Original Article

# Dual trigger increases the number of top quality embryos in normal responders

Priyanka Kalra[1], M Gouri Devi[2], Meeta Sharma[3]

[1]Fellow in Clinical ART, Ridge IVF, Gouri Hospital, Delhi, India
[2]Director, Ridge IVF, Gouri Hospital, Delhi, India
[3]Clinical Director, Ridge IVF, Gouri Hospital, Delhi, India

## Abstract

**Context:** Human chorionic gonadotropin (hCG) has been used for years for oocyte maturation in IVF cycles, however, with the risk of a few complications like ovarian hyperstimulation. The introduction of gonadotropin-releasing hormone (GnRH) agonist along with hCG as a dual trigger has helped to reduce the incidence of such complications. **Aims:** The study aims to compare the outcomes of IVF cycles in hCG versus hCG plus GnRH (dual trigger). **Settings and Design:** Retrospective center-based study. **Materials and Methods:** Retrospective center-based study to compare the effect of hCG and dual trigger on the cycle outcomes in normal responders. **Statistics:** Data analysis was done using ClinCalc online calculator through chi-square test, two-independent $t$ test. $P < 0.05$ was considered to indicate statistical significance. **Results:** The number of oocytes and clinical pregnancy rates was higher in the dual trigger group. A higher number of top quality embryos were formed in the dual trigger group with statistical significance. **Conclusions:** Dual trigger can help to improve the outcomes of IVF in normal responders too.

**Keywords:** Dual trigger, embryo, gonadotropin-releasing hormone, human chorionic gonadotropin

**Address for correspondence:** Dr Priyanka Kalra, 2GF1, ATS Dolce, Zeta 1, Greater Noida - 201310, UP, India. E-mail: priya18won@gmail.com
**Submission:** 20–01–2022, **Revised:** 17–02–2022, **Accepted:** 03–05–2022, **Published:** 29–June–2022

## INTRODUCTION

Human chorionic gonadotropin (hCG) is used conventionally for triggering fertilization cycles after controlled ovarian stimulation.[1] Although the use of an hCG trigger in IVF is associated with a higher Live birth rate (LBR) after fresh embryo transfer (ET), it is associated with a higher rate of ovarian hyperstimulation syndrome (OHSS).[2] The use of a gonadotropin-releasing hormone (GnRH) agonist as an alternative to the age-old hCG trigger has been a strategy to reduce the risk of OHSS. Triggering with GnRHa has minimized the risk of OHSS, but the pregnancy rate is adversely affected due to the impaired luteal function.[3] In order to rescue the luteal phase, a number of strategies such as aggressive post-retrieval progesterone and estrogen supplementation and adding a reduced dose of hCG either at oocyte retrieval or intermittently during the luteal phase have been devised.[4] Then the concept of "dual trigger," which is GnRHa plus a reduced dose of hCG, emerged as another means to compensate for the deficient luteal phase after GnRHa triggering.

Several studies have demonstrated significant improvements in ongoing pregnancy rates and live birth rates when a dual trigger was used instead of a lone trigger of a GnRHa, in high responders all without

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.fertilityscienceresearch.org |
| | DOI: 10.4103/fsr.fsr_1_22 |



**How to cite this article:** Kalra P, Gouri Devi M, Sharma M. Dual trigger increases the number of top quality embryos in normal responders. Fertil Sci Res 2022;9:46-50.

conferring a remarkable increase in the incidence of OHSS.[5] However, there is insufficient evidence regarding the impact of a dual trigger on the reproductive outcome in normal responders.

However, there is not much evidence available regarding the impact of a dual trigger on the reproductive outcome in normal responders. Some studies have shown a markedly improved ongoing pregnancy rate and live birth rate in fresh embryo transfer (FET) cycles for a dual trigger group compared with the hCG-trigger group in normal responders.[6] On the other hand, several studies have demonstrated that the dual trigger of oocyte maturation was not associated with a change in the live birth rate in FET cycles for normal ovarian responders.

The present study was conducted with an aim to investigate whether differences were present in the number of oocytes collected, the number of top quality embryos, and the live birth rate in normal responders triggered by GnRHa with hCG (a dual trigger) and hCG alone.

## MATERIALS AND METHODS

This was a retrospective study conducted at an IVF clinic in New Delhi and the study period extended from January 2017 to December 2021. Out of all the patients who underwent intracytoplasmic sperm injection cycles with an antagonist protocol during the study period, a sample size of 120 was selected in each of the two groups from the hospital database. Group A, was the hCG alone trigger while group B was the dual trigger, hCG plus GnRH a group.

Cases of female factor infertility (tubal factor, pelvic adhesions, or mild endometriosis), mild male factor infertility, and unexplained infertility were included in the study. A normal response was defined as the aspiration of 6 to 14 oocytes and a peak oestradiol level lower than 2500 pg/mL.

The **exclusion criteria** were: third day FSH >10, anti-mullerian hormone <1.1, high risk of ovarian hyperstimulation, endocrine disorders such as diabetes mellitus, hyperprolactinemia, thyroid dysfunction, polycystic ovarian syndrome, an anomaly confirmed by Hysterosalpingography (HSG) or hysteroscopy in the uteri, severe male factor (azoospermia needed Testicular sperm extraction (TESE) or Percutneous Epididymal Sperm Extraction (PESA)), previous history or existing signs of ovarian hyperstimulation syndrome, a body mass index over 30 kg/m$^2$, and poor or hyper-response to ovarian stimulation.

## OVARIAN STIMULATION PROTOCOLS

Transvaginal ultrasound was done on the third day of menstruation for all participants. Then ovarian stimulation began with a fixed dosage of 225 IU daily recombinant FSH (Gonal–F; Merk Sereno SPA) for 5 days. On the sixth day of ovarian stimulation, FSH dosage was adjusted if needed according to the ovarian response by monitoring transvaginal ultrasound for follicular development. On the sixth day of stimulation, subcutaneous injection of Cetrorelix (Cetrotide; Merk sereno, Brazil) began at 0.25 mg per day along with recombinant FSH. When at least two follicles had reached 17 mm, 0.5 mg of Lupride (Leuprolide) plus 5000 IU hCG (Choragon, Ferring) was administered to the A group and 10,000 IU hCG to the B group. Oocytes were retrieved after 34 to 36 hours of triggering under transvaginal ultrasound guidance. The retrieved oocytes were incubated in the cultured medium. Then oocytes were classified according to their maturation level. Oocytes with the first polar body at the metaphase II stage were considered to have matured and were used for the intracytoplasmic sperm injection procedure. Three parameters considered for embryos on the second (41–44 hours after insemination) and the third days (66–71 hours after insemination) were (1) fragmentation (2) blastomeres number (3) multinucleated blastomere number. The morphology score of the embryos was as follows: four for regular blastomeres, no fragments, and no multinucleated blastomeres; three for regular blastomeres, <20% fragments, and no multinucleated blastomeres; two for unequal sized blastomeres or >50% fragments; and one for >80% fragmentation or no visible blastomeres. Top quality embryos were determined by 3 or 4 scores and contained at least four cells on the second day. Best quality embryos were selected for transfer on the third day of oocyte retrieval. The additional embryos were cryopreserved by vitrification. Luteal phase support by 50 mg intramuscular aqueous progesterone daily with 10 mg Dydrogesterone twice a day started on the day of oocyte retrieval.

A positive pregnancy test was based on serum β-hCG levels measured 2 weeks after ET on two occasions 48 hours apart. The clinical pregnancy rate was defined as a pregnancy diagnosed via the ultrasonographic appearance of one or more gestational sacs. The implantation rate was calculated by dividing the total sac number in ultrasound

by the total number of transferred embryos. We recorded the number of matured, fertilized, and retrieved oocytes and top quality and cryopreserved embryos for each patient. The primary outcomes were the live birth rate, the number of good quality embryos, and the number of mature oocytes.

## STATISTICAL ANALYSIS

The sample size was calculated by keeping the power of the study 0.8 and increasing the live birth rate by 20%. Data analysis was done using ClinCalc online calculator through a chi-square test and two-independent *t* test. Multiple logistics regression was used to adjust the potential confounders. $P < 0.05$ was considered to indicate statistical significance.

## RESULTS AND DISCUSSION

Both the groups were comparable in baseline and demographic parameters, including age, body mass index, cause of infertility, and baseline hormone levels [Table 1]. All patients in both the groups were normal responders from whom between 6 and 14 oocytes were collected. Table 2 shows the outcomes.

There was no statistically significant difference seen in both groups in terms of the duration of ovarian stimulation, peak estradiol levels achieved, number of oocytes collected, number of retrieved MII oocytes, and fertilization rates. The total number of good quality embryos obtained in the dual trigger group was higher than in the hCG group. The clinical pregnancy rates were higher in group B. No cases of OHSS were found in any of the groups.

This retrospective center-based study was conducted to assess the impact of the trigger of final oocyte maturation in antagonist co-treated Assisted reproductive technologies (ART) cycles in normal responders. Two trigger methods were compared: an hCG trigger and a dual trigger involving GnRHa and hCG.

hCG has been used conventionally in routine ART cycles for final oocyte maturation, for ages. Usually, a bolus of 5000 to 10,000 IU of hCG is administered to promote final oocyte maturation and ovulation before the ovum picks up. hCG primarily binds with LH receptors to facilitate the maturation of oocytes.

The hCG owing to its luteotrophic effect supports the luteal phase but then at the same time also increases the risk of the complication of OHSS.[1] However, during a natural cycle, in order to trigger ovulation, both LH and FSH will surge; hCG doesn't have FSH receptor activity. hCG induces LH receptor formation on granulosa cells to promote oocyte maturation and cumulus expansion and its trigger does not totally act like a natural oocyte maturation and ovulation.[1] On the contrary, GnRHa trigger results in both LH and FSH surges.[7] Hence, the result is more similar to natural ovulation. GnRH receptors exist in several sites such as the

**Table 1: Baseline characteristics of the study groups**

| S. No. | Variables | hCG group AMean (SD) | Dual trigger group BMean (SD) | *P* |
|---|---|---|---|---|
| 1 | Age (yrs) | 31.4(4.2) | 32.1(4.5) | 0.188 |
| 2 | BMI (kg/m$^2$) | 24.8(3.3) | 25.1(3.2) | 0.166 |
| 3 | Basal E2 Estradiol (pg/mL) | 44.3(2.8) | 47.6(3) | 0.091 |
| 4 | Baseline FSH (IU/mL) | 7.5(2.2) | 8.1(2.8) | 0.571 |
| 5 | Baseline LH (IU/mL) | 4.1(1.8) | 3.7(2) | 0.442 |
| 6 | Duration of infertility (yrs) | 6.4(2.1) | 5.8(2.2) | 0.55 |
| 7 | Female factor infertility | 16% | 23% | |
| | | | 21% | |
| 8 | Male factor | 20% | | |
| 9 | Unexplained infertility | 64% 56% | | |

**Table 2: Comparison of cycle characteristics and outcome parameters between the study groups**

| S. No. | Variable | hCG group A*n* ± SD | Dual trigger group Bn ± SD | *P* |
|---|---|---|---|---|
| 1 | Duration of stimulation(days) | 9.3 ± 1.3 | 9.5 ± 2.2 | 0.913 |
| 2 | Peak estradiol levels (pg/mL) | 1877 ± 932 | 1,916 ± 1,049 | 0.098 |
| 3 | Number of MII oocytes | 5.6 ± 3.7 | 7.2 ± 4.7 | 0.095 |
| 4 | Number of topquality embryos | 3.2 ± 2.1 | 4.4 ± 2.1 | **0.014** |
| 5 | Fertilization rate (%) | 64 ± 22 | 70 ± 33 | 0.55 |
| 6 | Clinical pregnancy rate (%) | 30.6 | 33.4 | 0.112 |
| 7 | Miscarriage rate (%) | 2.4 | 3.4 | 0.148 |

endometrium, fallopian tube, myometrium, ovaries, placenta, and pre-implanting embryo, all of which get activated after a GnRHa trigger. GnRH has multiple roles that are added to endometrial receptivity and embryo implantation.[8]

However, the most important drawback of a lone GnRHa trigger is the associated low clinical pregnancy and high miscarriage rates that deprive its use in fresh transfer cycles. The LH surge following a GnRHa trigger peaks rapidly and thus has a shorter duration and amplitude than the LH surge in a natural cycle. This short surge results in oocyte maturation and ovulation but is not sufficient to support the corpus luteum. The utilization of new luteal support strategies, such as intensive progesterone support, adjuvant low-dose hCG at the same time as GnRHa administration (dual trigger) or on the day of oocyte retrieval, and adjuvant very low-dose hCG in the luteal phase, led to the better outcomes in cycles using dual triggers.[9] Hence came the concept of the use of a dual trigger in normal responders where fresh transfer cycles are usually done.[10]

The clinical pregnancy rates in our research were also higher in the dual trigger group but without any statistical significance. ($P = 0.112$)

There have been studies in the past that have shown results similar to our research.

Griffin *et al*. showed that the use of a dual trigger (GnRH agonist plus hCG) for oocyte maturation can improve implantation, clinical pregnancy, and live birth rates compared to the GnRH agonist trigger alone.[11] In our study, there was no significant difference in the number of retrieved metaphase II oocytes in the two groups ($P = 0.095$). They found that the number of good quality embryos obtained in the dual trigger group however was higher than that in the hCG group with statistical significance. ($P = 0.014$)

Maybe the reason was that the total number of oocytes and the number of metaphase II were more in the dual trigger group. So there were more good quality oocytes for injection. Decleer *et al*. found an increase in good quality embryos after dual trigger with no effect on ongoing pregnancy rate. Maybe dual trigger also affects embryo morphology, but at the same time also it causes a decrease in endometrial receptivity because of the negative effect of high LH levels and additional FSH surge on the endometrium.

They have advised further research on the subject.[12]

Similarly, in a retrospective study, Zhou *et al*. also found that a dual trigger results in greater numbers of embryos available and high-quality embryos. They also reported trends toward higher implantation, clinical pregnancy, and live delivery rates in the dual trigger group compared to the hCG-trigger group; however, it was without statistical significance.[13]

Schachter *et al*. found that GnRH agonist had a higher tendency to bind to the receptors than GnRH antagonist. They also in their research suggested that it can displace the antagonists from their receptors in the endometrium and also in gonadotropin-producing cells. They suggested that after displacement, activation of the previously blocked GnRH receptor might lead to late post-receptor effects too in endometrial cells, improving implantation.[14] This is probably a reason why the dual trigger group showed better quality embryos and comparatively higher pregnancy rates than hCG alone.

A limitation of our study was the small sample size and retrospective nature. That's why we could not prove that dual trigger has a significant effect on a number of oocytes retrieved and the pregnancy rates. One reason was also that the study period included the COVID-19 effects too and its impact on overall fertility is still not established. It is suggested that if this same study is conducted with larger sample size and prospective and longer duration of follow-up, statistical significance can be achieved.

## CONCLUSION

In this study, there was a higher rate of good quality embryos yielded in the dual trigger group compared to the hCG group. However, there was no significant difference in the number of metaphase II oocytes, clinical pregnancy, and implantation rates despite higher numbers and rates in the dual trigger group. This can be attributed to our small sample size. Further randomized controlled trials with a larger sample size will be helpful to analyze the association between the kind of final oocyte triggering and oocyte and embryo quality and pregnancy rates.

### Acknowledgments

We thank Dr Geeta Goswami, our Senior Embryologist, for all her support during the study.

### Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES


1. Griffin R, Feinn L, Engmann J, Nulsen T, Budinetz C, Benadiva. Dual trigger with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin to improve oocyte maturity rates. Fertil Steril 2014;102:405–9.
2. Casper RF. Ovarian hyperstimulation: effects of GnRH analogues. Does triggering ovulation with gonadotrophin-releasing hormone analogue prevent severe ovarian hyperstimulation syndrome? Hum Reprod 1996;11:1144–46.
3. Kolibianakis EM, Schultze-Mosgau A, Schroer A, *et al*. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of hCG in patients undergoing IVF with GnRH antagonists. Hum Reprod 2005;20:2887–92.
4. Humaidan P, Ejdrup Bredkjaer H, Westergaard LG, Yding Andersen C. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. Fertil Steril 2010;93:847–54.
5. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Comparison of "triggers" using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. Fertil Steril 2011;95:2715–17.
6. Lin MH, Wu FS, Lee RK, *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. Fertil Steril 2013;100:1296–302.
7. Engmann L, Benadiva C, Humaidan P. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. Reprod Biomed Online 2016;32:274–85.
8. Jelodar G, Gholami S, Jafarpour F. Effect of GnRH on guinea pig endometrium at preimplantation stage. Indian J Exp Biol 2007;45:242.
9. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Thomas S. Gonadotropin-releasing hormone agonist combined with a reduced dose of human chorionic gonadotropin for final oocyte maturation in fresh autologous cycles of in vitro fertilization. Fertil Steril 2008;90:231–33.
10. Andersen CY, Elbaek HO, Alsbjerg B, *et al*. Daily low-dose hCG stimulation during the luteal phase combined with GnRHa triggered IVF cycles without exogenous progesterone: a proof of concept trial. Hum Reprod 2015;30:2387–95.
11. Griffin D, Benadiva C, Kummer N, Budinetz T, Nulsen J, Engmann L. Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders. Fertil Steril 2012;97:1316–20.
12. Decleer K, Osmanagaoglu B, Seynhave S, Kolibianakis B, Tarlatzis P, Devroey. Comparison of hCG triggering versus hCG in combination with a GnRH agonist: a prospective randomized controlled trial. Facts. Views Vis. Obgyn. 2014;6:203–9.
13. Zhou X, Guo P, Chen X, Ye D, Liu Y, Chen S. Comparison of dual trigger with combination GnRH agonist and hCG versus hCG alone trigger of oocyte maturation for normal ovarian responders. Int J Gynaecol Obstet 2018;141:327–31.
14. Schachter M, Friedler S, Ron-El R, *et al*. Can pregnancy rate be improved in gonadotropin-releasing hormone (GnRH) antagonist cycles by administering GnRH agonist before oocyte retrieval? A prospective, randomized study, Fertil Steril 2008;90:1087–93.