Original Article

Natural Cycle Frozen Embryo Transfer: Evaluating Optimal Protocols for Preparation and Timing

Kai N. Holder, Jessica S. Mormol[1], Jennifer B. Bakkensen[2], Mary Ellen Pavone[2], Kara N. Goldman[2], Chen Yeh[3], Lutfiyya N. Muhammad[3], Lia A. Bernardi[2]

Northwestern University Feinberg School of Medicine, [2]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Northwestern University Feinberg School of Medicine, [3]Department of Preventive Medicine, Division of Biostatistics, Northwestern University Feinberg School of Medicine, Chicago, IL, [1]Department of Obstetrics and Gynecology, The Ohio State University, Columbus, OH, USA

Abstract

Background: While natural cycle frozen embryo transfer (NC-FET) is becoming increasingly common, significant practice variation exists in the use of ovulation induction medications, administration of ovulation trigger, and timing of embryo transfer without consensus as to the optimal protocol. **Aims:** The objective of this study is to evaluate the association of key aspects of the NC-FET protocol with implantation, pregnancy and live birth. **Settings and Design:** This was a retrospective cohort study of blastocyst stage NC-FET cycles from October 2019 to July 2021 at a single academic fertility centre. **Materials and Methods:** Protocols varied between cycles across three key parameters which were evaluated as primary predictors of cycle outcomes: (1) use of letrozole for mild ovarian stimulation/ovulation induction, (2) administration of exogenous ovulation trigger versus spontaneous luteinising hormone surge and (3) transfer timing based on ovulation trigger versus sequential progesterone monitoring. Primary outcomes included implantation rate, clinical pregnancy and ongoing pregnancy. **Statistical Analysis Used:** Generalised estimating equations were fitted to obtain adjusted odds ratios or rate ratios as appropriate with 95% confidence intervals for each outcome across the three primary predictors. **Results:** A total of 183 cycles from 170 unique patients were eligible for inclusion. The average implantation rate was 0.58, resulting in an overall clinical pregnancy and ongoing pregnancy rate of 59.0% and 51.4%, respectively. After adjusting for age at embryo freeze and history of a failed embryo transfer, there were no significant associations between any predictor and implantation rate, clinical pregnancy, ongoing pregnancy, or live birth. **Conclusion:** In NC-FET, a variety of preparation and timing protocols may lead to comparable cycle outcomes, potentially allowing for flexibility on the basis of patient and physician preference. These findings warrant validation in a larger, randomised trial.

Keywords: *Endometrial preparation, frozen embryo transfer, natural cycle*

Introduction

As the use of frozen embryo transfer (FET) continues to rise, the optimal endometrial preparation protocol has yet to be determined. While programmed cycles have traditionally been used, natural cycle FET (NC-FET) has become increasingly common in light of data suggesting that NC-FET cycles are equally effective in achieving pregnancy and live birth[1-5] while reducing maternal and neonatal morbidity when compared with programmed cycles as evidenced by lower rates of pre-eclampsia, pre- and post-term birth, caesarean

Address for correspondence: Ms. Kai N. Holder, Northwestern University Feinberg School of Medicine, 420 E, Superior St., Chicago, IL 60611, USA.
E-mail: kai.holder@northwestern.edu

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section and postpartum haemorrhage.[6-14] For these reasons, NC-FET is an attractive option for patients and clinicians pursuing FET.

Despite this increase in popularity, there is currently no clear consensus on the optimal protocol for NC-FET. For example, some centres use oral agents such as letrozole for mild ovarian stimulation among ovulatory patients or ovulation induction among anovulatory patients. Preliminary data suggest conflicting results on whether outcomes differ with the use of letrozole, with some studies suggesting improved outcomes with letrozole use and others finding no benefit.[3,15,16]

Furthermore, there is debate surrounding the use of 'modified' natural cycles, in which ovulation is induced by the administration of an exogenous trigger, usually in the form of human chorionic gonadotropin (hCG). Modified natural cycles are thought to enhance the control of NC-FET, as triggering is done when the leading follicle is between 16 and 20 mm in diameter, and reduce the likelihood of cycle cancellation as compared with 'true' natural cycles; in a true natural cycle, FET scheduling is dependent on the timing of spontaneous ovulation, which rely on a patient's endogenous luteinising hormone (LH) surge for spontaneous trigger of ovulation, which makes this method less flexible.[17] The use of exogenous trigger has been studied with conflicting results. There are studies that have demonstrated lower pregnancy and live birth rates (LBRs) in patients receiving an exogenous trigger compared to a spontaneous trigger.[18-20] However, the majority of data suggests the use of ovulation triggers demonstrates no significant difference in cycle outcomes compared to true natural cycle but can lead to a possible increase in cost-effectiveness and convenience.[21-26]

Finally, the timing of embryo transfer is the subject of much debate. It has been proposed that embryo transfer should occur 7 days following an exogenous hCG trigger or 6 days following one's endogenous LH surge.[22,27] While transfer timing following exogenous hCG trigger may be carried out with some precision, the timing following one's endogenous LH surge may be less clear, given significant variation in amplitude and duration of the LH surge among women.[28-31] Citing this concern, some physicians at our centre monitor progesterone levels following either hCG trigger or LH surge and schedule embryo transfer on the basis of a pre-specified progesterone threshold. While this method reduces the need to precisely identifying the LH surge and ensures a post-ovulatory progesterone rise, it has not previously been studied as a validated method of embryo transfer timing in NC-FET.

Overall, there is a lack of a consensus in the existing literature on NC-FET timing and techniques. This study aimed to evaluate key aspects of the NC-FET protocol to determine their association with implantation, pregnancy and LBRs. We aim to evaluate if outcomes differ based on the use of letrozole for ovarian stimulation/ovulation induction, the administration of an hCG trigger, or the method of embryo transfer timing based on hCG trigger versus sequential progesterone monitoring.

Materials and Methods

This was a retrospective cohort study at a single academic fertility centre. All autologous, blastocyst-stage NC-FET cycles from October 2019 to July 2021 were included in the analysis. Exclusion criteria included cycles cancelled before transfer and cycles from patients with uterine factor infertility. Cycles cancelled before transfer and cycles from patients with uterine factor infertility were excluded from the analysis. This study was approved by the Northwestern Institutional Review Board: IRB ID: STU00215931. The authors have adhered to the Principles of the Helsinki Declaration in the creation and implementation of this research study. Given the retrospective nature of the study design, informed consent was not obtained.

Clinical and laboratory protocols

Controlled ovarian hyperstimulation was performed utilising GnRH antagonist, GnRH agonist, or low-dose GnRH agonist flare protocols as previously described.[32] Following retrieval, oocytes were either inseminated or underwent intracytoplasmic sperm injection. On day 5, embryos were either cryopreserved or cultured for a further 24 h for re-evaluation of cryopreservation suitability on day 6. If pre-implantation genetic testing (PGT) was indicated, a biopsy was performed at the blastocyst stage before cryopreservation. Indications for PGT included aneuploidy screening (PGT-A), testing for monogenic disorders and testing for chromosomal structural rearrangements. Blastocysts were cryopreserved individually and warmed using the manufacturer-recommended warming protocol.

At the time of the NC-FET cycle, baseline testing with ultrasound and serum oestrogen (E2), and progesterone (P4) was performed on cycle day (CD) 1–3 following either spontaneous menses or induced withdrawal bleed. Based on clinician preference, a subset of patients began letrozole 2.5–7.5 mg daily × 5 days on CD 3 due to a history of ovulatory dysfunction or for mild ovarian stimulation per physician preference. Patients began urinary LH monitoring on CD 8. If a surge was detected, serum estradiol, progesterone and LH were drawn, and ultrasound was performed to

confirm ovulation. If no surge was detected by CD 12, patients began monitoring with serial bloodwork and ultrasound until a surge was detected (LH >17 IU/L and × 2.5 the baseline LH), with subsequent monitoring of serum progesterone daily until a threshold of 5 ng/mL was reached, at which time embryo transfer was scheduled 3 days later. Alternately, ovulation could be triggered by hCG administration with a dominant follicle >17 mm and estradiol >150 pg/mL per physician discretion. For these cycles, embryo transfer could be scheduled 7 days after hCG trigger or by sequential progesterone monitoring as above, per physician preference. Methods for timing of embryo transfer are outlined in Figure 1.

Exogenous progesterone could be administered as luteal support per physician preference. If given, luteal support was initiated in one of the following forms: endometrin 100 mg vaginal inserts administered twice daily (Ferring Pharmaceuticals), crinone 8% 90 mg vaginal gel daily (Actavis Pharma, Inc.,), prometrium 200 mg vaginally daily (Virtus Pharmaceuticals, Inc.,), or intramuscular progesterone in oil daily.

Outcomes

Primary outcomes included implantation rate (defined as the number of gestational sacs on ultrasound divided by the number of embryos transferred), clinical pregnancy (defined by the presence of one or more gestational sacs on ultrasound or a foetal heartbeat on ultrasound at 6–7 weeks' gestational age), and ongoing pregnancy (defined by the presence of a foetal heartbeat on ultrasound on discharge from the practice between 8 and 10 weeks' gestational age).[33] LBR was also calculated among the subset of patients for whom a final pregnancy outcome was available at the time of analysis.

Sample size

A sample size calculation was performed. In order to detect a 15% difference in ongoing pregnancy rate at an alpha level of 0.05 with 80% power, 324 cycles would be required in the analysis.

Statistical analysis

Descriptive statistics were calculated for demographics and cycle characteristics. Categorical variables were summarised with the use of counts and percentages. Continuous variables were reported as means and standard deviations (SDs) or medians and interquartile range, as appropriate.

A generalised estimating equation (GEE) poisson regression model was fitted to determine the rate ratio and corresponding 95% confidence interval (CI) for the association between implantation rate and each predictor. The predictors of interest were the use of letrozole for mild ovarian stimulation/ovulation induction, administration of exogenous ovulation trigger and transfer timing based on ovulation trigger versus sequential progesterone monitoring. For the remaining binary outcomes, GEEs for binary data with a logit link were constructed to obtain the odds ratio and 95% CI between each outcome and predictor. GEEs were used to account for correlations between multiple cycles from the same woman. Adjusted analyses included patient age at embryo freeze and prior history of a failed embryo transfer as potential confounders determined *a priori*. Statistical analyses were performed using SAS version 9.4 (Cary, NC).

Results

A total of 183 cycles from 170 unique patients were eligible for inclusion. Demographic and cycle characteristics are outlined in Table 1. The mean patient age at the time of embryo freeze was 35.2 years (SD 2.6 years). The majority of cycles (76.0%) were among patients with no prior history of failed transfer, and 90% were among ovulatory women. Across all cycles, over half (68.9%) utilised PGT-A, and all but 10 were

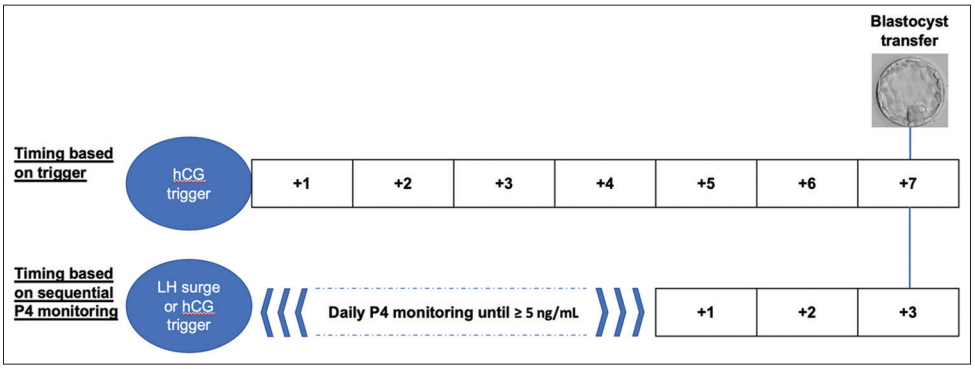

Figure 1: Clinical methods for embryo transfer timing. hCG = Human chorionic gonadotropin, P4 = Progesterone. For timing based on trigger, blastocyst transfer occurs seven days after hCG trigger (hCG + 7). For timing based on sequential progesterone monitoring, blastocyst transfer occurs three days after progesterone crosses a threshold of 5 ng/mL. LH: Luteinizing hormone

single embryo transfers. Most cycles (96.2%) involved some form of luteal support, with vaginal Endometrin and vaginal Crinone 8% gel being the most frequent formulations (81.4% and 13.1% of cycles, respectively).

Amongst all 183 cycles, 59.0% resulted in a clinical pregnancy at 6–7 weeks' gestation and 51.4% resulted in an ongoing pregnancy at the time of discharge from the practice. At the time of analysis, the final pregnancy outcome was available for 156 cycles, of which 66 (42.31%) had had a live birth. An additional 27 patients had ongoing pregnancies at that time but had not yet had a live birth and thus are not reflected in the analysis. Overall, for the 183 cycles, 50.8% resulted in a live birth or ongoing pregnancy.

Table 1: Demographics and cycle characteristics (*n*=183 cycles)

Characteristic	*n* (%), mean ± SD, or median (IQR)
Age at time of freeze (years)	35.2±3.6
Gravidity	1.0 (0.0–2.0)
Parity	0.0 (0.0–1.0)
Prior spontaneous abortion	89 (48.6)
BMI (kg/m^2)	26.2±6.4
History of failed transfer	44 (24.0)
Ovulatory dysfunction	18 (9.8)
Preimplantation genetic testing for aneuploidy	126 (68.9)
Single embryo transfer	173 (94.5)
Supplemental luteal support	176 (96.2)
Type of luteal support	
Endometrin 100 mg vaginal insert twice daily	149 (81.4)
Crinone 8% 90 mg vaginal gel daily	24 (13.1)
Prometrium 200 mg vaginally daily	1 (0.6)
Intramuscular progesterone	1 (0.6)
None	8 (4.3)
Peak endometrial thickness (mm)	8.8±2.1
Letrozole utilised	79 (43.2)
Trigger administered	54 (29.5)
Transfer timing	
hCG trigger	38 (20.8)
Sequential progesterone monitoring	145 (79.2)

All values expressed as *n* (%), mean±SD, or median (25th percentile–75th percentile). SD: Standard deviation

Cycle outcomes by each outcome are summarised in Tables 2-5. Letrozole was used for ovarian stimulation or ovulation induction in *n* = 79 cycles (43.2%). While implantation rate and clinical pregnancy, ongoing pregnancy and LBRs were persistently higher among the cycles that did not utilise letrozole, there was no significant difference across any of these outcomes in the adjusted analyses. Similarly, there were no significant differences in odds of implantation, clinical pregnancy, ongoing pregnancy or live birth between cycles with hCG trigger (*n* = 54, 29.5%) or without. Finally, transfers timed using the sequential progesterone monitoring technique (*n* = 145, 79.2%) showed comparable outcomes across all measures when compared to those timed 7 days after hCG trigger.

Discussion

Our results suggest that outcomes of NC-FET are remarkably stable across a wide range of protocols practiced within a single academic fertility centre. Specifically, we found no difference in implantation rate or clinical pregnancy, ongoing pregnancy or LBRs regardless of the use of letrozole for ovarian stimulation/ovulation induction, the use of hCG trigger, or the use of progesterone monitoring for timing of embryo transfer.

Few prior studies have evaluated outcomes following NC-FET when letrozole is used for ovarian stimulation or ovulation induction. The largest study to examine this question was a 2017 retrospective cohort study of 110,722 FET cycles from the Japanese national ART registry which compared 2409 cycles using letrozole to 41,470 natural cycles without letrozole and 66,843 hormone replacement cycles.[16] The authors found that the letrozole group had a higher clinical pregnancy and LBR and a lower miscarriage rate when compared with the natural cycles without letrozole or the hormone replacement cycles. While this study had the advantage

Table 2: Implantation rate by key cycle parameters

Variable	Implantation rate[a]	aRR (95% CI)	*P*
Letrozole use			
Letrozole (*n*=79)	0.54	1.20 (0.93–1.57)	0.17
No letrozole (*n*=104)	0.62		
Administration of exogenous hCG trigger			
Trigger (*n*=54)	0.57	1.07 (0.80–1.43)	0.66
No trigger (*n*=129)	0.59		
Transfer timing			
Trigger timing (*n*=38) (hCG +7 days)	0.54	1.15 (0.81–1.61)	0.43
Sequential progesterone (*n*=145)	0.59		

[a]Expressed as the number of gestational sacs divided by the number of embryos transferred. aRR=Adjusted rate ratio, CI=Confidence interval, hCG=Human gonadotropin

Table 3: Clinical pregnancy by key cycle parameters

Variable	Clinical pregnancy, *n* (%)	aOR (95% CI)	*P*
Letrozole use			
Letrozole (*n*=79)	44 (55.7)	1.35 (0.76–2.40)	0.31
No letrozole (*n*=104)	64 (61.5)		
Administration of exogenous hCG trigger			
Trigger (*n*=54)	32 (59.3)	1.10 (0.58–2.09)	0.78
No trigger (*n*=129)	76 (58.9)		
Transfer timing			
Trigger timing (*n*=38) (hCG +7 days)	21 (55.3)	1.35 (0.68–2.69)	0.44
Sequential progesterone (*n*=145)	87 (60.0)		

aOR=Adjusted odds ratio, CI=Confidence interval, hCG=Human gonadotropin

Table 4: Ongoing pregnancy by key cycle parameters

Variable	Ongoing pregnancy, *n* (%)	aOR (95% CI)	*P*
Letrozole use			
Letrozole (*n*=79)	36 (45.6)	1.62 (0.93–2.83)	0.10
No letrozole (*n*=104)	58 (55.8)		
Administration of exogenous hCG trigger			
Trigger (*n*=54)	28 (51.9)	1.91 (1.07–3.41)	0.70
No trigger (*n*=129)	66 (51.2)		
Transfer timing			
Trigger timing (*n*=38) (hCG +7 days)	18 (47.4)	1.50 (0.75–3.01)	0.32
Sequential progesterone (*n*=145)	76 (52.4)		

aOR=Adjusted odds ratio, CI=Confidence interval, hCG=Human gonadotropin

Table 5: Live birth rate by key cycle parameters

Variable	Live birth[a], *n* (%)	aOR (95% CI)	*P*
Letrozole use			
Letrozole (*n*=79)	26 (37.1)	1.49 (0.81–2.76)	0.20
No letrozole (*n*=104)	40 (46.5)		
Administration of exogenous hCG trigger			
Trigger (*n*=54)	18 (40.0)	1.36 (0.62–2.99)	0.51
No trigger (*n*=129)	48 (43.2)		
Transfer timing			
Trigger timing (*n*=38) (hCG +7 days)	11 (34.4)	1.36 (0.62–2.99)	0.51
Sequential progesterone (*n*=145)	55 (44.4)		

[a]Calculated among the subset of cycles with known live birth outcome (*n*=32 for trigger timing, *n*=124 for sequential progesterone).
aOR=Adjusted odds ratio, CI=Confidence interval, hCG=Human gonadotropin

of the inclusion of a large number of cycles, it was limited by the lack of information concerning reasons for selecting the specific FET method and important patient characteristics, including parity and the number of prior ART failures. Furthermore, no information was available regarding the dose and duration of letrozole intake. A more recent single-centre retrospective cohort study compared cycle outcomes between 2916 cycles with letrozole and 3958 natural cycles without letrozole.[15] In an attempt to control for differences in baseline characteristics between the two groups, including age, body mass index (BMI), duration of infertility, diagnosis, embryo quality and number of prior failed transfers, a 1–1 propensity score matching model was established. By analysing the data this way, the authors found no significant difference in clinical pregnancy or LBR between groups. These results are in line with those of the current study and suggest that the use of letrozole may be considered on an individual basis per physician and patient preference without compromising cycle outcomes.

Similarly, we found no effect of the use of an exogenous hCG trigger on NC-FET outcomes, although our results are limited by the small number of patients that received an exogenous hCG trigger relative to those who underwent spontaneous ovulation. Prior retrospective studies comparing the results of spontaneous versus triggered natural cycles have yielded mixed results, with

some showing improved outcomes among spontaneous cycles[18,19] and others showing no difference.[21,23-26,34] Two randomised controlled trials have addressed this question, including a small 2011 study by Weissman *et al*. (n = 60) and a more robust 2020 trial by Mackens *et al*. (n = 260), and found no significant differences in outcomes between spontaneous ovulation ('true' natural) cycles versus riggered ('modified' natural) cycles.[24-26] While the results of the latter trial were limited by a high drop-out rate in the 'modified' natural cycles given one-third of patients underwent spontaneous ovulation before receiving the intended hCG trigger, the results persisted in both intention to treat and per protocol analyses. Both studies also noted the use of an hCG trigger led to fewer clinic visits per patient, a potentially important benefit of the use of hCG trigger in NC-FET.

Finally, our study found no significant difference in cycle outcomes between two different methods of embryo transfer timing. Traditional methods of embryo transfer timing have been based on the idea of a 'window of implantation', believed to occur 6 days after the post-ovulatory progesterone surge and lasting 2–4 days.[35] While some physicians at our centre will target this window of implantation on the basis of a precisely defined hCG trigger, as has been previously described,[22,27] challenges in identifying the precise timing of the LH surge[28-29] as well as concerns over inadequate progesterone rise[36] have prompted others to rely on sequential progesterone monitoring for embryo transfer timing, particularly in the setting of a 'true' natural cycle with spontaneous LH surge. This latter approach has not previously been described but may represent a clinically useful protocol for other centres, particularly when ovulation is suspected, but the timing of the LH surge cannot be precisely identified. In these cases, our results suggest that it may be reasonable to monitor progesterone levels sequentially and to base transfer timing according to a progesterone threshold as opposed to the day of LH surge. The ability to rely on this approach may increase flexibility and decrease the frequency of required monitoring visits for patients, addressing important limitations of NC-FET.

Limitations of our study include its retrospective design, with protocols selected per physician discretion. Furthermore, the small sample size precluded adjustment for several potentially important confounders, including parity, BMI, and use of pre-implantation genetic testing for aneuploidy, and may have limited power to detect differences in outcomes. While our results suggest a high degree of flexibility in NC-FET protocols, larger randomized controlled trials are needed to provide further data validation.

Conclusion

In all, our results suggest a high degree of flexibility and potential for protocol individualisation at the discretion of the patient and physician without compromising cycle outcomes. As the benefits of NC-FET continue to surface and popularity for this method of preparation for embryo transfer grows, this flexibility may be critical and clinicians may increasingly incorporate NC-FET into clinical practice.

Author's Contributions

KNH – Data collection, analysis, and manuscript writing; JSM – Data collection, analysis, manuscript writing; JBB – Concept and design, data collection and analysis; MEP – Data collection and data contribution; KNG – Data collection and data contribution; CY – Contributed analysis tools and data analysis; LNM – Contributed analysis tool; LAB – Concept an design; data collection and analysis.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, K.N.H., upon reasonable request.

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