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Live birth rates after different endometrial preparation methods in frozen cleavage-stage embryo transfer cycles: a randomized controlled trial

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Abstract

Purpose This study aimed to compare the clinical outcomes in different endometrial preparation methods prior to frozen embryo transfer (FET) in women with normal menstrual cycles.

Methods A total of 471 eligible patients were randomly allocated into four groups of endometrial preparation prior to FET: natural cycle with spontaneous ovulation ($n=120$), natural cycle with human chorionic gonadotropin (hCG) for ovulation induction ($n=117$), hormone replacement cycle (HRC) ($n=113$) and HRC with pre-treatment with GnRH-a ($n=121$). Natural cycle with hCG also received hCG in luteal phase. The primary outcome was live birth rate. The secondary outcomes included implantation, biochemical and clinical pregnancy, ongoing pregnancy, and late miscarriage rates. Data analysis included *t* test, ANOVA and χ^2.

Results There were no statistically significant differences in the mean age ($p=0.31$), duration ($p=0.43$) and cause of infertility ($p=0.77$) and the number ($p=0.33$) and quality ($p=0.21$) of embryos transferred between the groups. No significant differences regarding the implantation rates per embryo transfer ($p=0.97$) and biochemical pregnancy rates ($p=0.90$) were observed between the groups. The rates of clinical pregnancy were 34.2%, 32.5%, 31% and 36.4% in the natural cycle, natural with hCG, HRC and HRC with GnRH-a groups, respectively ($p=0.83$). Ongoing pregnancy ($p=0.89$) and miscarriage ($p=0.33$) rates were comparable between groups. The rate of live birth was 30.8% in the natural group, 30% in the natural with hCG, 27.4% in the HRC and 31.4% in the HRC with GnRH-a groups ($p=0.91$).

Conclusion Four different types of endometrial preparation methods for FET cycles appear to be equally effective in terms of implantation, pregnancy, miscarriage and live birth rates in women with normal menstrual cycles.
Clinical Trial Registration Number: NCT02251925.

Keywords Endometrial preparation · Frozen embryo transfer · Hormone replacement cycle · Natural cycle · Pregnancy outcomes

Introduction

From the first report of successful frozen embryo transfer (FET) in 1983 [1], cryopreservation of embryos has been an important complementary process and the essential part of IVF routine programs. The recent policy of limiting the number of transferred embryos in a fresh IVF cycle, the role of FET in prevention of OHSS and the advanced cryopreservation techniques have increased the value of embryo freezing over the last decades [2–4]. Evidence indicates that frozen-thawed embryo transfer can be associated with higher success rates compared to the fresh embryo transfer in normal and poor responders in IVF cycles [5].

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It has been demonstrated that the optimum interaction of embryo and maternal endometrium has a great role in the success of implantation process [6]. Indeed, the low rate of success in many IVF/ET programs can be due to transferring of embryo to a non-receptive endometrium [7]. Therefore, applying the most appropriate preparation method to improve the endometrial receptivity would be one of the major clinical factors affecting pregnancy outcomes in FET [8, 9]. Previous data showed similar implantation, pregnancy and live birth rates between natural and hormonally programed cycles for FET [10–14], while other studies emphasized on natural cycles as the best option [15, 16].

In a randomized controlled trial, pregnancy outcomes were comparable between hormonal replacement cycles (HRC) with and without prior suppression with GnRH-a [17]. Similarly, Queenan et al. noted that pre-treatment with GnRH-a in artificial endometrial preparation cycles is not needed [18], while another trial confirmed the importance of down-regulation with GnRH-a in HRC to achieve favorable pregnancy and birth outcomes [19]. However, to date there is no definite evidence supporting any of these approaches in terms of improving pregnancy outcomes. This trial aimed to compare the pregnancy outcomes in four different endometrial preparation methods prior FET in women with normal menstrual cycles.

Materials and methods

Study design and population

This randomized controlled trial was carried out on 471 infertile women who underwent IVF/ICSI and FET in Reproductive Biomedicine Research Centre at Royan Institute, between September 2011 and August 2017. The study was approved by the Institutional Review Board and Ethics Committee of Royan Institute. Our study was registered in the Clinical Trial Website (www.clinicaltrials.gov, number NCT02251925).

Inclusion criteria were age 20–37 years, normal menstrual cycle, BMI less than 30 and the first FET cycle. Exclusion criteria were oocyte or embryo donation cycles, uterine malformations, hyperprolactinemia, thyroid disorders, ovulation disorders, history of recurrent miscarriage, tuberculosis and severe endometriosis.

Endometrial preparation

Eligible patients were randomly allocated into four groups: natural cycle, natural with human chorionic gonadotropin (hCG) to trigger ovulation, HRC and HRC with pre-treatment with GnRH-a.

In the natural cycle, urine luteinizing hormone (LH) was measured daily by patients from the eighth day of the cycle using LH detection kits (ABON Biopharm, Hangzhou, China). Five days after positive LH surge, in case of ultrasound evidence of collapsed follicles and endometrium thickness ≥ 7 mm, frozen-thawed embryos were transferred.

In the natural cycle with hCG, after ultrasound evidence of mature follicles (≥ 17 mm) and endometrium thickness ≥ 7 mm, 10,000 IU urinary hCG (Choriomon, IBSA, Lugano, Switzerland) was administered to trigger ovulation and 3–5 days later, thawed embryos were transferred. Luteal phase was supported with 2500 IU hCG (Choriomon, IBSA, Lugano, Switzerland) every 3 days.

In the HRC, endometrial preparation was started from the second day of menstrual cycle with daily administration of 6 mg oral estradiol valerate (Aburaihan Co, Tehran, Iran) for 10 days.

In down-regulated HRC, GnRH-a (Superfact, Aventis, Frankfurt, Germany) was administered at a subcutaneous daily dose of 0.5 mg commencing in the preceding midluteal phase. On days 2 and 3 of the menstrual cycle, an ultrasound scan and E_2 measurement was performed to confirm pituitary desensitization and if endometrial thickness was less than 5 mm and serum oestradiol level was less than 50 pg/ml, endometrial preparation was started using 4 mg oestradiol valerate daily. In both HRC groups, after 10 days of oestradiol administration, if favorable thickness of endometrium (≥ 7 mm) was confirmed by ultrasound, oestradiol valerate was continued with the same dose and 50 mg progesterone (Progestin®, Aburaihan Pharmaceutical Co., Tehran, Iran) intramuscularly was administered for 2 days and embryos were transferred. Otherwise, the dosage of oestradiol was increased to 8 mg/day until the favorable thickness of endometrium was achieved.

Luteal phase was supported by administration of intramuscular progesterone (Progestin®, Aburaihan Pharmaceutical Co., Tehran, Iran).

According to the patients' age, up to three frozen embryos were thawed and transferred at cleavage stage. In HRC groups, hormone therapy was continued until pregnancy test was performed and in case of positive pregnancy, administration of estradiol valerate and progesterone continued until 10 weeks of gestation. All the pregnant women were followed by the end of pregnancy. Permuted block randomization was carried out by a statistician according to a computer-generated list. The patients' enrolment and assignment to intervention and control groups were performed by a research midwife in the clinic. Each patient was participated in the study only once.

Outcome measures

The primary outcome was live birth rate. The secondary outcomes included implantation, biochemical and clinical pregnancy, ongoing pregnancy, and late miscarriage rates. Implantation rate was calculated with the number of observed gestational sacs divided by the number of embryos transferred for each patient. Clinical pregnancy was defined as the presence of a gestational sac with fetal heart rate on ultrasound. Late miscarriage was defined as the spontaneous loss of a clinical pregnancy between 14 and 20 weeks of gestation. The ongoing pregnancy rate was defined as the number of pregnancies confirmed by ultrasound scan and continued for at least 21 weeks after embryo transfer. Live birth referred to the birth of a live fetus regardless of the duration of pregnancy.

Calculation of sample size

Sample size calculation was based on the findings from a pilot study. Assuming a 13% difference in pregnancy rate, a sample size of 230 patients was needed in each group of natural and hormonal at a significance level (alpha level) of 0.05 and a power of 80%.

Sample size was also calculated based on the live birth rate as the primary outcome considering a medium effect size of 0.4. The number of subjects needed in each arm with $\alpha = 0.05$ and $\beta = 0.20$ (power 80%) was considered 100 participants. Due to the expected drop out, over 100 patients were considered in each group.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 23.0. The continuous variables were analyzed using independent *t* test and one way analysis of variance (ANOVA) test and were reported as mean ± standard deviation (SD). Categorical variables were analyzed by Chi-square test and reported as numbers/percentages. A p value < 0.05 was considered statistically significant.

Results

The flow diagram of the subjects according to the Consolidated Standards of Reporting Trials (CONSORT) guideline is shown in Fig. 1. During the study period, 541 infertile patients participated in the study. Of these,

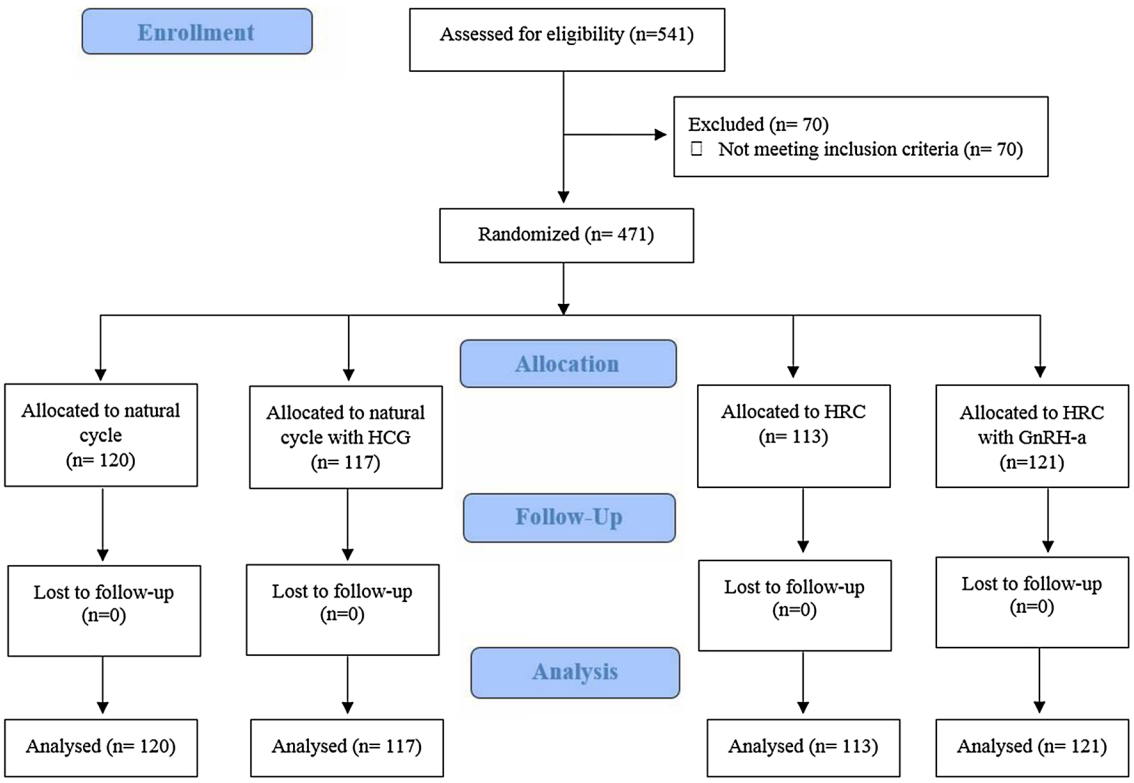

Fig. 1 CONSORT flow diagram

70 women were excluded due to non-eligibility for entering the study and a total of 471 women were randomly assigned to natural cycle with spontaneous ovulation ($n=120$), natural cycle with hCG for ovulation induction ($n=117$), HRC ($n=113$) and HRC with pre-treatment with GnRH-a ($n=121$). The baseline characteristics of patients (Table 1) and the number ($p=0.33$) and quality of embryos transferred ($p=0.21$) were similar between groups. Endometrial thickness was greater in natural versus hormonal cycles, although it did not reach the statistical significance ($p=0.05$) (Table 2).

Clinical outcomes for the randomized groups are shown in Table 2. There were no significant differences in implantation ($p=0.97$), biochemical ($p=0.90$) and clinical pregnancy rates per ET ($p=0.83$) between the groups. The rate of ongoing pregnancy was 31.7% for the natural, 30% for the natural plus hCG, 27.4% for the hormonal and 31.4% for the hormonal plus GnRH-a groups. The difference was not significantly different ($p=0.89$).

The miscarriage rates were comparable between four groups ($p=0.33$). The rate of live birth was 30.8% in the natural cycle, 30% in the natural cycle with hCG, 27.4% in the HRC and 31.4% in the HRC-GnRH-a ($p=0.91$) groups (Table 2).

Pregnancy outcomes were also compared between two groups of natural and hormonal and no significant differences were observed in terms of biochemical ($p=0.72$), clinical ($p=0.94$), and ongoing pregnancy ($p=0.75$),

Table 1 Patients' characteristics in the study groups

	Natural ($n=120$)	Natural+hCG ($n=117$)	Hormonal ($n=113$)	Hormonal+GnRH-a ($n=121$)	*P* value
Age (years)	28.49 ± 3.92	29.30 ± 4.01	28.33 ± 4.36	28.66 ± 4.60	0.31
BMI (kg/m^2)	23.41 ± 2.67	24.32 ± 2.84	24.00 ± 2.43	23.56 ± 2.76	0.08
Duration of infertility (years)	5.47 ± 3.61	6.28 ± 3.66	5.15 ± 2.92	5.88 ± 3.41	0.43
Causes of infertility					0.77
Male	(81.7) 98	94 (80.3)	91 (80.5)	97 (80.2)	
Tubal and male	2 (1.7)	1 (0.9)	2 (1.8)	2 (1.7)	
Tubal	5 (4.2)	1 (0.9)	1 (0.9)	4 (3.3)	
Endometriosis	0 (0)	0 (0)	1 (0.9)	1 (0.8)	
Unexplained	15 (12.5)	21 (18)	18 (16)	17 (14)	
Type of cycle					0.86
IVF	1 (0.8)	2 (1.7)	1 (0.9)	1 (0.8)	
ICSI	92 (76.7)	96 (82.1)	91 (80.5)	100 (82.6)	
IVF and ICSI	27 (22.5)	19 (16.2)	21 (18.6)	20 (16.5)	

Values are reported as means ± standard deviations or numbers (percentages)

Table 2 Comparison of the cycle and pregnancy outcomes among cycle regimens used for FET

	Natural ($n=120$)	Natural + hCG ($n=117$)	Hormonal ($n=113$)	Hormonal + GnRH-a ($n=121$)	*P* value
No. of embryos transferred	2.51 ± 0.50	2.59 ± 0.49	2.48 ± 0.50	2.50 ± 0.50	0.33
No. of good embryos transferred	1.08 ± 1.05	0.97 ± 0.96	1.04 ± 1.06	1.24 ± 1.00	0.21
No. of excellent embryos transferred	1.10 ± 1.12	1.29 ± 1.00	1.12 ± 1.02	0.93 ± 1.07	0.07
Endometrial thickness(mm)	10.25 ± 1.81	10.36 ± 1.50	9.90 ± 1.61	9.91 ± 1.53	0.05
Implantation rate (%)	20.3 (61/301)	18.8 (57/303)	19.3 (54/280)	19.2 (58/302)	0.97
Biochemical pregnancy rate per ET (%)	40.8 (49/120)	43.6 (51/117)	39 (44/113)	42.1 (51/121)	0.90
Clinical pregnancy rate per ET (%)	34.2 (41/120)	32.5 (38/117)	31 (35/113)	36.4 (44/121)	0.83
Ongoing pregnancy rate per ET (%)	31.7 (38/120)	30 (35/117)	27.4 (31/113)	31.4 (38/121)	0.89
Multiple pregnancy rate (%)	36.6 (15/41)	34.2 (13/38)	34.3 (12/35)	22.7 (10/44)	0.51
Late miscarriage rate (%)	0 (0/41)	8 (3/38)	11.4 (4/35)	11.4 (5/44)	0.33
Live birth rate (%)	30.8 (37/120)	30 (35/117)	27.4(31/113)	31.4 (38/121)	0.91

Values are reported as means ± standard deviations, or as numbers (percentages)

miscarriage ($p = 0.07$) and live birth rates ($p = 0.83$). The patient characteristics were not statistically different between these two groups.

Discussion

This is the first randomized controlled trial comparing four different endometrial preparation methods for FET cycles including spontaneous natural cycle, natural with hCG to trigger ovulation and HRC with or without down-regulation with GnRH-a. We found no significant differences in pregnancy outcomes between natural and hormonal stimulated cycles.

Natural cycle provides the advantage of using the endogenous steroids for endometrial preparation without exposing to the risks of exogenous hormones. In this method, the time of embryo transfer is determined by LH surge or triggering ovulation with hCG [13]. Several retrospective studies suggested natural cycle with or without hCG as the best option for endometrial preparation before FET in women with normal ovulatory cycles [12, 15, 16]. However, women undergoing natural cycle require several cycle monitoring for ovulation timing to prevent the risk of cycle cancelation [13].

The appropriate timing of ovulation and the subsequent period of endometrial receptivity are of great importance in implantation success. Implantation window, the period that endometrium has the highest receptivity for blastocyst, is regulated by the effect of estrogen and progesterone on endometrium [20, 21]. Obviously, in cases of spontaneous ovulation, the accurate time of implantation window is difficult to identify that may put women at risk of cycle cancelation [13]. Triggering ovulation with hCG in a natural cycle offers the advantage of no daily LH monitoring and less number of visits than the cycles without hCG [22, 23]. Chang et al. [15] demonstrated that administration of hCG creates favorable endometrial thickness. They showed higher clinical and ongoing pregnancy rates in both spontaneous natural cycle and natural cycle using hCG versus hormonal group in a vitrified blastocyst transfer program, whereas Fatemi et al. [23] reported a lower rate of ongoing pregnancy when hCG was administered for ovulation induction compared to a spontaneous natural cycle (14% versus 31%, respectively). To date, the role of hCG in improvement of clinical outcomes in FET cycles has not been well understood and further trials are required.

Endometrial preparation with artificial HRC using exogenous estrogen and progesterone [18, 24] would help to determine the accurate time of ovulation and planning for embryo transfer with significant reduction of cycle cancelation, particularly in women with irregular menstruation [25, 26]. Less cycle monitoring and easier planning for embryo transfer have provided more convenience for both patients and staff [17]. However, the superiority of artificial cycle over the natural cycle in terms of the pregnancy outcomes has not yet been confirmed.

Two large retrospective cohort studies concluded improved pregnancy and live birth rates in a hormonally controlled cycle with or without prior administration of GnRH-a compared to a natural cycle in FET cycles [27, 28]. In contrast, a retrospective study on 1235 FET cycles over a period of 12 years, greater endometrial thickness and higher implantation and pregnancy rates were observed in natural versus exogenous HRT [29]. However, in a recent prospective trial comparing these groups, no significant differences were observed in pregnancy and birth outcomes, although artificial cycle was associated with higher cycle cancelation. In addition, the cost of treatment in both methods was comparable [30], while natural cycle can reduce the total treatment cost compared to the HRC due to the absence of medication [31]. "In our infertility center, natural cycle patients are required more visits to the clinic compared to women in hormonal protocol. Considering that pharmaceutical products are more costly than clinical visits in our country, the overall cost of natural cycle is still cheaper for the patients".

Down-regulated GnRH-a cycles can decrease the risk of premature ovulation and incorrect timing of FET and prevent cycle cancelation [13]. Although in Queenan et al. study, despite the lack of pituitary suppression, no premature ovulation was reported and only 2% of the cycles were canceled that was not related to endometrial thickness. Applying the exogenous hormones without GnRH-a can be associated with less side effects and costs [18]. Similarly, two other studies did not agree with pre-treatment with GnRH-a [17, 26]. This treatment may be associated with hypoestrogenic risk prior to hormonal administration [24].

In contrast, in a randomized controlled trial on 234 patients undergoing FET, higher pregnancy and live birth rates were achieved in women who received estradiol valerate from the first day of stimulation (6 mg/d) after pituitary desensitization with GnRH-agonist (Superfact nasal spray) compared to women without prior ovarian suppression [19].

Our data are consistent with other trials indicating successful pregnancy outcomes in endometrial preparation with both natural and hormonal cycles [10–12, 26]. Givens et al. failed to show any significant differences in birth rates between women who underwent natural and hormonal cycles [32]. Similarly, in a recent pilot randomized trial on 159 women with regular menstrual cycles, similar live birth rates were observed between natural and hormonally stimulated cycles, although the lack of significant difference between groups may be due to the small sample size [31]. Two recent meta-analyzes also could not show the superiority of one endometrial preparation method over others among women

with normal cycles [13, 14]. However, in a retrospective study, lower estrogen levels and greater endometrial thickness in natural cycle was associated with higher pregnancy rate (37%) compared to the artificial methods using exogenous hormones (23%) [16]. In the present study, endometrial thickness was higher in natural cycle than hormonal group ($p=0.05$). However, the rates of implantation and pregnancy were similar between groups. Indeed, endometrial histology and microenvironment may be a stronger predictor of implantation than endometrial thickness that is regulated by ovarian hormones, estrogen and progesterone [15]. Therefore, natural cycle would be the best endometrial preparation method for women with normal ovulatory cycles, as exogenous hormones may change the physiologic hormonal levels possibly leading to poor pregnancy outcomes [16].

Our study found comparable late miscarriage rate between hormonally controlled and natural cycles that was consistent with a previous study [26]. Although, in a retrospective analysis on 666 natural and 466 hormonal FET cycles, the miscarriage rate was significantly higher in HRC (23%) compared to the natural cycle (11.4%) [33].

The novelty of research comparing four different methods of endometrial preparation and large sample size were the key strengths of this study. The potential weakness of the current trial includes the lack of blindness and a control arm treated with placebo. However, women's age, the number and quality of transferred embryos and endometrial thickness were not significantly different between groups to avoid any possible bias selection. These factors have been reported as the main factors affecting FET outcomes [9].

Conclusion

This study shows that all endometrial preparation methods for frozen-thawed embryo transfer programs were equally effective in improvement of pregnancy and birth outcomes in women with normal menstrual cycles. As such, the natural cycle protocols would be the preferred method of endometrial preparation among women with normal ovulatory cycles due to ease of use, less side effects and lower cost. However, the findings of this study may provide a basis for further larger clinical trials.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All the procedures performed in studies involving human participants were in accordance with the ethical standards committee of the Royan Institute and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethics Approval Code EC/91/1087.

Informed consent Informed consent was obtained from all the individual participants included in the study.

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