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Original Article

The Effect of Uterine Contractions on Fertility Outcomes in Frozen Embryo Transfer Cycles: A Cohort Study

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Abstract

Background: The relationship between uterine peristalsis before embryo transfer and the success of assisted reproductive techniques (ARTs) has not been properly investigated. In this study, the effect of uterine contractions on embryo implantation in frozen embryo transfer (FET) cycles was investigated to determine whether the frequency of uterine contractions can be used as a quantitative marker to assess endometrial receptivity.

Methods: In this cohort study of 68 eligible FET candidates, one hour before embryo transfer (ET), frequency of uterine contractions was assessed with transvaginal ultrasonography. Patients were followed up for 20 weeks. The association between FET outcomes including clinical pregnancy, abortion, and ectopic pregnancy with uterine contractions was evaluated. Binary logistic regression was conducted to test the association between clinical pregnancy outcomes in different groups. The p<0.05 were considered statistically significant.

Results: Of 68 patients, 25 (36.8%) experienced clinical pregnancy. Multiple logistic regression for omitted confounders (age, BMI, duration, type and cause of infertility) revealed that patients with uterine peristaltic wave frequency less than 2≤ *wave/min* had higher chance of successful pregnancy compared to those with ≥4 *wave/min* (odds ratio: 10.8; 95% confidence interval: 1.5-79.4, p=0.019). The Pearson's correlation showed a statistically significant relationship between the frequency of uterine contraction and endometrial thickness (r= 0.42, p=0.002).

Conclusion: Patients with uterine peristalsis of <4.0 *wave/min* before embryo transfer had a higher chance of successful implantation and pregnancy compared with those with higher contraction frequencies. It seems that measuring uterine contraction frequency before embryo transfer might help to predict pregnancy outcomes.

Keywords: Assisted reproductive technology, Clinical pregnancy, Uterine peristalsis.
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Introduction

Despite widespread advances in assisted reproductive techniques (ARTs), infertility still affects a large number of patients and is distributed in approximately 10-15% of couples (1). Various methods have been used to overcome to the fertility problems of young infertile couples. In vitro fertilization (IVF) is one of the most commonly used modalities (2). Success rate of IVF depends on multiple factors especially the age of the woman, as well as the cause of infertility such as male factors, immunological factors, anatomical factors, etc. (3) which sometimes lead to planning multiple IVF cycles (4, 5). Recurrent implantation failure (RIF) is a serious challenge in infertility management (6, 7).

IVF is performed by both fresh (ET) and frozen embryo transfer (FET) (8). Studies have shown that the probability of fertility success following

FET is higher than that of fresh ET (9). However, a large number of FET-transferred embryos are not implanted in the first few days (10). Identifying the factors that play critical role in FET cycles failure can increase the likelihood of success and reducing the risk of miscarriage and thus preventing repeated IVF.

The peristaltic contractions of the uterus is defined as wave-like movements in the sub-endometrial layer that can be detected by ultrasound scanning (11). These contractions vary during the menstrual cycle (12). So that at the beginning of the follicular phase, the frequency of these contractions is one to two per minute with small amplitudes around 30 *mmHg*. Thereafter, the frequency increases to about 3 to 4 *per/min* towards the time of ovulation. During the luteal phase, both the frequency and amplitude of contractions are decreased to facilitate the implantation of embryo. In ART cycles in which the ovary is stimulated using exogenous hormones compared with normal cycles, the uterine activity appears slightly more in the early luteal phase (4-6 contractions *per/min* versus 2-4 *per/min*) (12); thus, a calm uterus is necessary in IVF cycles for embryo implantation (13).

Recent studies have shown that there is an inverse association between uterine contractions and IVF success rate. Therefore, the more uterine contractions, the more embryo expulsion from the uterine cavity (14, 15). However, the number of studies in this area is small and there is still no comprehensive agreement regarding the role of uterine contractions in IVF success. In this study, the role of uterine contractions was investigated in pregnancy outcome in FET cycles to determine whether the frequency of uterine contractions can be used as a quantitative marker to assess endometrial receptivity.

Methods

In this cohort study, 68 eligible FET cycles candidates who were referred to Akbar-Abadi hospital from April 2019 to April 2020 were studied. Patients were those who had previously undergone ovulation stimulation cycles due to various infertility causes and now were candidates for frozen-thawed embryo transfer. Inclusion criteria were women aged 25 to 40 years. To limit the interference of confounding variables affecting embryo quality and uterine receptivity, patients were only selected if they had a normal uterus, had at least two good-quality (A or B) day 3-5 embryos with at least 7 cells, with no apparent morphological abnormalities, and fragmentation ≤20%. Women who had received donor eggs and those with recurrent implantation failure (RIF) did not participate in the study. Moreover, women with major systemic or immunologic diseases, intrauterine lesions (polyps and intracavitary myoma), and adenomyosis were excluded. Patients underwent a planned artificial cycle. In "artificial cycle" of FET, the patient is treated with estrogen and progesterone to mimic the natural cycle. All the participants in the study had undergone the FET protocol of the Akbar-Abadi infertility center. In this protocol, they received increasing doses of oral estrogen (estradiol valerate 2 *mg*, Aburaihan Pharmaceutical Co, Iran), two to three times daily, started from day one of the cycle in which the endometrium is too thin and uterine lining should be improved. Endometrial thickness was monitored by transvaginal ultrasound before adjusting the doses of estradiol valerate (added up to 8-10 *mg* daily). If the endometrial thickness is ≥7 *mm*, micronized progesterone (Vaginal suppository, Cyclogest 400 *mg*, Actavis, Iran) once per day, is administered to the regime. About 4-5 days later, thawing and transferring the embryo is commenced, according to the stage of cryopreservation. Luteal support is continued until 1 month after confirmation of intrauterine pregnancy or until pregnancy is ruled out by negative serum HCG pregnancy test.

One *hr* before embryo transfer, endometrial thickness was measured by an expert radiologist using a SuperSonic Mach 20 vaginal probe (Hologic, US). The probe was then gently inserted into the vagina by the same radiologist to prevent cervical irritation. After scanning the mid-sagittal plane of the uterus, the probe was introduced into the vagina as gently as possible to avoid stimulating the cervix. The probe was fixed as steady as possible while a 5-*min* video of uterine peristalsis was recorded simultaneously. The records were analyzed at 4× regular speed using a VLC media player (VideoLAN, France) by two blind experienced radiologists. The uterine peristaltic wave frequency was the mean of the results reported by each observer. The next step was the embryo transfer, under the guidance of abdominal ultrasound to locate the catheter correctly and prevent unwanted trauma to the endometrium. All the embryo transfers were performed by an expert who was blind to the study design. Patients were followed up to 20 weeks of ongoing pregnancy, whether they ended in normal pregnancy or abortion. Clinical pregnancy was defined as ultrasonographic visualization of gestational sac, 2-3 weeks after the positive chemical pregnancy test and around 7 weeks after the embryo transfer, and miscarriage was defined as an abortion between 7-20 weeks. The mean frequency of uterine contractions, which was documented by an expert radiologist, was categorized into three subgroups: ≤2, 2.1-4, and ≥4 *wave/min*. The leading purpose of the study was measuring the uterine contraction frequency before the frozen embryo transfer and analyzing its association with the embryo transfer outcomes including clinical pregnancy as the primary outcome and abortion as the secondary outcome. In addition, the association of other factors such as age, body mass index (BMI), duration of infertility, and previous pregnancy history was assessed based on pregnancy outcomes. It should be noted that luteal phase support was continued for one month after confirmation of intrauterine pregnancy or until pregnancy was ruled out by a negative serum HCG measurement result.

Statistical analysis and sample size calculation: SPSS *vs*. 16 (IBM, US) was used for data analysis. Mean and standard deviation were used to express quantitative descriptive data and frequency and percentage for qualitative findings. Independent t-test and chi-square were used to analyze normal data and Mann-Whitney U test for abnormally distributed ones. Multiple logistic regression with backward approach was used to test the relations between dependent parameters (pregnancy and abortion) and independent parameters (frequency and confounders), and also Pearson test was used to evaluate the correlations. A p-value below 0.05 was considered statistically significant. The sample size was calculated using the findings of previous studies and G–Power software with a power of 90% (19).

The participants' information was used without disclosing their identities. Also, a written informed consent was obtained from all patients. They also had the right to withdraw from the study whenever they wished. This study was approved by the ethics committee of Iran University of Medical Sciences, Tehran, Iran (Code IR.IUMS.FMD.REC.1398.266).

Results

The mean age of study population was 34.50±5.60 years (25 to 40 years). The mean body mass index was 26.63±3.83 kg/m^2. In addition, the mean duration of infertility was 6.17 years (1 to 15 years). A total of 41 (60.3%) patients had primary infertility and 27 (39.7%) had secondary infertility. Of 68 patients, 33(48.5%) had undergone ART before. Among participants, 27 (39%) had a history of a prior successful pregnancy, 17 (25%) had prior abortions, and only one (3.7%) had experienced ectopic pregnancy before. The mean numbers of embryos transferred, in pregnant and non-pregnant subjects, were 2.3±0.7 and 2.2± 0.9, respectively (p=0.727).

Of 68 participants, 25 (36.8%) had successful embryo transfer and became clinically pregnant. Ectopic pregnancy was not observed in any of these women. Seven (28.0%) women with clinical pregnancy experienced abortion in the 20th week of follow-up. In most abortions (71.4%), higher uterine frequencies (more than 3 *wave/min*) were reported. The range of uterine frequency was reported between one to eight *wave/min*. There was also no significant association between demographic factors and pregnancy outcomes in the study participants (Table 1).

All patients were subjectively divided into three groups according to uterine peristaltic wave frequency on embryo transfer day: ≤2.0, 2.1–4, and ≥4.0 *waves/min*. Frequency in most patients (60%) who became pregnant was equal to or below 2 *wave/min*, and only 2 (8.0%) had higher frequencies. In this study, those who had frequencies higher than 4 *waves/min* demonstrated the lowest pregnancy rate. The mean of uterine peristaltic wave frequency in the pregnant group was lower in comparison to non-pregnant group (2.2±1.6 *vs*. 3.3±1.2 *waves/min*, p=0.002).

Binary logistic regression revealed that lower uterine peristaltic wave frequency on the day of embryo transfer was significantly related to the chance of clinical pregnancy. Binary logistic regression demonstrated that patients with uterine peristaltic wave frequency less than 2≤ *wave/min* (odds ratio: 5.8; 95% confidence interval: 1.1-31.3, p=0.042) and those with 2 to 4 *wave/min* (odds ratio: 2.0; 95% confidence interval: 0.4-11.2, p=0.431) had higher chance of successful pregnancy compared to those with 4≥ *wave/min* (Table 2). Multiple logistic regression for omitted confounders (age, BMI, cause and type of infertality) revealed that patients with uterine peristaltic wave frequency less than 2≤ *wave/min* (odds ratio: 10.8; 95% confidence interval: 1.5-79.4, p=0.019) had higher chance of successful pregnancy compared to those with 4≥ *wave/min*. The endo-

Table 1. Comparison of demographic characteristics based on pregnancy outcomes

Variables		Clinical outcome of FET cycles		
		Negative n=43	Positive n=25	p-value
Age (yr.), mean±SD		33.9±6.2	35.3±4.6	0.337 *
BMI (*kg/m²*), mean±SD		26.8±4.1	26.4±3.5	0.653 *
Number of embryo transfers, mean±SD		2.2±0.9	2.3±0.6	0.727 *
Endometrial thickness (*mm*), mean±SD		8.1±1.9	9.0±2.6	0.631 *
Infertility duration (yr.), mean±SD		6.5±4.3	5.7±3.6	0.531 **
Infertility type, n (%)	Primary	26 (60.5)	15 (60.0)	0.970 ≠
	Secondary	17 (39.5)	10 (40.0)	
Previous ART, n (%)	Yes	20 (46.5)	13 (52.0)	0.662 ≠
	No	23 (53.5)	12 (48.0)	
Previous pregnancy, n (%)	Yes	18 (41.9)	9 (36.0)	0.634 ≠
	No	25 (58.1)	16 (64.0)	

Note: BMI: Body Mass Index, ART: Assisted Reproductive Techniques, SD: Standard Deviation
* Independent t-test, ** Mann-Whitney test, # Chi-square test

Table 2. Pregnancy outcomes in different uterine peristaltic wave frequency groups

Uterine peristaltic wave frequency (*wave/min*)		Clinical outcome of FET cycles			p-value
		Negative n=43	Positive n=25	OR (95%)	
Wave frequency (*wave/min*), n (%)	≥4, n=12	10 (23.3)	2 (8.0)	1	-
	2.1-4, n=28	20 (46.5)	8 (32.0)	2.0 (0.4-11.2)	0.431
	≤2, n=28	13 (30.2)	15 (60.0)	5.8 (1.1-31.3)	0.042

Dependent variable: pregnancy=1, OR: Odds Ratio, CI: Confidence Interval

metrial thickness ranged from 7 *mm* to 15 *mm*, and the mean endometrial thickness was 9.02±2.2 *mm*. There was no significant difference in the mean of endometrial thickness between the groups of pregnant and non-pregnant ones (9.03±2.58 *mm* *vs*. 8.05±1.90 *mm*, p=0.631) as well as who experienced abortion (p=0.38). Furthermore, the Pearson's correlation showed a significant correlation between uterine contraction frequency and endometrial thickness (r=0.420, p=0.002).

Discussion

Improvement in controlled ovarian stimulation and laboratory advances have provided high-quality embryos for infertile couples, leading to a steady increase in IVF success over the past years. Mock embryo transfer studies have also demonstrated the important role of uterine peristalsis in controlling the migration of analogs (14, 16, 17). Lesny et al. observed that the transferred fluid, after touching the uterine fundus with a catheter, can be removed from the uterine cavity with severe uterine contractions (18). Another study also showed that the frequency of uterine peristaltic waves was positively correlated with the distance that fluids move after deposition in the uterine cavity (14). Since uterine peristalsis can affect analog drift during the implantation window, it may also affect the outcome of subsequent clinical pregnancies during the actual embryo transfer cycle. Therefore, it was decided to assess the association between uterine peristalsis before embryo transfer and the rate of clinical pregnancy by frozen embryo transfer. There is still controversy regarding the effect of uterine contractions on pregnancy outcomes after IVF. In some studies, a lower frequency of uterine contractions has been associated with better outcomes in successful clinical pregnancies. Fanchin et al. prospectively studied 209 infertile women undergoing 220 cycles of controlled ovarian stimulation (19). They showed that the high frequency of uterine contractions on the day of embryo transfer affects the IVF outcomes, possibly by expelling the embryo from the uterine cavity. In addition, in Chung et al.'s study, live births were found to be significantly reduced in those with higher uterine contractions 5 *min* after embryo transfer, whereas uterine contractions before embryo transfer did not affect pregnancy outcomes (20). In another study by Zhu et al., the effect of uterine peristalsis before embryo transfer on the likelihood of clinical pregnancy in fresh and frozen-thawed embryo transfer cycles was investigated and it was revealed that clinical pregnancy rate was the highest when less than 3 waves per minute were recorded (17). With increasing peristaltic wave frequency, the rate of clinical pregnancy decreased significantly, especially at frequencies greater than 3. In contrast, the overall results of our study showed that women with a frequency of uterine peristalsis less than 4 waves *per/min* before embryo transfer were more likely to become pregnant than those with a higher frequency. In a pilot study by Blank et al., it was found that uterine activity after embryo transfer, characterized by high frequency and low amplitude, may be beneficial to embryo implantation (21). In our study, women who became pregnant after successful embryo transfer had lower uterine contraction frequency on the day of embryo transfer. In addition, an inverse correlation between the frequency and clinical pregnancy was observed in frequencies higher than 4 per minute. This is in line with some previous studies which emphasized an optimal range of contraction frequencies for successful implantation (17, 20). Another factor affecting the consequences of pregnancy following embryo transfer cycles is the direction of uterine contractions (14, 20, 22). In the study of Kim et al., pregnancy outcome was significantly correlated with endometrial movements related to the number of contractions, intense movement, cervicofundal direction, and hyperechoic change; so a higher rate of cervicofundal movement was reported in the pregnant group (22). However, this was not evaluated in our investigation. Endometrial thickness is the most widely used prognostic indicator to predict endometrial acceptance. Although observations show that the association between endometrial thickness and IVF results is still controversial (23, 24), there is a general agreement that thin endometrium is associated with lower pregnancy rates. In addition, several studies have shown that there is an association between endometrial thickness and clinical pregnancy after fresh embryo transfer (25, 26). It seems that an appropriate range of endometrium thickness is more effective than just a thicker endometrium in implantation success. Since embryo transfer was done when endometrial thickness was ≥7 *mm* similar to previous studies (27), no statistical relationship between pregnant and non-pregnant cases was seen based on the endometrial thickness. Incidentally, in our study, a positive correlation between endometrial thickness and uterine contraction frequency was seen and the two subjects with the highest endometrial thickness, ≥ 14 *mm*, showed the highest frequencies and both experienced transfer failure. It might be another evidence which indicates the inverse correlation between frequency and the embryo transfer outcomes. Nonetheless, the number of subjects with the thickest endometrium was very low and further studies should be conducted. Moreover, our study shows statistically significant inverse correlation between the contraction frequency and the occurrence of abortion in those who became pregnant, which illustrates the role of high uterine contraction frequencies even after implantation, that may interfere with final success of pregnancy. It is known that very thin endometrial thickness is not appropriate for implantation and it may lead to abortion. On the other hand, abortion might occur in the endometrium with the lowest contraction frequency. In fact, our sample size with thin endometrium was limited and further studies are required to be conducted to prove the claim (28). Moreover, no cases of ectopic pregnancy after fresh embryo transfer were reported in our study.

Conclusion

In this study, the optimal range of uterine contraction frequency was effective in successful implantation and ongoing pregnancy rate before embryo transfer. In fact, very high frequencies had adverse effects on the embryo transfer outcomes. A new finding from the current study was the association of endometrial thickness with uterine contractions. Thus, measuring the frequency of uterine contraction before embryo transfer seems to have some diagnostic values in predicting pregnancy outcomes. However, in addition to frequency, other indicators such as amplitude and directions of uterine contractions should be evaluated in future studies to expand our knowledge about the role of uterine contractions in IVF.

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Conflict of Interest

Authors declare no conflict of interest.

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