A Retrospective Analysis on the Clinical Efficiency of Dual Trigger During Vitro Fertilization and Embryo Transfer (IVF-ET) Treatment for Women with Diminished Ovarian Reserve

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Abstract

Background: Due to poor number and quality of oocytes, the reproductive prognosis of diminished ovarian reserve (DOR) patients is obstinately disapproving. This study will compare the effects of double trigger and hCG trigger on the culture outcome and implantation outcome during the IVF treatment for DOR patients.

Methods: a total of 857 controlled ovarian stimulation (COS) cycles and 366 frozen embryo transfer (FET) cycles were included in this study. The difference in retrieval cancellation, oocyte retrieval, number of oocytes, maturation oocytes, fertilization, embryos were evaluated between the two groups in the COS dataset, while implantation, clinical pregnancy, ongoing pregnancy, and spontaneous abortion were analyzed based on FET dataset. To robustly evaluate the marginal effect of the study factor as well as other covariates, a cluster-weighted GEE model was exploited to fit the uniqueness of IVF-ET data.

Results: Neither the retrieval outcome, nor cultural or implantation outcomes were improved after dual-trigger. The OR values were 1.08 (95% CI: 0.41 ~2.78) for retrieval cancellation, 1.33 (95% CI: 0.89 ~2.00) for oocyte harvest, 0.99 (95% CI: 0.97 ~1.02) for oocyte maturation, 1.03 (95% CI: 0.94 ~1.12) for fertilization, 1.04 (95% CI: 0.94 ~1.15) for viable embryo, and 1.03 (95% CI: 0.88 ~1.19) for top quality embryo. As to the transfer outcome, no difference was identified between the two groups, with the ORs 0.90 (95% CI: 0.62 ~1.30) for implantation and 0.97 (95% CI: 0.56 ~1.69) for clinical pregnancy. This insignificance remained unchanged after adjusting for the covariates such as age, BMI, infertility duration, etc.

Conclusions: Dual triggering the final oocyte maturation combined of GnRH-a and standard dose of hCG cannot significantly improve the retrieval, cultural or implantation outcomes of IVF-ET treatments in women with DOR.

Background

Decreased ovarian reserve (DOR) has been posing stubborn challenges to most reproductive clinicians in recent years. Even worse, this problem is exacerbated by the delayed reproductive age in human. In conventional in vitro fertilization and embryo transfer (IVF-ET) practice, the outcome of patients with DOR is unsurprisingly suboptimal due to meagrely follicles, poor oocyte quality, and subsequently limited number of transferable embryos. To this end, certain modifications have been attempted to different aspects of IVF-ET treatment in order to gain a better outcome, including pretreatment with various drugs, COS protocols as well as various supplements [1]. However, most of the measures had limited success, raising the necessity that additional modification be investigated.

Trigger of the final maturation of oocyte is one of the most key aspects during IVF-ET procedure. Human chorionic gonadotropin (hCG) and gonadotropin releasing hormone agonist (GnRHa) are two commonly used tools trigger. The former acts as a trigger by binding to luteinizing hormone (LH) receptor and mimicking the biological role of LH based on structural and biological similarities, [2], and the latter by

promoting the pulsed release of endogenous LH and follicle-stimulating hormone (FSH) through binding to pituitary GnRH receptor [3, 4]. Three trigger protocols have been developed based on the two drugs, including hCG alone (hCG trigger), GnRH alone (GnRH trigger) and GnRH plus reduced dose of hCG (dual trigger) [5]. It is generally acknowledged that harvesting enough mature oocytes and transferable embryos is one decisive factor for the success of IVF-ET treatment, underlining the importance of trigger protocols, especially for DOR patients.

A bulk of evidence have suggested that dual trigger has several significant advantages over hCG or GnRH protocol, such as better luteal phase support and lower risk of OHSS in normal and/or high respond patients [6-8]. More importantly, some reports showed that dual trigger may improve oocyte retrieval and maturation, cultural outcome, as well as embryo implantation in subjects with normal ovarian reserve [9, 10]. In view of this, the application of dual trigger to DOR patients has been proposed, especially for its potential in promising number and viability of embryos [11-14]. However, current conclusions remained inconsistent, with some studies believing that double trigger can significantly improve the rate of fertilized eggs, viable embryos, or embryo implantation [11, 12], while another two studies failing to identify any significance in these endpoints [13, 14]. Moreover, pitifully few evidence has been available for the improvement of oocyte retrieval and maturation rate by dual trigger [14]. Thus, more researches are needed to comprehensively evaluate the clinical efficiency of dual trigger in DOR.

Additionally, one of the biggest challenges in discriminating the potential factors that influencing clinical outcomes of IVF-ET treatment is to seek a suitable statistical model for the data with evident cluster dependence such as repeated cycles, as well as to properly estimate marginal effects of each potential covariate under the conditions that the clinical outcome is influenced by multiple factors. In this retrospective analysis, we carried out a cluster weighted generalized estimating equation (WGGE) to evaluate the contribution of trigger protocol to the outcomes of IVF-ET treatment in DOR patients.

Materials And Methods

Study design and participants

This is a retrospective analysis on the IVF-ET cycles from January, 2016 to August, 2021 at the Reproductive Center of Zhejiang Provincial People's Hospital, China. The study protocol was approved by the Ethics Committee of Zhejiang Provincial People's Hospital.

Decreased ovarian reserve (DOR) was defined according the POSEIDON criteria, and fulfilled both following criteria: anti-Müllerian hormone (AMH) < 1.2 ng/ml and antral follicle count [AFC (n)] < 5. IVF cycles with controlled ovarian stimulation GnRH antagonist, PPOS or mild stimulation protocols were included. Exclusion criteria were those with chromosome abnormality (carrier of balanced translocation or inversion), chemotherapy or radiotherapy treatment, BMI > 30 kg/m², or basic FSH > 20 IU/L. After the final recruitment by the inclusion and exclusion criteria, a total of 381 patients, who finished 857 COS cycles, were selected into present study, and divided into hCG trigger and dual trigger groups.

Controlled ovarian stimulation and oocyte maturation trigger

For mild stimulation protocol, clomiphene citrate (CODAL SYNTO LTD., Cyprus) was administered from cycle day 2 at a daily dose of 50-100mg combined with 75-150U human menopausal gonadotropin (hMG) (Lizhu Pharmaceutical, China). From cycle day 8, GnRH-antagonist (Cetrorelix, Merck Serono, Germany) was added when serum LH exceeded the level of 10 IU/mL. For progestin-primed ovarian stimulation (PPOS) protocol, medroxyprogesterone acetate (MPA) (Zhejiang Xianju Pharmaceutical Co., China) at a daily dose of 6-8 mg and hMG at 75 to 225 IU per day were given from cycle day 2 until the trigger day. For GnRH-antagonist regimen, gonadotropins including recombinant follicle stimulating hormone (Gonal-f; Merck Serono, Germany) or hMG (Lizhu Pharmaceutical Factory, China) were administered in a flexible way from cycle day 2 at a dose of 100-300U per day, and adjusted according to ovarian response. When the diameter of leading follicles reached 12 mm, a daily dose of 0.25 mg GnRH-antagonist was injected subcutaneously. When at least one follicle diameter 18 mm or two follicles diameter 17 mm, final oocyte maturation was triggered by either recombinant hCG (0.25mg; Merck Serono, Germany) alone, or combination of hCG with 0.1 mg triptorelin (Decapeptyl, Ferring Pharmaceuticals).

Embryo culture and score

About 36 hours after oocyte trigger, the cumulus-oocyte complexes (COCs) were aspirated by transvaginal puncture under ultrasound guidance. Depending on the semen quality or previous IVF history, the obtained oocytes were inseminated by conventional IVF or intracytoplasmic sperm injection (ICSI). Embryos at day 3 were graded by the blastomere symmetry and fragmentation according to the Istanbul consensus on embryo assessment [15]. Day 3 embryos graded as 'good' with blastomere number were defined as top-quality embryos, and directly cryopreserved by vitrification, while rest non-top-quality embryos underwent a continuous blastocyte culture. Only viable blastocysts with inner cell mass or trophectoderm graded above 'B' according to Gardner score system were cryopreserved on day 5 or day 6 [16]. All vitrification and warming procedures were conducted according to the instructions of reagent manufacturer (Vitrolife, Sweden).

Endometrium preparation, frozen embryo transfer (FET)

Either a natural cycle, hormone replacement (HRT) cycle, or induced ovulation cycle was used for endometrium preparation. Briefly, for natural cycles, progesterone treatment started from the ovulation day or 2 days after LH surge. For HRT cycle, oral estradiol (estradiol valerate, Delpharm Lille S.A.S, France) was administered at 6 mg/day from cycle day 3 onwards, and at day 7 after HRT initiation, 5g/day estradiol gels (Besins Manufacturing Belgium) were added for another 5-7 days if endometrial lining <7mm. Once the endometrium reached a satisfactory thickness, 40mg progesterone was injected. A maximum of two embryos were thawed on the morning of the transfer day, and transferred under ultrasound guidance. Once pregnancy was achieved, the progesterone and estradiol supplementation were continued until 10 weeks of gestation.

Outcome measures

The primary endpoints of present study included retrieval cancel rate, cycle rate of oocyte harvesting, oocyte harvesting rate, oocyte maturation rate, viable embryo rate and implantation rate. Here, retrieval cancel rate was defined as the number of cycles cancelled due to premature ovulation divided by the number of total cycles, cycle rate of oocyte harvesting as the number of cycles with successful oocyte harvesting divided by the number of oocyte pick-up (OPU) cycles, oocyte harvesting rate as the number of total harvested oocytes divided by the number of follicles aspirated (diameter 12 mm) on the OPU day, oocyte maturation and viable embryo rate as the ratio of MII oocyte number and viable embryo to total number of collected oocytes, respectively, and the implantation rate as the number of gestational sacs visualized under ultrasound examination divided by the number of embryos transferred. The secondary endpoints were clinical pregnancy rate and early miscarriage rate. A clinical pregnancy referred to a gestational sac under ultrasound, with or without heartbeat, and early miscarriage rate to the spontaneous loss of a clinical pregnancy before 12th weeks of gestation.

Statistical analysis

The data were analyzed by SPSS statistical software (version 21.0, IBM Corp., USA). The continuous variables were expressed as mean $\pm$ standard deviation ($\pm$ SD), and the category one as n (%). The comparison between the two groups was conducted with student t test or Fisher’s exact test. To identify the genuine association of trigger protocols with oocyte retrieval, culture outcomes and implantation endpoints, a cluster-weighted generalized estimating equation (GEE) models with a log link were used to account for within person correlations between repeated cycles in presence of nonignorable cluster size [17, 18]. During GEE models, a binomial distribution with log link function were specified for retrieval cancel rate, cycle rate of oocyte harvesting, clinical and ongoing pregnancy, and a poisson distribution with log link function for oocyte harvesting rate, culture outcomes and implantation rate. In poisson distribution models, a logarithm of the number of punctured follicles or transferred embryos were taken as the offset. The inverse of the number of oocytes or repeated cycles was taken as the weighting coefficient. Results were presented as a weighted average over levels of categorical variables according to their relative frequency. Statistical significance was defined as $P < 0.05$.

Results

Present study included a total of 381 patients, who finished 857 COS cycles. Of all included cycles, 21 canceled before oocyte retrieval due to premature ovulation, and 124 failed to harvest oocytes. Thus, 836 cycles fulfilled oocyte pick-up, and 733 cycles underwent in vitro culture procedure. The baseline characteristics for the patients and cycles included were summarized in Table 1 and 2, respectively. There was no significant difference in age, BMI, AMH, basic serum FSH, infertility duration or infertility diagnosis, whereas a significance existed in COS protocol ($p = 0.0004$), total dose of gonadotrophins ($p < 0.0001$), and days of gonadotrophins administration ($p < 0.0001$).

Table 1. Baseline characteristics of included couples

Parameter	Values
No. of couples (n)	381
Female age (yrs)	37.94±6.14
BMI (Kg/m ²)	22.10±2.90
AMH (ng/mL)	0.50±0.33
Basic FSH (IU/L)	9.27±4.89
Antral follicle count (AFC, n)	2.56±1.09
Infertility duration (yrs)	3.72±3.44
Primary infertility, n(%)	113 (29.66)
Male factor, n(%)	166 (43.57)
Endometriosis, n(%)	47 (12.34)
Tubal factor, n(%)	56 (14.70)
Ovulation disorder, n(%)	29 (7.61)
Unexplained, n(%)	25 (6.56)

Table 2. Baseline and treatment characteristics of studied subjects.

	Total (n=857)	hCG (n=258)	Dual trigger (n=599)	P values
Female age (yrs)	38.62±6.25	38.38±6.48	38.72±6.15	0.454
BMI (Kg/m ²)	22.01±2.75	21.88±2.78	22.06±2.74	0.388
AMH (ng/mL)	0.47±0.32	0.46±0.34	0.47±0.31	0.655
Basic FSH(IU/L)	9.45±5.09	9.14±5.08	9.59±5.1	0.233
AFC (n)	2.54±1.08	2.44±1.04	2.58±1.09	0.092
Infertility duration (yrs)	4.06±3.89	3.69±3.26	4.22±4.12	0.065
Gn dose (IU)	1334.89±789.14	1063.59±739	1448.95±782.24	<.0001
Days of Gn (d)	8.56±3.38	7.53±3.49	9±3.24	<.0001
Primary infertility, n(%)	253 (29.52)	72 (27.91)	181 (30.22)	0.497
Controlled ovarian stimulating protocol				
PPOS, n(%)	329 (38.39)	84 (32.56)	245 (40.90)	0.0004
Mini-stimulation, n(%)	355 (41.42)	101 (39.15)	254 (42.40)	
GnRH-ant, n(%)	139 (16.22)	55 (21.32)	84 (14.02)	
Others, n(%)	34 (3.97)	18 (6.98)	16 (2.67)	
Infertility diagnosis, n (%)				
Male factor	382 (44.92)	115 (44.57)	270 (45.08)	0.892
Endometriosis	96 (11.20)	34 (13.18)	62 (10.35)	0.229
Ovulation disorder	55 (6.42)	19 (7.36)	36 (6.01)	0.458
Tubal factor	127 (14.82)	40 (15.50)	87 (14.52)	0.711
Unexplained	59 (6.88)	20 (7.75)	39 (6.51)	0.510
Retrieval cancel, n(%)	21 (2.45)	6 (2.33)	15 (2.50)	0.877
Oocyte not harvestcycle, n(%)	124 (14.47)	44 (17.05)	80 (13.36)	0.158
ICSI, n (%)	265 (36.15)	76 (35.51)	189 (36.42)	0.909

BMI, body mass index; AMH, anti-Mullerian hormone; AFC, antral follicle count; PPOS, progestin primed ovarian stimulation protocol; ICSI, Intracytoplasmic sperm injection.

*P value was calculated based on student *t* test for continuous variable and Fisher exact test for categorical variables.

The mean number of punctured follicles (2.18 ± 1.49 and 2.31 ± 1.41), retrieved oocytes (1.86 ± 1.21 and 1.94 ± 1.16), MII oocytes (1.79 ± 1.18 and 1.85 ± 1.18), fertilized eggs (1.21 ± 1.08 and 1.29 ± 1.04), viable embryos (1.10 ± 1.07 and 1.20 ± 1.01) and top-quality embryos (0.75 ± 0.94 and 0.8 ± 0.87) were slightly higher in the dual trigger groups as compared with the hCG, but this difference did not reach a statistical significance (Table 3). To ascertain the robustness of our results, we also evaluated the difference in the relative value of above endpoints between the two groups, and draw a consistent conclusion (Table 3). Further, we employed a cluster-weighted GEE model, a statistical method compatible with repeated measurement data and count data, to calculate the edge effect of studied variable with or without adjusting for confounding factors such as age, BMI, primary infertility, COS protocols et al. Our robust analysis did not show any superior or inferior influence on oocyte retrieval or in vitro culture outcomes for dual trigger as related to HCG group, which was coincided with above result (Table 4).

Table 3. Outcomes of oocyte retrieving, in vitro fertilization and embryo culture.

	Total (n=733)	hCG (n=214)	Dual trigger (n=519)	P value
No. of follicles punctured per cycle	2.27 ± 1.43	2.18 ± 1.49	2.31 ± 1.41	0.271
Oocyte retrieved, n (%)	1595 (83.99)	399 (85.44)	1007 (83.99)	0.463
No. of oocytes retrieved per cycle	1.92 ± 1.18	1.86 ± 1.21	1.94 ± 1.16	0.429
Mature oocytes, n (%)	1507 (94.48)	382 (95.74)	953 (94.64)	0.475
No. of mature oocyte per cycle	1.83 ± 1.18	1.79 ± 1.18	1.85 ± 1.18	0.009
2PN eggs, n (%)	1044 (65.45)	258 (64.66)	670 (66.53)	0.722
No. of 2PN eggs per cycle	1.27 ± 1.05	1.21 ± 1.08	1.29 ± 1.04	0.316
Viable embryos, n (%)	957 (60.00)	236 (59.15)	621 (61.67)	0.382
No. of viable embryos per cycle	1.17 ± 1.03	1.10 ± 1.07	1.20 ± 1.01	0.262
Top-quality embryo, n (%)	643 (40.31)	160 (40.10)	415 (41.21)	0.702
No. of top-quality embryos per cycle	0.78 ± 0.89	0.75 ± 0.94	0.80 ± 0.87	0.474

*P value was calculated with one-way ANOVA test for continuous variables, and Fisher exact test for categorical variables. 2PN, two pronucleus.

Table 4. Weighted GEE analysis on the effect of trigger protocol on the endpoint of oocyte retrieving, in vitro fertilization and embryo culture.

	Model 1			Model 2		
	ORs	95% CI	P value	ORs	95% CI	P value
Retrieval cancellation	1.08	0.41 ~2.78	0.877	1.1	0.40 ~3.03	0.851
Cycle rate of oocyte harvest	1.33	0.89 ~2.00	0.159	1.32	0.87 ~2.00	0.195
Oocyte harvest per follicle	1.01	0.95 ~1.06	0.829	1.01	0.95 ~1.06	0.789
No. of mature oocytes per retrieved oocyte	0.99	0.97 ~1.02	0.567	0.99	0.96 ~1.01	0.359
No. of 2PN eggs per retrieved oocyte	1.03	0.94 ~1.12	0.52	1.02†	0.93 ~1.11	0.623
No. of viable embryo per retrieved oocyte	1.04	0.94 ~1.15	0.411	1.03†	0.93 ~1.14	0.548
No. of top-quality embryo per retrieved oocyte	1.03	0.88 ~1.19	0.719	1.02†	0.88 ~1.19	0.814

Effect of trigger protocol was evaluated as risk ratio(95% CI) with dual trigger protocol as the reference.

Model1, weighted GEE analysis model for data from repeated cycles; model2, weighted GEE analysis adjusted for female age, infertility diagnosis, infertility duration, primary infertility, AMH, basic FSH and AFC.

† Additional adjusted for male factor and ICSI procedure.

A total of 366 FET cycles were included into the transfer dataset for evaluating the influence of trigger protocols on implantation outcomes. There was no significant difference between the two groups in terms of baseline characteristics, such as women's age, BMI, endometrium thickness as well as the number of transferred embryos (Table 5). Our results showed that transferring the embryos from hCG trigger might produce higher rates of implantation (26.09% and 22.62%), clinical pregnancy (32.35% and 29.17%) and ongoing pregnancy (23.53% and 18.56%), while lower early miscarriage (24.24% and 35.06%) as compared to those in the dual trigger group, however, neither univariate Chi square test nor robust cluster-weighted GEE model identified a statistical significance (Table 5 and 6).

Table 5 Characteristics and outcomes of embryo transfer cycles

	Total (n=366)	hCG (n=102)	Dual trigger (n=264)	P value
Female age (yrs)	38.64±5.84	37.95±6.78	38.91±5.42	0.160
BMI (Kg/m ²)	21.91±2.75	22.07±2.74	21.85±2.75	0.495
AMH (ng/mL)	0.55±0.32	0.53±0.35	0.56±0.31	0.403
Basic FSH (IU/L)	8.17±3.93	8.05±4.31	8.22±3.78	0.719
AFC (n)	2.75±1.09	2.78±1.06	2.75±1.09	0.763
Infertility duration (yrs)	4.10±3.84	3.84±3.59	4.2±3.94	0.428
No. of embryos transferred	1.44±0.50	1.35±0.48	1.47±0.51	0.040
No. of top embryos transferred	1.10±0.64	1.02±0.6	1.14±0.65	0.116
Endometrial thickness (mm)	9.41±2.46	9.41±2.46	9.67±2.36	0.358
Male factor, n(%)	167 (45.63)	48 (47.06)	119 (45.08)	0.733
Unexplained factor, n(%)	30 (8.20)	9 (8.82)	21 (7.95)	0.786
Controlled ovarian stimulation				
PPOS, n(%)	126 (34.43)	28 (27.45)	98 (37.12)	0.055
Mini-stimulation, n(%)	171 (46.72)	47 (46.08)	124 (46.97)	
GnRH-ant, n(%)	53 (14.48)	19 (18.63)	34 (12.88)	
Others, n(%)	16 (4.37)	8 (7.84)	8 (3.03)	
ICSI, n(%)	127 (34.70)	34 (33.33)	93 (35.23)	0.771
Embryo implantation, n(%)	124 (20.60)	36 (26.09)	88 (22.62)	0.410
Clinical pregnancy, n(%)	110 (30.05)	33 (32.35)	77 (29.17)	0.551
ongoing pregnancy, n(%)	73 (19.95)	24 (23.53)	49 (18.56)	0.286
Early miscarriage, n(%)	35 (31.82)	8 (24.24)	27 (35.06)	0.264
Ectopic Pregnancy, n(%)	2 (1.82)	1 (3.03)	1 (1.30)	0.551

*P value was calculated based on one-way ANOVA test for continuous variable and Fisher exact test for categorical variables.

Table 6 Weighted GEE analysis on the effect of trigger protocol on clinical outcomes after embryo transfer

	Model 1			Model 2		
	OR value	95% CI	P value	OR value	95% CI	P value
Clinical pregnancy	0.97	0.56 ~1.69	0.911	1.12	0.68 ~1.85	0.636
Implantation‡	0.90	0.62 ~1.30	0.576	1.00	0.71 ~1.41	0.993
Early miscarriage	1.64	0.53 ~5.00	0.384	1.89	0.59 ~6.25	0.283
ongoing pregnancy	0.85	0.45 ~1.59	0.615	0.85	0.42 ~1.69	0.639

Effect of trigger protocol was evaluated as risk ratio(95% CI) with dual trigger protocol as the reference.

† Adjusted for female age, BMI, primary infertility, infertility duration, embryo stage and endometrium thickness.

‡Poisson model was used to estimate OR when GEE log binomial models did not converge.

Discussion

This retrospective analysis is the first to employ a robust statistical model (cluster-weighted GEE model) to compare the clinical efficiency of commonly used trigger protocols, hCG and dual trigger, in poor ovarian responders. Our results showed that dual trigger might not have any superiorities over hCG trigger in terms of oocyte retrieval cancellation, cycles rate of oocyte harvesting, and number of retrieved oocytes, mature oocyte, fertilized eggs, viable embryos as well top-quality embryos. Furthermore, dual trigger didn't improve the outcome of embryo transfer yet—including implantation, clinical pregnancy or early miscarriage. Our results indicated that addition of GnRHa to the trigger regimen cannot better the outcome of IVF-ET for DOR patients.

Due to the structural similarity to LH (sharing the same α subunit and 85% of the amino acid sequence of the β subunit), and consequent vigorous binding and activating of LH/HCG receptor[2], hCG has been routinely used to induce oocyte maturation and ovulation for several decades in artificial reproductive treatment. With a stronger affinity (6~8 times that of LH), and longer half-life, it displays a violent power in triggering oocyte maturation, but concurrently, raises a higher risk of OHSS as well as impaired embryo quality[6, 19]. By binding to pituitary GnRH receptor and exerting a "flare up" role, GnRHa can induce an endogenous natural-like mid-cycle hormone surge (including LH peak and FSH secretion) [3]. In view of the biological role of FSH in regulation of LH receptor expression, one potential benefit proposed for GnRHa or dual trigger is improved maturation and quality of oocytes, and subsequently, more viable embryos [20-23]. This improvement has been extensively checked in high responders[7, 24, 25], normal responders [9, 10, 26, 27], and poor responder[11-14], however, the current viewpoints remain controversy. Despite several prospective studies showed GnRHa trigger result in a higher number and percentage of mature oocytes as compared with hCG[24, 28], a pooled analysis including four eligible random controlled trials failed to show any difference in the number of either retrieved oocytes, or mature

oocytes[29]. Thus, it is not unexpected that our study did not identify any significance in oocyte retrieval or oocyte maturation between the two triggers.

From the biological basis, it is unilateral to consider an indisputable superiority for GnRHa in triggering oocyte maturation. Indeed, GnRHa can induce an endogenous mid-cycle FSH surge, which might endow it with an advantage in cumulus expansion and consequent oocyte maturation, whereas the peak time, duration, and profile of the induced gonadotrophins secretion is distinct from that of the natural cycle[4, 30]. For these non-physiological features, we should furtherly consider certain unfavourability of GnRHa when using it to trigger the oocyte maturation process. It is widely acknowledged that, under COS conditions, poor oocyte quality and embryo development partly lie in a stubborn asynchrony between nuclear and cytoplasmic maturation process of oocytes[31, 32]. Despite the explicit underlying mechanism underlying this asynchrony remains elusive, a premature resumption of oocyte meiosis induced by exogenous pharmacological drugs, including GnRHa or HCG may presumably play an important role. Basic researches have indicated that the transportation of signal molecules, nutrients, and energy substrates through the gap junction, from cumulus cell to oocytes, be requisite for not only oocyte nuclear maturation, but also its cytoplasmic maturation [33, 34]. In response to the LH surge and its downstream ovulatory signals, such as EGF-like peptides (including AREG, EREG and BTC), EGFR-ERK1/2 as well as NPPC-NPR1 signal pathways[35-37], a diminished phosphorylation of Cx43 and a consequential closure of gap junction between oocytes and cumulus cells will emerge in concurrence with the meiosis resumption and progress, i.e., nuclear maturation[38-40]. In terms of the pharmacokinetics, the peak time of hCG after administration is about 12h [41], while the LH peak induced by GnRHa only 4h[30], which is even precocious in relation to the natural cycles. This relative premature LH surge may induce an untimely interruption of the junction between oocytes and cumulus cells, which in turn, eliminate the accumulation of oocyte quality factors such as signal molecules, nutrients, and energy substrates, and eventually aggravate the asynchrony between nuclear and cytoplasmic maturation. On the other way, cumulus granulosa cell expansion induced by mid-cycle FSH surge also mechanically destroys the gap junctions[42]. Another concern for GnRHa trigger lies in the reduced amount of released gonadotrophins. The area under the gonadotrophins curve (AUC) after GnRHa administration has been reported to be significantly decreased as compared with that in natural cycle, or HCG[4], which raises the risk of insufficient maturity trigger. Thus, a preconceived proposal that GnRHa or dual trigger should acquire to a superior outcome still lack solid biological basis. All these complexities in the biological roles of GnRHa trigger may partly explained the contradictory conclusion of current works. Our study, together with others, suggest that the biological advantages of dual trigger in theory may be of no clinical significance for DOR patients[13, 43]. Our additional analysis with the embryo transfer dataset yet failed to find any difference in embryo implantation, furtherly implying that dual trigger have no role in improving the oocyte quality.

One divergence of our analysis from others was embodied in the diversity of ovarian stimulating protocols, including mini-stimulation, PPOS, and GnRH-antagonist protocols. Inclusion of single COS protocol, the way of most previous studies, seems to be partly driven by the consideration that COS protocols may impose an essential impact on the clinical outcomes such as oocyte retrieval, oocyte

maturation, fertilization, embryo development and implantation. Indeed, the COS protocols distributed unevenly between the two groups. Nevertheless, the analysis focusing on the marginal effect demonstrated that COS protocols do not affect either cultural or implantation outcomes [data not shown]. Then the diversity in COS may not impair the robustness of present study, and furtherly strengthen the extrapolation for our conclusion.

When choosing an appropriate model for statistical analysis, we should consider certain tricky features of the IVF-ET data. Firstly, repeated observation at the levels of cycles, oocytes or embryos lead to an evident correlation within these observation objectives, making the data usually deviating from normal distribution or lognormal distribution. This data type is beyond the reach of traditional statistical methods, such as univariate model, logistic or multiple linear regressions [44]. Secondly, the cluster size, i.e., the number of retrieved oocytes, embryos, or repeated cycles of individuals in IVF practice, has been universally acknowledged to be informative for the clinical outcome, for example, the count of repeated cycles may contribute to the variance of response variables, such as implantation rate and top-quality embryos. Henceforth, a cluster-weighted statistical model considering the nonignorable cluster size is necessary in this context, for example, cluster-weighted GEE or mixed effect models. Finally, some confounding factors, such as age and available follicles, have so tremendous an influence on reproductive outcomes that even slight baseline unevenness (despite no significance) may give rise to a poor comparison between studied groups. This covariates should be adjusted in a multivariate analysis model [45]. Nevertheless, most of the current studies have not fully considered above factors, and might give unrobust, and even inconsistent conclusions. In present study, we exploited a cluster-weighted GEE model to evaluate the marginal effects of trigger protocols and related covariates, which is suitable for multivariate analysis of non-normal, repeated observational or cluster data, and might produce a considerably reliable result.

Taken together, from present study, we preliminarily believe that dual trigger be not superior to conventional hCG protocol in term of either embryo outcomes or implantation outcomes during IVF-ET for DOR patients. However, because this is merely a retrospective investigation, further large-sample, prospective research is expected to provide a more solid conclusion.

Conclusions

Dual triggering the final oocyte maturation combined of GnRH-a and standard dose of hCG cannot significantly improve the retrieval, cultural or implantation outcomes of IVF-ET treatments in women with DOR.

Abbreviations

DOR diminished ovarian reserve

COS controlled ovarian stimulation

FET frozen embryo transfer

IVF-ET in vitro fertilization and embryo transfer

hCG human chorionic gonadotropin

GnRHa gonadotropin releasing hormone agonist

LH luteinizing hormone

FSH follicle-stimulating hormone

WGGE weighted generalized estimating equation

BMI, body mass index;

AMH anti-Mullerian hormone

AFC antral follicle count

hMG human menopausal gonadotropin

PPOS progestin-primed ovarian stimulation

MPA medroxyprogesterone acetate

COCs cumulus–oocyte complexes

ICSI intracytoplasmic sperm injection

HRT hormone replacement

OPU oocyte pick-up

GEE generalized estimating equation

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of supporting data

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

Competing interests

The authors declare no competing interests in this study.

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Authors' contributions

Ling Zhang, Li-mei Wu wrote the main manuscript text and Meng-xia Ji, Shi-shi Li, Lin Zhang, Zhen Jin, Yi-er Zhou, Chong-yi Shu prepared figures 1-6. Jing Shu and Wei-hai Xu achieved the assessment of the quality of the literature. All authors reviewed the manuscript.

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References

1. Ubaldi, F., et al., *Management of Poor Responders in IVF: Is There Anything New?* BioMed Research International, 2014. **2014**: p. 352098.
2. Kessler, M., et al., *Structure of N-glycosidic carbohydrate units of human chorionic gonadotropin*. The Journal of biological chemistry, 1979. **254**: p. 7901-8.
3. Dosouto, C., T. Haahr, and P. Humaidan, *Gonadotropin-releasing hormone agonist (GnRHa) trigger - State of the art*. Reprod Biol, 2017. **17**(1): p. 1-8.
4. Humaidan, P., et al., *GnRH agonist for triggering of final oocyte maturation: time for a change of practice?* Hum Reprod Update, 2011. **17**(4): p. 510-24.
5. Ortega, I., J.A. García-Velasco, and A. Pellicer, *Ovarian manipulation in ART: going beyond physiological standards to provide best clinical outcomes*. Journal of assisted reproduction and genetics, 2018. **35**(10): p. 1751-1762.
6. Engmann, L., C. Benadiva, and P. Humaidan, *GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis*. Reproductive BioMedicine Online, 2016. **32**(3): p. 274-285.
7. Li, S., et al. *Dual trigger of triptorelin and HCG optimizes clinical outcome for high ovarian responder in GnRH-antagonist protocols*. Oncotarget, 2018. **9**, 5337-5343 DOI: 10.18632/oncotarget.23916.
8. Orvieto, R., *Triggering final follicular maturation- hCG, GnRH-agonist or both, when and to whom?* Journal of Ovarian Research, 2015. **8**(1): p. 60.
9. Haas, J., et al., *GnRH agonist and hCG (dual trigger) versus hCG trigger for final follicular maturation: a double-blinded, randomized controlled study*. Human Reproduction, 2020. **35**(7): p. 1648-1654.

10. Lin, M.-H., et al., *Dual trigger with combination of gonadotropin-releasing hormone agonist and human chorionic gonadotropin significantly improves the live-birth rate for normal responders in GnRH-antagonist cycles*. Fertility and Sterility, 2013. **100**(5): p. 1296-1302.
11. Mutlu, I., et al., *Dual trigger with the combination of gonadotropin-releasing hormone agonist and standard dose of human chorionic gonadotropin improves in vitro fertilisation outcomes in poor ovarian responders*. Journal of Obstetrics and Gynaecology, 2021: p. 1-6.
12. Chern, C.-U., et al., *Dual-trigger improves the outcomes of in vitro fertilization cycles in older patients with diminished ovarian reserve: A retrospective cohort study*. PloS one, 2020. **15**(7): p. e0235707-e0235707.
13. Lin, M.-H., et al., *Dual trigger with gonadotropin releasing hormone agonist and human chorionic gonadotropin significantly improves live birth rate for women with diminished ovarian reserve*. Reproductive Biology and Endocrinology, 2019. **17**(1): p. 7.
14. Zhang, J., et al., *Dual trigger of final oocyte maturation in poor ovarian responders undergoing IVF/ICSI cycles*. Reproductive BioMedicine Online, 2017. **35**(6): p. 701-707.
15. Medicine, A.S.i.R. and E.S.I.G.o. Embryology, *The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting†*. Human Reproduction, 2011. **26**(6): p. 1270-1283.
16. Gardner, D.K. and W.B. Schoolcraft, *Culture and transfer of human blastocysts*. Current Opinion in Obstetrics and Gynecology, 1999. **11**(3).
17. Missmer, S.A., et al., *Analysis of multiple-cycle data from couples undergoing in vitro fertilization: methodologic issues and statistical approaches*. Epidemiology (Cambridge, Mass.), 2011. **22**(4): p. 497-504.
18. Williamson, J.M., H.-Y. Kim, and L. Warner, *Weighting Condom Use Data to Account for Nonignorable Cluster Size*. Annals of Epidemiology, 2007. **17**(8): p. 603-607.
19. Valbuena, D., et al., *Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo*. Fertility and Sterility, 2001. **76**(5): p. 962-968.
20. RICHARDS, J.S., et al., *Ovarian Follicular Development in the Rat: Hormone Receptor Regulation by Estradiol, Follicle Stimulating Hormone and Luteinizing Hormone*¹. Endocrinology, 1976. **99**(6): p. 1562-1570.
21. ZELEZNIK, A.J., A.R. MIDGLEY, JR, and L.E. REICHERT, JR, *Granulosa Cell Maturation in the Rat: Increased Binding of Human Chorionic Gonadotropin Following Treatment with Follicle-Stimulating Hormone in Vivo*¹. Endocrinology, 1974. **95**(3): p. 818-825.
22. Eppig, J.J., *FSH stimulates hyaluronic acid synthesis by oocyte-cumulus cell complexes from mouse preovulatory follicles*. Nature, 1979. **281**(5731): p. 483-484.
23. Strickland, S. and W.H. Beers, *Studies on the role of plasminogen activator in ovulation. In vitro response of granulosa cells to gonadotropins, cyclic nucleotides, and prostaglandins*. Journal of Biological Chemistry, 1976. **251**(18): p. 5694-5702.
24. Humaidan, P., et al., *GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study*. Human Reproduction, 2005. **20**(5): p. 1213-1220.

25. Griffin, D., et al., *Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders*. Fertility and sterility, 2012. **97**(6): p. 1316-1320.
26. Gao, F., et al., *Effect of a "Dual Trigger" Using a GnRH Agonist and hCG on the Cumulative Live-Birth Rate for Normal Responders in GnRH-Antagonist Cycles*. Frontiers in Medicine, 2021. **8**(759).
27. Zhang, Y., et al., *Outcomes comparison of IVF/ICSI among different trigger methods for final oocyte maturation: A systematic review and meta-analysis*. The FASEB Journal, 2021. **35**(7): p. e21696.
28. Oktay, K., I. Türkçüoğlu, and K.A. Rodriguez-Wallberg, *GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation*. Reproductive BioMedicine Online, 2010. **20**(6): p. 783-788.
29. Griesinger, G., et al., *GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis*. Human reproduction update, 2006. **12**(2): p. 159-168.
30. Itskovitz, J., et al., *Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist**Presented in part at the International Symposium on GnRH Analogues in Cancer and Human Reproduction, Geneva, Switzerland, February 18 to 21, 1988, and at the 6th World Congress of In Vitro Fertilization and Alternative Assisted Reproduction, Jerusalem, Israel, April 2 to 7, 1989*. Fertility and Sterility, 1991. **56**(2): p. 213-220.
31. Sundström, P. and B.O. Nilsson, *Meiotic and cytoplasmic maturation of oocytes collected in stimulated cycles is asynchronous*. Human Reproduction, 1988. **3**(5): p. 613-619.
32. Pereira, N., et al., *The role of in-vivo and in-vitro maturation time on ooplasmic dysmaturity*. Reproductive BioMedicine Online, 2016. **32**(4): p. 401-406.
33. Carabatsos, M.J., et al., *Oocyte–Granulosa Cell Heterologous Gap Junctions Are Required for the Coordination of Nuclear and Cytoplasmic Meiotic Competence*. Developmental Biology, 2000. **226**(2): p. 167-179.
34. Landschaft, D., *Gaps and barriers: Gap junctions as a channel of communication between the soma and the germline*. Seminars in Cell & Developmental Biology, 2020. **97**: p. 167-171.
35. Park, J.-Y., et al., *EGF-Like Growth Factors As Mediators of LH Action in the Ovulatory Follicle*. Science, 2004. **303**(5658): p. 682-684.
36. Zhang, M., et al., *Granulosa Cell Ligand NPPC and Its Receptor NPR2 Maintain Meiotic Arrest in Mouse Oocytes*. Science, 2010. **330**(6002): p. 366-369.
37. Xi, G., et al., *The mRNA-destabilizing protein Tristetraprolin targets "meiosis arrester" Nppc mRNA in mammalian preovulatory follicles*. Proceedings of the National Academy of Sciences, 2021. **118**(22): p. e2018345118.
38. Granot, I. and N. Dekel, *Phosphorylation and expression of connexin-43 ovarian gap junction protein are regulated by luteinizing hormone*. Journal of Biological Chemistry, 1994. **269**(48): p. 30502-30509.

39. Cai, H., et al., *Involvement of PKC ϵ in FSH-induced connexin43 phosphorylation and oocyte maturation in mouse*. Biology Open, 2018. **7**(8).
40. Sela-Abramovich, S., et al., *Mitogen-Activated Protein Kinase Mediates Luteinizing Hormone-Induced Breakdown of Communication and Oocyte Maturation in Rat Ovarian Follicles*. Endocrinology, 2005. **146**(3): p. 1236-1244.
41. Chan, C.C.W., et al., *Bioavailability of hCG after intramuscular or subcutaneous injection in obese and non-obese women*. Human Reproduction, 2003. **18**(11): p. 2294-2297.
42. Atef, A., et al., *The potential role of gap junction communication between cumulus cells and bovine oocytes during in vitro maturation*. Molecular Reproduction and Development, 2005. **71**(3): p. 358-367.
43. Eftekhari, M., et al., *A comparison of dual triggering (by administration of GnRH agonist plus HCG) versus HCG alone in poor ovarian responders in ART outcomes*. Middle East Fertility Society Journal, 2018. **23**(4): p. 350-353.
44. Chiu, Y.H., et al., *Serum omega-3 fatty acids and treatment outcomes among women undergoing assisted reproduction*. Human reproduction (Oxford, England), 2018. **33**(1): p. 156-165.
45. Farquhar, C.M., et al., *Female subfertility*. Nature Reviews Disease Primers, 2019. **5**(1): p. 7.