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Nonlinear association of trigger-day serum estradiol with metaphase II oocyte count after adjustment for follicle-size distribution: a retrospective ICSI cohort study

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Abstract

Objective To assess whether trigger-day serum estradiol (E2) was associated with metaphase II (MII) oocyte count after accounting for the size distribution of follicles ≥ 10 mm, and whether this association was nonlinear. We also examined the number of oocytes retrieved and the MII oocyte maturation rate.

Methods This single-center retrospective cohort study included 869 first eligible ICSI oocyte retrieval cycles recorded between January 2022 and December 2025. The primary outcome was MII oocyte count. We used negative binomial regression and modeled $\log_2$(E2) with a three-knot restricted cubic spline. Models adjusted for five trigger-day follicle-size categories and prespecified patient- and treatment-related covariates. The number of oocytes retrieved was analyzed with negative binomial regression, and the MII oocyte maturation rate with quasi-binomial regression. Temporal replication and sensitivity analyses assessed robustness.

Results Likelihood ratio tests showed evidence of an overall association between trigger-day serum E2 and MII oocyte count (χ^2 = 188.49, P < 0.001) and of nonlinearity (χ^2 = 48.72, P < 0.001). The adjusted MII oocyte counts at the 25th percentile of E2 (1,472 pg/mL) and the 75th percentile (4,826 pg/mL) were 7.71 and 12.70, respectively. The absolute difference was 4.99 oocytes (95% CI, 4.33–5.69; P < 0.001). The corresponding adjusted difference in the number of oocytes retrieved was 5.73 (95% CI, 4.99–6.50; P < 0.001). No overall association was observed between E2 and the MII oocyte maturation rate (P = 0.356). The period interaction was not statistically significant (P = 0.471), and sensitivity analyses yielded consistent results.

Conclusions Trigger-day serum E2 was nonlinearly associated with MII oocyte count after accounting for the complete size distribution of follicles ≥ 10 mm and prespecified covariates. This association paralleled variation in the number of oocytes retrieved rather than the MII oocyte maturation rate. Trigger-day serum E2 may therefore provide information complementary to ultrasonographic follicle assessment when estimating MII oocyte count in ICSI cycles.

Introduction

Obtaining an adequate number of metaphase II (MII) oocytes is an immediate objective of oocyte retrieval in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Clinicians generally determine the timing of final oocyte maturation by integrating follicle size and number, serum hormone concentrations, and patient characteristics [1, 2]. Follicular development is asynchronous, however, and follicles of different sizes often coexist within the same patient. The follicle diameter that should prompt triggering therefore remains uncertain. Some studies found that larger follicles were more likely to yield mature oocytes, whereas others reported no clear differences across follicle diameters. Yang et al. attributed some of this inconsistency to patient characteristics and reported age-dependent associations between trigger-day follicle-size distribution and MII oocyte or embryological outcomes[3]. In natural-cycle IVF, Helmer et al. found that follicle diameter was associated with MII oocyte recovery, whereas serum estradiol (E2) was associated with both oocyte retrieval and MII oocyte recovery [4]. Lawrenz et al. reported that smaller trigger follicle diameters may be appropriate in modified natural-cycle IVF among patients with severely diminished ovarian reserve [5]. Together, these findings support evaluating the entire follicle-size distribution alongside patient characteristics, rather than relying only on a few leading follicles.

Trigger-day serum E2 reflects the endocrine activity of the follicle cohort and may provide information beyond follicle number and diameter. Previous studies reported positive correlations between trigger-day serum E2 and both the number of oocytes retrieved and MII oocyte count in gonadotropin-releasing hormone (GnRH) antagonist cycles [6]. Malathi et al. reported similar correlations with mature follicle count, the number of oocytes retrieved, and MII oocyte count in ICSI cycles [7]. These studies, however, relied mainly on simple correlation analyses. They did not fully account for the distribution of follicles across size categories or evaluate nonlinearity in the E2–MII association. Among patients without polycystic ovary syndrome (PCOS) undergoing fresh embryo transfer, Xu et al. reported a nonlinear, plateau-shaped association between trigger-day E2 and live birth [8]. A single linear coefficient or simple categorization may therefore inadequately represent the relationship between E2 and assisted reproduction outcomes. Moreover, MII oocyte count depends on both the total number of oocytes retrieved and the MII oocyte maturation rate. A positive E2–MII association may thus reflect more oocytes retrieved rather than a greater maturation probability per oocyte. Ovarian stimulation parameters may also relate differently to MII oocyte count and the proportion of mature oocytes [9].

We therefore evaluated the overall and nonlinear associations between trigger-day serum E2 and MII oocyte count after accounting for five size categories of follicles ≥ 10 mm and prespecified covariates. We also examined the number of oocytes retrieved and the MII oocyte maturation rate to distinguish oocyte yield from maturation efficiency.

Materials and Methods

Study design and participants

This single-center retrospective observational cohort study included 869 patients treated at the Department of Reproductive Health, Qinzhou Maternal and Child Health Hospital, between January 2022 and December 2025. Patients were eligible if fertilization in their in vitro fertilization cycle was performed by intracytoplasmic sperm injection (ICSI). Only the first eligible ICSI oocyte retrieval cycle recorded for each patient during the study period was included.We excluded cycles canceled before triggering, cycles without completed oocyte retrieval or an ascertainable outcome, and cycles without a trigger-day serum estradiol (E2) measurement. Extracted clinical data included trigger-day serum E2, trigger-day follicle sizes, the number of oocytes retrieved, and metaphase II (MII) oocyte count.The Medical Ethics Committee of Qinzhou Maternal and Child Health Hospital approved the study protocol (approval no. QSFYL[2026]08002). Table 1 summarizes the study population.

Controlled ovarian stimulation and assessment of oocyte maturity

Controlled ovarian stimulation protocols included a long gonadotropin-releasing hormone (GnRH) agonist protocol, a GnRH antagonist protocol, progestin-primed ovarian stimulation (PPOS), and a gonadotropin-only protocol. Clinicians individualized the protocol and gonadotropin (Gn) preparation according to patient age, body mass index (BMI), ovarian reserve markers, and ovarian response.Starting Gn preparations were classified as follicle-stimulating hormone (FSH) alone, human menopausal gonadotropin (HMG) alone, or HMG combined with FSH. The starting Gn dose was the first-day prescription recorded in the medical record. Follicular response was monitored by transvaginal ultrasonography and serum hormone measurements, and subsequent doses were adjusted when clinically indicated.

When center-specific criteria were met, final oocyte maturation was triggered with human chorionic gonadotropin (hCG) or a GnRH agonist. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h later.Retrieved cumulus–oocyte complexes were placed in pre-equilibrated fertilization medium (G-IVF PLUS; Vitrolife, Sweden) and cultured in a tri-gas incubator at 37 °C, 6% CO_2, and 95% humidity. Cumulus cells were removed 2–6 h after retrieval, and oocyte maturity was assessed under an inverted microscope. Oocytes with an extruded first polar body were classified as MII oocytes, and the MII oocyte count was recorded.

Trigger-day follicle measurements and serum E2 assessment

Transvaginal ultrasonography was performed on the day of trigger, and follicle sizes were recorded. Follicle diameter was defined as the mean of the two longest perpendicular diameters in the maximal cross-sectional plane.Ultrasound examinations used a system equipped with a transvaginal transducer operating at ≥7 MHz. Physicians trained in reproductive ultrasonography performed all examinations. Scanning and measurements of each ovary were reviewed at least once to improve accuracy.The analysis included follicles recorded in the database as ≥10.0 mm. Follicles were assigned to five mutually exclusive categories: 10.0–14.0, >14.0–16.0, >16.0–18.0, >18.0–20.0, and >20.0 mm. A follicle measuring 14.0 mm was assigned to the first category. Follicles measuring exactly 16.0, 18.0, or 20.0 mm were assigned to the immediately preceding category.Serum E2 was measured on the day of trigger using the same model of automated chemiluminescence immunoassay analyzer (Kangrun, China). Concentrations were reported in pg/mL.

Exposure and outcome variables

The primary exposure was trigger-day serum E2. Because its distribution was right-skewed, E2 was $\log_2$-transformed for regression modeling. A one-unit increase in $\log_2$(E2) corresponds to a twofold increase in the original E2 concentration.The primary outcome was MII oocyte count per cycle. Secondary outcomes were the number of oocytes retrieved and the MII oocyte maturation rate, calculated as MII oocyte count divided by the number of oocytes retrieved.

Classification of starting Gn preparations and doses

Starting Gn preparations were classified as FSH alone, HMG alone, or HMG combined with FSH. Because nominal doses were not directly comparable across classes, the recorded starting dose was standardized within each class to generate GnDoseZ. A one-unit increase in GnDoseZ indicates a starting dose 1 standard deviation higher within the same Gn class.

Statistical analysis

Multivariable model construction

MII oocyte count and the number of oocytes retrieved were overdispersed count outcomes relative to a Poisson distribution. We therefore analyzed both outcomes using negative binomial regression with a log link. The MII oocyte maturation rate was analyzed using quasi-binomial regression with a logit link.The base model (M1) excluded E2. It included counts in the five follicle-size categories, age, body mass index (BMI), ln-transformed anti-Müllerian hormone (AMH), polycystic ovary syndrome (PCOS) status, ovarian stimulation protocol, starting gonadotropin (Gn) class, GnDoseZ, and treatment year.The simplified linear model (M2L) added $\log_2$(E2) to M1 and estimated the average incidence rate ratio (IRR) associated with a twofold increase in E2. The primary model (M2S) added $\log_2$(E2) to M1 using a three-knot restricted cubic spline (RCS), allowing the association to vary across the E2 range. Knots were fixed at the 10th, 50th, and 90th percentiles of the $\log_2$(E2) distribution in the 2022–2024 data.

Overall association and nonlinearity of E2

Model comparisons proceeded in two stages. First, a likelihood ratio test compared M1 with M2S and jointly tested whether both E2 spline parameters were zero.This comparison evaluated the overall E2–MII association after adjustment for the five follicle-size categories and other prespecified covariates. The models differed by 2 degrees of freedom.Second, a likelihood ratio test compared M2L with M2S and tested whether the additional nonlinear parameter was zero. This comparison evaluated whether a linear function adequately described the E2–MII association. The models differed by 1 degree of freedom.For each model, we reported the log-likelihood and Akaike information criterion (AIC). Pearson dispersion statistics and residual diagnostics were also examined to assess model fit (Table 2).

Adjusted curves and marginal standardization

M2S was used to plot the continuous association between E2 and adjusted MII oocyte count with 95% confidence intervals (CIs). Marginal standardization estimated adjusted MII oocyte counts at the 25th, 50th, and 75th percentiles of E2, corresponding to 1,472, 2,507, and 4,826 pg/mL, respectively.For each target value, we set every patient's E2 concentration to that value while retaining the observed values of all other covariates. M2S then generated an individual predicted MII oocyte count, and predictions were averaged across all 869 patients.We calculated adjusted absolute differences between the 75th and 25th percentiles and between each percentile and the median E2 concentration. The 95% CIs and two-sided P values were obtained from 8,000 parameter draws from the asymptotic multivariate normal distribution of the estimated coefficients (Table 4).

Temporal replication and sensitivity analyses

For temporal replication, the same M2S specification was fitted separately to the 2022–2024 and 2025 data. In the full cohort, we added interactions between the period indicator (2025 versus 2022–2024) and both RCS basis functions. A likelihood ratio test jointly evaluated whether the complete E2–MII association curve differed between periods.Likelihood ratio tests were used for negative binomial sensitivity analyses, whereas the robust Poisson analysis used a joint Wald test. All sensitivity analyses retained the fixed RCS knots and representative P25 and P75 E2 values. The 95% CIs were obtained from 5,000 parameter draws (Table 5).All tests were two-sided, with $P < 0.05$ used as the threshold for statistical evidence. Effect estimates are reported with 95% CIs. Analyses were performed in R version 4.6.1.

Results

Characteristics of the study population

The analysis included 869 patients, each contributing one eligible ICSI oocyte retrieval cycle. The numbers treated in 2022, 2023, 2024, and 2025 were 82, 267, 271, and 249, respectively.Mean age was 35.23 ± 5.29 years, and mean BMI was 22.95 ± 3.47 kg/m². Median AMH was 2.35 ng/mL (interquartile range, 1.03–4.10). Overall, 95 patients (10.9%) had PCOS.Starting Gn preparations

were FSH alone in 507 patients (58.3%), HMG alone in 141 (16.2%), and HMG combined with FSH in 221 (25.4%). GnDoseZ had a mean of 0.00 and standard deviation of 1.00, as expected after within-class standardization.Median trigger-day serum E2 was 2,507 pg/mL (interquartile range, 1,472–4,826). Median numbers of oocytes retrieved and MII oocytes were 11 (5–19) and 9 (4–16), respectively. Median MII oocyte maturation rate was 85.7% (75.0%–100.0%) (Table 1).

Table 1. Characteristics of the study cohort

Variable	Overall (N = 869)
Age, years	35.23 ± 5.29
Body mass index, kg/m²	22.95 ± 3.47
Anti-Müllerian hormone, ng/mL	2.35 [1.03, 4.10]
Antral follicle count	12 [6, 19]
Polycystic ovary syndrome	
No	774 (89.1%)
Yes	95 (10.9%)
Ovarian stimulation protocol	
Long GnRH agonist protocol	745 (85.7%)
GnRH antagonist protocol	52 (6.0%)
Progestin-primed ovarian stimulation	33 (3.8%)
Gn-only protocol	39 (4.5%)
Starting Gn class	
FSH alone	507 (58.3%)
HMG alone	141 (16.2%)
HMG + FSH	221 (25.4%)
GnDoseZ (starting dose standardized within Gn class)	0.00 ± 1.00
Starting Gn dose: FSH alone, IU	225 [150, 225]
Starting Gn dose: HMG alone, IU	300 [225, 300]
Starting Gn dose: HMG + FSH, IU	300 [300, 300]
Total Gn dose, IU	2700 [1875, 3300]
Duration of Gn stimulation, days	11 [10, 12]
Trigger agent	
GnRH agonist	66 (7.6%)
Recombinant hCG (Ovitrelle)	803 (92.4%)
Treatment year	
2022	82 (9.4%)
2023	267 (30.7%)
2024	271 (31.2%)
2025	249 (28.7%)
Number of 10.0–14.0-mm follicles	4 [2, 7]
Number of >14.0–16.0-mm follicles	2 [1, 4]
Number of >16.0–18.0-mm follicles	2 [1, 4]
Number of >18.0–20.0-mm follicles	3 [2, 5]
Number of >20.0-mm follicles	1 [0, 1]

Variable	Overall (N = 869)
Trigger-day serum E2, pg/mL	2507 [1472, 4826]
Number of oocytes retrieved	11 [5, 19]
MII oocyte count	9 [4, 16]
MII oocyte maturation rate, %	85.7 [75.0, 100.0]

Note: Approximately normally distributed continuous variables are presented as mean ± standard deviation, skewed continuous variables as median [interquartile range], and categorical variables as n (%). The MII oocyte maturation rate was calculated as MII oocyte count divided by the number of oocytes retrieved.

Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; E2, estradiol; FSH, follicle-stimulating hormone; Gn, gonadotropin; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; HMG, human menopausal gonadotropin; MII, metaphase II; PCOS, polycystic ovary syndrome.

Primary association between E2 and MII oocyte count

The AIC values for M1, M2L, and M2S were 4,719.75, 4,581.97, and 4,535.26, respectively. Compared with M1, adding E2 as an RCS in M2S improved model fit (LR χ^2 = 188.49, df = 2, P < 0.001). This result provided evidence of an overall E2–MII association beyond the five follicle-size categories and other prespecified covariates.M2S also improved model fit relative to M2L (LR χ^2 = 48.72, df = 1, P < 0.001), indicating that a constant linear effect did not adequately summarize the E2–MII association. The Pearson dispersion statistic for M2S was 0.881, with no evidence of residual overdispersion (Table 2A and B).

In M2L, the average IRR for $\log_2$(E2) was 1.301 (95% CI, 1.249–1.356; P < 0.001). Under the linear specification, a twofold higher E2 concentration was associated with a 30.1% higher expected MII oocyte count. Because the association was nonlinear, this IRR is an average across the observed E2 range and should not be interpreted as constant within every interval (Table 2C).

In the fully adjusted M2S model, follicle counts in the 10.0–14.0, >14.0–16.0, >16.0–18.0, and >18.0–20.0 mm categories were positively associated with MII oocyte count. The >20.0 mm follicle count was not clearly associated with the outcome (P = 0.141). HMG alone was associated with a lower expected MII oocyte count than FSH alone after covariate adjustment (Table 3).

Table 2. Overall and nonlinear associations of trigger-day serum E2 with MII oocyte count and model fit

A. Overall association and test for nonlinearity

Model comparison	LR χ^2	df	P value
M1 vs M2S: overall association of E2	188.49	2	<0.001
M2L vs M2S: nonlinearity of E2	48.72	1	<0.001

B. Model fit and comparison

Model	N	No. of parameters	Log-likelihood	AIC	ΔAIC vs M1	Pearson dispersion	LR comparison	LR P value
M1	869	20	−2339.87	4719.75	0.00	0.869	—	—
M2L	869	21	−2269.99	4581.97	−137.77	0.887	M2L vs M1: χ^2 = 139.77, df = 1	<0.001
M2S	869	22	−2245.63	4535.26	−184.49	0.881	M2S vs M2L: χ^2 = 48.72, df = 1	<0.001

C. Average effect in the simplified linear model

Measure	β	SE	IRR	95% CI	P value
Average effect of $\log_2$(E2) in M2L	0.263	0.021	1.301	1.249–1.356	<0.001

Note: M1 excluded E2. M2L added $\log_2$(E2) as a linear term to M1, whereas M2S added $\log_2$(E2) using a three-knot restricted cubic spline. The IRR from M2L summarizes the average association per twofold increase in E2. Interpretation of the primary association was based on the M2S curve and adjusted absolute estimates.

Abbreviations: AIC, Akaike information criterion; CI, confidence interval; E2, estradiol; IRR, incidence rate ratio; LR, likelihood ratio; MII, metaphase II; RCS, restricted cubic spline; SE, standard error.

Table 3. Fully adjusted results from the primary negative binomial regression model (M2S)

Variable	Unit of comparison or reference	Adjusted IRR	95% CI	P value
Number of 10.0–14.0-mm follicles	Per 1-follicle increase	1.025	1.017–1.032	<0.001
Number of >14.0–16.0-mm follicles	Per 1-follicle increase	1.037	1.025–1.050	<0.001
Number of >16.0–18.0-mm follicles	Per 1-follicle increase	1.038	1.024–1.052	<0.001
Number of >18.0–20.0-mm follicles	Per 1-follicle increase	1.052	1.037–1.067	<0.001
Number of >20.0-mm follicles	Per 1-follicle increase	1.018	0.994–1.042	0.141
Age	Per 1-year increase	0.999	0.993–1.005	0.777
Body mass index	Per 1-kg/m² increase	1.009	1.000–1.017	0.041
ln(AMH)	Per 1-unit increase	1.041	0.991–1.094	0.108
PCOS: no	Reference	1.000	Reference	—
PCOS: yes	Yes vs no	0.920	0.845–1.001	0.054
Long GnRH agonist protocol	Reference	1.000	Reference	—
GnRH antagonist protocol	Vs long GnRH agonist protocol	0.936	0.808–1.084	0.378
Progestin-primed ovarian stimulation	Vs long GnRH agonist protocol	0.790	0.573–1.089	0.150
Gn-only protocol	Vs long GnRH agonist protocol	0.751	0.593–0.952	0.018
Starting Gn class: FSH alone	Reference	1.000	Reference	—
Starting Gn class: HMG alone	Vs FSH alone	0.806	0.724–0.896	<0.001
Starting Gn class: HMG + FSH	Vs FSH alone	0.937	0.876–1.003	0.061
GnDoseZ	Per 1-SD higher within Gn class	1.024	0.994–1.055	0.122
Treatment year: 2022	Reference	1.000	Reference	—
Treatment year: 2023	Vs 2022	1.000	0.908–1.101	0.998
Treatment year: 2024	Vs 2022	0.928	0.841–1.023	0.134
Treatment year: 2025	Vs 2022	1.010	0.914–1.115	0.850

Note: The outcome was MII oocyte count. For continuous variables, the incidence rate ratio is interpreted per unit shown; for categorical variables, it is interpreted relative to the stated reference group. Trigger-day serum E2 was modeled as $\log_2$(E2) using a three-knot restricted cubic spline. Spline-basis coefficients are not shown because they are not directly interpretable.

Abbreviations: AMH, anti-Müllerian hormone; CI, confidence interval; FSH, follicle-stimulating hormone; Gn, gonadotropin; GnRH, gonadotropin-releasing hormone; HMG, human menopausal gonadotropin; IRR, incidence rate ratio; MII, metaphase II; PCOS, polycystic ovary syndrome; SD, standard deviation.

Adjusted MII oocyte count at representative E2 levels

Marginal standardization yielded adjusted MII oocyte counts of 7.71 (95% CI, 7.28–8.18), 10.29 (95% CI, 9.90–10.73), and 12.70 (95% CI, 12.30–13.16) at P25, P50, and P75 of E2, respectively. These percentiles corresponded to 1,472, 2,507, and 4,826 pg/mL.The adjusted absolute P75–P25 difference was 4.99 MII oocytes (95% CI, 4.33–5.69; P < 0.001). Relative to median E2, the adjusted differences at P25 and P75 were −2.58 (95% CI, −2.92 to −2.25; P < 0.001) and 2.41 MII oocytes (95% CI, 1.96–2.87; P < 0.001), respectively. The slope of the positive E2–MII association varied across the observed E2 range (Table 4 and Figure 1A).

Adjusted number of oocytes retrieved and MII oocyte maturation rate at representative E2 levels

E2 was associated with the number of oocytes retrieved overall (LR χ^2 = 203.28, df = 2, P < 0.001), with evidence of nonlinearity (LR χ^2 = 50.68, df = 1, P < 0.001). Adjusted numbers at P25, P50, and P75 were 9.49 (95% CI, 9.03–10.02), 12.45 (95% CI, 12.02–12.93), and 15.22 (95% CI, 14.78–15.71), respectively. The adjusted P75–P25 difference was 5.73 oocytes (95% CI, 4.99–6.50; P < 0.001) (Table 4 and Figure 1B).

In contrast, quasi-binomial regression showed no evidence of an overall association between E2 and the MII oocyte maturation rate (F(2, 848) = 1.03, P = 0.356) or of nonlinearity (F(1, 848) = 0.49, P = 0.485).Adjusted maturation rates at P25, P50, and P75 were 81.80%, 82.94%, and 83.75%, respectively. The adjusted P75–P25 difference was 1.95 percentage points (95% CI, −0.75 to 4.75; P = 0.166) (Table 4 and Figure 1C). Thus, the E2–MII association paralleled variation in the number of oocytes retrieved, whereas no clear association with maturation efficiency was observed.

Table 4. Adjusted outcomes at representative trigger-day serum E2 levels

A. Marginally standardized absolute estimates and representative contrasts

Outcome	E2 level	E2, pg/mL	Adjusted estimate	95% CI	Difference from median	95% CI for difference	P for difference	P values for overall association/nonlinearity
MII oocyte count	P25	1472	7.71	7.28–8.18	−2.58	−2.92 to −2.25	<0.001	Overall <0.001; nonlinear <0.001
MII oocyte count	P50 (median)	2507	10.29	9.90–10.73	Reference	—	—	
MII oocyte count	P75	4826	12.70	12.30–13.16	2.41	1.96–2.87	<0.001	
MII oocyte count	P75 vs P25	4,826 vs 1,472	—	—	4.99	4.33–5.69	<0.001	
Number of oocytes retrieved	P25	1472	9.49	9.03–10.02	−2.96	−3.32 to −2.59	<0.001	Overall <0.001; nonlinear <0.001
Number of oocytes retrieved	P50 (median)	2507	12.45	12.02–12.93	Reference	—	—	
Number of oocytes retrieved	P75	4826	15.22	14.78–15.71	2.77	2.29–3.26	<0.001	
Number of oocytes retrieved	P75 vs P25	4,826 vs 1,472	—	—	5.73	4.99–6.50	<0.001	
MII oocyte maturation rate, %	P25	1472	81.80%	79.62–83.48%	−1.14 percentage points	−2.79 to 0.42 percentage points	0.156	Overall 0.356; nonlinear 0.485
MII oocyte maturation rate, %	P50 (median)	2507	82.94%	81.23–84.16%	Reference	—	—	
MII oocyte maturation rate, %	P75	4826	83.75%	81.75–85.15%	0.81 percentage points	−0.64 to 2.27 percentage points	0.262	
MII oocyte maturation rate, %	P75 vs P25	4,826 vs 1,472	—	—	1.95 percentage points	−0.75 to 4.75 percentage points	0.166	

B. Overall and nonlinear tests for the three outcomes

Outcome	Overall test statistic	Overall P value	Nonlinearity test statistic	P for nonlinearity
MII oocyte count	LR χ^2 = 188.49, df = 2	<0.001	LR χ^2 = 48.72, df = 1	<0.001
Number of oocytes retrieved	LR χ^2 = 203.28, df = 2	<0.001	LR χ^2 = 50.68, df = 1	<0.001
MII oocyte maturation rate	F = 1.03, df = 2,848	0.356	F = 0.49, df = 1,848	0.485

Note: P25 and P75 are representative values within the observed E2 distribution and are not clinical cutoffs. The P75–P25 contrast quantifies the adjusted absolute difference across the interquartile range. MII oocyte count and the number of oocytes retrieved were analyzed using negative binomial regression with likelihood ratio tests. The MII oocyte maturation rate was analyzed using quasi-binomial regression with F tests. Maturation-rate differences are expressed in percentage points.

Abbreviations: CI, confidence interval; E2, estradiol; LR, likelihood ratio; MII, metaphase II; P25, 25th percentile; P50, 50th percentile; P75, 75th percentile.

Temporal replication and sensitivity analyses of the E2–MII association

When M2S was fitted separately to the 2022–2024 and 2025 data, the adjusted P75–P25 differences in MII oocyte count were 5.04 (95% CI, 4.30–5.79; P < 0.001) and 4.57 (95% CI, 3.00–6.18; P < 0.001), respectively (Table 5 and Figure 2).The full-cohort period interaction was not statistically significant (LR χ^2 = 1.51, df = 2, P = 0.471). We therefore found no evidence that the complete E2–MII association curve differed between 2022–2024 and 2025.

Across sensitivity analyses, adjusted P75–P25 differences remained positive and ranged from 3.86 to 5.52 MII oocytes. No 95% CI included zero, and all tests for the overall association and nonlinearity yielded P < 0.001.The adjusted difference was 5.05 without GnDoseZ, 5.02 after additional adjustment for total Gn dose and stimulation duration, and 4.97 when the interaction between Gn class and the original starting dose was modeled. Additional adjustment for trigger agent also yielded a difference of 4.97. These estimates were close to the primary estimate of 4.99 MII oocytes.After excluding patients with PCOS, the adjusted difference decreased to 3.86 MII oocytes, but its direction was unchanged. Robust Poisson regression yielded an adjusted difference of 5.52 MII oocytes. Overall, findings remained consistent across analyses addressing treatment year, Gn dose, ovarian reserve markers, trigger agent, extreme E2 values, and count-model specification (Table 5).

Table 5. Temporal replication and sensitivity analyses of the association between trigger-day serum E2 and MII oocyte count

A. Temporal replication and sensitivity analyses

Analysis	N	P75–P25 adjusted difference in MII oocyte count	95% CI	P value for difference	P value for overall E2 association	P value for nonlinearity
Full cohort, M2S (primary analysis)	869	4.99	4.33–5.69	<0.001	<0.001	<0.001
2022–2024	620	5.04	4.30–5.79	<0.001	<0.001	<0.001
2025	249	4.57	3.00–6.18	<0.001	<0.001	<0.001
Excluding 2022	787	4.93	4.23–5.67	<0.001	<0.001	<0.001
Without GnDoseZ	869	5.05	4.37–5.73	<0.001	<0.001	<0.001
AFC substituted for AMH	869	5.01	4.34–5.70	<0.001	<0.001	<0.001
Additional adjustment for total Gn dose and duration of stimulation	869	5.02	4.35–5.71	<0.001	<0.001	<0.001
Gn class × original Gn starting dose	869	4.97	4.31–5.68	<0.001	<0.001	<0.001
Excluding PCOS	774	3.86	3.15–4.57	<0.001	<0.001	<0.001
Additional adjustment for trigger agent	869	4.97	4.29–5.66	<0.001	<0.001	<0.001
Excluding the lowest and highest 1% of E2	851	4.97	4.27–5.71	<0.001	<0.001	<0.001
Robust Poisson regression (HC3)	869	5.52	4.56–6.48	<0.001	<0.001	<0.001

B. Period interaction test

Test	Interaction term	LR χ^2	df	P value	Interpretation
Interaction: 2025 vs 2022–2024	RCS linear and nonlinear basis functions × period (joint test)	1.51	2	0.471	Tests whether the entire E2–MII association curve differs between the two periods

Note: In each analysis, the effect measure was the marginally standardized absolute difference in MII oocyte count for E2 at P75 (4,826 pg/mL) versus P25 (1,472 pg/mL). The same representative E2 values and RCS knot locations were used throughout. The overall P value jointly tests the linear and nonlinear E2 terms. The P value for nonlinearity tests the nonlinear term, and the interaction test evaluates whether the complete E2–MII curve differed between 2022–2024 and 2025.

Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; CI, confidence interval; E2, estradiol; Gn, gonadotropin; GnDoseZ, starting Gn dose standardized within Gn class; HC3, heteroskedasticity-consistent type 3 covariance estimator; MII, metaphase II; PCOS, polycystic ovary syndrome; RCS, restricted cubic spline.

Discussion

Trigger-day serum E2 showed a nonlinear association with MII oocyte count. After adjustment for five follicle-size categories and prespecified covariates, adding E2 improved model fit. The adjusted P75–P25 difference was 4.99 MII oocytes. A similar pattern was observed for the number of oocytes retrieved, whereas the MII oocyte maturation rate showed no clear association with E2.

E2 is synthesized primarily by follicular granulosa cells and contributes to follicular growth and maturation [10]. Trigger-day ultrasonography captures follicle number and diameter, whereas follicular function also reflects granulosa cell differentiation, steroidogenesis, and the local microenvironment [10,11]. Patients with similar follicle-size distributions may therefore have different E2 concentrations. The E2–MII association after adjustment for follicle-size distribution may reflect functional differences beyond follicle number and size. However, follicular-fluid hormones, granulosa cell function, and individual follicle–oocyte pairing were unavailable. This mechanistic interpretation remains hypothetical and cannot be established from these data.

Previous studies generally reported positive correlations between trigger-day E2 and the numbers of oocytes retrieved and MII oocytes. Godoy Morales et al., Malathi et al., and Chopra et al. found that higher E2 concentrations accompanied greater oocyte retrieval or MII oocyte counts [6,7,12]. These studies mainly used correlation analyses or retrospective category-based comparisons and did not fully account for follicle-size distribution. In natural-cycle IVF, Helmer et al. found that E2 remained associated with oocyte retrieval and MII outcomes after accounting for follicle diameter [4].The present study extends this evidence by modeling five follicle-size categories simultaneously, using negative binomial regression for count outcomes, and allowing a nonlinear E2–MII association. The key finding is not simply that higher E2 accompanied more MII oocytes. Rather, E2 remained associated with MII oocyte count after accounting for the complete size distribution of follicles ≥10 mm.

Follicle-size distribution itself remained associated with MII oocyte count. Bosdou et al. reported that counts of follicles ≥16 mm and ≥18 mm were informative for estimating minimum numbers of oocytes retrieved and MII oocytes, respectively[14] . In a multicenter explainable artificial intelligence analysis, Hanassab et al. found that intermediate-sized follicles contributed most to MII oocyte count[15] . Yang et al. further suggested that the optimal follicle-size distribution may vary with age[3].We entered counts from all five follicle-size categories simultaneously rather than grouping patients by the proportion of follicles in one category. This approach accounted for the complete cohort of follicles ≥10 mm. Counts in all categories from 10.0 to 20.0 mm were positively associated with MII oocyte count, whereas the >20.0 mm category was not statistically significant. This result does not imply that follicles >20.0 mm lack clinical value. Because all categories were modeled together, each coefficient represents the conditional association of one additional follicle in that category while all other category counts remain constant. It does not represent the overall contribution of that follicle-size category.

The E2–MII association primarily paralleled variation in the number of oocytes retrieved rather than the MII oocyte maturation rate. Between P25 and P75 of E2, the adjusted difference was 5.73 oocytes retrieved. By contrast, the maturation-rate difference was 1.95 percentage points, and its confidence interval included zero. The higher MII oocyte count at higher E2 concentrations may therefore reflect a greater total number of oocytes retrieved. It does not necessarily indicate a higher maturation probability for each retrieved oocyte.Previous studies suggested that Gn dose and trigger protocol may be associated with the number of oocytes

retrieved, MII oocyte count, or maturation-related outcomes. Findings, however, varied across studies and outcomes[9,16-20]. Our results therefore do not support interpreting higher E2 as evidence of superior oocyte maturation competence or oocyte quality.

E2 should complement, rather than replace, ultrasonographic assessment of follicle number and size. It should not be used alone to determine trigger timing. Previous studies combined patient characteristics, ovarian stimulation medications, follicle sizes, and E2 in tools for predicting MII oocyte count or optimal trigger timing [13,21]. Their clinical benefit requires further validation.The European Society of Human Reproduction and Embryology guideline on ovarian stimulation does not recommend routinely adding E2 measurement to ultrasound monitoring as a universal strategy [22]. Our study therefore characterizes the conditional E2–MII association in completed ovarian stimulation cycles. It does not show that increasing E2 would increase MII oocyte count, and it does not support changing Gn dose or delaying triggering based solely on E2.

Several limitations warrant consideration. First, the retrospective single-center design could not exclude unmeasured confounding, and the findings represent conditional associations rather than causal effects. We included only cycles that proceeded to triggering and completed oocyte retrieval. The findings may not generalize to cycles canceled because of extremely poor ovarian response, a premature luteinizing hormone (LH) surge, or other reasons.Second, the first cycle was defined as the first eligible ICSI cycle recorded in the study database. We could not confirm that patients had never received assisted reproductive treatment before the study period. Follicles <10 mm were not recorded, and individual follicles could not be paired with their corresponding oocytes. Ultrasonographic measurement error may also have affected the results.Only one trigger-day serum E2 measurement was available, precluding evaluation of longitudinal changes. Other trigger-day hormones, including LH and progesterone, were not analyzed. We also did not evaluate fertilization, embryo development, cumulative live birth, or ovarian hyperstimulation syndrome (OHSS). Finally, the 2025 analysis was a temporal replication within the same center and cannot replace independent external validation.

Conclusion

After adjustment for the complete size distribution of follicles ≥10 mm and prespecified covariates, trigger-day serum E2 was nonlinearly associated with MII oocyte count. This association paralleled variation in the number of oocytes retrieved, whereas no clear association was observed with the MII oocyte maturation rate.Trigger-day serum E2 may provide information complementary to ultrasonographic follicle assessment when estimating MII oocyte count. These findings do not indicate that increasing E2 would increase the number of mature oocytes, and E2 should not be used alone to determine trigger timing.Prospective multicenter studies incorporating longitudinal E2 measurements, follicle–oocyte pairing, and cumulative live-birth outcomes are needed to validate these findings.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Qinzhou Maternal and Child Health Hospital (approval No. [QSFYL[2026]08002]). The requirement for written informed consent was waived by the Ethics Committee because of the retrospective nature of the study and the use of anonymized clinical data. All procedures were performed in accordance with the Declaration of Helsinki.

Consent for publication

N/A

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available because they contain clinical information from patients, but are available from the corresponding author on reasonable request and with permission from Qinzhou Maternal and Child Health Hospital.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

J.H. and J.P. contributed equally to this work. J.H. and J.P. conceived and designed the study. J.H. and J.P. collected and organized the clinical data. J.H. and L.S. performed the statistical analyses and prepared the tables and figures. J.H. drafted the main manuscript text. H.W. supervised the study and critically revised the manuscript. All authors reviewed and approved the final manuscript.

Authors' information

N/A

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Figures

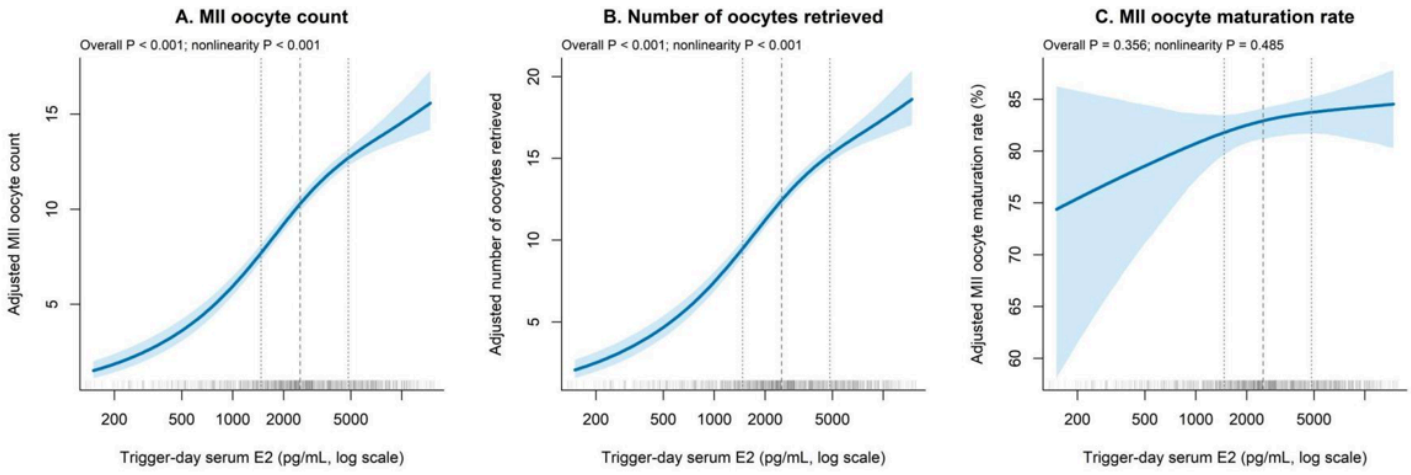


Figure 1

Adjusted associations of trigger-day serum E2 with the three study outcomes.Solid lines show marginally standardized estimates, and shaded bands show 95% confidence intervals. The x-axis displays E2 in the original clinical unit (pg/mL) on a logarithmic scale. Vertical lines mark P25, P50, and P75 of E2 and do not represent clinical cutoffs. Panels A and B were estimated using negative binomial regression; panel C was estimated using quasi-binomial regression.

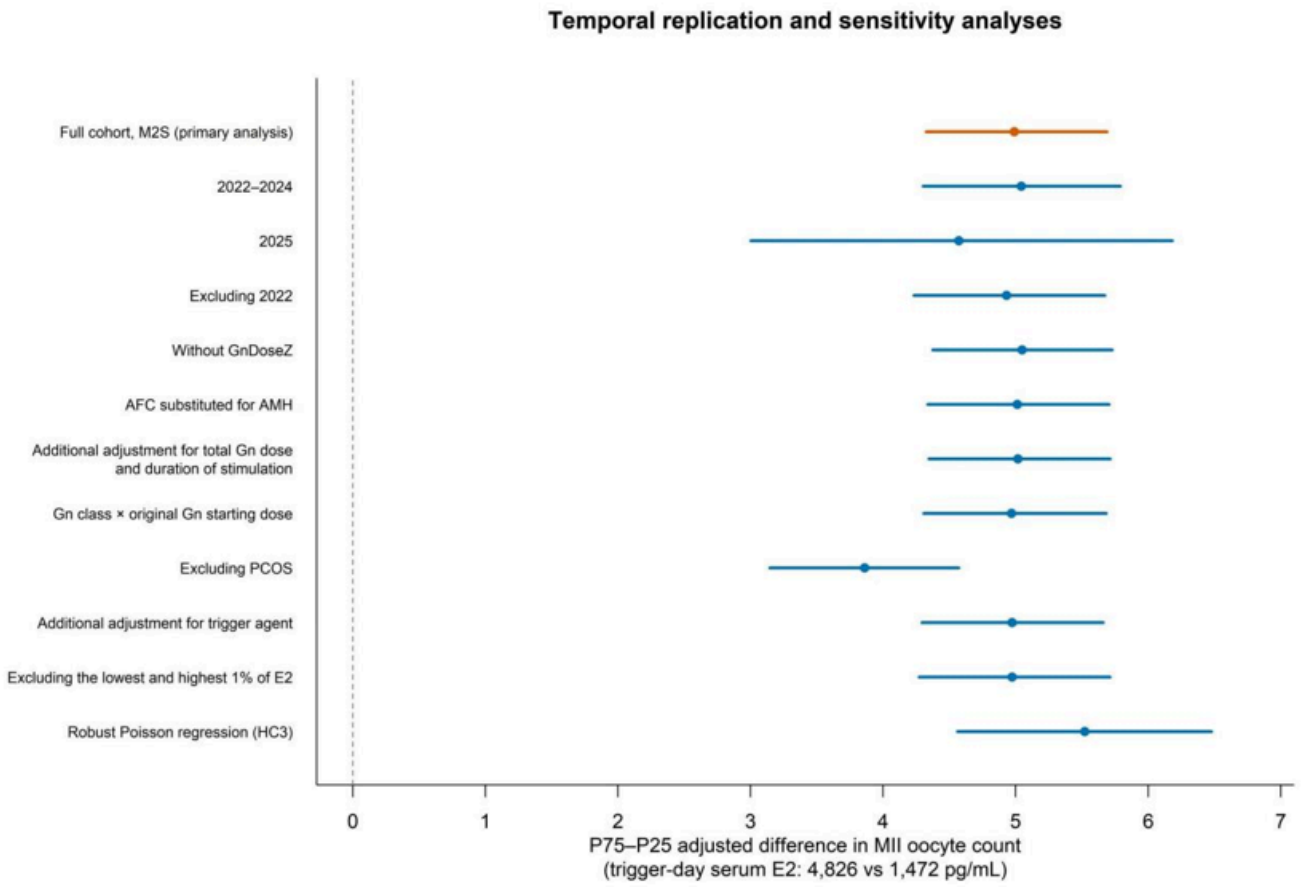


Figure 2

Temporal replication and sensitivity analyses of the association between trigger-day serum E2 and MII oocyte count.Points show marginally standardized P75–P25 differences in MII oocyte count, comparing E2 concentrations of 4,826 and 1,472 pg/mL. Horizontal lines show 95% confidence intervals. Orange denotes the primary analysis, and blue denotes temporal replication and sensitivity analyses.