International Journal of Multiphysics
Volume 18, No. 3, 2024
ISSN: 1750-9548

Hormonal Monitoring and Ovulation Timing in Frozen Embryo Transfer: A Focused Narrative Review

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Abstract

Background: Accurate identification of ovulation is important for timing embryo transfer in natural and stimulated FET cycles. Objective: To review luteinizing hormone (LH) surge definitions, endocrine monitoring, and ultrasound assessment of ovulation.

Methods: A focused narrative synthesis was prepared from the supplied hormonal monitoring review, preserving its cited observations. Conclusion: Variation in LH surge definitions and serum–urine detection may influence scheduling; combined hormonal and ultrasound assessment remains clinically relevant.

Keywords: frozen embryo transfer; LH surge; ovulation; hormonal monitoring; ultrasound

Introduction

One of the challenges in natural and stimulated cycles is that spontaneous ovulation may occur so determining the day of ovulation is crucial for timing of embryo transfer to maximize the LBR.The current practice is mixed and relies on LH surge documentation by daily/frequent endocrine monitoring, including serum LH, estradiol (E2)and progesterone(P4), combined or not with serial ultrasound assessments to confirm ovulation once the leading follicle attains a mean diameter of 15mm[(1)].While the definition of the onset of the LH surge should be standardized in t-NCFET cycles to optimize reproductive outcomes, aconsensus definition is lacking in the available literature.Currently, some clinics rely on the onset and others on the peak of the LH surge [(2)].

Definitions of the LH Surge

Different criteria were proposed to define the onset of the LH surge. A different fold increase in LH level from the baseline LH measurements, ranging from1.8- to 6-fold, was used to define the onset of the LH surge [(3)].The baseline LH level used to define the fold increase differed across studies; the LH level on the preceding day [(4)], all preceding days [(5)], follicular phase levels [(6)], the mean of the preceding four[(7)] or six measurements [(8)] and the mean of the preceding 5 [(3)]or7days[(9)].

The definition for the onset of the LH surge, as described by **Testart *et al.*[(7)]**, is the most commonly employed criterion in the literature where the onset of the LH surge defined as any LH level equal to or exceeding 180% of the mean value from the preceding four measurements. Rather than a fold-increase, studies used an increase of at least two to three SDs [(10)] above the mean of the preceding measurements to define the onset of the LH surge. **Hoff *et al.*[(11)]** defined the onset of the LH surge as the first LH value exceeding the mean +2SD of six preceding values. In the study by **Kesner *et al.*[(10)]**, with once-daily urinary LH sampling, an increase >3SD over the mean of the seven preceding days was used to define the onset of the LH surge.

LH Surge and Ovulation Timing

In the earliest study, the duration between the onset of the LH surge and ovulation was estimated by histologic dating of the corpus luteum [(12)]. By studying the serum concentrations of FSH, LH and P4 in8-h intervals from eight subjects during the mid-cycle, the authors reported that ovulation occurred 32.8h, with a range of 28–44h, following the onset of the LH surge [(12)].In this study, ovulation occurred at 17.6h, with a rangeof16–24h following the LH peak. Serum samples were taken every 8-h to measure levels of E2, LH, FSHandP4.Thefirst

significant rise in LH, denoting the onset of a surge, was the first value which was 1.5times the mean of the preceding baseline values; however, the definition of preceding baseline values was not provided[13]. The median duration between the onset of the LH surge and ovulation was 32.0h (95%CI¼23.6–38.2h), ranging from 24to56h.

The time between the LH peak and ovulation was 16.5 h (95% CI: 9.5–23.0), with arrange of 8–40 h.The authors concluded that the onset of the LH surge, rather than the peak, would be the best indirect parameter of impending ovulation. The mean duration between the onset of LH surge and ovulation was 33.91h (95%CI=30.79–37.03) in six studies[8].

Currently, since the timing of FET in a t-NC is most commonly based on the onset of the LH surge, i.e. +4 days and +6 days for warmed cleavage stage or stage blastocyst transfer, respectively [14], differences in definitions of the onset of the LH surge may impact the timing of embryo transfer. Of note, different definitions for the onset of the LH surge have been used in NC studies, including: first attainment of LH 17 mIU/ml during the follicular phase with a 30% drop in E2 levels the following day [2]; LH >10 mIU/ml [15]; LH > 15 mIU/ml[16]; and LH > 20 mIU/ml [17]. Moreover, single point arbitrary thresholds may not be entirely correct because there might be an overlap in LH levels between the 90th percentile of the pre-surge levels (e.g. 10–15 mIU/ml) and 10th percentile of surge levels (9.9 mIU/ml) [18]. To our knowledge, there isno comparative study evaluating the differences in embryo transfer timing and their impact on reproductive outcomes in a t-NC FET cycle, using different definitions for the onset of the LH surge.

Only three studies used frequent endocrine and ultrasound monitoring [8]. On the basis of the available data, it appears that there is a marked interindividual variation in the time interval between the onset of the LHsurge and ovulation; the mean duration in hours was 33.91 (95% CI=30.79–37.03: six studies, 187 cycles), ranging from 22 to 56 h.This considerable variation could be relevant for planning and timing of FET in t-NC because the standard practice for scheduling FET in a tNC is to rely on the onset of the LH surge rather than documentationof ovulation by ultrasound.

Physiology and LH Surge Patterns

During an NC, the rise of E2 originating from the dominant follicle, exceeding 200–300 pg/ml for a minimum of 50 h, triggers the LH surge[19]. Although an increase in serum P4, 12 h before the onset of the LH surge was defined approximately four decades ago [11], it has not been considered to play a critical role in the physiology of ovulation. However, a recent interest in the LH-independent rise in circulating P4, characterized by a precipitous increase in serum P4to 0.5ng/ml, as the trigger for the LH surge has emerged [20].

Urinary Versus Serum LH Testing

From a practical standpoint, detecting ovulation in urine is more convenient and patient-friendly than testing in serum. However, several disadvantages exist for urine testing, as discussed below.Testing LH in urine is dependent on the assay used, as different urinary LH assays measure different molecules [18].

A wide variation exists in the sensitivity of the urinary LH assays. The lowest threshold levels of five different urinary LH assays have been reported to range from 25.5 to 48.7 mIU/ml [21]. Accordingly, for those assays with detection limits<40 mIU/ml, false-negative results will be encountered in patients with low peakLH levels (<40 mIU/ml), which might affect up to 35% of ovulatory cycles [22].

When urinary testing is compared with serum testing, a time delay should be taken into account owing to the prolonged urinary clearance of LH [23].

In one study [24], peaks and nadirs of the individual profiles of urinary estrogens and LH were always delayed by at least 1 day and often 2 days compared with the corresponding serum profiles. In contrast, in another study including a total of 33 patients, the detection of the onset of the LH surge was simultaneous in serum and urine in 11 of 33 patients [23], whereas in the remaining 22 patients, the onset of the LH surge in urine was delayed by 3–21 h when compared with the onset of the serum LH surge.

Ultrasound Assessment and Transfer Timing

Documentation of ovulation by ultrasound may be used instead of or in addition to detection of the LH surge for FET timing in t-NC. The LH surge is an indirect surrogate for ovulation as not all LH surges result in

ovulation and, in fact, 3–4% of women with regular cycles and documented LH surges are anovulatory [3]. Furthermore, the absence of a secretory endometrium in endometrial histological specimens following urinary LH surges hasbeen reported in a total of 7% of cycles [25]. In contrast, documentation of ovulation by ultrasound is a direct measurement and as such, highly reliable. The ultrasonographic signs of ovulation have been closely related to ovulation [5], and follicular collapse is the most predictive sign of ovulation [26], resulting in either disappearance of the follicle, reduction of its volume with thickening of the follicle wall, or replacement of the follicle by an area of 'spongy' appearance [5].

Timing of thawed embryo transfer in a t-NC

Considering the day of the LH surge as Day 0, the usual practice to perform t-NC FET at the cleavage and blastocyst stages is LH surge + 4 days and LH surge + 6 days, respectively [1]. Of note, only three retrospective studies compared reproductive outcomes employing different criteria for timing of FET in t-NC [27]. Of those three studies, LH surge testing was performed in serum in two studies [28] and in urine in the remaining one study [27]. In a retrospective setting, t-NC was performed in patients who underwent warmed blastocyst transfer after either pre-implantation genetic testing for aneuploidy (PGT-A) (n=365 women, 407 cycles) or without PGT-A(n=247 women, 284 cycles) [2]. No luteal phase support was administered. Patients in the PGT-A and non-PGT-A groups were further divided into two sub-groups. Group A included patients in whom the LH surge was defined as the first attainment of LH17 mIU/ml with a 30% drop in E2 levels the following day. Group B included patients whose LH level continued to rise, and the surge was defined as the highest serum LH level occurring 1 day after LH 17 mIU/ml, despite 30% drop in E2levels. Among the non-PGT-A cycles, Group A was associated with significantly higher implantation rates (48.7% versus 38.1%; P=0.01; adjusted odds ratio (OR): 1.6(95% CI, 1.1–2.3)) and LBRs (52.9% versus 40.1%; P=0.01; adjusted OR: 1.7 (95% CI, 1.1–2.8)) compared to Group B. In contrast, among the PGT-A cycles, Groups A and B had comparable implantation rates(57.4% versus 63%, respectively; P=0.39) and LBRs (56.7% versus63.4%, respectively; P=0.37). The authors speculated that the lower success rate among non-PGT-A patients in Group B might be attributed to a higher rate of embryo-endometrial asynchrony owing to a relatively lengthy exposure of the endometrium to P4. The authors further speculated that the lack of a negative impact on LBR in PGT-A cycles could be caused by earlier implantation of tested blastocysts following zona breaching during biopsy [29].

The retrospective study design, single-point assessment for the LH surge and lack of cluster analysis are important limitations of the study.In another retrospective study, the impact of timing of warmed blastocyst transfer in t-NC was evaluated in 341 cycles [28]. As in the previous study [2], heterogeneity existed in the timing of FET, as for some cycles a serum LH 20 mIU/ml was used to define the LH surge while in other cycles the LH peak was used. Each cycle was classified by the timing of FET according to the LH surge, which was defined as the first attainment of serum LH 20 mIU/ml: Group 1 (n=211; 61.9%), LH 20 mIU/ml lasting for 1 day in whom FET was performed 6 days later; Group 2 (n=60;17.6%), LH 20 mIU/ml lasting for two consecutive days in whom FET was performed 6 days after the LH surge; Group 3 (n=70;20.5%), LH 20 mIU/ml lasting for two consecutive days in whom FET was performed 7 days after the LH surge. Vaginal progesterone was used for luteal phase support 3 or 4 days prior to FET, while a minority received intramuscular progesterone based on patient preference and provider practice. The authors reported that in the three groups implantation, clinical and ongoing pregnancy rates were comparable. Owing to the arbitrary nature of choosing an LH cut-off point of 20 mIU/ml, the authors explored various other thresholds. For example, the transfer 6 or 7 days after the LH surge achieved comparable ongoing pregnancy rates in relation to LH cut-off points of 15, 16, 17, 18, 19, 20 and 25 mIU/ml. It was concluded that timing of blastocyst transfer in t-NC after the LH surge is flexible within 24 h as outcomes were equally good with embryo transfers performed on days 6 or 7 after the LH surge. Limitations of the study relate to the retrospective design, lack of serum hormone measurements 1 day after LH 20 mIU/ml in some patients, and a single-point assessment for the LH surge the reproductive outcomes of vitrified warmed blastocyst transfer performed 5, 6 or 7 days after detecting the LH surge in urine werecompared in a retrospective study enrolling 2080 cycles [27].

Urine LH testing every morning was commenced when the leading follicular diameter was 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. Luteal phase support was administered as 400 mg/day of micronized vaginal progesterone immediately after FET. 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 retrospective study design and lack of cluster analysis to account for the inclusion of more than one cycle for a patient are the limitations of this study.

Conclusion:

Collectively, until now conflicting and limited data are available, comparing the effect of different timings of FET in t-NC on the reproductive outcome. Further randomized controlled trials evaluating the impact of different timings on reproductive outcomes in t-NC FET cycles are clearly required.

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