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Evaluation of Results of Frozen Embryo Replacement Cycles Based on Spinnbarkeit and Endometrial Thickness

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ABSTRACT

Background & Objective: Frozen embryo replacement (FER) cycle is a procedure that assists in, in vitro fertilization (IVF) outcome by using the storage and transfer of excess embryos. This study evaluated the success of FER using Spinnbarkeit and endometrial thickness.

Materials & Methods: This cross-sectional study was performed on 97 infertile women at the Imam Khomeini hospital of Ahvaz, from March 2019 to March 2020. Endometrium was prepared with Estradiol valerate from the third day of menstruation and followed by serial vaginal ultrasound till the thickness of the endometrium attained seven millimeters to eight millimeters. The cervical secretions were collected from the external Os and were estimated based on centimeters. Also, endometrial thickness was recorded.

Results: no substantial difference between endometrial thickness and spinnbarkeit in the FER cycles with pregnancy outcomes was observed (P>0.05).

Conclusion: Some variables such as endometrial thickness, spinnbarkiet, estradiol dose intake, and quality of embryos have no relationship with the success of FER cycles.

Keywords: Infertility, Frozen embryo replacement cycles, Spinnbarkeit, Endometrial thickness

Introduction

Infertility is the inability to get pregnant or to carry a child after regular sexual intercourse **(1, 2)**. Several treatment strategies have been evaluated to improve fertility in couples with infertility **(3, 4)**. In vitro fertilization (IVF) is a complex process outside the body that enhances the chance of fertilization, embryo development, and implantation **(5, 6)**. Several factors, including embryo quality, endometrial thickness, and the fresh/frozen method of embryo transfer have been found to impact IVF results **(7)**. Based on previous studies, the use of frozen embryos increased the chances of a successful pregnancy **(8, 9)**. Frozen embryo replacement (FER) cycles are an efficient cryopreservation method that enhances IVF outcome **(10, 11)**.

The endometrium is a unique, elastic, dynamic, and steroid-sensitive tissue that undergoes periodic changes during the menstrual cycle, including proliferation, differentiation, destruction, and repair **(12)**. Therefore, improving endometrial receptivity appears to be the most important bottleneck in the treatment of reproductive problems **(13)**. Several factors such as echogenic pattern, reflection pattern, blood flow, and endometrial thickness can affect endometrial receptivity **(14, 15)**. Low endometrial thickness reduces endometrial receptivity, therefore, some experiments have examined endometrial acceptability by measuring its thickness (6 to 11 mm) **(16)**. Transvaginal ultrasound has been used to measure endometrial thickness in patients with abnormal uterine bleeding and women undergoing infertility treatment with assisted reproductive techniques **(17, 18)**. The cervical mucus can also be important for female fertility **(19)**. Cervical secretions are often evaluated with the spinnbarkeit (mucus stretchability) test around the time of ovulation **(20)**. This study aimed to assess

the results of FER cycles based on spinnbarkiet and endometrial thickness in patients who underwent endometrial preparation by estrogen.

Methods

This cross-sectional study was performed on 97 infertile women at the Imam Khomeini hospital of Ahvaz, from March 2019 to March 2020. Inclusion criteria were women undergoing FER cycles by hormone therapy (estrogen and progesterone). Women with vaginismus, unwillingness to cooperate, and with endometrial thickness less than 6 mm were excluded from the study. Information on infertile women was collected by a researcher based on a checklist.

Endometrium was prepared by natural or modified cycles and the use of GnRH agonist such as estradiol valerate (Aburayhan, Iran) from the third day of menstruation and followed by serial vaginal ultrasound until the endometrium thickness reached between seven mm to eight mm.

Next, the viscoelastic properties and spinnability (Spinnbarkeit) of the cervical secretions was measured using a simple apparatus from the external Os and determined by drawing the mucus out vertically **(21)**. The mean value was recorded in cm.

Other variables such as doses of estradiol valerate, the day of embryo transfer, age and grade of the embryo, and the number of embryo transfer were also recorded.

Before embryo transfer, all patients received 100 mg progesterone (for 3-5 days). Embryo transportation was conducted in lithotomy position by cook catheter embryo transfer (Cook medical, Ireland,) in the operating room. The initial estimate of pregnancy results was described in accordance to if β-HCG serum concentration was more than five IU/l (14 days after embryo transfer).

This research was accepted by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.HGOLESTAN.REC.1399.064).

Statistical analysis

Statistical analysis was assessed with the SSPS software (version 22.0, SPSS Inc., Chicago, Illinois, USA). For quantitative data with a normal distribution, an independent sample t-test was used and data was informed as a mean standard deviation. For qualitative data, the Chi-squared and Fisher-exact tests were used. To compare the mean variables among the two groups, the Mann-Whitney non-parametric test was used. P-value less than 0.05 was statistically substantial.

Results

In the present study, among 97 patients, 73 cases (75.3%) had no history of childbirth, 23 cases (24%) had a history of delivery, 12 cases (12.4%) had a vaginal delivery, and 11 cases (11.3%) had the history of cesarean delivery. There was no statistically significant between the type of childbirth with the pregnancy success rate (P=0.808). Of the total women, 72 cases (74.2%) had no history of abortion, 15 cases (15.5%) had experienced at least one spontaneous abortion, and 10 cases (10.3%) had a history of curettage. These findings were summarized in Table 1. We found that there was no statistically significant between estradiol dose, endometrial thickness, spinnbarkiet, transmission day in FER cycles, and the success rate of the pregnancy outcome (P>0.05) (Table 2). The quality of the transferred embryo was divided into three categories (A, B, C). The results showed that the highest frequency was related to the transfer of 2 embryos with B-quality (28.9%) (Table 3).

Table 1. Details of data of FER cycles

		Pregnancy outcome					P-value
	Categories	No		Yes		Total	
		No. (%)		No. (%)			
History of Childbirth	No history of childbirth	42	57.5	31	42.5	73	0.808
	Vaginal delivery	8	66.7	4	33.3	12	
	Cesarean section	6	54.5	5	45.5	11	
History of Abortion	No history of abortion	43	59.7	29	40.3	72	0.838
	Spontaneous abortion	9	60.0	6	40.0	15	
	History of curettage	5	50.0	5	50.0	10	
Transmission embryo	One	10	55.6	8	44.4	18	0.034
	Two	34	72.3	13	27.7	47	
	Three	13	41.9	18	58.1	31	

	Categories	Pregnancy outcome					P-value
		No		Yes		Total	
		No. (%)		No. (%)			
Embryo A	Four	0	0.0	1	100.0	1	0.661
	Not transferred	54	58.7	38	41.3	92	
	One transferred	2	50.0	2	50.0	4	
	Two transferred	1	100.0	0	0.0	1	
Embryo B	Not transferred	11	57.9	8	42.1	19	0.891
	One transferred	19	54.3	16	45.7	35	
	Two transferred	21	63.6	12	36.4	33	
	Three transferred	6	60.0	4	40.0	10	
Embryo C	Not transferred	36	65.5	19	34.5	55	0.289
	One transferred	8	53.3	7	46.7	15	
	Two transferred	11	50.0	11	50.0	22	
	Three transferred	1	25.0	3	75.0	4	

FER cycles: **Freeze** embryo replacement cycles

Table 2. The descriptive statistics of pregnancy outcomes.

	Pregnancy outcome						
	NO=57			Yes=40			
Variables	**Median**	**Mean**	**SD***	**Median**	**Mean**	**SD**	**p-value****
Estradiol dose (mg)	50.0	63.02	23.31	54.00	61.30	18.82	0.997
Endometrial Thickness (mm)	8.30	8.34	1.09	8.40	8.51	1.14	0.761
Spinnbarkiet (cm)	8.00	7.96	0.83	8.00	7.99	0.98	0.683
Transmission day	16.00	16.28	2.78	16.00	15.60	3.12	0.186

*: Standard deviation; **: Mann Whitney test.

Table 3. Number of embryos to transfer according to the quality of embryos

Frequency	Embryo quality	Percent (%)
1.0	A	1
1.0	A, A	1
13.4	B	13
1.0	B, A	1
9.3	B, C	9
12.4	B, 2C	12
28.9	2B	28
2.1	2B, A	2
2.1	2B, C	2
1.0	2B, 2C	1
10.3	3B	10
4.1	C	4

9.3	2C	9
4.1	3C	4
100.0	Total	97

Discussion

The present study was performed on 97 infertile women. We evaluated the effect of endometrial thickness and spinnbarkeit after endometrial preparation by estrogen on the FER cycle success rates. This study for the first time evaluated the relation between endometrial thickness and spinnbarkiet simultaneously with the outcome of FER cycles.

In the present study, the increase of endometrial thickness and spinnbarkiet could not improve pregnancy rates. Besides, the relationship between dosages of the estradiol and the number of embryos replacement with pregnancy outcome were evaluated. We displayed that none of the mentioned variables had an impact on the success of FER cycles. In addition, the effect of embryo transfer quality on pregnancy rates was assessed, and no major relationship was seen among the groups.

In our study, cervical mucus was tested for spinnbarkeit after the endometrial thickness reached to more than 7.5 to 8 mm in vaginal ultrasound. It has been reported that cervical mucus should be decreased before embryo transfer to promote the rates of pregnancy **(22, 23)**.

Previous studies assessed the relation that exists among the endometrial thickness and the pregnancy outcome **(24-26)**. Although endometrial thickness is a crucial factor for pregnancy and thin endometrium (in most cases <7 mm) is predictable for poor pregnancy outcome **(27, 28)**, some studies reported that endometrial thickness had no relation with pregnancy outcome **(29)**.

Our finding also showed that the increase of endometrial thickness (until 8 mm) had no significant effect on pregnancy outcome. Recently, a cohort study of more than 40,000 participants from Canada, and another study in the UK with more than 20,000 participants, showed similar results **(14, 15)**. Some variables such as the day of β-hCG administration, ovulation, and embryo transfer may have an effect on the endometrial thickness and pattern during each IVF cycle **(30)**. In some experiments, the thickness of the endometrium was measured considering the proliferation phase prior to prescribing o progesterone **(31)**. Nevertheless, the normal activity of endometrial growth is distinct in the follicular and luteal phases. In the follicular phase, the endometrium is sensitive to estrogen that helps to elevate the endometrial thickness and speeds the linear growth of endometrial glands and blood vessels. In the presence of progesterone, endometrial proliferation decreased two to three days following ovulation **(32)**.

Haas et al. examined the difference between endometrial thickness when the estrogen phase finishes and on the day of embryo transportation in 274 FER cycles and found a very major relationship among pregnancy results and endometrial thickness variation **(33)**. Abdominal ultrasound was employed on the day of embryo transfer and vaginal ultrasound when estrogen phase finished.

For endometrial thickness, transvaginal ultrasound proved to be more precise than abdominal ultrasound. Also, they only assessed the rate of current pregnancy in the FET cycle and didn't enumerate the association that exists between EMT changes in relation to progesterone prescription and birth rates. All of these abnormalities might be the major reasons for inconsistencies with our findings. Zhang et al. demonstrated that additional estradiol stimulation and endometrial thickness may improve pregnancy outcomes **(34)**.

In our study, for the first time, we evaluated two parameters endometrial thickness and spinnbarkiet before embryo transfer. However, the measurement of spinnbarkiet was done after detection of the endometrial thickness (7 to 8 mm) and not less than 7 mm. Besides, embryo transfer was based on endometrial thickness, not spinnbarkiet.

Conclusion

In this study, we demonstrated that some variables such as endometrial thickness, spinnbarkiet, estradiol dose intake, and quality of embryos have no relationship with the success of FER cycles.

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Availability of data and materials

The datasets used and/or evaluated on the course of the present research are accessible from the corresponding author on sensible request.

Authors' contributions

R. N.: designed and conducted experiments, and co-wrote the paper. M.Z.: conducted experiments and co-wrote the paper. A. M.: conducted the experiments, co-wrote the paper, and oversaw the study. M.S.: analyzed

data and edited the paper. All researchers studied and validated the manuscript.

Ethics approval and consent to participate

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Conflict of interests

No competing interest was reported from the authors.

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