Predicting successful induction of oocyte maturation after gonadotropin-releasing hormone agonist (GnRHa) trigger

N.E. Kummer¹, R.S. Feinn², D.W. Griffin¹, J.C. Nulsen¹, C.A. Benadiva¹, and L.L. Engmann¹,*

¹Center for Advanced Reproductive Services, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Connecticut Health Center, Dowling South Building, 263 Farmington Avenue, Farmington, CT 06030-6224, USA
²Department of Biostatistics, University of Connecticut Health Center, Farmington, CT 06030-6224, USA

*Correspondence address. Tel: +1-860-679-4580; Fax: +1-860-679-1499; E-mail: lengmann@uchc.edu

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STUDY QUESTION: Are there factors predicting the number of total and mature oocytes retrieved after controlled ovarian hyperstimulation (COH) utilizing a gonadotropin-releasing hormone (GnRH) antagonist protocol and a GnRH agonist (GnRHa) to induce oocyte maturation?

SUMMARY ANSWER: Peak estradiol (E₂) level, post-trigger LH and progesterone and the magnitude of LH rise are independent predictors of the total number of oocytes and mature oocytes retrieved.

WHAT IS KNOWN ALREADY: Despite multiple follicular development in high responders, oocyte retrieval after a GnRHa trigger in a small subset of patients fails to obtain a substantial number of total oocytes or mature oocytes.

STUDY DESIGN, SIZE AND DURATION: A retrospective chart review of all autologous and oocyte donation cycles utilizing a GnRHa antagonist protocol where GnRHa was used for the induction of oocyte maturation between 1 April 2003 and 31 December 2011.


MAIN RESULTS AND THE ROLE OF CHANCE: Peak E₂ on the day of trigger (r = 0.19, P < 0.001), post-trigger LH (r = 0.12, P = 0.009) and progesterone (r = 0.47, P < 001) and LH rise (r = 0.18, P < 0.001) all positively correlated with the number of total and mature oocytes retrieved. The true incidence of empty follicle syndrome was 1.4% (7/508). There was no post-trigger LH or progesterone cut-off value for the prediction of oocyte yield. However, all cases of empty follicle syndrome occurred in patients with post-trigger LH <15 IU/l and P ≤ 3.5 ng/ml. The findings of this study may also be due to chance since it was a retrospective study and not prospectively designed.

LIMITATION, REASONS FOR CAUTION: This is a retrospective chart review and therefore subject to bias. Serum hormone measurements were performed between 8 and 12 h after GnRHa trigger rather than a standardized time period following trigger administration. Therefore, peak levels of LH may have been missed due to the short ascending limb of LH rise lasting approximately 4 h after GnRHa trigger.

WIDER IMPLICATIONS OF THE FINDINGS: The results of this study can be generalized to high responders utilizing a GnRHa antagonist protocol for COH and a GnRHa for the induction of oocyte maturation. The use of alternative stimulation regimens or medications will limit the ability to generalize the results of this study to other populations.

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Key words: GnRH agonist / GnRH antagonist / LH surge / GnRHa trigger / empty follicle syndrome
Introduction

Administration of hCG is routinely used for the induction of oocyte maturation during IVF cycles. Unfortunately, the use of hCG contributes to the development of ovarian hyperstimulation syndrome (OHSS) (Golan et al., 1989; Echalat and Schenker, 1997; Fauser et al., 1999; Whelan and Vlahos, 2000) due to its stimulatory effect on multiple corpora lutea and increased half-life when compared with LH (Darmewood et al., 1989). A GnRH agonist (GnRHa) may alternatively be used for the induction of oocyte maturation (Itskovitz et al., 1991; Itskovitz-Eldor et al., 2000; Kol, 2004). The GnRHa trigger induces an endogenous LH and FSH flare (Itskovitz et al., 1991) that effectively results in the induction of final oocyte maturation (Fauser et al., 2002) and ovulation (Emperaire and Ruffie, 1991; Kulikowski et al., 1995). Moreover, due to rapid luteolysis following its use, the GnRHa trigger significantly reduces or eliminates the risk of OHSS (Kol, 2004; Engmann et al., 2008; Di Luigi et al., 2010; Humaidan et al., 2011).

Some clinicians are reluctant to use GnRHa for oocyte maturation as some studies have reported compromised pregnancy rates after its use (Humaidan et al., 2005; Kolibianakis et al., 2005), although several publications have reviewed alternate luteal phase supplementation strategies to optimize pregnancy rates (Engmann et al., 2008; Humaidan, 2011; Humaidan et al., 2011; Engmann and Benadiva, 2012). Moreover, anecdotal concerns regarding the effectiveness of GnRHa to induce optimal oocyte yield and mature oocytes, as well as previous reports of empty follicle syndrome (EFS) after GnRHa trigger (Griesinger et al., 2011; Honnma et al., 2011; Castillo et al., 2012) may contribute to the reluctance of its routine use for the prevention of OHSS. It is therefore important to determine factors that may predict the oocyte yield after GnRHa induction of oocyte maturation. Such reference values will aid clinicians in deciding whether a patient should progress to oocyte retrieval after a GnRHa trigger or if a cycle cancellation is warranted due to a reduced likelihood of obtaining oocytes.

Despite multiple follicular development in high responders, oocyte retrieval after a GnRHa trigger in a small subset of patients fails to obtain a substantial number of total oocytes or mature oocytes, although it has been shown that there are no differences in EFS rates between GnRHa and hCG triggers (Castillo et al., 2012). A rise in serum LH and progesterone after a GnRHa trigger indicates that an endogenous flare has occurred and oocyte maturation has been initiated, but serum LH or progesterone thresholds, which may predict successful oocyte retrieval, have not been previously characterized. The aim of this study therefore was to evaluate if the magnitude of the endogenous LH surge and progesterone rise after the GnRHa trigger are predictive of the total number of oocytes and mature oocytes retrieved.

Materials and Methods

Study design and participants

This was a retrospective database review conducted at the Center for Advanced Reproductive Services at the University of Connecticut Health Center. Approval for this study was obtained from the Institutional Review Board at the University of Connecticut Health Center. All autologous and oocyte donation cycles utilizing a GnRH antagonist protocol where GnRHa was used for the induction of oocyte maturation between 1 April 2003 and 31 December 2011 were considered for analysis. Cycles that were triggered with both GnRHa and hCG were excluded from the analyses.

The controlled ovarian hyperstimulation (COH) treatment protocol used has been previously described (Engmann et al., 2008). In brief, patients underwent COH consisting of co-treatment with a GnRH antagonist (Ganirelix, Merck & Co., NJ, USA) in a daily dose of 0.25 mg subcutaneously added once the leading follicle was ≥14 mm and the serum estradiol (E2) exceeded 350 pg/ml. Oocyte maturation was achieved with 1 mg of leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL) administered subcutaneously at least 12 h after the last dose of the GnRH antagonist, followed by transvaginal ultrasound-guided oocyte retrieval 35 h later. Oocyte maturation was induced with a GnRHa if patients were deemed to be at high risk of OHSS development due to the presence of >13 follicles measuring >11 mm in diameter on the day of the trigger (Papanikolaou et al., 2006). To have a history of previous cycle cancellation for the risk of OHSS, or a history of OHSS development in a previous cycle. Serum E2 and LH were measured on the day of the trigger and E2, LH and progesterone levels were measured 8–12 h after GnRHa administration to ensure that an LH surge had occurred.

Measurement of serum E2, LH and progesterone

Serum E2, LH and progesterone were measured by a paramagnetic particle chemiluminescent immunoassay (Access® Immunoassay Systems, Beckman Coulter, Fullerton, CA, USA) with analytical sensitivities of 20 pg/ml, 0.2 IU/ml and 0.08 ng/ml, respectively. The intra-assay and inter-assay coefficients of variation were 4.4 and 7.6% for E2, 3.8 and 6.4% for LH and 6.1 and 7.5% for progesterone, respectively.

Outcome variables

The primary outcome variable was the total number of eggs. The secondary outcome variables were the number of mature oocytes and the proportion of oocytes that were mature. Mature oocytes were those observed to have entered metaphase II, and therefore only cycles utilizing ICSI were included in the analyses for mature oocytes. The proportion of mature oocytes was calculated by dividing the number of mature oocytes by the total number of oocytes retrieved.

Data analysis

The SPSS statistical package (Release 20.0; SPSS, Chicago, IL) was used for statistical evaluation. The data were normally distributed and therefore an independent sample t-test was used for continuous variables and Fisher’s exact test for categorical variables where appropriate. Data are presented as mean ± SD unless otherwise stated. Pearson correlations were used to analyze if factors such as age, BMI, oocyte donor status or diagnosis of polycystic ovarian syndrome, serum peak E2, post-GnRHa trigger LH or progesterone and the magnitude of LH rise correlated with the number of total or mature oocytes retrieved or the proportion of mature eggs. LH rise was defined as the LH on the day after the trigger divided by the LH on the day of the trigger. Pearson correlation was also used to determine whether the BMI was correlated to post-trigger LH and progesterone values.

Multiple linear regression was used to determine the independent predictive value of these factors on the total number of eggs and the number of mature eggs (both outcomes approximated normality). The generalized linear model (GLM) with a binary logistic response (logit link) was used to model the above factors on the proportion of mature oocytes (number of
mature eggs out of the total number of eggs retrieved). This model was chosen over a linear regression modeling the proportion of eggs as the outcome because the proportion of eggs was negatively skewed and women varied in the total number of eggs retrieved, which makes using an unweighted proportion a bias estimate. P-values < 0.05 were considered statistically significant.

Results

A total of 508 cycles received GnRHa for induction of oocyte maturation during the time period considered and were all included in the calculation of the true risk of EFS. Four cycles were excluded from the analyses evaluating the predictive factors for oocyte yield. This is because in one cycle the patient received a rescue hCG protocol before retrieval and three cycles did not proceed with oocyte retrieval because of the suboptimal LH surge but these cycles have been described in Table I. Therefore, a total of 504 COH cycles, consisting of 413 autologous and 91 donor IVF cycles underwent oocyte retrieval and are therefore included in data for the analyses of predictive factors for oocyte yield. Two cycles did not have data available for post-trigger LH and progesterone and although included in the final analyses, they did not have data for analysis of the effect of post-trigger LH and progesterone on oocyte yield. Only ICSI cycles (n = 432) were included in the analysis of mature oocytes.

The mean age of the women in the study was 31.7 years (SD: 4.6; range: 21.7–43.1 years) and the mean BMI was 26.1 kg/m² (SD: 5.9; range: 17.5–51.7 kg/m²). The mean number of oocytes retrieved was 25.7 (SD: 11.1; range: 0–80). The mean post-trigger LH was 59.1 IU/l (SD: 36.2; range: 7.65–226.6 IU/l) and mean post-trigger progesterone was 9.1 ng/ml (SD: 5.1; range: 0.7–33.7 ng/ml). None of the patients in the study developed OHSS.

The lowest post-trigger LH and progesterone values to yield oocytes were 7.65 IU/l and 0.7 ng/ml, respectively. BMI is negatively correlated with both LH rise (\(r = -0.26, P < 0.001\)) and post-trigger progesterone (\(r = -0.22, P < 0.001\)). This indicates that the lower the BMI the higher the LH rise and post-trigger progesterone.

Patients with EFS or cycle cancellation due to suboptimal LH surge

Table I consists of four women (Subjects 1, 2, 3 and 4) who had sub-optimal LH surges and were included in the estimation of the true EFS rate but not included in the analyses of predictive factors for oocyte yield, as well as three women (Subjects 5, 6 and 7) who did not have any oocytes retrieved. Subject 1 received a rescue hCG protocol and retrieval 35 h later yielded 15 oocytes. Subjects 2, 3 and 4 were oocyte donors and therefore their cycles were canceled without re-trigger with hCG.

Three cycles (3/504, 0.6%) had no oocytes at the time of retrieval. The retrieval was terminated after follicular aspiration of one ovary in Subject 5. A rescue hCG protocol was administered and subsequent retrieval 35 h later yielded 13 oocytes. This subject was included in the analysis and considered as no oocytes retrieved. Subject 6 underwent follicular aspiration from both ovaries with no oocytes retrieved. Subject 7 had hypothalamic dysfunction and was inadvertently triggered with GnRHα. She received a second dose of GnRHα 12 h after the initial injection due to a lack of optimal LH surge or progesterone rise and no oocytes were obtained. However, when the data analyzed comprised all patients triggered with GnRHa, including those who did not proceed with oocyte retrieval and those who received rescue hCG because of suboptimal LH rise, the true incidence of EFS is estimated as 1.4% (7/508), since we assumed that these seven patients would not have had any oocytes obtained at retrieval.

Factors determining number of total, mature and proportion of mature oocytes retrieved

Binary correlation analyses revealed that the age (\(r = -0.20, P < 0.001\)) and the BMI (\(r = -0.15, P = 0.001\)) were both negatively correlated with the number of oocytes retrieved, although donor status and PCOS diagnosis did not correlate with oocyte yield. Peak E2 on the day of the trigger (\(r = 0.19, P < 0.001\)) and post-trigger LH (\(r = 0.12, P = 0.009\)) and progesterone (\(r = 0.47, P < 0.001\)) and LH rise (\(r = 0.18, P < 0.001\)) all positively correlated with the number of total oocytes retrieved. A multiple linear regression, controlling for the age and the BMI, resulted in all four factors above independently predicting the total number of oocytes. As seen in Table II under the total number of oocytes column, post-trigger progesterone was the strongest predictor of oocyte yield (\(β = 0.500\)).

Binary correlation analyses revealed that the age (\(r = -0.19, P = 0.005\)) and the BMI (\(r = -0.20, P < 0.001\)) were both negatively correlated with the number of mature oocytes retrieved. Peak E2 on the day of the trigger (\(r = 0.13, P = 0.008\)) and post-trigger progesterone (\(r = 0.47, P < 0.001\)) and LH rise (\(r = 0.18, P < 0.001\)) all positively correlated with the number of mature oocytes retrieved. Multiple linear regression analysis demonstrated that peak E2 on the day of trigger, post-trigger LH and progesterone, and LH rise independently predicted mature oocyte yield, after controlling for age and BMI. As seen in Table II under the number of mature oocytes column, post-trigger P was the strongest predictor of mature oocyte yield (\(β = 0.484\)).

Bivariate GLM logistic models showed that a lower BMI is related to obtaining a larger proportion of mature oocytes (\(P = 0.023\)). Additionally, donors produced a higher ratio of mature oocytes than subjects undergoing an autologous cycle (\(P = 0.014\)). The multivariate GLM model found that only the BMI demonstrated a significant unique association with the proportion of mature oocytes retrieved. Table II shows that a higher BMI was associated with decreased odds in mature oocytes retrieved [odds ratio (OR) = 0.99, \(P = 0.018\)].

Progesterone and LH threshold values

Data from the 502 cycles were plotted on locally weighted scatterplot smoothing (LOESS) curves in order to determine if there exists a post-trigger LH or progesterone threshold below which predicts the retrieval of less oocytes or above which predicts a larger oocyte yield (Fig. 1a and b): no such thresholds were evident. However, using a receiver operating characteristics (ROC) curve it was found that the area under the curve (AUC) for post-trigger LH was 0.985 and that a cutoff post-trigger LH value of 15 IU/l has a sensitivity of 97.4% and specificity of 100% for predicting EFS. Moreover, a post-trigger progesterone AUC was 0.973 and a cut-off value of 3.5 ng/ml has a sensitivity of 100% and specificity of 69% for predicting EFS. However, as there were only three cycles where no eggs were
Table I Characteristics of cycles with EFS and/or suboptimal LH surge.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>BMI (kg/m²)</th>
<th>Peak E₂ (pg/ml)</th>
<th>P post-trigger (ng/ml)</th>
<th>LH post-trigger (IU/ml)</th>
<th>LH rise</th>
<th>Retrieval/comments</th>
<th>Reason for EFS and/or suboptimal LH surge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>32.7</td>
<td>Unexplained infertility</td>
<td>20.0</td>
<td>3453</td>
<td>0.5</td>
<td>1.15</td>
<td>0.91</td>
<td>HCG 10 000 IU administered due to lack of LH surge. Post-HCG injection retrieval yielded 15 oocytes. No conception</td>
<td>Most likely human error in administration of GnRHa</td>
</tr>
<tr>
<td>2a</td>
<td>22.9</td>
<td>Oocyte donor</td>
<td>23.6</td>
<td>2979</td>
<td>9.6</td>
<td>3.79</td>
<td>5.74</td>
<td>Retrieval canceled because of lack of LH surge</td>
<td>Unknown</td>
</tr>
<tr>
<td>3a</td>
<td>26.0</td>
<td>Oocyte donor</td>
<td>18.6</td>
<td>2332</td>
<td>0.4</td>
<td>&lt;0.01</td>
<td>0.14</td>
<td>Retrieval canceled. Patient had previously done three cycles with GnRHα trigger and had optimal LH surges and oocytes retrieved</td>
<td>Most likely human error in administration of GnRHa</td>
</tr>
<tr>
<td>4a</td>
<td>30.0</td>
<td>Oocyte donor</td>
<td>24.4</td>
<td>3324</td>
<td>2.7</td>
<td>2.6</td>
<td>2.45</td>
<td>Retrieval canceled. Subsequently had a cycle with GnRHα trigger and had optimal LH surge and oocytes retrieved</td>
<td>Most likely human error in administration of GnRHa</td>
</tr>
<tr>
<td>5b</td>
<td>36.7</td>
<td>Anovulation</td>
<td>24.9</td>
<td>5906</td>
<td>3.5</td>
<td>10.9</td>
<td>7.03</td>
<td>No oocytes from one ovary. HCG 10 000 IU injection followed by second retrieval 35 h later yielded 13 oocytes. No conception</td>
<td>Unknown</td>
</tr>
<tr>
<td>6b</td>
<td>32.9</td>
<td>Unexplained infertility</td>
<td>21.4</td>
<td>2760</td>
<td>2.4</td>
<td>14.3</td>
<td>143</td>
<td>No oocytes retrieved from both ovaries</td>
<td>Unknown</td>
</tr>
<tr>
<td>7b</td>
<td>29.4</td>
<td>Hypothalamic amenorrhea</td>
<td>20.9</td>
<td>1327</td>
<td>1</td>
<td>13.3</td>
<td>66.5</td>
<td>Received a second dose of GnRHα 12 h apart due to lack of surge. No oocytes retrieved</td>
<td>Hypothalamic amenorrhea</td>
</tr>
</tbody>
</table>

* Included in the estimation of the rate of empty follicle syndrome, but not in analysis of predictive factors of oocyte yield.

* Included in both the estimation of the rate of empty follicle syndrome and the analysis of predictive factors of oocyte yield.

Table II Factors predicting oocyte yield using multiple linear regression analysis for ‘total number of oocytes’ and ‘number of mature oocytes’ as well as generalized linear model with logit link for ‘proportion of mature oocytes’.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Total number of Oocytes</th>
<th>Number of mature oocytes</th>
<th>Proportion of mature Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard coefficient β</td>
<td>P-value</td>
<td>Standard coefficient β</td>
</tr>
<tr>
<td>Age</td>
<td>-0.391</td>
<td>&lt;0.001</td>
<td>-0.142</td>
</tr>
<tr>
<td>BMI</td>
<td>0.011</td>
<td>0.783</td>
<td>-0.039</td>
</tr>
<tr>
<td>Peak E₂</td>
<td>0.200</td>
<td>&lt;0.001</td>
<td>0.151</td>
</tr>
<tr>
<td>Post-trigger LH</td>
<td>0.198</td>
<td>&lt;0.001</td>
<td>0.168</td>
</tr>
<tr>
<td>Post-trigger Progesterone</td>
<td>0.500</td>
<td>&lt;0.001</td>
<td>0.484</td>
</tr>
<tr>
<td>LH rise</td>
<td>0.090</td>
<td>0.025</td>
<td>0.091</td>
</tr>
</tbody>
</table>
obtained in the study, firm conclusions cannot be made using the ROC curve. This finding prompted us to dichotomize post-trigger LH values into two groups (<15 and ≥15 IU/l). These groups were compared with respect to their relationship to oocyte yield and hormone characteristics (Table III). Women with a post-trigger LH value ≥15 IU/l had significantly more total oocytes and mature oocytes retrieved.

### Table III Cycle characteristics based on post-trigger LH using independent t-test and Fisher’s exact test where appropriate.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LH (IU/l)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;15 (n = 16)</td>
<td>≥15 (n = 486)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.3 ± 6.2</td>
<td>31.7 ± 4.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 5.8</td>
<td>26.1 ± 5.8</td>
</tr>
<tr>
<td>Total oocytes</td>
<td>16.3 ± 10.7</td>
<td>26.1 ± 11.1</td>
</tr>
<tr>
<td>Empty follicle syndrome (%)</td>
<td>3/16 (18.8)</td>
<td>0/486 (0)</td>
</tr>
<tr>
<td>Mature oocytes</td>
<td>13.1 ± 9.7</td>
<td>19.8 ± 8.9</td>
</tr>
<tr>
<td>Proportion of mature oocytes (%)</td>
<td>78 ± 28.2</td>
<td>76.2 ± 14.9</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>71.9 ± 14.4</td>
<td>73.1 ± 17.7</td>
</tr>
<tr>
<td>Peak E2 (pg/ml)</td>
<td>2564 ± 1257</td>
<td>3242 ± 1233</td>
</tr>
<tr>
<td>LH day of trigger (IU/l)</td>
<td>1 ± 1.4</td>
<td>2.1 ± 1.9</td>
</tr>
<tr>
<td>E2 day after trigger (pg/ml)</td>
<td>3650 ± 1599</td>
<td>4143 ± 1616</td>
</tr>
<tr>
<td>LH day after trigger (IU/l)</td>
<td>12.2 ± 2.1</td>
<td>60.6 ± 35.8</td>
</tr>
<tr>
<td>Progesterone day after trigger (ng/ml)</td>
<td>7.9 ± 3.6</td>
<td>9.1 ± 5.2</td>
</tr>
<tr>
<td>LH rise</td>
<td>42.4 ± 45.4</td>
<td>65.3 ± 84.3</td>
</tr>
</tbody>
</table>

*NA—P-value not calculated since the groups were based on post-trigger LH cut-off.

### Discussion

The findings of this study demonstrate that the magnitude of LH increase and post-trigger LH and progesterone are independent predictors of the number of total oocytes and mature oocytes retrieved. Moreover, post-trigger progesterone was the strongest predictor of oocyte yield. Although there was no post-trigger LH or progesterone cut-off for predicting the oocyte yield, all cases of EFS occurred in patients with post-trigger LH <15 IU/l and progesterone ≤3.5 ng/ml.

GnRHa displaces GnRH antagonists from pituitary receptors and results in a flare in both LH and FSH (Itskovitz-Eldor et al., 2000; Fauser et al., 2002). The physiological LH surge lasts ≏48 h (Hoff et al., 1983), which is in contrast to the GnRHa-induced endogenous LH surge, which lasts ≏24–36 h (Itskovitz et al., 1991; Fauser et al., 2002). These differences may have an effect on periovulatory events (Seibel et al., 1982; Chandrasekher et al., 1991; Zelinski-Wooten et al., 1991, 1992).

Several studies have shown that the GnRHa trigger is effective in inducing oocyte maturation and the number of oocytes and mature oocytes retrieved are similar to that of the hCG trigger in autologous (Fauser et al., 2002; Kolibianakis et al., 2005; Engmann et al., 2008) and oocyte donor (Acevedo et al., 2006; Galindo et al., 2009; Melo et al., 2009; Sismanoglu et al., 2009) cycles. However, EFS may occur after a GnRHa trigger and it was reported in 0.6% of patients who underwent oocyte retrieval and 1.4% of all patients in this study, which is similar to that reported after an hCG trigger of 0.1–2% (Ben-Shlomo et al., 1991; Zegers-Hochschild et al., 1995; Quintans et al., 1998; Baum et al., 2011; Mesen et al., 2011). This is therefore reassuring and implies that EFS is not an inherent and exclusive problem to the

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**Figure 1** LOESS curves showing that there is no post-trigger LH (a) and progesterone (b) that will predict total number of oocytes. The three cycles where no oocytes were retrieved can be seen on the bottom left of each figure. (P, progesterone; LOESS, locally weighted scatterplot smoothing).
Predicting oocyte yield after GnRHa trigger

GnRHa trigger. However, our reported incidence of EFS appears to be slightly lower than the 3.5% recently reported by Castillo et al. (2012) after a GnRHa trigger. This may be due to the fact that serum LH and progesterone were not measured after the trigger to identify cases of suboptimal LH surge and also because they reported the true existence of EFS since no oocyte retrievals were canceled and no rescue hCG was administered.

The exact etiology of EFS is not clearly understood; however, several theories have been proposed to explain this anomaly after an hCG trigger (Ben-Shlomo et al., 1991; Zegers-Hochschild et al., 1995; Quintans et al., 1998; Baum et al., 2011; Mesen et al., 2011). Genuine EFS (GEFS) and false EFS (FEFS) have been proposed as the two main mechanisms to explain failure to retrieve oocytes after an hCG trigger (Stevenson and Lashen, 2008) and are applicable to the GnRHa triggers; although some differences may exist because the mechanisms of action are not similar. Moreover, differences between the physiological LH surge and endogenous LH surge after the GnRHa trigger described above (Hoff et al., 1983; Itskovitz et al., 1991; Zegers-Hochschild et al., 1991; Zelinski-Wooten et al., 1996; Stavrou et al., 1998) may result in GEFS. It has also been suggested that GEFS may be secondary to abnormal folliculogenesis and early oocyte atresia (Stevenson and Lashen, 2008), although there is no strong scientific evidence to support this. None of the patients in our study fulfilled the criteria for GEFS.

On the contrary, FEFS occurs after a failure of induction of optimal endogenous LH surge and/or progesterone rise after the GnRHa trigger. FEFS accounted for all the cases of EFS in this study and is likely to be amenable to a rescue hCG protocol. The potential reasons may include, among others, human error (Quintans et al., 1998), abnormality in the in vivo biological activity of some batches of commercially available GnRHa (Zegers-Hochschild et al., 1995), hypothalamic dysfunction or GnRH receptor mutations (Chevrier et al., 2011). It is logical that inappropriate drug administration as a result of human error would result in a suboptimal LH surge. The use of a 2.8 ml multiple dose vial containing 5 mg/ml of leuprolide acetate may prevent this error. An improperly compounded aliquot of GnRHa may result in abnormalities of the in vivo biological activity of some batches of commercially available GnRHa, accounting for a lack of adequate flare effect of the medication. Two of the donors reported in Table 1 had either previously or subsequently used the GnRHa trigger with an optimal LH surge and oocyte yield, which is highly suggestive of human error in administration or abnormalities in the in vivo biological activity of some batches of medications used.

It may also be postulated that a high BMI and excess subcutaneous tissue may prevent adequate serum absorption of the medication, resulting in lack of effectiveness. Although we showed that the higher the BMI, the lower is the LH rise and post-trigger progesterone BMI does not independently predict oocyte yield and the number of mature oocytes. Moreover, it has only a very weak independent negative prediction of the proportion of mature oocytes. Therefore, based on these findings, one cannot propose a higher dose of GnRHa for patients with a high BMI.

Patients with hypothalamic dysfunction are not appropriate candidates for a GnRHa trigger since this method of ovulation induction relies upon a functional pituitary–ovarian axis. In hypogonadotrophic hypogonadism individuals, the pituitary gland may be suppressed from lack of endogenous GnRH stimulation and thus may not be able to respond to GnRHa administration. This is clearly demonstrated in this study when a patient with hypothalamic amenorrhea was inadvertently given GnRHa for trigger, resulting in a suboptimal flare effect and no oocytes retrieved. It is also conceivable that patients with undiagnosed subtle hypothalamic dysfunction may not respond to a GnRHa trigger. Moreover, patients with GnRH receptor mutations (Chevrier et al., 2011) may not respond appropriately to GnRHa administration resulting in a suboptimal endogenous LH surge.

An adequate time interval between the last dose of GnRHa antagonist administration and the GnRHa trigger may ensure optimal endogenous LH surge since the GnRHa antagonist acts by competitive inhibition at the GnRHa receptor sites (Chillik et al., 1987; Felberbaum et al., 1995). Fauser et al. (2002) have previously shown that GnRHa administered 12 h after the last antagonist injection is sufficient to displace the GnRHa antagonist from the receptor sites and results in an optimal LH surge, which is therefore our standard practice. Finally, although LH-β subunit polymorphisms may be associated with a less bioactive LH molecule (Altmae et al., 2011) resulting in EFS, studies have suggested that patients with such aberrations are usually hypo-responders to ovarian stimulation (Alviggi et al., 2011) rather than hyper-responders, which is the population that requires a GnRHa trigger.

The ability to predict EFS or suboptimal oocyte yield is useful clinically to determine when to proceed with or cancel the oocyte retrieval after the GnRHa trigger. Unfortunately, we could not identify a cut-off post-trigger LH or progesterone that will be useful clinically in predicting the oocyte yield. This is contrary to the study by Shapiro et al. (2011) who showed that a low oocyte yield and maturity corresponded to an LH surge 12 h after the GnRHa trigger (LH,12) of <52 IU/l. However, such a cut-off may not be useful clinically since it was derived by a statistical calculation. There are several reasons to explain the lack of a cut-off post-trigger LH to predict the oocyte yield. The threshold amplitude of the LH surge required to initiate oocyte maturation in a natural cycle is not known (Shoham et al., 1995), although it has been shown in rats that only 5% of the normal LH surge amplitude is necessary for oocyte maturation (Peluso, 1990). There is also variability in the amplitude and length of the LH curve as 12% of normal ovulatory women have peak LH levels of <20 IU/l, while 23% will have peak values of 21–40 IU/l (Arici et al., 1992). Moreover, our study was retrospective in design. Thus, the LH measurements were performed at different time intervals ranging between 8 and 12 h and not at a standardized time interval following the GnRHa trigger. Moreover, a single time point estimation of serum LH may not be useful since the duration of the LH surge is a better predictor of oocyte maturation (Chandrasekher et al., 1991; Zelinski-Wooten et al., 1991, 1992), although the measurement of the LH duration is not clinically practical.

Although there was no LH cut-off for predicting the oocyte yield in this study, we showed that the probability of EFS in patients with post-
trigger LH of <15 IU/ml is 18.8% and all the cases of EFS occurred in this group. This post-trigger LH cut-off has sensitivity of 97.4% and specificity of 100% for predicting EFS. Although these estimates were based on only three patients having the event, it is unlikely that EFS will occur in patients with post-trigger LH levels ≥15 IU/ml. This finding is consistent with results from a previous prospective study, which showed that a post-trigger LH of <15 IU/ml was associated with a lower oocyte yield (Chen et al., 2012). This is useful clinically for counseling patients prior to oocyte retrieval. As shown in this study, a rescue hCG protocol could be administered in patients exhibiting no LH rise that could result in successful retrieval of oocytes. Moreover, the retrieval can be terminated if no eggs are obtained from one ovary in patients with a suboptimal LH surge and a rescue dose of hCG given followed by a second retrieval 35 h later with successful retrieval of oocytes, as shown in this study as well as others (Honnma et al., 2011). One should however, caution that there is a potential risk that the use of a rescue hCG protocol may result in a reduced chance of conception due to a premature progesterone rise or may result in a significant risk of OHSS.

This is the first study to evaluate the value of both post-trigger LH and progesterone in predicting oocyte yield. Although there is no post-trigger LH or progesterone cut-off to predict the number of oocytes retrieved, all patients with EFS had low serum LH levels of <15 IU/l and progesterone levels of <3.5 ng/ml. The measurement of post GnRHa trigger serum LH and progesterone is therefore useful in counseling patients prior to oocyte retrieval regarding the likelihood of EFS and also in deciding appropriate timing of rescue hCG.

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Authors’ roles

N.E.K.: participation in study design, data acquisition and interpretation, data analysis, manuscript preparation and final approval of the version to be published. R.S.F.: analysis and interpretation of data; revision of manuscript and final approval of the version to be published. D.W.G.: data acquisition and interpretation; revision of manuscript and final approval of the version to be published. J.C.N.: participation in study design, and interpretation of data; revision of manuscript and final approval of the version to be published. C.A.B.: participation in conception, design and acquisition of data; analysis and interpretation of data; drafting and revision of manuscript and final approval of the version to be published.

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