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Increasing dominant follicular proportion was associated with adverse IVF/ICSI outcomes in low-prognosis women undergoing GnRH antagonist protocol: a retrospective cohort study

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Abstract

Purpose This study aimed to examine the correlation between different dominant follicle proportions (DFPs) and outcomes of in-vitro fertilization or intracytoplasmic sperm injection (IVF/ICSI) among patients classified under POSEIDON Groups 3 and 4, who underwent gonadotropin-releasing hormone antagonist (GnRH-ant) protocols. Additionally, it sought to determine the optimal DFP threshold for trigger timing.

Methods A retrospective analysis was performed on patients classified under POSEIDON Groups 3 ($n=593$) and 4 ($n=563$) who underwent GnRH-ant protocols for controlled ovarian hyperstimulation (COH) between 2016 and 2022. These patients were categorized into two groups based on their DFPs, defined as the ratio of ≥ 18-mm dominant follicles to ≥ 12-mm follicles on the trigger day (DFP ≤ 40% and DFP ≥ 40%). Statistical analyses, including restricted cubic spline (RCS) and multivariate logistic regression, were employed to assess the relationship between DFP and IVF/ICSI outcomes.

Results Demographic characteristics of patients were similar across groups. In POSEIDON Groups 3 and 4, DFP > 40 was associated with a significant decrease in the number (No.) of oocytes retrieved, cleaved embryos, and available embryos. Moreover, following the GnRH-ant cycle, the clinical pregnancy and live birth rates in fresh embryo transfer (ET) were notably reduced in the DFP > 40 group compared with the DFP ≤ 40 group, whereas no significant differences were observed in the pregnancy outcomes of the first frozen-thawed embryo transfer (FET) between the groups. In POSEIDON Group 3, the cumulative clinical pregnancy rate (CCPR) and cumulative live birth rate (CLRB) were significantly higher in the DFP ≤ 40 subgroup than in the DFP > 40 subgroup, with a notable decrease in CLRB observed with increasing DFP levels. However, in POSEIDON Group 4, no significant differences in CCPR and CLRB

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were found between the groups. Logistic regression analysis identified age and the No. of oocytes retrieved as pivotal factors influencing CLRB in Group 4.

Conclusion For patients in POSEIDON Group 3, maintaining a DFP ≤ 40 mm is crucial to achieve optimal laboratory and pregnancy outcomes by avoiding delayed triggering. However, for patients in POSEIDON Group 4, age remains a critical factor influencing CLRB regardless of DFP, although a higher No. of oocytes retrieved and available embryos with DFP ≤ 40 is beneficial.

Keywords GnRH antagonist protocol, POSEIDON criteria, DFP, Trigger timing, Embryo transfer (ET), Cumulative live birth rate, Restricted cubic spline

Introduction

The gonadotropin-releasing hormone antagonist (GnRH-ant) regimen, characterized by a shorter duration of gonadotropin (Gn) use, shows comparable pregnancy rates to the GnRH agonist regimen [1], with a lower risk of ovarian hyperstimulation syndrome (OHSS) [2, 3]. Currently, the GnRH-ant regimen is widely utilized in assisted reproductive treatment (ART). During controlled ovarian hyperstimulation (COH) with GnRH-ant, these agents directly inhibit the endogenous luteinizing hormone (LH) peak before ovulation. Consequently, the use of drugs to simulate the effect of endogenous LH peak and induce the final maturation of oocytes during follicle development, known as triggering, becomes necessary [4]. Determining the optimal trigger timing is crucial for obtaining sufficient number (No.) of high-quality oocytes and ensuring the success of the ART process.

Low-prognosis ovarian response refers to an inadequate response to Gn stimulation during ART [5], characterized by high Gn dosage, limited follicular development, few retrieved oocytes, high cycle cancellation rates, and poor clinical outcomes [6, 7]. In 2016, researchers proposed the POSEIDON criteria, an individualized oocyte number-based management strategy for women with low prognosis [8]. These criteria classify patients into four groups based on age, antral follicle count (AFC), anti-Müllerian hormone (AMH) level, and previous ovarian response to Gn [8, 9], allowing to distinguish between those with adequate ovarian reserve but poor response to standard ovarian stimulation (Groups 1 and 2) and those with poor ovarian reserve (Groups 3 and 4). Patients classified as POSEIDON Group 3 are aged < 35 years, whereas those classified as POSEIDON Group 4 are aged > 35 years. Currently, effective treatments for low-prognosis patients undergoing in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) remain elusive, posing a challenge for reproductive physicians.

The most commonly used trigger criteria in reproductive centers globally are when there are ≥3 follicles with a diameter of ≥17 mm or ≥2 follicles with a diameter of ≥18 mm [10–15]. However, in clinical practice, physicians often opt to delay the trigger to promote the development of as many dominant follicles as possible exceeding 2, aiming to obtain more potentially mature oocytes [16]. Despite the prevalence of this approach, there exists no consensus or universal standard regarding the optimal trigger timing for patients with low prognosis [17, 18]. Thus, further exploration is warranted to ascertain whether the generally accepted trigger timing is suitable for such patients.

Some studies have indicated that during IVF cycles for women with advanced age, a maximum follicular diameter between 16 mm and 18 mm is associated with a higher clinical pregnancy rate than those with a diameter > 18 mm. However, follicular growth and development during COH often occur asynchronously, and relying solely on the development of individual mature follicles to determine trigger timing may be somewhat simplistic [16]. Hence, it is essential to consider the overall developmental status of follicular cohorts. Dominant follicular proportion (DFP) serves as a more effective and objective indicator for assessing the optimal trigger timing [11, 17]. To investigate the suitable trigger timing in women with low prognosis receiving GnRH-ant protocols and its impact on reproductive outcomes, we analyzed the effects of DFP on laboratory and pregnancy outcomes among patients in POSEIDON Groups 3 and 4 undergoing the GnRH-ant regimen.

Materials and methods

Study design

This hospital-based cohort study recruited a total of 1156 patients who underwent IVF/ICSI cycles using the GnRH-ant protocol at Women's Hospital of Nanjing Medical University between January 2016 and December 2022. The patients were diagnosed according to the POSEIDON criteria of Groups 3 or 4 (AFC ≤ 5 or AMH < 1.2 ng/ml). The exclusion criteria included: (1) The number of oocytes retrieved in this IVF/ICSI cycle was less than 3, (2) polycystic ovarian syndrome, endometriosis, history of ovarian surgery, metabolic or endocrine abnormalities, (3) Abnormal parental karyotypes, (4) preimplantation genetic diagnosis (PGT) cycle, (5) recurrent implant failure or spontaneous abortions, congenital or acquired uterine malformations, (6) missing

cycle data or follow-up. This study adhered to the Declaration of Helsinki and was approved by the ethics committee of Nanjing Maternity and Child Health Care Hospital (NJFY-2023KY-018). The study was retrospective and analyzed patient data anonymously, eliminating the need for informed patient consent. The study flowchart was shown in Figure S1.

Assessment of ovarian reserve

During the days 2 to 4 of natural menstrual cycle, ovarian reserve assessments were meticulously conducted, occurring 1 to 3 months preceding the commencement of ovarian stimulation procedures.

The AFC, defined as the cumulative number of follicles measuring 2 to 10 mm in diameter within the ovary, was meticulously measured using two-dimensional transvaginal ultrasound. This assessment was performed by a team of highly skilled reproductive medicine experts at our reproductive center. Each member of the team has undergone rigorous training in ultrasonography and reproductive medicine, boasting a minimum of 5 years of professional expertise. This ensures the utmost precision and reproducibility of the AFC measurements.

The serum concentration of AMH was accurately measured utilizing the Beckman DX1800 chemiluminescence analyzer (serial no. 607564). The assay employed the Beckman AMH reagent (batch no. 971017) and calibrator (batch no. 989302) to ensure precision. For quality control, Preci Control AMH (batch no. 42628901) was utilized to safeguard the accuracy and reproducibility of the results. Blood specimens were obtained from the patient in the morning, during the early follicular phase of the menstrual cycle, to capture the most representative AMH levels.

Definition of DFP

DFP was defined as the ratio of the number of follicles measuring≥18 mm to the number of follicles measuring≥12 mm on the trigger day. Our study exclusively focused on patients with a poor ovarian response, with a median follicle count of 5 on the trigger day. Meanwhile, existing GnRH-ant protocol guidelines recommend triggering when there are ≥2 follicles measuring≥18 mm. Therefore, we adopted a DFP threshold of 40% (2/5) for patient stratification, dividing them into DFP≤40% and DFP>40% groups.

Ovarian stimulation protocol

All patients participating in the study underwent a flexible GnRH-ant protocol. On the second or third day of their menstrual cycle, blood samples were collected to assess baseline serum levels of follicle-stimulating hormone (FSH), LH, and estradiol (E2), progesterone. Considering age, body mass index (BMI), AFC and AMH levels, the initial dose of Gn was tailored for each patient and was injected daily from the second or third day of the menstrual cycle. The Gn category encompasses recombinant FSH for injection (r-FSH, GONAL-f, Merck Serono, Italy; PUREGON, Merck Sharp & Dohme, Germany), as well as human menopausal gonadotropin (HMG, Menotropins for Injection, Lizhu Pharmaceutical Group, China).Once the diameter of the dominant follicle reached 12–14 mm or the E2 levels surpassed 300 ng/L, subcutaneous administration of GnRH antagonists (Cetrorelix, Merck Serono, Darmstadt, Germany) commenced, with dosages ranging from 0.125 to 0.25 mg/day. These dosages were tailored to each patient's weight and serum LH levels on the initial day of GnRH-ant protocol, and were maintained until the trigger day. Follicular growth was closely monitored by ultrasound and sex hormone levels every 2–3 days, enabling precise gonadotropin dosing adjustments.

The trigger time was determined according to the diameter and number of dominant follicles, the time of using Gn and the level of hormone. Final oocyte maturation was triggered by either HCG (Lizhu Pharmaceutical Factory, China) alone or with a dual trigger comprising 2000 IU HCG and GnRH agonist (0.2 mg Decapeptyl, Ferring International Center SA). Oocyte retrieval was scheduled 36 h later, ensuring optimal conditions for successful fertilization and subsequent embryo development. Oocytes were inseminated approximately 4–6 h after follicular aspiration by IVF or ICSI, depending on sperm quality. Morphologic criteria were used for embryo scoring. According to our previously published article [19], the embryos were cultured in vitro for 3 to 6 days for fresh embryo transfer (ET) cycle or cryopreservation.

Embryo transfer and luteal phase support

For fresh ET, the following criteria must be met: endometrial thickness should be at least 8 mm with a uniform echo pattern, progesterone levels should remain below 1.5 μg/L, and without any relevant medical history. On day 3, one to two available cleavage embryos with high score are selected for ET. For frozen-thawed embryo transfer (FET), patients with embryo freeze-all strategy or patients who did not reach live delivery after fresh ET performed with endometrial preparation protocol for FET, including the natural/stimulated cycle and the artificial cycle, depending on the characteristics and preferences of each woman. One or two thawed embryos were transferred depending on the age, BMI, embryo quality, and personal will of each subject. For luteal phase support (LPS), intramuscular progesterone at a dose of (40 mg, Xianju Pharmaceutical Factory) and oral Duphaston (30 mg, Abbott Healthcare Products B.V.) were administered once daily. If a positive pregnancy test

was obtained two weeks after ET, progesterone therapy was maintained until the 8th to 10th week of gestation.

Outcome assessment

The serum β-hCG test was performed 2 weeks post-FET. The implantation rate was calculated as the number of gestational sacs divided by the number of embryos transferred. Clinical pregnancy was defined as the presence of an intrauterine gestational sac with or without a fetal heartbeat, observed through transvaginal ultrasound after 6 weeks of gestation. Early miscarriage was defined as pregnancy loss before 12 weeks of gestation, whereas late miscarriage was defined as pregnancy loss after 12 weeks but before 28 weeks of gestation. Live birth was defined as a fetus born alive after 28 weeks of pregnancy. The main pregnancy outcomes were cumulative clinical pregnancy rate (CCPR) and cumulative live birth rate (CLBR). CCPR was calculated as the number of clinical pregnancy cycles divided by the number of first oocyte retrieval cycles, and CLBR was calculated as the number of live birth cycles divided by the number of first oocyte retrieval cycles. Secondary pregnancy outcomes were chemical pregnancy, clinical pregnancy, miscarriage, and live birth rates. Laboratory outcomes measured included the No. of oocytes retrieved, 2 pronuclei (PN), cleavages, available embryos, blastocysts, and high-quality blastocysts and the ratio of available embryos, blastocysts, and high-quality blastocysts.

Sample size estimation

The sample size estimation was conducted using PASS software, which based on the two primary outcomes, No. of oocytes retrieval and CLBR. It was estimated that the No. of oocytes retrieval in DFP≤40% was about 5, while the group of DFP>40% was 4. With a power of 0.8, the alpha of 0.05, the sample size ratio of 0.6, the mean difference of 1, and the standard deviation of 1.5, the estimated sample size for each group was 6 vs. 4. Regarding the CLBR, it was assumed to be around 60% and 45% in the DFP≤40% and DFP>40% group in Poseidon Group 3, and the rate was expected to be around 35% and 20% in the DFP≤40% and DFP>40% group in Poseidon Group 4. The sample size required was 257 vs. 129 (Poseidon Group 3) and 191 vs. 96 (Poseidon Group 4), with a power of 0.8, the alpha of 0.05, and the sample size ratio of 0.5. The sample size is basically enough to detect the main results difference between the two groups.

Statistical analysis

Statistical analyses were conducted using SPSS 27.0 software and R 4.2.1 statistical software. Continuous variables are presented as medians with interquartile ranges, and categorical variables as numbers/total numbers (percentages). Independent samples t-test was conducted to compare the arithmetic means of the two groups, while the χ^2-test was applied to analyze the frequencies of attributive features. Restricted cubic splines (RCS) were used to visualize dose-response associations between DFP and reproductive outcomes, with continuous confounders (female age, male age, infertility type, infertility duration, BMI, basal FSH, basal LH, AMH, total Gn dose, total GnRH-ant dose, trigger drugs, sperm density, and insemination method) included. The RCS model incorporated three knots positioned at the 5th, 50th, and 95th percentiles. Multivariate logistic regression analysis was performed to examine the independent effects of clinical characteristics on CLBR, with adjusted OR (aOR) and 95% confidence intervals (CIs) calculated. All tests were two-tailed, and a P-value of <0.05 was considered statistically significant.

Results

Characteristics of baseline and COH cycles

The study enrolled a total of 1,156 patients, categorized into POSEIDON Groups 3 ($n=593$) and 4 ($n=563$), according to the established POSEIDON criteria. This categorization aimed to meticulously assess the impact of different DFPs on laboratory and pregnancy outcomes in patients undergoing the GnRH-ant protocol within distinct POSEIDON subtypes. Patients were divided into two groups based on DFP on the trigger day: DFP≤40 and DFP>40. The sample sizes for the two groups were 376 and 217 in POSEIDON Group 3 and 371 and 192 in Group 4, respectively.

Table 1 demonstrates that among patients in POSEIDON Group 3, no significant differences were observed in baseline and cycle characteristics between the DFP≤40 and DFP>40 groups ($P>0.05$). In POSEIDON Group 4, the DFP≤40 group exhibited significantly lower levels of E2 on the trigger day than the DFP>40 group ($P=0.019$), with no other statistically significant differences noted between the two groups ($P>0.05$).

Laboratory outcomes of COH cycles

To analyze the laboratory outcomes of COH cycles, we assessed differences between the DFP≤40 and DFP>40 groups among patients in POSEIDON Groups 3 and 4. Univariate analysis revealed similar laboratory outcomes between the DFP≤40 and DFP>40 groups in both POSEIDON Groups 3 and 4, including the No. of oocytes retrieved, 2PN, cleaved embryos, blastocysts, and available embryos, as well as the ratio of available embryos and blastocysts (Table 2).

Subsequently, RCS incorporating linear regression models were utilized to explore nonlinear relationships between DFP and laboratory outcomes in POSEIDON Group3. The models demonstrated an association between DFP and No. of oocytes retrieved

Table 1 Characteristics of baseline and controlled ovarian hyperstimulation cycles among Poseidon Group 3 and Group 4 patients grouped by DFP

	POSEIDON Group 3			POSEIDON Group 4		
	DFP ≤ 40	**DFP>40**	***P***	**DFP ≤ 40**	**DFP>40**	***P***
N	376	217		371	192	
Female age (years)	30.22 ± 2.99	28.77 ± 3.06	0.084	39.20 ± 3.21	38.99 ± 3.44	0.462
Male age (years)	31.29 ± 3.87	31.23 ± 4.12	0.860	40.5 ± 5.45	39.7 ± 5.74	0.105
Infertility duration (years)	3.15 ± 2.48	3.00 ± 2.13	0.446	4.21 ± 4.04	3.79 ± 3.59	0.221
Infertility type (%)			0.606			0.308
Primary	191/376 (50.8)	115/217 (53.0)		54/371 (14.6)	22/192 (11.5)	
Secondary	185/376 (49.2)	102/217 (47.0)		317/371 (85.4)	170/192 (88.5)	
BMI (kg/m^2)	22.74 ± 3.38	22.33 ± 3.22	0.154	22.90 ± 2.87	23.07 ± 3.10	0.497
Basal FSH (IU/L)	9.28 ± 2.98	9.01 ± 2.34	0.335	9.40 ± 2.65	9.45 ± 2.88	0.850
Basal LH (IU/L)	3.78 ± 1.80	3.67 ± 1.51	0.449	3.80 ± 1.81	4.00 ± 2.51	0.282
AMH (ng/mL)	1.03 ± 1.02	1.06 ± 1.23	0.759	0.97 ± 0.78	0.87 ± 0.75	0.171
AFC	6.18 ± 3.16	5.82 ± 2.99	0.178	5.41 ± 2.50	5.66 ± 2.72	0.300
Total Gn dose (IU)	2461.90 ± 578.86	2417.67 ± 603.31	0.378	2500.27 ± 535.38	2451.43 ± 512.57	0.298
Duration of Gn (day)	9.01 ± 1.53	8.82 ± 1.66	0.158	8.74 ± 1.58	8.60 ± 1.72	0.363
Total GnRH-ant dose (IU)	0.74 ± 0.36	0.71 ± 0.39	0.365	0.75 ± 0.33	0.77 ± 0.38	0.702
Duration of GnRH-ant (day)	3.53 ± 1.28	3.50 ± 1.55	0.820	3.53 ± 1.26	3.53 ± 1.40	0.980
E2 on trigger day (pg/mL)	1999.60 ± 1100.86	2062.36 ± 1002.98	0.490	1821.57 ± 1019.90	2043.10 ± 1122.05	***0.019***
P on trigger day (ng/mL)	0.98 ± 0.50	1.00 ± 0.47	0.737	1.00 ± 0.70	1.00 ± 0.72	0.273
LH on trigger day (IU/L)	3.26 ± 2.10	3.20 ± 2.03	0.730	3.82 ± 2.75	3.57 ± 2.19	0.926
Trigger drugs (%)			0.085			0.538
HCG	5/376 (1.3)	7/217 (3.2)		7/371 (1.9)	2/192 (1.0)	
GnRHa	3/376 (0.8)	5/217 (2.3)		3/371 (0.8)	3/192 (1.6)	
HCG + GnRHa	368/376 (97.9)	205/217 (94.5)		361/371 (97.3)	187/192 (97.4)	
Insemination Method (%)			0.891			0.799
IVF	278/375 (74.1)	157/216 (72.7)		230/370 (62.2)	120/192 (62.5)	
ICSI	91/375 (24.3)	56/216 (25.9)		136/370 (36.8)	71/192 (37.0)	
IVF + Rescue ICSI	6/375 (1.6)	3/216 (1.4)		4/370 (1.1)	1/192 (0.5)	
Sperm density	6.84 ± 2.35	6.67 ± 2.40	0.406	6.69 ± 2.49	6.51 ± 2.58	0.449

Continuous variables presented as mean ± SD. Categorical variables presented as n/N (%)

Abbreviations: DFP, dominant follicular percentage; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, anti-Mullerian hormone; AFC, antral follicle count; Gn, gonadotropin; GnRH-ant, gonadotropin releasing hormone antagonist; HCG, human chorionic gonadotropin; GnRHa, gonadotrophin-releasing hormone analogs; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection

Table 2 Laboratory outcomes among Poseidon Group 3 and Group 4 patients grouped by DFP

	POSEIDON Group 3			POSEIDON Group 4		
	DFP ≤ 40	**DFP>40**	***P***	**DFP ≤ 40**	**DFP>40**	***P***
N	376	217		371	192	
No. of oocytes retrieved	4.91 ± 1.80	4.75 ± 1.85	0.302	4.54 ± 1.67	4.39 ± 1.62	0.295
No. of 2PN	3.61 ± 1.82	3.60 ± 1.79	0.955	3.48 ± 1.68	3.26 ± 1.66	0.143
No. of cleavage embryos	3.71 ± 1.87	3.68 ± 1.84	0.846	3.53 ± 1.69	3.32 ± 1.70	0.155
No. of 2PN cleavage embryos	3.59 ± 1.85	3.54 ± 1.83	0.726	3.45 ± 1.68	3.24 ± 1.67	0.177
No. of available embryos	3.33 ± 1.85	3.21 ± 1.77	0.433	3.22 ± 1.60	2.98 ± 1.62	0.098
Ratio of available embryos (%)	89.68 ± 20.99	87.02 ± 21.26	0.144	91.43 ± 18.86	89.82 ± 21.21	0.362
No. of blastocysts	1.46 ± 1.42	1.58 ± 1.53	0.426	1.27 ± 1.45	1.42 ± 1.24	0.308
Ratio of blastocysts	28.98 ± 37.25	49.63 ± 37.23	0.869	45.78 ± 36.97	47.56 ± 36.93	0.559

Continuous variables presented as mean ± SD

Abbreviations: DFP, dominant follicular percentage; No., Number; PN, pronucleus

(*P*-overall < 0.001, *P*-nonlinear = 0.114), cleavage embryos (*P*-overall = 0.039, P-nonlinear = 0.443), and available embryos (*P*-overall = 0.040, *P*-nonlinear = 0.626). When DFP was ≤40, there was no notable variation in these outcomes as DFP increased. Conversely, when DFP was >40, these laboratory outcomes significantly decreased with increasing DFP (Fig. 1, Figure S2.A-C).

In POSEIDON Group4, the RCS model identified associations between DFP and laboratory outcomes such as the No. of oocytes retrieved (*P*-overall = 0.013, *P*-nonlinear = 0.029), 2PN (*P*-overall = 0.009, *P*-nonlinear = 0.018), cleavage embryos (*P*-overall = 0.012, *P*-nonlinear = 0.028), and available embryos (*P*-overall = 0.011, *P*-nonlinear = 0.047). Specifically, a gradual increase in these outcomes was observed when DFP was ≤40, whereas a significant decrease occurred when DFP was >40 (Fig. 2, Figure S2.D-F).

Clinical outcomes of the first ET cycle

Analysis of the first ET cycle after COH in the POSEIDON Group 3 included a total of 447 ET cycles, comprising 92 fresh ET cycles and 335 FET cycles. Among patients undergoing fresh ET cycles, the DFP ≤ 40 group (*n* = 55) demonstrated significantly higher rates of embryo implantation (38.5% vs. 21.9%, *P* = 0.018), biochemical pregnancy (67.3% vs. 45.9%, *P* = 0.042), clinical pregnancy (63.6% vs. 35.1%, *P* = 0.007), and live birth (52.7% vs. 29.7%, *P* = 0.029) than the DFP > 40 group (*n* = 37; Table 3). Conversely, in the first FET cycles, no significant differences were observed in pregnancy outcomes between the DFP ≤ 40 and DFP > 40 groups (Table S1).

Analysis of 381 first ET cycles following COH in the POSEIDON Group 4 included 82 fresh ET cycles and 299 FET cycles. Among patients undergoing fresh ET cycles, more high-quality embryos were transferred (1.49 ± 0.77 vs. 0.96 ± 0.93, *P* = 0.010) in DFP ≤ 40 group (*n* = 59) than in the DFP > 40 group (*n* = 23) and pregnancy outcomes were better, including higher clinical pregnancy rate (35.6% vs. 13.0%, *P* = 0.044) and live birth rate (22.0% vs. 0.0%, *P* = 0.015), than the DFP > 40 group (*n* = 23; Table 3). However, similar to Group 3, in the first FET cycles, no

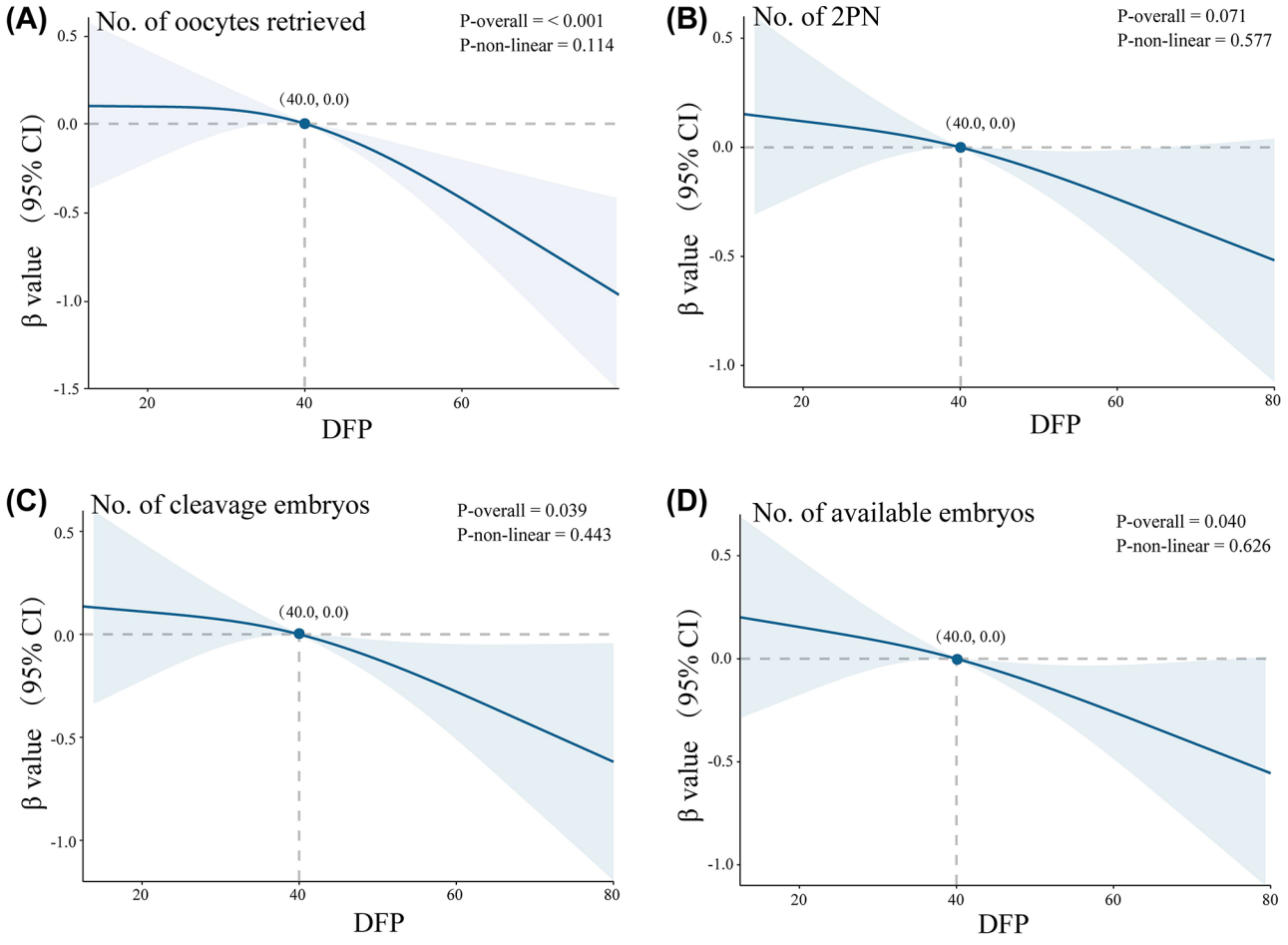

Fig. 1 Association between the DFP on trigger day and laboratory outcomes among POSEIDON Group 3 population. Relationship with the number (No.) of oocytes retrieved (**A**), 2 pronucleus (PN) (**B**), cleavage embryos (**C**), and available embryos (**D**). β value are indicated by solid lines and 95% confidence intervals (CIs) are indicated by shaded areas

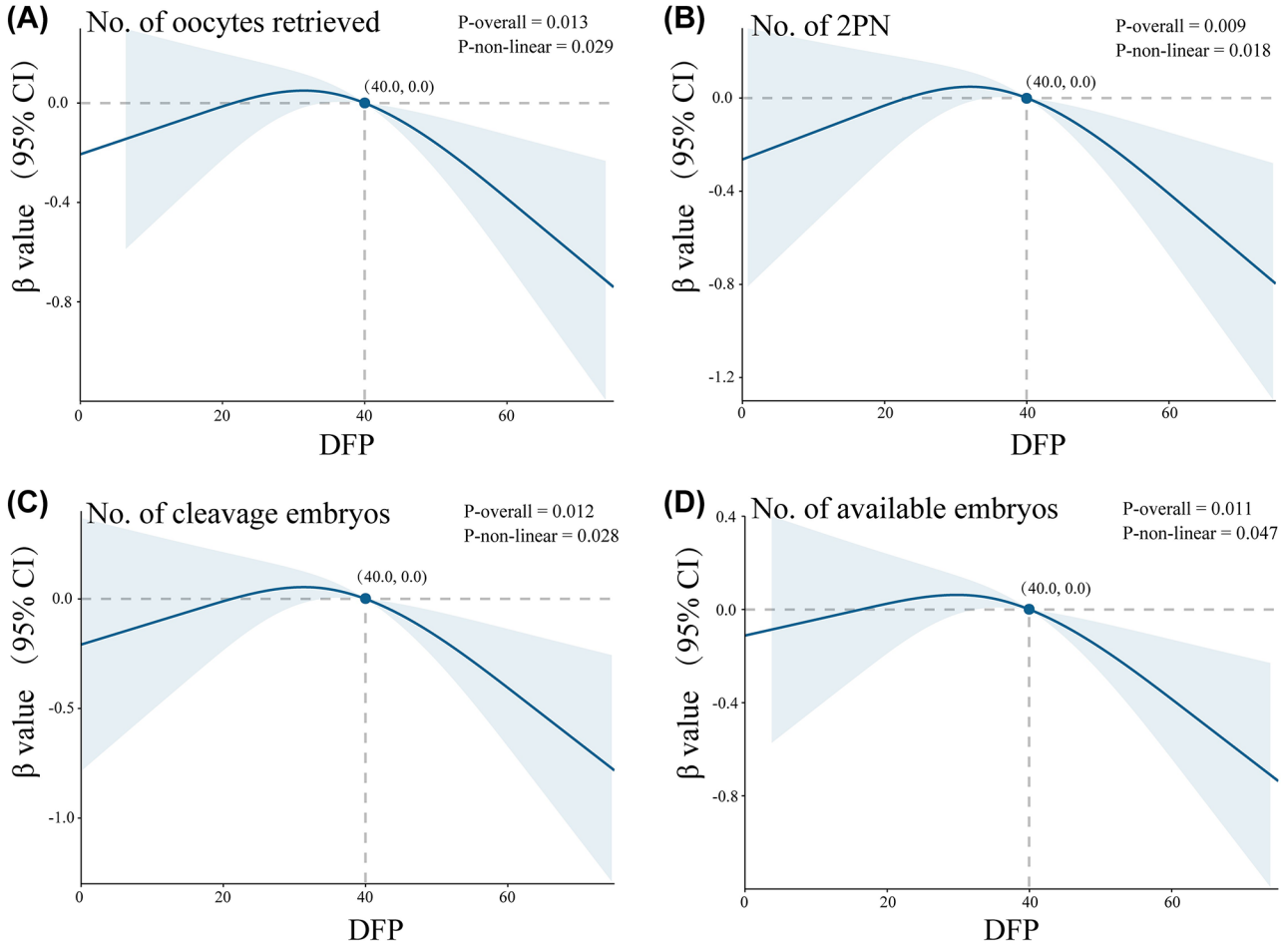

Fig. 2 Association between the DFP on trigger day and laboratory outcomes among POSEIDON Group 4 population. Relationship with the number (No.) of oocytes retrieved (**A**), 2 pronucleus (PN) (**B**), cleavage embryos (**C**), and available embryos (**D**). β value are indicated by solid lines and 95% confidence intervals (CIs) are indicated by shaded areas

significant differences were noted in pregnancy outcomes between the DFP ≤ 40 and DFP > 40 groups (Table S1).

Relationship between DFP and CCPR/CLRB

To further evaluate the impact of trigger timing on reproductive outcomes in subsequent ET cycles of GnRH-ant protocols, we analyzed the relationship between DFP and CCPR as well as CLRB (Table 4; Fig. 3). For this analysis, patients who achieved live births through embryo transplantation derived from the current ovulation stimulation cycle or did not achieve live births despite transplanting all available embryos were included.

In POSEIDON Group 3, a higher clinical pregnancy rate was observed in the DFP ≤ 40 group than in the DFP > 40 group (62.6% vs. 50.0%, $P=0.014$) during the first ET cycle (Table 4). Although the live birth rate was slightly higher in the DFP ≤ 40 group, this difference did not reach statistical significance (52.4% vs. 43.9%, $P=0.101$). Furthermore, both the clinical pregnancy rate (67.1% vs. 54.1%, $P=0.010$) and live birth rate (58.1% vs. 47.3%, $P=0.037$) in the cumulative three ET cycles were significantly higher in the DFP ≤ 40 group than in the DFP > 40 group. RCS incorporating logistic regression models revealed an association between DFP and CCPR (P-overall < 0.001, P-nonlinear = 0.114), as well as between DFP and CLBR (P-overall = 0.040, P-nonlinear = 0.626) in patients in POSEIDON Group 3. Notably, when DPF was > 40, both CCPR and CLBR significantly decreased as DPF increased (Fig. 3.A-B). Logistic regression models demonstrated that DFP ≤ 40 (OR 1.636, 95% CI 1.060–2.526, $P=0.026$) and the No. of oocytes retrieved (OR 1.215, 95% CI 1.607–1.384, $P=0.003$) were independent protective factors for CLBR (Table 5).

Similarly, in POSEIDON Group 4, although the clinical pregnancy (44.1% vs. 38.0%, $P=0.306$) and live birth (35.5% vs. 30.0%, $P=0.339$) rates slightly increased in the DFP ≤ 40 group compared with the DFP > 40 group in cumulative three ET cycles, this difference did not reach statistical significance in RCS model (P-overall > 0.05; Fig. 3.C-D). Logistic regression models identified female

Table 3 Outcomes of fresh embryo transfer cycle among Poseidon Group 3 and Group 4 patients grouped by DFP

Fresh ET cycle	POSEIDON Group 3				POSEIDON Group 4			
	DFP ≤ 40	DFP>40	*P*	*95%CI*	DFP ≤ 40	DFP>40	*P*	*95%CI*
N	55	37			59	23		
Female age (years)	30.33 ± 3.16	30.11 ± 3.12	0.743	-1.106, 1.544	39.03 ± 36.3	40.00 ± 4.04	0.298	-2.801, 0.869
BMI (kg/m^2)	21.64 ± 2.74	21.41 ± 2.66	0.693	-0.914, 1.370	22.53 ± 2.98	22.52 ± 3.21	0.992	-1.481, 1.496
Basal FSH (IU/L)	9.68 ± 2.71	9.36 ± 2.03	0.544	-0.721, 1.358	9.23 ± 2.18	9.42 ± 1.87	0.714	-1.219, 0.839
AMH (ng/mL)	1.01 ± 0.40	0.99 ± 1.31	0.948	-0.395, 0.422	1.08 ± 0.79	0.75 ± 0.63	0.076	-0.036, 0.700
Embryos transferred type (%)			1.000	0.040, 11.004			0.280	0.000, 0.000
Cleavage stage	1/55 (1.8)	1/37 (2.7)			0/59 (0.0)	1/23 (4.3)		
Blastocyst	54/55 (98.2)	36/37 (97.3)			59/59 (100.0)	22/23 (95.7)		
No. embryos transferred	1.98 ± 0.30	1.97 ± 0.29	0.889	-0.117, 0.134	2.08 ± 0.43	1.96 ± 0.64	0.294	-0.114, 0.370
No. of good-quality embryos	1.49 ± 0.74	1.46 ± 0.80	0.847	-0.293, 0.355	1.49 ± 0.77	0.96 ± 0.93	***0.010***	0.134, 0.936
Endometrium thickness (mm)	9.76 ± 1.59	9.34 ± 1.49	0.199	-0.229, 1.080	9.36 ± 1.32	9.33 ± 1.44	0.932	-0.672, 0.731
Implantation rate (%)	42/109 (38.5)	16/73 (21.9)	***0.018***	1.136, 4.388	21/59 (35.6)	4/23 (17.4)	0.108	0.788, 8.739
Chemical pregnancy rate (%)	37/55 (67.3)	17/37 (45.9)	***0.042***	1.026, 5.701	27/123 (22.0)	4/45 (8.9)	0.053	0.948, 8.764
Clinical pregnancy rate (%)	35/55 (63.6)	13/37 (35.1)	***0.007***	1.353, 7.714	21/59 (35.6)	3/23 (13.0)	***0.044***	0.979, 13.866
Miscarriage rate (%)	5/35 (14.3)	2/13 (15.4)	1.000	0.155, 5.433	8/21 (38.1)	3/3 (100.0)	0.082	0.000, 0.000
Early miscarriage rate (%)	4/35 (11.4)	2/13 (15.4)	0.656	0.114, 4.431	7/21 (33.3)	2/3 (66.7)	0.533	0.019, 3.254
Late miscarriage rate (%)	1/35 (2.9)	0/13 (0.0)	1.000	0.000, 0.000	1/21 (4.8)	1/3 (33.3)	0.239	0.004, 2.287
Live birth rate (%)	29/55 (52.7)	11/37 (29.7)	***0.029***	1.029, 6.366	13/59 (22.0)	0/23 (0.0)	***0.015***	0.000, 0.000

Continuous variables presented as mean ± SD. Categorical variables presented as n/N (%)

Abbreviations: DFP, dominant follicular percentage; CI, Confidence Interval; BMI, body mass index; FSH, follicle-stimulating hormone; AMH, anti-Mullerian hormone; No., number

Table 4 Cumulative clinical pregnancy rate and cumulative live birth rate among Poseidon Group 3 and Group 4 patients grouped by DFP

	POSEIDON Group 3			POSEIDON Group 4		
	DFP ≤ 40	DFP>40	*P*	DFP ≤ 40	DFP>40	*P*
N	246	148		220	100	
No. average ET cyles	1.15 ± 0.38	1.21 ± 0.44	0.147	1.20 ± 0.41	1.27 ± 0.49	0.186
First ET cycle						
No. of ET cycles	246	148		220	100	
Clinical pregnancy rate (%)	154/246 (62.6)	74/148 (50.0)	***0.014***	85/220 (38.6)	32/100 (32.0)	0.253
Miscarriage rate (%)	23/154 (14.9)	9/74 (12.2)	0.572	18/85 (21.2)	9/32 (28.1)	0.427
Live birth rate (%)	129/246 (52.4)	65/148 (43.9)	0.101	67/220 (30.5)	23/100 (23.0)	0.169
Cumulative two ET cycles						
No. of ET cycles	34	23		42	25	
Clinical pregnancy rate (%)	165/246 (67.1)	80/148 (54.1)	***0.010***	97/220 (44.1)	38/100 (38.0)	0.306
Miscarriage rate (%)	11/165 (6.7)	10/80 (12.5)	0.126	11/97 (11.3)	8/38 (21.5)	0.144
Live birth rate (%)	143/246 (58.1)	70/148 (47.3)	***0.037***	78/220 (35.5)	30/100 (30.0)	0.339
Cumulative three ET cycles						
No. of ET cycles	2	2		1	2	
Clinical pregnancy rate (%)	165/246 (67.1)	80/148 (54.1)	***0.010***	97/220 (44.1)	38/100 (38.0)	0.306
Miscarriage rate (%)	11/165 (6.7)	10/80 (12.5)	0.126	11/97 (11.3)	8/38 (21.5)	0.144
Live birth rate (%)	143/246 (58.1)	70/148 (47.3)	***0.037***	78/220 (35.5)	30/100 (30.0)	0.339

Continuous variables presented as mean ± SD. Categorical variables presented as n/N (%)

Abbreviations: DFP, dominant follicular percentage; No., number; ET, embryo transfer

age (OR 0.770, 95% CI 0.695–0.852, P=0.000) as an independent risk factor and the No. of retrieved oocytes (OR 1.218, 95% CI 1.026–1.446, P=0.024) as an independent protective factor for CLRB (Table 5).

Discussion

This study is the first attempt to evaluate how trigger timing affects laboratory and pregnancy outcomes in patients in POSEIDON Groups 3 and 4, using the DFP metric. Our findings indicate that when DFP was >40, the No. of oocytes retrieved and available embryos

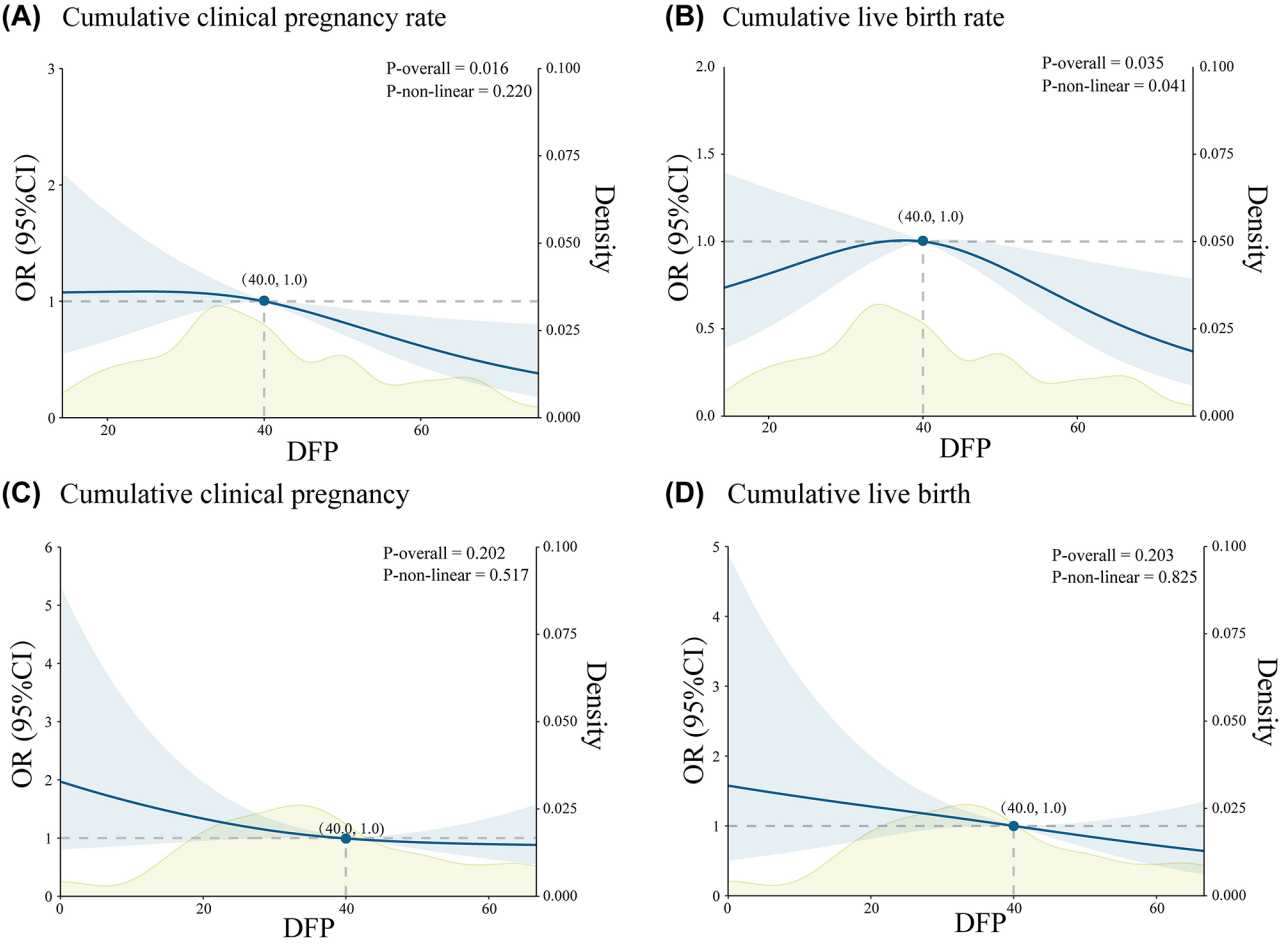

Fig. 3 Association between the DFP on trigger day and pregnancy outcomes among POSEIDON Group 3 and 4 population. Relationship with the cumulative clinical pregnancy rate (CCPR) (**A**), and cumulative live birth rate (CLRB) (**B**) in patients with POSEIDON Group 3. Relationship with the CCPR (**A**), and CLRB (**B**) in patients with POSEIDON Group 4. Solid lines show the estimation of the difference in laboratory outcomes when using DFP = 40 as the odds ratios. 95% confidence intervals (CIs) are indicated by shaded areas

Table 5 Logistic regression analysis of factors related to cumulative live birth rate among Poseidon Group 3 and Group 4 patients

Cumulative live birth	POSEIDON Group 3				POSEIDON Group 4			
	B	*P*	*aOR*	*95%CI*	B	*P*	*aOR*	*95%CI*
DFP (>40% as Ref)	***0.492***	***0.026***	***1.636***	***(1.060, 2.526)***	0.463	0.115	1.588	(0.893, 2.825)
Female age (years)	-0.023	0.540	0.977	(0.909, 1.051)	**-0.262**	**0.000**	**0.770**	**(0.695, 0.852)**
BMI (kg/m^2)	0.014	0.689	1.014	(0.946, 1.087)	0.019	0.689	1.020	(0.927, 1.121)
Basal FSH (IU/L)	-0.042	0.316	0.959	(0.883, 1.041)	0.044	0.425	1.045	(0.938, 1.165)
AMH (ng/mL)	0.004	0.969	1.004	(0.808, 1.248)	0.266	0.131	1.304	(0.924, 1.842)
AFC	-0.001	0.986	0.999	(0.926, 1.078)	0.121	0.041	1.129	(1.005, 1.268)
Total Gn dose (IU)	0.000	0.526	1.000	(1.000, 1.001)	0.000	0.476	1.000	(0.999, 1.000)
Sperm density	0.063	0.166	1.605	(0.974, 1.164)	0.000	0.998	1.000	(0.896, 1.115)
No. of oocytes retrieved	***0.195***	***0.003***	***1.215***	***(1.607, 1.384)***	**0.197**	**0.024**	**1.218**	**(1.026, 1.446)**

Abbreviations: aOR, adjusted odds ratio; DFP, dominant follicular percentage; BMI, body mass index; FSH, follicle-stimulating hormone; AMH, anti-Mullerian hormone; AFC, antral follicle count; Gn, gonadotropin; No., Number

decreased significantly in both POSEIDON Groups 3 and 4. Concurrently, clinical pregnancy rates and live birth rates for fresh ETs declined significantly, while no significant impact was observed on the first FET following COH. Additionally, we utilized CCPR and CLRB to assess treatment efficacy of the GnRH-ant protocol in this low-prognosis population. In POSEIDON Group 3, both CCPR and CLRB decreased significantly when DFP was >40. In contrast, CLRB in POSEIDON Group 4 was only associated with age and the No. of oocytes retrieved.

Optimal trigger timing during ovarian stimulation is crucial for obtaining sufficient No. of high-quality oocytes [20–22]. This premature trigger can result in close adhesion of smaller cumulus cells to the follicle wall, which subsequently hinders oocyte maturation and retrieval [23]. Conversely, a delayed trigger can cause excessive oocyte maturation, which is evident through chromatin condensation, ultimately leading to oocyte aging and subsequent cell death. Over-mature oocytes exhibit an elevated proportion of smooth endoplasmic reticulum, leading to a significantly lower pregnancy rate [24, 25]. The traditional approach for determining the trigger time for GnRH-ant protocols relies on achieving 3 follicles measuring 17 mm or 2 follicles measuring 18 mm. However, this method is limited because it fails to account for the variable ovarian responses among patients and potential differences in follicular synchronization, even within standardized treatment regimens. Thus, it is not universally suitable to rely solely on the number of mature follicles to determine trigger timing. Usually, a dominant follicle diameter of 18–22 mm indicates follicular maturation, and DFP on the trigger day is closely related to pregnancy [11, 16, 17, 26]. Hence, it is imperative to investigate the optimal DFP in patients with low prognosis to facilitate personalized trigger strategies.

In both POSEIDON Groups 3 and 4, when DFP exceeded 40, the No. of oocytes retrieved, cleavage embryos, and available embryos significantly decreased, whereas no significant differences were observed between No. of blastocysts and rate of available embryos and blastocysts. One study revealed increased apoptosis of granulosa cells among the elderly or POI patients following standard trigger procedures (the dominant follicular diameter reached 19–21 mm), which was primarily ascribed to downregulation of the FSH receptor and upregulation of the LH chorionic gonadotropin receptor (LHCGR) [27]. Furthermore, the gonadotropin surge-attenuating factor (GnSAF), primarily secreted by small- and medium-sized follicles, can inhibit the secretion of endogenous FSH and LH in women [28–30]. Decreased levels of GnSAF in older women, with a limited number of follicles, makes them more prone to premature endogenous LH surges. In POSEIDON Groups 3 and 4, elevated DFP was linked to adverse laboratory outcomes, likely due to increased granulosa cell apoptosis and oocyte aging from delayed triggers, leading to failed oocyte retrieval after follicular aspiration or retrieval of poor-quality or degraded oocytes.

Furthermore, we analyzed the outcomes of the first ET cycle following COH in different DFP groups. Our results showed that, among patients in POSEIDON Groups 3 and 4, the clinical pregnancy and live birth rates following fresh ET were significantly higher in the DFP≤40 group than in the DFP>40 group. This suggests that delaying trigger timing may negatively impact endometrial receptivity. As antagonist use duration increases, increasing progesterone levels may lead to asynchrony between embryo and endometrial development, as well as decreased endometrial receptivity, which negatively affects embryo implantation [31, 32]. However, when the first transfer following COH was an FET cycle, pregnancy outcomes were similar between the DFP≤40 and DFP>40 groups, as the ovarian stimulation effect on endometrial receptivity was eliminated. Therefore, for patients in POSEIDON Groups 3 and 4, it is advisable to cancel fresh ET if DFP is >40 on the trigger day with the GnRH-ant protocol, to prevent embryo-endometrium asynchrony and rare embryo waste.

Recently, FET technology has gained popularity, allowing more reasonable evaluation of complete IVF/ICSI stimulation cycles using CLRB as an important quality control indicator. Analyzing pregnancy outcomes of all ET cycles in different DFP groups revealed that in POSEIDON Groups 3 and 4, most individuals underwent only one ET cycle, whereas few had a second ET cycle. Only four and three patients, respectively, underwent a third ET cycle and none of them achieved pregnancy. Notably, in POSEIDON Group 3, when DFP exceeded 40, both CCPR and CLRB decreased significantly with increasing DFP. Logistic regression analysis further indicated that DFP≤40 and the No. of oocytes retrieved were independent protective factors against CLRB. This could be attributed to the fact that patients in POSEIDON Group 3 are typically younger, and DFP≤40 allows for a higher count of oocytes retrieved and available embryos, thereby increasing the chances of achieving a live birth. In the POSEIDON Group 4 population, although CCPR and CLRB were slightly higher in the DFP≤40 group than in the DFP>40 group, these differences were not statistically significant. Logistic regression analysis revealed that age was the primary independent risk factor for CLRB in patients in POSEIDON Group 4. A previous study found that embryo euploidy rates were similar between POSEIDON Groups 1 and 3, whereas they were significantly lower in Groups 2 and 4 and further decreased with advancing age. This suggests that the primary factor determining embryo quality is female age, rather than ovarian reserve, which is consistent with the findings of our study [33, 34]. Thus, although the No. of oocytes retrieved and available embryos were higher when DFP was ≤40, it did not significantly improve the CLRB of GnRH-ant stimulation cycle, due to poor-quality embryos in patients in POSEIDON Group 4.

Our study possessed several merits. First, to the best of our knowledge, this is the first study to assess the impact of GnRH-ant trigger timing in low-prognosis patients (POSEIDON Groups 3 and 4) using the DFP metric.

Second, we employed RCS models to explore the nonlinear relationship between DFP and clinical outcomes, adjusting for potential confounding factors to ensure the reliability of our results. Finally, this study focused on CLRB as the primary outcome, providing robust clinical data supporting trigger strategies in low-prognosis populations. However, our study also had limitations as being a single-center retrospective study with a relatively small sample size, which may have potentially introduced selection bias. Future prospective randomized controlled trials with larger sample sizes are necessary to validate and refine our findings.

Conclusion

In conclusion, our findings underscore the importance of trigger timing in patients belonging to POSEIDON Groups 3 and 4. Triggering when DFP exceeds 40 leads to unfavorable laboratory outcomes and adverse effects on pregnancy outcomes in fresh ET cycles. Specifically, in Group 3, elevated DFP correlates with reduced CLRB, highlighting the necessity of avoiding delayed triggers. Conversely, in Group 4, DFP did not significantly impact CLRB, with age emerging as the primary determinant. Hence, timely intervention and management are imperative, particularly for older women with low prognosis.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

Study design was done by Ling XF, and Shen R; data collection by Xie QJ and Yan N; statistical analysis by Xie QJ, Wei Y, and Ni DY; manuscript drafting by Xie QJ, and Jiang W; and research supervision by Ling XF, Yang Y, and Zhao C. All authors contributed to manuscript revision and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Ethics approval and consent to participate Ethics approval was obtained from the the Ethics Committee of Nanjing Maternity and Child Health Care Hospital without the need for informed consent (NJFY-2023KY-018).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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