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Retrieval-to-transfer interval and autologous frozen embryo transfer outcomes: age-stratified and joint associations with oocyte retrieval age

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Background: The retrieval-to-transfer interval (RTI) is the elapsed time between oocyte retrieval and frozen embryo transfer (FET), which in an autologous freeze-all cohort equals the duration of embryo cryostorage. Whether RTI is associated with FET outcomes independently of oocyte retrieval age, whether this association differs across retrieval-age strata, and whether the two risks combine additively or synergistically have not been formally evaluated in a large autologous FET cohort.

Methods: In a single-center retrospective cohort of 57,282 women undergoing their first autologous vitrified FET (January 2018–August 2025), maternal ages at retrieval and transfer were each categorized (<30, 30–34, 35–39, and ≥40 years) and cross-classified into eight age-combination groups, two of which combined small age-discordant subgroups. RTI was modeled both continuously and categorically (≤1, 2–4, and ≥5 months) across four retrieval-age strata using stratified restricted cubic splines, and effect modification was assessed on multiplicative and additive scales. The primary outcome was live birth; secondary outcomes were clinical pregnancy and clinical pregnancy loss (analyzed only among clinical pregnancies).

Results: Adjusted live birth was lowest when both oocyte retrieval and embryo transfer occurred at ≥40 years (OR 0.24, 95% CI 0.20–0.28, versus both at 30–34 years). After adjustment for retrieval age, RTI ≥5 months (versus ≤1 month) remained associated with lower live birth (OR 0.88, 95% CI 0.83–0.93) and clinical pregnancy (OR 0.79, 95% CI 0.75–0.84), and with higher clinical pregnancy loss (OR 1.19, 95% CI 1.08–1.30; all $P < 0.001$), greatest in the 30–39-year retrieval strata; in the 35–39-year stratum, adjusted live birth fell from 35.8% at 3 months to 28.6% at 24 months. A multiplicative interaction was detected for clinical pregnancy; odds-ratio-scale additive interaction showed no departure from additivity for any outcome.

Conclusions: A longer RTI was associated, in a dose-dependent manner, with less favorable FET outcomes, most markedly when oocytes were retrieved at 30–39

years. When advanced retrieval age and a long RTI occurred together, the associated risks combined approximately additively rather than synergistically. Because this design cannot separate maternal aging, cryostorage duration, and reasons for delay, these findings represent associations rather than causal effects; RTI merits consideration alongside retrieval age when counseling on transfer timing.

KEYWORDS

clinical pregnancy loss, cryostorage duration, frozen-thawed embryo transfer, live birth, oocyte retrieval age, retrieval-to-transfer interval

1 Introduction

The rising age at first childbirth has driven widespread adoption of freeze-all strategies, in which embryos are cryopreserved after retrieval and transferred in subsequent cycles (1, 2). This creates a temporal separation between two ages that are otherwise identical: oocyte retrieval age, which is fixed once embryos are vitrified, and embryo transfer age, which continues to advance. We refer to the elapsed time between them as the retrieval-to-transfer interval (RTI). RTI is used here as a neutral temporal descriptor rather than a mechanistic construct: it makes the contribution of oocyte age at retrieval and of elapsed time to transfer separately estimable within a single autologous frozen embryo transfer (FET) cohort, which is a prerequisite for establishing whether elapsed time carries risk of its own and, if so, in which women. Because, in an autologous freeze-all cohort, this interval is also the duration of embryo cryostorage, RTI bundles continued maternal aging, cryostorage, and the circumstances of delay, which this design cannot separate.

That maternal age impairs FET outcomes is well established (3, 4); the consequential question is no longer whether age matters, but whether its two temporally separable components—oocyte age at retrieval, which is fixed once oocytes are vitrified, and the elapsed time to transfer, which continues to accrue afterward—contribute to outcome and whether that contribution is uniform across the reproductive lifespan. Conventional analyses measure maternal age as a single fused value at the time of treatment: they can quantify the level of risk associated with being older but cannot isolate the marginal risk of additional elapsed time once oocyte age is held fixed nor detect whether that marginal risk varies by age stratum.

Of these two components, the oocyte component is well characterized: aneuploidy rates rise from approximately 30% to more than 90% in women over 40, mediated by cohesion fatigue and spindle checkpoint dysfunction (5). Declining endometrial receptivity is one candidate contributor to the post-retrieval component; it is mechanistically supported (6, 7) but remains clinically contested: oocyte donation studies suggest a residual association with uterine aging independent of oocyte quality (8, 9), yet whether the association with elapsed time varies by retrieval age stratum and whether it combines with retrieval age on the multiplicative or the additive scale have not, to our knowledge, been formally tested in a large autologous FET cohort.

A second, complementary literature has framed this same interval as embryo cryostorage duration and has likewise not resolved the question. Prior studies of cryostorage duration have yielded inconsistent results (10–16). Both literatures share the same difficulty: in an autologous cohort, storage duration, elapsed time, and maternal age at transfer advance together (r = 0.987 between retrieval and transfer age in the present cohort), and the reasons for delay are rarely recorded. A recently published retrospective cohort of 1,837 women undergoing FET after fresh cycle cancellation provided preliminary age-stratified evidence that shorter intervals were associated with more favorable reproductive outcomes in younger women and that prolonged delay increased clinical pregnancy loss risk in women aged 35–40 years (17); however, that analysis was restricted to post-cancellation cycles and did not formally evaluate whether the associations with oocyte age and post-retrieval elapsed time combine additively or synergistically across retrieval age strata.

To address these gaps, we analyzed 57,282 first autologous FET cycles, parameterizing transfer age as the sum of retrieval age and RTI in order to characterize the dose–response association of RTI across retrieval age strata, to test its interaction with oocyte retrieval age on both the multiplicative and the additive scale, and to estimate its association with live birth, clinical pregnancy, and clinical pregnancy loss. We thereby examine whether any association with RTI is independent of oocyte retrieval age, whether it is concentrated within specific age windows rather than uniform across the reproductive lifespan, and whether it is additive or synergistic with oocyte retrieval age. The present study therefore asks a descriptive question that is directly relevant to counseling: given the age at which oocytes were retrieved, how are outcomes associated with the time that elapses before transfer, does this association differ across retrieval age strata, and does it combine with retrieval age additively or multiplicatively? Because RTI bundles maternal aging, cryostorage, and the circumstances of delay, we quantify the combined time-related association without attributing it to a specific mechanism.

2 Methods

2.1 Study design and participants

We conducted a single-center retrospective cohort study at Sichuan Jinxin Xinan Women and Children's Hospital. We enrolled all patients undergoing their first FET cycle with autologous oocytes and vitrified embryos between January 2018 and August 2025. The

exclusions were chromosomal abnormality in either partner, significant uterine pathology (congenital malformations, intrauterine adhesions grades III–IV, or severe endometriosis stage IV), recurrent miscarriage (≥3 losses), donor sperm use, untreated hydrosalpinx, or a missing value for any key variable (maternal age at retrieval, maternal age at transfer, endometrial preparation protocol, embryo stage at transfer, number of embryos transferred, or pregnancy outcome). After exclusions, 57,282 cycles were included (Figure 1). Data were de-identified by two independent coordinators; the study was reported per STROBE guidelines (18).

2.2 Study procedures

Ovarian stimulation, retrieval, and vitrification (Cryotop method, KITAZATO; day 3 or day 5/6) protocols are detailed in Supplementary Methods S1. Endometrial preparation used one of four protocols: natural cycle, hormone replacement therapy, ovarian stimulation, or downregulation. One or two embryos were transferred per cycle under ultrasound guidance, with luteal-phase progesterone support continued through the first trimester. Serum hCG was measured 10 days post-transfer, and clinical pregnancy was confirmed by transvaginal ultrasound at 6 to 7 weeks.

2.3 Outcome measures and definitions

Terminology was adapted from the International Glossary on Infertility and Fertility Care (19); the operational definitions applied in this cohort are specified below. Live birth (primary outcome) was defined as delivery of ≥1 liveborn neonate at ≥24 completed weeks of gestation. Clinical pregnancy required ≥1 gestational sac with fetal cardiac activity on ultrasound at 6 to 7 weeks; serum hCG positivity alone was insufficient. Clinical pregnancy loss was defined as spontaneous miscarriage after confirmed clinical pregnancy (miscarriages/clinical pregnancies ×100%). Consistent with this definition, clinical pregnancy loss was modeled only among cycles that achieved a clinical pregnancy (n = 34,466), whereas live birth and clinical pregnancy were modeled in the full cohort. Because this restriction conditions on an intermediate outcome, loss estimates may be affected by selection bias and are interpreted with that caveat.

2.4 Exposure and adjustment variables

Maternal age at retrieval and transfer were each categorized into four strata (<30, 30–34, 35–39, and ≥40 years) and cross-classified into eight groups after merging small-cell combinations (G1–G8;

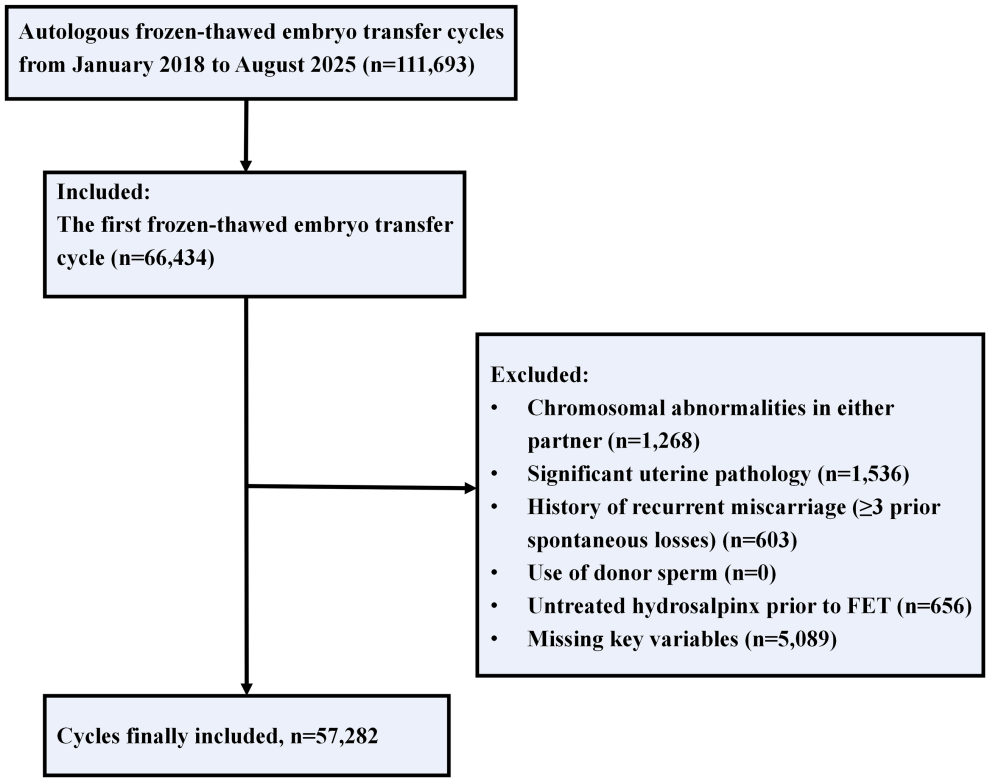

FIGURE 1
Study flowchart of patient selection and inclusion. Flowchart depicting the stepwise selection of participants included in the final analysis. A total of 111,693 autologous frozen–thawed embryo transfer cycles were performed at Sichuan Jinxin Xinan Women and Children's Hospital between January 2018 and August 2025. After restricting to each patient's first FET cycle, 66,434 cycles were eligible for further screening. Cycles were subsequently excluded for the following reasons: chromosomal abnormality in either partner (n = 1,268), significant uterine pathology (n = 1,536), history of recurrent miscarriage (≥3 prior spontaneous losses) (n = 603), use of donor sperm (n = 0), untreated hydrosalpinx prior to FET (n = 656), and missing key variables (n = 5,089). A final cohort of 57,282 autologous first FET cycles was included in the analysis. FET, frozen–thawed embryo transfer.

Supplementary Table S1), with G4 (retrieval and transfer both 30–34) as the reference.

The retrieval-to-transfer interval (RTI) was derived as the interval in months between oocyte retrieval and embryo transfer. Because retrieval and transfer ages are near-perfectly collinear (r = 0.987, Supplementary Table S4), transfer age was reparameterized as retrieval age + RTI, allowing the associations with retrieval age and with RTI to be estimated simultaneously and interpreted separately. RTI is measured as elapsed calendar time only and carries no mechanistic assumption. In an autologous freeze-all cohort, the elapsed time between retrieval and transfer is numerically identical to the duration of embryo cryostorage, so the two exposures cannot be separated in this design; any association reported here may therefore reflect continued maternal aging, cumulative effects of cryostorage on the embryo, the clinical or personal reasons that caused the delay, or a combination of these. We accordingly report associations with RTI without attributing them to a specific biological pathway and address this limitation explicitly in the "Discussion".

Covariates were selected *a priori* via directed acyclic graph (DAG; Supplementary Figure S1) and comprised female body mass index (BMI), male age, infertility duration, infertility type, infertility factors, and anti-Müllerian hormone (AMH). Treatment-related variables (endometrial preparation protocols, embryo stage, number transferred, and endometrial thickness) were added in the fully adjusted model (overadjustment potential assessed in sensitivity analyses; Supplementary Methods S2). Among embryo-related characteristics, embryo stage at transfer (cleavage-stage vs. blastocyst) and the number of embryos transferred were recorded and adjusted for; morphological grade, day 5 versus day 6 blastocyst designation, fertilization method, post-warming survival, embryo selection order, and the number of preceding retrieval cycles were not captured in the database. Apart from post-warming survival, these characteristics are fixed before cryopreservation and are not expected to be systematically related to how long transfer was subsequently deferred, so substantial confounding of the RTI association is less likely, although residual confounding cannot be excluded. Prespecified sensitivity analyses restricted separately to single-embryo transfers and to blastocyst-stage transfers, which together reduce heterogeneity in embryo number and developmental stage, were performed to examine this (Supplementary Tables S7, S8). No cycle in this cohort underwent preimplantation genetic testing for aneuploidy (PGT-A).

2.5 Statistical analysis

All analyses were performed in R (v4.4.1). Continuous data are reported as mean ± SD or median (IQR) and categorical data as n (%). Group differences were tested by ANOVA or Kruskal–Wallis and chi-square or Fisher's exact test. Missing covariate data (AMH ~20%; all others <5%) were imputed by random forest imputation with predictive mean matching (missRanger v2.6.1; seed = 123), which yields a single completed dataset and therefore does not propagate imputation uncertainty into the standard errors. Two analyses address this: multiple imputation by chained equations (m = 20, combined by Rubin's rules) and a complete-case analysis restricted to the 45,558 cycles with directly measured AMH. Multivariable logistic regression estimated adjusted ORs (95% CI) and absolute risk differences (ARDs by marginal standardization) under unadjusted (model 1) and fully adjusted (model 2) specifications. Clinical pregnancy loss was modeled only among cycles achieving a clinical pregnancy. Dose–response associations were characterized categorically (≤1 [reference], 2–4, ≥5 months) and continuously (per-month OR). Stratified restricted cubic spline (RCS) analyses across four retrieval age strata (knots selected by Akaike information criterion [AIC]) assessed nonlinearity and heterogeneity of the association across strata. Effect modification was quantified on multiplicative (product-term logistic regression) and additive (relative excess risk due to interaction [RERI], attributable proportion [AP], synergy index [SI]; delta-method 95% CIs) scales; because odds ratios overstate risk ratios for common outcomes, additive interaction was additionally quantified on the risk-difference scale by marginal standardization (Supplementary Methods S2). Eight sensitivity analyses, including merging of the two smallest age-combination groups (G3 into G2 and G7 into G6) and a complete-case analysis restricted to cycles with directly measured AMH, are described in Supplementary Methods S2. All P-values are two-tailed (α = 0.05); Benjamini–Hochberg false discovery rate (FDR) corrections were computed as a supplementary check. No *a priori* power calculation was performed (the cohort was predefined); *post hoc* calculations for the two smallest age-combination groups, G3 (n = 61) and G7 (n = 275), showed that only large differences from G4 were detectable (Supplementary Methods S2). Estimates for these two groups are flagged as unstable and interpreted descriptively throughout.

3 Results

3.1 Baseline characteristics of participants

A total of 57,282 autologous FET cycles were included and classified into eight age-combination groups (Supplementary Table S1), of which G4 was the largest (n = 22,107). Baseline characteristics differed significantly across groups (all P < 0.001; Table 1), with ovarian reserve markers and blastocyst transfer rates generally declining with advancing age (median AMH, 4.97 to 1.77 ng/mL; antral follicle count (AFC), 21 to 9; blastocyst rate, 84.9% to 36.5%, G1 to G8). The RTI was substantially longer in age-discordant groups (median 89 months in G3, 27 in G2, 21 in G5, and 12 in G7; Supplementary Figure S2). Crude live birth rates ranged from 50.2% (G1) to 13.3% (G8), excluding G3 (n = 61); clinical pregnancy loss ranged from 12.9% to 40.3% (Table 1).

3.2 Stratum-specific associations of oocyte retrieval age and embryo transfer age with FET outcomes

Stratified analyses indicated that retrieval age and transfer age were each associated with outcomes (Supplementary Tables S2, S3). Within the ≥40-year transfer stratum, retrieval at 35–39 versus ≥40

TABLE 1 Baseline characteristics of study participants according to the eight age-combination groups.

Characteristic	Overall	G1	G2	G3	G4	G5	G6	G7	G8	*P*-value
N (%)	57,282	18,261	800	61	22,107	817	10,939	275	4,022	
Female BMI (*n*, %)										<0.001
<18.5	5,243 (9.2)	2,130 (11.7)	84 (10.5)	11 (18.0)	2,081 (9.4)	78 (9.5)	724 (6.6)	16 (5.8)	119 (3.0)	
18.5–23.9	37,770 (65.9)	11,735 (64.3)	527 (65.9)	39 (63.9)	14,974 (67.7)	551 (67.4)	7,245 (66.2)	177 (64.4)	2,522 (62.7)	
≥24	14,269 (24.9)	4,396 (24.1)	189 (23.6)	11 (18.0)	5,052 (22.9)	188 (23.0)	2,970 (27.2)	82 (29.8)	1,381 (34.3)	
Male age (years)	33.36 ± 4.93	29.52 ± 3.33	30.25 ± 3.28	29.50 ± 3.13	33.38 ± 3.49	34.20 ± 3.51	37.17 ± 4.01	38.30 ± 4.21	40.49 ± 4.13	<0.001
Infertility duration (years)	3.00 (2.00, 5.00)	2.00 (1.00, 4.00)	3.00 (2.00, 5.00)	4.00 (3.00, 6.00)	3.00 (2.00, 5.00)	4.00 (2.00, 6.00)	3.00 (2.00, 6.00)	4.00 (2.00, 7.00)	3.00 (1.00, 5.00)	<0.001
Infertility type (*n*, %)										<0.001
Secondary	29,140 (50.9)	7,203 (39.4)	449 (56.1)	43 (70.5)	10,860 (49.1)	537 (65.7)	6,922 (63.3)	176 (64.0)	2,950 (73.3)	
Primary	28,142 (49.1)	11,058 (60.6)	351 (43.9)	18 (29.5)	11,247 (50.9)	280 (34.3)	4,017 (36.7)	99 (36.0)	1,072 (26.7)	
Infertility factors (*n*, %)										<0.001
Male factor	18,406 (32.1)	6,224 (34.1)	311 (38.9)	26 (42.6)	6,723 (30.4)	322 (39.4)	3,374 (30.8)	103 (37.5)	1,323 (32.9)	
Female factor	23,169 (40.4)	7,125 (39.0)	278 (34.8)	17 (27.9)	8,973 (40.6)	281 (34.4)	4,792 (43.8)	99 (36.0)	1,604 (39.9)	
Combined male/female	8,792 (15.3)	2,790 (15.3)	113 (14.1)	8 (13.1)	3,541 (16.0)	151 (18.5)	1,564 (14.3)	35 (12.7)	590 (14.7)	
Unknown	6,915 (12.1)	2,122 (11.6)	98 (12.2)	10 (16.4)	2,870 (13.0)	63 (7.7)	1,209 (11.1)	38 (13.8)	505 (12.6)	
Parity (*n*, %)										<0.001
Primipara	48,367 (84.4)	17,361 (95.1)	752 (94.0)	59 (96.7)	19,363 (87.6)	727 (89.0)	7,868 (71.9)	196 (71.3)	2,041 (50.7)	
Multipara	8,915 (15.6)	900 (4.9)	48 (6.0)	2 (3.3)	2,744 (12.4)	90 (11.0)	3,071 (28.1)	79 (28.7)	1,981 (49.3)	
AFC (*n*)	18.00 (10.00, 23.45)	21.00 (14.00, 26.00)	18.78 (13.00, 22.47)	19.70 (17.42, 21.35)	18.00 (11.00, 23.43)	15.00 (10.00, 19.38)	14.00 (8.00, 21.84)	10.00 (6.00, 15.08)	9.00 (5.00, 17.00)	<0.001
Hormone levels										
AMH (ng/mL)	4.05 (2.01, 5.67)	4.97 (2.85, 6.56)	4.55 (2.82, 5.77)	5.11 (4.26, 5.54)	4.15 (2.16, 5.73)	3.39 (1.92, 4.79)	3.06 (1.49, 5.20)	1.98 (0.96, 3.40)	1.77 (0.82, 4.05)	<0.001
FSH (mIU/mL)	7.04 (5.85, 8.39)	6.69 (5.60, 7.89)	7.01 (5.83, 8.27)	6.88 (5.68, 7.86)	6.99 (5.85, 8.27)	7.36 (6.10, 8.61)	7.44 (6.13, 8.73)	8.11 (6.82, 9.03)	8.32 (6.76, 9.29)	<0.001
E2 (pg/mL)	37.00 (28.00, 49.00)	36.81 (27.35, 48.55)	42.00 (31.00, 53.00)	46.45 (35.62, 58.40)	37.00 (28.00, 49.00)	41.00 (29.00, 51.01)	37.00 (27.70, 49.00)	37.00 (27.16, 51.00)	40.00 (28.00, 51.35)	<0.001
T (ng/mL)	0.53 (0.36, 0.75)	0.56 (0.38, 0.80)	0.55 (0.38, 0.79)	0.56 (0.44, 0.75)	0.52 (0.36, 0.74)	0.53 (0.36, 0.74)	0.50 (0.34, 0.71)	0.51 (0.33, 0.72)	0.49 (0.32, 0.70)	<0.001
No. of oocytes retrieved	11.00 (7.00, 17.00)	15.00 (10.00, 20.00)	11.00 (8.00, 16.00)	11.00 (8.00, 15.00)	12.00 (8.00, 17.00)	9.00 (6.00, 13.00)	8.00 (5.00, 13.00)	5.00 (2.00, 9.00)	4.00 (3.00, 8.00)	<0.001
RTI (months)	2.00 (1.00, 3.00)	2.00 (1.00, 3.00)	27.00 (8.00, 48.00)	89.00 (78.00, 107.00)	2.00 (1.00, 3.00)	21.00 (8.00, 49.00)	2.00 (1.00, 4.00)	12.00 (7.00, 30.00)	2.00 (1.00, 4.00)	<0.001
Endometrial preparation protocols (*n*, %)										<0.001

(Continued)

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TABLE 1 Continued

Characteristic	Overall	G1	G2	G3	G4	G5	G6	G7	G8	*P*-value
OS	5,251 (9.2)	1,458 (8.0)	76 (9.5)	4 (6.6)	2,130 (9.6)	85 (10.4)	1,121 (10.2)	19 (6.9)	358 (8.9)	
DR	7,160 (12.5)	1,566 (8.6)	89 (11.1)	8 (13.1)	2,566 (11.6)	160 (19.6)	1,786 (16.3)	79 (28.7)	906 (22.5)	
HRT	39,013 (68.1)	13,813 (75.6)	531 (66.4)	41 (67.2)	14,988 (67.8)	467 (57.2)	6,693 (61.2)	149 (54.2)	2,331 (58.0)	
NC	5,858 (10.2)	1,424 (7.8)	104 (13.0)	8 (13.1)	2,423 (11.0)	105 (12.9)	1,339 (12.2)	28 (10.2)	427 (10.6)	
Endometrial thickness at transfer (mm)	9.00 (8.00, 11.00)	10.00 (8.00, 11.00)	9.94 (8.00, 11.00)	10.00 (9.00, 11.00)	9.55 (8.00, 11.00)	9.00 (8.00, 10.00)	9.00 (8.00, 11.00)	9.00 (8.00, 10.00)	9.00 (8.00, 10.00)	<0.001
Embryo stage at transfer (*n*, %)										<0.001
Cleavage embryo	13,965 (24.4)	2,749 (15.1)	131 (16.4)	6 (9.8)	4,576 (20.7)	186 (22.8)	3,636 (33.2)	128 (46.5)	2,553 (63.5)	
Blastocyst	43,317 (75.6)	15,512 (84.9)	669 (83.6)	55 (90.2)	17,531 (79.3)	631 (77.2)	7,303 (66.8)	147 (53.5)	1,469 (36.5)	
No. of embryos transferred (*n*, %)										<0.001
Single	28,412 (49.6)	8,461 (46.3)	449 (56.1)	26 (42.6)	11,159 (50.5)	504 (61.7)	5,721 (52.3)	161 (58.5)	1,931 (48.0)	
Double	28,870 (50.4)	9,800 (53.7)	351 (43.9)	35 (57.4)	10,948 (49.5)	313 (38.3)	5,218 (47.7)	114 (41.5)	2,091 (52.0)	
Live birth rate (*n*, %)										<0.001
No	32,817 (57.3)	9,097 (49.8)	445 (55.6)	29 (47.5)	11,894 (53.8)	528 (64.6)	7,127 (65.2)	208 (75.6)	3,489 (86.7)	
Yes	24,465 (42.7)	9,164 (50.2)	355 (44.4)	32 (52.5)	10,213 (46.2)	289 (35.4)	3,812 (34.8)	67 (24.4)	533 (13.3)	
Clinical pregnancy rate (*n*, %)										<0.001
No	22,816 (39.8)	5,920 (32.4)	297 (37.1)	15 (24.6)	8,089 (36.6)	377 (46.1)	5,057 (46.2)	165 (60.0)	2,896 (72.0)	
Yes	34,466 (60.2)	12,341 (67.6)	503 (62.9)	46 (75.4)	14,018 (63.4)	440 (53.9)	5,882 (53.8)	110 (40.0)	1,126 (28.0)	
Clinical pregnancy loss rate (*n*, %)										<0.001
No	29,044 (84.3)	10,750 (87.1)	415 (82.5)	37 (80.4)	12,027 (85.8)	360 (81.8)	4,700 (79.9)	83 (75.5)	672 (59.7)	
Yes	5,422 (15.7)	1,591 (12.9)	88 (17.5)	9 (19.6)	1,991 (14.2)	80 (18.2)	1,182 (20.1)	27 (24.5)	454 (40.3)	

Group definitions (G3 and G5 each combine small age-discordant subgroups; see also Supplementary Table S1): G1—oocyte retrieval age <30 years and embryo transfer age <30 years (*n* = 18,261); G2—oocyte retrieval age <30 years and embryo transfer age 30–34 years (*n* = 800); G3—oocyte retrieval age <30 years and embryo transfer age ≥35 years, combined from the original subgroups with transfer age 35–39 or ≥40 years (*n* = 61); G4—oocyte retrieval age 30–34 years and embryo transfer age 30–34 years (*n* = 22,107); G5—oocyte retrieval age 30–34 years and embryo transfer age ≥35 years, combined from the original subgroups with transfer age 35–39 or ≥40 years (*n* = 817); G6—oocyte retrieval age 35–39 years and embryo transfer age 35–39 years (*n* = 10,939); G7—oocyte retrieval age 35–39 years and embryo transfer age ≥40 years (*n* = 275); G8—oocyte retrieval age ≥40 years and embryo transfer age ≥40 years (*n* = 4,022). BMI categories are based on Chinese/Asian-specific cutoffs (underweight: <18.5 kg/m^2; normal: 18.5–23.9 kg/m^2; overweight/obese: ≥24 kg/m^2). Outcome definitions: Clinical pregnancy loss rate refers to the proportion of spontaneous miscarriages among cycles with confirmed clinical pregnancy. Data are presented as mean ± standard deviation, median (interquartile range), or (*n*, %). *P*-values were calculated using analysis of variance, the Kruskal–Wallis test, or the χ^2 test, as appropriate. $P < 0.05$ was considered statistically significant.

BMI, body mass index; AFC, antral follicle count; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; E2, estradiol; T, testosterone; RTI, retrieval-to-transfer interval; HRT, hormone replacement therapy; OS, ovarian stimulation; NC, natural cycle; DR, down-regulation cycle.

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years was associated with significantly higher live birth (OR 1.88, 95% CI 1.38–2.54) and clinical pregnancy (OR 1.54, 95% CI 1.18–2.01) and lower clinical pregnancy loss (OR 0.52, 95% CI 0.32–0.82; all $P < 0.01$), indicating that younger retrieval age remained associated with better outcomes even within the oldest transfer stratum. Conversely, within the 30–34-year retrieval stratum, transfer at ≥35 years was associated with lower live birth (OR 0.70) and clinical pregnancy (OR 0.74; both $P < 0.001$).

Across the eight groups, FET outcomes were consistently less favorable in the older groups relative to the reference group G4 (Table 2; Supplementary Figure S3). Compared with G4, adjusted live birth ORs were significantly reduced in G5 (0.61), G6 (0.69), G7 (0.57), and G8 (0.24; all $P < 0.05$), with G8 showing the largest absolute reduction (ARD −27.4%). Estimates for the two smallest groups, G3 and G7 (n = 61 and 275), were unstable, with wide confidence intervals, and should be interpreted with caution; merging G3 into G2 and G7 into G6 left the remaining estimates essentially unchanged, although the merged G2 showed a higher clinical pregnancy loss (OR 1.40, 95% CI 1.11–1.75; Supplementary Table S17). Women in G8 faced approximately 27–29 percentage-point lower adjusted probabilities of live birth and clinical pregnancy and nearly 20 percentage-point higher clinical pregnancy loss (OR 3.09, 95% CI 2.43–3.92) relative to G4. Adjusted predicted rates across the 4×4 retrieval × transfer matrix are shown in Supplementary Figure S4.

3.3 Adjusted and dose–response associations of RTI with FET outcomes

After adjustment for oocyte retrieval age and other covariates, an RTI ≥5 months remained associated with lower live birth (OR 0.88, 95% CI 0.83–0.93), clinical pregnancy (OR 0.79, 95% CI 0.75–

TABLE 2 Association of age-combination groups at oocyte retrieval and embryo transfer with clinical outcomes of frozen–thawed embryo transfer cycles.

Outcomes	Groups	Model 1 OR (95% CI)	*P*-value 1	Model 2 OR (95% CI)	*P*-value 2	ARD (95% CI)
Live birth	G1	1.17 (1.13, 1.22)	<0.001	1.04 (0.96, 1.12)	0.330	0.93% (-1.59%, 3.45%)
	G2	0.93 (0.81, 1.07)	0.310	0.89 (0.70, 1.15)	0.377	-2.71% (-10.69%, 5.27%)
	G3[a]	1.29 (0.78, 2.14)	0.329	1.78 (0.75, 4.55)	0.203	14.34% (-14.97%, 43.66%)
	G4	Reference		Reference		Reference
	G5	0.64 (0.55, 0.74)	<0.001	0.61 (0.47, 0.80)	<0.001	-11.23% (-18.87%, -3.59%)
	G6	0.62 (0.59, 0.65)	<0.001	0.69 (0.63, 0.75)	<0.001	-8.73% (-11.51%, -5.95%)
	G7[a]	0.38 (0.28, 0.49)	<0.001	0.57 (0.36, 0.89)	0.016	-12.68% (-25.29%, -0.06%)
	G8	0.18 (0.16, 0.20)	<0.001	0.24 (0.20, 0.28)	<0.001	-27.43% (-30.71%, -24.15%)
Clinical pregnancy	G1	1.20 (1.15, 1.25)	<0.001	1.05 (0.97, 1.14)	0.242	1.15% (-1.42%, 3.72%)
	G2	0.98 (0.85, 1.13)	0.758	0.98 (0.76, 1.27)	0.867	-0.53% (-8.91%, 7.84%)
	G3[a]	1.77 (1.01, 3.28)	0.055	2.45 (0.90, 8.58)	0.109	18.78% (-6.27%, 43.83%)
	G4	Reference		Reference		Reference
	G5	0.67 (0.59, 0.78)	<0.001	0.69 (0.54, 0.89)	0.004	-9.00% (-17.39%, -0.61%)
	G6	0.67 (0.64, 0.70)	<0.001	0.79 (0.72, 0.86)	<0.001	-5.76% (-8.70%, -2.81%)
	G7[a]	0.38 (0.30, 0.49)	<0.001	0.55 (0.36, 0.83)	0.005	-14.78% (-28.53%, -1.03%)
	G8	0.22 (0.21, 0.24)	<0.001	0.30 (0.26, 0.35)	<0.001	-28.86% (-32.97%, -24.74%)
Clinical pregnancy loss	G1	0.89 (0.83, 0.96)	0.002	1.03 (0.90, 1.17)	0.650	0.37% (-1.77%, 2.50%)
	G2	1.28 (1.01, 1.61)	0.039	1.19 (0.78, 1.77)	0.399	2.26% (-5.20%, 9.73%)
	G3[a]	1.47 (0.66, 2.91)	0.302	1.83 (0.51, 5.17)	0.293	8.92% (-17.66%, 35.50%)
	G4	Reference		Reference		Reference
	G5	1.34 (1.04, 1.71)	0.019	1.65 (1.08, 2.45)	0.016	7.17% (-1.85%, 16.18%)
	G6	1.52 (1.40, 1.64)	<0.001	1.38 (1.20, 1.60)	<0.001	4.39% (1.59%, 7.19%)
	G7[a]	1.97 (1.25, 3.00)	0.002	1.76 (0.83, 3.44)	0.116	8.23% (-7.98%, 24.44%)
	G8	4.08 (3.59, 4.64)	<0.001	3.09 (2.43, 3.92)	<0.001	19.48% (12.60%, 26.36%)

Group definitions (G3 and G5 each combine small age-discordant subgroups; see also Supplementary Table S1): G1—oocyte retrieval age <30 years and embryo transfer age <30 years (n = 18,261); G2—oocyte retrieval age <30 years and embryo transfer age 30–34 years (n = 800); G3—oocyte retrieval age <30 years and embryo transfer age ≥35 years, combined from the original subgroups with transfer age 35–39 or ≥40 years (n = 61); G4—oocyte retrieval age 30–34 years and embryo transfer age 30–34 years (n = 22,107); G5—oocyte retrieval age 30–34 years and embryo transfer age ≥35 years, combined from the original subgroups with transfer age 35–39 or ≥40 years (n = 817); G6—oocyte retrieval age 35–39 years and embryo transfer age 35–39 years (n = 10,939); G7—oocyte retrieval age 35–39 years and embryo transfer age ≥40 years (n = 275); G8—oocyte retrieval age ≥40 years and embryo transfer age ≥40 years (n = 4,022). ARD represents the absolute difference in predicted probabilities compared with the reference group, derived from model 2 (fully adjusted model).

Model 1, unadjusted (crude model); model 2, adjusted for female body mass index (BMI), male age, infertility duration, infertility type, infertility factors, anti-Müllerian hormone level (AMH), endometrial preparation protocols, embryo stage at transfer, number of embryos transferred, and endometrial thickness at transfer; CI, confidence interval; OR, odds ratio; ARD, absolute risk difference.

[a]Results for G3 (n = 61) and G7 (n = 275) should be interpreted with caution owing to the small sample size.

0.84), and higher clinical pregnancy loss (OR 1.19, 95% CI 1.08–1.30; all $P < 0.001$ vs. an RTI ≤1 month; Table 3). An interval of 2–4 months showed no significant association with clinical pregnancy or clinical pregnancy loss; a modest positive association with live birth (OR 1.08, 95% CI 1.03–1.12) was attenuated after calendar-year adjustment (Supplementary Table S13), suggesting residual confounding. When modeled continuously, each additional month was associated with slightly lower odds of live birth and clinical pregnancy (both OR 0.994, 95% CI 0.992–0.996) and higher odds of clinical pregnancy loss (OR 1.006, 95% CI 1.002–1.009; all $P < 0.001$; Table 3). Because the spline models departed significantly from linearity in several strata, we report adjusted probabilities at 1, 3, 6, 12, and 24 months within each retrieval age stratum (Table 4) rather than an annualized slope.

Stratified RCS analyses (Figure 2) showed that dose–response profiles varied by retrieval age stratum. In the 30–34-year and 35–39-year strata (referred to jointly hereafter as the 30–39-year retrieval window), a longer RTI was associated with a near-monotonic decline in live birth and clinical pregnancy rates (P-overall and P-nonlinear both < 0.001 for both outcomes). In the <30-year stratum, significant nonlinear associations were observed for clinical pregnancy (P-overall = 0.001, P-nonlinear < 0.001) and clinical pregnancy loss (P-overall < 0.001), but not for live birth. In the ≥40-year stratum, a predominantly linear association was detected for clinical pregnancy only (P-overall = 0.006); live birth and clinical pregnancy loss showed no significant overall association.

3.4 Effect modification of RTI by oocyte retrieval age

Having shown that the association with RTI varied by retrieval age stratum and was most marked in the 30–39-year window, we next tested formally whether oocyte retrieval age modifies the magnitude of that association. For the primary interaction analysis, retrieval age was dichotomized at 35 years (20) and RTI at 6 months, the nearest clinically interpretable boundary to the empirically identified inflection point (≥5 months) and consistent with the freeze-all literature (11). A statistically significant multiplicative interaction was detected for clinical pregnancy ($P = 0.016$; Table 5): among women with retrieval age ≥35 years, adjusted ORs were 0.67 (95% CI 0.64–0.70) for a short RTI and 0.48 (95% CI 0.44–0.53) for a long RTI relative to the <35 years/short-RTI reference. No significant interaction was detected for live birth ($P = 0.497$) or clinical pregnancy loss ($P = 0.074$). Continuous interaction models corroborated this finding ($P < 0.001$; Supplementary Table S5). Additive interaction analyses showed RERI, attributable proportion, and synergy index near null and non-significant across all outcomes on the odds-ratio scale (Table 5). On the absolute (risk-difference) scale, the joint association of advanced retrieval age and a longer interval did not exceed the sum of the individual associations for any outcome; for clinical pregnancy, it was modestly sub-additive (risk-difference–scale RERI −3.5 percentage points, 95% CI −6.2 to −0.9), while deviations for live birth and clinical pregnancy loss were non-significant (Table 5). The absence of departure from additivity does not, however, imply that the combination is clinically unimportant: women aged ≥35 years with an RTI ≥6 months had an adjusted live-birth probability of 29.9% versus 46.2% in the reference category (risk difference −16.3 percentage points, 95% CI −18.5 to −14.3) and a corresponding difference of −17.0 points (95% CI −19.5 to −14.9) for clinical pregnancy (Table 5). The two exposures therefore combine approximately additively, and their joint absolute burden is substantial.

3.5 Sensitivity analyses

The direction and approximate magnitude of the primary findings were largely unchanged across the eight sensitivity analyses (Supplementary Tables S6–S18; Supplementary Figure S5),

TABLE 3 Association between RTI and FET outcomes: categorical and continuous analyses.

Outcomes	RTI	Model 1 OR (95% CI)	*P*-value 1	Model 2 OR (95% CI)	*P*-value 2
Live birth	≤1 month (ref)	1.00 (Ref)	—	1.00 (Ref)	—
	2–4 months	1.11 (1.07, 1.16)	<0.001	1.08 (1.03, 1.12)[a]	<0.001
	≥5 months	0.86 (0.81, 0.91)	<0.001	0.88 (0.83, 0.93)	<0.001
	Per 1-month increase	0.995 (0.993, 0.997)	<0.001	0.994 (0.992, 0.996)	<0.001
Clinical pregnancy	≤1 month (ref)	1.00 (Ref)	—	1.00 (Ref)	—
	2–4 months	1.03 (0.99, 1.07)	0.176	0.99 (0.95, 1.03)	0.486
	≥5 months	0.76 (0.72, 0.80)	<0.001	0.79 (0.75, 0.84)	<0.001
	Per 1-month increase	0.995 (0.993, 0.997)	<0.001	0.994 (0.992, 0.996)	<0.001
Clinical pregnancy loss	≤1 month (ref)	1.00 (Ref)	—	1.00 (Ref)	—
	2–4 months	1.01 (0.94, 1.08)	0.765	1.00 (0.93, 1.07)	0.990
	≥5 months	1.25 (1.14, 1.37)	<0.001	1.19 (1.08, 1.30)	<0.001
	Per 1-month increase	1.004 (1.001, 1.007)	0.011	1.006 (1.002, 1.009)	<0.001

The retrieval-to-transfer interval (RTI) was defined as the interval in months between oocyte retrieval and embryo transfer. Categorical analyses used three groups: ≤1 month (reference), 2–4 months, and ≥5 months. Continuous analyses estimated the association with each additional month of RTI. Odds ratios for continuous RTI are presented to three decimal places to preserve meaningful precision given the small per-unit change in odds. Reference categories are denoted by "—" (OR = 1.00 by definition; P-value not applicable).

Model 1: Unadjusted (crude model). Model 2: Adjusted for oocyte retrieval age, female body mass index (BMI), male age, infertility duration, infertility type, infertility factors, anti-Müllerian hormone level (AMH), endometrial preparation protocols, embryo stage at transfer, number of embryos transferred, and endometrial thickness at transfer.

[a]This association may reflect residual confounding from freeze-all cycle timing rather than a causal benefit of delay; interpret with caution.

RTI, retrieval-to-transfer interval; OR, odds ratio; CI, confidence interval; FET, frozen–thawed embryo transfer.

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TABLE 4 Adjusted absolute probabilities of frozen–thawed embryo transfer outcomes at increasing RTI, stratified by oocyte retrieval age.

Oocyte age group	No.	Adjusted probability, % (95% CI), by RTI (months)					P^a	P^b
		1	3	6	12	24		
Live birth								
<30	19,122	49.8 (48.7, 50.9)	50.3 (49.4, 51.3)	50.3 (49.1, 51.5)	49.8 (48.6, 51.0)	48.9 (47.3, 50.5)	0.099	0.366
30–34	22,924	45.9 (45.0, 46.9)	46.6 (45.8, 47.4)	43.7 (42.2, 45.3)	42.3 (40.6, 44.0)	41.1 (39.3, 42.9)	<0.001	<0.001
35–39	11,214	34.7 (33.3, 35.9)	35.8 (34.7, 36.9)	32.4 (30.5, 34.2)	30.5 (28.3, 32.5)	28.6 (25.0, 31.8)	<0.001	<0.001
≥40	4,022	14.3 (12.6, 15.9)	13.0 (11.8, 14.4)	12.2 (10.6, 13.9)	11.7 (8.9, 13.6)	10.7 (4.8, 15.7)[c]	0.192	0.329
Clinical pregnancy								
<30	19,122	68.6 (67.7, 69.6)	66.7 (65.9, 67.5)	65.1 (63.5, 66.6)	64.6 (62.8, 66.3)	65.2 (63.4, 66.9)	0.001	<0.001
30–34	22,924	64.3 (63.3, 65.3)	63.2 (62.4, 64.0)	59.5 (58.0, 61.1)	58.3 (56.5, 60.0)	58.1 (56.5, 60.0)	<0.001	<0.001
35–39	11,214	55.4 (54.0, 56.8)	53.7 (52.6, 54.9)	48.9 (46.8, 50.8)	46.9 (44.6, 49.0)	45.8 (42.4, 49.1)	<0.001	<0.001
≥40	4,022	29.9 (27.7, 32.1)	28.0 (26.4, 29.7)	26.2 (24.0, 28.4)	23.9 (20.7, 26.6)	19.8 (12.9, 26.4)[c]	0.006	0.464
Clinical pregnancy loss								
<30	12,890	12.0 (11.2, 12.8)	13.5 (12.8, 14.2)	14.9 (13.4, 16.3)	15.5 (13.9, 17.2)	15.9 (14.2, 17.6)	<0.001	0.005
30–34	14,458	13.7 (12.8, 14.6)	14.7 (14.0, 15.4)	15.0 (14.1, 16.0)	15.1 (14.1, 16.1)	15.2 (13.7, 16.8)	0.175	0.106
35–39	5,992	19.9 (18.4, 21.6)	20.2 (18.9, 21.6)	20.5 (18.8, 22.2)	21.1 (18.9, 22.9)	22.2 (17.7, 26.0)	0.576	0.889
≥40	1,126	39.8 (35.6, 44.1)	41.1 (37.4, 45.2)	40.8 (36.3, 45.6)	39.1 (31.2, 45.3)	35.6 (19.1, 49.8)[c]	0.707	0.527

Values are adjusted absolute probabilities (%) with 95% confidence intervals, estimated by marginal standardization (g-computation) from oocyte-age-stratified logistic models with a restricted cubic spline for RTI (three or four knots selected by AIC); confidence intervals are from 1,000 bootstrap replicates. Models were adjusted for female body mass index, male age, infertility duration, infertility type, infertility factors, anti-Müllerian hormone, endometrial preparation protocols, embryo stage at transfer, number of embryos transferred, and endometrial thickness at transfer. Clinical pregnancy loss was analyzed only within clinical pregnancies, for which No. denotes the number of clinical pregnancies.

[a]*P* for overall association.

[b]*P* for nonlinearity; both from likelihood ratio tests.

[c]In the ≥40-year stratum, an RTI of 24 months lies at or slightly beyond the 99th percentile of the observed distribution; these estimates rest on few cycles and should be read with the width of their confidence intervals in mind. The numbers of cycles with an RTI of ≥12 and ≥24 months were 1,010 and 678 (<30 years), 1,102 and 603 (30–34 years), 492 and 158 (35–39 years), and 230 and 45 (≥40 years).

RTI, retrieval-to-transfer interval; CI, confidence interval; No., number of cycles.

including reanalysis using the original 10-group classification, restriction to single-embryo or blastocyst-only transfers, reduced adjustment, truncation of RTI at the 99th percentile, calendar-year adjustment, and multiple imputation. Merging the two smallest groups (G3 into G2 and G7 into G6) did not materially change the remaining estimates (Supplementary Table S17). In the complete-case subset with directly measured AMH (n = 45,558; Supplementary Table S18), the live-birth and clinical-pregnancy associations were concordant with the main analysis, whereas the clinical-pregnancy-loss association was attenuated and no longer statistically significant although unchanged in direction. FDR correction did not alter any statistical conclusion (Supplementary Table S16). All primary models demonstrated adequate calibration (Supplementary Figure S6); area under the receiver operating characteristic curve (AUC) values were 0.652–0.659 for live birth and clinical pregnancy and 0.595–0.601 for clinical pregnancy loss. These values are typical of etiological models built from routinely collected clinical variables and indicate that the models are not intended and should not be used for individual-level prediction.

4 Discussion

Advancing maternal age is an established determinant of live birth after FET. Less certain is whether that risk is fully captured by oocyte age alone, whether it is uniform across the reproductive lifespan, and whether it compounds with oocyte age or contributes separately. In this cohort of 57,282 first autologous FET cycles, three principal findings emerged. First, advancing transfer age was independently associated with lower live birth and clinical pregnancy within fixed retrieval age strata, while younger retrieval age remained associated with better outcomes even in the oldest transfer stratum. Second, a longer RTI was associated with all three outcomes in a dose-dependent manner after adjustment for retrieval age, most markedly, for live birth and clinical pregnancy, in the 30–39-year window. Third, a multiplicative interaction was detected for clinical pregnancy (P = 0.016), whereas the additive-interaction indices showed no departure from additivity for any outcome on the odds-ratio scale and only a modest sub-additive deviation for clinical pregnancy on the risk-difference scale. The associated risks of older retrieval age and of a longer RTI therefore combined approximately additively rather than synergistically; the design, however, does not allow us to identify the biological processes responsible. Our contribution is therefore descriptive rather than mechanistic: an age-stratified, dose–response characterization of the time-related association after retrieval, its joint absolute burden with retrieval age, and stratum-specific absolute probabilities that can inform counseling on transfer timing.

The outcome gradient across age-combination groups is consistent with the near-exponential rise in aneuploidy with advancing maternal age (21). Critically, younger oocytes retained a statistically

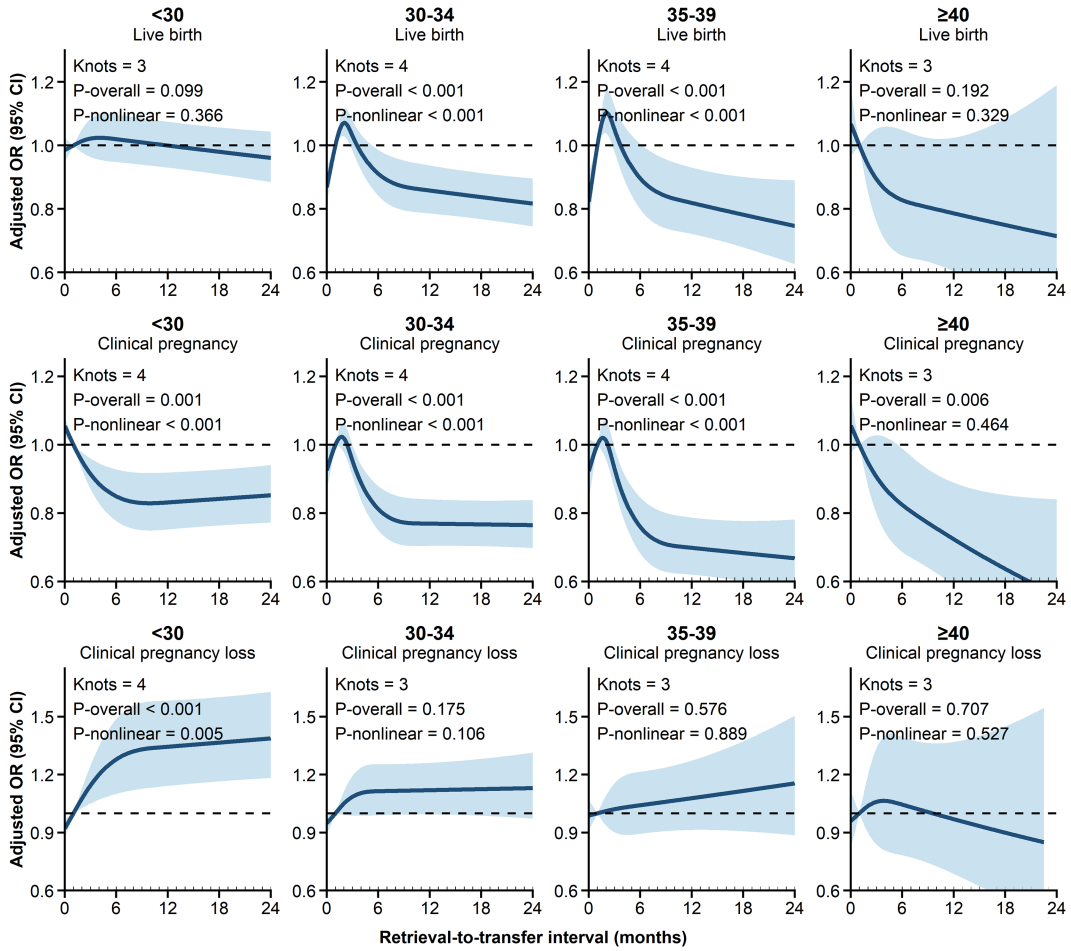

FIGURE 2
Restricted cubic spline analyses of RTI stratified by oocyte retrieval age group. Restricted cubic spline models were used to evaluate the adjusted associations between RTI and three frozen–thawed embryo transfer outcomes (live birth, clinical pregnancy, and clinical pregnancy loss) within four oocyte retrieval age groups (<30 years, *n* = 19,122; 30–34 years, *n* = 22,924; 35–39 years, *n* = 11,214; ≥40 years, *n* = 4,022). Curves represent adjusted odds ratios (ORs) and shaded areas the 95% confidence intervals (CIs); the horizontal dashed line indicates an OR of 1.00. RTI = 1 month was used as the reference value in all spline models (OR = 1.00). Curves are plotted only up to the 99th percentile of RTI in each stratum, although all models were fitted to the full cohort; a sensitivity analysis in which the cohort itself was restricted to RTI ≤ the 99th percentile before refitting is shown in Supplementary Figure S5. The number of knots (three or four) was selected by AIC, with knot positions at default quantiles (three knots: 10th, 50th, and 90th; four knots: 5th, 35th, 65th, and 95th percentiles). *P*-overall and *P*-nonlinear were derived from likelihood ratio tests. Models were adjusted for female body mass index (BMI), male age, infertility duration, infertility type, infertility factors, anti-Müllerian hormone (AMH), endometrial preparation protocols, embryo stage at transfer, number of embryos transferred, and endometrial thickness at transfer. Within each outcome, the x-axis (0–24 months) and y-axis were held constant across the four strata to allow direct comparison; y-axis ranges differ between outcomes (live birth, 0.6–1.2; clinical pregnancy, 0.6–1.1; clinical pregnancy loss, 0.6–1.7) to accommodate the wider CIs of the latter, which does not affect within-outcome comparisons. RTI, retrieval-to-transfer interval; FET, frozen–thawed embryo transfer; OR, odds ratio; CI, confidence interval.

significant advantage even within the oldest transfer stratum, and advancing transfer age was associated with lower live birth and clinical pregnancy within fixed retrieval age strata. Differences in oocyte quality alone are unlikely to explain these associations fully. This residual association is consistent with declining endometrial receptivity: a meta-analysis of donor-oocyte cycles confirmed lower live birth and higher miscarriage rates at advanced age independent of oocyte quality (9), with mechanistic support from evidence of decidualization defects, immune dysregulation, and senescence-associated secretory phenotype (SASP)-mediated cellular senescence (6). Continued endometrial aging is therefore one plausible explanation for the residual association with RTI since vitrification suspends embryonic development while the maternal environment continues to change. It is not, however, the only one: cumulative effects of cryostorage on the embryo and the clinical circumstances that led to the delay remain equally compatible with our data, and the present design cannot distinguish between them. The age-stratified pattern described below is likewise compatible with each of these explanations.

Prior cryostorage studies have yielded inconsistent results (10–16): some cohort studies have reported graded, storage-time-dependent declines in outcomes (11, 12), whereas a dose–response meta-analysis and other cohort studies have found no association (13–15). Whether cryostorage duration per se adversely affects embryo viability therefore remains unresolved, and our data cannot resolve it. The literature and the present study face the same difficulty: storage duration, elapsed time, and maternal age at transfer advance together, and the reasons for delay are seldom

TABLE 5 Multiplicative and additive interaction between oocyte retrieval age and the retrieval-to-transfer interval (RTI) on FET outcomes, with additive interaction assessed on both the odds-ratio and risk-difference scales.

Outcomes	Oocyte retrieval age	RTI (months)	Adjusted OR (95% CI)	*P*-value	*P* for interaction	Additive interaction indices (OR scale)		
						RERI	AP	SI
Live birth	<35	<6	Reference		0.497			
	<35	≥6	0.83 (0.78, 0.89)	<0.001				
	≥35	<6	0.60 (0.57, 0.63)	<0.001				
	≥35	≥6	0.48 (0.43, 0.53)	<0.001				
	Additive interaction		—	—	—	0.05 (-0.03, 0.12)	0.10 (-0.06, 0.25)	0.92 (0.80, 1.05)
	Joint absolute burden (≥35 and ≥6 vs. reference)		Adjusted RD -16.3 (-18.5, -14.3) pp					
	RERI (risk-difference scale)		-0.4 (-2.6, 2.0) pp					
Clinical pregnancy	<35	<6	Reference		0.016			
	<35	≥6	0.83 (0.78, 0.88)	<0.001				
	≥35	<6	0.67 (0.64, 0.70)	<0.001				
	≥35	≥6	0.48 (0.44, 0.53)	<0.001				
	Additive interaction		—	—	—	-0.02 (-0.09, 0.06)	-0.03 (-0.19, 0.12)	1.03 (0.89, 1.19)
	Joint absolute burden (≥35 and ≥6 vs. reference)		Adjusted RD -17.0 (-19.5, -14.9) pp					
	RERI (risk-difference scale)		-3.5 (-6.2, -0.9) pp					
Clinical pregnancy loss	<35	<6	Reference		0.074			
	<35	≥6	1.27 (1.14, 1.40)	<0.001				
	≥35	<6	1.56 (1.44, 1.70)	<0.001				
	≥35	≥6	1.65 (1.39, 1.96)	<0.001				
	Additive interaction		—	—	—	-0.18 (-0.49, 0.13)	-0.11 (-0.31, 0.09)	0.79 (0.50, 1.23)
	Joint absolute burden (≥35 and ≥6 vs. reference)		Adjusted RD + 7.1 (4.3, 10.1) pp					
	RERI (risk-difference scale)		-2.2 (-5.8, 1.1) pp					

Oocyte retrieval age was dichotomized at 35 years and RTI at 6 months; the reference group comprised women aged <35 years with an RTI <6 months. Adjusted odds ratios (ORs) were estimated by multivariable logistic regression adjusted for female body mass index, male age, infertility duration, infertility type, infertility factors, anti-Müllerian hormone, endometrial preparation protocols, embryo stage at transfer, number of embryos transferred, and endometrial thickness at transfer. Multiplicative interaction was assessed with a cross-product term (likelihood ratio test). Additive interaction on the odds-ratio scale was quantified by the relative excess risk due to interaction (RERI), the attributable proportion (AP), and the synergy index (SI); values near 0 (RERI, AP) or 1 (SI) indicate no additive interaction. Absolute-scale estimates: The last two rows of each outcome were obtained by marginal standardization (g-computation) from the same interaction model, with 95% CIs from 500 bootstrap resamples. Adjusted probabilities for the four exposure categories (<35 and <6, <35 and ≥6, ≥35 and <6, ≥35 and ≥6) were, respectively: live birth 46.2%, 41.9%, 34.6%, and 29.9%; clinical pregnancy 63.4%, 59.2%, 54.1%, and 46.3%; clinical pregnancy loss 14.0%, 17.1%, 20.2%, and 21.1%. Joint absolute burden is the risk difference between the doubly exposed and reference categories. RERI on the risk-difference scale is $p_{11} - p_{10} - p_{01} + p_{00}$; computed from the unrounded probabilities, where 0 denotes exact additivity; it is reported because odds ratios overstate risk ratios for common outcomes, so odds-ratio-based indices may misrepresent additivity on the risk scale.

OR, odds ratio; CI, confidence interval; RTI, retrieval-to-transfer interval; RERI, relative excess risk due to interaction; AP, attributable proportion; SI, synergy index; RD, risk difference; pp, percentage points.

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recorded; thus, differences in study populations, adjustment strategies, and units of analysis may all have contributed to the inconsistency. What the present analysis adds is age-stratified information: dose–response profiles differed across retrieval age strata, with a longer RTI accompanied by a near-monotonic decline in live birth and clinical pregnancy in the 30–39-year window, attenuated associations below age 30, and no detectable association with live birth above age 40. This pattern is compatible with more than one explanation, including age-dependent endometrial susceptibility, age-dependent vulnerability of embryos to prolonged storage, and differing reasons for delay by age; low baseline live-birth rates in the ≥40-year stratum may also limit what can be detected there. Pooling across strata may mask such heterogeneity, but these data do not indicate which mechanism underlies it. Our parameterization (transfer age = retrieval age + RTI) allows the two ages to be interpreted separately, but it does not separate RTI from cryostorage duration. The directional age stratification in our findings is consistent with a recent smaller cohort restricted to post-cancellation FET cycles (17); unlike that study, however, the present analysis provides formal multiplicative and additive interaction testing and a continuous dose–response characterization across four retrieval age strata.

The dose–response was continuous and near-monotonic rather than threshold-based, with associations most pronounced in the 30–39-year retrieval window; to our knowledge, this age-specific pattern has not been reported in previous interval-based FET analyses (16) and requires confirmation in independent cohorts. One possible explanation is that this represents a transitional period in which functional uterine reserve begins to decline yet remains responsive to incremental elapsed time, whereas women under 30 retain greater functional reserve (9, 22) and women over 40 may already have substantially reduced reproductive capacity. Age-dependent vulnerability of embryos to prolonged storage or differences in the reasons for delay across age groups could produce a similar pattern; none of these explanations is demonstrated by the present data.

We are not aware of a previous autologous FET cohort of comparable size that has formally tested both multiplicative and additive interaction between retrieval age and RTI. We report interaction on both scales, as currently recommended (23). The largely additive joint associations indicate that the risks associated with older oocytes and with a longer interval mostly add together rather than amplifying one another, with only a modest sub-additive deviation for clinical pregnancy on the absolute scale. This pattern is compatible with two separate contributions. The first is oocyte aging, acting through chromosomal nondisjunction and reduced developmental competence (24, 25). The second is a post-retrieval component, which may reflect maternal aging, cryostorage effects, or the circumstances of delay; if maternal aging is responsible, SASP dysregulation (6, 26), impaired decidualization (27), and altered immune–endometrial crosstalk (28) have been proposed. The present data cannot confirm any of these mechanisms, and an absence of additive interaction does not by itself establish separate biological pathways.

Clinically, freeze-all strategies fix oocyte age at retrieval, a determinant of embryo developmental competence (29), but do not suspend the woman's continued aging or the passage of storage time; the continuous decline observed here, concentrated in the 30–39-year retrieval window, suggests that the retrieval-to-transfer interval is not prognostically neutral, whatever its underlying cause. For women aged 30–39 undergoing freeze-all cycles for non-medical reasons, these data are compatible with avoiding unnecessary elective deferral where this is clinically convenient, but they do not establish that earlier transfer is beneficial, and convenience should not override a medical indication for delay. In women under 30, no association with live birth was detected in the primary analysis, although one emerged after truncation of extreme intervals (Supplementary Figure S5). This should not, therefore, be taken as an assurance that deferral is without consequence since a longer interval in this stratum was still accompanied by a lower clinical pregnancy rate and a higher clinical pregnancy loss rate. Several interventions target endometrial receptivity: individualized progesterone supplementation (30), endometrial priming optimization (31, 32), and personalization of transfer timing based on endometrial transcriptomic profiling, an approach that remains biologically motivated but clinically unproven (33, 34). Whether any of these would modify the associations reported here was not examined in this study and cannot be inferred from it; such questions would require randomized evaluation.

Strengths include the large well-powered cohort (57,282 first FET cycles) enabling stratified analyses across eight age-combination groups and four retrieval age strata; the stratified RCS framework, which reveals dose–response heterogeneity that pooled analyses cannot detect; formal testing of both multiplicative and additive interaction; providing a joint assessment of interaction on both scales in autologous FET; and a parameterization (transfer age = retrieval age + RTI) that allows the associations with retrieval age and with elapsed time to be interpreted separately, although it cannot make the two exposures statistically independent or separate RTI from cryostorage duration. Eight sensitivity analyses supported the robustness of the primary findings, with the clinical-pregnancy-loss association attenuated in the complete-case AMH subset. Because RTI bundles cryostorage duration, additional maternal aging, and the circumstances of delay, our estimates describe the combined time-related association that a patient deferring transfer would experience, irrespective of which component is responsible.

Several limitations should be emphasized. First and most importantly, the retrieval-to-transfer interval and the duration of embryo cryostorage are numerically identical in an autologous freeze-all cohort and cannot be separated in this design; the association we report is therefore equally compatible with continued maternal aging, with cumulative effects of cryostorage on the embryo or with both, and our data cannot adjudicate between them. This inseparability is compounded by a field-wide uncertainty: whether cryostorage

duration itself carries an independent adverse effect on embryo viability remains an open question even among large, well-designed studies (10–16), and the present design does not allow us to resolve it either. Second, the clinical and personal reasons for delaying transfer were not recorded. These include further medical treatment, additional retrieval cycles, financial or personal decisions, and service disruption during the COVID-19 period. Confounding by indication, therefore, cannot be excluded despite calendar-year adjustment. Third, several embryo-related characteristics (morphological grade, day 5 versus day 6 blastocyst designation, fertilization method, post-warming survival, embryo selection order, and the number of preceding retrieval cycles) were not available for adjustment, and no cycle underwent PGT-A, so the contribution of embryo-related mechanisms cannot be quantified (35). Fourth, clinical pregnancy loss was analyzed only among cycles achieving a clinical pregnancy, which conditions on an intermediate outcome and may introduce selection bias. Finally, the design was retrospective and single-center; restriction to first FET cycles introduces survivor selection; and the two smallest groups (G3 and G7) yielded unstable estimates that should not be used to draw independent conclusions.

Future studies should incorporate prospective assignment of transfer timing, restriction to euploid embryo transfers to remove chromosomal contributions, analyses of donor-oocyte or embryo-donation cycles in which storage duration can vary independently of the recipient's age, systematic recording of the reasons for delay, and validated biomarkers of endometrial aging. Randomized trials of endometrial-targeting protocols stratified by RTI and retrieval age would be needed to determine whether the associations reported here are modifiable.

5 Conclusions

In this large single-center cohort of 57,282 first autologous FET cycles, a longer RTI was associated in a dose-dependent manner with lower live birth and clinical pregnancy and with higher clinical pregnancy loss after adjustment for oocyte retrieval age, with the first two most markedly in the 30–39-year retrieval window. Formal interaction testing revealed a significant multiplicative interaction for clinical pregnancy but little additive interaction overall, suggesting that the associated risks of older retrieval age and longer RTI combine approximately additively rather than synergistically. Because the interval cannot be separated from cryostorage duration or from the reasons for delay in this design, these results should be read as associations rather than demonstrated effects. They nonetheless support considering RTI alongside retrieval age in individualized patient counseling, with greatest relevance in the 30–39-year retrieval window.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Institutional Review Boards of Sichuan Jinxin Xinan Women & Children's Hospital (No. 2023001) and Chongqing Medical University (No. 2022132). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the retrospective nature of the study.

Author contributions

HMZ: Conceptualization, Formal analysis, Methodology, Writing – original draft. RLL: Conceptualization, Formal analysis, Methodology, Data curation, Writing – review & editing. QFL: Conceptualization, Methodology, Data curation, Writing – review & editing. XJW: Conceptualization, Data curation, Investigation, Writing – review & editing. LQF: Data curation, Investigation, Validation, Writing – review & editing. ZHZ: Data curation, Investigation, Writing – review & editing. CYT: Conceptualization, Supervision, Writing – review & editing. LT: Methodology, Validation, Writing – review & editing. YLH: Data curation, Investigation, Validation, Resources, Writing – review & editing. LJF: Conceptualization, Methodology, Data curation, Investigation, Resources, Funding acquisition, Supervision, Writing – review & editing. YBD: Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing. XBW: Conceptualization, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2026.1968086/full#supplementary-material

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