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# Trigger-to-retrieval interval and cumulative live birth in 25,669 in vitro fertilization cycles: mechanisms

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## Abstract

# Background

To determine whether the trigger-to-oocyte-retrieval interval affects cumulative live birth in in vitro fertilization (IVF), the laboratory-chain mechanisms involved, and modification by trigger-day follicle spectrum or trigger regimen.

# Methods

Retrospective single-center cohort study with patient-level cluster-robust inference across repeated cycles. All 46,145 oocyte retrieval cycles (January 2016–September 2026) at a single academic center were screened; 25,669 outcome-determined cycles with complete covariates were analyzed. The trigger-to-retrieval interval was categorized a priori as < 34h, 34–36h (reference), 36–38h, or ≥ 38h. The primary outcome was cumulative live birth per retrieval cycle; fourteen laboratory-chain secondary outcomes (retrieval, maturation, fertilization, cleavage, day-3 embryo quality, usable embryos, blastocyst formation, etc.) were analyzed as rates and absolute counts.

# Results

Cumulative live birth was 47.4%. Versus 34–36h, adjusted ORs were 0.72 (95% CI 0.54–0.95) before 34h, 1.07 (1.01–1.13) at 36–38h, and 1.00 (0.85–1.18) at ≥ 38h. Before 34h, deficits concentrated in oocyte maturation and quantity; only one-tenth of the live-birth deficit was mediated by mature-oocyte count. Beyond 38h, fertilization was preserved but cleavage and day-3 embryo quality declined, offset by quantity gains. Adjusted absolute differences were − 7.4 percentage points before 34 h and + 1.5 points at 36–38h. Without any follicle > 18mm at trigger, early retrieval reduced live-birth odds by two-thirds (OR 0.30) and 36–38h increased them (OR 1.19); the 36–38h gain was nominally confined to low-dose hCG triggers. Findings were robust under Firth penalization, inverse-probability weighting, within-patient discordant-pair analysis, extreme-case bounds, temporal validation, and restriction to first cycles.

# Conclusions

Retrieval before 34h should be avoided; 34–38h is safe across protocols and trigger regimens; deliberate extension toward 36–38h is most defensible after a young trigger-day follicle spectrum or a low-dose trigger.

# Clinical trial registration

Not applicable.

# Background

The interval between the ovulation trigger and oocyte retrieval, the trigger-to-retrieval interval (TTR), is one of the few remaining procedural variables in assisted reproduction that is fixed by convention rather than by evidence. Most programs schedule retrieval at 34–36 hours after human chorionic gonadotropin (hCG) administration, a practice inherited from the earliest days of in vitro fertilization (IVF) and supported only by modest-sized studies with oocyte maturation, fertilization, or fresh-transfer pregnancy endpoints [1, 2, 3, 4, 5]; only very recently have large cohorts revisited the question, suggesting that optimal timing may differ between agonist and hCG triggers [6, 7, 8]. The convention must be long enough for the luteinizing hormone (LH)-equivalent stimulus to complete meiotic maturation, yet short enough to prevent post-maturity and incipient luteinization. It is also shaped by logistics, such as operating-room schedules and weekend staffing. Small deviations in either direction are common and presumed negligible.

Whether such deviations carry any consequence for the outcome that matters most to patients, the cumulative live birth rate (CLBR) per retrieval cycle, remains essentially unknown. The question has acquired new weight with the shift toward freeze-all strategies and single-embryo transfer, in which a cycle's value is captured by the cumulative endpoint rather than any single fresh transfer [9]. Moreover, the laboratory mechanisms through which interval length could act have never been systematically characterized. Because cumulative live birth depends fundamentally on the number of oocytes and usable embryos produced per cycle [10, 11], any interval effect must be traced through the entire oocyte-to-embryo chain (retrieval, maturation, fertilization, cleavage, embryo quality, blastocyst formation) rather than read from the cumulative endpoint alone.

Two biologically distinct hypotheses frame the question. On one side, oocytes may require a minimum interval of LH-equivalent exposure to complete meiotic maturation and cumulus expansion; retrieval too early risks recovering a cohort enriched for immature oocytes [2, 4, 12]. On the other side, excessive prolongation has been linked to follicular over-maturation, post-mature oocytes of compromised developmental competence, and incipient follicular luteinization [5, 13]. The net effect on CLBR should therefore follow an inverted-U pattern. Yet the location and width of the plateau, and whether it shifts with the stimulation protocol, the trigger agent and dose, dual-trigger use, or the size composition of the follicle cohort at trigger, cannot be resolved by small fresh-transfer studies underpowered for the cumulative endpoint and unable to separate quantity from quality effects.

In the first two studies of this series, the trigger-day follicle spectrum, in particular the proportion of follicles measuring 13–18mm, predicted cumulative live birth in a dose-dependent manner, an effect transmitted entirely through the quantity pathway of the laboratory chain, with oocyte and embryo quality uniformly robust (unpublished observations), as independently shown by a multicentre explainable-artificial-intelligence analysis of 19,082 treatment-naïve patients, in which intermediately sized follicles contributed most to the number of mature oocytes retrieved and maximizing their proportion was associated with higher live-birth rates [14]. Those findings identified when to trigger given a follicle

cohort; they did not address how long to wait afterwards. The two questions are mechanistically linked. A cycle triggered at a “younger” spectrum (a high proportion of 13–18mm follicles, none yet beyond 18mm) contains oocytes that are on average less advanced in maturation and may benefit disproportionately from additional hours of in vivo maturation. Conversely, the interval is the one trigger-side parameter that could plausibly affect oocyte and embryo quality, the domain in which the follicle-spectrum studies found uniformly null effects. A study connecting the two questions would link trigger timing to retrieval timing.

We therefore analyzed 25,669 outcome-determined retrieval cycles with four pre-specified aims: (i) estimating the TTR-band–CLBR association; (ii) tracing it through the complete laboratory chain, in rates and absolute counts; (iii) decomposing the effects at both ends by formal counterfactual mediation; and (iv) testing modification by stimulation protocol, trigger type and dose, dual-trigger use, and trigger-day follicle spectrum.

## Methods

# Study design and population

This retrospective single-center cohort study included all oocyte retrieval cycles recorded in the institutional database between January 2016 and September 2026. Trigger and retrieval times are documented to the minute in the electronic medical record and all laboratory outcomes derive from the same system, so exposure and outcomes were measured independently of this analysis. Both first and repeated cycles per patient were eligible (25,513 patients contributed 1–34 cycles each; 38.6% contributed more than one), with patient-level clustering handled in inference. From 46,145 retrieval cycles, exclusions in order were: no documented trigger-to-retrieval interval (natural and minimal-stimulation cycles without a recorded trigger, or missing records); interval outside the plausible 22–42-hour window; undetermined cumulative live birth outcome; incomplete covariates, leaving 25,669 cycles in the analysis set (flow in Supplementary Fig. 1). Reporting follows the STROBE guidelines [15]. The study was approved by the institutional ethics committee with a waiver of informed consent [approved by the Ethics Committee of Shanghai First Maternity and Infant Hospital (approval no.KS26285;2026)].

**Supplementary Fig. 1.** Cohort flow diagram

STROBE-style flow of cycle selection: from 46,145 oocyte retrieval cycles to the primary analysis set of 25,669 outcome-determined cycles, distributed across interval bands (< 34 h, n = 239; 34–36 h, n = 12,762; 36–38 h, n = 11,953; ≥38 h, n = 715). The follicle-spectrum subset (n = 17,728) underlies stratification and effect-modification analyses.

## Exposure

The trigger-to-retrieval interval was computed from the documented time of trigger administration to the documented time of oocyte retrieval and, per the pre-registered statistical analysis plan, analyzed in four bands: <34hours, 34–36hours (reference, the institutional convention), 36–38hours, and ≥ 38hours. Restricted cubic splines (3 knots) display the dose–response; categorical models are reported as primary because spline Wald tests were unstable under the multi-level year covariate (ill-conditioned covariance matrix).

## Outcomes

The primary outcome was CLBR per retrieval cycle, defined as at least one live birth from all embryos (fresh and frozen) derived from that cycle [16]; cycles with undetermined status were excluded from the primary analysis and examined in a bounds analysis. A cumulative rather than fresh-transfer endpoint was chosen because the interval acts on the oocyte before any transfer decision and is carried by all embryos of the cohort; a fresh-transfer endpoint would entangle interval timing with endometrial and scheduling factors. Fourteen laboratory-chain secondary outcomes were analyzed as rates and absolute counts: rates isolate per-oocyte competence, whereas counts capture the material yield on which cumulative live birth depends. The chain comprised oocyte retrieval (per follicle ≥ 13 mm at trigger), oocyte maturation (per retrieved oocyte; pure-ICSI(Intracytoplasmic Sperm Injection) cycles add maturation per injected cohort), normal fertilization (two-pronuclear, per mature oocyte), cleavage, day-3 good-embryo rate, usable-embryo rate, and blastocyst formation (each per zygote or cultured embryo), plus the corresponding counts per cycle.

## Covariates and trigger-regimen classification

The pre-specified core adjustment set (unpublished observations) comprised female age, gonadotropin-stimulation days, follicle count at trigger, stimulation protocol (six groups: antagonist; long gonadotropin-releasing hormone agonist; short protocol; progestin-primed ovarian stimulation; minimal stimulation/natural cycle; other)[17], fertilization method (IVF, ICSI, mixed), and calendar year. Trigger regimens were classified from prescription records into five dose groups (recombinant hCG 250 μg, approximately 6,500 IU, including combined urinary regimens of 6,000–7,999 IU; urinary 4,000–5,999 IU; urinary 1,000–3,999 IU; urinary ≥ 8,000 IU, including 10,000-IU regimens; and agonist-only trigger, triptorelin 0.1–0.2 mg), with concurrent agonist co-administration (dual trigger) as a separate binary covariate [6, 18, 19]. Implausible records above 20,000 IU (n = 4) never entered interval models. The trigger-day follicle spectrum (proportion of 13–18 mm follicles, p13–18; number of follicles > 18 mm, n > 18; available for 17,728 cycles) served for stratification and effect-modification analyses (unpublished observations).

# Statistical analysis

Continuous variables are summarized as mean (SD) or median (IQR), categorical variables as counts (%). TTR-band associations with CLBR used logistic regression with patient-level cluster-robust standard errors; rate outcomes, binomial models (denominator as frequency weights); count outcomes, Poisson models (cluster-robust). Multiplicity across the 14 chain outcomes was controlled within each contrast by Benjamini–Hochberg false-discovery-rate (FDR) correction.

Formal counterfactual mediation (VanderWeele framework) decomposed the < 34-hour effect through the number of mature oocytes and the ≥ 38-hour effect through cleavage rate and day-3 good-embryo rate (linear mediator model, logistic outcome model with exposure–mediator interaction; 300 patient-cluster bootstrap replicates). Effect modification was tested by likelihood-ratio tests of nested models with and without interaction terms (robust to separation), with stratified estimates reported for each subgroup.

The robustness program comprised: (i) Firth penalized regression against separation and small-sample bias in the sparse < 34-hour stratum; (ii) inverse-probability weighting for outcome determination and a within-patient analysis of discordant-outcome patients, addressing informative outcome missingness and stable unmeasured confounders; (iii) extreme-case bounds reassigning all 10,809 outcome-undetermined cycles; (iv) temporal validation, fitting on 2016–2022 and evaluating 2023–2026; (v) sensitivity analyses (first cycles; follicle-spectrum adjustment; 33–40-hour window; weekend retrievals excluded); and (vi) E-values for the primary contrasts [20, 21, 22, 23]. Adjusted absolute risk differences were computed by covariate-standardized prediction. Two-sided $P < 0.05$ was considered significant. Analyses used Python 3.12 (statsmodels 0.14, patsy, NumPy, pandas).

## Results

# Cohort

The primary analysis set comprised 25,669 outcome-determined cycles (flow in Supplementary Fig. 1). TTR was < 34 hours in 239 cycles (0.9%), 34–36 hours in 12,762 (49.7%), 36–38 hours in 11,953 (46.6%), and ≥ 38 hours in 715 (2.8%); cycle CLBR was 47.4%. The bands differed in their clinical context (Table 1): cycles retrieved before 34 hours were older on average (mean female age 33.9 vs 32.5–32.8 years), had fewer follicles at trigger (median 2 vs 4–6), and far more often followed minimal-stimulation or natural-cycle protocols (18.0% vs 3.4%–12.3%), whereas the ≥ 38-hour band was enriched for progestin-primed ovarian stimulation (18.6%) and low-dose urinary hCG triggers (22.9%). These imbalances, expected if interval length tracks the developmental stage of the cohort, motivated the extensive adjustment and sensitivity analyses that follow.

Table 1
Baseline and cycle characteristics by trigger-to-retrieval interval band (n = 25,669).

| Characteristic | < 34 h (n = 239) | 34–36 h (n = 12,762) | 36–38 h (n = 11,953) | ≥ 38 h (n = 715) |
|---|---|---|---|---|
| Female age, years, mean ± SD | 33.9 ± 5.3 | 33.3 ± 4.7 | 32.8 ± 4.4 | 32.5 ± 4.3 |
| BMI, kg/m², mean ± SD † | 22.3 ± 3.2 | 22.3 ± 3.3 | 22.3 ± 3.3 | 22.6 ± 3.4 |
| AMH, ng/mL, median (IQR) † | 2 (1–4) | 3 (1–5) | 3 (2–5) | 3 (2–5) |
| Gonadotropin days, median (IQR) | 10 (8–13) | 9 (8–11) | 9 (8–11) | 9 (8–11) |
| Gn total dose, IU, median (IQR) | 2100 (1575–3000) | 1800 (1350–2325) | 1800 (1500–2362) | 2025 (1575–2625) |
| Follicles at trigger, n, median (IQR) | 2 (0–9) | 4 (0–9) | 6 (0–10) | 5 (0–10) |
| Stimulation protocol, n (%) | | | | |
| Antagonist | 60 (25.1%) | 6320 (49.5%) | 5341 (44.7%) | 199 (27.8%) |
| Long GnRH-agonist | 112 (46.9%) | 3618 (28.3%) | 3624 (30.3%) | 260 (36.4%) |
| Luteal-phase stimulation | 1 (0.4%) | 168 (1.3%) | 382 (3.2%) | 21 (2.9%) |
| Minimal stimulation/natural | 43 (18.0%) | 1564 (12.3%) | 413 (3.5%) | 24 (3.4%) |
| Other | 2 (0.8%) | 117 (0.9%) | 167 (1.4%) | 27 (3.8%) |
| PPOS | 6 (2.5%) | 556 (4.4%) | 1024 (8.6%) | 133 (18.6%) |
| Short protocol | 15 (6.3%) | 419 (3.3%) | 1002 (8.4%) | 51 (7.1%) |
| Fertilization method, n (%) | | | | |
| ICSI | 92 (38.5%) | 5085 (39.8%) | 4460 (37.3%) | 222 (31.0%) |
| IVF | 131 (54.8%) | 7003 (54.9%) | 6844 (57.3%) | 465 (65.0%) |
| Mixed | 15 (6.3%) | 615 (4.8%) | 613 (5.1%) | 26 (3.6%) |
| Unknown | 1 (0.4%) | 59 (0.5%) | 36 (0.3%) | 2 (0.3%) |
| Trigger regimen, n (%) | | | | |
| rhCG 250 μg (≈ 6,500 IU) | 202(84.5%) | 9055 (71.0%) | 7085 (59.3%) | 366 (51.2%) |
| hCG 1,000–3,999 IU | 7 (2.9%) | 766 (6.0%) | 1380 (11.5%) | 164 (22.9%) |

| Characteristic | < 34 h (n = 239) | 34–36 h (n = 12,762) | 36–38 h (n = 11,953) | ≥ 38 h (n = 715) |
|---|---|---|---|---|
| hCG 4,000–5,999 IU | 16 (6.7%) | 1541 (12.1%) | 2290 (19.2%) | 158 (22.1%) |
| hCG ≥ 8,000 IU | 7 (2.9%) | 961 (7.5%) | 732 (6.1%) | 17 (2.4%) |
| GnRH-a only | 7 (2.9%) | 439 (3.4%) | 466 (3.9%) | 10 (1.4%) |
| Missing | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Dual trigger, n (%) | | | | |
| No | 213 (89.1%) | 11475(89.9%) | 10603(88.7%) | 617 (86.3%) |
| Yes | 26 (10.9%) | 1287 (10.1%) | 1350 (11.3%) | 98 (13.7%) |
| Calendar year, mean ± SD | 2021.7 ± 2.1 | 2021.2 ± 2.6 | 2021.2 ± 2.5 | 2021.6 ± 2.7 |

† BMI restricted to plausible values (14–60 kg/m²); AMH was available in 51.7% of cycles.

# Primary outcome

Relative to 34–36 hours, adjusted odds of cumulative live birth were lower before 34 hours (OR 0.72, 95% CI 0.54–0.95; *P* = 0.023), modestly higher at 36–38 hours (1.07, 1.01–1.13; *P* = 0.017), and unchanged at ≥ 38 hours (1.00, 0.85–1.18) (Table 2; Fig. 1). A restricted cubic spline confirmed an overall dose–response (likelihood-ratio *P* = 0.002; Supplementary Fig. 2): the standardized adjusted live-birth rate rose from 32.8% at 30 hours to a peak of 48.5% at 37.1 hours; the harm below 34 hours and the plateau across 34–38 hours were both visible without categorization (nonlinearity *P* = 0.10).

Table 2
Trigger-to-retrieval interval and cumulative live birth: crude rates, adjusted odds ratios, Firth-verified estimates and E-values.

| TTR band | n | Crude CLBR | Adjusted OR (95% CI) | P | Firth OR | E-value |
|---|---|---|---|---|---|---|
| < 34 h | 239 | 37.2% | 0.72 (0.54–0.95) | 0.023 | 0.72 (0.54–0.95) | 2.14 |
| 34–36 h | 12,762 | 45.2% | 1.00 (ref) | — | — | — |
| 36–38 h | 11,953 | 49.9% | 1.07 (1.01–1.13) | 0.017 | 1.06 (1.01–1.12) | 1.34 |
| ≥ 38 h | 715 | 48.5% | 1.00 (0.85–1.18) | 0.97 | 1.01 (0.86–1.19) | 1.34 |

Restricted cubic spline (3 knots) for the trigger-to-oocyte-retrieval interval fitted by cluster-robust logistic regression and standardized to the cohort covariate distribution; the shaded band is the 95% confidence interval from 200 patient-cluster bootstrap resamples. Shaded vertical spans mark the four a priori interval bands. Overall association likelihood-ratio $P = 0.002$; nonlinearity $P = 0.10$.

# Laboratory chain

Before 34 hours, deficits concentrated in maturation and quantity: ICSI maturation OR 0.67 (0.49–0.91; FDR q = 0.032), mature-oocyte count IRR 0.77 (q = 0.001), usable-embryo count IRR 0.84 (q = 0.001); all seven nominally significant signals survived FDR correction (Supplementary Table 1; Fig. 2). At 36–38 hours, FDR-robust improvements spanned retrieval rate (OR 1.66 per follicle), ICSI maturation (1.16), normal fertilization (1.08), and counts of two-pronuclear zygotes (IRR 1.07), day-3 good embryos (1.09), usable embryos (1.03), and usable blastocysts (1.06); the usable-embryo *rate* declined slightly (OR 0.93) while the *count* rose, a denominator effect of the larger zygote pool. At ≥ 38 hours, fertilization was preserved (OR 1.24) but an embryonic-quality penalty survived FDR correction: cleavage OR 0.65 (0.52–0.82), day-3 good-embryo rate 0.86, usable-embryo rate 0.80, and blastocyst formation 0.87, with counts unchanged. The flat CLBR in this band is thus a quality loss offset by quantity gains, not equivalence.

# Mediation

For < 34 hours, the indirect effect through mature-oocyte number was significant (OR 0.968, 95% CI 0.938–0.995) but explained only 10.0% of the effect; the direct effect remained strong (OR 0.745, 0.549–0.986) (Supplementary Table 2). For ≥ 38 hours, the near-null total effect (OR 0.97) decomposed into a significant indirect effect through cleavage rate (OR 0.989, 0.981–0.996; 40.9% mediated) and a borderline effect through day-3 good-embryo rate (OR 0.974, 0.950–1.001), the direct pathway fully offsetting the quality loss; the blastocyst pathway was null, consistent with selection into blastocyst culture.

Effect modification (interaction with interval band; adjusted OR, 95% CI vs 34–36 h):

| Analysis | Estimate (95% CI) | P |
|---|---|---|
| n > 18 = 0: <34 h vs 34–36 h | 0.30 (0.14–0.65) | 0.002 |
| n > 18 = 0: 36–38 h vs 34–36 h | 1.19 (1.03–1.39) | 0.022 |
| Interaction, continuous p13–18 × <34 h | — | 0.035 |
| Interaction, protocol (6 groups) | — | 0.15 |
| Interaction, trigger-regimen groups | — | 0.59 |
| Interaction, dual trigger | — | 0.74 |

# Effect modification

The interval effect was homogeneous across protocol (*P* = 0.15), trigger-regimen (*P* = 0.59), and dual-trigger (*P* = 0.74) groups; no subgroup showed harm from extension, and only the urinary low-dose hCG (approximately 1,000–3,000 IU) subgroup showed a nominally significant 36–38-hour gain (OR 1.24, 1.02–1.52); this is hypothesis-generating given the null interaction test (Supplementary Tables 3 and 4). In contrast, the trigger-day follicle spectrum modified the association (continuous p13–18 × <34-hour interaction *P* = 0.035; overall interaction *P* = 0.13). In cycles without any follicle > 18 mm (n = 3,525), < 34-hour retrieval reduced cumulative-live-birth odds by two-thirds (OR 0.30, 0.14–0.65) whereas 36–38-hour retrieval increased them (OR 1.19, 1.03–1.39); cycles with one or two follicles > 18 mm showed no interval effect (Supplementary Table 2; Fig. 1).

Interaction likelihood-ratio test, interval × dual trigger: P = 0.641. A reduced covariate set (female age, gonadotrophin days, follicles at trigger, protocol group) is used in this stratification because dual-trigger status is structurally collinear with the trigger-dose factor (the agonist-only group is undefined within the non-dual stratum).

# Robustness

Firth penalized regression confirmed all three primary estimates (< 34 hours: OR 0.72, 0.54–0.95; 36–38 hours: 1.06, 1.01–1.12; ≥38 hours: 1.01, 0.86–1.19), excluding small-sample bias and separation in the sparsest strata. Inverse-probability weighting for outcome determination yielded essentially identical estimates (< 34 hours: OR 0.72, 0.54–0.95; 36–3hours: 1.07, 1.01–1.13; ≥38hours: 1.00, 0.86–1.18), arguing against informative outcome missingness, and a within-patient analysis of the 4,792 discordant-outcome cycles confirmed both ends of the interval (< 34 hours: OR 0.40, 0.20–0.79; 36–38 hours: 1.42, 1.26–1.60; ≥38 hours: 1.42, 0.98–2.06), showing the associations are not confounded by stable patient characteristics. Standardized to the cohort covariate distribution, retrieval < 34hours was associated with an adjusted absolute CLBR difference of − 7.4 percentage points relative to 34–36 hours (36–38 hours: +1.5 points; ≥38 hours: +0.1 points). Under extreme-case reassignment of all 10,809 outcome-undetermined cycles, the < 34-hour estimate ranged from 0.70 to 0.95 and the 36–38-hour estimate from 1.01 to 1.10; both findings were preserved under all but the most extreme missingness assumption (Supplementary Table 5). Temporal validation showed the < 34-hour penalty throughout, more pronounced in 2023–2026 (OR 0.55, 0.34–0.89 vs 0.82, 0.57–1.17 earlier), while the 36–38-hour gain was similar in magnitude but significant only in the earlier era (1.07, 1.00–1.15 vs 1.06, 0.97–1.16); ≥38 hours remained CLBR-neutral in both eras. Sensitivity analyses restricted to first cycles (36–38 hours OR 1.09, 1.01–1.17), with follicle-spectrum adjustment (< 34 hours OR 0.58, 0.41–0.80), in a 33–40-hour window, and excluding weekends (< 34 hours OR 0.69, 0.50–0.95; cf. [24]) were all directionally consistent (Supplementary Table 6; Supplementary Fig. 3). E-values were 2.14 (CI limit 3.12) for the < 34-hour contrast and 1.34 (CI limit 1.51) for the 36–38-hour contrast.

Adjusted ORs (95% CI) for cumulative live birth by interval band across eight analyses: primary analysis, inverse-probability weighting for outcome determination, Firth penalization, first-cycle restriction,

temporal validation (2016–2022 and 2023–2026), follicle-spectrum adjustment, and within-patient analysis of discordant pairs. All estimates are directionally consistent with the primary analysis.

Under the most extreme assumption in favour of reversal (every undetermined cycle a live birth), the < 34 h contrast loses significance; under the converse assumption it strengthens. The 36–38 h contrast remains significant when undetermined outcomes are assigned to live birth and is directionally preserved otherwise. The ≥ 38 h contrast is null under both bounds. The primary conclusions are therefore preserved under all but the most extreme missingness assumption.

Weekend-retrieval restriction and non-dual-trigger stratification were additionally examined and were directionally consistent with the primary analysis (weekend-excluded < 34 h OR 0.69, 0.50–0.95; non-dual-trigger stratum 36–38 h OR 1.09, 1.03–1.15); they are omitted from the summary forest plot for parsimony.

Adjusted absolute risk differences (standardized to the cohort covariate distribution): <34 h − 7.4 percentage points; 36–38 h + 1.5 points; ≥38 h + 0.1 points.

# Discussion

In 25,669 outcome-determined retrieval cycles, the trigger-to-retrieval interval showed a clear safety pattern: a plateau spanning 34–38 hours, a significant penalty below 34 hours, and a modest cumulative benefit at 36–38 hours. The benefit was homogeneous across stimulation protocols and trigger regimens and was concentrated after a younger trigger-day follicle spectrum. The two ends acted on different links of the laboratory chain: retrieval too early compromised oocyte maturation and downstream quantity, only about one-tenth of the CLBR deficit being mediated through mature-oocyte count; retrieval beyond 38 hours preserved fertilization but impaired cleavage and day-3 quality, with a significant indirect effect through cleavage rate. The flat CLBR in this band therefore reflects a quantity–quality trade-off, not equivalence, a distinction visible only because the cumulative endpoint was traced through the complete laboratory chain.

Prior evidence consists largely of small cohorts with maturation or fresh-pregnancy endpoints [1, 2, 3, 4, 5, 13]; a randomized comparison in poor responders favoured a 34-hour over a 36-hour schedule in that selected population [25]. A recent 59,206-cycle analysis concluded that optimal trigger timing differs between agonist and hCG triggers [6]; although our trigger-regimen interaction tests were null, our agonist-only subgroup likewise showed no benefit from extension (OR 0.99), a compatible signal. Our results reconcile with, rather than contradict, that literature: in the bulk of the cohort with average prognosis the plateau is wide, and shifting within it is harmless, while the population in which timing matters is defined not by ovarian response per se but by the trigger-day follicle spectrum.

The effect-modification pattern (cycles without a follicle > 18 mm were harmed by early retrieval and helped by extension) is the direct mechanistic complement of our previous finding that a high 13–18 mm proportion is favourable (unpublished observations): the same “young” cohort that benefits from

early triggering also benefits from more post-trigger maturation time. To our knowledge, this is the first study connecting follicle-spectrum-guided trigger timing to interval optimization, and the first to show that the interval is the trigger-side parameter that affects embryonic quality, where spectrum effects were uniformly null. Unlike the endometrial effects of late-follicular progesterone elevation, which are recoverable by cryopreservation [26, 27, 28, 29], the beyond-38-hour quality penalty is an oocyte-intrinsic defect that cryopreservation cannot undo, a distinction that matters in an era of freeze-all strategies.

One subgroup signal deserves framing: the 36–38-hour gain was nominally confined to low-dose urinary hCG triggers, while the overall interaction test was null; this is hypothesis-generating, not evidence of effect modification [20]. The hypothesis is biologically coherent: after a low-dose hCG trigger the LH-equivalent stimulus is weaker and of shorter duration [30], so completion of meiotic maturation may depend more heavily on the duration of in vivo exposure, whereas a full-dose trigger (recombinant hCG 250 µg, approximately 6,500 IU, or ≥ 8,000 IU) provides a strong maturation stimulus and leaves little marginal value in additional waiting. This reasoning mirrors randomized evidence that delaying the maturation trigger itself by one day increases the metaphase-II yield [12] and is consistent with our mediation finding that most of the < 34-hour harm bypasses the mature-oocyte count and acts through retrieved-but-immature oocytes, the population a low-dose trigger leaves most exposed. We therefore state the prediction explicitly for prospective testing: cycles triggered with low-dose hCG (≤ 3,999 IU) may derive cumulative benefit from deliberate extension toward 36–38 hours, whereas adequately dosed cycles may not.

Three conclusions follow. First, retrieval should not be scheduled before 34 hours: the penalty is the study's strongest and most robust signal (Firth-stable, E-value 2.14, more pronounced in the recent era) and clinically material at − 7.4 percentage points of absolute CLBR. Second, the 36–38-hour band is safe for every protocol and regimen examined and may be actively preferred after a young-spectrum trigger (no follicle > 18 mm), where adjusted odds of cumulative live birth rose by approximately 19%. Third, intervals ≥ 38 hours should not be adopted routinely: CLBR was preserved on average in every era, but the FDR-robust embryonic-quality penalties argue against deliberate extension; when delay threatens, the retrieval should be scheduled earlier, because the in vivo quality penalty cannot be undone by cryopreservation. Within the plateau, interval choice can be made for logistical reasons without measurable cost; when the cohort is young on the final monitoring scan, planning retrieval toward 36–38 hours is a low-risk maneuver with a plausible modest benefit, particularly after a low-dose trigger.

The era analysis is consistent: the 36–38-hour gain was era-stable in magnitude but significant only in the earlier era. The era-stable message is the safety window, not the size of the gain: as practice improves, interval timing becomes less a means of optimization than a safeguard against harm outside it.

The strengths of this study address specific weaknesses of the earlier literature. The 25,669 outcome-determined cycles from a decade of practice are the largest sample analyzed for this question to our knowledge, orders of magnitude larger than the cohorts on which the 34–36-hour convention rests.

Repeated cycles were retained with patient-level cluster-robust inference rather than discarded. The analysis plan was pre-specified, with interval bands fixed before outcome inspection. The laboratory chain was represented in rates *and* counts, with FDR control across 14 outcomes per contrast, so quantity and quality effects can be told apart rather than conflated. Counterfactual mediation was applied symmetrically at both ends of the interval, effect modification was tested by likelihood-ratio tests valid under separation (relevant to the sparse extreme bands), and the robustness program (Firth penalization, inverse-probability weighting, within-patient discordant-pair analysis, extreme-case bounds, temporal validation, and E-values) addresses the main technical objections in advance.

Several limitations merit emphasis. The single-centre retrospective design is principal: although the < 34-hour contrast is difficult to explain away (E-value 2.14), the 36–38-hour gain is weak (E-value 1.34) and rests on the coherence of the chain pattern rather than the primary estimate alone; external validation in an independent cohort is planned as the next study of this series. Indication bias is a specific concern, as extension may reflect judgement about a slower-developing cohort, but spectrum adjustment *strengthened* the < 34-hour effect (OR 0.58). This argues against full confounding by indication, although residual confounding cannot be excluded. The < 34-hour (n = 239) and ≥ 38-hour (n = 715) bands are modest, so extreme-band estimates in both directions should be read as exploratory. Oocyte maturation is observable only in ICSI cycles, and maturation in IVF-inseminated cycles is unknown. Trigger records were missing in a minority of cycles (concentrated in 2019–2022; addressed by year adjustment), and 10,809 cycles had undetermined outcomes (addressed by bounds). The follicle spectrum serves both as a stratifier and, in sensitivity analysis, as a confounder; the two models are fitted independently and should not be conflated. Finally, the mediated proportions, though formally significant, carry wide bootstrap intervals and are best read as qualitative evidence of pathway structure rather than precise decomposition.

# Conclusions

A trigger-to-retrieval window of 34–38 hours is safe across protocols, trigger agents and doses. Retrieval before 34 hours was associated with lower cumulative live birth, chiefly through impaired oocyte maturation; retrieval beyond 38 hours trades embryonic quality for quantity; deliberate extension toward 36–38 hours is most defensible after a younger follicle spectrum. Interval timing (the only trigger-side parameter shown to affect embryonic quality) completes the follicle-spectrum-guided framework for individualizing the final steps of ovarian stimulation; whether its benefit is confined to low-dose-triggered, young-spectrum cycles requires prospective confirmation.

# Abbreviations

IVF
in vitro fertilization
TTR
trigger—to—retrieval interval

CLBR
cumulative live birth rate
ICSI
Intracytoplasmic Sperm Injection
hCG
human chorionic gonadotropin
LH
luteinizing hormone

## Declarations

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee of Shanghai First Maternity and Infant Hospital with a waiver of informed consent (approval No. KS26285; 2026). All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

**Consent for publication**

Not applicable.

# Competing interests

The authors declare that they have no competing interests.

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# Author Contribution

Author roles were as follows: JQ — Conceptualization, Methodology, Formal analysis, Writing – original draft; ML — Resources, Writing – review & editing; FH — Investigation, Data curation; QY — Investigation, Data curation; EC — Software, Validation; ZC — Conceptualization, Methodology, Project administration, Writing – review & editing, Supervision.

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Not applicable.

# Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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# Figures

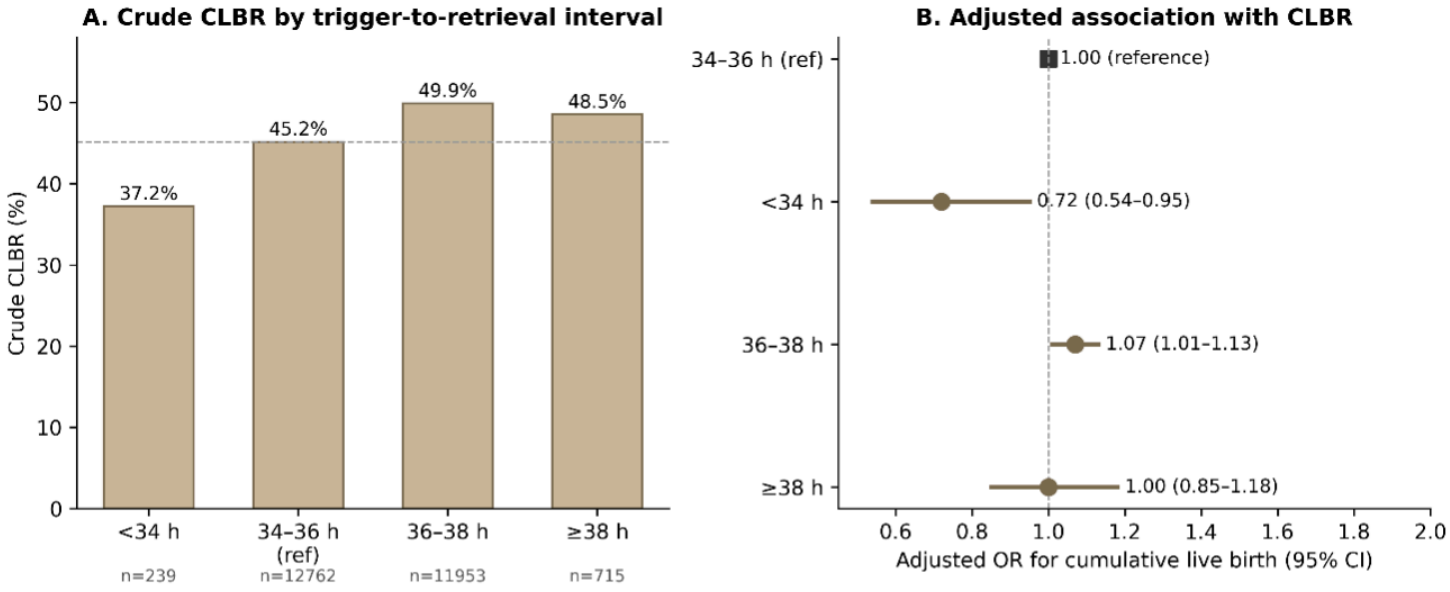


**Figure 1**

Trigger-to-retrieval interval and cumulative live birth.

(A) Crude CLBR by interval band (reference 34–36 h, dashed line). (B) Forest plot of adjusted CLBR odds ratios by interval band (reference 34–36 h). Estimates derive from cluster-robust logistic regression

adjusted for female age, stimulation duration, trigger-day follicle count, protocol, fertilization method, hCG dose, trigger agent and year.

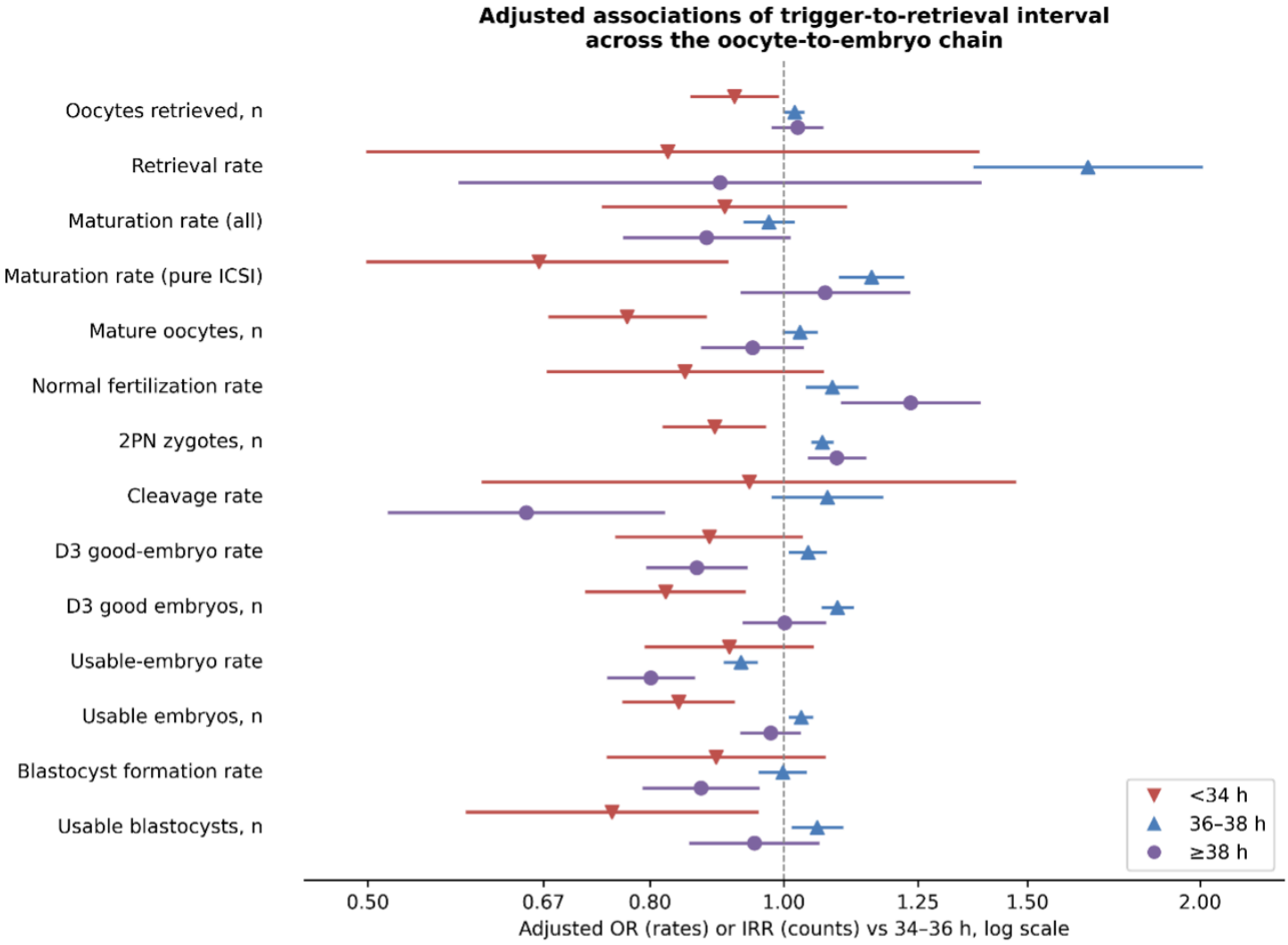


**Figure 2**

**Laboratory-chain forest plot.** Adjusted odds ratios (rates) and incidence-rate ratios (counts) for 14 chain outcomes, contrasting <34 h, 36–38 h and ≥38 h with 34–36 h; filled symbols denote Benjamini–Hochberg q < 0.05; exact estimates, confidence intervals and q-values are given in Supplemental Table 1.

## Supplementary Files

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