

# Prediction of Assisted Reproductive Technology Outcome by Day 5 Estradiol (E2) Levels in Antagonist Cycles

Naga P Indrakanti<sup>1</sup>, Anjali S Mudkur<sup>2</sup>, Vidyashree G Poojari<sup>3</sup>, Mehtab Kaur<sup>4</sup>, Dhanya Vikhnan<sup>5</sup>, Pratap K Narayan<sup>6</sup>, Prashanth K Adiga<sup>7</sup>

Received on: 13 May 2024; Accepted on: 24 July 2024; Published on: 23 October 2024

## ABSTRACT

**Aim and background:** Follicle monitoring and hormone levels, particularly those of estradiol (E2), progesterone (P4), follicle-stimulating hormone (FSH), and luteinizing hormone, are critical factors in determining the effectiveness of *in vitro* fertilization (IVF). Based on their day-5 E2 readings, patients are divided into three groups. Group I comprises values less than 400 pg/mL, group II ranges from 400 to 900 pg/mL, and group III exceeds 900 pg/mL. The study's objectives were to construct a prediction formula for the total number of retrieved oocytes, analyze the mean number of mature and total oocytes, and determine the incidence of ovarian hyperstimulation syndrome (OHSS) in three groups.

**Materials and methods:** The Department of Reproductive Medicine and Surgery at a tertiary care facility in coastal Karnataka carried out this retrospective observational research. The study included 140 patients who underwent IVF stimulation using the antagonist protocol between May 2022 and April 2023, a duration of 1 year. Simple linear regression techniques and receiver operating curve (ROC) curves are utilized in statistical analysis.

**Results:** The average age, duration of infertility, BMI, antral follicle count (AFC), and anti-mullerian hormone (AMH) were in that order 31.51 years, 5.04 years, 24.44 kg/m<sup>2</sup>, 17.67, and 3.63 ng/mL. Based on the day 5 E2 values and the difference between the day 5 and the trigger E2 values, the quantity and mature oocytes were predicted with statistical significance. The E2 threshold levels on day 5 and the day of trigger to day 5 E2 difference were 946 pg/mL and 2758 pg/mL, respectively, to forecast and prevent OHSS.

**Conclusion:** The current study's measurement of the day-5 E2 level is significant since it aids in the prediction of the number and maturity of oocytes as well as the risk of OHSS.

**Keywords:** Antagonist, Estradiol, M-II oocytes, Ovarian hyperstimulation syndrome.

*Journal of South Asian Federation of Obstetrics and Gynaecology* (2024): 10.5005/jp-journals-10006-2507

## INTRODUCTION

The European Society of Human Reproduction and Embryology (ESHRE) has widely recommended gonadotropin releasing hormone (GnRH) antagonist protocols as the first-line method for controlled ovarian stimulation (COH).<sup>1</sup> The benefits of antagonist protocols include avoiding pituitary downregulation, reducing the total dosage and duration of gonadotropins, and preventing complications such as ovarian hyperstimulation syndrome (OHSS).<sup>2</sup> Hormonal tests and follicular evaluation are used to monitor ART cycles. There are two ways to initiate a GnRH antagonist protocol: fixed or flexible. Day 5 E2 is known to be important for initiating antagonists and frequently indicates how early follicles respond to stimulation.<sup>3</sup> Day 5 low serum E2 levels have been linked to increased cancellation rates.<sup>4</sup> Estradiol levels aid in the prediction of the stimulation cycle since follicular growth is dependent on an environment rich in estrogen. This aids in patient counseling on the clinical result of recovered oocytes and aids in identifying individuals who may be at risk for OHSS. It is helpful to choose the course of therapy in advance if the day 5 E2 value can forecast the likely number of mature and total oocytes that are recovered and can determine a threshold value above which the odds of developing OHSS are high. We postulated that at an early stage of stimulation, the day 5 E2 value and the difference between the day 5 and the day of trigger E2 value can predict the number of oocytes and problems such as OHSS. Thus, it helps in adjusting the dose and duration of required gonadotropin treatment, assessing the response to stimulation, deciding the type of trigger (HCG or

<sup>1-5</sup>Department of Reproductive Medicine and Surgery, Kasturba Medical College, Manipal, Karnataka, India

<sup>6,7</sup>Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal, Karnataka, India

**Corresponding Author:** Prashanth K Adiga, Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal, Karnataka, India, Phone: +91 9035036832, e-mail: Prashanth.adiga@manipal.edu

**How to cite this article:** Indrakanti NP, Mudkur AS, Poojari VG, *et al.* Prediction of Assisted Reproductive Technology Outcome by Day 5 Estradiol (E2) Levels in Antagonist Cycles. *J South Asian Feder Obst Gynae* 2024;16(5):542–545.

**Source of support:** Nil

**Conflict of interest:** None

agonist) using preventive measures in patients at risk for OHSS and finally, on fresh or frozen embryo transfer.

## MATERIALS AND METHODS

The present study is a retrospective observational study conducted at the Department of Reproductive Medicine and Surgery at a tertiary care center in coastal Karnataka. All patients who underwent IVF stimulation by flexible antagonist protocol from May 2022 to April 2023 were included in the study. The inclusion criteria were as follows: women 1) age 20–40 years; (2) undergoing 1st IVF stimulation, (3) with regular cycles from 25 to 35 days,

and (4) both ovaries intact. Exclusion criteria were as follows: (1) cycles abandoned for various reasons, (2) patients who underwent agonist protocols, and (3) donor IVF cycles.

### Ovarian Stimulation Protocol

Included were all patients who underwent COH utilizing the flexible GnRH antagonist regimen. On day two or three of the cycle, gonadotropin (Gn), either human menopausal gonadotropin (HMG), recombinant HMG, or follicle-stimulating hormone (FSH), was begun. Starting dose is tailored based on the patient's baseline parameters and ranging from 100 to 450 IU/day. Transvaginal ultrasonography (TVS) measurements of follicular development and hormone levels were taken on days 5–7 after stimulation. When serum E2 was >400 pg/mL and the leading follicle measured  $\geq 14$  mm or six follicles measuring  $\geq 11$  mm, GnRH antagonist 0.25 mg was started. TVS performed daily for follicular monitoring. When three or more lead follicles had a diameter of 17 mm, or when the lead follicle diameter was  $\geq 18$  mm, the trigger was determined. Based on the number of follicles ( $\geq 13$  in each ovary) and E2 readings ( $\geq 3000$  pg/mL), the kind of trigger was selected. If the aforementioned cut-off levels were reached, an agonist trigger is administered to reduce the chance of OHSS. Oocytes were extracted transvaginally 35–36 hours after the trigger.

### Hormonal Assays

Serum levels of FSH, Luteinizing hormone (LH), E2, progesterone-4 (P4) were measured on the day of stimulation. Repeat estimation of LH, P4 on day 5 and LH, E2 and P4 on the day of trigger was done. Repeat E2 was done the next day if the day 5 E2 value was <400 pg/mL. Blood assays were performed using corresponding kits and an automatic chemiluminescence system.

### Outcome Measures

Female age, infertility duration, type of infertility, body mass index (BMI), basal hormone levels, antral follicle count (AFC), Anti-mullerian hormone (AMH), total Gn dose, duration of stimulation, serum hormone levels on day 5 and day of trigger, and type of trigger were the demographic and cycle characteristics that were analyzed. The difference between day 5 and the day of trigger E2 was calculated. The outcome variables analyzed were the total no. of oocytes retrieved, no. of mature oocytes, and incidence of OHSS (mild/moderate) irrespective of the trigger.

Patients were divided into three groups based on day 5 E2 levels. Group I with values <400 pg/mL, group II between 400 and 900 pg/mL, group III with values >900 pg/mL. The mean no. of total oocytes and mature (MII) oocytes were observed in each group. The incidence of OHSS in each group was analyzed. The other outcomes observed were to evaluate the predictive value of day 5 E2, the difference of day 5 from day of trigger E2 on the number and maturity of oocytes. To assess the predictive value of day 5 E2, the difference of day 5 from the day of trigger E2 on prevention of mild/moderate OHSS.

### Statistical Analysis

Continuous variables were summarized using mean  $\pm$  standard deviation (SD). Statistical analysis was carried out using R software. Categorical variables were expressed in terms of frequencies and percentages. Scatter diagrams were used to understand the correlation between day 5 E2 and no. of total and mature

**Table 1:** Demographic variables

Variable	Mean	SD
Age (YRS)	31.51	3.88
INF (YRS)	5.04	2.78
BMI (kg/m <sup>2</sup> )	24.44	3.75
AFC	17.67	7.62
AMH (ng/mL)	3.63	2.08
Day 2 E2	45.78	15.05
Day 5 E2	827.23	453.52
Day 9 E2	3582.82	2787.67
Day 9 to Day 5 E2	2755.88	2472.37
Follicles	22.36	12.14
Oocytes	10.91	5.62
M II	9.28	5.06

**Table 2:** Day 5 E2 values divided into three groups

Day 5 E2 (pg/mL)	Mean oocytes	Mean M II	OHSS incidence
<400	7	5	0
400–900	9	8	3
>900	14	12	8

oocytes. A prediction formula was derived to calculate the no. of oocytes retrieved. The receiver operating curve (ROC) was used to understand the diagnostic accuracy of day 5 E2 in predicting OHSS.

## RESULTS

A total of 140 patients were analyzed who underwent flexible GnRH antagonist protocol COH due to varied infertility reasons like an ovulation, male factor, unexplained, tubal, combined, etc. Mean and SD values were analyzed for the variables female age, duration of infertility, BMI, AFC, and AMH. The mean age was  $31.51 \pm 3.88$  years and the mean duration of infertility was  $5.04 \pm 2.78$  years, and the mean BMI was  $24.44 \pm 3.75$  kg/m<sup>2</sup>. The mean number of AFC was  $17.67 \pm 7.62$ , mean AMH was  $3.63 \pm 2.08$  ng/mL. Mean day 5 E2 was  $827.23 \pm 453.52$  pg/mL, and the mean day of trigger E2 was  $3582.8 \pm 2787.67$  pg/mL (Table 1).

### Serum E2 Levels and ART Outcome

All the patients were divided into three categories based on day 5 estradiol values. The mean no. of total oocytes in group I were 7.33, and mature oocytes were 5.86. The incidence of OHSS was zero. In group II, the mean no. of total oocytes was 9.54, and of mature oocytes was 8.29, the incidence of OHSS incidence was 3. In group III, the mean number of total oocytes was 14.41, of mature oocytes was 12.09, the incidence of OHSS was 8 (Table 2).

Relationship between day 5 E2 and difference of day 5 from the day of trigger E2 with the total number and mature oocytes was analyzed.

Scatter diagrams were used to understand the correlation between day 5 E2 and the number of total mature oocytes. Simple linear regression was used to estimate the relationship between the two quantitative variables. Scatter diagrams and regression equations explain the strong positive correlation between the day 5 values with total and mature oocytes as the *p*-value is <0.001 (Figs 1 and 2).

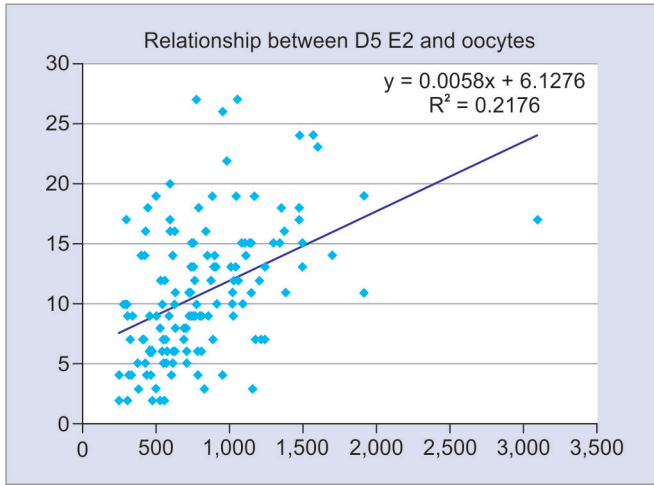


Fig. 1: Relationship between day 5 E2 and oocytes

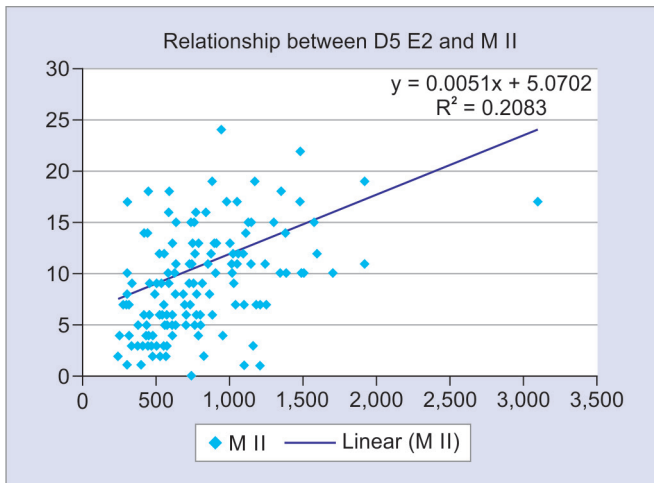


Fig. 2: Relationship between day 5 and MII

Adjusted R2 (adjusted coefficient of determination) value explains that the quantity of number of total and M II oocytes can be predicted with day-5 E2 with highly significant *p*-values (Table 3). Similarly, the difference in the day of the trigger to day 5 E2 value is also highly significant in predicting the number of total and mature oocytes (Figs 3 and 4).

Prediction formula to estimate the total oocyte number and M II oocytes. Total number of oocytes =  $6 \times /1000 + 6$ ; *x* is the day 5 E2 value:

$$[0.0058 x + 6.1276]$$

Total number of M II oocytes =  $5 \times /1000 + 5$ ; *x* is the day 5 E2 value:

$$[0.0051 x + 5.0702]$$

Total number of oocytes =  $xx/1000 + 8$ ; where *xx* is the difference between the day of the trigger and the day 5 E2 value:

$$[0.0012 xx + 7.6039]$$

Total number of M II oocytes =  $xx/1000 + 6$ ; where *xx* is the difference between the day of the trigger and the day 5 E2 value:

$$[0.001 xx + 6.5247]$$

Table 3: Simple linear regression results

Prediction based on simple linear regression	Adj R <sup>2</sup> value	<i>p</i> -value
Oocytes on D5 E2	0.22	0.000000007
Maturity on D5 E2	0.21	0.00000001
Maturity on D9 and D5 difference E2	0.24	0.00000009
Follicles on D9 and D5 difference E2	0.49	0.000000002
Oocytes on D9 and D5 difference E2	0.28	0.000000024

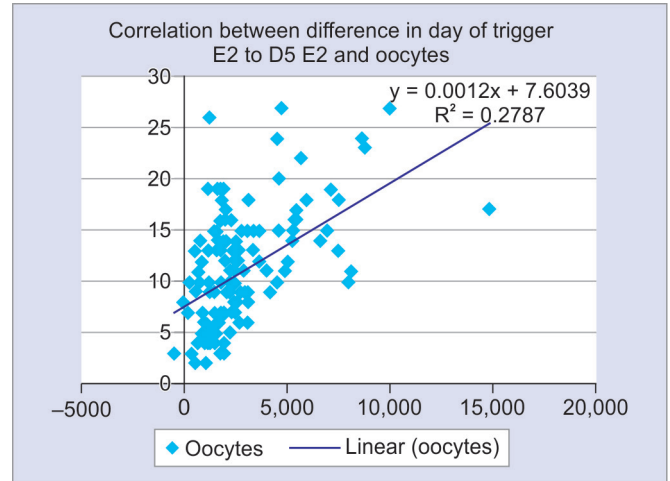


Fig. 3: Correlation between the difference in the day of trigger E2 to Day 5 E2 and oocytes

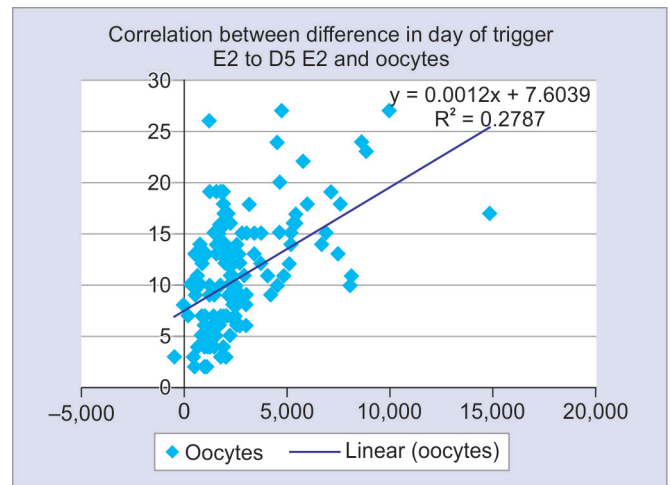


Fig. 4: Correlation between the difference in the day of trigger E2 to Day 5 E2 and MII

### Prediction of OHSS

The difference between the day of trigger and day 5 E2 in predicting the likelihood of OHSS was also examined using the ROC curve to determine the diagnostic accuracy of day 5 E2. A probability curve is the ROC curve. The trade-off between sensitivity and specificity is displayed on the ROC curve. The degree or level of separability is represented by the area under curve (AUC). As a generic measure of accuracy, AUC functions well. AUC of 0.5 means there is no discrimination, 0.7–0.8 means it is reasonable, and 0.8–0.9

**Table 4:** Threshold values for mild and moderate OHSS

Prediction based on ROC curve	AUC	Sensitivity (%)	Specificity (%)	Threshold (pg/mL)	p-value
Mild OHSS on day 5	0.636	70	71	946	0.002
Moderate OHSS on D5 E2	0.728	72	73	977	0.001
OHSS on D9 to D5 difference	0.679	60	72	2758	0.04

means it is quite accurate. Patients with and without illness can be distinguished more effectively with higher AUC (OHSS).

There was no case of severe or critical OHSS as agonist trigger was given in high-risk patients and received prophylactic cabergoline, hydroxyethyl starch solution (HESS), antagonist even after oocyte pick-up, and low molecular weight heparin.

When AUC is 0.7, there was a 70% chance that the model will be able to distinguish between the positive and negative classes. The threshold value of day 5 E2 above which mild OHSS may develop is 946 pg/mL, and moderate OHSS is 977 pg/mL. The threshold value of the day of trigger and day 5 E2 difference above which the mild or moderate OHSS may develop is 2758 pg/mL (Table 4).

## DISCUSSION

It is commonly known that the E2 level on the trigger day is significant since it signals follicular maturation. Granulosa cells have elevated aromatase activity and high estrogen levels during follicular growth, which is correlated with follicular quality. As a result, E2 levels represent follicular function and USG shows follicular expansion. In our investigation, estimating day 5 E2 for outcome prediction—specifically, the number of oocytes and OHSS risk—allows for considerably earlier analysis of the stimulation response, supporting the notion of preventative counseling.<sup>5</sup> In terms of oocyte number, in relation to day 5 E2 and the difference from the day of trigger to day 5 E2 values, the results of this study are comparable to those of Chun-Xiao Wei et al.<sup>6</sup>

In order to aid with the planning of the trigger, the dosage and duration of stimulation, and the fresh or frozen embryo transfer, we developed a formula in our study to estimate the number of oocytes on day 5 of the stimulation. In terms of mature oocytes, day 5 values had a linear connection as of the trigger day, similar to Wang et al.<sup>3</sup> In contrast to Wertheimer et al., this work shows that follicles with high E2 on the day of GnRH-ant start<sup>7</sup> exhibit sub-optimal synchronization, which impairs oocyte maturation.

Our research indicates that day 5 E2 is a more accurate predictor than the day of trigger E2 since it cannot estimate the number of mature oocytes because there is no difference in E2 production across follicles that range in size from 11 to 18 mm or more.

When estimating the risk of OHSS, the day-5 E2 value threshold level aids in adjusting the stimulation regimen, arranging the trigger, and transferring the embryo. Patients can get counseling on post-oocyte pick-up monitoring, freezing, and OHSS prophylactic measures. Follicle monitoring is more important than the day of trigger E2 in predicting OHSS, as Papanikolaou et al.<sup>8</sup> showed. When it comes to estimating the danger at a very early stage of stimulation, our work is well ahead.

Our study's relatively small sample size is one of its limitations. We were unable to rule out other problems such as male factor infertility, laboratory issues, and other related pathologies in order to assess the embryo status, thus we were unable to evaluate the

final outcome of ART treatment in terms of clinical pregnancy rate and live birth rate. Cycles of embryo transfer were thus not taken into account.

Additionally, increasing the sample size and employing the prediction method to determine the likely number of oocytes and OHSS risk based on the threshold values may yield the intended live birth rate findings.

## CONCLUSION

The physical and financial strain on the couple is lessened when the ART outcomes are predicted during the first phase of COH. It is demonstrated that day 5 E2 is crucial for interpreting the early follicular response and forecasting the final result.

## Clinical Significance

The current work aids in considerably early estimation of the number of oocytes during the controlled ovarian stimulation phase of antiretroviral therapy. Determining the type of trigger and opting for frozen embryo transfer can be aided by forecasting the likelihood of OHSS on day 5 after stimulation.

## ORCID

Naga P Indrakanti  <https://orcid.org/0009-0006-6970-3427>

Anjali S Mudkur  <https://orcid.org/0000-0002-4389-3676>

Vidyashree G Poojari  <https://orcid.org/0000-0001-5972-9945>

Pratap K Narayan  <https://orcid.org/0000-0002-7245-1746>

## REFERENCES

1. The ESHRE guideline group on ovarian stimulation, Bosch E, Broer S, et al. Erratum: ESHRE guideline: Ovarian stimulation for IVF/ICSI. *Hum Reprod Open* 2020;2020:hoaa067. DOI: 10.1093/hropen/hoaa067.
2. Al-Inany HG, Youssef MA, Aboulghar M, et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011;(5):CD001750. DOI: 10.1002/14651858.CD001750.pub3.
3. Wang Y, Xu X, Yang AM, et al. Optimizing estradiol level for gonadotrophin-releasing hormone antagonist initiation among patients with simple tubal factor infertility. *Front Endocrinol (Lausanne)* 2022;13:915923. DOI: 10.3389/fendo.2022.915923.
4. Khalaf Y, Taylor A, Braude P. Low serum estradiol concentrations after five days of controlled ovarian hyperstimulation for in vitro fertilization are associated with poor outcome. *Fertil Steril* 2000;74(1):63–66. DOI: 10.1016/S0015-0282(00)00569-0.
5. El Maghraby HA, Mahdi M, El Arab MS, et al. Stimulation day-six serum estradiol: A predictive indicator for the probability of embryo cryopreservation in IVF/ICSI cycles. *J Obstet Gynaecol Res* 2009;35(2):326–329. DOI: 10.1111/j.1447-0756.2008.00940.x.
6. Wei CX, Zhang L, Pang CH, et al. Effect of the ratios of estradiol increase on the outcome of in vitro fertilization-embryo transfer with antagonist regimens: A single center retrospective cohort study. *BMC Pregnancy Childbirth* 2023;23(1):134. DOI: 10.1186/s12884-023-05438-3.
7. Wertheimer A, Danieli-Gruber S, Hochberg A, et al. The association between treatment parameters on the day of gonadotropin-releasing hormone antagonist initiation during a flexible protocol and oocyte maturation rate. *Reprod Biol* 2020;20:127–131. DOI: 10.1016/j.repbio.2020.04.004.
8. Papanikolaou EG, Pozzobon C, Kolibianakis EM, et al. Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist in vitro fertilization cycles. *Fertil Steril* 2006;85(1):112–120. DOI: 10.1016/j.fertnstert.2005.07.1292.