Association between serum estradiol level decline in the days preceding ovulatory trigger and assisted reproductive technology outcomes

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Objective: To study whether a decline in serum estradiol level in the days leading up to the ovulatory trigger in gonadotropin-releasing hormone antagonist ovarian stimulation cycles impacts cycle outcomes.
Design: Retrospective cohort study.
Subjects: Patients undergoing autologous retrieval cycles using a gonadotropin-releasing hormone antagonist protocol between 2010 and 2021 at a multicenter infertility practice network in the United States. Patients were excluded if an estradiol level was not measured on the 4 days before the ovulatory trigger or if they had conventional insemination.
Exposure: Patients with a documented decrease of at least 1 pg/mL in serum estradiol on any of the 4 days before ovulatory trigger. The referent group experienced a consistent rise in serum estradiol each day before the trigger.
Main Outcome Measures: The primary outcome was the number of usable blastocysts, defined as the sum of blastocysts used for fresh transfer (if any) and the remaining cryopreserved blastocysts. Secondary outcomes included oocyte maturity, fertilization, ≥ 1 usable blastocyst, positive human chorionic gonadotropin, biochemical pregnancy, spontaneous abortion, clinical pregnancy, and live birth.
Results: A total of 6,945 cycles met the inclusion criteria. Among this cohort, 5,653 demonstrated a consistent rise in serum estradiol, 1,103 cycles experienced one drop, and 189 cycles experienced 2–4 drops in estradiol during the 4 days preceding the ovulatory trigger. After adjusting for antimüllerian hormone and female age, there was no difference in the average number of usable blastocysts between the referent group (4.9 ± 4.0) and cycles with a single drop in estradiol (5.2 ± 4.8) (mean ratio 1.04, 95% confidence interval [CI] 0.97–1.10) and those with 2–4 drops in estradiol (mean ratio 0.98, 95% CI 0.85–1.14). There were no statistical differences in any of the secondary outcomes, including live birth. Live birth was observed at 41.1% for the consistent rise group, 36.8% for the one-drop group (risk ratio 0.91, 95% CI 0.81–1.02), and 42.1% for the 2–4 drops group (risk ratio 0.98, 95% CI 0.74–1.28).
Conclusion: The findings underscore that a decline in serum estradiol in the 4 days before the ovulatory trigger does not compromise oocyte quality. Patients with an estradiol decline preceding oocyte retrieval had comparable outcomes to those with a continued rise in estradiol. (Fertil Steril® 2026;126:86–95. ©2026 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Estradiol decline, GnRH antagonist ovarian stimulation, usable blastocysts, live birth

During the in vitro fertilization (IVF) process, the ovarian follicular response is monitored with interval transvaginal ultrasounds and serum hormonal levels of estradiol and progesterone to assess follicular development (1). The integration of multiple clinical datapoints over the course of an IVF cycle is used to evaluate a patient's response to controlled ovarian hyperstimulation with the goal of a successful oocyte retrieval (1). Serum estradiol is typically measured at the beginning of the IVF cycle to establish the baseline hormonal

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level. It is subsequently measured during each monitoring appointment to evaluate follicular growth, based on the assumption that increasing estradiol levels are reflective of sufficient gonadotropin levels and continued healthy follicular development (2). Despite its widespread use, the role of serum estradiol trends in estimating IVF cycle success remains incompletely understood. Unfavorable estradiol trends have been cited as a potential factor contributing to the risk of a poor cycle outcome and may result in cycle cancellation (1–5).

During an ovarian stimulation cycle, the level of serum estradiol can decrease before ovulatory trigger, with previous literature noting a decline in estradiol immediately after antagonist administration (1, 5). In 2005, Shapiro et al. (5) analyzed serum estradiol patterns from the day of antagonist initiation before its administration to the day after the second dose in 225 cycles involving both normal and poor prognosis patients. They found that changes in estradiol levels immediately after antagonist administration were not predictive of IVF cycle success (5). The study observed comparable implantation rates across groups with either a decline, plateau, or rise in serum estradiol (5). Based on these findings, the investigators concluded that frequent monitoring of serum estradiol during antagonist cycles may be unnecessary, as estradiol trends do not appear to significantly influence cycle success (5). In contrast, Lindheim and Morales (1) reported that a decrease in estradiol levels in closer proximity to the ovulatory trigger negatively impacted IVF outcomes, with cycles showing a decline in estradiol levels associated with lower implantation and pregnancy rates. However, the findings of the study were based on 40 donor oocyte cycles, lacked a standardized definition of estradiol decline, and included variable start dates after the cessation of combined oral contraceptives (1). Altogether, the current body of literature is limited by the lack of large-scale studies that evaluate the clinical significance of serum estradiol drops between the initiation of the gonadotropin-releasing hormone (GnRH) antagonist and the ovulatory trigger, highlighting the need for further research to clarify their association with cycle outcomes.

Given the gap in the literature, our study aims to evaluate whether a decline in serum estradiol level in the days leading up to the ovulatory trigger in GnRH antagonist ovarian stimulation cycles is related to cycle outcomes. We hypothesized that there is no difference in the number of usable blastocysts in patients with a decrease in serum estradiol levels compared with those with a consistent increase in estradiol levels in the days preceding the ovulatory trigger. We anticipate these results will inform whether estradiol trends are meaningfully associated with IVF outcomes independently of other risk factors and should be considered when making decisions regarding cycle cancellation and patient counseling.

MATERIALS AND METHODS

Study design

This was a retrospective cohort study including all patients undergoing their initial autologous retrieval cycles using a GnRH antagonist protocol between 2010 and 2021 at a multicenter infertility practice network in the United States. The first ovarian stimulation with oocyte retrieval was included for each patient, and therefore each patient was only included once in the study. Patients were excluded if they did not have an estradiol level measured on the 4 days before the ovulatory trigger (including the trigger day) because of an inability to classify such patients into the correct estradiol trend group. For patients in the study cohort who underwent a fresh embryo transfer, the pregnancy outcomes were additionally included to evaluate the association between the estradiol change before the ovulatory trigger and fresh embryo transfer outcomes. The study group was comprised of patients with a documented decrease of at least 1 pg/mL in serum estradiol and was further separated into how many drops in estradiol that were appreciated. The study group was compared with the referent group, which consisted of patients experiencing a consistent rise in serum estradiol each day over the same timeframe. The median rise as well as the average percent rise of estradiol between days leading up to the ovulatory trigger for the reference group is shown in Supplemental Table 1 (available online). Demographics, cycle characteristics, and cycle and pregnancy outcomes were obtained through an electronic medical database. This study received institutional review board approval (IRB Pro00058669).

Study population

Inclusion criteria for this study were patients undergoing a gonadotropin-stimulated antagonist cycle who had serum estradiol levels measured on each of the 4 days preceding the ovulatory trigger. Only cycles utilizing intracytoplasmic sperm injection were included. Exclusion criteria included use of aromatase inhibitors or selective estrogen receptor modulators such as letrozole or clomiphene citrate, use of nonantagonist stimulation protocols, cycles not proceeding with oocyte retrieval, or incomplete estradiol monitoring in the final 4 days of stimulation. These criteria ensured that all participants had complete late-follicular phase estradiol data and underwent comparable stimulation protocols. Additionally, patients were excluded if conventional fertilization was used to reduce heterogeneity and avoid introducing unmeasured confounding.

Study definitions

Estradiol decline, drop, and decrease all refer to any cycle in which there was at least one instance of a ≥ 1 pg/mL decrease in serum estradiol during the 4 days preceding oocyte retrieval. In contrast, a consistent rise refers to the reference group, defined as cycles in which estradiol levels increased each day over the same 4-day period.

The primary outcome was the number of usable blastocysts, defined as the sum of blastocysts used for fresh transfer (if any) and the remaining blastocysts that were cryopreserved. Embryos were considered for cryopreservation if they developed into usable blastocysts on either day 5, 6, or 7 after fertilization. Usable blastocysts were defined as having an expansion grade of 4, 5, or 6, an inner cell mass score of A or B, and a trophectoderm grade of A, or B using

the simplified Society for Assisted Reproductive Technology grading scale. Embryos graded AA or AB were categorized as good and BA or BB as fair (6).

Secondary outcomes included oocyte maturity rate, fertilization rate, ≥1 usable blastocyst, positive human chorionic gonadotropin (hCG), chemical pregnancy, spontaneous abortion, clinical pregnancy, and live birth. Oocyte maturity rate was defined as the number of mature oocytes divided by the number of oocytes retrieved. Fertilization rate was defined as the number of normally fertilized oocytes (2 pronuclei [2PN]) divided by the number of mature oocytes. More than or equal to one usable blastocyst was defined as having at least one blastocyst that was suitable for transfer. Usable blastocyst rate was defined as the number of usable blastocysts divided by the number of 2PN. A positive hCG result was characterized as an hCG level >5 mIU/mL confirmed through serum testing, or a positive result from a home pregnancy test 10 days after fresh embryo transfer. Chemical pregnancy was defined as an initial positive hCG level with a subsequent hCG level below 5 mIU/mL before a pregnancy could be visualized via ultrasound (7). Clinical pregnancy was defined as the presence of an intrauterine pregnancy, confirmed by the detection of at least one gestational sac on ultrasound. Spontaneous abortion was defined as the unintentional loss of a clinical pregnancy before completing 22 weeks of gestation (7). Live birth was defined as delivery of a viable fetus after 22 weeks of completed gestation (7).

Clinical protocols

All cycles included involved autologous oocyte retrieval, which was obtained via controlled ovarian stimulation with injectable gonadotropins. Patients were treated with a GnRH antagonist for suppression of luteinizing hormone release. The GnRH antagonist was started when the lead follicle was ≥14 mm in size or the estradiol level was >400 pg/mL (8). Patients were monitored with serial transvaginal ultrasound and serum estradiol and progesterone levels, which were obtained using a standard electrochemiluminescence immunoassay using the Roche Cobas Pro e801 analyzer. The ovulatory trigger injection was administered once the lead follicles were ≥17–18 mm. The ovulatory trigger was either hCG, leuprolide acetate, or a combination of both (dual trigger), as determined by the patient's reproductive history and provider discretion. Ultrasound-guided transvaginal oocyte retrieval was conducted 35–36 hours after the ovulatory trigger injection. Mature oocytes were then fertilized using intracytoplasmic sperm injection, and a fertilization evaluation was performed 16–18 hours later. Fertilized oocytes were then placed into extended culture media and monitored up to 7 days after retrieval and vitrified if they developed into usable blastocysts on either day 5, 6, or 7 after fertilization.

Patients were candidates for a fresh embryo transfer if their serum progesterone was <2 ng/mL on the day of ovulatory trigger or cumulatively <4.5 ng/mL over the last 3 days of stimulation (9, 10). Luteal support was provided with daily intramuscular progesterone in oil or a combination of daily vaginal progesterone and intramuscular progesterone every third day (11). Fresh embryo transfer occurred on day 5 after retrieval with the number of embryos transferred determined by American Society for Reproductive Medicine guidelines (12). Serum hCG levels were then measured 10 days after the embryo transfer. If positive, serial hCG levels were obtained to evaluate for an appropriate rise. Transvaginal ultrasound was then performed between 5 and 6 weeks of gestation to confirm an intrauterine pregnancy, followed by a repeat ultrasound 1–2 weeks later. If normal gestational development was observed, patients were discharged to their obstetrician at approximately 8 weeks of gestation.

Statistical analysis

Patient demographics, baseline characteristics, and cycle outcomes were recorded and compared across categories of estradiol trajectory with means and SDs, medians and interquartile ranges, or N and percents as appropriate.

In the primary analysis, patients were categorized into the referent group if they demonstrated a consistent rise in serum estradiol levels each day over the 4 days leading up to the ovulatory trigger. Patients who experienced at least 1 day with an estradiol drop over this time period were stratified into two groups: those with only one drop and those with 2–4 days of an estradiol drop. Primary and secondary outcomes were compared across estradiol trajectory groups. Mean ratios (for number of oocytes and metaphase II [MII] oocytes retrieved, number of 2PNs and usable embryos, oocyte maturity, fertilization, and usable embryo percentages) and risk ratios (RRs; for transfer outcomes) with 95% confidence intervals (CIs) were generated using Poisson regression models fitted with generalized estimating equations, adjusting a priori for female age and antimüllerian hormone (AMH). These covariates were selected because they represent nonmodifiable patient characteristics that may also confound the association between estradiol drops and outcomes. Given the potential utility of estradiol trajectories in deciding whether to cancel a cycle, we aimed to identify whether estradiol drops were associated with outcomes specifically after accounting for the influence of characteristics that would already be known and fixed for a given patient during an IVF cycle attempt, and therefore would not be useful in determining whether to cancel the cycle.

A subanalysis was conducted to evaluate patients with diminished ovarian reserve, defined as a baseline AMH level <1 ng/mL, and those with optimal ovarian reserve, defined as a baseline AMH level >3 ng/mL. This analysis was performed due to the distinct characteristics of these patient cohorts, as their differing baseline ovarian reserve likely reflects multiple underlying factors that could contribute to a decline in serum estradiol. Again, mean ratios and RR with 95% CI were estimated using Poisson regression models fitted with generalized estimating equations, adjusting a priori for female age. Exploratory analyses using percentage-based thresholds (≥10% or ≥20%) were completed for the primary outcome of the number of usable blastocytes, which is shown in Supplemental Table 2.

RESULTS

Baseline characteristics

A total of 6,945 patients met the inclusion criteria, and therefore 6,945 cycles were included, of which 5,653 demonstrated a consistent rise in serum estradiol levels during the 4 days leading up to the ovulatory trigger. A total of 1,103 cycles experienced exactly one drop in estradiol before trigger on any of the 4 days preceding trigger. As the total number of cycles with 2–4 drops in estradiol was only 189, these groups were consolidated for analysis. The distribution of these 189 cycles included 179 patients with two drops in estradiol, 8 patients with three drops, and two patients with four drops in serum estradiol levels during the 4 days leading up to the ovulatory trigger.

Table 1 describes the baseline characteristics of each cohort. There was no difference in age, body mass index, racial demographics, follicle-stimulating hormone, or luteinizing hormone between any of the estradiol drop groups. Individuals who experienced multiple occurrences of a drop in serum estradiol demonstrated a higher AMH at baseline, lower estradiol level on the day of ovulatory trigger, and a lower total dose of gonadotropins.

Cycle outcomes

The cycle outcomes for the total cohort are described in Table 2. In the unadjusted analysis the number of usable embryos was increased as the number of estradiol drops increased (mean ± SD: consistent rise: 4.9 ± 4.0, 1 drop: 5.2 ± 4.8, 2–4 drops: 5.9 ± 4.3; unadjusted *P* value for trend = .0007). After adjusting for AMH and female age, this association was no longer present (mean ratio, 1 drop vs. consistent rise: 1.04, 95% CI 0.97–1.10; mean ratio, 2–4 drops vs. consistent rise: 0.98, 95% CI 0.85–1.14; adjusted *P* value for trend = 0.58). The proportion of patients with ≥1 usable embryo was similarly comparable across all estradiol patterns (94.7% for consistent rise, 94.2% for 1 drop [RR 0.99, 95% CI 0.97–1.01], and 94.2% for 2–4 drops [RR 0.98, 95% CI 0.94–1.02]). The secondary outcome analysis similarly demonstrated no significant differences between cycles with a continuous rise in estradiol and those with one drop or 2–4 drops. Specifically, the number of oocytes retrieved, number of MII oocytes, number of 2PNs, oocyte maturity rate, fertilization rate, and usable blastocyst rate were comparable across groups.

Table 3 describes the cycle outcomes for patients with a baseline AMH <1 ng/mL. Cycles with a consistent rise in estradiol (n = 637) produced 2.2 ± 1.8 usable blastocysts, whereas cycles with one drop in estradiol (n = 148) produced 2.0 ± 1.9 usable blastocysts (mean ratio: 0.92, 95% CI: 0.78–1.09). There was also no difference between the average number of usable blastocysts between those with a consistent rise and those who had 2–4 drops in serum estradiol (n = 9) (mean ratio 0.94, 95% CI 0.42–2.12). Similarly, the number of patients with at least one usable embryo was comparable between groups (84.8% among consistent rise cycles, 78.4% among cycles with 1 drop [RR 0.93, 95% CI 0.85–1.01], and 55.6% among cycles with 2–4 drops [RR 0.66, 95% CI 0.37–1.17]). However, cycles with a single drop in serum estradiol exhibited a slightly lower average number of oocytes retrieved (mean ratio: 0.85, 95% CI 0.75–0.95) and MII oocytes (mean ratio: 0.84, 95% CI 0.74–0.95). This observation was not observed for the same population experiencing 2–4 drops in serum estradiol. Additionally, there was no difference in the oocyte maturity rate, fertilization rate, or usable blastocyst rate.

The outcomes for the subanalysis of cycles with a baseline AMH >3 ng/mL are also described in Table 3. A total of 2,423 cycles were included in referent group, which was compared with 482 cycles with at least one drop in serum estradiol and 109 cycles with 2–4 drops in serum estradiol.

TABLE 1

Demographic and cycle characteristics between patients with consistent estradiol rise and estradiol decreases.

Characteristics	Consistent rise (n = 5,653)	1 drop (n = 1,103)	2–4 drops (n = 189)
Age (y)[a]	34.6 ± 4.4	34.7 ± 4.4	33.6 ± 4.1
BMI (kg/m^2)[a]	25.7 ± 5.5	25.7 ± 5.7	25.7 ± 5.6
Baseline testing[b]			
FSH (mIU/mL)	6.8 (5.3–8.4)	6.2 (4.6–8.1)	5.6 (4.1–7.0)
LH (mIU/mL)	5.1 (3.6–7.2)	5.1 (3.3–7.3)	5.6 (4.0–8.4)
AMH (ng/mL)	3.1 (1.6–5.5)	3.3 (1.5–6.6)	6.8 (3.5–11.9)
Progesterone (ng/mL)[b]			
Initiation of antagonist	0.4 (0.3–0.7)	0.5 (0.3–0.8)	0.5 (0.3–0.7)
Day of trigger	1.3 (0.9–1.7)	1.1 (0.7–1.6)	0.8 (0.5–1.3)
Estradiol (pg/mL)[b]			
Initiation of antagonist	839 (525–1,176)	1,026.9 (642–1,527)	1,181 (879–1,720)
Day of trigger	3,798 (2,779–4,997)	3,215 (2,288–4,393)	2,728 (1,793–3,916)
Days of ovarian stimulation[a]	10.9 ± 2.1	11.3 ± 2.4	11.4 ± 2.4
Days of antagonist[a]	5.8 ± 2.4	5.9 ± 2.7	6.3 ± 3.8
Total gonadotropin dose (mIU/mL)[b]	2,959.0 (1,933.0–4,575.0)	2,762.5 (1,817.3–4,725.0)	2,037.5 (1,550.0–2,737.5)

Note: AMH = antimüllerian hormone; BMI = body mass index; FSH = follicle-stimulating hormone; LH = luteinizing hormone.
[a] Mean ± SD.
[b] Median (interquartile range [25%–75%]).

Lindner. Estradiol drop before ovulatory trigger. Fertil Steril 2026.

TABLE 2

Oocyte, fertilization, and embryo outcomes, total cohort.

Outcome	Consistent rise (n = 5,653)	1 drop (n = 1,103)	2–4 drops (n = 189)
No. of oocytes retrieved	17.9 ± 10.1	18.0 ± 10.8	20.2 ± 11.5
	Ref	0.98 (0.94–1.02)	0.99 (0.88–1.11)
No. of MII oocytes	13.4 ± 8.1	13.6 ± 8.7	15.2 ± 9.2
	Ref	0.98 (0.94–1.02)	0.99 (0.87–1.12)
No. of 2PNs	10.4 ± 6.8	10.7 ± 7.6	12.0 ± 7.9
	Ref	1.00 (0.95–1.05)	1.00 (0.87–1.14)
No. of usable blastocyst	4.9 ± 4.0	5.2 ± 4.8	5.9 ± 4.3
	Ref	1.04 (0.97–1.10)	0.98 (0.85–1.14)
Oocyte maturity rate (ICSI only)	77.4% (65.8%–87.5%)	77.8% (66.7%–88.2%)	77.8% (64.3%–87.5%)
	Ref	1.00 (0.99–1.02)	1.0 (0.97–1.04)
Fertilization rate (ICSI only)	80.0% (66.7%–90.5%)	81.5% (66.7%–92.0%)	81.5% (65.4%–94.4%)
	Ref	1.01 (0.97–1.05)	1.00 (1.00–1.00)
Usable blastocyst rate	50.0% (30.0%–66.7%)	50.0% (33.3%–66.7%)	50.0% (36.0%–66.7%)
	Ref	1.03 (1.0–1.07)	0.98 (0.90–1.06)
≥1 usable embryo,[a] n (%)	5,356 (94.7%)	1,039 (94.2%)	178 (94.2%)
	Ref	0.99 (0.97, 1.01)	0.98 (0.94, 1.02)

Note: Mean ± SD or median (IQR) with effect estimate given as mean ratio (95% confidence interval). Usable blastocysts: Sum of transferred and cryopreserved blastocysts.
Oocyte maturity rate: Number of mature oocytes/Number of oocytes retrieved.
Fertilization rate: Number of 2PN/Number of mature oocytes.
Usable blastocyst rate: Number of usable blastocysts/Number of 2PN.
Usable embryo defined as a blastocyst suitable for embryo transfer.
ICSI = intracytoplasmic sperm injection; IQR = interquartile range; MII = metaphase II; 2PN = 2 pronuclei.
[a] N (%) with effect estimate as relative risk (95% confidence interval).

Lindner. Estradiol drop before ovulatory trigger. Fertil Steril 2026.

For cycles with a consistent rise in estradiol, the mean number of usable blastocysts was 6.6 ± 4.6. Cycles with one drop in estradiol had a significantly increased mean of 7.3 ± 5.6 usable blastocysts (mean ratio: 1.08 [1.01–1.16]), whereas those with 2–4 drops had a comparable mean of 7.1 ± 4.6 usable blastocysts (mean ratio: 0.99 [0.87–1.12]). However, the number of patients with at least one usable embryo was comparable between groups (97.5% among consistent rise cycles, 97.9% among 1-drop cycles [RR 1.0, 95% CI 0.99–1.02], and 97.2% among 2–4 drop cycles [RR 0.99, 95% CI 0.96–1.03]). Additionally, there were no significant differences in secondary outcomes, including the number of oocytes retrieved, MIIs, 2PNs, oocyte maturity rate, fertilization rate, or usable blastocyst rate.

Fresh transfer outcomes

Results for fresh embryo transfer outcomes are described in Table 4. The results indicate that outcomes were generally comparable across the groups with a consistent rise in estradiol, one drop, or 2–4 drops. Positive hCG rates were 57.6% for the consistent rise group, 55.7% for the one-drop group (RR 0.98, 95% CI 0.90–1.06), and 63.6% for the 2–4 drops group (RR 1.07, 95% CI 0.90–1.27). Live birth rates were 41.1% for the consistent rise group, 36.8% for the one-drop group (RR 0.91, 95% CI 0.81–1.02), and 42.1% for the 2–4 drops group (RR 0.98, 95% CI 0.74–1.28). Chemical pregnancy and spontaneous abortion rates were also comparable between groups.

DISCUSSION

The aim of this study was to evaluate whether a decline in serum estradiol levels before ovulatory trigger in patients undergoing IVF treatment in antagonist protocols with gonadotropin stimulation is associated with the resulting number of usable blastocysts. In the unadjusted analysis, patients who experienced greater drops in estradiol had the highest number of usable embryos. However, this absolute increase is primarily driven by AMH levels, because patients in the 2–4 drop group had a higher baseline AMH compared with all other groups. However, after adjusting for known confounders such as AMH and female age we observed that patients who experienced one estradiol drop and patients who experienced 2–4 estradiol drops both achieved a comparable usable blastocyst rate and likelihood of achieving one usable blastocyst when compared with patients who had a daily estradiol rise leading up to the ovulatory trigger. Further, among patients who had a fresh embryo transfer, the chance of live birth was comparable for each group. These results offer reassurance to both patients and providers encountering cycles marked by a decline in serum estradiol. For patients of a given age and AMH level, drops in estradiol do not indicate poorer prognosis.

Prior studies looking at this question have conflicting results and are distinctly different from our study. A smaller study including only 40 cycles showed that there was a difference in pregnancy probability (1). Although this study also used the same criteria to define the decrease in estradiol level the most reason for a difference in the concluded results is that this study had a larger sample size and had used the primary exposure of number of estradiol drops may be associated with IVF outcomes. Shapiro et al. (5) applied a >10% decline threshold to define a decline in estradiol; although this differed from our definition our results aligned with their findings that there were no statistically significant effects between estradiol drops and IVF outcomes. However, it is important to note that prior study was also limited by sample size including only 225 cycles in their evaluation (5). In

TABLE 3

Oocyte, fertilization, and embryo outcomes, subanalysis of low and high baseline AMH level subgroups.

Outcome	AMH < 1 ng/mL consistent rise (n = 637)	AMH < 1 ng/mL 1 drop (n = 148)	AMH < 1 ng/mL 2–4 drops (n = 9)
No. of oocytes retrieved	8.5 ± 5.2	7.0 ± 4.8	6.8 ± 3.7
	Ref	0.85 (0.75–0.95)	0.81 (0.59–1.13)
No. of MII oocytes	6.2 ± 4.3	5.1 ± 3.8	4.2 ± 3.3
	Ref	0.84 (0.74–0.95)	0.70 (0.44–1.10)
No. of 2PNs	4.6 ± 3.5	3.9 ± 3.4	3.0 ± 2.8
	Ref	0.86 (0.74–1.00)	0.66 (0.38–1.15)
No. of usable blastocysts	2.2 ± 1.8	2.0 ± 1.9	2.0 ± 2.7
	Ref	0.92 (0.78–1.09)	0.94 (0.42–2.12)
Oocyte maturity rate (ICSI only)	75.0% (60.0%–85.8%)	75.0% (55.4%–93.1%)	68.8% (28.6%–80.0%)
	Ref	0.99 (0.94–1.05)	0.86 (0.67–1.10)
Fertilization rate (ICSI only)	77.8% (60.0%–100.0%)	83.3% (63.8%–100.0%)	72.7% (0.0%–85.7%)
	Ref	0.95 (0.8–1.14)	0.99 (0.91–1.07)
Usable blastocyst rate	50.0% (28.6%–80.0%)	56.3% (33.3%–78.8%)	50.0% (50.0%–78.1%)
	Ref	1.06 (0.95–1.19)	1.43 (0.96–2.15)
≥1 usable embryo,[a] n (%)	540 (84.8%)	116 (78.4%)	5 (55.6%)
	Ref	0.93 (0.85–1.01)	0.66 (0.37–1.17)
	AMH>3 ng/mL	AMH>3 ng/mL	AMH>3 ng/mL
	Consistent rise (n = 2,423)	1 drop (n = 482)	2–4 drops (n = 109)
No. of oocytes retrieved	22.9 ± 10.3	23.6 ± 10.5	24.5 ± 11.6
	Ref	1.01 (0.97–1.05)	0.98 (0.90–1.08)
No. of MII oocytes	17.3 ± 8.2	17.8 ± 8.6	18.5 ± 9.4
	Ref	1.01 (0.96–1.06)	0.99 (0.89–1.09)
No. of 2PNs	13.5 ± 7.1	14.3 ± 7.9	14.6 ± 8.2
	Ref	1.03 (0.98–1.09)	0.99 (0.89–1.10)
No. of usable blastocysts	6.6 ± 4.6	7.3 ± 5.6	7.1 ± 4.6
	Ref	1.08 (1.01–1.16)	0.99 (0.87–1.12)
Oocyte maturity rate (ICSI only)	78.2% (66.7%–87.5%)	77.8% (66.7%–88.2%)	78.6% (66.7%–86.7%)
	Ref	1.00 (0.98–1.02)	1.00 (0.97–1.04)
Fertilization rate (ICSI only)	81.3% (69.2%–89.7%)	81.8% (70.5%–91.3%)	83.3% (66.0%–94.1%)
	Ref	1.00 (0.96–1.05)	1.00 (0.96–1.05)
Usable blastocyst rate	50.0% (33.3%–66.7%)	50.0% (33.3%–66.4%)	50.0% (37.8%–63.6%)
	Ref	1.05 (1.00–1.09)	1.00 (0.92–1.08)
≥1 usable embryo,[a] n (%)	2,348 (97.5%)	468 (97.9%)	106 (97.2%)
	Ref	1.0 (0.99–1.02)	0.99 (0.96–1.03)

Note: Mean ± SD or median (IQR) with effect estimate given as mean ratio (95% confidence interval). Usable blastocysts: Sum of transferred and cryopreserved blastocysts.
Oocyte maturity rate: Number of mature oocytes/Number of oocytes retrieved.
Fertilization rate: Number of 2PN/Number of mature oocytes.
Usable blastocyst rate: Number of usable blastocysts/Number of 2PN.
Usable embryo defined as a blastocyst suitable for embryo transfer.
AMH = antimüllerian hormone; ICSI = intracytoplasmic sperm injection; IQR = interquartile range; MII = metaphase II; 2PN = 2 pronuclei.
[a] N(%) with effect estimate as relative risk (95% confidence interval).

Lindner. Estradiol drop before ovulatory trigger. Fertil Steril 2026.

contrast, our study included a substantially larger cohort (n = 6,945) and adjusted a priori for both age and AMH using multivariable statistical models. This likely improves precision and comparability between groups, reduces confounding, and allows more robust interpretation of null findings. Further, the stimulation goals and protocols in our study cohort are more aligned with modern stimulation approaches with a large proportion of patients undergoing embryo

TABLE 4

Fresh transfer outcomes, total cohort.

	Consistent rise (n = 2,819)		1 drop (n = 584)		2–4 drops (n = 84)	
Outcome	N (%)	RR (95% CI)	N (%)	RR (95% CI)	N (%)	RR (95% CI)
Positive hCG	1,540 (57.6)	Ref	302 (55.7)	0.98 (0.90–1.06)	49 (63.6)	1.07 (0.90–1.27)
Chemical pregnancy	200 (13.0)	Ref	38 (12.6)	0.97 (0.70–1.34)	8 (16.3)	1.27 (0.66–2.42)
Spontaneous abortion	200 (13.0)	Ref	48 (15.9)	1.21 (0.90–1.62)	7 (14.6)	1.20 (0.59–2.44)
Clinical pregnancy	1,319 (49.4)	Ref	253 (46.7)	0.96 (0.87–1.05)	41 (53.2)	1.04 (0.84–1.29)
Live birth	1,096 (41.1)	Ref	199 (36.8)	0.91 (0.81–1.02)	32 (42.1)	0.98 (0.74–1.28)

Note: Adjusted for age.
Positive human chorionic gonadotropin (hCG): hCG >5 mIU/mL 10 days after FET.
Chemical pregnancy: Positive hCG with return of hCG to <5 mIU/mL before the visualization of pregnancy on ultrasound.
Spontaneous abortion: Loss of a clinical pregnancy before 22 weeks of gestation (restricted to positive hCG pregnancies).
Live birth: Delivery of a viable fetus at >22 completed weeks of gestation per transfer.
CI = confidence interval; FET = frozen embryo transfer; RR = risk ratio.

Lindner. Estradiol drop before ovulatory trigger. Fertil Steril 2026.

cryopreservation and the routine use of Lupron-only or dual trigger. Thus, our results may be more generalizable to current practice compared with older studies on this topic. Taken together, differences in statistical methodology, adjustment for confounders, and sample size may explain some of the discrepancies between prior reports and our current results. More recently, a retrospective cohort study investigated spontaneously decreasing estradiol levels during IVF with a primary outcome of number of oocytes retrieved (13). Their study found that spontaneous decreases in estradiol was associated with a decreased number of oocytes retrieved if the spontaneous decrease was >20% (13). This study was also limited by sample size as it included 107 cycles for analysis and therefore our study provides more patients to evaluate and better evaluate our secondary outcomes such as live birth.

Our study differed from prior studies in that our exposure was the number of estradiol drops observed in the 4 days before oocyte retrieval rather than a specific percentage threshold for the decline. However, assessing the magnitude of decline could provide additional insight into whether larger decreases are associated with greater risk. Our exploratory analysis using percentage-based thresholds ($\geq$ 10% or $\geq$ 20%) showed no statistically significant differences in either the mean number of usable embryos or usable embryo rate compared with cycles without such drops. These results are consistent with our findings when the exposure remains the number of drops and supports the conclusion that decreases in estradiol before oocyte retrieval is not associated with poor IVF outcomes.

Concerns regarding poor outcomes in patients with a decline in serum estradiol can be further extrapolated from literature evaluating the effects of coasting in patients at risk for ovarian hyperstimulation syndrome (OHSS), a practice which often results in an estradiol decline before ovulatory trigger. Three studies concluded that coasting for 3 or more days resulted in reduced pregnancy rates, implantation rates, and fewer oocytes retrieved compared with patients with <3 days of coasting (14–16). The largest and most recent of these studies included a total of 1,223 patients who underwent coasting for ovarian hyperstimulation risk (15). Analyzing patients with coasting $\leq$3 days (n = 983) compared with those >3 days (n = 240), the patients who were coasted for >3 days had two fewer oocytes retrieved and a significantly decreased chance of pregnancy (36% vs. 52%) (15). However, there are multiple potential etiologies for the decline in outcomes in the prolonged coasting group including a premature increase in progesterone. Hill et al. (17) studied how GnRH antagonist rescue, a different iatrogenic cause of estradiol decline, during controlled ovarian stimulation might impact cycle outcomes. Switching a GnRH agonist with a GnRH antagonist effectively stops any endogenous gonadotropin release and rapidly lowers serum estradiol in patients at high risk of OHSS, with mean decrease of estradiol concentration of 35% within the first day of use (17). This study showed that the induced decrease in serum estradiol was not associated with oocyte maturity, fertilization, blastocyst development, and most importantly live birth which is different from the outcomes found in the coasting literature (17). Although coasting and GnRH antagonist rescue may share the similarity of a decline in estradiol with the patients included in this study, these results are not generalizable to patients with an unexpected or unexplained estradiol decline in the setting of continued daily gonadotropin administration. These studies differ from ours, as the patient population in our study did not have a protocol change with the intent to drop the serum estradiol level.

In addition to the primary analysis, we performed a subanalysis to examine patients with baseline AMH levels <1 ng/mL and >3 ng/mL, as it was hypothesized that the etiology of a drop in serum estradiol might differ between these groups. Patients with AMH <1 ng/mL are indicative of suspected poor responders, potentially predisposing them to decreases in serum estradiol because of alterations in the follicular environment and developmental capacity either causing or because of a reduced number of oocytes. It is understood that patient's with diminished ovarian reserve are more likely to experience premature ovulation during GnRH antagonist cycles (18). A decrease in estradiol may indicate the possibility of premature ovulation, particularly in the subanalysis of patients with AMH <1. However, this study was designed to exclude cases of premature ovulation by omitting patients who did not undergo oocyte retrieval. This approach was chosen because the clinical management of obvious premature ovulation is well established and typically involves either cycle cancellation or, depending on the timing, proceeding directly to oocyte retrieval. A single drop in serum estradiol was associated with a decrease in the number of oocytes retrieved and in the number of mature oocytes in this specific cohort. Despite this, the developmental competence of the oocytes remained intact, as the proportion of oocytes retrieved that were mature was comparable between groups as were the proportion of mature oocytes that successfully fertilized. Interestingly, even patients experiencing 2–4 drops in estradiol in the diminished ovarian reserve subgroup maintained favorable outcomes, achieving an average of approximately two usable blastocysts per cycle, underscoring the resilience of these cycles to declines in estradiol.

A subanalysis was also conducted to evaluate patients with a baseline AMH >3 ng/mL, a patient subgroup that is more likely to hyperrespond to stimulation and is at an increased risk for OHSS). Thus, it was hypothesized that some patients in this subgroup might experience an unintended decline in serum estradiol levels due to conservative use of gonadotropins to mitigate the risk of OHSS; however, we are unable to completely assess if this is the sole cause of why there was the highest amount of estradiol drops in this group of patients. There were no observed differences in the outcomes between patients with a consistent rise of serum estradiol and patients with one or multiple drops in serum estradiol if their baseline AMH was >3 ng/mL. This reinforces the finding that drops in estradiol levels are not associated with adverse cycle outcomes in this high-responder subgroup.

This study has several limitations to acknowledge. First, to objectively quantify changes in estradiol we selected any

decrease in estradiol, 1 pg/mL or greater, to signify a decline in estradiol. However, due to interassay variability, it is possible that some patients with a small decrease or a small increase in estradiol may have been misclassified as serum estradiol levels were measured from each of the 4 days were separately run each day, as is the standard in clinical practice, rather than run together at the same time as would be done in an ideal design. It is also important to address that the patients selected for this study had serum estradiol levels obtained each day for the 4 days preceding the ovulatory trigger. This is particularly significant as it addresses the limitation in our study's generalizability, given that not all clinics routinely measure serum estradiol with the same frequency. Additionally, including only patients who had an estradiol measurement recorded for each of the 4 days preceding oocyte retrieval may exclude patients who had a decrease in estradiol but only had 1–3 measurements obtained before oocyte retrieval. Another limitation is that our dataset only included cycles that progressed to oocyte retrieval, we were unable to assess the impact of unfavorable estradiol trends on cycles that were cancelled, which may underestimate any negative association between estradiol decline and IVF outcomes. It is important to note that the group with multiple drops in serum estradiol could not be separated further due to the small sample size of patients who experienced three drops or four drops in serum estradiol, limiting the ability to assess potential differences in outcomes based on the frequency of estradiol declines. Finally, other potential factors that could influence estradiol fluctuations include certain lifestyle/medical factors (e.g., smoking, polycystic ovary syndrome status), although these were not consistently available in our cohort. Future studies with more granular data could incorporate these variables to further improve group comparability and strengthen inferences.

CONCLUSION

In conclusion, our findings underscore that a decline in serum estradiol in the 4 days leading up to the ovulatory trigger has comparable outcomes to patients in whom a continued increase occurs, this remained true in both high and expected poor responders and when looking at the magnitude of estradiol decrease. This offers reassurance to both patients and providers in the setting of unfavorable serum estradiol trends during controlled ovarian stimulation cycles.

CRediT Authorship Contribution Statement

Peter Lindner: Writing – original draft, Investigation, Data curation, Conceptualization. **Kerry Flannagan:** Writing – review & editing, Formal analysis, Conceptualization. **Charlene Echague:** Writing – review & editing, Investigation, Conceptualization. **Amalia Namath:** Writing – original draft, Investigation. **Jerry Wang:** Investigation. **Jeanne E. O'Brien:** Writing – review & editing, Project administration, Conceptualization. **Phillip Romanski:** Writing – review & editing, Project administration, Conceptualization.

Declaration of Interests

P.L. has nothing to disclose. K.F. is an employee of US Fertility, which provided the data for this manuscript. C.E. has nothing to disclose. A.M. has nothing to disclose. J.W. has nothing to disclose. J.E.O. has nothing to disclose. P.R. has nothing to disclose.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be found online at https://doi.org/10.1016/j.fertnstert.2026.02.030.

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Asociación entre la disminución de los niveles deestradiol sérico los días previos a la inducción de la ovulación y los resultados de las técnicas de reproducción asistida

Objetivo: Evaluar si una disminución de los niveles séricos de estradiol en los días previos al trigger ovulatorio, en ciclos de estimulación ovárica con antagonistas de la de la hormona liberadora de gonadotropina, influye en los resultados del ciclo.

Diseño: Estudio de cohorte retrospectivo.

Pacientes: Pacientes sometidas a ciclos de aspiración folicular autóloga tras protocolo de antagonistas de la de la hormona liberadora de gonadotropina entre 2010 y 2021 en una red multicéntrica de centros de infertilidad en Estados Unidos. Se excluyeron las pacientes a las que no se les midió el nivel de estradiol en los 4 días previos a la inducción de la ovulación o que se sometieron a inseminación convencional.

Exposición: Pacientes que presentaron un descenso documentado de al menos 1 pg/mL en los niveles séricos de estradiol en cualquiera de los 4 días previos al trigger ovulatorio. El grupo de referencia mostró un aumento sostenido del estradiol sérico cada día antes del trigger.

Variables de resultado: El resultado principal fue el número de blastocistos utilizables, definido como la suma de los blastocistos transferidos en fresco (cuando correspondiera) y los blastocistos criopreservados restantes. Las variables secundarias incluyeron madurez ovocitaria, la tasa de fecundación, la obtención de al menos un blastocisto utilizable, la positividad de gonadotropina coriónica humana, embarazo bioquímico, aborto espontáneo, embarazo clínico y nacimiento vivo.

Resultados: Un total de 6,945 ciclos cumplieron con los criterios de inclusión. De ellos, 5,653 presentaron un aumento sostenido del estradiol sérico, 1,103 ciclos evidenciaron una única caída y 189 ciclos presentaron entre 2 y 4 descensos en los 4 días previos al trigger ovulatorio. Tras ajustar por hormona antimülleriana y edad materna, no se observaron diferencias en el número promedio de blastocistos utilizables entre el grupo de referencia (4.9 $\pm$ 4.0) y los ciclos con una caída de estradiol (5.2 $\pm$ 4.8) (razón de medias 1.04, intervalo de confianza 95% [CI] 0.97–1.10), ni en aquellos con 2–4 descensos (razón de medias 0.98, 95% CI 0.85–1.14). Tampoco se evidenciaron diferencias estadísticamente significativas en ninguna de las variables secundarias, incluyendo la tasa de recién nacido vivo. Se observó una tasa de recién nacido vivo del 41.1 % en el grupo de aumento constante, del 36.8% en el grupo con una caída (riesgo relativo 0.91, 95% CI 0.81–1.02) y 42.1% en el grupo con 2–4 descensos (riesgo relativo 0.98, 95% CI 0.74–1.28).

Conclusión: Los resultados indican que una disminución del estradiol sérico en los 4 días previos al trigger ovulatorio no compromete la calidad ovocitaria. Las pacientes que presentaron un descenso de estradiol previo a la captación ovocitaria mostraron resultados comparables a aquellas con un aumento sostenido del estradiol.