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Clinical outcome of vitrified-warmed blastocyst transfer performed on days 5, 6, and 7 after the luteinizing hormone surge detection using urine tests: A retrospective cohort study with propensity score matching

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Abstract

Research question: This retrospective study aimed to determine whether the clinical outcomes of vitrified-warmed blastocyst transfer were comparable if performed on days 5, 6, and 7 after detecting the luteinising hormone (LH) surge using urine tests.

Design: Between 2013 and 2019, 2080 vitrified-warmed blastocyst transfers in a true natural cycle were performed and later analysed at the Department of Reproductive Medicine, University Medical Centre Maribor. Urine LH tests were performed twice daily to monitor LH surge onset. Frozen embryo transfer (FET) was performed on days 5 (group 1), 6 (group 2) and 7 (group 3) after LH surge in 18.3%, 77.4% and 4.3% of cycles, respectively. The patient and cycle characteristics among the groups were compared using the Cochran–Mantel–

Haenszel test and respective Generalized Linear Mixed models. Propensity score matching was used to adjust for potential differences among the groups.

Results: There were no statistically significant differences in the groups 1, 2 and 3 respectively in the cycle and patient characteristics, clinical pregnancy rate (37.6% vs. 39.3% vs. 31.1%), implantation rate (37.6% vs. 39.3% vs. 31.1%), miscarriage rate (7.1% vs. 8.6% vs. 6.7%) and delivery rate (30.5% vs. 30.8% vs. 24.4%). The day of FET after LH surge detected using urine test was not significantly associated with live births.

Conclusions: The results of our study suggested that the vitrified-warmed blastocyst transfer could be scheduled on days 5, 6, or 7 after positive LH urine tests without having a significant impact on the clinical outcome.

Keywords

vitrified blastocyst, embryo transfer, natural cycle, LH surge, live birth rate

Abbreviations

FET: vitrified-warmed blastocyst transfer; LH:luteinising hormone; tNC: true natural cycle, HCG: human chorionic gonadotropin, mNC: modified natural cycle, IVF: *in-vitro* fertilization, WOI: window of implantation, PG: progesteron

Introduction

In recent years, vitrified-warmed blastocyst transfer (FET) has become an important part of the *in vitro* fertilisation program. Elective single embryo transfer and freeze-all strategy, together with advanced cryopreservation techniques, provide important benefits for the patients and primarily improve the safety of the treatment and a higher cumulative live birth rate. Various endometrial preparation strategies have been introduced to optimise the success of FET; however, the optimal method has not yet been established. The most frequently used cycle regimens include a natural ovulatory cycle protocol with luteinising hormone (LH) detection (true natural cycle, tNC), human chorionic gonadotropin application (modified natural cycle, mNC) and the artificial cycle. Each has advantages and disadvantages. The benefit of NC FETs is to avoid medication and the disadvantage is a need for the precise monitoring and limited flexibility during the timing of FET (Reljič and Knez, 2018).

In tNC, the timing of FET is determined after spontaneous LH surge and/or detection of ovulation. FET should be performed when the endometrium is receptive during the window of implantation (WOI). In the textbook 28-day cycle, WOI opens on day 19 and remains open 4–5 days in the endometrial cycle at the time of maximal serum progesterone concentration (Lessey and Young, 2019; Navot et al., 1991; Psychoyos, 1986). Vitrified-warmed blastocyst transfer on day 6 after the LH surge has been proposed to achieve synchronisation between embryonic and endometrial development and optimal implantation rate (Mackens et al., 2017).

The LH surge usually begins between midnight and 7:30 in the morning in two-thirds of women; consequently, follicle rupture occurs 34–36 h later, while the LH surge itself lasts 48–50 h (Glass RH Speroff L, 1994; Hoff et al., 1983). Since urine LH increases with a delay of 12–36 h after the detection of the blood LH surge, synchronisation could also be achieved if FET is performed on day 5 after a positive LH urine test (Martinez et al., 1986). However, there are currently no studies in the literature on this topic.

This study aimed to establish whether the clinical outcomes of vitrified-warmed blastocyst transfer were comparable if FET was performed on days 5, 6, and 7 after the LH surge detection using urine tests.

Material and methods

All vitrified-warmed blastocyst transfers performed in tNC between 2013 and 2019 at the Department of Reproductive Medicine, University Medical Centre Maribor, were included in this retrospective study. Women with uterine pathology, hydrosalpinxes visible on ultrasound and endocrinological disorders, such as polycystic ovarian syndrome and premature ovarian insufficiency, were excluded from the analysis.

Women below 43 years of age, with regular menstrual cycles (24–35 days), were included in the study. Ovulatory status was confirmed during a diagnostic workup (dominant follicle in the preovulatory phase on ultrasound and progesterone in the mid-luteal phase).

Cycle monitoring

Vaginal ultrasound was performed in all patients on day 8–10 of the cycle. If the leading follicle was selected and accompanied by adequate thickening and ultrasonographic appearance of the endometrium, the patient was instructed to perform a urine LH test (RapiTest LH, MD Doctors Direct GmbH, Zürich, Switzerland) every morning from the day of the anticipated follicular diameter of 15 mm. Growth of the follicular diameter by 2 mm/day was assumed. When a patient had a positive LH test in the morning,, she was instructed to repeat the test in the evening of the same day to confirm the LH surge. If the LH surge was undetected or if the results were inconclusive, the FET was cancelled. FETs were routinely performed on day 6 after the LH surge and carried out 7 days a week. Occasionally FET was scheduled on day 5 or 7 after the LH surge, to avoid transfer onbusy days or to modify workload for the staff during weekends.

Blastocyst thawing and evaluation

All expanded blastocysts were vitrified on days 5 or 6 using a combination of dimethyl sulfoxide and ethylene glycol cryoprotectants. Before vitrification, blastocysts were graded according to our established grading system (Kovacic et al., 2004; Kovačič B, 2012; Martinez et al., 1986). After thawing, blastocysts were cultured in a recovery medium (Blast Assist System, Origio, Denmark) for at least 4 h before transferring into the uterus.

Only blastocysts that had at least 50% intact blastomeres after thawing and started to re-expand were assessed suitable for transfer (Kovačič B, 2012). Preimplantation genetic testing for aneuploidy was not performed in the included cycles.

One or two vitrified-warmed blastocysts were transferred using Labotect catheters (Labotect GmbH, Labor-Technik-Göttingen, Germany). The number of embryos transferred in each case depended on the quality of the available embryos, the number of previous treatments, the number of embryos frozen in the same straw and the patient-doctor agreement.

Embryo transfer and outcome

A pelvic ultrasound examination was performed immediately before embryo transfer to measure the endometrial thickness and evaluate the endometrial pattern.

Progesterone supplementation (400 mg/day of micronised vaginal progesterone) was initiated immediately after FET. The serum human chorionic gonadotropin (HCG) test was performed 2 weeks after FET, and clinical pregnancy, defined as ultrasonographic documentation of at least one foetus with a discernible heartbeat, was evaluated 2 weeks later. The clinical pregnancy rate was calculated as the number of clinical pregnancies divided by the number of transfer cycles. Similarly, the implantation rate was calculated as the number of gestational sacs observed divided by the number of embryos transferred, where both values were expressed as percentages. Miscarriage was defined as the number of spontaneous loss of clinical pregnancies before 22 completed weeks of gestation divided by the total number of clinical pregnancies. Additionally, the live birth rate was calculated as the number of deliveries that resulted in at least one live birth per all embryo transfers.

The patient characteristics and clinical data were collected from our software database.

Statistical analyses

Reproductive outcomes, cycle and patient characteristics of FET on days 5, 6, and 7 after the LH surge were compared using the Cochran–Mantel–Haenszel test for factorial variables. Power and sample size calculations for the Cochran–Mantel–Haenszel test were performed. According to literature-based success rates, 58, 253 and 16 cycles were required on days 5, 6 and 7, respectively, for the desired power of 0.9. Univariate analysis was performed to consider the effect of successive cycles by building respective generalised linear models with the couples' unique ID as a blocking variable. Furthermore, the three groups were propensity score matched to minimise potential bias. The propensity scores were calculated for all pairs of the three groups using binary logistic regression based on the woman's age, number of transferred embryos, and the blastocyst quality. Success rates were compared using the Cochran–Mantel–Haenszel test. Statistical analyses were performed with RStudio using R version 4.0.3 (R Core Team 2020; R: Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 2731 FETs were performed. After removing missing data, 2080 vitrified-warmed blastocyst transfers were included in the analysis. Following positive LH urine tests, 1610 (77.4%), 380 (18.3%) and 90 (4.3%)

FETs were performed on days 6, 5 and 7. Differences in women's age, cause of infertility, number of previous *in-vitro* fertilization (IVF) attempts, medical history regarding previous IVF cycles and their outcome, average menstrual cycle length, and menstrual cycle variability were not observed. Furthermore, differences in the FET cycle characteristics on the day of LH surge, endometrial thickness on the day of FET, and endometrial morphology were not found (Table 1). The number of blastocysts transferred in all groups was the same; the proportion of difficult embryo transfers, transferred blastocysts vitrified on day 5, and the proportion of morphologically optimal blastocyst transfers also did not differ among the cycles with FET on days 5 and 6 and day 7 after the LH surge(Table 1).Patients undergoing a FET on day 5, 6 or 7 after the LH surge showed no statistically significant differences concerning clinical pregnancy rate, implantation rate, miscarriage rate, and live birth rate (Table 2).Propensity score matching was performed using age, the number of transferred embryos and blastocyst quality to account for potential differences among the groups (Figure 1); the Cochran–Mantel–Haenszel test was used again to compare FET cycle outcomes between the respective groups. No statistical difference was found in live birth rates (day 5 vs. day 6, p=0.98; day 6 vs. day 7, p=0.31; day 5 vs. day 7, p=0.40).

Discussion

The number of FET cycles performed in recent years has increased drastically due to the trend of transferring fewer embryos in a fresh IVF cycle and improved laboratory techniques (Groenewoud et al., 2013). In a spontaneous cycle in patients with regular menstrual cycles, FET seems to be a reasonable choice because of good results and no medication requirement before FET. Vaginal progesterone supplementation is suggested after FET in tNC (Casper et al, 2016).

True natural cycle FET is the preferred approach to FET in women with regular menstrual cycles at our centre, and we performed 2731 FETs between 2013 and 2019 in tNC. The problem of FETs in an NC is the lack of flexibility.

FET timing in NC can be determined by the detection of spontaneous LH or ovulation induction using HCG. In the latter case (mNC), careful cycle monitoring using ultrasonography and hormone assessment to identify the endometrial receptive period is required. HCG is usually administered when the dominant follicle reaches a size indicating its maturity (16–20 mm), considering or regardless of LH and progesterone levels (Casper and Yanushpolsky, 2016; Greco et al., 2016; Groenewoud et al., 2013, 2012)(Casper and Yanushpolsky, 2016; Greco et al., 2016; Groenewoud et al., 2013, 2013, 2012). Earlier data speak against the use of HCG because of the initially unsatisfying results; however, recent studies have not confirmed earlier conclusions, although there might be a benefit of tNC attributed to LH-induced changes in the endometrium that favour implantation (Fatemi et al., 2010; Groenewoud et al., 2016; Huberlant et al., 2018; Kyrou et al., 2012, Mackens et al, 2020). In a true natural cycle, the LH surge is accompanied by a follicle-stimulating hormone surge, the role of which is still not fully understood; it might influence the function of the corpus luteum through the induction of LH receptors in the luteinising granulosa cells (Humaidan et al., 2011). Early progesterone rise before ovulation probably contributes to the induction of WOI in NC (Mackens et al., 2017).

There is no unanimous definition of an LH surge. Some clinicians define the LH surge as a 180% increase above the basal level (Fatemi et al., 2010; Groenewoud et al., 2016; Huberlant et al., 2018; Humaidan et al., 2011;

Kyrou et al., 2012; Park et al., 2007), others use the serum LH concentration of 10 IU/L or more (Groenewoud et al., 2013). An abrupt increase of LH with a doubling time of 2 h, duration of 48 h, the second rapid rise of progesterone 36 h after the beginning of the LH surge and 12 h before its termination have been reported (Hoff et al., 1983). Studies show high variability in configuration, amplitude and duration of the LH surge (Direito et al., 2013; Park et al., 2007). The LH surge onset precedes ovulation by 34–36 h but sometimes up to 44 hours.. For that reason, the timing of FET can be assumed to be flexible (Glass RH Speroff L, 1994; Su HW et al, 2017). Makens et al. suggested that FET should be performed on days of HCG +7 in mNC and LH +6 in tNC based on the different time spans to ovulation (Mackens et al., 2017). Since a delay in the detection of peak hormone levels was described, it seems reasonable to assume that FET on day +5 after a positive urine LH test might be successful (Cekan et al., 1986).

Monitoring for a spontaneous LH surge using the serum analysis can burden the patient with repeated monitoring visits (Fatemi et al., 2010; Weissman et al., 2011). An alternative to vencipuncture is the urine LH test, an inexpensive, easy-to use option, making timing of FET patient-friendly.A urine LH test is positive 12–36 h after the LH surge in plasma (Martinez et al., 1986). The challenge with urine LH testsis that the ovulation time cannot be determined precisely because of the high physiological variability; hence, the beginning of WOI cannot be precisely determined. However, the outcomes were not significantly different in FET performed in the range of 3 days. Ovulation sets in motion processes leading to a period of optimal endometrial receptivity (Hoff et al., 1983). Associations were identified between clinical pregnancy and various endometrial receptivity markers, indicating their poor ability to predict pregnancy (Craciunas et al., 2019). Considering similar ultrasonographic endometrial characteristics and results obtained regardless of the day of FET, there might be some extent of individual variability in the WOI length. Consequently, endometrial receptivity tests are likely relevant only for patients with recurrent implantation failure.

We planned FET solely based on the day of urine LH surge without sonographic evidence of ovulation that may occur up until the second morning after the detection of the urine LH surge (Pearlstone and Surrey, 1994). While comparing the FET cycle outcomes during the embryo transfer on days 5, 6, and 7, the difference in the clinical pregnancy rate, implantation rate, miscarriage rate and live birth rate were not observed among the groups when adjusted for age, number and quality of transferred blastocysts. Bartels et al. also found equally good results with day +6 and +7 FETs, although there was some decline on day +7, consistent with our study observation (Bartels et al, 2019). The reason might be either coincidence due to the low number of transferred blastocysts or the decline might suggest that WOI could be closing.

The main limitation of our study is the nature of its design. It is a single-centre retrospective analysis, and despite an acceptable number of included cycles and robust methodological approaches, the presence of potential selection bias could not be completely excluded. Systematic differences among the three groups, not captured in cycle characteristics analysis, might exist and not be evident because the cycles were analysed and not the patients. In this study the groups consisted of non-homogeneous patients, including single and double embryo transfers and a wide array of blastocyst qualities. The issue was addressed using propensity score matching, aiming to eliminate potential bias. A properly designed prospective randomised study is needed to confirm our findings. Another limitation of this study is that several FET cycles were performed for the same couple. Since the study consisted of neither independent nor repeated measures, specific statistical methods were used

to counter the issue. The Cochran-Mantel-Haenszel test compared factorial variables among different groups while considering the successive number of embryo transfers in the respective couple. As no appropriate method exists for continuous variables in the matter, the respective GLM model was built for each variable using the couple's unique ID as a random blocking variable. In this way, the potential bias of repeated cycles in the same couple was eliminated to the best. A prospective, randomised study is needed to confirm our findings.

Conclusion

The results of our study suggest that vitrified-warmed blastocysts can be transferred to tNC on days +5, +6 or +7 based on the positive LH urine test without impacting the clinical outcome.

Study funding

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Conflict of interests

The authors have no competing interests to declare.

Ethics approval and consent to participate

As all the data were collected as part of quality control with no direct influence on the treatment, ethics approval was not required.

Authors' contribution

Milan Reljič conceived and designed the study; Nejc Kozar and Milan Reljič performed the statistical analyses and participated in drafting the manuscript. Vida Gavrić Lovrec wrote the manuscript. All three authors participated in data interpretation. All authors read and approved the final manuscript.

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Author biography

Vida Gavrić Lovrec was awarded a PhD by the University of Ljubljana in the field of perifollicular blood flow characteristics in IVF. She is involved in several research projects covering different fields of in vitro fertilization at the Department of Reproductive Medicine and Gynecologic Endocrinology at University Medical Centre Maribor.

Key message

Transfer of vitrified-warmed blastocyst may be scheduled on day 5, 6 and day 7 after the luteinizing hormone surge detected by urine tests without significantly affecting either clinical pregnancy or live birth rates.

Table 1. Comparison of patient and cycle characteristics between vitrified-warmed blastocyst transfers performed on days +5, +6 and +7 after the urine LH surge

	FET after the LH surge			p-value
	Day +5	Day +6	Day +7	
Number	380	1610	90	
Age (median, IQR)	34 (31–37)	34 (7)	34 (7)	NS
Unexplained infertility (N, %)	168 (45.28)	658 (41.59)	32 (35.96)	NS
Tubal factor infertility (N, %)	66 (17.79)	310 (19.59)	20 (22.47)	NS
Male factor infertility (N, %)	153 (40)	669 (42)	35 (39)	NS
No of previous IVF/ICSI cycles (median, IQR)	2 (1–2)	2 (1)	2 (1)	NS
Proportion of cycles with birth after fresh ET (N, %)	65 (18.62)	279 (19.14)	8 (9.30)	NS
Proportion of FET after freeze-all cycles (N, %)	48 (13.75)	200 (13.72)	17 (19.77)	NS
Menstrual cycle length (days) (median, IQR)	28 (28–29)	28 (1)	28 (1)	NS
Menstrual cycle variability (days) (median, IQR)	3 (2–5)	4 (3)	5 (4)	NS
Day of the LH surge using urine LH test (median, IQR)	13 (12–15)	13 (3)	13 (4)	NS
Endometrium thickness on the day of FET (mm) (median, IQR)	10 (8–11)	10 (3)	10 (2)	NS
Secretory endometrium pattern (N, %)	203 (53)	978 (61)	57 (63)	NS
No. of blastocysts transferred (median, IQR)	1 (1–2)	1 (1-2)	1 (1–2)	NS
Proportion of difficult ET (N, %)	7,1 (27)	4.9 (80)	3.3 (3)	NS
Proportion of BC vitrified on day 5 (N, %)	226 (59)	982 (61)	62 (69)	NS
Proportion of ET of morphologically optimal BC (N, %)	66 (18.80)	258 (18.60)	12 (14.12)	NS

ET embryo transfer, BC blastocyst, FET vitrified-warmed blastocyst transfer, IQR interquartile range, IVF *in-vitro* fertilization, ICSI Intra-cytoplasmic sperm injection, LH luteinizing luteinising hormone.

Table 2. Comparison of outcomes between vitrified-warmed blastocyst transfers performed on days +5, +6 and +7 after the urine LH surge

	FET after the LH surge			p-value
	day +5	day +6	day +7	
Number	380	1610	90	
Clinical pregnancy rate (N, %)	143 (37.6)	633 (39.3)	28 (31.1)	NS
Implantation rate (%)	34.3	35.6	30.9	NS
Miscarriage rate (N, %)	27 (7.1)	138 (8.6)	6 (6.7)	NS
Live birth rate (N, %)	116 (30.5)	495 (30.7)	22 (24.4)	NS

FET vitrified-warmed blastocyst transfer, LH luteinising hormone.

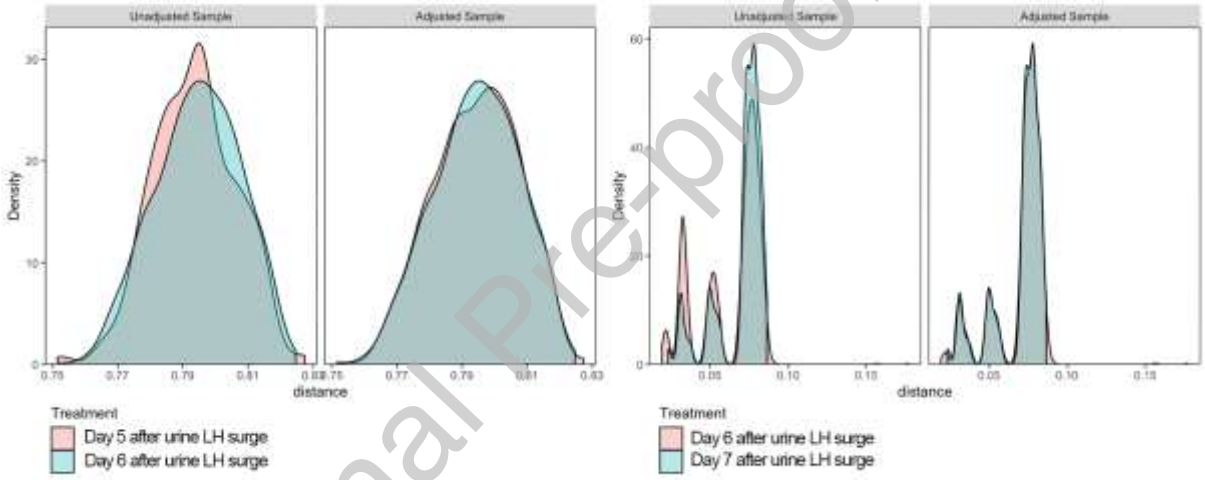

Figure 1: The distribution of propensity scores before and after propensity score matching.