ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Is There a Difference Between Clinical Outcomes of the Spontaneous Natural Cycle and Hormone Replacement Treatment in Frozen-Thawed Human Embryo Transfer?

Dondurulmuş-Çözülmüş İnsan Embriyo Transferinde Klinik Sonuçlar Açısından Spontan Doğal Siklus ve Hormon Replasman Tedavisi Arasında Fark Var mı?

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ABSTRACT Objective: Frozen-thawed embryo transfer can be accomplished during a natural cycle after spontaneous ovulation or after artificial preparation of endometrium with exogenous steroids. The implantation, pregnancy and live birth rates following frozen-thawed embryo transfer (FET) were compared between in a natural and hormonal control cycle. **Material and Methods:** This single-center, retrospective trial was implemented on data derived from a series of 244 women who had successful FET in our tertiary care center between January 2012 and June 2015. Two groups were constituted: Group 1 consisted of 101 women who underwent FET after spontaneous ovulation; while 143 women had FET after endometrial preparation with hormone replacement therapy. Rates of implantation, clinical pregnancy, and live birth were compared between two groups. **Results:** Two groups were similar on baseline characteristics (maternal ages at time of freezing and transfer of embryos, the number of previous fresh and frozen embryo transfer cycles, the number of oocytes and mature oocytes obtained in natural cycles) ($p>0.05$). There was no difference between 2 groups regarding rates of clinical pregnancy ($p=0.13$), implantation ($p=0.19$) and live birth ($p=0.26$). **Conclusion:** The findings of this study indicated that the clinical outcomes were comparable between the spontaneous natural cycle and hormone replacement treatment in FET.

Keywords: Embryo transfer; embryo implantation; pregnancy rate; live birth; hormone replacement therapy

ÖZET Amaç: Dondurulmuş-çözülmüş embriyo transferi ovulasyon sonrası doğal siklus veya endometriyumun dışarıdan steroidler ile hazırlanması sonrası gerçekleştirilebilir. Doğal siklus ve hormonal kontrollü siklus arasında dondurulmuş-çözülmüş embriyo transferi sonrası implantasyon, gebelik ve canlı doğum oranları karşılaştırıldı. **Gereç ve Yöntemler:** Bu tek merkezli, retrospektif çalışma bizim üçüncü basamak merkezimizde, Ocak 2012-Haziran 2015 tarihleri arasında başarılı şekilde donmuş embriyo transferi yapılmış olan 244 hasta verisinden gerçekleştirildi. Çalışmada 2 grup oluşturuldu: Grup 1 spontan ovulasyon sonrası donmuş embriyo transferi yapılmış olan 101 kadını içerirken; Grup 2 hormonal replasman tedavisi ile endometriyal hazırlık sonrası donmuş embriyo transferi yapılan 143 hastayı içermektedir. Gruplar implantasyon, klinik gebelik ve canlı doğum oranları açısından karşılaştırıldı. **Bulgular:** Temel özellikler açısından iki grup birbirine benzerdi (embriyo dondurma ve embriyo transferi sırasındaki maternal yaş, önceki taze ve dondurulmuş embriyo transfer siklus sayısı, doğal sikluslarda elde edilen oosit ve olgun oosit sayıları) ($p>0,05$). İki grup arasında klinik gebelik ($p=0,13$), implantasyon ($p=0,19$) ve canlı doğum ($p=0,26$) oranları açısından fark yoktu. **Sonuç:** Bu çalışmanın bulguları dondurulmuş embriyo transferi işleminde doğal siklus ve hormon replasman tedavisinin klinik sonuçlarının benzer olduğunu gösterdi.

Anahtar Kelimeler: Embriyo transferi; embriyo implantasyonu; gebelik oranı; canlı doğum; hormon replasman tedavisi

Frozen–thawed embryo transfer (FET) is an assisted reproductive technique which improves clinical outcome by the preservation of spare embryos, prevention of endometrial abnormalities during collection of oocytes, avoidance of ovarian hyperstimulation syndrome (OHSS), and adjusting the synchronization of endometrial and embryonic ripening. Factors that may affect the success of FET are the quality of embryo, synchronization of endometrial and embryonic developmental processes as well as receptivity of the endometrium. In this context, preparation of endometrium prior to FET can be important for achievement of clinical pregnancy in selected patients. Common strategies for this purpose involve ovulation induction cycling, hormone replacement therapy (HRT) cycling and natural cycling.[1,2]

Frozen-thawed embryo transfer can be accomplished during a natural cycle after spontaneous ovulation or after artificial preparation of endometrium with exogenous steroids. Since the natural cycle protocol does not necessitate administration of exogenous hormones, it is still preferred by many patients. However, problems frequently exist after this protocol is used. The cycle needs to be monitored thoroughly to determine since ovulation entails higher costs and discomfort. The exact time of ovulation may not be documented precisely, and failure to determine the date of FET can constitute a problem in centers that do not operate seven days a week. Thereby, preparation of endometrium with exogenous steroids offers some remarkable advantages. Medical staff or patients can select the date of FET, and the likelihood of cycle cancellation can be notably diminished, reducing the anxiety of the patient. This protocol is especially useful for women with irregular cycles.[3-5]

Previous publications reported the successful use of exogenous estrogens and progesterone without previous ovarian suppression by GnRH agonist for the artificial preparation of endometrium in women with functioning ovaries who were undergoing FET.[5,6] Although the women used different formulations and doses of estradiol, the results were the same and comparison of endometrial preparation with and without previous GnRH agonist suppression yielded similar success rates.[3,5]

The objective of the current study was to evaluate and compare the therapeutic outcomes in FET after natural cycle and after preparation of endometrium with HRT in women with normal ovarian function. Moreover, we attempted to analyze whether there is a correlation between clinical characteristics, laboratory data and treatment outcomes of patients receiving FET after natural cycle and after HRT.

MATERIAL AND METHODS

STUDY DESIGN

This single-center, retrospective study was implemented in the in-vitro fertilization (IVF) unit of the obstetrics & gynaecology department of our tertiary care center. The approval of the local Institutional Review Board had been obtained before the study. All interventions had been executed in accordance with principles announced in Helsinki Declaration.

STUDY POPULATION

A total of 244 women planning to receive FET in the IVF unit of our institution were recruited in this trial. Inclusion criteria were age < 40 years at the time embryos were frozen, regular ovulatory cycles, the presence of at least one blastocyst and at most two previous FET cycles. Women were excluded from participation in the trial more than once. Both treatment protocols were currently standard regimens in our department at that time. Exclusion criteria consisted of hyperstimulated ovarian follicles, diseases like adenomyosis, endometrial polyps, intrauterine adhesions, uterine submucosal myomas, anovulation, age ≥40 years of age, embryos frozen for preimplantation screening and loss to follow-up.

PROCEDURE

Patients willing to participate the treatment who fulfill the inclusion criteria were invited to IVF unit between 1st and 5th days of their monthly cycle for a baseline scan and enrollment in the study. Two groups were constituted out of a total of 244

women: Group 1 was composed of 101 women (41.8%) to receive FET after a natural cycle, whereas Group 2 consisted of 143 women (58.2%) that underwent HRT prior to FET. Participants in Group 1 had ultrasound assessment between 10th and 13th days of their cycle to evaluate follicular growth and endometrial thickness. If necessary, additional ultrasound examination was performed in subsequent days. Impending ovulation was monitored by every patient via blood Luteinizing hormone (LH) level which was defined as the day on which the LH level was above 15 IU/L and more than double the average of the LH levels over the past 3 days. Determination of LH surge was followed by information of the IVF unit and planning the FET one week later depending on the stage of embryo development at freezing. The transfer day was seven days later for blastocysts. In case endogeneous LH surge could not be observed, further visits were scheduled. Luteal support (LH and progesterone) was not provided for participants in Group 1. One or two thawed frozen embryos were transferred to the uterus under abdominal ultrasound guidance. A urinary pregnancy test was performed at home after 11-14 days with respect to the stage of the embryo and IVF unit was informed about the result. If the pregnancy test result was positive, further follow-up visits were planned to identify (via detection of fetal heart activity) and follow-up pregnancy. Pregnancy outcomes were recorded regarding implantation, clinical pregnancy, and live births.

NATURAL CYCLE

As recommended in the relevant literature, patients in the natural cycle group were invited to the clinic 18 days prior to the next expected period of the determination of serum levels of E2 and LH until surge of LH. A surge of LH was described as the day on which LH level was >15 IU/L and more than twice the average level of LH over the last three days. FET was performed on the 3rd day after surge of LH.[7]

Hormone replacement treatment in Group 2 consisted of administration of estradiol tb (Estrofem®, Novo Nordisk Healthcare, Istanbul, Turkey) twice daily, for eight days starting from the 3rd day of the cycle. The dose of estrofem tb was increased to 3 times a day for at least four days. Subsequent to the achievement of an endometrial thickness of 7 mm, maintenance dose for estrofem was shifted to 2 tablets daily, and a vaginal gel containing 90 mg of progesterone (Crinone® 8% vaginal gel, Serono, Istanbul, Turkey) was added to the regimen. Transfer of blastocysts was performed on the 6th day, and the same treatment was continued until 10th week if pregnancy was confirmed clinically. One or two embryos were transferred with respect to the preference of the patient.[8]

All embryos were transferred or cryopreserved at blastocyst stage. Embryos with >50% of blastomeres existent after thawing were transferred. In our center, two embryos were stored in one straw for cryopreservation and up to 2 embryos will be transferred in one treatment cycle. Ultrasonography performed on 8th week was used to determine clinical pregnancy and implantation. Live birth rates were investigated by means of an interview with patients by phone calls.

EMBRYO TRANSFER TECHNIQUE

Embryo transfers were all performed on day five. Patients presented with a full bladder, which would provide an acoustic window for visualization of the uterus, in preparation for the cavity measurements and ultrasound-guided transfer. All procedures were implemented in a similar fashion while abdominal ultrasonography was performed via a 5-MHz probe (GE Logiq 400 Pro Series, General Electric Company, Pewaukee, WI, USA). The tip of the catheter was loaded with the embryos and was placed to a level of 1.0–2.0 cm below the apex of the endometrial cavity as confirmed by transabdominal ultrasound. If necessary, the outer sheath was angled manually to approximate the angle of the cervix to help navigate the cervical canal. Efforts were spent to avoid contact of the transfer catheter with the uterine fundus.[6]

STATISTICAL ANALYSIS

Data was analyzed by means of IBM SPSSStatistics 20 program. Normal distribution of variables was tested with Kolmogorov-Smirnov test. Variables

with normal distribution were evaluated with parametric tests while non-parametric tests were utilized for variables without normal distribution. Two independent groups were compared by means of Independent-Samples T test and Mann-Whitney U test. For comparison of more than two groups, One-way ANOVA, a parametric test, was used and homogeneous groups were constituted by means of Tukey test. In the same purpose, Kruskal-Wallis test, a non-parametric test, was used while Mann-Whitney U test was utilized. Assessment of categorical variables was carried out by Pearson ChiSquare test. Quantitative variables are expressed as mean, standard deviation, median, interquartile range, minimum and maximum. The confidence interval was 95% and level of significance was set at $p<0.05$.

RESULTS

Clinical pregnancy was detected in 137 women (56.1%). No implantation was observed in 105 patients (43.0%), whereas a number of singleton and twin implantations were 114 (46.7%) and 25 (10.2%), respectively. Live birth could not be accomplished in 125 patients (51.2%); numbers of singleton and twin live births were 103 (42.2%) and 16 (6.6%). Ages of patients at the time of embryo freezing and during FET procedure, a number of previous fresh and FET cycles, numbers of oocytes and mature oocytes obtained in natural cycles, numbers of initially frozen and transferred embryos as well as rates of clinical pregnancy, implantation and live births were noted in two groups under investigation. Table 1 presents a comparative overview of aforementioned parameters (Table 1). Two groups were similar on average maternal ages at time of freezing (p=0.91) and transfer of embryos (p=0.85), the number of previous fresh (p=0.16) and frozen ET cycles (p=0.58), the number of oocytes (p=0.82) and mature oocytes (p=0.91) obtained in natural cycles. Similarly, there was no

TABLE 1: Comparison of descriptive, clinical and laboratory parameters in patients receiving frozen-thawed embryo transfer after natural cycle and hormone replacement therapy.

Variable	Group		p Value
	Natural cycle (n=102)	HRT (n=142)	
Age at freezing‡	30.0±4.3	30.0±5.3	0.91
Age at time of FET‡	30.8±4.1	30.9±.0	0.85
No. of previous fresh ET cycles	1.0-1.0	2.0-1.0	0.16
No. of previous frozen ET cycles	0.0-1.0	0.0-1.0	0.58
No. of oocytes obtained in natural cycles	13.0-9.0	13.0-7.0	0.82
No. of mature oocytes obtained in natural cycles	10.0-6.0	10.0-7.0	0.91
No. of initially frozen embryos	3.0-2.0	3.0-2.0	0.73
No. of transferred embryos	2.0-1.0	1.0-1.0	0.19
Clinical pregnancy			
Yes	63 (61.8%)	74 (52.1%)	0.13
No	39 (38.2%)	68 (47.9%)	
Implantation			
None	37 (36.3%)	68 (47.9%)	0.19
Singleton	53 (52.0%)	61 (43.0%)	
Twin	12 (11.8%)	13 (9.2%)	
Live birth			
None	46 (45.1%)	79 (55.6%)	0.26
Singleton	48 (46.6%)	55 (38.7%)	
Twin	8 (7.8%)	8 (5.6%)	

(Abbreviations: *: statistically significant; HRT: hormone replacement therapy; FET: frozen-thawed embryo transfer; ET: embryo transfer; ǂ: expressed in mean±standard deviation; other parameters are expressed in median (min-max).

TABLE 2: Comparison of maternal ages at time of embryo freezing and FET procedure in patients with various outcomes for clinical pregnancy, implantation, and live births.

Variable	Age (years)	
	At time of freezing	At time of FET
Clinical pregnancy		
No	31.00±4.94	31.90±4.60
Yes	29.20±4.76	30.10±4.56
p Value	0.006*	0.003*
Implantation		
None	31.00±4.99	31.90±4.63
Singleton	29.10±4.64	30.10±4.48
Twin	30.20±5.15	31.00±4.87
p Value	0.016*	0.010*
Live birth		
None	30.90±4.88	31.70±4.56
Singleton	29.10±4.80	30.00±4.59
Twin	29.10±4.90	30.00±4.72
p Value	0.020*	0.011*

(Abbreviations: FET: frozen–thawed embryo transfer; *: statistically significant).

difference between 2 groups with respect to number of initially frozen (p=0.73) and transferred (p=0.19) embryos.

There was no difference between two groups in terms of rates of clinical pregnancy (p=0.13), implantation (p=0.19) and live birth (p=0.26). Number of embryos transferred were significantly higher in patients with twin implantation (p<0.001).

Table 2 demonstrates the comparison of maternal ages at time of embryo freezing and FET procedure in patients with various outcomes of clinical pregnancy, implantation and live births (Table 2). Accordingly, age at the time of embryo freezing (p=0.006) and age at FET procedure (p=0.003) was more advanced in patients without clinically confirmed pregnancy. Similarly, patients with failed implantation were at more advanced ages at the time of freezing (p=0.016) and during FET (p=0.010). Ages at time of embryo freezing (p=0.020) and during FET (p=0.011) were higher in patients without live births compared to women with singleton live births.

DISCUSSION

Our results indicated that therapeutic outcomes regarding rates of clinical pregnancy, implantation, and live birth are similar in patients receiving FET in the natural cycle and after HRT.

Frozen-thawed embryo transfer has been successfully carried out in natural cycles subsequent to spontaneous ovulation and in cycles in which the endometrium is prepared with exogenous steroids.[9,10] The absence of hormonal therapy in the natural cycle protocols is preferential for women, but some problems may arise after the use of this protocol. The timing of ovulation may bring about difficulties in women with irregular cycles, and this may lead to higher rates of cancellation. The date of embryo thaw and transfer may not be planned exactly with confidence, and this may pose a problem for particularly centers that do not operate continuously. Thus, workload issues related with the unpredictability and daily number of FET cycles may result in a reduction of the number of patients accepted for treatment.[9]

Achievement of implantation and pregnancy are associated with the interactions and synchronization between embryo development and endometrial receptivity.[9,11] The success of FET is linked with the receptivity of the endometrium for embryos, which have been created in a previous treatment cycle. Artificial preparation of the endometrium with estrogen and progesterone following pituitary desensitization with a GnRH agonist may play a crucial role.[12]

Synchronization between embryo development and endometrial receptivity can be provided in a natural cycle after spontaneous ovulation or after artificial preparation of the endometrium with exogenous steroids.[3,13] Contemporary protocols prepare endometrium with exogenous estrogens and progesterone after pituitary down-regulation with a GnRH analog to avoid spontaneous ovulation. The main advantage offered by such a protocol is a reduction of the risk of the cycle; thereby, the date of embryo thaw and transfer may be determined by either the medical team

or the patient. In contrast, this protocol possesses disadvantages such as high cost, the risk of hypoestrogenic side effects before hormonal replacement and long preparation period.[3] Recently, successful use of exogenous estrogens and progesterone without previous ovarian suppression by GnRH agonist during artificial preparation of endometrium in women with functioning ovaries has been reported for FET.[4,14]

Our study is important since it particularly focusses on outcomes of blastocysts transfer. Our results demonstrated that endometrial preparation with HRT seems not to provide any advantage on therapeutic outcomes of FET.

Dal Prato et al. reported that endometrial preparation with exogeneous steroids without pretreatment with GnRH agonists did not reduce the success rate of FET.[3] Since these findings were consistent with previous reports [14,15] indicating that suppression with a GnRH agonist is not required for endometrial preparation, we do not routinely administer GnRH agonists for FET. Thereby, avoidance of administration of a GnRH agonist makes the procedure simpler and cheaper.

We preferred a starting dose of 4 mg estradiol for HRT. In the relevant literature, the dose of estradiol for preparation of endometrium was reported to be not that important.[15,16] Dose and composition of HRT may vary in different institutions. Our results remind that advanced age may be an important factor that may diminish the success of FET. Thus, early diagnosis and timely intervention are critical for the achievement of satisfactory outcomes.

Li et al. suggested that endometrial thickness and clinical pregnancy rates in the HRT group were lower.[1] No remarkable differences were noted regarding endometrial thickness and clinical pregnancy rates between patients undergoing HRT or natural cycling.[17] Various cycle regimens for FET, including natural ovulatory cycle, artificial cycle and ovulation induction cycles using clomiphene citrate or gonadotrophins, were compared in a Cochrane review, and there was no significant difference in the pregnancy outcome among different cycle regimens.[18] By this data, our results support natural cycles in ovulatory women. Similar with our results, Fatemi et al. suggested that the natural cycle was superior compared with the natural cycle controlled by hCG administration in cryothawed ET cycles.[19] In contrary, Hill et al. reported that the synthetic hormone protocol was associated with a higher live-birth rate when compared with a natural cycle protocol for frozen-thawed blastocyst-stage ET cycles.[6]

Hormone replacement therapy offers the advantage of cycle control, and this may aid in planning the workforce in the timing of ET. This is the main underlying reason for preference of HRT for the vast majority of FET. On the other hand, natural cycles that avoid the use of exogenous steroids prevent the side effects of down-regulation. Shorter duration and diminished financial cost are other advantages of natural cycle approach.[7] No endocrine monitoring is necessary after pituitary suppression has been confirmed, and thus fewer visits to the center are necessary. The day of embryo transfer can be programmed in advance. This is convenient to the woman and benefits the planning of the workload within an IVF unit, especially if not operating seven days a week.[9]

Notably, patients who report regular cycles may not be necessarily ideal candidates for a natural cycle protocol. In addition, patients must be informed on the difficulties for detection of ovulation and variability in the lengths of the cycle and ovulation.[7] Further trials are warranted to establish criteria for selection of appropriate patients for FET with natural cycle and HRT. Our data indicated that increased number of embryos transferred was higher in patients with twin implantation. Therefore, determination of the number of embryos must be made on an individualized basis depending on the reproductive history of the patient.

Main weaknesses of the current study include retrospective design, data limited to the experience of a single institution and possibility of bias. Main strengths of our trial were the selective inclusion of blastocyst transfer in a series with an adequate number of patients.

Based on the current literature it is not possible to say that using natural or hormonal control cycle in FET for endometrial preparation is more effective than other. Therefore, both methods appear to be equally effective in terms of clinical outcomes. Results of the present study indicated that the implantation, pregnancy and live birth rates following FET were similar between in a natural and hormonal control cycle. In spite of increased workload and need for close follow-up, therapeutic outcomes of FET during the natural cycle are comparable to that of FET after endometrial preparation with HRT. Further randomised trials should not only address pregnancy rates but also consider convenience, cost efficiency, possible serious adverse events and side-effects of medication, and physician and patients' preferences.

Conflict of Interest

Authors declared no conflict of interest or financial support.

Authorship Contributions

Conception and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, supervision: *Turgut Aydın;* ***Conception and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content:*** *Hüseyin Aksoy;* ***Conception and design, statistical analysis:*** *Özge İdem Karadağ;* ***Conception and design, acquisition of data;*** *Ülkü Aksoy;* ***Conception and design, administrative technial or material support:*** *Elif Çınar;* ***Conception and design, acquisition of data:*** *Mustafa Taş.*

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