0.056
Endometrial thickness groups				
Gross compaction	-	Reference group	-	-
Any expansion	1.14	3.1	(1.50-6.55)	0.002
Minor compaction or no change	0.43	1.5	(0.63-3.74)	0.34

BMI: body mass index; OR: Odds ratio; CI: confidence interval.

and that it was not associated with any improvement in the clinical pregnancy rate.

The endometrial proliferation ends approximately three days after ovulation physiologically resulting from the peak level of serum progesterone; and EMT is supposed to remain unchanged at this time period as the glands and blood vessels are well-developed, which is associated with elevated density rather than endometrial height (3,14). Therefore, it is more physiologically probable that endometrial compaction may be a valid indicator of endometrial receptivity (3). Although it has been suggested that ongoing endometrial growth during the secretory phase may be due to progesterone resistance and a sign of the insufficient environment for implantation of the embryo, there is still limited scientific evidence on this area and further studies are necessary to explore it (3).

To the best of our knowledge, four previous studies assessed the effect of endometrial compaction on ongoing pregnancy (9-11) and live birth rates (3) in FET cycles. At first, Haas et al reported that the cycles with endometrial compaction were associated with a higher ongoing pregnancy rate, and that the rate of ongoing pregnancy was elevated significantly with an increase in the percentage of compaction (9). In their cohort (9), the rate of decreased thickness of endometrium (endometrial compaction) was 42.4%, which was significantly higher than that reported in the study by Riestenberg et al (16.6%) (3) and even in our study (27.3%). In the second study by Zilberberg et al, similar results were obtained (10). The rate of endometrial compaction ≥5% was 43.1%, and a higher ongoing pregnancy rate was found after single euploid FET cycles with artificial endometrial preparation and any endometrial compaction at the time of ET (10). Two subsequent studies, in contrast, showed controversial findings (3,11). Bu et al in a study with a large sample size (i.e., 1757 medicated and 1334 natural blastocyst FET cycle) demonstrated that clinical pregnancy rate was higher in the cycles with the endometrial lining expanded after progesterone initiation in both types of endometrial preparation (11). Our findings were in agreement with the results from Bu et al study in this regard. In this cohort, the rates of endometrial compaction were 26.2% and 19.6% in the natural and medicated cycles, respectively (11). Later, Riestenberg et al evaluated 259 single euploid medicated FET cycles by conducting a prospective study and found that the majority of cycles (83.4%) showed no decreased thickness of endometrium, and it was not predictive for live birth or spontaneous abortion rates (3).

The contradictory results in these studies may have been attributed to differences in the protocols of endometrial preparation, methods, and time scheduling of two ultrasound evaluations (3). In the first two studies by the same group, the ultrasound at the end of the E_2 phase was a trans-vaginal ultrasound, whereas the second ultrasound at the time of ET was a transabdominal one. In present study and two other studies, all ultrasound studies were done transvaginally and in majority of cycles endometrial compaction was not seen. In all previous studies (9-11) as well as in our study, the second phase of EMT measurement was performed on the day of ET, whereas it was performed one day prior to ET in the study by Riestenberg et al (3). It is worth noting that in all previous studies, either the blastocyst embryos (9,11) or cycles with a single euploid embryo was considered for ET (3,10). In the present study, the highest quality cleavage embryos were selected for ET; however, this was a limitation because, in our medical center, blastocyst embryo and preimplantation genetic testing for aneuploidy were considered only for cases with repeated implantation failure diagnosis. The strengthen of the present study lies in the fact that a multivariate

regression analysis was carried out in this study in order to investigate the confounding factors affecting clinical pregnancy rates.

The available evidence in this area is still limited and, therefore, it cannot be decided whether to cancel or change the program based on the occurrence of endometrial compression on the day of ET. Since performing an ultrasound assessment on the day before or in the morning of ET day is a safe and low-cost procedure, it was suggested that further prospective studies should be carried out to assess the predictive value of endometrial compaction for FET outcomes in both programmed and natural cycles.

Conclusions

It was concluded endometrial compaction had no significant positive effect on clinical or ongoing pregnancy rates in cleavage-stage frozen-thawed ET cycles with artificial endometrial preparation.

Authors' Contribution

Conceptualization: Mina Jafarabadi.
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Validation: Mina Jafarabadi.
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Data Curation: Mina Jafarabadi.
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Writing—review and editing: Mina Jafarabadi.
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Supervision: Mina Jafarabadi.
Project administration: Fedyeh Haghollahi.
Funding acquisition: Maedeh Ahmadi Dastjerdi.

Conflict of Interests

Authors have no conflict of interest.

Ethical Issues

The study was approved by the Institutional Review Board and Ethics Committee of the Tehran University of Medical Sciences (ethics approval code: IR.TUMS.IKHC.REC.1399.273).

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