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ARTICLE

Vitrified–warmed blastocyst transfer timing related to LH surge in true natural cycle and its impact on ongoing pregnancy rates

BIOGRAPHY

Murat Erden is a final year resident at Hacettepe University, School of Medicine, Department of OB/GYN. He is pursuing a master's degree in clinical trials at the London School of Hygiene and Tropical Medicine. His research area is the natural cycle for vitrified–warmed embryo transfer.

Murat Erden[1], Mehtap Polat[2,3], Sezcan Mumusoglu[1], Irem Yarali Ozbek[2], Gonca Ozten Dere[2], Lale Karakoc Sokmensuer[4], Sandro C. Esteves[5,6], Peter Humaidan[6,7], Hakan Yarali[1,2,*]

KEY MESSAGE

In patients undergoing warmed blastocyst transfer employing the true natural cycle protocol, differences in embryo transfer timing related to the LH surge (LH surge +6/+7/+8/+9 days) are associated with comparable ongoing pregnancy rates, reflecting the flexibility of the window of implantation for 1–3 days.

ABSTRACT

Research question: Does the timing of warmed blastocyst transfer in true natural cycle (tNC) differ according to six different commonly used definitions of LH surge, and do differences in timing have any impact on ongoing pregnancy rate (OPR)?

Design: Prospective monitoring, including repeated blood sampling and ultrasound analyses of 115 warmed blastocyst transfer cycles performed using tNC between January 2017 and October 2021.

Results: The reference timing of follicular collapse +5 days would be equivalent to LH surge +6 days in only 5.2–41.2% of the cycles employing the six different definitions of the LH surge. In contrast, the reference timing was equivalent to LH surge +7 days in the majority of cycles (46.1–69.5%) and less commonly to LH surge +8 days (1.8–38.3%) and +9 days (0–10.4%). For each definition of the LH surge, the OPR were comparable among the different warmed blastocyst transfer timings related to the LH surge (LH surge +6/+7/+8/+9 days). When logistic regression analysis was performed to evaluate the independent effect of variation of warmed blastocyst transfer timing (LH surge +6/+7/+8/+9 days) on OPR and taking LH surge +6 days as the reference, change in timing was not an independent predictor of OPR for any of the definitions of the LH surge.

Conclusions: Employing a policy of performing warmed blastocyst transfer on follicular collapse +5 days and using six different definitions of the LH surge, vitrified–warmed embryo transfer timing is indeed equivalent to LH surge +7/+8 and even +9 days in a significant proportion of tNC with comparable reproductive outcomes.

[1] Hacettepe University School of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey
[2] Anatolia IVF and Women's Health Center, Ankara, Turkey
[3] Atilim University Vocational School of Health Services, Department of Medical Services and Techniques, First and Emergency Aid Program, Ankara, Turkey
[4] Hacettepe University School of Medicine, Department of Histology and Embryology, Ankara, Turkey
[5] Androfert, Andrology and Human Reproduction Clinic, Referral Center for Male Reproduction, Campinas SP, Brazil
[6] Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[7] The Fertility Clinic, Skive Regional Hospital Resenvej 25, Skive, Denmark

KEYWORDS

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*Corresponding author. E-mail address: hyarali@anatoliatupbebek.com.tr (H. Yarali). https://doi.org/10.1016/j.rbmo.2022.04.018 1472-6483/
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INTRODUCTION

Efficient and safe embryo vitrification techniques have contributed to a marked worldwide increase in frozen embryo transfer (FET) cycles during the last decade (*De Geyter et al., 2020*; *Roque et al., 2019b*). Currently, low-quality evidence suggests that the hormone replacement therapy (HRT) protocol is associated with lower live birth rates (LBR) than the natural cycle for endometrial priming during FET (*Mumusoglu et al., 2021*; *Wu et al., 2021*). Moreover, it has been suggested that the natural cycle is associated with more favourable maternal, obstetric and perinatal outcomes than the HRT protocol (*Asserhøj et al., 2021*; *Ginström Ernstad et al., 2019*; *Hu et al., 2021*; *Litzky et al., 2018*; *Makhijani et al., 2020*). Therefore, a 'back to nature' approach, which advocates an expanded use of natural cycle FET, has been suggested by various authors (*Lawrenz et al., 2020*; *Roque et al., 2019a*).

Pinpointing the day of ovulation is crucial for the timing of FET in the true natural cycle (tNC) to maximize reproductive success. The usual practice relies on the LH surge documentation to schedule warmed blastocyst transfer at LH surge +6 days (*Mackens et al., 2017*; *Mumusoglu et al., 2021*). However, there is no consensus on the definition of the LH surge (*Erden et al., 2022*). Some physicians rely on the onset, whereas others rely on the peak of the LH surge (*Irani et al., 2017*). Our local approach uses 'direct' evidence of ovulation rather than the LH surge. Accordingly, the follicular collapse confirmed by transvaginal ultrasonography is used to schedule the warmed blastocyst FET as follicular collapse +5 days. Naturally, using different criteria to define the LH surge results in differences in the day of FET in tNC, which might potentially impact the window of implantation and so the reproductive outcome. Currently, there is a paucity of data in this area and only three retrospective studies have compared reproductive outcomes using different timings of warmed blastocyst transfer in tNC, reporting contradictory results (*Bartels et al., 2019*; *Irani et al., 2017*; *Lovrec et al., 2021*).

On this basis, the study centre's database of tNC FET cycles was queried to determine the impact of different definitions of LH surge, as commonly used in the existing literature, on the date of warmed blastocyst transfer. In addition, the ongoing pregnancy rates (OPR) after tNC FET were compared according to different definitions of the LH surge.

MATERIALS AND METHODS

Inclusion criteria

The main criteria to perform a tNC FET in this study were the presence of regular menstrual cycles and the fact that patients needed to live locally to permit frequent endocrine and ultrasonographic monitoring. Consecutive tNC FET cycles performed at Anatolia IVF and Women's Health Center, Ankara, Turkey, from January 2017 to October 2021 were prospectively monitored, including repeated blood sampling and ultrasound analyses (FIGURE 1). Of the 294 cycles identified, 179 were excluded: 115 due to lack of three or more consecutive daily serum LH measurements before follicle rupture, 39 due to luteinized unruptured follicle (LUF) syndrome, 23 due to cleavage-stage transfer, and finally, two due to vaginal bleeding or thyroid disease (FIGURE 1). LUF cycles were excluded because the follicular collapse was the criteria to pinpoint the day of ovulation and hence assign the warmed blastocyst transfer day. A total of 115 tNC cycles were finally included in the analysis

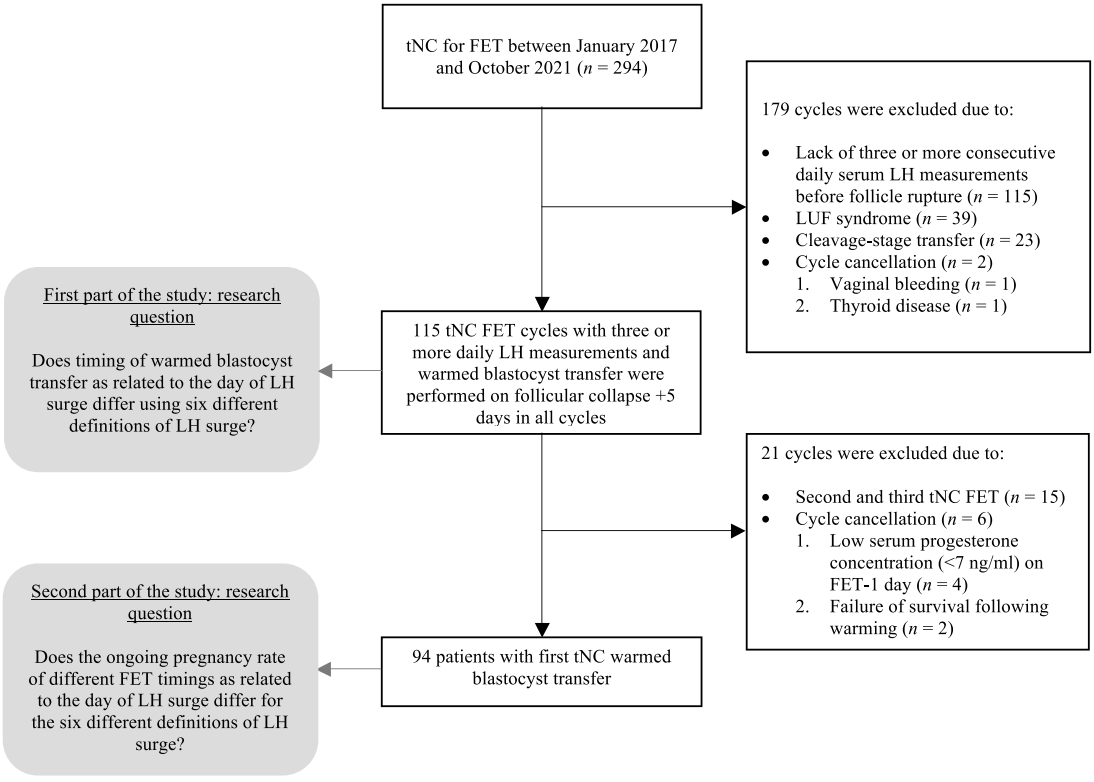

FIGURE 1 Flow diagram for the study population.

to determine the impact of different definitions of the LH surge on the date of warmed blastocyst transfer (FIGURE 1).

For the second part of the study, comparing the OPR after tNC blastocyst FET according to different definitions of the LH surge, 21 cycles from the first part of the study were excluded, leaving 94 cycles to be analysed (FIGURE 1). Specifically, because each patient was included only once, 15 cycles were excluded as these cycles constituted second or third tNC FET cycles. Cycle cancellation due to low serum progesterone (<7 ng/ml) on the FET-1 day (n = 4) or failure of survival following warming (n = 2) were other reasons for exclusion (FIGURE 1).

The Institutional Review Board of Hacettepe University approved the study protocol on 27 October 2021 (KA-21116).

tNC protocol

On day 2 or 3 of menses, transvaginal ultrasonography was performed to rule out any cysts or corpora lutea prevailing from the previous cycle. In the presence of persistent corpora lutea, cycle cancellation was undertaken in cycles with serum progesterone >1.5 ng/ml measured on day 2 or 3 of menses if tNC was scheduled immediately after a failed fresh transfer or a freeze-all cycle. Transvaginal ultrasonographic monitoring started on cycle days 8–10. Daily endocrine monitoring commenced using serum oestradiol, LH and progesterone measurements alongside daily transvaginal ultrasonographic monitoring when the leading follicle reached a mean diameter of approximately 14–15 mm. Following frequent endocrine and ultrasonographic monitoring, the day of ovulation was precisely documented by follicular collapse as defined by the complete disappearance of the follicle or the reduction of its volume with thickening of the follicle wall (*Wetzels and Hoogland, 1982*). Embryo transfer timing was scheduled as follicular collapse +5 days for blastocyst-stage transfers in all patients. All included cycles were tNC without human chorionic gonadotrophin trigger or luteal phase support (LPS) administration.

Laboratory procedures

Ovarian stimulation, triggering of final oocyte maturation, oocyte retrieval, embryo culture, vitrification, warming and embryo transfer were conducted according to standard protocols, as described previously (*Mumusoglu et al., 2017*). Vitrification was performed on day 5 or 6 based on the development of each embryo. Blastocyst grading was performed using Gardner's criteria (*Gardner and Schoolcraft, 1999*); only 3–6 A/B blastocysts were vitrified. In order to adjust for blastocyst grading, which is a confounding variable, blastocysts were divided into four groups according to their morphological grading before cryopreservation: excellent (≥3AA), good (3–6AB, 3–6BA, 1–2AA), average (3–6BB, 3–6AC, 3–6CA, 1–2AB, 1–2BA) and poor (1–6BC, 1–6CB, 1–6CC, 1–2BB).

LH was measured using the Cobas e601 analyser employing the Elecsys LH immunoassay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). The assay uses a sandwich test principle and a measuring range of 0.100–200 IU/l, as defined by the lower detection limit and the maximum of the master curve. The coefficients of variation for repeatability and intermediate precision were 0.6–1.2% and 1.6–2.2%, respectively.

LH surge definition

Because serum sampling was routinely performed in the current study, the six most commonly used definitions for LH surge in serum were specified (*Bartels et al., 2019*; *Erden et al., 2022*; *Groenewoud et al., 2012*; *Irani et al., 2017*; *Testart et al., 1981*; *Ursillo et al., 2021*; *Wetzels and Hoogland, 1982*). Of these definitions, some were based on the results of the authors' own data to develop the criteria for the LH surge (*Testart et al., 1981*; *Wetzels and Hoogland, 1982*), whereas others used empirical thresholds (*Bartels et al., 2019*; *Groenewoud et al., 2012*; *Irani et al., 2017*; *Ursillo et al., 2021*). Moreover, modifications as to the frequency of serum sampling were used in some studies (*Lee et al., 2017*; *Mackens et al., 2020*). For example, the definition for the onset of the LH surge described by *Testart et al. (1981)* is one of the most commonly employed criteria in the literature. In this study, serum LH sampling was performed four times daily from when 'LH release was considered imminent'. The authors defined the term LH 'surge-initiating rise' (SIR), which corresponded to the onset of the LH surge, as any LH concentration equal to or exceeding 180% of the mean value for the preceding four measurements (e.g. mean LH of the four preceding values = 3.2 IU/l; LH SIR concentration = 1.8 × 3.2 = 5.8 IU/l). Because this frequency of serum sampling is not practical in daily life, others used once-a-day measurements (*Lee et al., 2017*; *Mackens et al., 2020*).

The present analysis aimed to include those LH surge criteria that were most commonly used in the available literature, including LH ≥10 IU/l (*Groenewoud et al., 2012*), LH ≥15 IU/l (*Ursillo et al., 2021*), LH ≥17 IU/l (*Irani et al., 2017*), LH ≥20 IU/l (*Bartels et al., 2019*), ≥180% of the mean of the preceding LH values (*Testart et al., 1981*) and LH >2 times higher than all preceding values (*Wetzels and Hoogland, 1982*).

Outcome measures

The outcome measure for the first part of the study was to assess how frequently and to what extent there would be a change in transfer date related to the day of LH surge, using the six different definitions of LH surge compared with follicular collapse +5 days. For each definition of the LH surge, the transfer date was expressed as LH surge +n days, considering the first day of the LH surge as day 0. To illustrate the timing of warmed blastocyst transfer related to LH surge, an example is given in FIGURE 2. In this case, with four consecutive once-daily late follicular phase LH measurements as 10.1, 16.3, 22.9 and 51.0 IU/l followed by follicular collapse the next day and warmed blastocyst transfer performed on collapse +5 days, the patient would be classified as having the warmed blastocyst transfer on LH surge +9 days using the ≥10 IU/l LH surge definition (*Groenewoud et al., 2012*). Using an LH surge definition as ≥15 IU/l (*Ursillo et al., 2021*), ≥17 IU/l (*Irani et al., 2017*), ≥20 IU/l (*Bartels et al., 2019*), ≥180% of the mean of preceding LH values (*Testart et al., 1981*) and >2 times higher than all preceding values (*Wetzels and Hoogland, 1982*), this patient would be classified as having the warmed blastocyst transfer on LH surge +8, +7, +7, +7 and +6 days, respectively (FIGURE 2).

The main outcome measure for the second part of the study was OPR, as defined by a gestational sac with fetal cardiac activity at 12 weeks of gestation. For each definition of the LH surge, the OPR of different FET timings related to the day of the LH surge were compared.

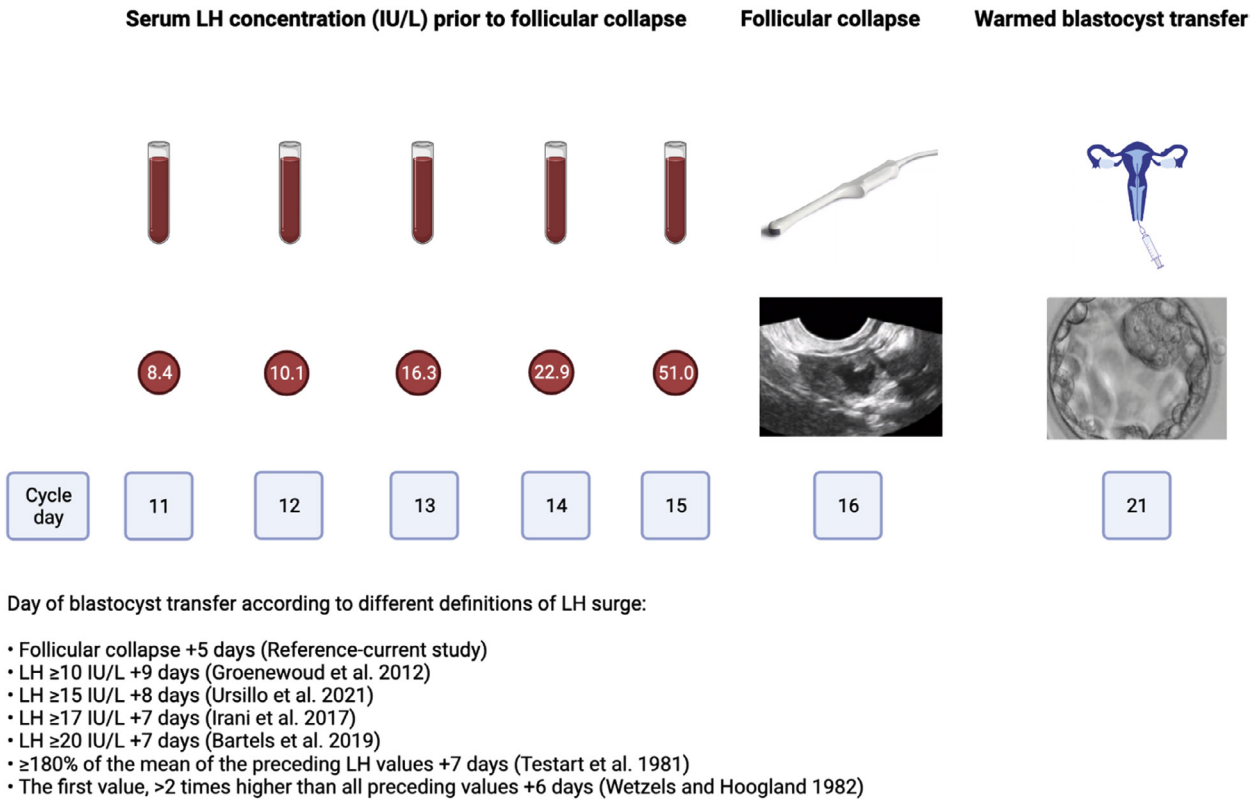

FIGURE 2 The day of blastocyst transfer according to different definitions of the LH surge for a patient using four consecutive daily late follicular phase LH measurements prior to follicular collapse.

Statistics

Distribution characteristics of variables were visually assessed, using histograms, box plots and Q–Q plots, and analysed with Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous variables with normal distribution were expressed as mean ± SD, whereas median (minimum–maximum) with non-Gaussian distribution. Chi-squared and Fisher's exact tests were used to compare the categorical variables. A linear-by-linear association chi-squared test was used to compare categorical variables of more than two groups. Logistic regression analysis was conducted to delineate whether the different definitions of LH independently affect OPR. SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses.

RESULTS

The baseline demographic features and cycle characteristics for the first part of the study are given in TABLE 1. Of the included 115 cycles, 26 (22.6%) were from freeze-all and 38 (33.0%) from preimplantation genetic testing (PGT) cycles (TABLE 1). Before the collapse, the mean follicular phase length and follicular diameter were 13.8 days and 19.8 mm, respectively (TABLE 1).

Timing of transfer according to the different definitions of LH surge versus follicular collapse +5 days

As dictated by the study centre's warmed blastocyst transfer timing policy (follicular collapse +5 days), if the first attainment of serum LH ≥10 IU/l was used to define the LH surge, the warmed blastocyst transfer timing would have been performed on LH surge +6 days in six cycles (5.2%), LH surge +7 days in 53 cycles (46.1%) and LH surge +8 days in 44 cycles (38.3%). In 12 cycles (10.4%), the warmed blastocyst transfer timing would have been performed on LH surge +9 days (TABLE 2).

With a presumed LH surge definition of LH ≥15 IU/l, the warmed blastocyst transfer timing would have been classified as LH surge +6 days, +7 days and +8 days in 30 cycles (26.1%), 66 cycles (57.4%) and 19 cycles (16.5%), respectively. With a presumed LH surge definition of LH ≥17 IU/l, the warmed blastocyst transfer timing would have been performed as LH surge +6 days in 33 cycles (28.7%), LH surge +7 days in 75 cycles (65.2%) and LH surge +8 days in seven cycles (6.1%).

The warmed blastocyst transfer timings according to the three remaining different definitions of LH surge are given in TABLE 2.

Reproductive outcome

The demographic features of the 94 patients included for the second part of the study are given in Supplementary Table 1.

For each definition of the LH surge, the OPR were comparable among the different FET timings related to LH surge (LH surge +6/+7/+8/+9 days) (TABLE 3).

Logistic regression analysis was performed to evaluate the independent effect of variation of warmed blastocyst transfer timing (LH surge +6/+7/+8/+9 days) on OPR for each definition of the LH surge (FIGURE 3). Female age, body mass index, previous childbirth, number of previous IVF attempts, number of blastocysts transferred, day of vitrification, PGT and blastocyst morphology were entered into the model as covariates. Taking LH surge +6 days as

TABLE 1 BASELINE DEMOGRAPHICS AND TNC CHARACTERISTICS OF 115 PATIENTS WHO WERE SCHEDULED TO UNDERGO WARMED BLASTOCYST TRANSFER ON FOLLICULAR COLLAPSE +5 DAYS (FIRST PART OF THE STUDY)

Characteristic	Patients
Female age, years	35.0 ± 5.8
Male age, years	37.4 ± 6.1
Female BMI (kg/m^2)	23.3 ± 4.1
Type of infertility	
Primary	89 (77.4)
Secondary	26 (22.6)
Duration of infertility, months	28 (2; 168)
Number of previous IVF cycles	3 (2; 11)
Previous childbirth	23 (20)
Number of patients with freeze-all strategy	26 (22.6)
Number of patients with PGT	38 (33.0)
Monitoring characteristics of tNC	
Follicular phase length, days	13.8 ± 2.6
Follicle diameter before collapse, mm	19.8 ± 2.0
Peak oestradiol concentration, pg/ml	346.7 ± 105.6
Peak LH concentration, IU/l	49.5 ± 19.1
Endometrial thickness, mm	10.5 ± 2.0
Progesterone concentration on FET-1 day, ng/ml	16.5 ± 6.1
Number of embryos transferred	1 (1; 2)
Number of cycles with single blastocyst transfer	78 (67.8)

Data are presented as mean ±SD, median (min; max) or *n* (%).

BMI = body mass index; FET = frozen embryo transfer; PGT = preimplantation genetic testing; tNC = true natural cycle.

the reference, change in timing was not an independent predictor of OPR for any definitions of the LH surge (FIGURE 3).

DISCUSSION

Considering the day of the LH surge as day 0, LH surge +6 days is commonly used to schedule the day of warmed blastocyst transfer in tNC (*Mackens et al., 2017*; *Mumusoglu et al., 2021*). Over the years, through different studies and set-ups, testing either in serum or urine, different definitions of the LH surge have been developed, but without reaching any consensus (*Godbert et al., 2015*). Importantly, differences in definitions of the LH surge may impact the timing of embryo transfer in tNC FET. Moreover, performing warmed blastocyst transfer on LH surge +6 days assumes that ovulation occurs in all cases 1 day after the LH surge. However, there may be marked interpersonal variations in the time interval between the onset of the LH surge and subsequent ovulation, ranging from 22 to 56 h (*Erden et al., 2022*). For the present study, it was therefore decided *a priori* to rely on follicular collapse to pinpoint the day of ovulation and hence assign the day of FET accordingly. It was noted that the reference timing of follicular collapse +5 days would be equivalent to LH surge +6 days in only 5.2–41.2% of the cycles employing six different definitions of the LH surge. In contrast, the reference timing was equivalent to LH surge +7 days in the majority of cycles (46.1–69.5%) and less commonly to LH surge +8 days (1.8–38.3%) and +9 days (0–10.4%). Importantly, for each different definition of the LH surge, the OPR were comparable among the LH surge +6/+7/+8/+9 timings, reflecting the high degree of flexibility of the window of implantation.

The LH surge is 'indirect', and not all LH surges result in ovulation as 3–4% of women with regular cycles and documented LH surges may be anovulatory (*Guermandi et al., 2001*; *Park et al., 2007*). Furthermore, the absence of a secretory endometrium in endometrial biopsies following urinary LH surges has been reported in 7% of cycles (*McGovern et al., 2004*). Hence, rather than LH surge +6 days,

TABLE 2 ASSESSMENT OF HOW FREQUENT AND TO WHAT EXTENT THERE WOULD BE A CHANGE IN TRANSFER DATE, AS RELATED TO THE DAY OF LH SURGE, USING THE SIX COMMONLY KNOWN DIFFERENT DEFINITIONS OF THE LH SURGE, WHEN WARMED BLASTOCYST TRANSFER WAS PERFORMED ON FOLLICULAR COLLAPSE +5 DAYS (*N* = 115)

Definition of the LH surge (Author, year)	LH +9 days	LH +8 days	LH +7 days	LH +6 days
LH ≥10 IU/l (*Groenewoud et al., 2012*)	12 (10.4)	44 (38.3)	53 (46.1)	6 (5.2)
LH ≥15 IU/l (*Ursillo et al., 2021*)	–	19 (16.5)	66 (57.4)	30 (26.1)
LH ≥17 IU/l (*Irani et al., 2017*)	–	7 (6.1)	75 (65.2)	33 (28.7)
LH ≥20 IU/l (*Bartels et al., 2019*)	–	6 (5.3)	61 (53.0)	47 (41.2)
≥180% of the mean of the preceding LH values (*Testart et al., 1981*)	–	9 (8)	80 (69.5)	24 (21.2)
The first value, >2 times higher than all preceding values (*Wetzels and Hoogland, 1982*)	–	2 (1.7)	76 (66.0)	34 (30.4)

Data are presented as *n* (%).

TABLE 3 ASSESSMENT OF THE IMPACT OF TRANSFER DATE, AS RELATED TO THE DAY OF LH SURGE, ON ONGOING PREGNANCY RATES[a] USING THE SIX COMMONLY KNOWN DIFFERENT DEFINITIONS OF THE LH SURGE, WHEN WARMED BLASTOCYST TRANSFER WAS PERFORMED ON FOLLICULAR COLLAPSE +5 DAYS (*N* = 94)

Definition of the LH surge (Author, year)	LH +9 days	LH +8 days	LH +7 days	LH +6 days	*P*-value[b]
LH ≥10 IU/l (*Groenewoud et al., 2012*)	8/11 (72.7)	21/37 (56.8)	20/41 (48.8)	2/5 (40.0)	0.125
LH ≥15 IU/l (*Ursillo et al., 2021*)	–	12/19 (63.2)	27/50 (54)	12/25 (48.0)	0.278
LH ≥17 IU/l (*Irani et al., 2017*)	–	4/7 (57.1)	34/59 (57.6)	12/27 (44.4)	0.237
LH ≥20 IU/l (*Bartels et al., 2019*)	–	3/6 (50.0)	29/49 (59.2)	18/38 (47.4)	0.444
≥180% of the mean of the preceding LH values (*Testart et al., 1981*)	–	6/8 (75.0)	36/67 (53.7)	8/17 (47.1)	0.240
The first value, >2 times higher than all preceding LH values (*Wetzels and Hoogland, 1982*)	–	1/1 (100.0)	37/64 (57.8)	11/26 (42.3)	0.124

Data are presented as *n/n* (%).

[a] Ongoing pregnancy rate is defined by a gestational sac with fetal cardiac activity at 12 weeks of gestation.

[b] Linear by linear association value is given.

follicular collapse +5 days was used to time the warmed blastocyst transfer in the current study. In general, follicular collapse is the most predictive sign of ovulation (*Marinho et al., 1982*), resulting in complete disappearance of the follicle or reduction of its volume with thickening of the follicle wall, or replacement of the follicle by an area of spongy appearance (*Wetzels and Hoogland, 1982*). However, it should be acknowledged that ultrasonographic documentation of ovulation has some drawbacks. First, the sensitivity and specificity of follicular collapse to predict ovulation have been reported to be 84.3% and 89.2%, respectively (*Ecochard et al., 2000*). Second, in some cycles, a typical accelerated growth pattern compatible with a LUF is noted instead of rupture, posing difficulty in assigning the date of transfer. Therefore, LUF cycles were excluded from the current series. From the present analysis, it is concluded that further prospective randomized trials are warranted to establish which of the markers, LH surge and/or ultrasonographic documentation of ovulation, should be used to optimally pinpoint the day of ovulation and FET timing to accomplish the highest reproductive outcome.

There are thought to be only three retrospective studies that have compared reproductive outcomes according to different criteria for timing of FET in tNC (*Bartels et al., 2019*; *Irani et al., 2017*; *Lovrec et al., 2021*). In the study

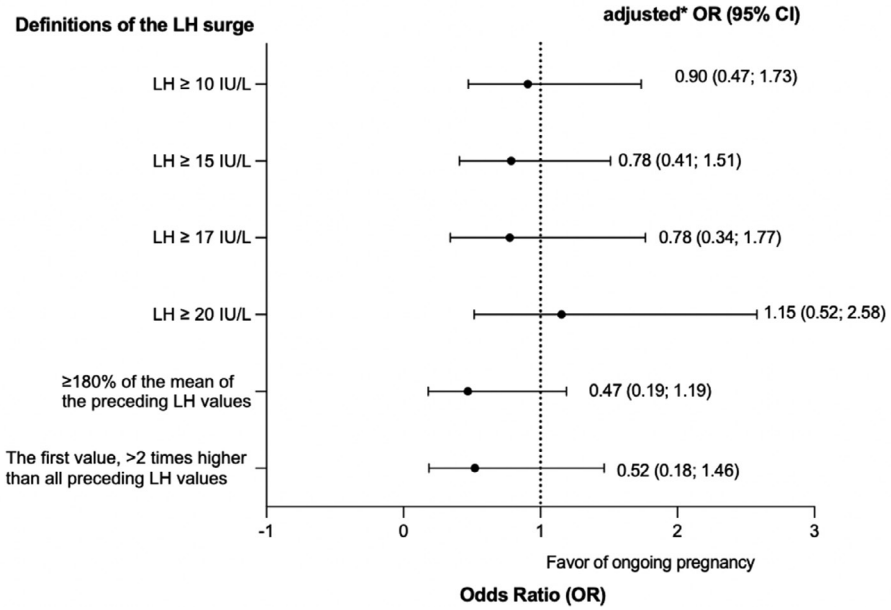

FIGURE 3 Logistic regression analysis to evaluate the independent effect of variation of warmed blastocyst transfer timing related to the LH surge, using the six commonly known different definitions of the LH surge, on ongoing pregnancy rates (*n* = 94). *A model including female age, body mass index, previous childbirth, number of previous IVF attempts, number of embryos transferred, day of vitrification, preimplantation genetic testing and blastocyst morphology as covariates to test the independent effect of using different definitions of the LH surge without changing timing of FET on ongoing pregnancy rates.

by *Irani et al. (2017)*, warmed blastocyst transfer in tNC was performed in 612 patients (691 cycles) 5 days after the documentation of the LH surge. Of those 612 patients, PGT for aneuploidies (PGT-A) was performed in 365 (407 cycles), and no LPS was administered. Patients in the PGT-A and non-PGT-A groups were further divided into two subgroups according to the physician's preference. Group A included patients in whom the LH surge was defined as the first attainment of LH ≥17 IU/l with a ≥30% drop in oestradiol concentrations the following day; Group B included patients in whom the LH concentration continued to rise, and the surge was defined as the highest serum LH concentration occurring 1 day after LH ≥17 IU/l despite a ≥30% drop in oestradiol concentrations. Among the non-PGT-A cycles, Group A was associated with significantly higher implantation rates (48.7% versus 38.1%; $P = 0.01$; adjusted OR 1.6, 95% CI 1.1–2.3) and LBR (52.9% versus 40.1%; $P = 0.01$; adjusted OR 1.7, 95% CI 1.1–2.8) compared with Group B. Among the PGT-A cycles, Groups A and B had comparable implantation rates (57.4% versus 63%, respectively; $P = 0.39$) and LBR (56.7% versus 63.4%, respectively; $P = 0.37$). The authors speculated that the lower success rate among non-PGT-A patients in Group B than Group A might be attributed to a higher rate of embryo–endometrium dys-synchrony caused by relatively longer exposure to the endometrium to progesterone. The authors further speculated that the lack of a detrimental effect in reproductive outcomes in PGT-A cycles might be due to earlier implantation of tested blastocysts following zona breaching during biopsy (*Liu et al., 1993*). The retrospective study design, single-point LH assessment and lack of cluster analysis are clearly the limitations of this study.

In another retrospective study, the impact of timing of warmed blastocyst transfer in tNC was evaluated in 341 cycles (*Bartels et al., 2019*). However, there was heterogeneity in physician practice for FET timing; some used a serum LH cut-off ≥20 IU/l, while others awaited the LH peak or analysed serum oestradiol and progesterone dynamics in the setting of an LH increase. Each cycle was classified by the timing of FET in relation to the LH surge, which was defined as the first attainment of serum LH ≥20 IU/l: Group 1 ($n = 211$; 61.9%), LH ≥20 IU/l lasting for 1 day in whom FET was performed 6 days later; Group 2 ($n = 60$; 17.6%), LH ≥20 IU/l lasting for two consecutive days in whom FET was performed 6 days after the LH surge; Group 3 ($n = 70$; 20.5%), LH ≥20 IU/l lasting for two consecutive days in whom FET was performed 7 days after the LH surge. LPS, either vaginal progesterone or less commonly intramuscular progesterone, was administered, based on the physician's discretion. The authors reported that implantation, clinical and OPR were comparable for the three groups. When Groups 1 and 2 were combined and compared with Group 3 (FET timing 6 or 7 days after the LH surge), the OPR were also comparable (66.4% and 62.9%). Due to the arbitrary nature of choosing an LH cut-off of 20 IU/l, various other thresholds were investigated; transferring 6 or 7 days after the LH surge achieved comparable OPR in relation to the LH cut-off of 15, 16, 17, 18, 19, 20 and 25 IU/l. The authors concluded that the timing of blastocyst transfer in tNC after the LH surge is flexible within 24 h as outcomes were equally good with embryo transfers on day 6 or 7 after the LH surge. The limitations of that study are the retrospective study design, lack of serum hormone measurements 1 day after LH ≥20 IU/l in some patients, and a single-point assessment for the LH surge.

Finally, the reproductive outcomes of vitrified–warmed blastocyst transfer performed 5, 6, or 7 days after detecting the LH surge in urine were compared in a recent retrospective study enrolling 2080 cycles (*Lovrec et al., 2021*). Urine LH testing every morning commenced when the leading follicle attained a mean diameter of 15 mm. Although warmed blastocyst transfer was performed most commonly 6 days after the urinary LH surge (1610 cycles, 77.4%), it was also scheduled 5 (380 cycles, 18.3%) or 7 (90 cycles, 4.3%) days after the LH surge, to avoid transfer on busy days or workload during weekends. LPS was administered as 400 mg/day of micronized vaginal progesterone immediately after blastocyst transfer. The clinical pregnancy, miscarriage, implantation and delivery rates of the vitrified–warmed blastocyst transfers performed 5, 6 and 7 days after the urinary LH surge were all comparable. The limitations of this study are the retrospective study design and lack of cluster analysis to account for the inclusion of more than one cycle for a patient.

There are several differences among the three available retrospective studies and the current series. LPS was administered in the studies by *Bartels et al. (2019)* and *Lovrec et al. (2021)*, but not in the current series. LH testing was performed in the urine rather than serum in the study by *Lovrec et al. (2021)*. In the study by *Irani et al. (2017)*, although warmed blastocyst transfer was performed 5 days after the LH surge in all patients, different definitions of the LH surge have been employed by different physicians even within the same clinic. Despite these differences, and in line with the studies by *Bartels et al. (2019)* and *Lovrec et al. (2021)*, for the six different LH surge definitions in the present study, comparable OPR were also noted among the various time subgroups related to the LH surge.

A limitation of the current study is the assignment of the warmed blastocyst transfer timings related to LH surge by the study centre's routine policy (follicular collapse +5 days), rather than by randomization. Other limitations include single daily measurements of LH, exclusion of LUF cycles and a limited sample size exploring reproductive outcomes in relation to transfer day, particularly for the second part of the study.

It is concluded that with a policy of performing warmed blastocyst transfer on follicular collapse +5 days, FET timing is indeed equivalent to LH surge +7/+8 or even +9 days in a significant proportion of tNC cycles, using six different definitions of the LH surge. Interestingly, these differences in FET timing related to the LH surge seem to be associated with comparable reproductive outcomes, reflecting the flexibility of the window of implantation for 1–3 days. Finally, more research is warranted to delineate which serum progesterone concentrations and duration of progesterone exposure are needed to open and secure the window of implantation.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2022.04.018.

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