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The LH surge and ovulation re-visited: a systematic review and meta-analysis and implications for true natural cycle frozen thawed embryo transfer

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BACKGROUND: Efficient and safe embryo vitrification techniques have contributed to a marked worldwide increase in the use of elective frozen embryo transfer (FET). Pinpointing the day of ovulation, more commonly by documentation of the LH surge and less commonly by ultrasonography, is crucial for timing of FET in a true natural cycle (t-NC) to maximize the reproductive outcome.

OBJECTIVE AND RATIONALE: The definition of the *onset* of the LH surge should be standardized in t-NC FET cycles; however, a clear definition is lacking in the available literature. The first search question concerns the definition of the onset of the LH surge in a natural cycle. The second search question relates to the duration between the onset of the LH surge and ovulation.

SEARCH METHODS: We searched PubMed, Web of Science and Cochrane Library databases for two search questions from inception until 31 August 2021. 'Luteinizing hormone'[MeSH] OR 'LH' AND 'surge' terms were used to identify eligible articles to answer the first question, whereas 'Luteinizing hormone'[MeSH] OR 'LH' AND 'surge' OR 'rise' AND 'ovulation'[MeSH] OR 'follicular rupture' OR 'follicular collapse' were the terms used regarding the second question. The included publications were all written in the English language, conducted in women of reproductive age with regular ovulatory cycles and in whom serial serum or urine LH measurement was performed. For the quality and risk of bias assessment of the included studies, the Strengthening the Reporting of Observational Studies in Epidemiology and modified Newcastle Ottawa Scale were used.

OUTCOMES: A total of 10 and 8 studies were included for search Questions 1 and 2, respectively. Over the years, through different studies and set-ups, testing in either serum or urine, different definitions for the onset of the LH surge have been developed without a consensus. An increase in LH level varying from 1.8- to 6-fold above the baseline LH level was used in seven studies and an increase of at least two or three standard deviations above the mean of the preceding LH measurements was used in two studies. An LH level exceeding the 30% of the amplitude (peak-baseline LH level) of the LH surge was defined as the onset day by one study. A marked inter-personal variation in the time interval between the onset of the LH surge and ovulation was seen, ranging from 22 to 56 h. When meta-analysis was performed, the mean duration in hours between the onset of the LH surge and ovulation was 33.91 (95% CI = 30.79–37.03: six studies, 187 cycles).

WIDER IMPLICATIONS: The definition of the onset of the LH surge should be precisely defined in future well-designed studies employing state-of-art laboratory and ultrasonographic equipment. The window of implantation in a natural cycle is still a black box, and future research is warranted to delineate the optimal interval to time the embryo transfer in t-NC FET cycles. Randomized controlled trials employing different precise endocrine and/or ultrasonographic criteria for timing of FET in a t-NC are urgently required.

Key words: LH surge, window of implantation, natural cycle, true natural cycle, ultrasound, ovulation, frozen embryo transfer

Introduction

Efficient and safe vitrification techniques in IVF have contributed to a marked worldwide increase in elective frozen embryo transfer (FET) cycles during the last decade (Roque *et al.*, 2019b; De Geyter *et al.*, 2020). Currently, low-quality evidence indicates that the hormone replacement treatment protocol is associated with a lower live birth rate (LBR), when compared with the natural cycle (NC) for endometrial priming during FET (Mumusoglu *et al.*, 2021; Wu *et al.*, 2021). Moreover, recent evidence indicates a more favorable maternal, obstetric and perinatal outcome when NC is used (Litzky *et al.*, 2018; Ginström Ernstad *et al.*, 2019; Makhijani *et al.*, 2020; Asserhøj *et al.*, 2021; Hu *et al.*, 2021). On this basis, some authors have suggested a 'back to nature' attitude, advocating the NC over the hormone replacement cycle for FET (Roque *et al.*, 2019a; Lawrenz *et al.*, 2020).

Pinpointing the day of ovulation is crucial for timing FET in a true-NC (t-NC) to maximize the LBR. The current practice is mixed and relies on LH surge documentation by daily/frequent endocrine monitoring, including serum LH, estradiol (E_2) and progesterone (P_4), combined or not with serial ultrasound assessments to confirm ovulation once the leading follicle attains a mean diameter of 15 mm (Bartels *et al.*, 2019; Mumusoglu *et al.*, 2021). While the definition of the onset of the LH surge should be standardized in t-NC FET cycles to optimize reproductive outcomes, a consensus definition is lacking in the available literature. Currently, some clinics rely on the onset and others on the peak of the LH surge (Irani *et al.*, 2017). Importantly, there is a paucity of data concerning reproductive outcomes when employing different criteria for timing of FET in t-NC (Irani *et al.*, 2017; Bartels *et al.*, 2019).

This systematic review aims at discussing the heterogeneity as regards the definition of the LH surge during an NC. The primary outcome measures are: the definition of the onset of the LH surge; and the duration between the onset of the LH surge and ovulation in an NC. The configuration, amplitude, duration, inter- and intra-personal variability of the LH surge are reviewed, and the pros and cons for LH surge determination in either serum or urine and the value of ultrasonographic signs for documenting ovulation are explored. Lastly, the evidence concerning timing of FET in t-NC, based on existing criteria, is presented.

Methods

Protocol and registration

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol for the review was registered (CRD42021277365) on the international prospective register for systematic reviews, PROSPERO (www.crd.york.ac.uk/prospero).

Eligibility criteria

Manuscripts were included if they met all of the following criteria: articles were written in the English language; studies were based on human subjects; studies comprised women of reproductive age and with regular ovulatory cycles; and serial serum or urine LH measurements were performed to define the onset of the LH surge or assess the duration between the onset of the LH surge and ovulation.

Articles were excluded if they were abstracts, conference proceedings, reviews, or publications without original data, or animal studies.

Information sources and search strategy

A comprehensive search of PubMed, Web of Science and Cochrane Library databases was performed to identify relevant studies. These databases were searched from inception until 31 August 2021. Two searches were performed. The first search was performed to elucidate the definition of the onset of the LH surge, whereas the second search related to the duration between the onset of the LH surge and ovulation. The full search strategies are provided in Supplementary Tables SI and SII. The filters used during the searches were manuscripts written in the English language and human subjects. Reference lists of the full-text articles were also checked in order to identify additional relevant studies.

Study selection and data extraction

All retrieved publications from databases were imported into EndNote X9 (Clarivate Analytics, Philadelphia, PA, USA). Duplicate publications were removed automatically via EndNote or manually when EndNote could not detect differences in the titles. Studies were screened using the eligibility criteria described above for titles via EndNote and their abstracts via Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia). Full-text articles were assessed for possible eligibility and several were excluded for reasons given in Figs 1 and 2. A total of 10 and 8 studies were included for search Questions 1 and 2, respectively.

The following data were extracted from each study: first author's last name, year of publication, country of study, sample size (No. of women or No. of cycles), female age range, inclusion–exclusion criteria, method and frequency of LH level sampling. In addition, for the first search question, data regarding the definition of baseline LH levels and the onset of the LH surge were extracted. Likewise, assessment of ovulation by various tools (laparoscopy, histologic dating of the corpus luteum and ultrasonography) and the duration between the onset of the LH surge and ovulation were extracted for the second search question.

The study selection and data extraction process were performed independently by two authors (M.E. and S.M.) and disagreements were resolved by discussion or consensus, involving a third investigator (H.Y.).

Quality assessment of included studies

The Strengthening the Reporting of Observational Studies in Epidemiology and modified Newcastle Ottawa Scale (m-NOS) were used for the quality assessment of studies included. The m-NOS assesses the risk of bias in three domains, i.e. participant selection, comparability of groups and outcome assessment; this system rates a score of 0 for the lowest quality and 8 for the highest quality (Supplementary File S1). The quality assessment of the included studies was performed independently by two authors (M.E. and S.M.), and disagreements were resolved by discussion or consensus involving a third investigator (H.Y.) (Supplementary File S1).

Synthesis of results

Given that the first search question did not permit a quantitative synthesis to be carried out, results are provided in a narrative format. A meta-analysis was performed for the second search question. Specifically, the meta-analysis was performed, using the Metafor package version 4.1.1 in R 3.0-2 software (R Foundation for Statistical Computing, Vienna, Austria). Cochran's *Q*-test, and Higgins' I^2 statistics were used to assess the heterogeneity among studies. Since heterogeneity was observed among studies, the random-effects model was used to estimate the pooled mean and 95% CI. For studies reporting median (minimum–maximum) values, the values were converted to mean and SD using the formula as suggested by Hozo *et al.* (2005). Sensitivity analyses were performed by removing individual studies to assess each study's influence on the pooled mean duration between the onset of the LH surge and ovulation.

Results

Definition of the onset of the LH surge

The main characteristics of the included studies are summarized in Table I (Johansson *et al.*, 1971; Moghissi *et al.*, 1972; Testart *et al.*, 1981; Wetzels and Hoogland, 1982; Hoff *et al.*, 1983; Fritz *et al.*, 1992; Kesner *et al.*, 1998; Reutman *et al.*, 2002; Park *et al.*, 2007; Direito *et al.*, 2013). These studies used their own data to develop the criteria for the onset of the LH surge. Of the 10 included studies, six were carried out in the USA (Moghissi *et al.*, 1972; Hoff *et al.*, 1983; Fritz *et al.*, 1992; Kesner *et al.*, 1998; Reutman *et al.*, 2002; Park *et al.*, 2007), two in France (Testart *et al.*, 1981; Direito *et al.*, 2013), one in the Netherlands (Wetzels and Hoogland, 1982), and one in Sweden (Johansson *et al.*, 1971). Furthermore, five of the studies were published between four and five decades ago, dating back to either before (Johansson *et al.*, 1971; Moghissi *et al.*, 1972) or to the first days of IVF (Testart *et al.*, 1981; Wetzels and Hoogland, 1982; Hoff *et al.*, 1983), whereas the remaining studies were published more recently (Fritz *et al.*, 1992; Kesner *et al.*, 1998; Reutman *et al.*, 2002; Park *et al.*, 2007; Direito *et al.*, 2013).

Serum was used to measure the LH surge in five studies; the frequency of serum LH level sampling was once a day in two studies (Moghissi *et al.*, 1972; Wetzels and Hoogland, 1982), and multiple daily measurements in three studies (Testart *et al.*, 1981; Hoff *et al.*, 1983; Fritz *et al.*, 1992). Urine samples were used in five studies with once-daily assessment in all five (Johansson *et al.*, 1971; Kesner *et al.*, 1998; Reutman *et al.*, 2002; Park *et al.*, 2007; Direito *et al.*, 2013).

Different criteria were proposed to define the onset of the LH surge across the 10 included studies. A different fold increase in LH level from the baseline LH measurements, ranging from 1.8- to 6-fold, was used to define the onset of the LH surge in seven studies (Johansson *et al.*, 1971; Moghissi *et al.*, 1972; Testart *et al.*, 1981; Wetzels and Hoogland, 1982; Fritz *et al.*, 1992; Reutman *et al.*, 2002; Park *et al.*, 2007). The baseline LH level used to define the fold increase differed across studies; the LH level on the preceding day (Johansson *et al.*, 1971), all preceding days (Wetzels and Hoogland, 1982), follicular phase levels (Moghissi *et al.*, 1972), the mean of the preceding four

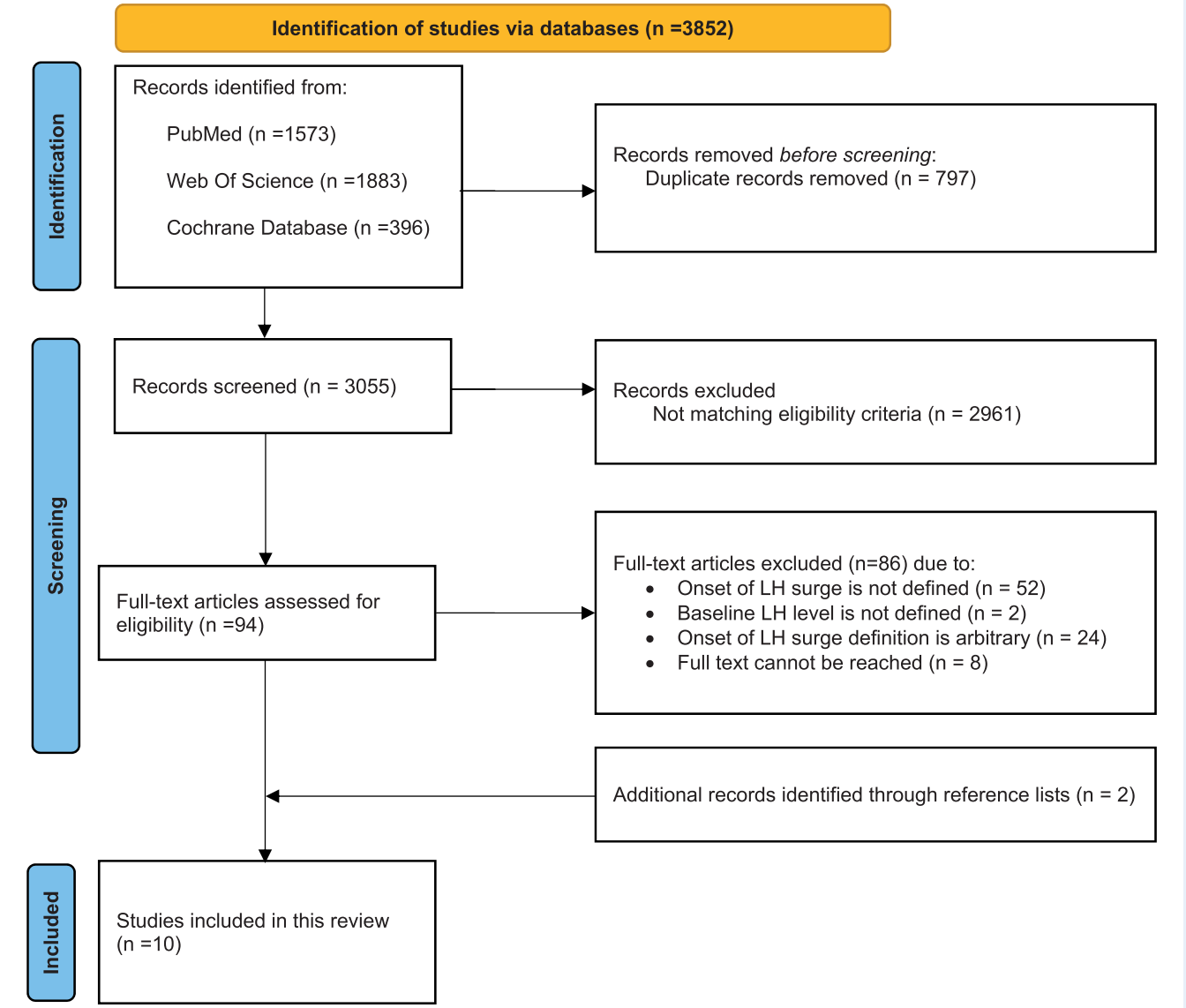


Figure 1. The PRISMA flow diagram for the definition of the onset of the LH surge. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

(Testart *et al.*, 1981) or six measurements (Fritz *et al.*, 1992) and the mean of the preceding 5 (Park *et al.*, 2007) or 7 days (Reutman *et al.*, 2002).

The definition for the onset of the LH surge, as described by Testart *et al.* (1981), is the most commonly employed criterion in the literature. Twenty-seven women (30 cycles), 25–40 years of age, underwent frequent serum sampling followed by laparoscopy. Of the included 30 cycles, 22 were NCs, and in 8 patients, clomiphene citrate was administered. Serum sampling for LH was performed four times daily from the time point when LH release was considered imminent. The authors introduced the term LH 'surge-initiating rise' (SIR), which corresponded to the onset of the LH surge, as any LH level equal to or exceeding 180% of the mean value from the preceding four measurements (e.g. mean LH of the four preceding values = 3.2 mIU/ml; LH SIR concentration = 1.8 × 3.2 = 5.8 mIU/ml).

Rather than a fold-increase, two studies used an increase of at least two (Hoff *et al.*, 1983) to three (Kesner *et al.*, 1998) SDs above the mean of the preceding measurements to define the onset of the LH surge. Hoff *et al.* (1983), in five women (seven cycles), assessed serum E_2, P_4, FSH and LH levels at 2-h intervals for five consecutive days during the periovulatory phase. These authors defined the onset of the LH surge as the first LH value exceeding the mean +2 SD of six preceding values (Hoff *et al.*, 1983). In the study by Kesner *et al.* (1998), with once-daily urinary LH sampling, an increase >3 SD over the mean of the seven preceding days was used to define the onset of the LH surge.

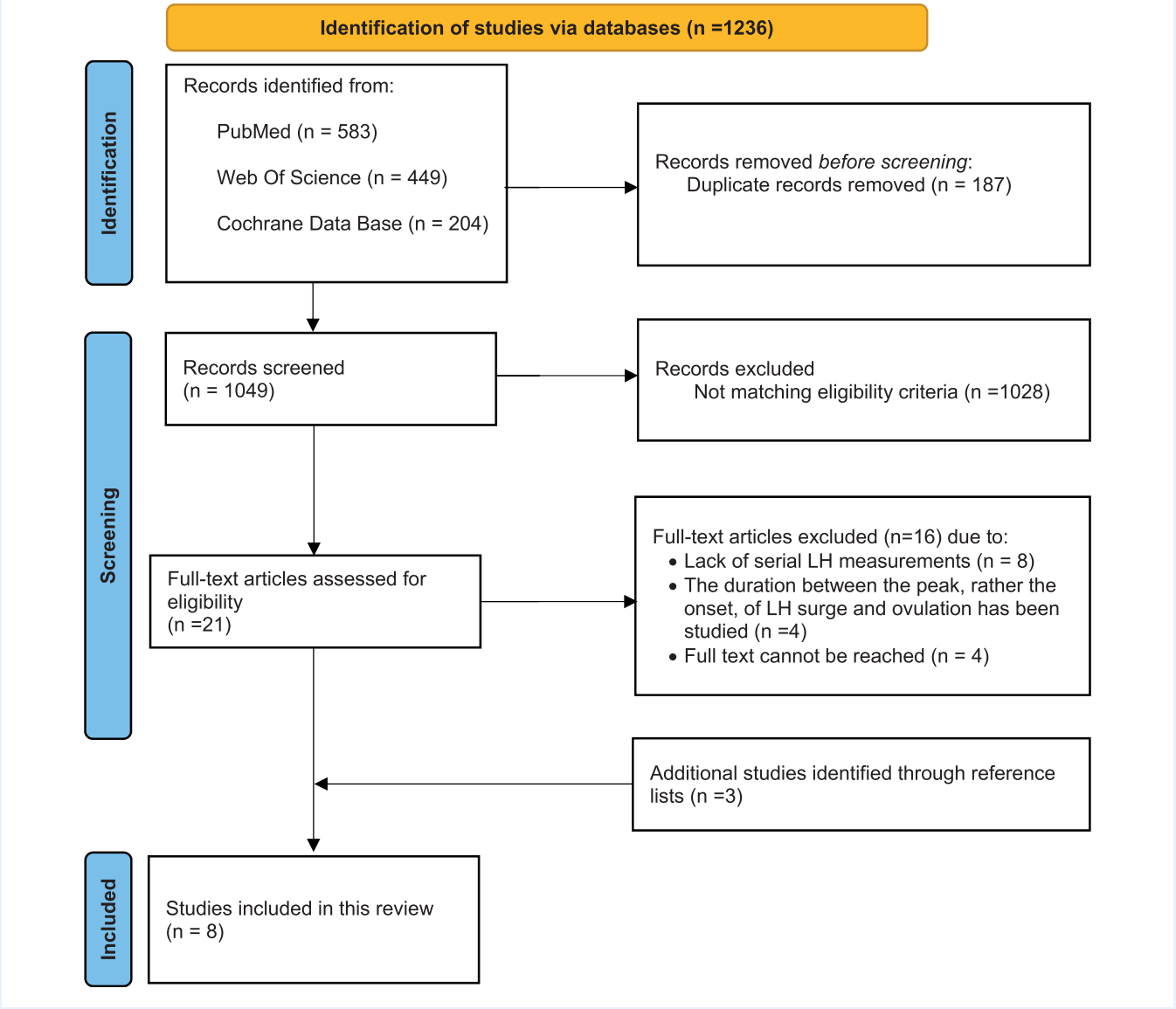


Figure 2. The PRISMA flow diagram for the duration between the onset of the LH surge and ovulation. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

A different criterion was developed by Direito *et al.* (2013), using once-daily urinary LH sampling. The authors drew a horizontal line at 30% of the LH peak amplitude. The amplitude was calculated by subtracting the mean of the five daily LH levels immediately before the onset of the LH surge from the LH peak, which was the maximum LH level attained during the surge. According to these authors, the onset of the LH surge was recorded as the first day when the LH surge was above the 30% line.

Risk of bias assessment

Of the 10 studies included, one had an m-NOS score of 3 (Wetzels and Hoogland, 1982), three had a score of 4 (Moghissi *et al.*, 1972; Testart *et al.*, 1981; Kesner *et al.*, 1998), one had a score of 5 (Johansson *et al.*, 1971), four had a score of 6 (Hoff *et al.*, 1983; Reutman *et al.*, 2002; Park *et al.*, 2007; Direito *et al.*, 2013) and one had a score of 7 (Fritz *et al.*, 1992). The main contributor to a higher score was the frequency of LH sampling; the only study with an m-NOS score of 7 had serum sampling every 3 h (Fritz *et al.*, 1992) (Supplementary File S1; Table I).

Duration between the onset of the LH surge and ovulation

The main characteristics of the eight studies included are summarized in Table II (Yussman and Taymor, 1970; WHO, 1980; de Crespigny *et al.*, 1981; Garcia *et al.*, 1981; Lemay *et al.*, 1982; Testart and Frydman, 1982; Taymor *et al.*, 1983; Fritz *et al.*, 1992). The included studies were conducted in five different countries: USA ($n = 4$), France

Table I Studies reporting the definition of the onset of the LH surge.

Author (Year) Country	No. of women (No. of cycles)	Age, years	Method and frequency of LH level sampling	Definition of onset of the LH surge	m-NOS quality score
Johansson et al. (1971) Sweden	25 (25)	20–31	Urine Once daily	4-fold increase from the previous day level	5
Moghissi et al. (1972) USA	10 (10)	19–40	Serum Once daily	6-fold increase from the follicular phase levels	4
Testart et al. (1981) France	27 (30)	25–40	Serum 4-times daily	≥180% of the mean of the preceding 4 values	4
Wetzels and Hoogland (1982) Netherlands	11 (28)	N/A	Serum Once daily	The first value, more than two times higher than all foregoing values	3
Hoff et al. (1983) USA	5 (7)	N/A	Serum Every 2 h	First LH value exceeding the mean + 2 SD of the 6 preceding values	6
Fritz et al. (1992) USA	7 (7)	21–35	Serum Every 3 h	≥100% increment over a running mean of the 6 preceding values	7
Kesner et al. (1998) USA	9 (14)	25–36	Urine Once daily	First rise of >3 SD above the mean of the previous 7 days	4
Reutman et al. (2002) USA	98 (98)	<42	Urine Once daily	First rise of >2.5-fold above the mean of the previous 7 days	6
Park et al. (2007) USA	46 (46)	20–35	Urine Once daily	2.5-fold increase from the mean of the preceding 5 days	6
Direito et al. (2013) France	107 (283)	18–45	Urine Once daily	The first day when LH is above the 30% of the amplitude[a]	6

m-NOS, Modified Newcastle–Ottawa Quality Assessment Scale; N/A, not available.
[a]Amplitude was defined as the difference between the peak and the baseline LH levels.

($n = 1$), Canada ($n = 1$), Switzerland ($n = 1$) and Australia ($n = 1$). Six were published in the early 1980s (WHO, 1980; de Crespigny et al., 1981; Garcia et al., 1981; Lemay et al., 1982; Testart and Frydman, 1982; Taymor et al., 1983), one in 1970 (Yussman and Taymor, 1970) and the latest in 1992 (Fritz et al., 1992).

Serum was used in seven studies (Yussman and Taymor, 1970; WHO, 1980; Garcia et al., 1981; Lemay et al., 1982; Testart and Frydman, 1982; Taymor et al., 1983; Fritz et al., 1992) and urine in a single study (de Crespigny et al., 1981). A sampling frequency of every 3–8 h was used to measure LH levels in all eight studies. The tools used to detect ovulation were histologic dating of the corpus luteum (Yussman and Taymor, 1970), laparoscopy (Garcia et al., 1981; Testart and Frydman, 1982; Taymor et al., 1983) or ultrasonography (de Crespigny et al., 1981; Lemay et al., 1982; Fritz et al., 1992). Furthermore, the histology of the corpus luteum and the impression of the surgeon were used in one study (WHO, 1980).

In the earliest study, the duration between the onset of the LH surge and ovulation was estimated by histologic dating of the corpus luteum (Yussman and Taymor, 1970). By studying the serum concentrations of FSH, LH and P_4 in 8-h intervals from eight subjects during the mid-cycle, the authors reported that ovulation occurred 32.8 h, with a range of 28–44 h, following the onset of the LH surge (Yussman and Taymor, 1970). In this study, ovulation occurred at 17.6 h, with a range of 16–24 h following the LH peak.

The World Health Organization Task Force study, with the largest sample size, studied the duration between the onset of the LH surge and ovulation in 177 women (WHO, 1980). Serum samples were taken every 8-h to measure levels of E_2, LH, FSH and P_4. The first significant rise in LH, denoting the onset of a surge, was the first value which was 1.5 times the mean of the preceding baseline values; however, the definition of 'preceding baseline values' was not provided. In the study, the surfaces of the ovaries were examined at laparotomy, and the mature follicle or corpus luteum were excised for histologic examination in 97 women. Whether ovulation had or had not occurred was assessed by the impression of the operating surgeon and verified by histology. The median duration between the onset of the LH surge and ovulation was 32.0 h (95% CI = 23.6–38.2 h), ranging from 24 to 56 h. The respective figure for the time between the LH peak and ovulation was 16.5 (95% CI = 9.5–23.0), with a range of 8–40 h. The authors concluded that the onset of the LH surge, rather

Table II Studies reporting the duration between the onset of the LH surge and ovulation.

Author (Year) Country	No. of cycles	Age, years	Method and frequency of LH sampling	Ovulation detection method	Time between the onset of LH surge and ovulation, hours	m-NOS quality score
Yussman and Taymor (1970) USA	8	N/A	Serum Every 8 h	Histologic	Mean= 32.8 Range = 28–44	3
WHO (1980) Switzerland	97	21–40	Serum Every 8 h	Histology and surgeon's impression	Median= 32.0 95% CI = 23.6–38.2 Range = 24–56	7
Garcia *et al.* (1981) USA	21	30.5 ± 3.0	Serum Every 4 h	Laparoscopic follicle aspiration	Mean ± SD= 27.2 ± 6.24 Range = 26.3–31	3
de Crespigny *et al.* (1981) Australia	4	N/A	Urine Every 3 h	Ultrasound	Median= 34.5 Range = 28–35	4
Lemay *et al.* (1982) Canada	20	23–35	Serum Every 3–5 h	Ultrasound	Range = 18–48	2
Testart and Frydman (1982) France	26	24–40	Serum 4 times daily	Laparoscopic follicle aspiration	Median= 37 Range = 35–47	4
Taymor *et al.* (1983) USA	4	N/A	Serum Every 4 h	Laparoscopic follicle aspiration	Median= 37 Range = 36–38	3
Fritz *et al.* (1992) USA	7	21–35	Serum Every 3 h	Ultrasound	Mean ± SEM= 37.6 ± 4.2 Range = 22–43.5	7

m-NOS, Modified Newcastle–Ottawa Quality Assessment Scale; N/A, not available; WHO, World Health Organization study.

than the peak, would be the best indirect parameter of impending ovulation.

Of the eight studies, six were included in the meta-analysis (Yussman and Taymor, 1970; WHO, 1980; de Crespigny *et al.*, 1981; Testart and Frydman, 1982; Taymor *et al.*, 1983; Fritz *et al.*, 1992), whereas two studies were not eligible (Garcia *et al.*, 1981; Lemay *et al.*, 1982) (Table II). Thus, the study by Lemay *et al.* (1982) was excluded because mean, median or individual data were not available. The study by Garcia *et al.* (1981) was excluded after the sensitivity analysis because it markedly decreased the pooled mean duration between the onset of the LH surge and ovulation. When a meta-analysis was performed, the mean duration between the onset of LH surge and ovulation was 33.91 h (95% CI = 30.79–37.03: six studies, 187 cycles) (Fig. 3).

Risk of bias assessment

The m-NOS scores of the included studies are presented in Table II. One study was assigned an m-NOS score of 2 (Lemay *et al.*, 1982), three studies a score of 3 (Yussman and Taymor, 1970; Garcia *et al.*, 1981; Taymor *et al.*, 1983), two studies a score of 4 (de Crespigny *et al.*, 1981; Testart and Frydman, 1982) and the remaining two studies a score of 7 (WHO, 1980; Fritz *et al.*, 1992). The frequency of sampling (Fritz *et al.*, 1992) and sample size (WHO, 1980) were the two main contributors for a high scoring (Supplementary File S1; Table II).

Discussion

Main findings

Definition of the onset of the LH surge

Over the years, through several studies using different designs, mainly measuring LH levels either in serum or urine, different definitions for the onset of the LH surge were developed without reaching a consensus. The onset of the LH surge definitions can be summarized as: an increase of 1.8- to 6-fold from the baseline LH level (Johansson *et al.*, 1971; Moghissi *et al.*, 1972; Testart *et al.*, 1981; Wetzels and Hoogland, 1982; Fritz *et al.*, 1992; Reutman *et al.*, 2002; Park *et al.*, 2007); an increase of at least two (Hoff *et al.*, 1983) or three (Kesner *et al.*, 1998) SD above the mean of the preceding measurements; and an LH level of 30% of the amplitude of the LH surge (Direito *et al.*, 2013).

When the 'LH surge' is referred to, it is critical to specify the time point during the surge, as to whether it may be detected at the onset/ascending limb, peak or descending limb of the LH surge. Unfortunately, the definition of the *onset* or *peak* of the LH surge has not been standardized in t-NC FET cycles. Since the LH peak is detected after ovulation in ~25% of women, the onset rather than the peak is more reliable to predict impending ovulation (Roos *et al.*, 2015). Thus, the onset of the LH surge should be used for timing in t-NC FET cycles.

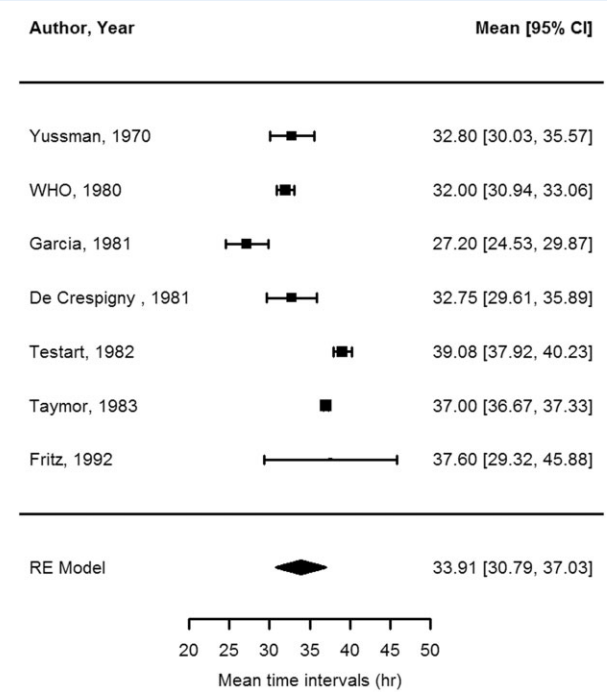


Figure 3. The mean duration (95% CI) between the onset of the LH surge and ovulation, in hours. Of the eight studies included in the systematic review, six were included for the meta-analysis. For studies reporting median (minimum–maximum) values, the values were converted to mean and SD using the formula as suggested by Hozo *et al.* (2005). Since heterogeneity was observed among studies, the random-effects model was used to estimate the pooled mean and 95% CI.

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 (Mackens *et al.*, 2017), 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 E_2 levels the following day (Irani *et al.*, 2017); LH >10 mIU/ml (Groenewoud *et al.*, 2012); LH > 15 mIU/ml (Kahraman and Sahin, 2020); and LH > 20 mIU/ml (Bartels *et al.*, 2019; Johal *et al.*, 2021). Apart from those included in the current systematic review, the above definitions were based on single and arbitrary LH level thresholds, which might be misleading. 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) (Johnson *et al.*, 2015). To our knowledge, there is no 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.

Duration between the onset of the LH surge and ovulation

Most available studies were conducted several decades ago, employing tools to detect ovulation (e.g. histological dating of the corpus luteum, laparoscopy) which are currently more of historical interest. Only three studies used frequent endocrine and ultrasound monitoring (de Crespigny *et al.*, 1981; Garcia *et al.*, 1981; Fritz *et al.*, 1992). On the basis of the available data, it appears that there is a marked inter-individual variation in the time interval between the onset of the LH surge 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 (Fig. 3). This considerable variation could be relevant for planning and timing of FET in t-NC because the standard practice for scheduling FET in a t-NC is to rely on the onset of the LH surge rather than documentation of ovulation by ultrasound.

The profile of the LH surge

Physiology

During an NC, the rise of E_2 originating from the dominant follicle, exceeding 200–300 pg/ml for a minimum of 50 h, triggers the LH surge (Young and Jaffe, 1976). Although an increase in serum P_4, 12 h before the onset of the LH surge was defined approximately four decades ago (Hoff *et al.*, 1983), 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 P_4, characterized by a precipitous increase in serum P_4 to 0.5 ng/ml, as the trigger for the LH surge has emerged (Dozortsev and Diamond, 2020).

The mid-cycle LH surge is indispensable for ovulation, resumption of the first meiotic division and luteinization of mural granulosa cells. Although the role of the mid-cycle FSH surge in the NC is not fully clear, it has been shown to promote LH receptor formation on the luteinizing granulosa cells, thus securing the function of the corpus luteum during the luteal phase, as well as nuclear maturation and cumulus expansion (Strickland and Beers, 1976; Eppig, 1979; Zelinski-Wooten *et al.*, 1995; Yding Andersen *et al.*, 1999; Yding Andersen, 2002).

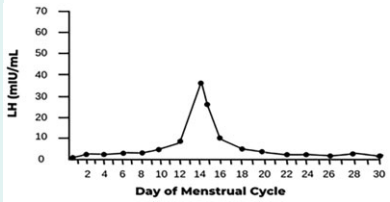
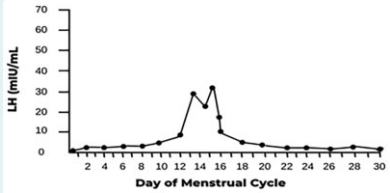
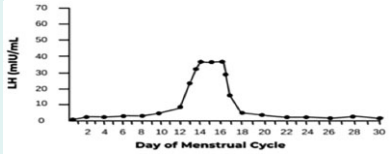
Configuration of the LH surge

LH surges resulting in ovulation are not of one type only; instead, they seem to be highly variable in configuration, amplitude and duration (Park *et al.*, 2007; Alliende, 2013; Direito *et al.*, 2013). Thomas *et al.* (1970) were the first to describe a midcycle bi-phasic pattern of the LH surge. Of the eight cycles evaluated by daily serum LH measurements, seven were noted to be bi-phasic (Thomas *et al.*, 1970). The first surge lasted for about 20 h and was followed by a decrease in serum LH; after that, a second peak was noted, ~24 h following the first surge (Thomas *et al.*, 1970).

The various configurations of the LH surge and their frequencies have been reported in studies using urine testing (Park *et al.*, 2007; Alliende, 2013; Direito *et al.*, 2013). According to these studies three LH surge configurations have been defined as spike, biphasic and plateau. The definitions and frequencies of these three types of LH surge configurations are given in Table III.

A natural question to be addressed in this context is the relevance of different types of LH surge configurations for reproductive outcomes in a natural or t-NC FET cycle. Direito *et al.* (2013), in the only available study assessing urinary samples, reported that cycles in which multiple peak LH surges were seen were associated with a smaller follicle size ($P = 0.01$) just before rupture and significantly lower urinary LH levels on the ultrasonographically verified day of ovulation

Table III Types and frequencies of urinary LH surge configurations.

Configuration	Park *et al.* (2007) (n = 46)	Direito *et al.* (2013) (n = 107)	Alliende (2013) (n = 25)[a]	Alliende (2013) (n = 25)[b]
Spike 	42% All values subsequent to the LH peak were less than that of the LH peak	48% LH peak was maintained over the horizontal line at 30% of the amplitude of the LH peak for ≤5 days	58%	42%
Bi-phasic 	44% First significant LH rise was followed by a decrease, then an increase in LH level which may or may not be greater than that of the first LH rise	41% LH surge was marked by troughs, i.e. days with a LH level below the 30% line Double-peaked (33%); multiple-peaked (8%)	25% Double-peaked (23%); multiple-peaked (2%)	42% Double-peaked (33%); multiple-peaked (9%)
Plateau 	14% LH levels remained at peak values for 2–3 days before decreasing	11% LH level was maintained over the 30% line for >5 days	17%	15%

[a]Secondary analysis of data according to criteria defined by Direito *et al.* (2013).
[b]Secondary analysis of data according to criteria defined by Park *et al.* (2007).

($P = 0.03$) when compared with single peak or plateau surges. Moreover, the authors reported that prolonged LH surges (lasting >3 days after documentation of ovulation by ultrasound) were characterized by lower urinary pregnanediol 3-alpha glucuronide levels (a metabolite of serum P_4), and a smaller corpus luteum, which could indicate luteal phase insufficiency. Importantly, with multiple peaks of LH, the use of the initial LH peak (pseudo-peak) could be misleading for the timing of FET in t-NC.

Urinary LH surge assay

LH surge assays measure different components of LH and its metabolites. The type of assay is critical to define the different LH surge profiles. In a study by Johnson *et al.* (2015), urinary LH was measured using two different assays in a total of 40 fertile women, aged 18–40 years, by collecting daily urine samples during the menstrual cycle. Of the two urinary LH assays, the Perkin Elmer assay detected intact LH, free-beta LH (LH-beta) and LH beta core fragment (LH-βcf). Of importance, LH-βcf is a degradation product of LH. The in-house urinary LH assay, on the other hand, measured only intact LH. In six patients, the in-house assay showed a single peak, whereas two peaks were noted with the Perkin-Elmer kit. The authors speculated that the variability in the LH surge configuration, amplitude and duration, as reported by Direito *et al.* (2013) and Park *et al.* (2007), both employing urinary kits measuring all three components (intact LH, LH-beta and LH-βcf), could be an artifact, as the inconsistency was not noted when only intact urinary LH was measured (Johnson *et al.*, 2019). Considerable inter-individual variation in the degradation pattern of LH might contribute to marked differences in urinary LH-βcf secretion and hence configuration of the LH surge (Johnson *et al.*, 2019).

The amplitude and duration of the LH surge

In an NC, an optimal P_4 output from the corpus luteum, originating from mono-follicular development, is crucial to establish and support a pregnancy (Hull *et al.*, 1982). In this aspect, it was recently shown that the serum P_4 level on the day or 1 day prior to warmed blastocyst embryo transfer in t-NC cycle impacts the LBR (Gaggiotti-Marre *et al.*, 2020). In theory, differences in the amplitude and duration of the LH surge might result in differences in the AUC for LH as the driving force of P_4 production by the corpus luteum and, hence, may have implications for the reproductive outcome in t-NC FET.

Regarding the duration of the LH surge, in a study by Hoff *et al.* (1983), using serum samples at 2-h intervals, the mean duration of the LH surge was found to be 48 h divided into three phases. The first phase consisted of a short ascending phase lasting for 14 h, followed by a rapid decline in E_2 and a continued rise in P_4. The second phase

was characterized by a peak plateau phase lasting for 14 h with a transient leveling of P_4, whereas during the third phase, a long descending phase lasting 20 h was associated with a second rapid rise in P_4.

The impact of the amplitude and duration of the LH surge on pregnancy rates was evaluated in 127 patients (382 cycles), undergoing IUI during an NC (Cohlen *et al.*, 1993). An LH surge was defined as a rise to ≥25 mIU/ml, and IUI was performed the next day. Cycles with LH surges lasting for 2 days (n = 152) were associated with higher pregnancy rates (22.4% versus 8.3%, respectively; $P < 0.001$) when compared with surges of 1-day duration (n = 230). Regarding the amplitude of the LH surge, the pregnancy rates in the low (LH < 43 mIU/ml; n = 131 cycles), middle (LH = 43–63 mIU/ml; n = 128) and high (LH> 63 mIU/ml; n = 123) LH groups were 8.4%, 14.8% and 18.7%, respectively ($P = 0.055$). The authors concluded that the duration of the LH surge was more important than its amplitude.

Soules *et al.* (1988) studied factors controlling corpus luteum function in 14 volunteers during the NC. The LH surge was quantified by determining the AUC of the LH levels on the day, 1 day before and 1 day after the LH peak. The integrated serum P_4 was calculated by multiplying the mean daily serum P_4 level per number of days from the mid-cycle LH peak to the first day of the next menstrual period. Although there was a significant positive correlation between mean follicle diameters and serum E_2 in the late follicular phase, these parameters did not correlate with P_4 production during the luteal phase. Moreover, there was no correlation between the AUC-LH surge and luteal P_4 secretion. These findings suggest that the size of the follicle and the amplitude of the LH surge are not quantitative determinants of corpus luteum function during NC. Concordant with the study by Soules *et al.* (1988) and Johnson *et al.* (2015), using urinary LH kits, reported that the LH amplitude differed between individuals, however, the variation was neither related to follicular phase length nor to the likelihood of pregnancy (Johnson *et al.*, 2015).

Collectively, data are limited and conflicting regarding the impact of the amplitude and duration of the LH surge on P_4 output by the corpus luteum and reproductive outcome of an NC.

The intra-personal variability of the LH surge

A diurnal rhythm exists for the onset of the LH surge, mainly occurring from 11 p.m. to 8 a.m. (Krotz *et al.*, 2005) with no particular variation by day of the week (Cahill *et al.*, 1998), and LH surge characteristics do not seem to be influenced by age (Direito *et al.*, 2013; Johnson *et al.*, 2019) or BMI (Johnson *et al.*, 2019). In the study by Direito *et al.* (2013), LH surge characteristics remained consistent from cycle to cycle in regularly cycling women. In contrast, Alliende (2013), did not find a clear repetition of the same LH profile, as only 44% of cycles showed the same profile.

Urinary testing versus serum testing for the LH surge: does it make a difference?

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 (Johnson *et al.*, 2015). Urinary assays measuring intact LH-only provide physiologically more relevant information than those measuring intact LH, LH-beta or LH-βcf. Moreover, inter-individual differences in the LH metabolism may contribute to differences in the LH-βcf level (Johnson *et al.*, 2015).

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 (Ghazeeri *et al.*, 2000). Accordingly, for those assays with detection limits <40 mIU/ml, false-negative results will be encountered in patients with low peak LH levels (<40 mIU/ml), which might affect up to 35% of ovulatory cycles (Arici and Byrd, 1992).

When urinary testing is compared with serum testing, a time delay should be taken into account owing to the prolonged urinary clearance of LH (Frydman *et al.*, 1984). In one study (Roger *et al.*, 1980), 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 (Frydman *et al.*, 1984), 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.

In a recent prospective study of 40 regularly cycling women, aged 18–40 years, with no history of infertility, the inter-individual variations in urinary and serum LH levels relative to ultrasound-observed ovulation were investigated (Roos *et al.*, 2015). Unlike, the studies discussed above (Roger *et al.*, 1980; Frydman *et al.*, 1984), urinary and serum LH showed an excellent agreement with a short delay for the urinary signal. The authors concluded that LH testing might be used interchangeably in serum and urine. The use of transvaginal ultrasound daily to confirm ovulation rather than laparoscopy (Roger *et al.*, 1980; Frydman *et al.*, 1984) was the strength of the study by Roos *et al.* (2015). Further studies are warranted to delineate whether a critical time delay exists for the detection of the onset of the LH surge when tested in serum or urine and, if a delay exists, whether it impacts reproductive outcomes in t-NC FET.

Urinary LH-kits may also demonstrate LH surges in the absence of ovulation, the so-called premature LH surges (Miller and Soules, 1996); these premature urinary LH surges appear to be a common phenomenon in regularly cycling women (Chan *et al.*, 1989; Ponto *et al.*, 1990; Miller and Soules, 1996; Krotz *et al.*, 2005). When comparing once-daily urinary LH testing with ultrasound, as many as 18% of women demonstrate LH surges in the absence of ovulation (Chan *et al.*, 1989; Ponto *et al.*, 1990; Miller and Soules, 1996). FET timing will be suboptimal if a premature LH surge is mistaken for a true ovulatory surge, decreasing pregnancy rates. Along these lines, the frequency and impact of a premature urinary LH surge on the reproductive outcome was evaluated in 188 regularly cycling IVF patients who underwent cleavage-stage t-NC FET (Krotz *et al.*, 2005). Timing of embryo transfer was calculated by adding 13 h and the embryonic age to the onset of the urinary LH surge. When a leading follicle >16 mm was identified, daily urinary specimens were collected at 2- to 4-h intervals from 6 a.m. to 11 p.m. LH secretion, expressed by milliunits/hour, was calculated by use of the formula 'LH level (mIU/ml) × total collection volume (ml)/total collection time (h)'. An ovulatory (or true) LH surge was defined as a urinary LH value of >800 mIU/h during three consecutive collections. However, a premature LH surge was defined as one urinary LH value of >800 mIU/h that was not sustained at repeat measurements. Eighty-eight (47%) of 188

regularly cycling women had premature LH surges, and 33 (37%) of those 88 women had multiple premature LH surges. Importantly, pregnancy rates per embryo transfer were similar between women with and without premature LH surges. Moreover, neither the presence nor the number of premature surges were independent predictors of pregnancy at logistic regression analysis (Krotz *et al.*, 2005).

Taking the transvaginal ultrasound as the gold standard to predict ovulation, the sensitivity, specificity and accuracy for urine LH testing have been reported to be 1.00, 0.25 and 0.97, respectively (Guermandi *et al.*, 2001). The positive predictive values of positive urine LH testing for follicular collapse within 24 or 48 h were 73% and 92%, respectively (Miller and Soules, 1996).

Different frequencies of urinary LH testing, ranging from one to three times daily, to detect ovulation is employed in current practice. As expected, increased frequency of urinary testing will improve the efficiency of identification of the urinary LH surge. A urinary LH surge was observed in nine out of 10 ovulatory cycles using twice daily testing (Nulsen *et al.*, 1987). Had morning-only daily testing been done, a urinary LH surge would have been observed in only six out of 10 ovulatory cycles. Conversely, if testing had been performed in the afternoon only, a urinary LH surge would have been observed in seven out of 10 ovulatory cycles (Nulsen *et al.*, 1987).

Collectively, there are pros and cons for the urinary versus serum testing for the LH surge. Future randomized controlled trials are warranted to compare these two methods regarding reproductive outcomes in t-NC.

Documentation of ovulation by ultrasound

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 (Guermandi *et al.*, 2001; Park *et al.*, 2007). Furthermore, the absence of a secretory endometrium in endometrial histological specimens following urinary LH surges has been reported in a total of 7% of cycles (McGovern *et al.*, 2004).

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 (Wetzels and Hoogland, 1982), and follicular collapse is the most predictive sign of ovulation (Marinho *et al.*, 1982), 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 (Wetzels and Hoogland, 1982). However, other ultrasonographic signs of ovulation have also been described, as discussed below.

Ecochard *et al.* (2000) evaluated the sensitivity and specificity of ultrasonographic signs of ovulation in a multicenter study enrolling 107 fertile women (271 cycles). Transvaginal ultrasound was performed in 58% of the cycles, whereas transabdominal ultrasound was performed in the remaining 42%. The estimated day of ovulation as determined by ultrasound (US-EDO) was defined as the day of maximal follicular enlargement, followed by rupture the following day. However, in some cases, the largest follicle persisted for 2 days before rupture; the US-EDO was assumed to be the first day of these 2 days for those follicles $\geq$8 mm in diameter and the second day for leading follicles $<$18 mm. The mean diameter of the leading follicle on US-EDO was 21.94 mm (90% CI, 21.33–22.55) with a maximum diameter of 39 mm. Ultrasonographic signs of ovulation included: disappearance or sudden decrease in size; increased echogenicity; irregularity of follicular walls; and appearance of free fluid in the pelvis. The sensitivity and specificity of disappearance or sudden decrease in size were 84.3% and 89.2%, respectively; the respective figures were 38.4% and 79.7% for increased echogenicity, 61.6% and 87.1% for irregularity of follicular walls and 71.0% and 88.2% for the appearance of free fluid in the pelvis. Increased echogenicity inside the follicle was not a reliable marker of ovulation because echoes were noted in 7%, 13% and 20% of the third-, second- and first-day preceding ovulation, respectively. Interestingly, a good correlation was noted between transabdominal and transvaginal ultrasound.

Luteinized unruptured follicle

In some cycles, a typical accelerated growth pattern compatible with a luteinized unruptured follicle (LUF) is noted instead of rupturing. LUF can be encountered in 9.4–46.7% fertile (Vanrell *et al.*, 1982; Donnez *et al.*, 1983) and 10.0% unselected infertile women (Hamilton *et al.*, 1985). However, LUF is usually not repetitive and may occur infrequently in every woman (Evers, 1993).

Although Stein and Leventhal (1935) first described the failure of follicle rupture in their classical manuscript on PCOS, only in 1978 was evidence of LUF as the absence of ovulation stigma suggested by laparoscopy performed during the early luteal phase (Koninckx *et al.*, 1978; Marik and Hulka, 1978). The three physiological events occurring following the LH surge in a natural ovulatory cycle are: resumption of the first meiotic division; luteinization of granulosa cells, culminating in the formation of a corpus luteum; and rupture of the follicle wall resulting in ovulation. These three events are all LH-dependent (Evers, 1993). However, the LH levels required for each of these three events differ. Resumption of meiosis occurs at low LH levels whereas adequate luteinization requires higher LH levels. In contrast, follicle rupture is only achieved at very high LH levels (Evers, 1993). In a rat model, the threshold LH level required for resumption of meiosis and P_4 secretion was only 5% of the peak level, whereas the threshold was $>$85% of the peak level for follicular rupture (Peluso, 1990). This hierarchic level-response effect of LH explains the lack of follicle wall rupture with blunted LH surges, despite luteinization and hence serum P_4 rise (Hamilton *et al.*, 1985; Schenken *et al.*, 1986; Hamilton *et al.*, 1987; Koskimies *et al.*, 1987). Moreover, LUF cycles are typically characterized by luteal phases of normal duration, however with lower mid-luteal serum P_4 levels (Murdoch and Dunn, 1983; Schenken *et al.*, 1986; Hamilton *et al.*, 1987; Koskimies *et al.*, 1987). Apart from a blunted LH surge, diminished LH receptor expression of the corpus luteum may also contribute to the development of LUF. Thus, in a study by Koskimies *et al.* (1987), a 60% lower LH receptor expression in the corpus luteum was reported in LUF patients compared to corpora lutea of fertile controls.

Documentation of ovulation by ultrasound is not a common practice during t-NC cycles for the timing of FET, and serum P_4 assessment on the day, or 1 day prior to warmed blastocyst transfer until now is rarely performed in t-NC FET, assuming that once ovulation occurs the resultant P_4 production by the corpus luteum would suffice in all cases, which might indeed not be the case. Since LUF is associated with decreased mid-luteal serum P_4 levels (Murdoch and Dunn, 1983; Schenken *et al.*, 1986; Hamilton *et al.*, 1987; Koskimies *et al.*,

1987), delineation of LUF cycles by ultrasound, although posing additional workload, could be of benefit. If serum P_4 levels are sub-optimal on the day or 1 day prior to warmed blastocyst transfer, either cycle cancellation or rescue protocols using exogenous progesterone supplementation might be employed, however, studies are needed to explore the efficacy of rescue protocols in t-NC FET.

The window of implantation

Embryo implantation involves the close interaction between a 'competent' blastocyst and a receptive endometrium, occurring during the 'window of implantation' (WOI). In most women, the WOI is open during the mid-luteal phase, 8–10 days after ovulation, driven by the sequential actions of estrogen and P_4 (Wilcox *et al.*, 1999).

To pinpoint the WOI in an NC, Wilcox *et al.* (1999) collected daily urine samples for up to 6 months from 221 women with no history of infertility, attempting to conceive. Timing of ovulation was identified by the ratio of urinary E_2 metabolite (estrone 3-glucuronide) to P_4 metabolite (pregnanediol 3-glucuronide) (Baird *et al.*, 1991). The timing of implantation was defined as the first detection of hCG in maternal urine. Of the 199 conceptions, sufficient data for analysis was present in 189 pregnancies. Of these 189 pregnancies, 141 (75%) lasted for at least 6 weeks past the last menstrual period, and the remaining 48 pregnancies (25%) ended before 6 weeks. Among the 141 pregnancies lasting 6 weeks or more, implantation occurred 6–12 days after ovulation; in particular, most (118 women; 84%) implantations occurred 8, 9 or 10 days after ovulation. Among the 102 conceptuses that implanted 9 days after ovulation, the early pregnancy loss rate was 13%. In contrast, the early pregnancy loss rates were 26%, 52% and 82% ($P < 0.001$) for pregnancies occurring 10, 11 and more than 11 days after ovulation. The authors discussed several reasons for the high early loss rates of late-implanting conceptuses, such as decreased receptivity during the luteal phase, a less hCG responsive corpus luteum or intrinsic embryonic factors resulting in a lower production of hCG. In most successful ongoing pregnancies, the embryo implanted 8–10 days after ovulation, coinciding with days 22–24 of the cycle (Wilcox *et al.*, 1999). A large sample size with a 6-month follow-up for each patient is the strength of this milestone study; however, determining the day of ovulation using urinary E_2 and P_4 metabolites without transvaginal ultrasound is a limitation.

In summary, the WOI continues to be a black box. However, based on the existing evidence, the WOI for best reproductive performance appears to be narrow, ~2 days coinciding with 8–10 days after ovulation, and an accurate determination of the WOI is essential to optimize the reproductive outcomes of frozen and warmed embryo transfer in t-NC FET.

Implications for clinical practice

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 (Mackens *et al.*, 2017; Mumusoglu *et al.*, 2021). Of note, only three retrospective studies compared reproductive outcomes employing different criteria for timing of FET in t-NC (Irani *et al.*, 2017; Bartels *et al.*, 2019; Lovrec *et al.*, 2021). Of those three studies, LH surge testing was performed in serum in two studies (Irani *et al.*, 2017; Bartels *et al.*, 2019) and in urine in the remaining one study (Lovrec *et al.*, 2021). 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) (Irani *et al.*, 2017). 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 LH ≥17 mIU/ml with a ≥30% drop in E_2 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 a ≥30% drop in E_2 levels. 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% versus 63.4%, 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 P_4. 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 (Liu *et al.*, 1993). 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 (Bartels *et al.*, 2019). As in the previous study (Irani *et al.*, 2017), 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 were compared in a retrospective study enrolling 2080 cycles (Lovrec *et al.*, 2021). 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.

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.

Strengths, limitations and future research

To our knowledge, this is the first systematic review and meta-analysis evaluating the definition of the onset of the LH surge and the duration between the onset of the LH surge and ovulation. Unfortunately, most included studies were old and did not use state-of-art technology for endocrine and ultrasound work-up. Studies including a large sample size employing high-resolution transvaginal ultrasound and endocrine monitoring are required to precisely define the onset of the LH surge and the duration between the onset of the LH surge and ovulation. Future research is also warranted to delineate the optimal P_4 level, exposure time and duration needed to open the WOI.

Conclusion

Testing either in serum or urine, different definitions for the onset of the LH surge have been developed without reaching a consensus. A marked inter-personal variation exists for the time interval between the onset of the LH surge and ovulation, with a mean duration of 33.91 h (95% CI = 30.79–37.03: six studies, 187 cycles) and ranging from 22–56 h. LH surges resulting in ovulation are not necessarily one type; rather, they may be extremely variable in configuration, amplitude and duration, which might have consequences for reproductive outcomes in t-NC FET. Urinary and serum testing of LH may be used interchangeably. Ultrasonographic documentation of ovulation and serum P_4 assessment on the day or 1 day prior to warmed blastocyst transfer is not a common practice worldwide during t-NC FET cycles. Since LUF is associated with decreased mid-luteal serum P_4 levels, delineation of LUF cycles by ultrasonography, although posing additional workload, might be of importance. Conflicting and limited data are available comparing the effect of different timings of FET in t-NC on the reproductive outcomes, warranting further randomized controlled trials.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Data availability

The present study and the corresponding search protocol were registered with the PROSPERO registry (http://www.crd.york.ac.uk/PROSPERO) as PROSPERO 2021: CRD42021277365. The data underlying this article are available in the article and in its online supplementary material.

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Authors' roles

Study conception and design: all authors; search strategy design and execution: M.E. and S.M.; data extraction: M.E. and S.M.; data interpretation: all authors; drafting of manuscript: M.E., S.M. and H.Y.; critical revision of manuscript: all authors; and manuscript approval for submission: all authors.

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None related to the current study.

References

Alliende ME. Luteinizing hormone surge in normally ovulating women. *Fertil Steril* 2013;**99**:e14–e15.

Arici AC, Byrd W. Comparison of two LH monitoring methods in women undergoing intrauterine insemination. In: *Proceedings of the 48th Annual Meeting of the American Fertility Society*. San Antonio, TX, S70. November 2–5, 1992.

Asserhøj LL, Spangmose AL, Aaris Henningsen AK, Clausen TD, Ziebe S, Jensen RB, Pinborg A. Adverse obstetric and perinatal outcomes in 1,136 singleton pregnancies conceived after

programmed frozen embryo transfer (FET) compared with natural cycle FET. *Fertil Steril* 2021;**115**:947–956.

Baird DD, Weinberg CR, Wilcox AJ, McConnaughey DR, Musey PI. Using the ratio of urinary oestrogen and progesterone metabolites to estimate day of ovulation. *Stat Med* 1991;**10**:255–266.

Bartels CB, Ditrio L, Grow DR, O'Sullivan DM, Benadiva CA, Engmann L, Nulsen JC. The window is wide: flexible timing for vitrified-warmed embryo transfer in natural cycles. *Reprod Biomed Online* 2019;**39**:241–248.

Cahill DJ, Wardle PG, Harlow CR, Hull MG. Onset of the preovulatory luteinizing hormone surge: diurnal timing and critical follicular prerequisites. *Fertil Steril* 1998;**70**:56–59.

Chan YF, Ho PC, So WW, Chan SY, Chan ST. Efficacy of the urinary hemagglutination test (Higonavis) and enzyme immunoassay (Ovustick) in detection of the spontaneous luteinizing hormone surge in an in vitro fertilization/gamete intrafallopian transfer (IVF/GIFT) program. *J In Vitro Fert Embryo Transf* 1989;**6**:218–221.

Cohlen BJ, Te Velde ER, Scheffer G, van Kooij RJ, Maria de Brouwer CP, van Zonneveld P. The pattern of the luteinizing hormone surge in spontaneous cycles is related to the probability of conception. *Fertil Steril* 1993;**60**:413–417.

de Crespigny LJC, O'herlihy C, Hoult IJ, Robinson HP. Ultrasound in an in vitro fertilization program. *Fertil Steril* 1981;**35**:25–28.

De Geyter C, Wyns C, Calhaz-Jorge C, de Mouzon J, Ferraretti AP, Kupka M, Nyboe Andersen A, Nygren KG, Goossens V. 20 years of the European IVF-monitoring Consortium registry: what have we learned? A comparison with registries from two other regions. *Hum Reprod* 2020;**35**:2832–2849.

Direito A, Bailly S, Mariani A, Ecochard R. Relationships between the luteinizing hormone surge and other characteristics of the menstrual cycle in normally ovulating women. *Fertil Steril* 2013;**99**:279–285.e3.

Donnez J, Langerock S, Thomas K. Peritoneal fluid volume, 17beta-estradiol and progesterone concentrations in women with endometriosis and/or luteinized unruptured follicle syndrome. *Gynecol Obstet Invest* 1983;**16**:210–220.

Dozortsev DI, Diamond MP. Luteinizing hormone-independent rise of progesterone as the physiological trigger of the ovulatory gonadotropins surge in the human. *Fertil Steril* 2020;**114**:191–199.

Ecochard R, Marret H, Rabilloud M, Bradaï R, Boehringer H, Girotto S, Barbato M. Sensitivity and specificity of ultrasound indices of ovulation in spontaneous cycles. *Eur J Obstet Gynecol Reprod Biol* 2000;**91**:59–64.

Eppig JJ. FSH stimulates hyaluronic acid synthesis by oocyte-cumulus cell complexes from mouse preovulatory follicles. *Nature* 1979;**281**:483–484.

Evers JL. The luteinized unruptured follicle syndrome. *Baillieres Clin Obstet Gynaecol* 1993;**7**:363–387.

Fritz MA, McLachlan RI, Cohen NL, Dahl KD, Bremner WJ, Soules MR. Onset and characteristics of the midcycle surge in bioactive and immunoactive luteinizing hormone secretion in normal women: influence of physiological variations in periovulatory ovarian steroid hormone secretion. *J Clin Endocrinol Metab* 1992;**75**:489–493.

Frydman R, Testart J, Feinstein MC, Roger M. Interrelationship of plasma and urinary luteinizing hormone preovulatory surge. *J Steroid Biochem* 1984;**20**:617–619.

Gaggiotti-Marre S, Álvarez M, González-Foruria I, Parriego M, Garcia S, Martínez F, Barri PN, Polyzos NP, Coroleu B. Low progesterone levels on the day before natural cycle frozen embryo transfer are negatively associated with live birth rates. *Hum Reprod* 2020;**35**:1623–1629.

Garcia JE, Jones GS, Wright GL Jr. Prediction of the time of ovulation. *Fertil Steril* 1981;**36**:308–315.

Ghazeeri GS, Vongprachanh P, Kutteh WH. The predictive value of five different urinary LH kits in detecting the LH surge in regularly menstruating women. *Int J Fertil Womens Med* 2000;**45**:321–326.

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**:126.e1–126.e18.

Groenewoud ER, Kollen BJ, Macklon NS, Cohlen BJ. Spontaneous LH surges prior to HCG administration in unstimulated-cycle frozen-thawed embryo transfer do not influence pregnancy rates. *Reprod Biomed Online* 2012;**24**:191–196.

Guermandi E, Vegetti W, Bianchi MM, Uglietti A, Ragni G, Crosignani P. Reliability of ovulation tests in infertile women. *Obstet Gynecol* 2001;**97**:92–96.

Hamilton CJ, Evers JL, de Haan J. Ovulatory disturbances in patients with luteal insufficiency. *Clin Endocrinol (Oxf)* 1987;**26**:129–136.

Hamilton CJ, Wetzels LC, Evers JL, Hoogland HJ, Muijtjens A, de Haan J. Follicle growth curves and hormonal patterns in patients with the luteinized unruptured follicle syndrome. *Fertil Steril* 1985;**43**:541–548.

Hoff JD, Quigley ME, Yen SS. Hormonal dynamics at midcycle: a reevaluation. *J Clin Endocrinol Metab* 1983;**57**:792–796.

Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005;**5**:13.

Hu KL, Zhang D, Li R. Endometrium preparation and perinatal outcomes in women undergoing single-blastocyst transfer in frozen cycles. *Fertil Steril* 2021;**115**:1487–1494.

Hull MG, Savage PE, Bromham DR, Ismail AA, Morris AF. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle ("ovulation") derived form treated and untreated conception cycles. *Fertil Steril* 1982;**37**:355–360.

Irani M, Robles A, Gunnala V, Reichman D, Rosenwaks Z. Optimal parameters for determining the LH surge in natural cycle frozen-thawed embryo transfers. *J Ovarian Res* 2017;**10**:70.

Johal JK, Bavan B, Zhang W, Gardner RM, Lathi RB, Milki AA. The impact of timing modified natural cycle frozen embryo transfer based on spontaneous luteinizing hormone surge. *J Assist Reprod Genet* 2021;**38**:219–225.

Johansson ED, Wide L, Gemzell C. Luteinizing hormone (LH) and progesterone in plasma and LH and oestrogens in urine during 42 normal menstrual cycles. *Acta Endocrinol (Copenh)* 1971;**68**:502–512.

Johnson S, Schiffner J, Freundl G, Bachmann N, Gnoth C. Luteinising hormone profiles in conception and non-conception natural cycles. *Eur J Contracept Reprod Health Care* 2019;**24**:140–147.

Johnson S, Weddell S, Godbert S, Freundl G, Roos J, Gnoth C. Development of the first urinary reproductive hormone ranges

referenced to independently determined ovulation day. *Clin Chem Lab Med* 2015;**53**:1099–1108.

Kahraman S, Sahin Y. Is there a critical LH level for hCG trigger after the detection of LH surge in modified natural frozen-thawed single blastocyst transfer cycles? *J Assist Reprod Genet* 2020;**37**: 3025–3031.

Kesner JS, Knecht EA, Krieg EF Jr, Wilcox AJ, O'Connor JF. Detecting pre-ovulatory luteinizing hormone surges in urine. *Hum Reprod* 1998;**13**:15–21.

Koninckx PR, Heyns WJ, Corvelyn PA, Brosens IA. Delayed onset of luteinization as a cause of infertility. *Fertil Steril* 1978;**29**:266–269.

Koskimies AI, Liukkonen S, Tenhunen A, Huhtaniemi I. Low LH receptor content in corpora lutea in luteinized unruptured follicle (LUF) syndrome. *Hum Reprod* 1987;**2**:367–369.

Krotz S, McKenzie LJ, Cisneros P, Buster J, Amato P, Carson S. Prevalence of premature urinary luteinizing hormone surges in women with regular menstrual cycles and its effect on implantation of frozen-thawed embryos. *Fertil Steril* 2005;**83**:1742–1744.

Lawrenz B, Coughlan C, Melado L, Fatemi HM. The ART of frozen embryo transfer: back to nature! *Gynecol Endocrinol* 2020;**36**:479–483.

Lemay A, Bastide A, Lambert R, Rioux JE. Prediction of human ovulation by rapid luteinizing hormone (LH) radioimmunoassay and ovarian ultrasonography. *Fertil Steril* 1982;**38**:194–201.

Litzky JF, Boulet SL, Esfandiari N, Zhang Y, Kissin DM, Theiler RN, Marsit CJ. Effect of frozen/thawed embryo transfer on birthweight, macrosomia, and low birthweight rates in US singleton infants. *Am J Obstet Gynecol* 2018;**218**:433.e431–433.e10.

Liu HC, Cohen J, Alikani M, Noyes N, Rosenwaks Z. Assisted hatching facilitates earlier implantation. *Fertil Steril* 1993;**60**:871–875.

Lovrec VG, Kozar N, Milan R. 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. *Reprod Biomed Online* 2021. https://doi.org/10.1016/j.rbmo.2021.12.008.

Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H, Blockeel C. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. *Hum Reprod* 2017;**32**:2234–2242.

Makhijani R, Bartels C, Godiwala P, Bartolucci A, Nulsen J, Grow D, Benadiva C, Engmann L. Maternal and perinatal outcomes in programmed versus natural vitrified-warmed blastocyst transfer cycles. *Reprod Biomed Online* 2020;**41**:300–308.

Marik J, Hulka J. Luteinized unruptured follicle syndrome: a subtle cause of infertility. *Fertil Steril* 1978;**29**:270–274.

Marinho AO, Sallam HN, Goessens LK, Collins WP, Rodeck CH, Campbell S. Real time pelvic ultrasonography during the periovulatory period of patients attending an artificial insemination clinic. *Fertil Steril* 1982;**37**:633–638.

McGovern PG, Myers ER, Silva S, Coutifaris C, Carson SA, Legro RS, Schlaff WD, Carr BR, Steinkampf MP, Giudice LC *et al.*; NICHD National Cooperative Reproductive Medicine Network. Absence of secretory endometrium after false-positive home urine luteinizing hormone testing. *Fertil Steril* 2004;**82**:1273–1277.

Miller PB, Soules MR. The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. *Obstet Gynecol* 1996;**87**:13–17.

Moghissi KS, Syner FN, Evans TN. A composite picture of the menstrual cycle. *Am J Obstet Gynecol* 1972;**114**:405–418.

Mumusoglu S, Polat M, Ozbek IY, Bozdag G, Papanikolaou EG, Esteves SC, Humaidan P, Yarali H. Preparation of the endometrium for frozen embryo transfer: a systematic review. *Front Endocrinol (Lausanne)* 2021;**12**:688237.

Murdoch WJ, Dunn TG. Luteal function after ovulation blockade by intrafollicular injection of indomethacin in the ewe. *J Reprod Fertil* 1983;**69**:671–675.

Nulsen J, Wheeler C, Ausmanas M, Blasco L. Cervical mucus changes in relationship to urinary luteinizing hormone. *Fertil Steril* 1987;**48**:783–786.

Park SJ, Goldsmith LT, Skurnick JH, Wojtczuk A, Weiss G. Characteristics of the urinary luteinizing hormone surge in young ovulatory women. *Fertil Steril* 2007;**88**:684–690.

Peluso JJ. Role of the amplitude of the gonadotropin surge in the rat. *Fertil Steril* 1990;**53**:150–154.

Ponto KL, Barnes RB, Holt JA. Quantitative and qualitative tests for urinary luteinizing hormone. Comparison in spontaneous and clomiphene-citrate-treated cycles. *J Reprod Med* 1990;**35**: 1051–1054.

Reutman SR, LeMasters GK, Kesner JS, Shukla R, Krieg EF Jr, Knecht EA, Lockey JE. Urinary reproductive hormone level differences between African American and Caucasian women of reproductive age. *Fertil Steril* 2002;**78**:383–391.

Roger M, Grenier J, Houlbert C, Castanier M, Feinstein MC, Scholler R. Rapid radioimmunoassays of plasma LH and estradiol-17 beta for the prediction of ovulation. *J Steroid Biochem* 1980;**12**:403–410.

Roos J, Johnson S, Weddell S, Godehardt E, Schiffner J, Freundl G, Gnoth C. Monitoring the menstrual cycle: Comparison of urinary and serum reproductive hormones referenced to true ovulation. *Eur J Contracept Reprod Health Care* 2015;**20**:438–450.

Roque M, Bedoschi G, Cecchino GN, Esteves SC. Fresh versus frozen blastocyst transfer. *Lancet* 2019a;**394**:1227–1228.

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* 2019b;**25**:2–14.

Schenken RS, Werlin LB, Williams RF, Prihoda TJ, Hodgen GD. Histologic and hormonal documentation of the luteinized unruptured follicle syndrome. *Am J Obstet Gynecol* 1986;**154**:839–847.

Soules MR, Clifton DK, Steiner RA, Cohen NL, Bremner WJ. The corpus luteum: determinants of progesterone secretion in the normal menstrual cycle. *Obstet Gynecol* 1988;**71**:659–666.

Stein I, Leventhal M. Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstetr Gynecol* 1935;**29**:181–191.

Strickland S, Beers WH. Studies on the role of plasminogen activator in ovulation. In vitro response of granulosa cells to gonadotropins, cyclic nucleotides, and prostaglandins. *J Biol Chem* 1976;**251**:5694–5702.

Taymor ML, Seibel MM, Smith D, Levesque L. Ovulation timing by luteinizing hormone assay and follicle puncture. *Obstet Gynecol* 1983;**62**:191–195.

Testart J, Frydman R. Minimum time lapse between luteinizing hormone surge or human chorionic gonadotropin administration and follicular rupture. *Fertil Steril* 1982;**37**:50–53.

Testart J, Frydman R, Feinstein MC, Thebault A, Roger M, Scholler R. Interpretation of plasma luteinizing hormone assay for the

collection of mature oocytes from women: definition of a luteinizing hormone surge-initiating rise. *Fertil Steril* 1981;**36**:50–54.

Thomas K, Walckiers R, Ferin J. Biphasic pattern of LH midcycle discharge. *J Clin Endocrinol Metab* 1970;**30**:269–272.

Vanrell JA, Balasch J, Fuster JS, Fuster R. Ovulation stigma in fertile women. *Fertil Steril* 1982;**37**:712–713.

Wetzels LC, Hoogland HJ. Relation between ultrasonographic evidence of ovulation and hormonal parameters: luteinizing hormone surge and initial progesterone rise. *Fertil Steril* 1982;**37**:336–341.

WHO. Temporal relationships between ovulation and defined changes in the concentration of plasma estradiol-17 beta, luteinizing hormone, follicle-stimulating hormone, and progesterone. I. Probit analysis. World Health Organization, Task Force on Methods for the Determination of the Fertile Period, Special Programme of Research, Development and Research Training in Human Reproduction. *Am J Obstet Gynecol* 1980;**138**:383–390.

Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med* 1999;**340**:1796–1799.

Wu H, Zhou P, Lin X, Wang S, Zhang S. Endometrial preparation for frozen-thawed embryo transfer cycles: a systematic review and network meta-analysis. *J Assist Reprod Genet* 2021;**38**:1913–1926.

Yding Andersen C. Effect of FSH and its different isoforms on maturation of oocytes from pre-ovulatory follicles. *Reprod Biomed Online* 2002;**5**:232–239.

Yding Andersen C, Leonardsen L, Ulloa-Aguirre A, Barrios-De-Tomasi J, Moore L, Byskov AG. FSH-induced resumption of meiosis in mouse oocytes: effect of different isoforms. *Mol Hum Reprod* 1999;**5**:726–731.

Young JR, Jaffe RB. Strength-duration characteristics of estrogen effects on gonadotropin response to gonadotropin-releasing hormone in women. II. Effects of varying concentrations of estradiol. *J Clin Endocrinol Metab* 1976;**42**:432–442.

Yussman MA, Taymor ML. Serum levels of follicle stimulating hormone and luteinizing hormone and of plasma progesterone related to ovulation by corpus luteum biopsy. *J Clin Endocrinol Metab* 1970;**30**:396–399.

Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP, Stouffer RL. Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. *Hum Reprod* 1995;**10**:1658–1666.