Int J Fertil Steril
International Journal of Fertility & Sterility
Homepage: https://www.ijfs.ir/
Original Article
Vol 17, No 3, July-September 2023, Pages: 195-200

Evaluation of Early Frozen Blastocyst Transfer in A True Natural Cycle Protocol in Comparison to A Hormone Replacement Protocol: A Single-Center Cohort Study

Jenna Gale, M.D., M.Sc., F.R.C.S.C.[1, 2*], Doron Shmorgun, M.D., F.R.C.S.C.[1, 2], Vanessa Bacal, M.D., M.Sc., F.R.C.S.C.,[1, 3], Marie-Claude Leveille, Ph.D.[1, 2, 3]

1. University of Ottawa, Department of Obstetrics and Gynecology, Ottawa, ON, Canada
2. Ottawa Fertility Centre, 100-955 Green Valley Crescent, Ottawa, ON, Canada
3. Ottawa Hospital Research Institute, Ottawa, ON, Canada

Abstract

Background: Timing of frozen embryo transfer (FET) within a purported window of implantation is of increasing interest, and there is a paucity of evidence surrounding the transfer of frozen embryos early within these frozen embryo transfer protocols. This study aimed to evaluate whether live birth rates were equivalent after FET of blastocysts 4 days after luteinizing hormone (LH) surge in a true natural cycle protocol, compared to a hormone replacement (HR) protocol.

Materials and Methods: Single-centre, retrospective cohort study involving patients undergoing autologous frozen blastocyst transfer from January 1st, 2013, to December 31st, 2016. Cycles were grouped according to their protocol: true natural cycle (hormonal detection of LH surge with FET scheduled four days later) versus HR cycle (luteal phase gonadotropin-releasing hormone agonist suppression, oral or vaginal estradiol and intramuscular progesterone starting five days before FET). A total of 850 cycles were included, 501 true natural cycles and 349 HR cycles. The primary outcome was the live birth rate, secondary outcomes included clinical pregnancy rate and miscarriage. Log-binomial regression models were performed adjusting for a priori selected variables.

Results: Adjusted resulted in live birth rates of 38.7 and 40.4%, [adjusted risk ratio (aRR): 0.96, 95% confidence interval (CI): 0.76-1.22, P=0.729] in the natural cycle and HR groups, respectively. The secondary outcome analyses did not demonstrate any statistically significant difference in the rate of positive human chorionic gonadotropin (hCG), clinical intrauterine pregnancy rate, or miscarriage rate.

Conclusion: The timing of the FET four days after LH surge in a true natural cycle protocol results in equivalent live birth rates compared to a HR protocol. Results of this study suggest that the window of implantation within the natural cycle may be less finite than currently believed and further prospective studies evaluating the timing of frozen embryo transfer are warranted.

Keywords: Blastocyst, Embryo Transfer, Hormone Replacement, Natural Cycle

Citation: **Gale J, Shmorgun D, Bacal V, Leveille MC. Evaluation of early frozen blastocyst transfer in a true natural cycle protocol in comparison to a hormone replacement protocol: a single-enter cohort study. Int J Fertil Steril. 2023; 17(3): 195-200. doi: 10.22074/IJFS.2022.544635.1238.**

Introduction

Overall rates of frozen embryo transfer (FET) have increased over time, likely as a result of more efficient cryopreservation strategies, increased number of good quality embryos following elective single embryo transfer policies, and elective freeze-all protocols (1). Many publications, including a recent meta-analysis, have reported that FET pregnancy rates may be superior to fresh, however, two recent large randomized control trials produced conflicting results and this remains to be elucidated (1-4). Several protocols exist for FET and it is not possible to identify one method as superior to another (5).

The two most employed protocols include the natural cycle (NC) and hormone replacement (HR) approach-

Received: 16/December/2021, Revised: 28/April/2022, Accepted: 16/May/2022
***Corresponding Address: University of Ottawa, Department of Obstetrics and Gynecology, Ottawa, ON, Canada**
Email: jgale@conceive.ca

Royan Institute
International Journal of Fertility & Sterility

es. In the NC approach, the FET is timed to ovulation in the patients' own cycle, often divided into 'true NC', where ovulation is allowed to occur spontaneously, or 'modified NC' (mNC) where ovulation is triggered with human chorionic gonadotropin (hCG) administration. In the HR approach, the patient is administered exogenous hormones and the FET is timed to the duration of exogenous progesterone. Typical HR protocols use progesterone supplementation for the equivalent number of days before transfer as the stage of development of the embryo is transferred (ie. 5 days for a day 5 blastocyst) (6). Pregnancy rates are lower, and the risk of early pregnancy loss is higher when transfer and implantation occur after greater than 6 days of progesterone administration for a day 5 blastocyst transfer (7-9). Conversely, there is a paucity of evidence evaluating the shorter duration of progesterone exposure. Given the relative importance of the outcomes associated with differing durations of progesterone exposure, it is of critical importance that this factor should be taken into consideration (10, 11).

We present a retrospective cohort analysis of an NC protocol with FET 4 days after luteinizing hormone (LH) surge and an HR protocol with transfer on the 5th day of progesterone administration from a single centre. Our study aimed to evaluate if live birth rates are equivalent between these two protocols. We will comment on the comparability of pregnancy and live birth rates to those reported after more standard FET protocols, given the paucity of evidence surrounding the early transfer.

Materials and Methods

Study population

Patients who started a frozen embryo transfer cycle between January 1st, 2013, and December 31st, 2016, at the Ottawa Fertility Centre in Ottawa, Ontario, Canada, were eligible for inclusion. The average age of patients at the time of FET was 34.7 years and the average body mass index (BMI) of patients included in this study was 24.4. Patients were identified through an in-house medical record system, and clinic linkage to the Canadian Assisted Reproductive Technologies Register (CARTR Plus) provided birth outcome data, which has been previously validated (12). The study protocol was reviewed by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) and deemed exempt from OHSN-REB review as a quality improvement initiative. Data was housed on a local secure server and analysis was available only to study authors.

Patients were included in the analysis if they underwent FET with blastocysts cryopreserved by vitrification, created from their oocytes with either partner or donor sperm, whether embryos cryopreserved were surplus after fresh embryo transfer or were cryopreserved in a freeze-all cycle. Patients were excluded if donor oocytes or a gestational carrier were utilized. Vitrification of blastocysts occurred on day 5 unless the cycle included pre-implantation genetic testing at which point blastocysts were vitrified on days 5 and 6. The vitrification-warming method was carried out using RapidVit and RapidWarm Blast kits (Vitrolife) and the Rapid-i vitrification system (Vitrolife) in accordance with the manufacturer's instructions (13).

Blastocysts were graded based on Gardner's scoring system (14). At our clinic, only good and best quality blastocysts (B1-3 and greater) were selected for cryopreservation, unless exceptional circumstances prevailed. During the duration of the study period, approximately 35% of *in vitro* fertilization (IVF) cycles performed at our clinic had resultant embryos to freeze (whether as surplus after a fresh embryo transfer or in a 'freeze-all' protocol to avoid ovarian hyperstimulation syndrome or in the case of PGT). The number of embryos transferred in the cycle was at the discretion of the physician in discussion with the patient and was pre-determined at a follow-up appointment before the FET cycle. The decision to transfer 1 vs. 2 embryos was made with the patient by considering the patient's age, the number of prior embryo transfers, and patient factors posing an additional risk in pregnancy given multiple gestations, with a tendency at our clinic toward elective single embryo transfer.

Natural cycle frozen embryo transfer protocol

The "true NC" approach was employed at our centre throughout this study period, whereby ovulation occurs spontaneously and was not triggered with exogenous hormones. Women were considered candidates for NC protocol if they had regular menstrual cycles, ranging in length between 27-32 days, a mid-luteal phase serum progesterone ≥30 nmol/L typically measured 6-8 days post urinary LH surge, and there was no luteal phase concern (ie. luteal phase spotting, or evidence of a short luteal phase). A patient's age and BMI were not considered as inclusion or exclusion criteria. The protocol involved daily serial morning bloodwork sampling for estradiol and LH, typically started 3-4 days prior to the expected LH surge, until the LH surge was observed. The LH surge was defined as the attainment of a serum LH ≥30 IU/L with a dropping estradiol, or the highest-level LH ≥30 IU/L given that a dropping serum estradiol was not a strict criterion. The day on which this was observed was considered day 0 of the cycle, as is standard within the FET literature (15).

Once a surge was identified, a pelvic ultrasound was performed to obtain a measurement of endometrial thickness. After a documented LH surge and endometrial thickness ≥7 mm, embryo transfer was scheduled on day 4. Exogenous progesterone was not administered for luteal phase support. If a patient did not meet these criteria, the cycle was cancelled, and the patient was scheduled for a follow-up with their physician to discuss

either another attempt at the NC protocol or switching to an HR protocol.

Hormone replacement frozen embryo transfer protocol

Patients were selected for HR FET if they did not meet the criteria for NC as outlined above, or if they elected to proceed with this approach for other reasons (ie. ease of scheduling and fewer visits for bloodwork and ultrasound). Gonadotropin-releasing hormone (GnRH) agonist pre-treatment was employed as a standard of care throughout this study period (Abbvie, Lupron depot, leuprolide acetate 3.75 mg intramuscular), which was administered prior to the onset of menses. Estrogen priming with an escalating oral or vaginal micronized estradiol (Acerus Pharmaceuticals Corportation, Estrace, 17β-estradiol tablets) administration was started between menstrual cycle days 3-5. Transvaginal estrogen administration proceeded as follows: 0.5 mg twice daily for 6-10 days, 1mg twice daily for 5-8 days, and 2mg three times daily for 5 days for a total of 16-23 days of estrogen prior to the ultrasound evaluation of endometrial thickness and serum estradiol and progesterone assessment. If patients met the requirements of the endometrial lining of ≥7 mm, serum estradiol ≥650 pmol/L, and progesterone <5 nmol/L, they were advised to start progesterone in oil IM 50 mg daily. The embryo transfer was scheduled for four days after the progesterone was begun. In cases of inadequate endometrial thickness or serum estrogen, ongoing estrogen supplementation, typically for an additional week at the same or higher doses, was employed. Endometrial thickness and serum estradiol were re-checked after additional estrogen and if adequate, progesterone was commenced, and FET scheduled. If inadequate, the cycle was either cancelled, or the patient could elect to proceed with progesterone and scheduling of FET after a discussion with the physician.

Embryo transfers were typically done between the hours of 10h00 – 13h00. The total number of hours of progesterone exposure with this protocol was 85-92. Estrogen and progesterone supplementation were then continued until either a negative serum pregnancy test or until 10 weeks' gestational age.

Outcome assessment

The primary outcome was live birth after FET. A live birth was defined as an infant born showing any signs of life, or at least ≥ 20 weeks' gestational age, or weighing 500 grams. Secondary outcomes included rate of positive serum hCG, clinical intrauterine pregnancy, miscarriage, ectopic and stillbirth pregnancy. Serum hCG was measured approximately 14 days after ET, and measurements ≥ 5 IU/L were considered positive. Clinical intrauterine pregnancy was defined as the presence of a gestational sac and yolk sac on transvaginal ultrasound. Miscarriage was defined as a birth outcome where a clinical pregnancy was diagnosed but no fetus development could be seen at <20 weeks' gestation. Stillbirth was defined as a pregnancy loss at ≥ 20 weeks' gestation.

Statistical analysis

Patient and cycle characteristics were described using frequencies and proportions for categorical variables and statistical comparisons were done with Fisher Exact test for non-parametric data and Chi-square for parametric data. We described normal continuous variables using means and standard deviations and compared groups using a two-sided t test. Overall live birth, positive hCG, clinical intrauterine pregnancy, miscarriage, ectopic and stillbirth pregnancy rates were compared between the two groups. We fit a multivariable log-binomial regression model with a priori variables for the primary and secondary outcomes, adjusting for patient age at oocyte retrieval, body mass index, polycystic ovarian syndrome (PCOS) or other ovulatory disorder as an indication for treatment, and the number of blastocysts transferred. Adjusted risk ratios with 95% confidence intervals were performed.

To detect a difference of 10% in the live birth rate between the two groups from a baseline of 35%, a sample size of 329 was required per group, with a power of 80% and an alpha of 0.05. A P<0.05 was considered statistically significant. Statistical analyses were performed using SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC).

Results

There were 850 frozen embryo transfer cycles from 614 patients between January 1st, 2013, and December 31st, 2016 meeting the inclusion criteria for this study. Demographic characteristics are presented in Table 1. Of the included cycles, 501 were from 354 patients within the NC group and 349 were from 267 patients within the HR group (there was a small amount of crossover between the two groups). The difference in the number of patients with more than 1 cycle included within each group was not statistically significant. There was a greater average BMI (P=0.023) and a higher percentage of patients with a diagnosis of the PCOS in the HR group [risk ratio (RR): 1.32, 95% confidence interval (CI): 1.24-1.41, P<0.001]. There were also more patients with a diagnosis of tubal factor (RR: 1.39, 95% CI: 1.01-1.92, P=0.048) and endometriosis (RR: 1.68, 95% CI: 1.02-2.79, P=0.018) within the NC group. The groups did not differ with respect to the number of prior embryo transfers.

Adjusted risk ratios for the primary outcome of live birth and the secondary outcomes are presented in Table 2. We found no significant difference between the NC and HR groups for the primary outcome or any of the secondary outcomes.

We performed a post-hoc sensitivity analysis stratifying each group by age. We found no statistically significant difference in the primary outcome of live birth rate between the two groups (P=0.729, Table 3).

Table 1: Baseline characteristics for the NC group versus the HR group

Demographic	NC group (n=501)	HR group (n=349)	P value
Age at FET (Y)	34.9 ± 3.7	34.4 ± 3.9	0.051
Age at oocyte retrieval (Y)	33.9 ± 3.7	33.5 ± 3.9	0.096
Body mass index (kg/m^2)	24.1 ± 4.1	24.8 ± 4.4	0.023
Prior pregnancies			0.048
0	198 (39.5)	114 (32.7)	
1	189 (37.7)	127 (36.4)	
≥2	114 (22.8)	108 (30.9)	
Prior births			0.123
0	314 (62.7)	233 (66.8)	
1	165 (32.9)	96 (27.5)	
≥ 2	22 (4.4)	20 (5.7)	
Indication for treatment			
Unexplained infertility	40 (8.6)	25 (7.6)	0.590
Male factor	349 (75.4)	234 (70.9)	0.160
Tubal factor	91 (19.7)	47 (14.2)	0.048
Endometriosis	47 (10.2)	18 (5.5)	0.018
PCOS/Other ovulatory disorder	6 (1.2)	102 (29.2)	<0.001
Other	12 (2.6)	7 (2.1)	0.669
Missing	38 (7.6)	19 (5.4)	0.220
Number of prior fresh cycles of IVF			0.849
1	355 (70.9)	241 (69.0)	
2	88 (17.6)	61 (17.5)	
≥3	58 (11.5)	47 (13.5)	
Number of prior ETs			0.059
0	70 (14.0)	69 (19.8)	
1	213 (42.5)	141 (40.4)	
≥2	218 (43.5)	139 (39.8)	
The number patients within the group with more than 1 FET cycle included	107 (21.4)	63 (18.1)	0.067
Use of PGT-A	7 (1.4)	5 (1.4)	1.000
Number of embryos transferred			
1	420 (83.8)	284 (81.4)	0.597
2	79 (15.8)	64 (18.3)	
3	2 (0.4)	1 (0.3)	
Number of embryos transferred (continuous outcome)	1.2 (0.4)	1.2 (0.4)	0.389
Endometrial thickness	9.6 (2.0)	9.5 (2.1)	0.433

Continuous data are presented as mean ± standard deviation (SD), and categorical data are presented as number (%). NC; Natural cycle, HR; Hormone replacement, FET; Frozen embryo transfer, PCOS; Polycystic ovarian syndrome, IVF; *In vitro* fertilization, and PGT-A; Preimplantation genetic testing for aneuploidy.

Ninety-nine patients were age 40 or over at the time of embryo transfer (in contrast to at the time of egg retrieval). Within this group, 60 (60.6%) and 39 (39.4%) utilized the NC and HR protocols, respectively (P=0.698). Seventeen of 60 (28.3%) patients within the NC protocol group achieved a live birth, and 8 of 39 (20.5%) patients within the HR protocol group achieved a live birth, which was not statistically significant (P=0.382).

Table 2: Pregnancy outcomes for NC versus HR group

Outcome	NC group (n=501)	HR group (n=349)	aRR [95% CI]	P value
Live birth	194/501 (38.7)	141/349 (40.4)	0.96 [0.76-1.22]	0.729
Positive hCG	273/501 (54.5)	205/349 (58.7)	1.05 [0.86-1.29]	0.617
Clinical intrauterine pregnancy	236/501 (47.1)	174/349 (49.9)	0.98 [0.82-1.16]	0.781
Miscarriage	39/236 (16.5)	33/174 (19.0)	0.95 [0.51-1.77]	0.876
Ectopic pregnancy	5/273 (1.8)	7/205 (3.4)	0.85 [0.21-3.39]	0.813
Stillbirth (>20 weeks)	3/236 (1.3)	0/174 (0)	---	---

Values are numbers (%). All analyses were performed using log-binomial regression adjusted for age at the time of retrieval, body mass index, diagnosis of PCOS or other ovulatory disorder, and the number of embryos transferred. NC; Natural cycle, HR; Hormone replacement, PCOS; Polycystic ovarian syndrome, aRR; Adjusted risk ratio, CI; Confidence interval, and hCG; Human chorionic gonadotropin.

Table 3: Live birth outcomes for NC versus HR group, according to patient age at the time of oocyte retrieval

Age group (Y)	NC group (n=501)	HR group (n=349)	aRR (95% CI)	P value
<35 (n=494)	118/284 (41.6)	99/210 (47.1)	0.83 [0.66-1.05]	0.122
35-37 (n=208)	49/127 (38.6)	26/81 (32.1)	1.12 [0.70-1.80]	0.629
38-39 (n=90)	20/57 (35.1)	13/33 (39.4)	0.98 [0.46-2.09]	0.967
≥40 (n=58)	7/33 (21.2)	3/25 (12.0)	-	-

Values are numbers (%). All analyses were performed using log-binomial regression adjusted for age at the time of retrieval, body mass index, diagnosis of PCOS or other ovulatory disorder, and the number of embryos transferred. NC; Natural cycle, HR; Hormone replacement, aRR; Adjusted risk ratio, and CI; Confidence interval.

Discussion

Patients who underwent a true natural cycle FET with transfer four days after LH surge did not have lower live birth rates compared to patients who underwent a hormone replacement FET with transfer on the 5th day of progesterone administration. This study demonstrates similar live birth outcomes when embryo transfer occurs relatively early within a true NC protocol, compared to the literature recommendation of transfer timing within this protocol (15). Results of this study suggest that it is likely that the purported 'window of implantation' may therefore include timing with a shorter duration of progesterone exposure.

The control group in this study was a HR protocol employing FET on the 5th day of progesterone administration. Standard HR embryo transfer protocols recommend transfer after progesterone exposure equivalent to the development of the embryo (6 days when considering a blastocyst) or less 1 day, as evidence

suggests these are equivalent (7, 8). The utilization of this HR protocol is additionally supported by the fact that pregnancy and live birth rates are similar to recent reports of FET on the 6th day of progesterone (16-18). There is a possibility that a shorter duration of progesterone exposure may be associated with an increased risk of miscarriage (19), however, within this study, we observed a low risk of miscarriage which did not differ between the two protocols.

We observed a slightly higher average BMI and a greater percentage of patients with a diagnosis of ovulatory disorder (PCOS) within the HR group, which was expected given that irregular menstrual cycles are an indication of a medicated FET cycle. While the higher average BMI in the HR group was statistically significant, this difference of 0.7 between the two groups may arguably not be clinically relevant. We do know that differences in BMI are linked to pregnancy outcomes, and BMI was taken into account as a confounder during the log-binomial regression analysis. Patients in the NC group were more likely to have a diagnosis of tubal factor or endometriosis, which was also expected given that these are anatomical factors that do not impact cycle regularity. We do not feel that these differences would have had a clinically important impact on the study outcomes.

The results of this study are highly generalizable given the limited exclusion criteria, representation of patients from all infertility diagnoses, and comparable proportions of natural cycles and HR protocols utilized. Additional strengths of this study include the large sample size, adjustment for important confounders including age, BMI, endometrial thickness, and the inclusion of a relatively large number of women over the age of 40 at the time of embryo transfer.

Our data suggest that a true NC may be a reasonable approach among women over the age of 40. This contrasts studies demonstrating a lower chance of live birth among patients greater than 40 years of age undergoing natural cycle FET compared to hormone replacement FET, and recent recommendations for a modified natural protocol (using hCG to trigger ovulation) in women over the age of 40 (16, 20). We need to interpret these last results with caution, as this was a secondary analysis of a much smaller sample size.

The main limitation of this study is its retrospective nature, the inherent selection bias, and confounding not addressed by statistical analysis. Additionally, it would be ideal to compare early transfer within a natural cycle FET protocol to more 'standard' transfer timing within the NC protocol for optimal evaluation of the early timing, however as this is not our standard practice this control group was not available. Finally, the outcomes of both groups in this study may represent a 'better prognosis' patient population given that only good and best quality embryos, based on the Gardner scoring criteria, are selected for freezing at our institution. However, this study does add to the literature given the substantial paucity of outcomes surrounding any transfer early within the purported window of implantation.

As a result of evidence indicating possible increased pregnancy rates and decreased maternal and neonatal morbidity among pregnancies conceived through FET relative to fresh transfer, it is likely we will continue to see an increase in frozen embryo transfer cycles (1, 21, 22). The NC approach is purported to have several benefits as it involves less (or no) medication, lower cost, and less discomfort for the patient (15, 23). Additionally, emerging evidence suggests that NC transfers, related to the presence of the corpus luteum, are associated with lower rates of pregnancy complications including hypertensive disorders of pregnancy, postpartum hemorrhage, macrosomia, and post-term birth (24, 25). Given that the optimal protocol within the NC has yet to be elucidated, further research in this area is required.

Conclusion

Timing of the FET four days after LH surge in a true NC protocol results in equivalent live birth rates compared to a HR protocol. The results of this study suggest that the window of implantation for frozen embryo transfer within the NC may be less finite than currently believed. When considering the probable future increase in the use of natural cycle FET protocols to optimize patient experience and pregnancy outcomes, these results fuel further important queries, specifically the need for prospective studies surrounding transfer timing within the NC protocol.

Acknowledgments

There is no financial support and conflict of interest in this study.

Authors' Contributions

J.G., D.Sh., V.B., M.-C.L.; Involved in the conception, and design of the manuscript, data acquisition, or review, contributed to the interpretation of the data, as well as drafting and revising the manuscript and approved the final draft. J.G., V.B.; Completed the data analysis. All authors read and approved the final manuscript.

References

1. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. Hum Reprod Update. 2014; 20(6): 808-821.
2. Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, et al. Transfer of Fresh versus Frozen Embryos in Ovulatory Women. N Engl J Med. 2018; 378(2): 126-136.
3. Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. Lancet. 2019; 393(10178): 1310-1318.
4. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. Hum Reprod Update. 2019; 25(1): 2-14.
5. Groenewoud ER, Cantineau AEP, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and

meta-analysis. Hum Reprod Update. 2013; 19(5): 458-470.
6. Casper RF, Yanushpolsky EH. Optimal endometrial preparation for frozen embryo transfer cycles: window of implantation and progesterone support. Fertil Steril. 2016; 105(4): 867-872.
7. Escribá MJ, Bellver J, Bosch E, Sánchez M, Pellicer A, Remohí J. Delaying the initiation of progesterone supplementation until the day of fertilization does not compromise cycle outcome in patients receiving donated oocytes: a randomized study. Fertil Steril. 2006; 86(1): 92-97.
8. Glujovsky D, Pesce R, Fiszbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev. 2010; (1): CD006359.
9. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. N Engl J Med. 1999; 340(23): 1796-1799.
10. Imbar T, Hurwitz A. Synchronization between endometrial and embryonic age is not absolutely crucial for implantation. Fertil Steril. 2004; 82(2): 472-474.
11. Theodorou E, Forman R. Live birth after blastocyst transfer following only 2 days of progesterone administration in an agonadal oocyte recipient. Reprod Biomed Online. 2012; 25(4): 355-357.
12. Bacal V, Fell DB, Shapiro H, Lanes A, Sprague AE, Johnson M, et al. The Canadian Assisted Reproductive Technologies Register (CARTR) Plus database: a validation study. Hum Reprod Open. 2020; 2020(2): hoaa005.
13. Cryopreservation. Vitrolife. Available from: https://www.vitrolife.com/products/cryopreservation/ (24 Feb 2022).
14. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril. 2000; 73(6): 1155-1158.
15. Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H, et al. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. Hum Reprod. 2017; 32(11): 2234-2242.
16. Alur-Gupta S, Hopeman M, Berger DS, Gracia C, Barnhart KT, Coutifaris C, et al. Impact of method of endometrial preparation for frozen blastocyst transfer on pregnancy outcome: a retrospective cohort study. Fertil Steril. 2018; 110(4): 680-686.
17. Cerrillo M, Herrero L, Guillén A, Mayoral M, García-Velasco JA. Impact of endometrial preparation protocols for frozen embryo transfer on live birth rates. Rambam Maimonides Med J. 2017; 8(2): e0020.
18. Mounce G, McVeigh E, Turner K, Child TJ. Randomized, controlled pilot trial of natural versus hormone replacement therapy cycles in frozen embryo replacement in vitro fertilization. Fertil Steril. 2015; 104(4): 915-920. e1.
19. van de Vijver A, Polyzos NP, Van Landuyt L, Mackens S, Stoop D, Camus M, et al. What is the optimal duration of progesterone administration before transferring a vitrified-warmed cleavage stage embryo? A randomized controlled trial. Hum Reprod Oxf Engl. 2016; 31(5): 1097-1104.
20. Kang HJ. Programmed versus natural frozen embryo transfer: which is the best nest? Fertil Steril. 2018; 110(4): 636-637.
21. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril. 2014; 101(1): 128-133.
22. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2012; 98(2): 368-377. e1-9.
23. Sathanandan M, Macnamee MC, Rainsbury P, Wick K, Brinsden P, Edwards RG. Replacement of frozen-thawed embryos in artificial and natural cycles: a prospective semi-randomized study. Hum Reprod. 1991; 6(5): 685-687.
24. Singh B, Reschke L, Segars J, Baker VL. Frozen-thawed embryo transfer: the potential importance of the corpus luteum in preventing obstetrical complications. Fertil Steril. 2020; 113(2): 252-257.
25. Ginström Ernstad E, Wennerholm UB, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: increased risks in programmed cycles. Am J Obstet Gynecol. 2019; 221(2): 126. e1-126. e18.