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Cryo-thawed embryo transfer. Artificial versus natural cycle

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Abstract

There has been a rise in the number of frozen embryo transfers (FET), ever since clinicians started adopting the "freeze-all" policy as a method of prevention for the hyperstimulation syndrome. Even though people have made great advances in this area, the pregnancy rates haven't changed much, so we constantly need to find new ways to improve in vitro fetilization outcomes. The purpose of our study is to evaluate the existing methods of endometrium preparation before the frozen-thawed embryo transfer: natural cycle (NC)-FET, modified NC- FET, artificial cycle (AC)-FET and AC- FET with gonadotropin releasing hormone (GnRH) agonist. The primary outcome the number of clinical pregnancies and the secondary outcomes were the number of cancellation cycles, implantation rate and live birth rate. The women that underwent NC- FET and AC- FET with pre-administration of GnRH agonist had similar outcomes, but the NC proved to have the best results.

Keywords: *frozen embryo transfer, natural cycle, modified natural cycle, artificial cycle, clinical pregnancy rate*

Introduction

More than 35 years have passed since Trounson and Mohr reported the first cryopreservation and thawing process of a human embryo after *in vitro* fertilization (IVF) treatment[1]. This moment was very important in the history of assisted reproduction techniques because it improved efficacy, promoted the transfer of fewer embryos inside the uterus and therefore, reduced the number of complications that relate to prematurity[2]. Ever since clinicians started adopting the 'freeze-all' policy in order to prevent the ovarian hyperstimulation syndrome and embryologists developed new techniques of freezing, there has been a rise in the number of frozen embryo transfers (FET)[3-5].

The success of an embryo transfer is influenced by three factors: the embryo quality, endometrial receptivity and balanced interaction between the embryo and the endometrium[6]. Nowadays, endometrial receptivity appears to be the bottleneck of the reproductive process. Two-thirds of implantation failures are a consequence of inadequate uterine receptivity. As we all know, the endometrium is hostile towards the embryo during most days of the menstrual cycle[7]. So the challenge appears to be finding the perfect synchronization between the endometrium and the developmental stage of the embryo.

There are several methods of endometrium preparation regarding frozen-thawed embryo transfer. The natural cycle frozen-thawed embryo transfer (NC-FET) looks like the simplest method of endometrium preparation, because the endometrium finds itself only under the action of endogenous sex steroids produced by the developing follicle[8]. It is essential to find the ovulation moment, in order to calculate the implantation window, when there is optimal endometrial receptivity[9]. The spontaneous luteinizing hormone (LH) peak can be estimated based on blood or urine exams. After the rise of serum LH, it is assumed that ovulation takes place after 36-40 h[10]. If the patient performs urine test, one has to consider a shorter period of time until ovulation, approximately 24 h. However, a further problem would be the variation between cycles and patients[11]. For the assessment of LH to be correct, it should be performed at least daily. The LH urine tests have different thresholds, which imply 30% false negative results[12]. Therefore, patients often complain about difficulties to interpret the results. One can't forget the false-positive results in patients with polycystic ovary syndrome.

In order to overcome the disadvantages of LH monitoring, clinicians often consider human chorionic gonadotropin (hCG) triggering. This method represents the modified NC-FET. This approach implies regular ultrasounds until the dominant follicle reaches the appropriate diameter for triggering (17-18 mm). Once the hCG is administered, the ovulation takes place 36-38 h later[10]. In both natural and modified natural cycles, the clinician should establish the moment of embryo transfer depending on the stage of the embryo[13]. The clinician can supplement the luteal phase with progesterone. There are studies that show an improved live birth rate after the supplementation with 400 mg vaginal progesterone as luteal phase support[14].

Another method of endometrium preparation is artificial cycle frozen embryo transfer (AC-FET), in which estrogen and progesterone are administered in a way to mimic the natural menstrual cycle. Initially, the patient receives estrogen for the proliferation of the endometrium, suppressing the development of a dominant follicle. The doctor measures the endometrium when performing the ultrasound and when it reaches 7-9 mm thickness, the progesterone supplementation starts. The embryo transfer is scheduled based on the number of days of progesterone. Complete suppression with estrogen and progesterone is not possible. Some studies report a 5% cycle cancellation rate due to the development of a dominant follicle[15,16]. If the follicle undergoes spontaneous luteinization, the endometrium is therefore exposed earlier to progesterone, leading to a miscalculation of the implantation window. In order to avoid this, a ginadotropin-releasing hormone (GnRH)

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agonist administration is possible before starting the hormone treatment (AC-FET with GnRH). The GnRH agonist offers complete pituitary suppression but doctors should not disregard the possible initial flare-up effect. Both of these artificial approaches require hormone treatment, which makes the embryo transfer easier to plan for the patient and the clinician. However, until present it is unclear whether natural or artificial cycle is better for the frozen-thawed embryo transfer.

This retrospective study was performed in order to assess the clinical pregnancy rate, abortion rate and cancellation rate after frozen-thawed embryo transfer, using different approaches of endometrium preparation.

Methods

Patient population and study design

We included in our study 294 women between 20 and 40 years old, who underwent FET procedures after conventional IVF or intracytoplasmatic sperm injection in the IVF Department of Clinical Hospital of Obstetrics and Gynecology "Prof. Dr. Panait Sarbu", Bucharest from Romania between June 2016 and December 2017. The exclusion criteria were the following: unilateral/bilateral hydrosalpinx, anatomical uterine anomalies and oocyte donation cycles. Patients willingly signed an informed consent. In order to avoid potential sources of bias, we included all women that matched the inclusion criteria in the above mentioned period.

NC-FET

The patients that underwent NC-FET attended for ultrasound evaluation of the dominant follicle between day 8-14 depending on each number of days of menstrual cycle. We usually performed an ultrasound 1-2 days before the presumed time of ovulation (Figures 1 and 2). If the follicle hadn't reached a diameter of 17-18 mm we scheduled the patient for another ultrasound depending on the size of the dominant follicle. When the follicle was mature enough (Figures 3 and 4), we requested blood exams (i.e. estradiol, LH, progesterone) or urine exams in order to establish the moment of ovulation, depending on womens' choice and financial status. All women administered 400 mg vaginal progesterone for luteal support.

Modified NC-FET

We performed ultrasound examinations on the patients that underwent modified NC-FET from day 8-14 of their menstrual cycle until the dominant follicle reached at least 17-18 mm. When the follicle was mature enough, hCG was

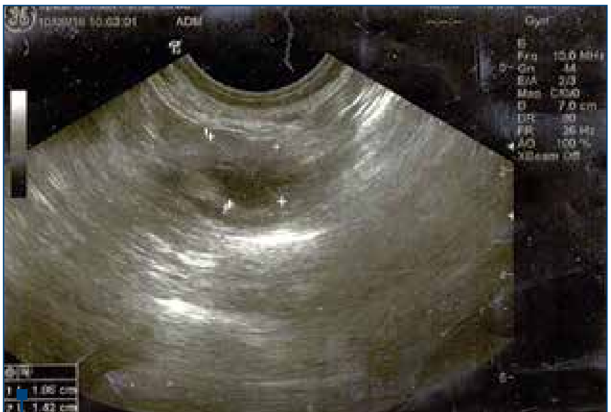

Figure 1. *Ultrasonographic aspect of a dominant follicle in day 12 of menstrual cycle (diameter 16.3 mm)*

Figure 2. *Ultrasonographic aspect of endometrium in day 12 of menstrual cycle (trilaminar aspect 9.1 mm)*

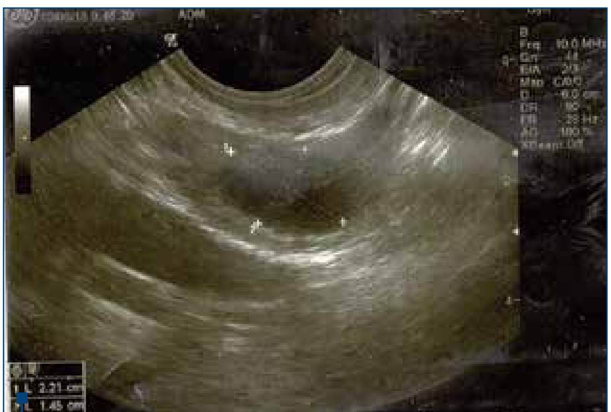

Figure 3. *Ultrasonographic aspect of a dominant follicle in day 14 of menstrual cycle (diameter 18.3 mm)*

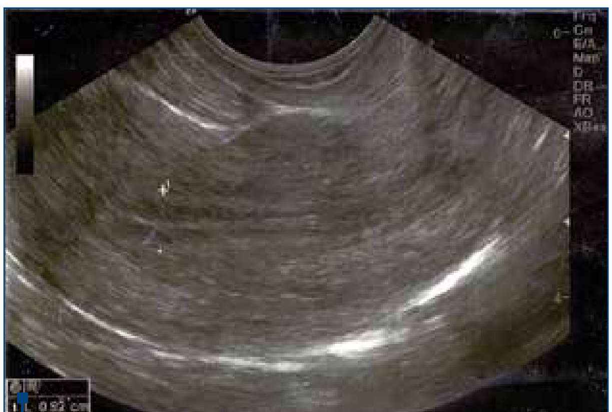

Figure 4. *Ultrasonographic aspect of endometrium in day 14 of menstrual cycle (trilaminar aspect 9.2 mm)*

given subcutaneously to trigger ovulation. There was no need for blood or urine tests. All women administered 400 mg vaginal progesterone for luteal support.

AC-FET

In AC-FET, oral estrogen (2 mg, three times daily) was commenced on the first day of the menstrual cycle. After 10-12 days, vaginal ultrasound was performed in order to establish that no dominant follicle had appeared and to measure endometrial thickness. When the endometrium was at least 7 mm, vaginal micronized progesterone was administered 4-6 times daily for 3 or 5 days, depending on the type of the embryo. If the endometrium wasn't thick enough, the estradiol dosage was raised to 8 mg per day and we repeated the ultrasound after 4 days. If there was no increase in the thickness of the endometrium at the next ultrasound, we added one transdermal patch with estrogen and we performed another ultrasound after 3 days. If there was still no increase in the endometrium thickness, the FET was cancelled. When a dominant follicle still emerged during the treatment, blood exams (i.e. estradiol, LH, progesterone) were performed. If ovulation was identified, we continued treatment and scheduled the FET depending on the endogenous LH peek of the patient.

AC-FET with GnRH agonist

Patients that underwent AC-FET with GnRH agonist for complete pituitary suppression received one single dose of 3.75 mg triptorelinum intramuscular on day 21-26 of the anterior menstrual cycle, depending on womans' wish and laboratory availability. After 10 days, we performed a vaginal ultrasound in order to establish complete pituitary suppression. Afterwards, we started the hormone therapy, as we described it above. The moment of embryo transfer was decided according to the stage of the embryo when frozen. Following the embryo transfer, patients continued the hormone treatment (i.e. estradiol until 8 weeks of pregnancy and progesterone until 12 weeks of pregnancy) and performed a serum pregnancy test (β-hCG) after 10 days.

Outcome measures

The primary outcome was the number of clinical pregnancies, which we defined as the presence of an intrauterine sac with fetal heart activity through vaginal ultrasound scan at four weeks after the embryo transfer, following a positive β-HCG test. Secondary outcomes were the number of cancellation cycles, implantation rate and live birth rate.

Statistical analysis

All the information has been analysed with the SPSS 20.0 for Windows statistical package. For the analysis of association between variables, we have used Student's t-test and Chi square test. For the aim of the present study, the statistical significance was settled at a p-value <0.05.

Results

Overall, we performed 294 cycles: 210 women underwent only one procedure, 30 women underwent 2 procedures and 8 women 3 procedures. We divided the procedures into 4 groups. Every group consists of a specific number of FET cycles in our clinic after different endometrium preparation as it follows: group A – 80 cycles – NC-FET, group B – 59 cycles – modified NC-FET, group C – 70 cycles – AC-FET and group D – 85 cycles – AC-FET with administration of GnRH agonist.

Table 1 illustrates the baseline characteristics of the patients enrolled in the study. The ages, body mass index (BMI) and cause of infertility did not differ very much in all four groups.

The outcomes are summarized in Table 2. Out of 285 cycles, we performed 266 frozen embryo transfers: 73 in group A, 57 in group B, 62 in group C and 74 in group D. The cancellation rate was more significant in group D, where patients underwent AC-FET (12.9%) and less significant in patients from group B, that underwent modified NC-FET (3.39%) (Table 2).

Furthermore, the final results were comparable between group A (NC-FET) and group D (AC-FET with GnRH), those from group A being slightly superior (implantation rate of 35.4% vs 34.4%, clinical pregnancy rate of 32.2% vs 29.7 and live birth rate of 27.4% vs 26.6%). The most discouraging results we obtained from group B, where patients underwent modified NC-FET (implantation rate 29.1%, clinical pregnancy rate 26.8% and live birth rate 22.3%).

Discussion

The transfer of frozen-thawed embryos has positively influenced the management of women undergoing conventional ovarian hyperstimulation for IVF. This strategy increases the cumulative pregnancy rate and reduces costs, by giving clinicians opportunities for further embryo transfer.

To our knowledge, this is the only retrospective study in our country that compares different methods of endometrium preparation before frozen embryo transfer.

Based on the presented data, it can be concluded that NC-FET is not inferior to AC-FET with regard to all outcomes of FET. The lowest rate of cancellation rate was seen among patients from group B, who underwent modified NC-FET. The most frequently reason of cycle cancellation were premature ovulation in group A, incomplete suppression of the pituitary gland in group C and insufficient endometrial thickness in group D.

Even though the outcomes in group A were the best in this study, one disadvantage for the patient can be the frequency of hospital visits, due to the fact that people come from all over Romania to Bucharest to treat infertility. Moreover, the awaiting for spontaneous LH surge can lead to uncertain planning, which can be bothersome for both patients and laboratories[18]. In the last years, some patients tend to refuse hormone treatment due to personal beliefs or history.

Consistent with our findings, a study compared the outcomes of FET after natural cycles or down-regulated hormone therapy cycles. This study show no significant differences in terms of clinical pregnancy and live birth rate[19].

We did not take into consideration the cost of the endometrium preparation method, which can also play an essential role in the choice of treatment, because infertility procedures in Romania are only partially financially sustained by the state. This could be another advantage of the NC-FET. Some studies have suggested a possible

Table 1 Baseline characteristics of the patients enrolled in the study

	Group A (*n*=80)	Group B (*n*=59)	Group C (*n*=70)	Group D (*n*=85)
Age (years)	32.7	31.5	33.1	32.8
BMI (kg/m²)	21.7	22.2	21.4	23
Unexplained infertility (%)	32	34	30	35
Male factor (%)	16	19	17.5	15
Tubal factor (%)	24	26	22	24
Polycystic ovarian syndrome(%)	14	16	18	15.3
Endometriosis (%)	14.8	5	12.5	10.7

BMI =body mass index

Table 2 Outcomes of FET

	Group A (*n*=80)	Group B (*n*=59)	Group C (*n*=70)	Group D (*n*=85)	*p* value
Total no. of FET	73	57	62	74	0.88
Cancellation rate (%)	8.75	3.39	11.43	12.9	0.005
Implantation rate (%)	35.4	29.1	30.5	34.4	0.89
Clinical pregnancy rate (%)	32.2	26.8	28.1	29.7	0.55
Live birth rate (%)	27.4	22.3	25.1	26.6	0.08

FET = frozen embryo transfer

detrimental effect of hCG on pregnancy rates in modified NC-FET, which is also in accordance with our findings[20,21].

Conclusions

The clinical pregnancy rate was comparable between group A and group D, but those that underwent natural cycle FET obtained better results in terms of cancellation and implantation rate. We consider the NC-FET a valid option that can be offered to patients that await for embryo transfer. Both patients' preference for treatment as well as logistics of the infertility clinic should be taken into account when choosing a certain method of endometrium preparation. ■

Conflict of interests: The authors declare no conflict of interests.

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