Original Article

Genuine Empty Follicle Syndrome: Role of Double Trigger and Delayed Oocyte Retrieval (DTDO)

Abdul Majiyd Noushin, Sankalp Singh, Aluvilayil Sonia, Swati Singh, Reema Basheer, Raiza Ashraf, Ahmed N. Waseem, Mohamed Ashraf

Department of Reproductive Medicine, CRAFT Hospital and Research Centre, Thrissur, Kerala, India

Abstract

Background: Empty follicle syndrome (EFS) is a condition of undetermined etiology where no oocytes are retrieved in an ART cycle despite adequate response to ovarian stimulation and diligent follicular aspiration. Because of the rarity of this condition, no much published strategies are available to tackle this. **Aim:** The aim of this study was to evaluate whether sequential administration of gonadotropin-releasing hormone agonist (GnRHa) and human chorionic gonadotropin (hCG) as a trigger at 40 h and 36 h, respectively, before oocyte retrieval (OCR) could correct genuine empty follicle syndrome (GEFS). **Study Setting and Design:** This retrospective observational cohort study was conducted in a tertiary fertility center over a period of 6 years from January 2014 to December 2019. Patients with a history of GEFS were administered GnRHa and recombinant hCG subcutaneously at 40 h and 36 h, respectively, before OCR, i.e., double trigger and delayed oocyte retrieval (DTDO) (n = 13). The primary outcome measures studied were number of mature oocytes retrieved, oocyte maturation index (OMI), number of fertilized oocytes, and number of embryos available for embryo transfer. The secondary outcome measures were clinical pregnancy rate (CPR), miscarriage rate (MR) and live birth rate (LBR) per first frozen embryo transfer (FET) cycle, incidence of inadvertent premature ovulation, and ovarian hyperstimulation syndrome. **Statistical Analysis:** Comparison between the groups was analysed by Fisher's exact test and paired t-test. **Results:** Patients in the DTDO group showed a significant improvement ($P < 0.01$) in the number of mature oocytes retrieved, OMI, number of fertilized oocytes, and number of embryos available for embryo transfer. In the first FET cycle, CPR (44.44%), LBR (44.44%), and MR (11.11%) were observed in the DTDO group. **Conclusion:** Our findings implicate that double trigger and delayed OCR (DTDO) is a safe and efficacious treatment strategy for GEFS.

Keywords: *Double trigger and delayed oocyte retrieval, DTDO, empty follicle syndrome, genuine empty follicle syndrome*

Introduction

Empty follicle syndrome (EFS) is a condition of undetermined etiology where no oocytes are retrieved in an ART cycle despite adequate ovarian response to stimulation with optimal estradiol levels and diligent follicular aspiration. It can be classified as genuine EFS (GEFS) and false EFS (FEFS), where GEFS is interpreted as a failure to retrieve oocytes after ovarian stimulation with an optimal posttrigger rise in serum beta-human chorionic gonadotropin (bhCG)

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Address for correspondence: Dr. Abdul Majiyd Noushin, Department of Reproductive Medicine, Craft Hospital and Research Centre, Thrissur - 680 664, Kerala, India.
E-mail: drnoshinashraf@gmail.com

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or luteinizing hormone (LH) levels, while FEFS is the failure to retrieve oocytes in the presence of low posttrigger serum bhCG/LH levels.[1] GEFS could be due to dysfunctional folliculogenesis, faulty technique of aspiration, or failure of the detachment of cumulus cell complex from the follicular wall. FEFS could be a consequence of manufacturer defect or faulty administration of the drug[2,3] and low bioavailability of administered trigger agent.[4] EFS is a rare event with an estimated incidence of 0.045%–3.5%. Thirty-three percent of EFS cases were identified as GEFS and 67% as FEFS.[1]

Various treatment strategies available for overcoming EFS are changing the stimulation protocol and the batch of hCG,[5,6] administering gonadotropic-releasing hormone agonist (GnRHa) as a trigger, and prolongation of the time interval between hCG trigger and oocyte retrieval (OCR).[7,8] However, there is little consensus on the efficacy of the treatment mentioned above.[9] Beck-Fruchter *et al*. in a single case report mentioned about the role of sequential administration of GnRHa and hCG trigger at 40 h and 34 h, respectively, before OCR to treat EFS.[3]

We, in our study, extended the *in vivo* oocyte maturation time interval and used two trigger agents sequentially for GEFS. The rationale behind this approach is that follicular rupture and oocyte maturation are time-bound processes, which varies in different women,[10] and addition of GnRHa trigger induces follicle-stimulating hormone (FSH) surge along with LH surge which is more physiological.[3]

The objective of our study is to answer the continuing question of whether extended *in vivo* exposure of the oocytes to trigger agents GnRHa and hCG at 40 h and 36 h, respectively, could correct GEFS, retrieve more mature oocytes, and improve the pregnancy outcome in patients who had a history of GEFS. Control group in our study had either hCG or GnRHa or a combination dual trigger (co-administration of hCG and GnRHa), thus ruling out a particular trigger agent dysfunction issue.

Materials and Methods

This retrospective observational cohort study was conducted in a tertiary fertility center over a period of 6 years from January 2014 to December 2019 after approval from the institutional ethics committee. All patients had consented for anonymized data to be used for educational and research purpose. We analyzed treatment records of 7238 cycles, and patients <40 years of age with a history of GEFS in the previous cycle at our center despite normal response to controlled ovarian stimulation (COS) were included for the study. In their previous stimulation cycle, these patients had received either hCG or GnRHa or dual trigger and posttrigger serum LH or bhCG levels were checked at our center to rule out FEFS. Subsequently, in the next intracytoplasmic sperm injection (ICSI) cycle, they had received double trigger and delayed oocyte retrieval (DTDO) *(n* = 13). Only those patients with <1-year time gap between both the COS cycles were included. Study participants who fulfilled the inclusion and exclusion criteria were selected from medical records [Figure 1].

Controlled ovarian stimulation

Flexible antagonist protocol was used for COS in both the groups. In both the groups, COS was started from day 2 or day 3 of the menstrual cycle after a baseline ultrasound, serum LH, estradiol (E2), and progesterone (P4) assessment. Stimulation was started with recombinant FSH (Gonal-F; Merck Serono). Based on the age of the patient, antral follicle count, anti-Mullerian hormone (AMH), body mass index (BMI), and response to previous COS, starting dose of gonadotropin varied in the range of 150–300 IU. Gonadotropin dosage was further adjusted according to the follicular growth and serum E2 levels. GnRH antagonist (Cetrotide 0.25 mg/day; Merck Serono) was added once the lead follicle reached a size of ≥14 mm or serum E2 was ≥600 pg/ml for the prevention of premature LH surge. The criteria followed for the administration of trigger for final oocyte maturation were when at least two follicles were ≥18 mm. Serum LH, E2, and P4 were measured on the day of trigger. We ensured that the drugs were refrigerated and administered properly at the right time to avoid pharmacological and human errors and were administered in hospital by a trained nurse.

Criteria for the trigger in the historical control group (first intracytoplasmic sperm injection cycle at our center)

- In patients with the trigger-day serum E2 <4000 pg/ml or when <14 follicles of ≥11 mm were seen, single-dose recombinant hCG (Ovitrelle 250 mcg; Merck Serono) was administered subcutaneously 36 h before OCR (normo responders or poor responders) (*n* = 3)[11]
- In patients with the trigger-day serum E2 ≥4000 pg/ml or when ≥14 follicles of >11 mm were seen, GnRH agonist (Decapeptyl 0.4 mg; Ferring) was administered subcutaneously 36 h before OCR (hyperresponder) (*n* = 4)[11]
- For patients with a history of EFS elsewhere undergoing the next cycle of COS at our center,

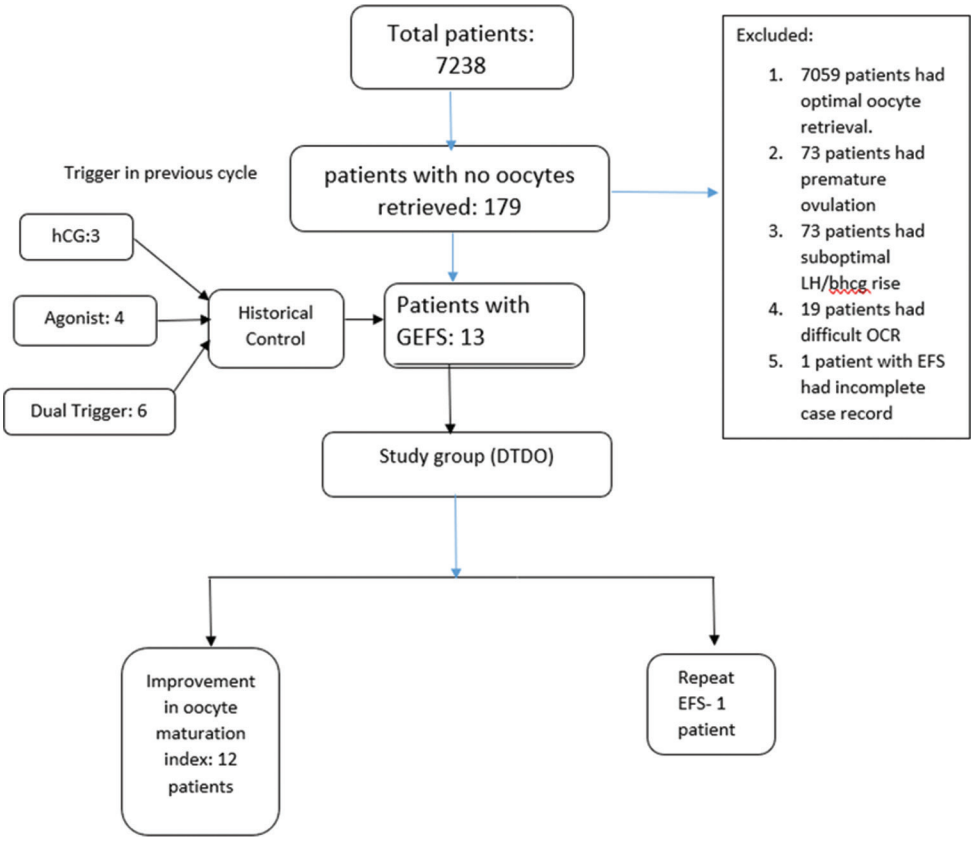

Figure 1: Flow diagram of the study participants

dual trigger (a combination of GnRH agonist 0.4 mg and recombinant hCG 250 mcg) was administered subcutaneously 36 h before OCR (n = 6).

Double trigger with delayed oocyte retrieval in the study group (second intracytoplasmic sperm injection cycle at our center)

All women who had GEFS in their first cycle at our center (historical control group) were administered GnRH agonist (Decapeptyl 0.4 mg; Ferring) and recombinant hCG (Ovitrelle 250 mcg; Merck Serono) subcutaneously at 40 h and 36 h, respectively, as the trigger before OCR.

Posttrigger

To confirm the action of the trigger, posttrigger LH and bhCG values were evaluated at 8 h and 12 h, respectively, according to the type of trigger. Before OCR, transvaginal ultrasound was done to rule out ovulation. If dominant follicle was not visualized, it was assumed that the ovulation had taken place. With all aseptic precautions, OCR was performed as per standard guidelines transvaginally under conscious sedation and ultrasound guidance, using an 18 G-330 mm single-lumen oocyte recovery needle (Wallace, Single Lumen Oocyte Recovery Systems) without flushing the follicle. Denudation of oocytes was done, and the nuclear maturity was assessed 2 h after the OCR. ICSI was performed in all patients so that oocyte maturity can be assessed and was followed up for fertilization check and embryo quality assessment.[12] As per the hospital protocol, elective freeze-all policy for the embryos was followed after counseling the patients with informed consent. Embryos were frozen on day 3 developmental stage. Hormonal evaluation, OCR technique, ICSI technique, embryo culture, and embryo transfer technique were standardized and were the same in both the groups.

Ovarian hyperstimulation syndrome monitoring

Patients were prescribed dopamine agonist – Cabergoline (CB-Lin; Serum Institute Of India Pvt. Ltd.) 0.5 mg/day from the day of trigger for 8 days[13] if the trigger-day E2 ≥4000 pg/ml, or ≥14 follicles of >11 mm size,[11] or if the number of oocytes retrieved was ≥20.[14] These patients were also administered GnRH antagonist 0.25 mg/day (Cetrotide 0.25 mg; Merck Serono) subcutaneously for 3 days for ensuring early luteolysis from the day of OCR.[15] Thromboprophylaxis was also started if needed after risk assessment. Moderate-to-severe ovarian hyperstimulation syndrome (OHSS) was diagnosed and graded as per the classification of OHSS by Golan A *et al*. 1992.[16]

Frozen embryo transfer protocol

Endometrial preparation was done by hormone replacement treatment protocol. Estradiol valerate (Progynova; Bayer Schering Pharma) was started on day 2 or 3 of the cycle at a daily dose of 6 mg orally, and the dose was further adjusted according to the response of the endometrium up to a maximum dose of 12 mg per day. Once endometrial thickness reached more than or equal to 8 mm, vaginal micronized progesterone (Capsule Susten 400 mg; Sun Pharma) twice daily was commenced for luteal phase support as per the hospital protocol along with estradiol valerate.

On day 4 of progesterone, two embryos of day 3 developmental stage were transferred under ultrasound guidance by a senior consultant in reproductive medicine. Luteal phase support was continued till 10 weeks if bhCG was positive. Confirmation of viability and intrauterine location of gestational sac was done by transvaginal ultrasound at 4–5 weeks after the embryo transfer.

The primary outcome measures studied were number of mature oocytes retrieved, oocyte maturation index (OMI) defined as the proportion of MII oocytes per number of oocytes retrieved, number of fertilized oocytes, number of embryos available for embryo transfer, and the incidence of inadvertent premature ovulation and OHSS. The secondary outcome measures were clinical pregnancy rate (CPR) defined as ultrasonographic confirmation of a live intrauterine pregnancy, miscarriage rate (MR) till 20 weeks, and live birth rate (LBR) defined as live birth of an infant after 24 weeks of gestation.[17] Analysis was restricted to only first frozen embryo transfer (FET) cycle.

Statistical analysis

Statistical analysis was performed with IBM, SPSS (version 20). For normally distributed data, mean and standard deviation were used to describe data location and dispersion. Comparison between the groups was evaluated by Fisher's exact test and paired *t*-test. A power calculation was not done because of the rarity of the condition, retrospective nature of the study, and the fact that no comparative studies are published so far on this aspect.

Results

All study participants had primary infertility. The mean age of the study participants was 31 years, their mean AMH was 3.1 ng/ml, and their mean BMI and duration of infertility were 25 kg/m^2 and 5.41 years, respectively. Since comparison was with the previous COS of the same patients and the time gap between both the COS was <1 year, baseline characteristics were matched. The response to stimulation was similar in both the groups [Table 1].

The incidence of GEFS in our study analyzing 7238 cycles analyzed was 0.17%. Eight-hour posttrigger LH levels and 12-h posttrigger bhCG levels were monitored in both the groups confirming the action of the trigger, thus excluding FEFS [Table 1]. Oocytes were successfully retrieved in 92.31% of cases (12 out of 13) with the DTDO protocol. Analyzing results of first FET cycle, a similar positive trend was observed in the OMI, fertilization rate, CPR, and LBR in the DTDO group when compared to the control group [Table 1]. One patient had repeat EFS (7.6%), one patient had poor quality embryos (7.6%), and thus, out of 13 patients, 2 (15.3%) in the study group did not reach the stage of embryo transfer after OCR. Two patients are waiting for first embryo transfer.

None of the patients in the DTDO group had an incidence of inadvertent premature ovulation, moderate-to-severe OHSS, or any untoward complications during the OCR or embryo transfer.

Discussion

To the best of our knowledge, this is the first study evaluating the role of double trigger and delayed OCR (DTDO) for GEFS, analyzing the risk of OHSS and premature ovulation. There was a significant improvement in the number of MII oocytes retrieved and OMI with DTDO. Similar improvement in terms of fertilization rate and cleavage rate ruled out the lagging cytoplasmic immaturity. These patients who had a history of EFS after DTDO protocol pregnancy rate, CPR, and LBR per first FET cycle were very much promising. The incidence of GEFS as per our study analyzing 7238 ICSI cycles was 0.17%.

For patients with a history of GEFS, a case report showed significant improvement in number of oocytes retrieved using dual trigger (combination of GnRHa and hCG trigger) at 36 h.[18] Dual trigger might combat EFS to a certain extent, but our observation from the analysis is that patients who had dual trigger at 36 h in the control group still experienced EFS and the extended *in vivo* maturation protocol could salvage the cycle.

The rationale behind our protocol is that oocyte maturation and follicular rupture is a time-bound process which might vary in different women. Hence, some women may exhibit a delayed expansion of cumulus and detachment of oocyte as compared to others.[17,19] Addition of agonist trigger to conventional hCG trigger causes induction of FSH surge along with LH surge; this

Table 1: Comparison between the groups

Variables	Historical controls (*n*=13)	DTDO group (*n*=13)	*P*
AFC	17.92±10.30	19.62±11.28	0.19
Weight (kg)	63.670±8.70	63.40±8.07	0.343
D2 LH (mIU/mL)	2.52±1.65	2.70±1.69	0.607
D2 E2 (pg/ml)	45.24±21.17	46.30±20.07	0.700
Total dose of gonadotropin (IU)	1854.37±378.52	1961±401.768	0.491
Days of stimulation	8.75±1.23	9.07±1.27	0.520
Trigger-day LH (mIU/mL)	1.78±1.31	2.03±0.94	0.607
Trigger-day E2 (pg/ml)	1580.92±786.62	2123.47±1430.37	0.155
Number of follicles≥14 mm on the day of trigger	9.45±2.25	10.36±4.45	0.346
8-h posttrigger serum LH	96.30±28.47	101.23±34.23	0.639
12-h posttrigger serum bhCG	111.42±18.57	115.69±33.41	0.536
Number of MII oocytes retrieved	0	11.44±4.95	0.001
OMI, *n* (%)	0	147/177 (83.05)	0.001
Number of fertilized oocytes	0	9.90±2.80	0.001
Number of embryos available for transfer	0	9.30±2.58	0.001
Positive bhCG per first FET cycle, *n* (%)	0	5/9 (55.55)	0.001
CPR per first FET cycle, *n* (%)	0	4/9 (44.44)	0.004
LBR per first FET cycle, *n* (%)	0	4/9 (44.44)	0.004
BP/MA per first FET cycle, *n* (%)	0	1/9 (11.11)	0.409

DTDO=Double trigger with delayed oocyte retrieval, AFC=Antral follicle count, LH=Luteinizing hormone, bhCG=Beta-human chorionic gonadotropin, OMI=Oocyte maturation index, FET=First frozen embryo transfer, CPR=Clinical pregnancy rate, LBR=Live birth rate, E2=Estradiol, BP/MA=Biochemical pregnancy/miscarriage rate, MII= Mature (metaphase II) oocytes

mimics a natural cycle and is more physiological when compared to only LH surge as seen in hCG trigger. FSH surge helps in nuclear maturation, resumption of meiosis, and cumulus expansion and also induces LH receptor formation on granulosa cells in the late follicular phase.[20,21] GnRH receptors were found on a wide variety of human tissues including granulosa cells. Overall, the interplay between FSH, LH, and GnRH (through receptors on granulosa cells) leads to retrieval of more mature oocytes.[22] These hypothetical benefits are confirmed on the mRNA expressions in mural granulosa cells. Similar case reports by Beck-Fruchter *et al*.[3] and Song and Sun[23] showed positive benefits of double trigger. In our study before OCR, the presence of dominant follicle was confirmed by transvaginal ultrasound to rule out premature ovulation. As per our analysis, none had premature ovulation, considering that it is potentially possible with the extended *in vivo* maturation protocol. Safety of DTDO was also reassuring with no incidence of moderate or severe OHSS, but elective freeze-all policy followed by us could partly be the reason for this. This study re-emphasizes that extending time interval is safe.

Inclusion of only GEFS cases for the study, by checking whether post trigger LH or hCG levels are adequate after triggering, monitoring the incidence of premature ovulation, and OHSS have strengthened the utilization of this protocol for GEFS. The study is limited by its small sample size, retrospective nature, and study participants being their historical controls. However, because GEFS is a rare entity and considering the noninvasive nature and safety profile of this protocol, the plausible benefits of such simple adjustments can outweigh the deleterious implications of EFS in couples. In the future, initiating a national registry for patients with EFS will help us to identify the etiology and risk factors of this infrequent condition, and a large prospective multicentric randomized controlled trial is warranted to generate further robust data.

Conclusion

Our findings implicate that double trigger and delayed OCR (DTDO) is a safe and efficacious treatment strategy for GEFS. The plausible benefits of such simple adjustments can outweigh the deleterious implications of EFS in couples.

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Conflicts of interest

There are no conflicts of interest.

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