ORIGINAL ARTICLE

Factors associated with the efficacy of mature oocyte production after dual-trigger controlled ovarian stimulation using a GnRH antagonist protocol

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Abstract

Background: The number of mature oocytes retrieved plays a significant role in determining embryo development and pregnancy outcomes of in vitro fertilization (IVF). However, studies investigating factors predictive of the efficacy of mature oocyte production (EMOP) after dual-trigger controlled ovarian stimulation (COS) are rare. This study aims to identify key predictors of EMOP during dual-trigger COS with a gonadotropin-releasing hormone (GnRH) antagonist protocol for IVF.

Methods: This retrospective cohort study included 359 first-time IVF patients undergoing dual-trigger COS with a GnRH antagonist protocol. EMOP was defined as the ratio of metaphase II (MII) oocyte count to antral follicle count (AFC). Based on EMOP results, patients were divided into two groups: group A (EMOP <70%; n = 232) and group B (EMOP ≥70%; n = 127).

Results: Multivariate logistic regression analysis revealed that day-2 follicle-stimulating hormone (FSH), stimulation duration, and total oocyte count were the most significant predictors of EMOP ($p < 0.05$; odds ratios: 1.637, 3.400, and 1.530, respectively). Receiver operating characteristic analysis demonstrated that total oocyte count <9.5 (area under the curve [AUC], 0.782; sensitivity, 76.2%; specificity, 69.2%; $p < 0.001$) and stimulation duration <9.5 days (AUC, 0.725; sensitivity, 63.5%; specificity, 66.7%; $p < 0.001$) significantly predicted EMOP <70%. Stimulation duration combined with total oocyte count exhibited the highest power in predicting EMOP <70% (AUC, 0.767; sensitivity, 92.3%; specificity, 42.4%).

Conclusion: Stimulation duration combined with total oocyte count was identified as the most important factor associated with the EMOP during dual-trigger COS in IVF using a GnRH antagonist protocol.

Keywords: Controlled ovarian stimulation; Dual trigger; Gonadotropin-releasing hormone antagonist protocol; In vitro fertilization; Mature oocyte

1. INTRODUCTION

Oocyte maturation is necessary for successful in vitro fertilization (IVF) outcomes. Mature oocytes progress from prophase I to metaphase II (MII), while immature oocytes remain at the germinal vesicle or metaphase I stage.[1] In current IVF practice, clinicians attempt to retrieve as many mature oocytes as possible during each controlled ovarian stimulation (COS) cycle to improve the chance of a live birth. The quality of oocytes can be assessed by evaluating their level of maturity, and the number of mature oocytes retrieved plays a critical role in the attainment of a live birth. However, both age and oocyte maturity are closely associated with the live birth rate. Even with a sufficient number of mature oocytes, a woman's age remains a significant factor in achieving a live birth. For instance, women aged ≤35 years who possess ten mature oocytes have a 60.5% chance of experiencing a live birth, whereas women over the age of 36 with the same number of mature oocytes have a lower probability of live birth (29.7%).[2] Unfortunately, aging is an unalterable factor that affects oocyte quality in the context of IVF practice. As a result, a variety of methods have been proposed to tailor ovarian stimulation protocols to obtain competent and mature oocytes.

The number of possible oocytes obtained during each COS cycle is determined by the ovarian response, which can be predicted from the antral follicle count (AFC) and antimüllerian hormone (AMH) level.[3] While the regulatory mechanisms that determine the extent of antral follicle sensitivity to follicle-stimulating hormone (FSH) remain unclear, several parameters have been proposed to predict the ovarian response to FSH. The follicle output rate (FORT) is an index that assesses the ratio of the number of responding preovulatory follicles after FSH administration to the preexisting pool of AFC.[4] A FORT value <30% indicates ovarian hyposensitivity to FSH, while that >80% suggests an adequate ovarian response to FSH. Another frequently used parameter is the follicle-to-oocyte index (FOI), which

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indicates the degree of ovarian sensitivity to FSH during COS.[5] FOI is calculated as the ratio of the number of oocytes collected at oocyte retrieval to the AFC at the start of ovarian stimulation. A low response to gonadotropins (FOI ≤0.5) can be caused by numerous factors, including a low gonadotropin starting dose, genetic or environmental factors, allochronic follicular development, and factors involved in triggering final oocyte maturation and oocyte retrieval.[5]

FORT and FOI, however, do not provide information on the actual efficacy of mature oocyte production (EMOP). FORT neither counts the actual number of oocytes retrieved nor the number of mature oocytes produced. Similarly, FOI relies on the total number of oocytes, including immature oocytes, which does not reflect the efficacy of mature oocyte retrieval in IVF.

In clinical practice, follicle size is usually used to predict oocyte maturity; the optimal time to induce the luteinizing hormone (LH) surge by human chorionic gonadotropin (hCG) is when the diameter of the leading follicle is ≥18 mm.[6] As a substitute for LH, hCG is the most commonly used drug for inducing final oocyte maturation. Alternatively, a gonadotropin-releasing hormone agonist (GnRHa) can also be used to trigger oocyte maturation by causing endogenous LH surges.[7] The concentrations of post-trigger LH and progesterone (P4) are proven independent predictors of the total number of oocytes and mature oocytes retrieved after GnRHa trigger.[8]

While most studies have focused on the total number of oocytes retrieved during COS, limited studies have investigated the EMOP during COS. The use of a dual trigger (hCG + GnRHa) for final oocyte maturation can increase the efficiency of mature oocyte production,[9] but immature oocytes are still inevitably retrieved. Therefore, this study aims to identify significant predictors for EMOP during dual-trigger COS using a GnRH antagonist protocol.

2. METHODS

2.1. Patient selection

This retrospective cohort, nonrandomized study enrolled 359 patients who completed the first COS using a GnRH antagonist protocol with dual trigger between October 2016 and March 2021. All patients included in this study completed clinical treatment at Cathay General Hospital, Taipei, Taiwan. The Ethics Committee of Cathay General Hospital, Taipei, Taiwan approved this study (Institutional Review Board no.: CGH-FJ105002), and we obtained written informed consent from all patients.

2.2. EMOP definition

We defined the EMOP as the percentage of the number of MII oocytes divided by the AFC, as follows:

$$\text{EMOP} = \text{MII oocyte count/AFC} \times 100\ \%$$

We used the EMOP because it is more practical in clinical IVF practice. When a patient enters an IVF cycle, AFC is measured on day 2 or 3, which could be used as a predictor of the number of oocytes retrieved after ovarian hyperstimulation. The study participants were categorized into one of two groups according to their EMOP results (group A, EMOP <70%; group B, EMOP ≥70%).

2.3. Inclusion and exclusion criteria

All the participants in this study were below 45 years of age. The body mass index (BMI) range was 18.0–35.0 kg/m^2. We only included patients who were undergoing their first IVF cycle, using a GnRH antagonist protocol with a dual trigger and a serum basal FSH level <15 mIU/mL. We excluded patients with chromosomal abnormalities. Patients were also excluded if they had ovarian pathologies, including ovarian endometrioma, cysts, teratoma, benign ovarian tumors, and ovarian cancers. Additional exclusion criteria included repeated egg retrieval, ovarian stimulation with a non-GnRH antagonist, diminished ovarian reserve (AMH <1.2 ng/mL), BMI <18.0 or >35.0 kg/m^2, history of sexually transmitted disease, and poor ovarian response (<3 eggs retrieved).

2.4. Measurements of serum hormones and AFC

The serum AMH of each participant was measured before COS. Serum basal FSH, LH, estradiol (E2), and P4 were recorded on day 2 of the treatment cycle. Serum E2, LH, and P4 were also recorded on trigger day. The concentrations of AMH, FSH, LH, and E2 were determined using a chemiluminescence-based immunometric assay (Abbott Biologicals B.V., Olst, Overijssel, The Netherlands) on a Roche Cobas E601 analyzer, and that of progesterone was determined using a Roche Cobas E411 analyzer. All hormone assays were performed as per the manufacturer's instructions.

Transvaginal ultrasound was performed on day 2 of the treatment cycle, and AFC was recorded by a single attending physician. This can minimize the interpersonal bias of measurement. AFC was measured using a General Electric Voluson P6 (General Electric Healthcare, Seongnam-si, Gyeonggi-do, Korea) ultrasound machine with a 4–10-MHz transvaginal transducer.

During oocyte retrieval from each patient, follicular fluid (FF) was aspirated from three preovulatory follicles and pooled for further analysis. Vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β) concentrations in the aspirated FF were determined using a human VEGF enzyme linked immunosorbent assay development kit (R&D Systems, Inc., Minneapolis, MN, USA) and the Bradford assay, respectively, with the optical density measured at 450 nm (reference wavelength for correction was set to 540 nm). The absolute VEGF concentration in FF was calculated from a standard curve.

2.5. Ovarian stimulation protocol

All patients were treated with a dual trigger and GnRH antagonist protocol. Treatment with human recombinant FSH (Gonal-F, Merck Serono, Modugno, Italy) and highly purified human menopausal gonadotropin (hMG-HP) (Menopur, Ferring, Saint-Prex, Switzerland) was initiated on day 2 or 3 of the menstrual cycle. The dose depended on the patient's age, body weight, AMH, and AFC. When at least one follicle exceeded a mean diameter of 14 mm or the serum E2 level was >500 pg/mL, cetrorelix acetate (0.25-mg) (Cetrotide, Merck Serono, Halle, Germany) was administered subcutaneously daily to prevent premature LH surge. When at least two leading follicles were >18 mm, ovulation was triggered via 250 μg choriogonadotropin alfa (Ovidrel, Merck Serono, Modugno, Italy) and 0.2 mg triptorelin (Decapeptyl, Ferring, Kiel, Germany). Transvaginal oocyte retrieval (TVOR) was performed 34 to 36 hours after dual trigger. Only follicles >12 mm in diameter were retrieved. The total number of oocytes and grade were recorded.

2.6. Statistical analysis

All data were analyzed using SPSS version 16.0 (Chicago, IL, USA). The following clinical characteristics were compared between groups using a two-sample *t* test: age; AMH; AFC; total number of oocytes; MII oocyte count; day-2 FSH, LH, E2, and P4 concentrations; hCG trigger-day E2, LH, and P4 concentrations; the ratio of concentrations of day-2 LH to hCG trigger-day LH; oocyte maturation rate; and fertilization

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rate. The Spearman rank-order correlation for nonparametric variables was used to identify variables that correlated significantly EMOP. For the significant variables, a multivariate logistic regression model was used for backward stepwise analysis of the critical parameters associated with EMOP. Differences with $p < 0.05$ were considered statistically significant. The distribution of variables was determined using receiver operating characteristic (ROC) curves drawn to establish the cutoff values for the most significant variables for predicting EMOP.

3. RESULTS

A total of 359 first-time IVF patients treated from October 2016 to March 2021 were divided into two groups: group A (EMOP <70%; n = 232) and group B (EMOP ≥70%; n = 127). The clinical and laboratory characteristics of the patients are presented in Table 1. No significant differences were observed between the two groups in terms of age; AFC; AMH; day-2 FSH, LH, and P4 levels; trigger-day P4 levels; D2 LH/DhCG LH; total FSH dose; total LH dose; intrafollicular VEGF; intrafollicular TGF-β; or fertilization rate. However, significant differences were noted in day-2 E2 levels; trigger-day E2, and LH; LH decline; stimulation duration; total oocyte count; MII oocyte count; 2 pronuclear (2PN) embryo count; and oocyte maturation rate between groups A and B ($p < 0.05$).

Spearman rank univariate correlation was used to further analyze the significant correlations with EMOP (Table 2), revealing that EMOP correlated significantly with age; AMH; day-2 FSH; total oocyte count; MII oocyte count; oocyte maturation rate; 2PN count; trigger-day E2, LH, and P4 levels; LH decline; and stimulation duration ($p < 0.05$). Additionally, multivariate logistic regression analysis revealed that D2 FSH, stimulation duration, and total oocyte count were the most significant predictors of EMOP ($p < 0.05$; odds ratios: 1.637, 3.400, and 1.530, respectively) (Table 3).

ROC analysis demonstrated that D2 FSH >6.095 mIU/mL (area under the curve [AUC], 0.481; sensitivity, 76.2%; specificity, 24.8%; $p = 0.681$), total oocyte count <9.5 (AUC, 0.782; sensitivity, 76.2%; specificity, 69.2%; $p < 0.001$), and stimulation duration <9.5 days (AUC, 0.725; sensitivity, 63.5%; specificity, 66.7%; $p < 0.001$) were significant predictors of EMOP <70% (Fig. 1). Strikingly, we observed that the AUC of stimulation duration <9.5 days plus total oocyte count <9.5 was up to 0.767 in predicting EMOP <70% (sensitivity, 92.3%; specificity, 42.4%). However, the combination of D2 FSH >6.095 mIU/mL, stimulation duration <9.5 days, and total oocyte count <9.5 exhibited lower power in predicting EMOP <70% (AUC, 0.714; sensitivity, 90.8%; specificity, 36.4%) (Fig. 2).

4. DISCUSSION

We observed that the production of mature oocytes after dual-trigger COS using a GnRH antagonist correlated significantly with patient age, AMH, day-2 FSH, oocyte count, MII oocyte count, oocyte maturation rate, 2PN count, trigger-day E2, LH and P4 level, LH decline, and stimulation duration. Logistic regression analysis showed that D2 FSH, stimulation duration, and total oocyte count were the most significant predictors for EMOP <70% ($p < 0.05$; odds ratios: 1.637, 3.400, and 1.530, respectively) (Table 3).

Table 1

Comparison of clinical characteristics and laboratory measurements according to EMOP

Variables	Group A[a] EMOP <70% (n = 232)[b]	Group B[a] EMOP ≥ 70% (n = 127)[b]	p[c]
Age (y)	37.6 ± 3.9	36.0 ± 3.9b	0.250
AFC	13.6 ± 8.1	13.5 ± 7.8	0.826
AMH (ng/mL)	4.0 ± 3.6	4.8 ± 4.0	0.06
D2 E2 (pg/mL)	34.9 ± 19.2	29.4 ± 13.4	<0.05
D2 FSH (mIU/mL)	12.6 ± 7.4	7.2 ± 2.0	0.450
D2 LH (mIU/mL)	5.4 ± 2.3	5.6 ± 2.9	0.521
D2 P4 (ng/mL)	0.4 ± 0.2	0.3 ± 0.2	0.288
DhCG E2 (pg/mL)	2654.1 ± 1786.3	3750.0 ± 2491.4	<0.05
DhCG LH (mIU/mL)	2.7 ± 2.1	2.0 ± 1.5	<0.05
DhCG P4 (ng/mL)	1.5 ± 6.9	1.2 ± 1.1	0.556
D2 LH/DhCG LH	2.2 ± 3.3	2.8 ± 3.3	0.129
LH decline (%)d	43.4 ± 59.3	56.1 ± 49.0	<0.05
Total FSH dose (IU)	2061.9 ± 755.9	2218.5 ± 892.7	0.149
Total LH dose (IU)	712.9 ± 508.2	674.8 ± 550.1	0.621
Stimulation duration (d)	9.2 ± 1.1	9.9 ± 1.2	<0.05
Intrafollicular VEGF (pg/mL)	1330.5 ± 872.1	2122.1 ± 2674.5	0.086
Intrafollicular TGF-β (pg/mL)	969.5 ± 282.7	863.3 ± 269.5	0.233
Oocyte count	9.5 ± 7.1	16.0 ± 9.6	<0.05
MII oocyte count	6.0 ± 4.9	13.6 ± 8.4	<0.05
2PN count	6.2 ± 3.9	11.1 ± 6.8	<0.05
Oocyte maturity rate (%)	64.4 ± 26.1	85.2 ± 14.0	<0.05
Fertilization rate (%)	69.7 ± 23.3	72.4 ± 22.1	0.326

2PN = 2 pronuclear embryos; AFC = antral follicle count; AMH = antimüllerian hormone; DhCG = day of hCG trigger; E2 = estradiol; EMOP = efficacy of mature oocyte production; FSH = follicle stimulation hormone; LH = luteinizing hormone; MII = metaphase II; P4 = progesterone; TGF-β = transforming growth factor-beta; VEGF = vascular endothelial growth factor.

[a]Two-sample, two-tailed Student t test.

[b]Data are expressed as the mean ± SD.

[c]$p < 0.05$ considered statistically significant.

[d]LH decline (%): (D2 LH–DhCG LH) × 100%/D2 LH.

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A previous studies demonstrated the influence of serum D2 FSH (basal FSH) levels on oocyte maturity before COS using the GnRH agonist protocol, showing that the serum basal FSH level at the start of gonadotropin stimulation correlated inversely with the number of mature oocytes.[2] Nonetheless, the specific threshold value of serum basal FSH level that affects oocyte maturity in the GnRH antagonist protocol remains undetermined. This study indicates for the first time that diminished production of mature oocytes (EMOP <70%) may occur if the serum D2 FSH levels surpass 6.095 mIU/mL during COS using the dual-trigger GnRH antagonist protocol. However, the predictive power did not reach statistical significance according to ROC analysis (AUC, 0.481; sensitivity, 76.2%; specificity, 24.8%; p = 0.681). Furthermore, a stimulation duration <9.5 days (AUC, 0.725; sensitivity, 63.5%; specificity, 66.7%; p < 0.001) and a total oocyte count <9.5 (AUC, 0.782; sensitivity, 76.2%; specificity, 69.2%; p < 0.001) emerged as significant factors in predicting reduced production of mature oocytes (EMOP <70%). These two factors are associated with the EMOP subsequent to COS using the dual-trigger GnRH antagonist protocol. Our findings offer a practical framework for clinicians in planning their COS strategies, encompassing the use of D2 FSH levels during initiation and the regulation of stimulation duration for the optimal production of mature oocytes in IVF using a dual-trigger GnRH antagonist protocol.

This study aimed to determine the key parameters that influence EMOP after dual-trigger COS with a GnRH antagonist. Oocyte maturation during COS is affected by numerous factors, including patient age, protocol type, final trigger agents, FSH and LH receptor polymorphisms, dosage or duration of FSH or LH stimulation, and the presence of polycystic ovarian syndrome (PCOS) or endometriosis.[5] The production of mature oocytes is crucial, as only these oocytes can be fertilized in vitro and have a higher chance of developing into healthy embryos that lead to live births. Conversely, an abundance of immature oocytes in the COS cycle often results in poor fertilization and embryo development. Therefore, identifying significant variables during ovarian stimulation that predict EMOP is vital for efficient fertilization of oocytes and the development of high-quality embryos. A greater number of immature oocytes have been noted in women ≥41 years of age.[10] Despite age, the trigger agent used for final oocyte maturation is crucial for obtaining mature oocytes. Clinically, the most well-known agent for triggering final oocyte maturation is hCG, which mimics the LH surge before ovulation. In patients with PCOS, a GnRH agonist is frequently used as a trigger agent, inducing endogenous FSH and LH secretion during COS with a GnRH antagonist protocol. Although the use of a dual trigger with both hCG and GnRHa was observed to increase the number of mature oocytes in poor, normal, and high responders, immature oocytes were still retrieved.[9,11] Most studies of oocyte maturation after COS have used a single trigger of hCG or GnRHa alone. These studies also did not focus on the effect of ovarian stimulation on the EMOP. Therefore, the current study was conducted to explore the EMOP after COS and to focus on the GnRH antagonist protocol using a dual trigger to minimize the effect of other confounding factors on final oocyte maturation.

According to the two-cell-two-gonadotropin theory, both LH and FSH are necessary for folliculogenesis and oocyte maturation. However, the role of LH in this process is still debated. In general, LH acts on the ovary by binding to LH receptors on the theca and/or granulosa cells.[12] Low LH levels at the start of ovarian stimulation have been linked to a reduced oocyte yield following a GnRHa trigger.[13] Measuring LH on the trigger day alone is not sufficient for predicting mature oocyte production because LH levels at this stage are downregulated by the administration of GnRH antagonists.[14] In this study, we discovered that the LH level on day 2 alone does not exhibit a significant correlation with EMOP. However, the decline in LH (the ratio of LH level on day 2 to that on trigger day) proved to be a predictive factor for the production of mature oocytes in COS using a GnRH antagonist protocol (Table 1). Our analysis of the association between the variables and EMOP revealed a significant positive correlation between AMH level and LH decline (Spearman's rho, 0.179;

Table 2

Correlation between the investigated variables and EMOP

Variables	Spearman's rho[a]	p^b
Age (y)	–0.183	<0.05
AFC	0.042	0.427
AMH (ng/mL)	0.234	<0.05
D2 E2 (pg/mL)	–0.097	0.161
D2 FSH (mIU/mL)	–0.155	<0.05
D2 LH (mIU/mL)	–0.007	0.909
D2 P4 (ng/mL)	–0.029	0.639
Oocyte count	0.540	<0.05
MII count	0.748	<0.05
Oocyte maturation rate (%)	0.533	<0.05
2PN count	0.572	<0.05
Fertilization rate (%)	0.070	0.212
DhCG E2 (pg/mL)	0.375	<0.05
DhCG LH (mIU/mL)	–0.208	<0.05
DhCG P4 (mIU/mL)	0.163	<0.05
D2 LH/DhCG LH	0.001	0.983
LH decline (%)c	0.124	<0.05
Intrafollicular VEGF (pg/mL)	–0.018	0.891
Intrafollicular TGF-β (pg/mL)	–0.020	0.902
Stimulation duration (d)	0.277	<0.05
Total FSH dose (IU)	0.045	0.483
Total LH dose (IU)	–0.096	0.176

2PN = 2 pronuclear embryos; AFC = antral follicle count; AMH = antimüllerian hormone; DhCG = day of hCG trigger; E2 = estradiol; EMOP = efficacy of mature oocyte production; FSH = follicle stimulation hormone; LH = luteinizing hormone; MII = metaphase II; P4 = progesterone; TGF-β = transforming growth factor-beta; VEGF = vascular endothelial growth factor.

[a]Spearman rank univariate correlation.

[b]p < 0.05 considered statistically significant.

[c]LH decline (%): (D2 LH–DhCG LH) × 100%/D2 LH.

Table 3

Logistic regression analysis of the parameters associated with EMOP

Variables	Odds ratio (95% CI)	p^a
Age (y)	0.893 (0.725-1.099)	0.285
AMH (ng/mL)	1.149 (0.628-2.099)	0.652
D2 E2 (pg/mL)	0.976 (0.932-1.021)	0.283
D2 FSH (mIU/mL)	1.637 (1.112-2.409)	<0.05
D2 LH (mIU/mL)	0.931 (0.647-1.339)	0.699
DhCG E2 (pg/mL)	1.000 (1.000-1.001)	0.508
DhCG LH (mIU/mL)	1.656 (0.849-3.232)	0.139
DhCG P4 (ng/mL)	1.021 (0.712-1.464)	0.910
LH decline (%)[b]	1.006 (0.984-1.029)	0.593
Stimulation duration (d)	3.400 (1.500-7.707)	<0.05
Oocyte count	1.530 (1.229-1.906)	<0.05

AMH = antimüllerian hormone; DhCG = day of hCG trigger; E2 = estradiol; EMOP = efficacy of mature oocyte production; FSH = follicle stimulation hormone; LH = luteinizing hormone; P4 = progesterone.

[a]p < 0.05 considered statistically significant.

[b]LH decline (%): (D2 LH–DhCG LH) × 100%/D2 LH.

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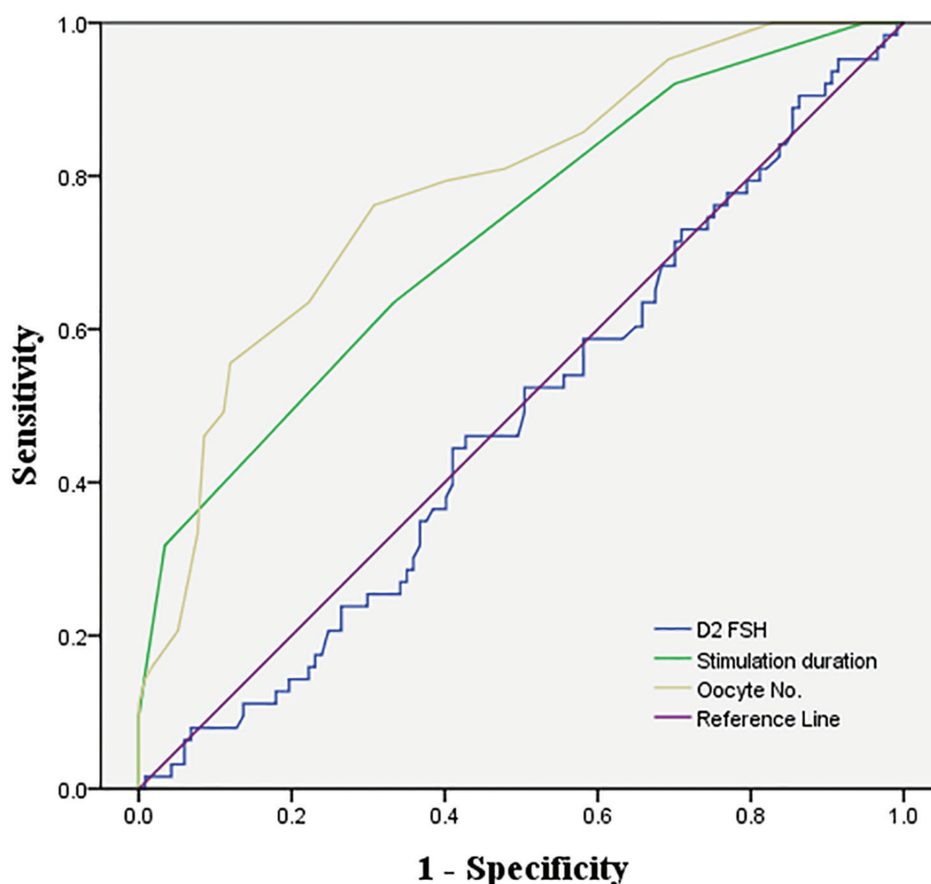

Fig. 1 ROC curves of the most significant predictors of EMOP. The computed ROC curves established the following cutoff values for the most significant predictors of EMOP <70%: D2 FSH >6.095 mIU/mL (AUC, 0.481; sensitivity, 76.2%; specificity, 24.8%; p = 0.681); total oocyte count <9.5 (AUC, 0.782; sensitivity, 76.2%; specificity, 69.2%; p < 0.001); and stimulation duration <9.5 d (AUC, 0.725; sensitivity, 63.5%; specificity, 66.7%; p < 0.001). AUC = area under the curve; EMOP = efficacy of mature oocyte production; FSH = follicle-stimulating hormone; ROC = receiver operating characteristic.

p = 0.002, <0.05). Therefore, higher AMH levels can result in a greater number of oocytes and higher peak E2 level, which increases the magnitude of LH decline. Similarly, lower AMH levels may indicate a lower degree of LH decline during ovarian stimulation. This phenomenon reflects the sensitivity of the hypothalamic-pituitary-ovary axis response to gonadotropin stimulation. A smaller LH decline during ovarian stimulation suggests that the hypothalamic-pituitary-ovary axis is less sensitive to gonadotropin stimulation through negative feedback, decreasing the likelihood of oocyte maturation. However, our multivariate logistic regression analysis demonstrated that serum AMH levels and LH decline were not significant predictors of EMOP (Table 3). Given that AMH is primarily secreted by granulosa cells, it is reasonable to infer that the conditions of intrafollicular hormones may be more closely associated with oocyte maturation than serum AMH and serum LH decline. However, the precise mechanism underlying the relationship between these variables and mature oocyte production remain unclear. Further research is needed to fully understand how parameters affect the production of mature oocytes after COS.

Our results show that the stimulation duration combined with total oocyte count has more power for predicting low mature oocyte production (EMOP <70%) (AUC, 0.767; sensitivity, 92.3%; specificity, 42.4%; p < 0.001). The optimal duration of ovarian stimulation for oocyte maturation varies between IVF patients with different COS responses. Yang et al[8] observed that the duration of ovarian stimulation has varied effects on oocyte maturation in both poor and normal responders in fresh IVF cycles with GnRH antagonist treatment. Their data showed an oocyte maturation rate of 85% when the stimulation duration was up to 9 or 10 days.[8] We also found that ovarian stimulation duration less than 9.5 days results in the retrieval of fewer mature oocytes (EMOP <70%). Our findings suggest that extending the duration of ovarian stimulation more than 9.5 days in a patient with a basal FSH level <6.095 mIU/mL may result in the production of more mature oocytes during dual-trigger COS with a GnRH antagonist protocol. This strategy for COS appears to be clinically practical and potentially beneficial to IVF outcomes.

The effect of intrafollicular VEGF and TGF-β on oocyte maturation has been studied extensively. Yin et al[15] reported that TGF-β might be a potent in vitro stimulator of oocyte maturation. TGF-β accelerates the growth of both follicle-enclosed oocytes and cumulus-oocyte complexes. Intrafollicular TGF-β promotes VEGF production in follicular granulosa cells via TGF-β receptor-mediated signaling pathways. The resulting release of VEGF leads to angiogenesis.[16] VEGF plays a vital role in angiogenesis, vascularization of the follicular, selection of the dominant follicle, corpus luteum development, and oxygenation of the follicle.[17] Further, Artini et al[18] reported that the serum level of VEGF did not significantly correlate with any IVF outcome. Additionally, no significant correlation was observed between follicular and serum VEGF levels. In the present study,

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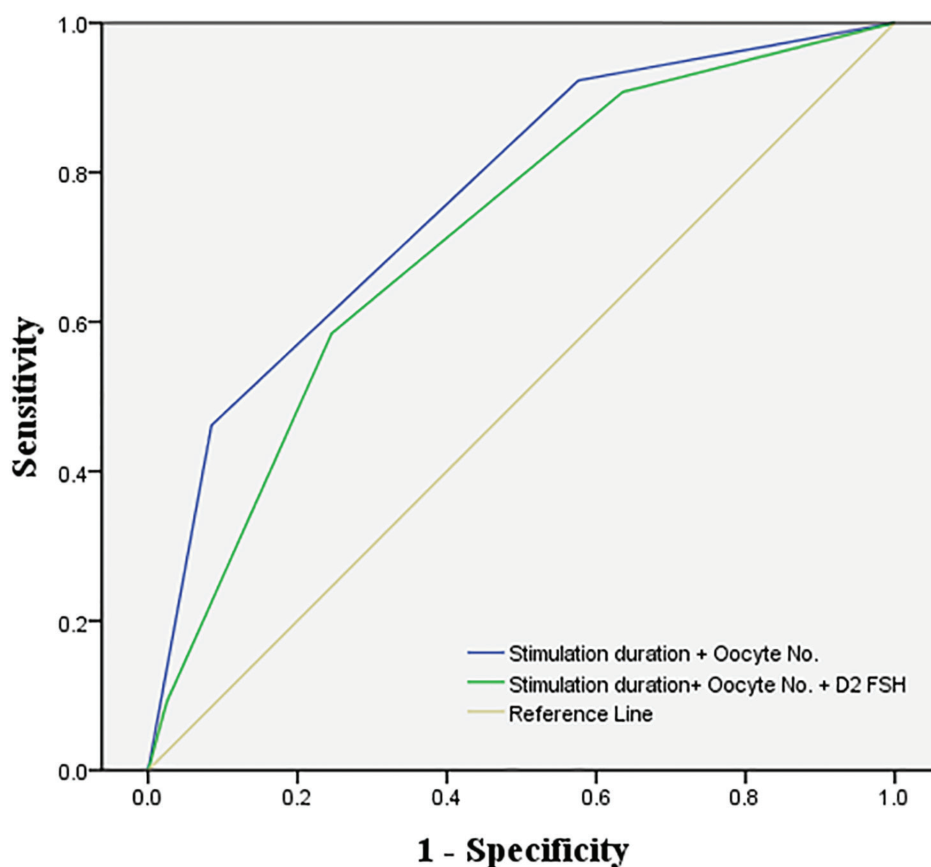

Fig. 2 ROC curve for the combination of stimulation duration and total oocyte count in predicting EMOP <70%. The computed ROC curve established that the cutoff value for stimulation duration <9.5 d plus total oocyte count <9.5 was the most crucial variable in predicting EMOP <70% (AUC, 0.767; sensitivity, 92.3%; specificity, 42.4%; $p < 0.001$). AUC = area under the curve; EMOP = efficacy of mature oocyte production; FSH = follicle-stimulating hormone; ROC = receiver operating characteristic.

we found that intrafollicular TGF-β and VEGF concentrations did not correlate with EMOP (Table 2).

To the best of our knowledge, this study is the first to identify the key predictors of low production of mature oocytes during dual-trigger COS using a GnRH antagonist protocol. The combination of two predictors—stimulation duration plus total oocyte count—showed good predictive ability for the suboptimal production of mature oocytes (EMOP <70%) during IVF under these conditions. However, this study has certain limitations. First, the retrospective nature of this cohort study can cause bias. Second, the sample size of the study was relatively small, which could limit the generalizability of the findings. Third, the study was conducted at a single center and did not include a comparison with other GnRH antagonist protocols and different trigger methods. Whether other COS protocols have the same results needs further verification. Finally, the study did not assess the effect of other factors such as endometriosis, PCOS, and FSH or LH receptor polymorphisms on EMOP. The precise mechanism underlying the observed associations with EMOP were not explored in the study. Further studies with larger sample sizes and comparisons with other GnRH antagonist protocols and different trigger methods are needed to validate the findings of this study.

In conclusion, this study is the first to identify independent predictors that correlate with EMOP during dual-trigger COS using a GnRH antagonist protocol. A stimulation duration <9.5 days together with a total oocyte count <9.5 was identified as the most important factor associated with efficacious production of mature oocytes. Prospective studies to validate our findings and clarify any associated clinical factors involved in EMOP should be conducted in the future.

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