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ASSISTED REPRODUCTION TECHNOLOGIES

Frozen blastocyst embryo transfer using a supplemented natural cycle protocol has a similar live birth rate compared to a programmed cycle protocol

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Abstract
Purpose The purpose of this study is to compare outcomes for a supplemented natural cycle with a programmed cycle protocol for frozen blastocyst transfer.
Methods A retrospective analysis was performed of frozen autologous blastocyst transfers, at a single academic fertility center (519 supplemented natural cycles and 106 programmed cycles). Implantation, clinical pregnancy, miscarriage, and live birth and birth weight were compared using Pearson's Chi-squared test, *T*-test, or Fisher's exact test.
Results There was no significant difference between natural and programmed frozen embryo transfers with respect to implantation (21.9 vs. 18.1 %), clinical pregnancy (35.5 vs. 29.2 %), and live birth rates (27.7 vs. 23.6 %). Mean birth weights were also similar between natural and programmed cycles for singletons (3354 vs. 3340 g) and twins (2422 vs. 2294 g)
Conclusion Frozen blastocyst embryo transfers using supplemented natural or programmed protocols experience similar success rates. Patient preference should be considered in choosing a protocol.

Keywords Frozen embryo transfer · Natural cycle · Pregnancy outcome · Programmed cycle

Capsule Supplemented natural cycle with hCG trigger and luteal progesterone support is equivalent to hormone replacement cycles for frozen blastocyst transfer.

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Introduction

Frozen embryo transfer (FET) is assuming a greater role in the practice of infertility than it has in the past. In 2012, 28.4 % of cycles reported to the Society for Assisted Reproductive Technology and the Center for Disease Control and Prevention were frozen embryo transfers compared with 16.1 % in 2003 [1]. FET has been primarily utilized when more good quality embryos were produced than could safely be transferred, if there was concern regarding risk of ovarian hyperstimulation syndrome, or when endometrial development during the fresh IVF cycle was inadequate. Indications for frozen embryo transfer have expanded. In more recent years, all embryos in a cohort may be frozen, with no fresh transfer performed in cycles utilizing preimplantation genetic diagnosis, in patients with a history of recurrent implantation failure, in cycles associated with a premature progesterone rise, and due to concerns regarding the effects of ovarian stimulation on the endometrium [2]. In addition, increasing emphasis on elective single embryo transfer (eSET) has also likely contributed to a greater number of embryos available for cryopreservation and future transfer after thaw.

Despite the high number of FETs performed each year, an optimal method of endometrium preparation for FETs remains

uncertain [3, 4]. Various protocols have been described for endometrial preparation in FET cycles, including dependence on endogenous hormones produced in a spontaneous ovulation cycle or the use of exogenous hormones, and downregulation with GnRH agonists followed by use of exogenous hormones [5–8]]. Some studies have shown equivalent ongoing pregnancy/live-birth rates between natural cycle protocols compared with protocols using exogenous hormone [6, 9–13]. In contrast, other authors have reported higher success rates in ovulatory cycles [14, 15] while still others have found that programmed cycles are superior [16, 17]. A Cochrane review prior to publication of many of these studies found no evidence in support of one protocol over another [5]. Given the range of reported outcomes and the importance of FET, further study of natural or supplemented natural versus programmed cycle FETs is warranted.

Our program has a large percentage of patients undergoing FETs in the context of spontaneous menstrual cycles, with some variance in protocol compared with what has been published in recent literature. Many of our patients tend to prefer the most natural approach to treatment. Natural cycle FET requires less medication and fewer injections, but it is less predictable compared with the programmed FET. With the increasing utilization of FET, it is imperative to continue working to optimize the protocol for embryo replacement. The goal of this study is to compare the live-birth rates and pregnancy outcomes following FETs conducted using our protocol for supplemented natural cycle with progesterone luteal phase support to those in which the endometrial preparation was achieved via a GnRH agonist suppression and administration of exogenous estradiol and progesterone.

Materials and methods

After obtaining approval from Stanford's Institutional Review Board, all FET cycles performed from January 21, 2007 to January 17, 2012 at Stanford University's IVF program were evaluated for potential inclusion in this study, with the goal of identifying transfers of thawed blastocysts from cycles which took place in either natural cycles or in programmed cycles, and which included GnRH agonist and exogenous hormone to prepare the endometrium. Cycles using embryos cryopreserved at the 2 PN stage or on day three (rather than as blastocysts) and cycles using donor oocytes were excluded. Programmed cycles without GnRH agonist down regulation were excluded as the population of patients undergoing such regimens was limited in our center during the timeframe of the study. There was no exclusion from analysis on the basis of indication for fertility treatment, patient age, and stimulation of preceding fresh cycle. However, FETs were almost exclusively performed with surplus embryos, not as a result of prior embryo banking without a fresh transfer. Only first FET attempts were included in this analysis. A total of 625 frozen-thawed blastocyst-stage FETs were identified that met inclusion and exclusion criteria. Generally, the treating physician recommended the supplemented natural cycle protocol to patients with ovulatory cycles, while programmed cycles were recommended to those with anovulatory cycles.

Supplemented natural cycle FET protocol

A baseline transvaginal ultrasound was performed during menses. Relative to a 28 days cycle, the next ultrasound was approximately performed on cycle day 11–12. In shorter and longer cycles, this ultrasound was performed earlier or later thus adjusting for cycle length. Patients were instructed to use a home urinary ovulation predictor kit beginning on cycle day 10–11 and to notify the clinic if the result was positive. When transvaginal ultrasound revealed a lead follicle of at least 18 mm and the patient reported a negative test on her home ovulation predictor kit, 250 μg of recombinant hCG was administered at approximately 9 PM in the evening. Four days after hCG was taken, patients began two daily doses of 100 mg vaginal progesterone. Seven days after the hCG was taken, the FET was performed. If patients detected an LH surge using the ovulation predictor kit, with a lead follicle of 16–20 mm and adequate lining, the FET was scheduled 6 days after the detection of the LH surge, and progesterone 100 mg twice daily was initiated 3 days after the LH surge. If there was any uncertainty regarding the kit, an hCG "booster" shot was administered the morning the positive LH surge was detected, and the FET was performed 6 days afterward. Vaginal progesterone supplementation 100 mg twice daily was continued until the positive pregnancy test, and up until 10–12 weeks gestation for those with ongoing pregnancy. Patients attempting FETs with natural cycles but not reaching and endometrial thickness of 7 mm or greater, were canceled. Typically these patients would be counseled to used programmed cycles in future attempts at FETs.

Programmed FET protocol

GnRH agonist suppression followed by exogenous estrogen treatment: Patients were given oral contraceptive pills (OCPs) as pre-treatment. After approximately 2 weeks of OCPs, daily subcutaneous leuprolide acetate (10 units (1 mg)) was begun after an ultrasound confirmed no functional cysts greater than 2 cm. OCPs were discontinued after a 7 days overlap with GnRH agonist. When menses occurred, increasing doses of 17β-estradiol were administered as follows; one 0.1 mg estradiol patch every alternate day for 4 days, followed by two 0.1 mg estradiol patches every alternate day for 4 days patches, followed by three 0.1 mg estradiol patches every alternate day until a pregnancy test. After about 2–4 days at the three patch dose, a transvaginal ultrasound was performed

to assess the uterine lining. If the uterine lining was inadequate (<7 mm), 2 mg of vaginal estradiol was added once or twice daily. When the lining was adequate, the GnRH agonist treatment was discontinued and 25 mg (0.5 cc) of intramuscular (IM) progesterone in oil was administered for 1 day, increasing to 50 mg (1 cc) the next day, and this IM progesterone was continued until the pregnancy test. In addition, vaginal progesterone, 100 mg three times daily was added the day after the first 50 mg IM dose, and also continued until the pregnancy test. FETs were performed on patients fifth full day of taking vaginal progesterone, with the first 50 mg IM counted as day 0. If the pregnancy test was positive, estradiol 0.1 mg patches three times daily, IM progesterone 50 mg, and vaginal progesterone 100 mg three times daily were all continued until the first obstetrics ultrasound at 6–7 weeks gestation. The IM progesterone dose was reduced to 25 mg the night prior to the first scheduled obstetric ultrasound. The estradiol patches and vaginal progesterone were continued until 10–12 weeks gestation, but the IM progesterone dose was discontinued between the seventh and ninth weeks at the physicians' discretion depending on patients' symptoms and serum progesterone levels.

Routine treatment process for natural or programmed transfer cycles

Blastocysts were thawed the day of embryo transfer approximately 1–3 h prior to transfer, only embryos with>50 % survival were transferred. Because of the variable time between thaw and transfer, expansion of blastocysts was not a requirement for transfer. A transabdominal ultrasound-guided embryo transfer was performed using a Tefcat or Echotip Softpass catheter (Cook Ob/Gyn, Spencer, IN). A pregnancy test was done 9–11 days after frozen blastocyst transfer, and considered to be positive if the β-hCG level was greater than 5 mU/L.

Statistical analysis

Numerical variables were summarized by mean and standard deviation. Two-sample t-tests were used to compare differences between natural and programmed cycles at the patient level as well as the cycle level. Gaussian assumption for the *t*-test was satisfied because of the adequate number of patients and cycles in the analyses. Categorical variables were summarized as proportions and compared using the Pearson's Chi-square test. The Fisher's exact test was also performed for categorical variables exhibiting sparse data. The results from the Fisher's exact tests were omitted from presentation because they led to the same inference as the Pearson's Chi-square tests. All statistical tests were evaluated at the 0.05 level, and all statistical analyses were performed using R 3.0.2.

Outcome variables The primary outcome variable of interest was the live birth rate (LBR), comparing natural with programmed cycles. Other secondary outcomes of interest were rates of clinical intrauterine pregnancy, implantation, biochemical pregnancy, and spontaneous abortion. Clinical intrauterine pregnancy (IUP) was defined as gestational sac seen on a transvaginal sonogram at 6–7 weeks gestational age with presence of a fetal heartbeat. Implantation rate was defined as the number of gestational sacs with a fetal heartbeat seen on transvaginal ultrasound divided by the number of embryos transferred. Biochemical pregnancy loss was defined as an initially positive β-hCG result that spontaneously declined with serial measurements, with no gestational sac on transvaginal sonogram, and no suspicion for ectopic pregnancy. Spontaneous abortion (SAB) was defined as loss of pregnancy after clinical IUP. Live birth data were collected by contacting patients and the live birth rate was defined as the number of live births divided by the total FET number of cycles which proceeded to embryo thaw.

Results

Medical records of all blastocyst FET cycles which met inclusion and exclusion criteria (n=625) were reviewed in detail, including 519 (83 %) cycles following a suplemented natural cycle protocol and 106 (17 %) cycles following a programmed cycle protocol. As described in Table 1, programmed and natural cycle patients groups were similar with respect to baseline pregnancy history (Table 1).

As noted in Table 2, the treatment parameters were similar between natural and programmed FET cycles. There were no differences between groups with respect to the number of embryos transferred and maximal endometrial thickness. Timing of the embryo transfer was determined by administration of hCG in 92.1 % of natural cycles.

There were no differences in treatment outcomes between natural and programmed FET cycles (Table 3). The rates of implantation, clinical intrauterine pregnancy, biochemical pregnancy, ectopic and live birth did not differ between groups. There was also no difference in the rate of spontaneous abortion between groups. The rate of twins was not different between groups. Finally, the birth weight did not differ between natural and programmed cycle FETs.

Discussion

We found no statistically significant difference in rates of clinical pregnancy and live birth between supplemented natural cycle FETs and programmed cycle FETs. Our results are in line with those of other studies in current literature, which have also found no difference [6, 9–12, 18].

Table 1 Demographic characteristics for patients undergoing supplemented natural FET and programmed FET cycles

Variable	Supplemented natural cycle (*N*=519 patients)	Programmed cycle (*N*=106 patients)	*p*-value
Age (mean±std)	36.10±4.11	35.44±4.94	0.20[1]
BMI (mean±std)	23.44±4.03	24.27±5.12	0.12[1]
Nulligravid (%)	35.8 %	36.8 %	0.94[2]
One or more prior full term births (%)	37.6 %	34.0 %	0.55[2]
Prior pre term birth (%)	0.6 %	0.9 %	1[2]
Prior SABs (%)	30.3 %	38.7 %	0.11[2]
Smoker (%)	2.5 %	3.8 %	0.69[2]

[1] two-sample t test

[2] Pearson's Chi-square test

Our finding of no difference between protocols contrasts with studies which found higher success rate with programmed cycles [16, 17]. Neither study used hCG trigger to time ovulation, instead they relied on home ovulation predictor kits, disappearance of dominant follicle or serum hormone analysis to time embryo transfer. These methods may be less precise and lead to embryo transfer outside of the window of implantation and could explain the lower pregnancy rates in natural cycle. With the use of a supplemented natural cycle, using hCG trigger, we are able to reduce the number of ultrasounds and blood tests and increase precision of timing of the frozen embryo transfer.

Our conclusion contrasts with studies which have shown higher success rates in ovulatory cycles [13–15] Levron et al. [14] reported ongoing pregnancy rates of 10.4 % with natural cycle vs 5.9 % with programmed cycle. These rates for both groups were much lower than ours, possibly due to the fact that they included only thawed cleaved embryos or due to differences in patient population. Morozov et al. [15] reviewed FETs for 162 patients who had day 3 embryos frozen and thawed. They noted a greater endometrial thickness in their natural cycles, a finding that we did not confirm. It is possible that differences in protocol for the natural cycle FET explain the differences between studies, as Veleva et al. [4] reported that compared with spontaneous cycles with luteal support, purely spontaneous cycles (OR 0.58, CI 0.40–0.84) and hormonally substituted FET (OR 0.47, CI 0.32–0.69) diminished the odds of pregnancy.

Our supplemented natural cycle, using ultrasound monitoring, hCG trigger and luteal support allows for the use of natural ovulation while minimizing the risk of timing errors or luteal dysfunction. A few studies in the literature have hypothesized that a detrimental effect of exogenous hCG on implantation results in lower success rates for patients in natural cycles who are administered hCG [19]. However, a randomized study [20] and a retrospective analysis [21] both found no difference in success rate with hCG trigger versus no trigger for timing a natural cycle FET. Given the uncertainty of home ovulation predictor kits [22] and added expense and stress of having daily blood draws, we find the certainty of the HCG trigger to time ovulation and embryo transfer when a mature follicle is present simplifies the process for patients undergoing supplemented natural cycle FET.

Our study has the limitations inherent with a non-randomized retrospective study. In analyzing the data at the cycle level there were slight differences between groups in age of questionable clinical significance. In addition, the direction of bias with age would favor the programmed cycle and the direction of bias for body mass index would favor the natural cycle. Patients who had suboptimal endometrial development with one protocol were allowed to switch to the other protocol.

Table 2 Treatment parameters in supplemented natural FET cycles compared with programmed FET cycles

Variable	Supplemented natural cycle (*N*=519 cycles)	Programmed cycle (*N*=106 cycles)	*p*-value
Number of embryos transferred (mean±std)	1.7±0.8	1.7±0.9	0.671[1]
Maximal endometrial thickness (mean±std)	9.1±1.6	8.8±2.0	0.161[1]
Use of hCG (%)	92.1 %	0 %	<0.012[2]

[1] two sample *t*-test

2 Pearson's Chi-square test

Table 3 FET treatment and pregnancy outcomes in protocols utilizing the supplemented natural cycle versus the programmed cycle. Data are displayed as number of cycles with percentage in parentheses

Treatment outcome	Supplemented natural cycles (*N*=519)	Programmed cycles (*N*=106)	*p*-value
			0.45[1]
Positive HCG (%)	45.9 %	43.4 %	
Clinical intrauterine pregnancy (%)	35.5 %	29.2 %	
Biochemical pregnancy (%)	9.8 %	13.2 %	
Ectopic pregnancy (%)	0.39 %	0.94 %	
Live birth (%)	27.7 %	23.6 %	0.45[1]
Implantation rate (%)	21.9 %	18.1 %	
Miscarriage rate (%)	19.0 %	19.4 %	>0.99[1]
Twins (%)	13.9 %	19.4 %	0.59[1]
Singletons birth weight in grams (mean±std)	3353.8±513.3 (*N*=174 infants)	3340.6±579.9 (*N*=25 infants)	0.77[2]
Twins birth weight in grams (mean±std)	2422.8±650.8 (*N*=56 infants)	2294.0±680.5 (*N*=12 infants)	0.91[2]

[1] Pearson's Chi-square test

[2] two sample *t*-test

Because we had patient in both groups switch to the other protocol, we do not think this biases our data in one direction.

We have recently published the hypothesis that maternal cardiovascular adaptation to pregnancy, fetal growth, and pregnancy outcome are optimal in the presence of a single corpus luteum [23]. In a programmed FET cycle, only estradiol and progesterone are replaced. However, the corpus luteum makes other vasoactive products which are not replaced in a programmed cycle such as relaxin and vascular endothelilal growth factor that may have important roles in the maternal cardiovascular adaptation to pregnancy. Given the increasing role of FET within the practice of IVF, future studies should examine the incidence of gestational hypertension and pre-eclampsia with natural versus programmed FETs.

Conclusion

In our population, programmed and natural cycles had similar outcomes in regard to clinical pregnancy rates, live-birth rates, and birth weights. Our findings contribute a large number of supplemented natural cycles to the literature examining natural versus programmed FET protocols. Prior studies have shown no difference, superiority of the natural cycle or higher success with the programmed cycle. Given this range of reported outcomes, it is certainly plausible that there in fact is no difference in outcome between programmed and supplemented natural cycle, consistent with our findings. Until further studies are performed which examine clinical outcomes such as incidence of pre-eclampsia in the presence versus absence of the corpus luteum, patient preference and medical history should be considered in protocol choice.

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