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ORIGINAL ARTICLE

Risk factors for empty follicle syndrome in assisted reproductive technology with gonadotropin-releasing hormone agonist trigger

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Abstract

Purpose: To analyze whether response to the GnRH test is a predictor of empty follicle syndrome (EFS) and to analyze independent risk factors for EFS.

Methods: The GnRH test results of 3765 patients from 2016 to 2018 were used to define the reference range of the GnRH test. Risk factors for EFS were estimated by multivariate logistic analysis of 5282 cycles (5247 oocyte-retrieved cycles with GnRH agonist trigger and 35 cycles of EFS) conducted from 2016 to 2019.

Results: GnRH testing showed basal hormone values as follows: median LH 5.2 (95 percentile; 1.3–12.6) mIU/mL, LH 30 min 22.0 (6.8–57.1), basal FSH 7.3 (3.0–20.5), FSH 30 min 11.5 (5.1–30.4) and FSH/LH ratio 1.5 (0.6–4.1). Independent risk factors for EFS were antral follicle count (adjusted odds ratio; 0.94, 95% CI; 0.89–0.99), basal LH (0.78, 0.66–0.90), and days duration of ovarian stimulation (1.41, 1.21–1. 60). The respective thresholds were 8 for AFC, 5.0 for basal LH, and 16 days for duration.

Conclusions: LH 30 min values of the GnRH test did not predict EFS. Independent risk factors for EFS were AFC, basal LH and days duration of ovarian stimulation.

KEYWORDS
empty follicle syndrome, gonadotropin-releasing hormone agonist, LH-RH, luteinizing hormone, risk factor

1 | INTRODUCTION

Empty follicle syndrome (EFS) is a condition where no eggs are collected after proper ovarian stimulation, despite multiple rounds of normal follicular development and elevated estradiol.[1] This syndrome can be caused by a gonadotropin-releasing hormone (GnRH)-agonist trigger or a human chorionic gonadotropin (hCG) trigger,[2] and the incidence of EFS is comparable between these.[3] A combination of controlled ovarian stimulation with the GnRH antagonist protocol and final oocyte maturation trigger with a GnRH agonist is recommended for the prevention of ovarian hyperstimulation syndrome (OHSS).[4,5] Prediction of EFS after GnRH agonist triggering would enable us to select the double (dual) trigger with GnRH agonist or the hCG trigger alone in advance, considering the physical and emotional burden on the patients.

However, little is known about the etiology of EFS. False EFS, that is, insufficient serum levels of hCG or luteinizing hormone (LH), where ovarian stimulation malfunctions, is presumed to stem from

medication issues, such as errors with medication dosages or methods, or technical issues, including drug production errors. Genuine EFS, in turn, is where no egg collection is possible despite the proper stimulation of ovulation and is thought to involve genetic issues, follicular developmental disorders, or dismissed or decreased ovarian reserve. However, it is often difficult to predict the onset of EFS from the patient's background before COS is started.[6] On this subject, prior research has shown that the LH level at the start of COS is an independent predictive factor for suboptimal egg collection after GnRH agonist triggering.[7]

The LH level at the start of COS is thus a candidate predictive factor for suboptimal egg collection or EFS. There are no reports, however, on whether the various hormones measured during routine screening for first-visit patients before treatment with COS could be predictive factors for EFS. We, therefore, studied whether the results of GnRH tests performed routinely at our hospital could be used to predict the response to ovarian stimulation with a GnRH agonist in COS and, in particular, whether they could be risk factors for EFS.

The purpose of the present study was to study whether results from GnRH tests, as part of the screening performed from the first visit up until the start of treatment, are useful for predicting egg collection and thus to show whether basal LH levels are a risk factor for EFS. These would allow the assessment of the usefulness of GnRH tests. If the GnRH test could be used to predict the onset of EFS, it would be possible to select the type and dose of oocyte maturation trigger in advance and to decide the optimal egg retrieval time for avoiding severe OHSS.

2 | MATERIALS AND METHODS

2.1 | Study design

The present study was a retrospective case–control study by chart review.

2.2 | Subjects

2.2.1 | Empty follicle syndrome group

The subjects were 35 patients (35 cycles) with EFS that occurred between January 2016 and December 2019. All subjects received COS with the GnRH antagonist protocol, with egg retrieval by a GnRH agonist trigger; cases where "egg collection was zero despite ample follicular development and elevated E2 levels" were diagnosed as EFS.

2.2.2 | Control group

The control group for this retrospective study comprised cases that satisfied the following criteria: (1) no missing values for any measurement items; (2) one or more successful egg collections with the GnRH agonist trigger; and (3) egg retrieval performed with the GnRH antagonist protocol between January 2016 and December 2019. This group contains 5247 people (5008 cycles).

2.2.3 | GnRH test

We obtained hormone data from GnRH tests conducted on menstrual cycle days (CD) 2–5 from first-visit patients with a chief complaint of infertility at our hospital between January 2016 and December 2018. These results were used to set reference ranges for stratification for statistical analysis. The stimulation was an intravenous injection of 0.1 mg of gonadorelin acetate (Nipro ES Pharma, Japan). The test data measured were basal LH, LH 30 min (levels 30 min after stimulation), basal follicle-stimulating hormone (FSH), and FSH 30 min (levels 30 min after stimulation); the basal FSH/basal LH ratio was also calculated.

2.2.4 | Treatment protocol

COS was performed from CD 2–5 using recombinant FSH (Gonalef; Merck Serono, Tokyo, Japan) 150–450 IU/person or human menopausal gonadotropin (hMG) 150–450 IU/person (Ferring Pharmaceuticals, Tokyo, Japan or ASKA Pharmaceutical, Tokyo, Japan). Ganirelix (Ganirest; MSD, Tokyo, Japan; 0.25 mg) or cetrorelix (Cetrotide; Merck Serono, Tokyo, Japan; 0.25 mg) was used as the GnRH antagonist and was administered once the dominant follicle had reached 14–16 mm in maximum diameter or when a premature LH surge was suspected. The final maturation of oocytes was induced when the dominant follicle diameter was 18 mm or larger, when at least 13 days had passed since the start of the COS, and when it was deemed possible to collect a mature egg based on the follicle diameter, hormone levels, etc. At 34–36 h after a nasal administration of 300 μg × 4 of buserelin acetate (Buserecur; Fuji Pharma, Tokyo, Japan) or subcutaneous administration of 1 mg of leuprolide acetate (Lucrin; AbbVie, IL, USA), a sedative and analgesic were administered to the patient, following which follicular fluid was suctioned with an ultrasound-guided transvaginal needle, and collection of the oocyte was performed. If no oocytes were retrieved, the follicle was flushed 3–5 times with buffer ranging from the same volume as the follicle to 2 mL. Where the risk of OHSS was deemed to be low, final oocyte maturation served as the hCG or dual trigger (hCG and GnRH agonist), depending on the follicular diameter, serum E2, patient age, and AMH.

The retrieved oocytes were fertilized by conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection. Split insemination was selected in cases with ≤6 metaphase II (M2) oocytes or oligozoospermia (defined as sperm concentrations postswim-up $\leq 10 \times 10^6$/mL). For a fertilized egg, embryo transfer was not performed in the same cycle to prevent OHSS. In principle, cryopreservation was to be performed at the pronuclear stage in all cases. However, where a certain number of fertilized eggs could not be

obtained, 4–12 were cryopreserved at the pronuclear stage, taking the patient's age into consideration, and the remainder were used for blastocyst culture. All embryo transfers were frozen–thawed embryo transfers in the hormone replacement cycle in the endometrial preparation protocol at a different cycle than the egg retrieval cycle.

2.2.5 | Exclusion criteria

Patients who underwent egg retrieval with hCG alone or the hCG + GnRH agonist dual (double) trigger and patients who had irregular menstrual cycles (<25 or >39 days cycle) or taking oral contraceptives within 6 months were excluded from the present study.

2.2.6 | Hormonal assay

Serum estradiol (E2) (pg/mL), serum FSH (mIU/mL), serum LH (mIU/mL), serum progesterone (P4) (pg/mL), and serum anti-Müllerian hormone (AMH) levels were measured via an automated electrochemiluminescence immunoassay (ECLIA) that was performed in-hospital using the Cobas e 411 Analyzer (Roche Diagnosis K. K., Tokyo, Japan).

2.3 | Statistical analysis

All statistical analyses were performed with EZR (Jichi Medical University, Saitama Medical Center),[8] which is based on R (R Foundation for Statistical Computing, Austria Vienna). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

2.4 | Data notation

The Kolmogorov–Smirnov test was performed for all data. Variables with $p \geq 0.05$ according to this test were considered to be normally distributed and are represented by the mean ± SD, while nonnormally distributed variables are presented as the median (25th–75th percentile). However, the 95th percentile was used only when the reference range of the GnRH test was set.

2.5 | Reference range of GnRH test

Ninety-five percentile of reference range was set for basal LH, LH 30 min, basal FSH, FSH 30 min, and basal FSH/basal LH ratio, and these were used as indices for comparing outcomes.

The correlation between LH levels at the start of stimulation and the basal LH levels in cases that underwent the antagonist protocol was tested with Spearman's rank correlation coefficient.

2.6 | Univariate analysis

The variables included in the univariate analysis were variables that were needed in principle for treatment and were routinely collected, with data obtained without an additional need for research. These were age, BMI, antral follicle count (AFC) (*n*) (total number of antral follicles measuring 2–10 mm in both ovaries), AMH, GnRH test (basal LH, LH 30 min, basal FSH, FSH 30 min, basal FSH/LH ratio) values, hormone values at CD 2–5 (serum E2, serum FSH, serum LH), duration of COS, and hormone values on the day of triggering (serum E2, serum FSH, serum LH, serum P4).

These items were included in the univariate logistic analysis [generalized linear model (GLM)] as a reference to identify the factors influencing the onset of EFS.

2.7 | Multivariate logistic regression analysis

Predictors were selected from items that were candidate risk factors for EFS based on the univariate logistic analysis; the 95% CI, *p* value, and area under the receiver operating characteristic (ROC) curve (AUC) were consulted. Variable selection was employed because of the diversity of items being measured. In this multivariate analysis, a two-tailed test was performed, with the level of statistical significance set to $p < 0.05$.

For EFS, there were no duplicate cases among the 35 cases, so no observed values were excluded, and all of the data were included. Multicollinearity was diagnosed by variance inflation factors (VIFs) >5, and conformity was assessed with the ROC curve.

3 | RESULTS

3.1 | Empty follicle syndrome versus control (patients with oocyte retrieval)

Table 1 presents each parameter measured for the EFS ($n = 35$) and control (patients with oocyte retrieval) ($n = 5247$) groups. Comparisons were made with the Mann–Whitney U test.

There was no significant difference for age, BMI, or AMH; only AFC [EFS 8.0 (4.0–9.0) vs. control 13.0 (9.0–18.0), $p < 0.001$] exhibited a significant difference.

3.2 | Stimulation test

3.2.1 | GnRH test

Significant differences were found for basal LH [2.7 (1.8–4.3) vs. 5.3 (3.6–7.2), $p < 0.001$], basal FSH [6.5 (4.3–7.8) vs. 7.0 (5.9–8.3), $p = 0.005$], and basal FSH/basal LH ratio [2.08 (1.50–3.16) vs. 1.33 (0.99–1.92), $p < 0.001$].

TABLE 1 Comparison between the empty follicle syndrome group and control group.

	Empty follicle syndrome ($n=35$)	Oocyte-retrieved patients ($n=5247$)	p value[a]
Age (y)	34.0 (33.0–38.0)	35.0 (32.0–38.0)	0.83
BMI (kg/m^2)	19.6 (19.0–21.9)	20.3 (19.1–22.2)	0.44
AMH (ng/mL)	4.0 (2.9–5.2)	3.4 (2.4–5.3)	0.27
Antral follicle count (n)	8.0 (4.0–9.0)	13.0 (9.0–18.0)	<0.001
GnRH test			
Basal LH (mIU/mL)	2.7 (1.8–4.3)	5.3 (3.6–7.2)	<0.001
LH 30 min. (mIU/mL)	22.4 (14.6–27.8)	24.8 (19.4–30.4)	0.19
Basal FSH (mIU/mL)	6.5 (4.3–7.8)	7.0 (5.9–8.3)	0.005
FSH 30 min (mIU/mL)	10.0 (7.1–12.5)	10.7 (9.0–12.7)	0.14
Basal FSH/basal LH	2.08 (1.50–3.16)	1.33 (0.99–1.92)	<0.001
Serum hormone concentration at the day of start			
E2 (pg/mL)	17.5(5.0–25.0)	25.0 (25.0–27.6)	<0.001
FSH (mIU/mL)	5.4 (1.2–8.1)	7.2 (6.6–7.8)	<0.001
LH (mIU/mL)	2.4 (1.2–6.1)	6.3 (5.0–7.8)	<0.001
Duration of controlled ovarian stimulation (days)	16.5 (15.0–18.0)	15.0 (14.0–16.0)	<0.001
Serum hormone concentration on the day of trigger			
E2 (pg/mL)	15 809 (10672–20 860)	13 287 (9808–17 749)	0.12
FSH (mIU/mL)	26.6 (21.8–28.8)	23.8 (23.0–24.6)	0.01
LH (mIU/mL)	0.2 (0.1–0.6)	0.7 (0.6–0.7)	<0.001
P4 (ng/mL)	4.4 (2.6–5.8)	3.2 (2.2–4.7)	0.03
No. of retrieved oocytes (A) (n)	0	20 (14–28)	n/a
No. of follicles (≧5 mm-) (B) (n)	30 (19–47)	28 (21–36)	0.12
Proportion of retrieved oocytes (A/B) (%)	0	73.9 (59.1–91.7)	n/a
No. of matured follicles (≧14 mm-) (C) (n)	19 (14–25)	18 (14–24)	0.48
Proportion of retrieved oocytes (A/C) (%)	0	110.0 (85.7–138.9)	n/a

Note: Data notation; median (IQR).

Abbreviations: AMH, anti-Müllerian hormone; n/a, not applicable.

[a]Mann–Whitney U test.

3.2.2 | Hormone levels at start of stimulation

Significant differences were observed for E2 at the start of stimulation [17.5 (5.0–25.0) vs. 25.0 (25.0–27.6), $p<0.001$], FSH at the start of stimulation [5.4 (1.2–8.1) vs. 7.2 (6.6–7.8), $p<0.001$], and LH at the start of stimulation [2.4 (1.2–6.1), 6.3 (5.0–7.8), $p<0.001$].

3.2.3 | Duration of stimulation

The duration of stimulation was significantly greater in the EFS group [16.5 (15.0–18.0) vs. 15.0 (14.0–16.0), $p<0.001$].

3.2.4 | Hormone levels at triggering (at first trigger for EFS)

There was no significant difference for peak E2; significant differences were observed for FSH levels [26.6 (21.8–28.8), $p=0.01$], LH levels [0.2 (0.1–0.6) vs. 0.7 (0.6–0.7), $p<0.001$], and P4 levels [4.4 (2.6–5.8) vs. 3.2 (2.2–4.7), $p=0.03$].

3.2.5 | Number of collected eggs

There were no significant differences observed between the number of follicles ≥5 mm and the number of follicles ≥14 mm on ultrasonography before egg retrieval; also, the number of eggs actually collected and the proportion collected in advance ultrasonography were not significantly different.

3.2.6 | Reference ranges and stratifications

Reference ranges based on the results of the GnRH test.

GnRH tests were performed on 3767 individuals with a mean age of 36.0 (32.0–39.0) years and BMI of 21.5 (18.0–40.0) kg/m^2. The Kolmogorov–Smirnov test was applied to all data, with results

showing a nonnormal distribution, so reference ranges were set with 95th percentile ranges as follows: basal LH 5.2 (1.5–12.6), LH 30 min 22.0 (7.0–56.3) (mIU/mL), basal FSH 7.3 (3.0–20.5), FSH 30 min 16.5 (5.1–30.4) (mIU/mL), and basal FSH/basal LH ratio 1.5 (0.6–4.1) (Table 2).

3.2.7 | Stratification by basal LH

The respective numbers of collected eggs were determined by dividing basal LH levels into three categories in accordance with the reference range obtained from the results of the GnRH test: Below (1.5), within (1.5–12.6), and above (12.6 < mIU/mL) the reference range. The number of collected eggs (with cycles) for each basal LH level was 16.0 (12.0–26.0) (99 cycles), 15.0 (10.0–24.0) (5070 cycles), and 10.5 (7.0–18.3) (78 cycles), respectively (Kruskal–Wallis test, $p < 0.001$); significant differences were found between below and above the reference range and between within and above the reference range with Bonferroni correction (Figure 1A)

3.2.8 | Stratification by LH 30 min

The respective numbers of retrieved eggs were determined by dividing LH 30 min levels into 3 categories (<7.0, 7.0–56.3, 56.3<, mIU/mL), according to the reference range obtained from the results of the GnRH test. The number of collected eggs (with cycles) for each LH 30 min level was 17.0 (12.0–24.0) (110 cycles), 15.0 (10.0–24.0) (5033 cycles), and 12.0 (9.0–19.8) (104 cycles), respectively (Kruskal–Wallis test, $p = 0.11$). There was no significant difference among LH 30 min levels with Bonferroni correction (Figure 1B).

TABLE 2 Descriptive statistics for LH and FSH before and after GnRH stimulations.

	GnRH test
	n = 3765
Age (y)	36.0 (32.0–39.0)[a]
BMI (kg/m^2)	21.5 (18.0–40.0)[a]
Basal LH (mIU/mL)	5.2 (1.3–12.6)[b]
LH 30 min. (mIU/mL)	22.0 (6.8–57.1)[b]
Basal FSH (mIU/mL)	7.3 (3.0–20.5)[b]
FSH 30 min. (mIU/mL)	11.5 (5.1–30.4)[b]
Basal FSH/basal LH ratio	1.5(0.6–4.1)[b]

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

[a]Results are described as the median (IQR: interquartile range).

[b]Results were described as 95%tile (0.025–0.975), respectively.

3.2.9 | Stratification by basal FSH/basal LH ratio

The respective numbers of collected eggs were determined by dividing values of the basal FSH/basal LH ratio into 3 categories (FSH/LH <0.6, 0.6–4.1, 4.1<), according to the reference range obtained from the results of the GnRH test. The Mann–Whitney U test comparing all 3698 cases (5247 cycles) with FSH/LH <0.6, 0.6–4.1, 4.1 < resulted in egg numbers of 13.0 (8.0–25.0), 15.0 (10.0–24.0), and 15.5 (11.0–24.0), respectively (not significant, $p = 0.41$) (Figure 1C).

3.2.10 | Correlation of basal LH and LH at the start of ovarian stimulation

The correlation between basal LH and LH at the start of ovarian stimulation was $\rho = 0.49$ ($p < 0.001$) according to Spearman's correlation test (Figure 2).

3.2.11 | Univariate analysis

To identify the risk factors for EFS from the EFS and control groups, univariate logistic regression analysis was performed on observation factors, without any modification of interaction and confounding factors. The results are presented in Table 3. The results showed that possible risk factors for EFS were AFC (odds ratio [OR] 0.91, 95% CI 0.87–0.97, AUC = 0.71), basal LH (0.72, 0.61–0.81, <0.001, 0.79), E2 at the start of ovarian stimulation (0.95, 0.93–0.97, <0.001, 0.70), LH at the start of ovarian stimulation (0.48, 0.41–0.59, <0.001, 0.77), and duration of COS (1.46, 1.29–1.62, <0.001, 0.75).

3.2.12 | Multivariate analysis

Significant factors from the univariate analysis were entered into multivariate analysis. The effects between factors were eliminated to identify independent risk factors for EFS. In the multivariate analysis, forced entry was used to select the factors. A basic model (Table 4) was created and included age, BMI, AFC, basal LH, and duration of COS. Table 4 presents the calculations of the adjusted OR (AOR) using the generalized linear model (GLM). Adjustment was performed by age, BMI, AFC, basal LH and duration of ovarian stimulation.

The results showed that AFC (AOR 0.94), basal LH (AOR 0.78), and duration of ovarian stimulation (AOR 1.41) were independent risk factors for EFS.

3.2.13 | ROC (AUC, specificity, sensitivity) and threshold value of each factor

The ROC curve of the model is presented in Figure 3. The logistic regression model had an AUC of 0.84 (95% CI 0.76–0.91). In terms

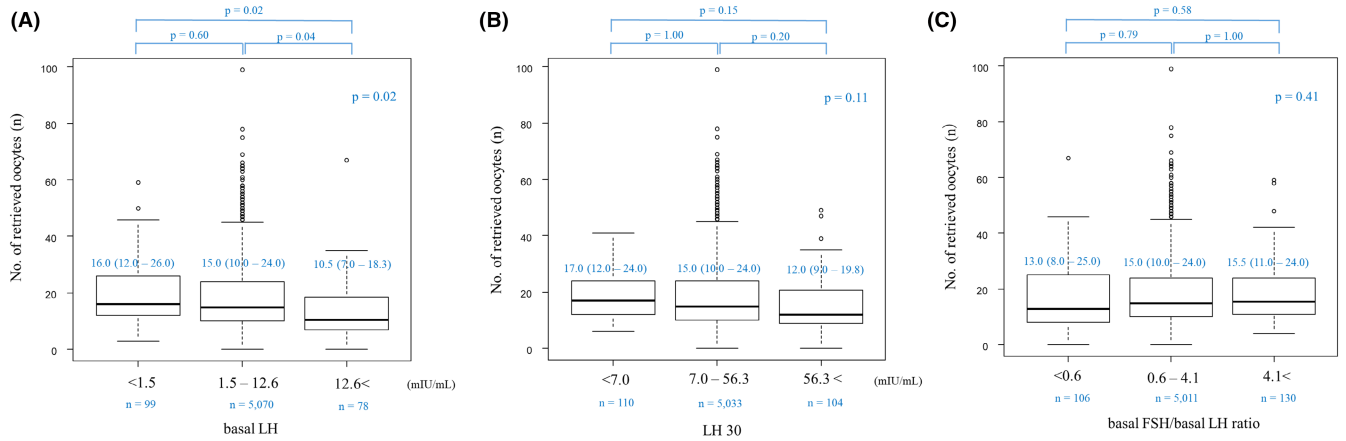

FIGURE 1 (A) Number of retrieved oocytes according to the basal LH categories. $p = 0.02$ (Kruskal–Wallis test). (B) Number of retrieved oocytes according to the LH 30 min categories. $p = 0.11$, not significant (Kruskal–Wallis test). (C) Number of retrieved oocytes according to the basal FSH/basal LH ratio categories. $p = 0.41$, not significant (Kruskal–Wallis test). FSH, follicle-stimulating hormone; LH, luteinizing hormone.

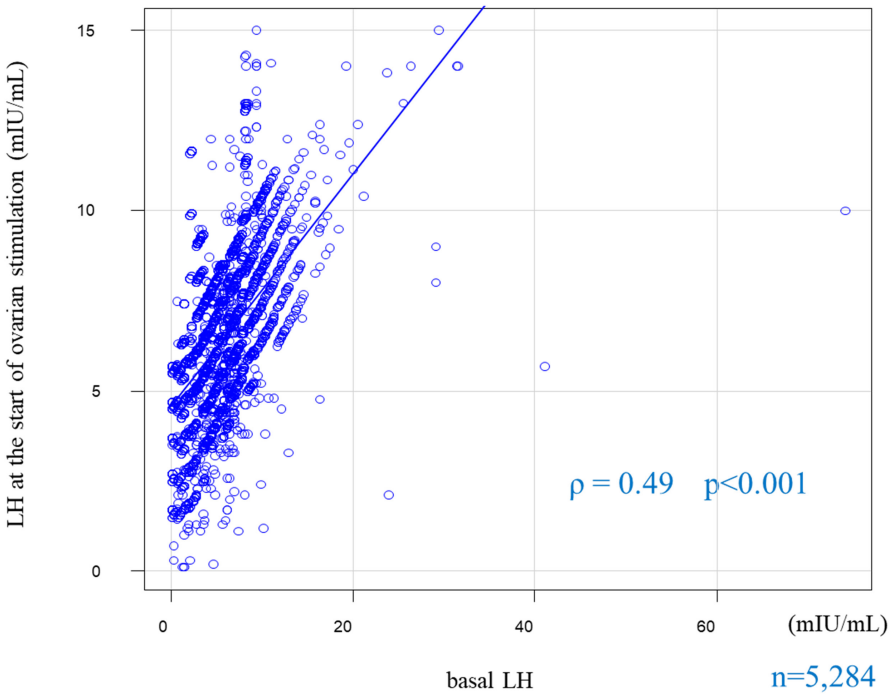

FIGURE 2 Correlation between basal LH and LH at the start of ovarian stimulation. $\rho = 0.49$, $p < 0.001$ (Spearman's rank correlation). LH, luteinizing hormone.

of threshold values and the sensitivity, specificity, and AUC regarding the risk factors for EFS, the threshold for AFC was 8 (sensitivity 0.622, specificity 0.788, AUC 072), basal LH was 5.0 mIU/mL (0.946, 0.538, 0.79), and the duration of COS was 16 days (0.722, 0.678, 0.75).

4 | DISCUSSION

In the present study, the reference range for basal LH was set to LH 1.5–12.6 mIU/mL, based on the results for the population of patients ($n = 3767$) that underwent the GnRH test at our institution. Applying the reference ranges to compare the number of collected eggs by category in the population ($n = 5282$) that underwent COS using the GnRH antagonist protocol and egg retrieval with the GnRH agonist trigger showed significant differences between below and above the reference range ($p = 0.02$) and between within and above the reference range ($p = 0.04$). The median number of eggs collected was 16, 15 and 10.5 for LH <1.5, 1.5–12.6, and 12.6 < mIU/mL, respectively; the oocyte yield decreased above the reference range ($p = 0.02$). The results from the multivariate risk factor analysis for EFS showed that basal LH levels, AFC, and duration of COS were independent risk factors for EFS.

A hormone stimulation test requires the collection of blood multiple times, which is disadvantageous in being costly and time-consuming and in imposing a major mental burden on the patient. It is, however, the gold standard for diagnosing precocious puberty in pediatrics[9] and is used in obstetrics and gynecology to diagnose the

TABLE 3 Univariate logistic regression analysis to identify the risk factors for empty follicle syndrome.

Variable	Odds ratio	95% CI	*p* value	AUC
Age (y)	1.01	0.93–1.09	0.84	0.49 (0.40–0.58)
BMI (kg/m^2)	0.99	0.87–1.11	0.73	0.54 (0.44–0.63)
AMH (ng/mL)	1.03	0.97–1.15	<0.001	0.53 (0.46–0.67)
AFC (*n*)	0.91	0.87–0.97	<0.001	0.71 (0.63–0.81)
GnRH test				
Basal LH (mIU/mL)	0.72	0.61–0.81	<0.001	0.79 (0.72–0.83)
LH 30 min. (mIU/mL)	1.00	0.96–1.01	<0.001	0.56 (0.47–0.66)
Basal FSH (mIU/mL)	0.75	0.63–0.87	<0.001	0.63 (0.51–0.74)
FSH 30 min. (mIU/mL)	0.97	0.89–1.04	<0.001	0.57 (0.47–0.68)
Basal FSH/basal LH	1.00	0.95–1.07	<0.001	0.71 (0.63–0.79)
Hormone value at the start of ovarian stimulation				
E2 (pg/mL)	0.95	0.93–0.97	<0.001	0.70 (0.59–0.81)
FSH (mIU/mL)	0.42	0.36–0.49	<0.001	0.66 (0.53–0.80)
LH (mIU/mL)	0.48	0.41–0.59	<0.001	0.77 (0.67–0.88)
Duration of COS (days)	1.46	1.29–1.62	<0.001	0.75 (0.66–0.81)
Hormone value on the day of trigger				
E2 (pg/mL)	1.01	1.00–1.00	<0.001	0.59(0.49–0.68)
FSH (mIU/mL)	1.21	1.13–1.28	<0.001	0.62 (0.48–0.77)
LH (mIU/mL)	0.53	0.18–1.47	<0.001	0.57 (0.46–0.68)
P4 (ng/mL)	1.08	1.00–1.19	<0.001	0.59 (0.51–0.70)

Abbreviations: AFC, antral follicle count; AMH, anti-müllerian hormone; AUC, area under the curve; BMI, body mass index; CI, confidence interval; COS, controlled ovarian stimulation; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P4, progesterone.

TABLE 4 Risk factors for empty follicle syndrome by multivariate logistic regression analysis.

	Vif	Estimate	SE	*z* value	Pr (>|z|)	AOR	95% CI
Intercept		−5.05	2.06	−2.45	0.01	0.01	0.001–0.37
Age	1.09	−0.06	0.05	−1.42	0.16	0.95	0.87–1.03
BMI	1.03	−0.06	0.06	−1.08	0.27	0.94	0.84–1.07
AFC	1.02	−0.07	0.04	−2.67	0.009	0.94	0.89–0.99
Basal LH	1,11	−0.27	0.08	−3,53	<0.001	0.78	0.66–0.90
Duration of ovarian stimulation	1.10	0.34	0.08	5.00	<0.001	1.41	1.21–1.60
						AUC = 0.84 (0.76–0.91)	

Abbreviations: AFC, antral follicle count; AOR, adjusted odds ratio; AUC, area under the curve; BMI, body mass index; CI, confidence interval; LH, luteinizing hormone; vif, variance inflation factor.

cause of amenorrhea as hypothalamic, pituitary, ovarian, or uterine. Thus, the hormone stimulation test is not essential but could be a useful examination for patient management. Apart from using the GnRH test to diagnose the cause of amenorrhea, we have attempted to use it to predict pituitary response to GnRH.

Reported parameters at the start of stimulation in COS, which are related to IVF outcomes, include LH levels,[7] FSH levels, and Day 3 FSH/LH ratio.[9,10] In the present study, however, we investigated whether results from screening all first-visit patients could be applied as predictive tools and investigated the usefulness of these results.

First, we investigated whether the basal LH level could be handled in the same manner as LH levels at the start of stimulation. Although the correlation between basal LH and LH at the start of stimulation was moderate (Spearman $\rho = 0.49$), it was significant ($p < 0.001$), suggesting that basal LH might be used as a predictive factor of IVF outcome, similar to LH levels at the start of stimulation.

The number of collected eggs decreased significantly with increased LH in this study. Regarding LH, it is essential to reach biologically optimal LH levels for the processes of follicular development, egg maturation, and ovulation. The concept of an LH window has been proposed, where the window is between a lowest value (LH

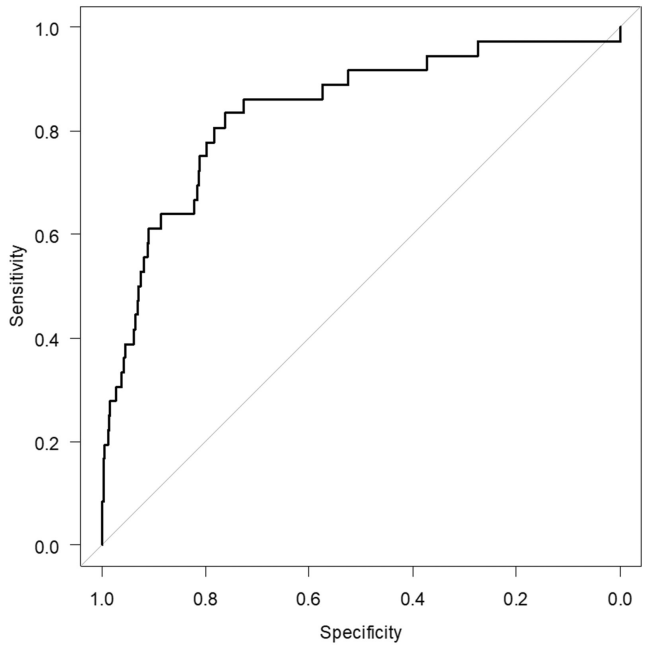

FIGURE 3 Receiver operating characteristic (ROC) curve of the multivariate logistic regression analysis. AUC = 0.84 (95% CI; 0.76–0.91). AUC, area under the ROC curve; CI, confidence interval.

threshold) and a highest value (LH ceiling) to allow for smooth progression through follicular development, granulosa cell and theca cell paracrine control, proliferation and functional differentiation of granulosa cells, follicle androgen/estrogen production, and follicle and egg maturation.[11,12] Levels less than the LH threshold (2.5 percentile in the present study; 1.5 mIU/mL) hinder granulosa cell and theca cell paracrine control, follicle androgen/estrogen production, and egg maturation, with the potential to substantially impair follicular development and ovulation. A representative example of this is hypothalamic malfunction. In the present study, EFS was often observed over this threshold (IQR of the basal LH level of the EFS population; 1.8–4.3 mIU/mL). Levels higher than the LH ceiling (97.5 percentile in the present study; 12.6 mIU/mL) result in reduced proliferation of granulosa cells, follicular atresia, early luteinization of the follicle, and impaired egg development. In the present study, the number of collected eggs in the higher LH group was significantly lower than that in the optimal LH subgroup. One reason for this inconsistency with the concept of the LH window may be that vesicular follicles in a high-LH environment outside the LH window express low levels of FSH receptors and are poorly responsive to FSH, and it has been suggested that later follicular development eventually ceases. In the present study, cases of antagonist with a poor response underwent egg retrieval with GnRH agonist and hCG dual (double) trigger or with hCG alone and, therefore, were among the excluded cases in the present study. For the LH 30 min values, there was no significant difference in the number of collected eggs between the underreference-range group and the within-reference-range group or between the within-reference-range group and the overreference-range group. Possible reasons for the lack of increase or decrease according to the stages of 30-minute levels include the fact that peak levels for LH 30 min have little direct relationship with GnRH agonist responsiveness and the fact that the overreference-range group for LH 30 min included PCOS cases[13] and cases with low ovarian reserve. Thus, the 30-minute levels for GnRH stimulation may not show the extent of the actual GnRH agonist trigger effect in parallel. In other words, although the GnRH test does not show a GnRH response to the pituitary gland, an effective GnRH agonist trigger and the ability to perform egg collection in a controlled ovarian cycle requires not only a pituitary response (elevated blood LH levels) but also complex relationships such as egg maturation, impaired cumulus cell development, increased apoptosis due to aging of the ovum, impaired follicle formation due to follicular atresia, defective granulosa cell function, strong binding of the cumulus cell complex to the follicular wall, or gene deficiency, such that GnRH stimulation at 30-minute levels may not be directly indicative of potency outside the pituitary gland. Thus, LH 30 min could not be a predictor of EFS. According to Itskovitz et al.,[14] an LH surge induced by a GnRH agonist lasts 24–36 h, with a short peak after 4 hours followed by a long decrease over 20 hours. Actual post-trigger hormone levels have been reported by Chang et al.,[15] who reported that after GnRH agonist triggering in 1878 cases, LH levels after 10.8 ± 2.0 h were 59.6 ± 36.9 mIU/mL. In other words, the GnRH 30 min does not reproduce the actual LH surge.

Regarding the discrepancy between basal FSH and FSH at the start of ovarian stimulation, it is known from previous findings that basal FSH values vary from cycle to cycle. In their report, Laszlo et al. state that the highest value of the two measurements is a more accurate predictor of poor response.[16] It has also been shown that the cycle with the highest basal FSH is a predictor of ovarian reserve, although the benefit has been described as limited.[17,18] Regarding the FSH/LH ratio, Seckin (2012) et al.[10] reported that an elevated Day 3 FSH/LH ratio (≥3) was useful for predicting the results of IVF in terms of egg collection volume and clinical pregnancy rate. Prasad et al.[11] made a similar report using an FSH/LH ratio ≥2 as the reference. In our results, creating the same categories with basal FSH/basal LH showed no significant change in egg yield as the basal FSH/basal LH ratio increased. Thus, the reference range was set to 0.6–4.1. A background where a higher FSH/LH ratio means a poorer response was described by Prasad et al., who described a strong correlation with LH levels, where a decrease in LH resulted in a higher ratio. Moreover, in other studies, an elevated ratio has been reported as being dependent not on a higher FSH but on a lower LH[19–21]; in the present study as well, basal FSH levels were lower in the EFS group than in the control group. In our research, an elevated FSH/LH ratio appeared to depend more on low LH levels than on high FSH.

In the present study, we identified AFC, basal LH, and duration of ovarian stimulation as independent risk factors for EFS (Table 4). Previously reported risk factors include advanced age, longer infertility duration, high baseline FSH, and lower E2 levels before hCG injection according to Baum et al.[22] The results of the present study indicated that LH levels had an AOR of 0.78 for event occurrence (EFS). High baseline FSH has also been identified as a risk factor for EFS in previous research,[23] but in our study, EFS instead involved

significantly lower basal LH values. One possible reason for the difference between previously published findings and those of the present study is as follows. Low ovarian reserve and high FSH levels are associated with an increased risk of EFS. However, the participants in this antagonist method study had high anti-mullerian hormone levels; thus, their ovarian reserve was conserved. These facts account for the abovementioned discrepancy. That is, in addition to hypothalamic–pituitary dysfunction, low basal LH levels appear to be a major risk factor, under the certain condition of maintenance of the ovarian reserve.

The AOR for event occurrence (EFS) was 0.94 for AFC and 1.41 for number of days of COS, meaning that the low AFC or long duration of stimulation might embody the risk of event occurrence. The AFC also reflects ovarian reserve, and it has been shown that a low AFC at the start of stimulation is associated with an increased risk of EFS. The long duration of stimulation increases the amounts of total gonadotropin/human menopausal gonadotrophin required until egg retrieval. This is why there is a risk of poor responses.

The present study has several limitations. First, it was a retrospective study. In particular, the subjects of the present study had an EFS probability of 0.7% (37/5284), making statistical analysis more likely to yield significant differences and possibly creating bias. There were also limitations in terms of screening. In this study, the results of the GnRH test lacked normality. In screening for healthy individuals, test data have normality, but our subjects underwent screening at an IVF clinic, making it impossible to exclude hypothalamic–pituitary disorders or PCOS. Thus, there is possibly a bias in the distribution of the population's data. Appropriate participants must be selected for the screening cohort; however, it proved impossible to completely exclude patients with characteristics such as PCOS. Furthermore, causes of infertility, for example, advanced age, endometriosis, and PCOS, affect development of EFS, but the infertility background has not been discussed in this study.

The other issue is external validity. At our clinic, patients who have maintained their ovarian reserve with low AMH and high FSH are not subject to the antagonist protocol approach from the beginning. In addition, although there was no significant difference between EFS and the control in E2 levels at the determination of the trigger, patients such as those with a lower peak E2 or poor response with E2 elevation, rather than the number of mature follicles, underwent egg retrieval with a dual (double) trigger, considering the risk of OHSS. The difference in design from previous research may potentially mean that the results of the present study cannot be applied to all populations.

Furthermore, the other limitation of this study lies in the fact that the oocyte maturation trigger involved using only the GnRH agonist following the above procedure, preventing OHSS, so it is not possible to strictly distinguish between genuine and false EFS.

In conclusion, LH 30 min values in the GnRH test alone did not predict EFS. This study failed to identify the predictor of EFS. However, it revealed that AFC, basal LH, and duration (days) of ovarian stimulation are independent risk factors for EFS. Each of the measurements prior to starting controlled ovarian stimulation may be factors that minimize the risk of EFS.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

This study was approved by the institutional review board of Asada Ladies Clinic and was conducted in accordance with the principles of the Helsinki Declaration of 1964. Informed consent was obtained from all patients before inclusion in the study.

ANIMAL STUDIES

This article does not contain any studies with human subjects performed by any of the authors.

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