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

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Objective: To investigate whether dual triggering of final oocyte maturation with a combination of gonadotropin-releasing hormone (GnRH) agonist and human chorionic gonadotropin (hCG) can improve the live-birth rate for normal responders in GnRH-antagonist in vitro fertilization/intracytoplasmic sperm injection (IVF-ICSI) cycles.
Design: Retrospective cohort study.
Setting: Infertility unit of a university-affiliated medical center.
Patient(s): Normal responders to controlled ovarian hyperstimulation who were undergoing IVF-ICSI with a GnRH antagonist protocol.
Intervention(s): Standard dosage of hCG trigger (6,500 IU of recombinant hCG) versus dual trigger (0.2 mg of triptorelin and 6,500 IU of recombinant hCG).
Main Outcome Measure(s): Live-birth, clinical pregnancy, and implantation rates per cycle.
Result(s): A total of 376 patients with 378 completed cycles with embryo transfer were enrolled (hCG trigger/control group: n = 187; dual trigger/study group: n = 191). The dual trigger group demonstrated statistically significantly higher implantation (29.6% vs. 18.4%), clinical pregnancy (50.7% vs. 40.1%), and live-birth (41.3% vs. 30.4%) rates as compared with the hCG trigger group. There was no statistically significant difference in terms of patient demographics, cycle parameters, or embryo quality.
Conclusion(s): Dual trigger of final oocyte maturation with a GnRH-agonist and a standard dosage of hCG in normal responders statistically significantly improves implantation, clinical pregnancy, and live-birth rates in GnRH-antagonist IVF cycles. (Fertil Steril® 2013;100: 1296–302. ©2013 by American Society for Reproductive Medicine.)
Key Words: Dual trigger, GnRH agonist, GnRH antagonist, normal responders

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Substituting human chorionic gonadotropin (hCG) with gonadotropin-releasing hormone (GnRH) agonist for triggering the final oocyte maturation was first introduced by Gonen et al. (1) more

than 20 years ago. Nevertheless, the concept did not generate much interest until the clinical introduction of a GnRH-antagonist protocol for in vitro fertilization (IVF). The initial goal of GnRH-agonist triggering was to eliminate the risk of ovarian hyperstimulation syndrome (OHSS) in GnRH-antagonist IVF cycles. Remarkably, there has been zero incidence of OHSS reported in series of randomized controlled trials (RCTs) involving high and/or normal responders triggered by GnRH-agonist in fresh IVF cycles with embryo transfer (2–9). From these results, triggering with GnRH-agonist is currently viewed as the most effective method of eliminating OHSS from clinical practice (10–13).

Nevertheless, when compared with the conventional hCG triggered cycles, significantly reduced implantation rate and higher abortion rate were observed for GnRH-agonist triggered cycles (3, 4). The inferior pregnancy outcomes were attributed to defective luteal phase function and decreased endometrial receptivity induced by the GnRH-agonist trigger (14). In a recent Cochrane review, the routine use of a GnRH agonist as the lone trigger for final oocyte maturation in fresh IVF autologous cycles was discouraged because of the significant decrease in live-birth rates (OR 0.44; 95% CI, 0.29–0.68; 4 RCTs) and ongoing pregnancy rates (OR 0.45; 95% CI, 0.31–0.65; 8 RCTs) compared with a conventional hCG trigger (15).

Therefore, modifications in luteal phase support have been suggested in IVF cycles triggered with a GnRH agonist. One method is intensive luteal progesterone and an estrogen supplement, as proposed by Engmann et al. (9). By maintaining the serum estradiol level above 200 pg/mL and progesterone level above 20 ng/mL, investigators have achieved a remarkable ongoing pregnancy rate of 53%. Another method that adds a reduced dosage of hCG either at oocyte retrieval (5, 6) or intermittently during luteal phase (16) has also showed promising results.

Recently, the concept of a "dual trigger" that combines a single bolus of GnRH-agonist with a reduced dosage of hCG at the time of triggering has been investigated for IVF high responders. Although the risk of OHSS was effectively minimized by triggering with a GnRH agonist, proper luteal function was also salvaged by the added hCG. Several studies focusing on high responders have demonstrated significant improvements in both ongoing pregnancy rates (17, 18) and live-birth rates (19) when a dual trigger was used instead of a GnRH-agonist trigger, all without conferring a significant increase in the OHSS rate.

For normal responder population who are not at high risk for OHSS, the role of adding a single-dose GnRH agonist to the standard dosage of hCG for triggering oocyte maturation also has been investigated as a method to further optimize pregnancy outcomes in cycles treated with a GnRH-antagonist protocol. In the first ever prospective randomized study on dual triggering in normal responders, Schachter et al. (20) demonstrated significantly improved ongoing pregnancy rates for the study group compared with the control group who received hCG triggering alone. However, Schachter did not show the live-birth rate data from that study.

In light of the promising results from the preliminary studies, further investigation regarding the efficacy of dual triggering for normal responders is warranted. We investigated whether dual triggering for final oocyte maturation by combining a single dose of GnRH-agonist with a standard dosage of hCG could improve live-birth rates for normal responders in GnRH-antagonist IVF-ICSI cycles.

MATERIALS AND METHODS

Study Design

A review of medical records from October 1, 2009, through July 31, 2011, was performed for all IVF-ICSI cycles with a GnRH-antagonist protocol at the Infertility Division of Mackay Memorial Hospital in Taipei City, Taiwan. The study protocol was approved by the institutional review board of Mackay Memorial Hospital.

Study Participants

Patients with either high or poor response to controlled ovarian hyperstimulation (COH) were excluded. Poor ovarian response was defined as a serum estradiol (E_2) level less than 500 pg/mL on the day of triggering or as the number of retrieved oocytes ≤ 3. High ovarian response was defined as an E_2 level greater than 4,000 pg/mL on the day of triggering or as the number of retrieved oocytes ≥ 20. Other exclusion criteria were advanced reproductive age (≥ 40 years), severe underweight or overweight status (body mass index <18 or >30 kg/m^2), occult ovarian failure (day-3 follicle-stimulating hormone [FSH] concentration of ≥ 10 IU/L or serum antimüllerian hormone [AMH] level of ≤ 1.0 ng/mL), presence of endocrine disorders (diabetes mellitus, hyperprolactinemia, thyroid dysfunction, congenital adrenal hyperplasia, Cushing syndrome, or polycystic ovary syndrome), or uterine anomaly confirmed by either hysterosalpingography or hysteroscopy. A total of 378 completed cycles with embryo transfer (ET) were included for final analysis (hCG trigger/control group: n = 187; dual trigger/study group: n = 191). Both groups contained two patients who had undergone two IVF cycles.

Ovarian Stimulation Protocols

All patients began ovarian stimulation with a flexible starting dosage of recombinant FSH (Gonal-F; Merck Serono S.p.A.) ranging from 150 to 225 IU on the third day of the menstrual cycle for 3 consecutive days. The starting dosage was determined by patient age, ovarian reserve, body mass index, and previous response to COH. The recombinant FSH dosage was then adjusted according to follicular growth, monitored by serial transvaginal ultrasound. After at least one follicle had reached 14 mm in diameter, patients also began subcutaneous injection of cetrorelix (Cetrotide; Merck Serono, Baxter Oncology GmbH) at a dosage of 0.25 mg per day along with the recombinant FSH. When at least two leading follicles had reached 18 mm in diameter, final oocyte maturation was triggered by either 250 μg of recombinant hCG (Ovidrel; Merck Serono S.p.A.) alone, which was equivalent to approximately 6,500 IU hCG according to the manufacturer's data, or by 250 μg of recombinant hCG plus 0.2 mg of triptorelin (Decapeptyl; Ferring GmbH).

The treatment arm assignment was based on the venue of administration of the trigger medicine. On the day of hCG trigger, the patients were given the option to choose the venue of administration of the trigger medicine. For patients who chose to self-administer the trigger medicine at home, 250 μg recombinant hCG alone was provided to simplify the injection procedure. For those who chose administration of the medicine at a hospital by a nurse, 250 μg recombinant hCG plus 0.2 mg of triptorelin were provided.

All oocyte retrievals were performed under transvaginal ultrasound guidance 35 to 36 hours after triggering. All embryo transfers were performed 72 hours after oocyte retrieval. The remaining viable embryos were cultured to the blastocyst stage and were cryopreserved by vitrification.

Luteal Phase Support and Confirmation of Pregnancy

The luteal phase support included daily intramuscular injection of 50 mg of progesterone along with vaginal supplementation of 300 mg micronized progesterone (Utrogestan; Besins International Belgique S.A., Belgium) starting on the day of oocyte retrieval. Serum β-hCG was measured 14 days after oocyte retrieval, and a value above 5 IU/mL was considered to be a positive pregnancy. The luteal support was then continued until the 10th week of gestation after the establishment of luteal-placental shift for all positive pregnancies.

Outcome Variables

The study's main outcome variable was the live-birth rate per cycle. Other analyzed variables included the clinical pregnancy rate, the implantation rate, the OHSS incidence, and the blastocyst progression rate. Live birth was defined as delivery of a viable fetus of ≥23 weeks' gestation. Clinical pregnancy was defined as a pregnancy confirmed by ultrasound visualization of the gestational sac between the 5th to 6th weeks of gestation. The implantation rate was calculated by dividing the total number of fetal cardiac activity detected by the total number of transferred embryos. The rate of blastocyst progression was calculated by dividing the total number of vitrified blastocysts by the total number of remaining embryos after ET.

Statistical Analysis

Statistical analysis was performed using MedCalc 10.2 (MedCalc Software). Continuous variables were presented as mean with standard deviation (SD). For categorical variables, the values were presented as raw frequencies with corresponding percentages, and the between-group differences were assessed either by a chi-square test with Yates correction if required, or by the Fisher exact test. $P<.05$ was considered statistically significant.

RESULTS

The baseline characteristics and demographics did not statistically significantly differ between the control and study groups (Table 1). The ovarian stimulation response and IVF-ICSI cycle outcome for each group are presented in Tables 2 and 3, respectively. We found no statistically significant differences in the total recombinant FSH dosage, duration of stimulation, duration of GnRH-antagonist treatment, endometrial thickness, or serum E_2 and P levels on

TABLE 1

Comparison of the standard and dual-trigger methods: baseline characteristics of patients.

	Control group (hCG)	Study group (hCG + triptorelin)	*P* value
Total no. of cycles	187	191	
Proportion of ICSI cycles (%)	49.7	53.4	NS
Age (y)	34.68 ± 3.44	34.81 ± 3.70	NS
Body mass index (kg/m^2)	22.0 ± 3.1	22.2 ± 5.4	NS
Day-3 FSH level (IU/L)	6.23 ± 2.51	6.68 ± 2.21	NS
AMH level (ng/mL)	2.78 ± 2.01	3.26 ± 4.04	NS
Infertility causes (%)			
Male factor	27.9	30.5	NS
Tubal factor	31.5	26.1	NS
Ovulation dysfunction	16.1	19.4	NS
Endometriosis	15.4	18.6	NS
Unexplained	8.1	5.4	NS

Note: Values are expressed as mean ± standard deviation or percentage. AMH = antimüllerian hormone; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; ICSI = intracytoplasmic sperm injection; NS = not statistically significant.

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TABLE 2

Comparison of the standard and dual-trigger methods: characteristics of ovarian stimulation.

Variable	Control group (hCG)	Study group (hCG + triptorelin)	*P* value
Total dose of gonadotropins (IU)	4,062.0 ± 1,555.7	3,850.6 ± 1,472.1	NS
Duration of stimulation (d)	10.3 ± 1.7	9.6 ± 1.7	NS
E_2 on trigger day (pg/mL)	2,138.9 ± 1,136.4	2,083.0 ± 1,371.4	NS
P on trigger day (ng/mL)	1.84 ± 0.95	2.09 ± 0.87	NS
Duration of GnRH-ant treatment (d)	3.5 ± 1.0	3.4 ± 1.1	NS
Endometrial thickness on trigger day (mm)	9.8 ± 1.4	10.1 ± 1.2	NS
No. of oocytes retrieved	10.10 ± 4.58	12.36 ± 6.64	<.01
No. of MII oocytes retrieved	8.03 ± 4.51	10.53 ± 6.47	<.01
No. of embryos obtained	5.3 ± 3.6	5.8 ± 3.8	NS
No. of top quality embryos obtained	2.9 ± 2.3	2.9 ± 3.1	NS
No. of embryos transferred	2.84 ± 0.85	2.79 ± 0.87	NS
No. of embryos cryopreserved	1.60 ± 0.49	1.97 ± 0.12	<.01

Note: Values are expressed as mean ± standard deviation. GnRH-ant = GnRH antagonist; hCG = human chorionic gonadotropin; MII = metaphase II.

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TABLE 3

Comparison of the standard and dual-trigger methods: outcomes of in vitro fertilization/intracytoplasmic sperm injection cycles.

Variable	Control group (hCG)	Study group (hCG + triptorelin)	*P* value
Implantation rate (%)	18.43 (106/575)	29.68 (160/539)	<.001
Clinical pregnancy rate per ET (%)	40.11 (75/187)	50.79 (97/191)	.047
Abortion rate (%)	18.67 (14/75)	16.49 (16/97)	NS
Live-birth rate per ET (%)	30.49 (57/187)	41.36 (79/191)	.042
Blastocyst progression rate (%)	55.6 (299/538)	52.9 (379/720)	NS
OHSS rate (%)	0.005 (1/187)	0 (0/191)	NS

Note: Values are expressed as percentage. ET = embryo transfer; hCG = human chorionic gonadotropin; OHSS = ovarian hyperstimulation syndrome.

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the day of trigger. The mean number of oocytes retrieved and mature metaphase II (MII) oocytes were both statistically significantly greater in the dual-trigger group. The mean number of embryos obtained and top quality embryos were similar between the two groups, as was the rate of blastocyst progression.

In terms of IVF-ICSI outcome, the dual-trigger group demonstrated a statistically significantly higher implantation rate (29.6% vs. 18.4%; $P<.001$), clinical pregnancy rate (50.7% vs. 40.1%; $P=.042$), and live-birth rate (41.3% vs. 30.4%; $P=.047$) in comparison with the hCG trigger group. The difference in abortion rate between the two groups was not statistically significant. No clinical pregnancy was achieved among the four patients who had had more than one IVF cycle who were included for final analysis. There was one case of moderate OHSS in the control group, but it did not require hospitalization; none occurred in the dual-trigger group.

DISCUSSION

The results from our study indicate that dual-triggered oocyte maturation with a GnRH-agonist and a standard dosage of hCG can be an effective strategy to optimize pregnancy outcome for normal responders in GnRH-antagonist cycles. Compared with the control group who received the conventional trigger of hCG alone, the dual-trigger group showed statistically significantly improved rates of implantation, clinical pregnancy, and live birth. With all the embryo quality parameters (proportions of top quality embryos and blastocyst progression) equivalent between the control and dual-trigger groups, the difference in cycle outcome was most likely related to the disparity in endometrial receptivity that resulted from the different triggering agent used.

With the identification of GnRH receptors in the extrapituitary sites such as the endometrium (21), myometrium (22), fallopian tube (23), ovaries (24, 25), placenta (26, 27), and preimplanting embryo (28), GnRH has been suggested to play multiple roles in the regulation of endometrial receptivity and embryo implantation (23, 28, 29). Not surprisingly, concerns have been raised regarding the impact of GnRH-antagonist exposure during the preimplantation stage (30). Gonadotropin-releasing hormone has been shown to modulate matrix metalloproteinases (MMPs) in the placental trophoblasts that mediate trophoblast cell invasion and extracellular matrix degradation (31, 32). Rackow et al. (33) demonstrated that the expression of endometrial HOXA10, a modulator of endometrial receptivity, was significantly decreased in endometrial stromal cells of GnRH-antagonist IVF cycles when compared with either natural or GnRH-agonist IVF cycles. A review by Devroey et al. (34) proposed that the reduced implantation rate in GnRH-antagonist cycles could be attributed to out-of-phase endometrium induced by a GnRH antagonist. Bukulmez et al. (35) found that even though GnRH-antagonist protocol patients had a lower overall clinical pregnancy rate compared with the GnRH-agonist protocol patients, the rates of blastocyst progression and good quality embryo were equivalent between the two groups. Further studies comparing the outcomes of frozen-thawed embryo transfer cycles noted statistically nonsignificant differences in implantation and ongoing pregnancy rates between embryos derived from either a GnRH-agonist or GnRH-antagonist protocol (36, 37). From these observations, it was deduced that the decrease in endometrial receptivity rather than poor embryo quality was the culprit for the inferior outcomes in GnRH-antagonist cycles.

In contrast, when Haouzi et al. (38) analyzed the difference in global gene expression profile during the endometrial receptive stages between natural cycles and stimulated cycles treated with either GnRH agonist or GnRH antagonist and found that the up-regulated genes under the GnRH-antagonist protocol were more similar to the genes up-regulated during the receptive stage of the natural cycle (38). In studies on endometrial receptivity for oocyte recipients, comparable implantation and pregnancy rates were noted among recipients who received either a GnRH agonist, a GnRH antagonist, or no GnRH analogue during endometrial preparation (39, 40). These results indicated that GnRH-antagonist exposure during the proliferative phase does not seem to confer a negative effect on implantation and pregnancy rates. Finally, the latest Cochrane review summing up the results of 45 randomized controlled trials did not find a statistically significant difference in terms of live-birth rate (OR 0.86; 95% CI, 0.69–1.08; 9 RCTs) or ongoing pregnancy rate (OR 0.87; 95% CI, 0.77–1.00; 28 RCTs) between the GnRH-agonist long protocol and GnRH-antagonist protocol recipients (41).

Overall, studies on the relationship between GnRH-antagonist exposure and endometrial receptivity have yielded conflicting results, and continuous efforts have been invested in optimizing the outcomes of GnRH-antagonist cycles. One method described by Schacter et al. (20) is a dual trigger with a GnRH agonist plus hCG for oocyte maturation. These investigators' hypothesis was that the binding of the GnRH antagonist to endometrial GnRH receptors could interfere with the postreceptor events that are critical for implantation (20). In that study, the group triggered with 0.2 mg of

triptorelin in combination with a standard dosage of hCG (5,000 IU) demonstrated statistically significantly improved implantation and ongoing pregnancy rates compared with the group triggered with the standard dosage of hCG alone. They theorized that the preovulatory administration of an GnRH agonist could displace the GnRH antagonist from endometrial GnRH receptors as well as from receptors in other gonadotropin-producing cells, thus enabling proper postreceptor actions for implantation.

In a recent meta-analysis by Oliveira et al. (42), the administration of a single-dose GnRH-agonist in the luteal phase significantly increased implantation rates in cycles treated with either a GnRH-agonist or GnRH-antagonist protocol, while significant improvement in clinical pregnancy and ongoing pregnancy rates were only noted in cycles treated with a GnRH antagonist protocol. These findings along with the results from our present study further confirm the beneficial effects of a GnRH agonist on embryo implantation and pregnancy outcomes in GnRH-antagonist cycles.

Another advantage of triggering with a GnRH agonist for oocyte maturation is the simultaneous induction of a midcycle FSH surge that is similar to the hormone surge in a natural cycle. Animal studies have confirmed the role of FSH in promoting the formation of luteinizing hormone (LH) receptor sites in rat granulosa cells (43, 44). The increase in LH receptors is crucial for preparing the maturing follicle for an LH surge that triggers the events of ovulation and subsequent luteinization of the granulosa cells. Furthermore, FSH has been shown to promote the resumption of oocyte meiosis (45, 46) and cumulus expansion (47, 48) in animal models.

The importance of the presence of FSH during human oocyte maturation process is also supported by studies in which the groups triggered with a GnRH agonist consistently resulted in retrieval of a significantly greater number of mature oocytes (4, 8, 49, 50). Our own study concurs with those observations. Lamb et al. (51) also investigated whether directly adding a single bolus of FSH to the hCG trigger could have similar positive effects. The group that had received a concomitant FSH bolus with hCG demonstrated significantly improved oocyte competence in terms of higher oocyte recovery and fertilization rates (51).

Another important point that needs to be emphasized is the inherent risk of OHSS associated with hCG triggering. Even though the women in our study did not fit the profile of a high-risk population for incurring OHSS, one case of moderate OHSS still occurred in the control group who received the standard dosage of hCG for a trigger. Previous literature has indicated that severe OHSS can occur even in normal responders (52), and some investigators have suggested that a GnRH-antagonist protocol in combination with a GnRH-agonist trigger should be used for all normal and high-responder patients undergoing their first IVF cycle (12). Therefore, clinicians must be aware of the danger of OHSS developing in normal responders after they have been triggered with a standard hCG dose, regardless of whether a GnRH agonist is coadministered. More prospective studies focusing on the minimal effective dose of hCG used as part of a dual-triggering regimen should provide further optimization for this promising protocol.

The limitation of our study was is its relatively small sample size. To achieve a desired power of 80% and an alpha value of 0.05, it would have required 386 subjects in each group (assuming 1:1 allocation) to be adequately powered to detect the 10% difference in live-birth rate that we observed in our present study. Though it was underpowered, the positive trend we observed of an improved live-birth rate in the dual-trigger group should nevertheless provide a topic of interest for future studies.

Dual trigger of final oocyte maturation with a GnRH agonist and the standard dosage of hCG in normal responders significantly improves implantation, clinical pregnancy, and live-birth rates in GnRH-antagonist IVF-ICSI cycles. The results we present here are another proof-of-concept that suggests a possible paradigm shift in ovulation-triggering agents in GnRH-antagonist cycles. To the best of our knowledge, our is the first study dedicated to a normal responder population to document the significantly improved live-birth rate in pregnancies achieved by dual triggering. Further prospective, randomized controlled studies are required to confirm the beneficial effects of dual triggering.

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