Evaluation of dual trigger with gonadotropin-releasing hormone agonist and human chorionic gonadotropin in improving oocyte maturity rates: A prospective randomized study

**ABSTRACT**

**BACKGROUND:** Mature oocytes are prerequisite for achieving the process of in vitro fertilization. Human chorionic gonadotropin (hCG) is the standard trigger used for stimulating ovulation but is associated with ovarian hyperstimulation syndrome (OHSS). Gonadotropin-releasing hormone agonist trigger achieves oocyte maturation and lowers the incidence of OHSS, but it has limitations of higher pregnancy loss rate and miscarriage rates. Coadministration of both hormones is found to improve the pregnancy rates and the number of mature oocytes retrieved. We aimed to assess if the dual trigger is better than the conventional hCG in triggering oocyte maturation. **METHODOLOGY:** The study included 76 female patients aged 24–43 years who were randomly divided into two groups with 38 patients in each arm. The study included patients with antimullerian hormone (AMH) <4 ng/ml, antral follicle counts (AFCs)/ovary <12. The study excluded high responders-AMH >4 ng/ml and AFC/ovary >12 to avoid OHSS risk with hCG trigger. **RESULTS:** The study showed statistically insignificant differences between dual group versus hCG group in terms of the number of oocytes retrieved (10.0 ± 5.6 vs. 8.7 ± 5.0; \(P = 0.2816\)), the number of mature oocytes recovered (8.4 ± 5.0 vs. 7.2 ± 4.0; \(P = 0.2588\)), fertilization rate (5.9 ± 4.2 vs. 5.6 ± 3.3; \(P = 0.7390\)), and the number of usable embryos on day 3 (4.0 ± 3.0 vs. 4.0 ± 2.4; \(P = 0.8991\)). **CONCLUSION:** The dual trigger is equivalent to hCG in triggering oocyte maturation.

**KEY WORDS:** Dual trigger, gonadotropin-releasing hormone agonist trigger, oocyte maturation

**INTRODUCTION**

The follicular phase of the menstrual cycle involves the hourly release of gonadotropin-releasing hormone (GnRH), which binds to GnRH receptors on the gonadotropes. This results in the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in hourly pulses that regulate follicular growth. At midcycle, rapidly rising estradiol from the dominant follicle and a small rise in progesterone (P) lead to a gonadotrophic surge. An increase in the amplitude of LH and FSH pulses initiates oocyte maturity and triggers ovulation approximately 36–40 h later.\(^1\)

Because of its similarity to LH and its long half-life >24 h,\(^2\) human chorionic gonadotropin (hCG) has been used traditionally to trigger ovulation. In 1973, Nakano *et al.* showed that a bolus of GnRH agonist (GnRHa) given intravenously could induce the LH surge.\(^3\) Subsequently, various authors confirmed that ovulation could be...
achieved successfully when a small bolus of GnRHa was given subcutaneously (s/c).

GnRHa trigger induces final maturation of follicles through an endogenous surge of LH and FSH, which closely resembles the natural midcycle surge. The additional FSH surge induced is believed to promote the resumption of oocyte meiosis, LH receptor formation, cumulus expansion, and release of proteolytic enzymes involved in ovulation.

GnRHa trigger has been demonstrated to result in the retrieval of a higher number of mature oocytes as compared with hCG; furthermore, it is believed to eliminate the risk of ovarian hyperstimulation syndrome (OHSS).

However, the use of GnRHa alone as a trigger results in a lower pregnancy rate and an extremely high early pregnancy loss rate due to a luteal phase insufficiency.

Studies attempted to test the concept of the dual trigger, which involves combining GnRHa with a low dose of hCG to trigger oocyte maturation. It was proposed that dual trigger approach provided better oocyte maturity, blastulation rates, and pregnancy rates.

Our study attempted to assess if the dual trigger is more efficacious than the conventionally used hCG in obtaining a higher number of mature oocytes and usable day 3 embryos.

**METHODOLOGY**

**Study population**

The study included 76 female patients aged 24–43 years with antimullerian hormone (AMH) < 4 ng/ml, antral follicle counts (AFCs)/ovary < 12 and those ready to sign an informed consent. The study excluded high responders-AMH > 4 ng/ml and AFC/ovary > 12 to avoid OHSS risk with hCG trigger.

**Ethical statement**

This randomized prospective pilot study was conducted at an infertility center. The study was approved by an Independent Ethics Committee. All the patients provided written informed consent before their enrollment in the study.

The study followed good clinical practices as required by the International Conference on Harmonization guidelines, the ethical guidelines for biomedical research on human subjects (ICMR, 2006), and the Declaration of Helsinki.

**Dosage schedule**

An antagonist protocol with gonadotropin stimulation was used. Starting dose of gonadotropin was based on age, AMH, AFC, and body mass index (BMI).

A combination of recombinant FSH GONAL-f (Merck Serono) for first 5 days followed by Menopure (Ferring Pharmaceuticals Pvt. Ltd.,) was used in all patients as per our standard protocol.

For ovulation trigger, the patients were divided into two groups: hCG group received an ovulation trigger of hCG 10,000 IU I/M (Bharat Serum and Vaccines Ltd.,) and dual group were triggered with luperide 1 mg s/c (Sun Pharmaceuticals Ltd.,) plus hCG 5000 IU (Bharat Serum and Vaccines Ltd.,) administered at the same time.

Intracytoplasmic sperm injection was performed in all the patients. Oocyte maturity and embryo grading were done as per the laboratory protocol.

**Monitoring**

Baseline estrogen (E₂) level, P level, and ultrasound were done on day 2 of the cycle. Ovarian stimulation was started when all the values were normal, i.e. endometrium < 5 mm, no follicle > 10 mm in the ovaries, E₂ < 50 pg/ml, and P < 0.9 ng/ml. The first monitoring was done on day 5 of stimulation. GnRH antagonist, cetorelix (0.25 mg, s/c; Intas Pharmaceutical Ltd.,) was started in a flexible regime when the lead follicle reached 13 mm, and/or endometrial thickness was > 6 mm – suggesting a rising E₂ level. The dose of gonadotropin was adjusted as per individual requirement. Ovulation trigger was given when at least three follicles were > 18 mm. E₂ and P levels were measured on the morning of the trigger. The primary outcome measure was to compare the number of Metaphase II (MII) oocytes and number of usable embryos (embryos good for transfer and cryopreservation) on day 3 in the two groups.

A subgroup analysis was performed for patients with AMH < 1.4 ng/ml.

**RESULTS**

A total of 76 patients were randomly assigned to either the dual group (n = 38) or the control hCG group (n = 38).

Simple randomization was performed using sequentially numbered sealed envelopes. The envelope was opened by our head nurse. The two groups were matched for age, BMI, basal hormone levels, and cause of infertility. The mean age of the patients in dual group versus hCG group was 32 ± 5 years (range: 24–42) and 33 ± 4 years (range: 25–43), respectively. The groups did not significantly differ with regard to the baseline characteristics such as age, BMI, basal FSH, LH, and AMH levels [Table 1]. Etiology of infertility in patients of both the groups was similar and is presented in Figure 1.

The outcome of ovarian stimulation and hormonal characteristics are presented in Tables 2 and 3, respectively. The duration of stimulation and the average total dose of gonadotropins given to the patients did not differ between the groups compared [Table 2]. Although the average number of total oocytes retrieved (10.0 ± 5.6 vs. 8.7 ± 5.0; P = 0.2816) and MII oocytes (8.4 ± 5.0 vs. 7.2 ± 4.0; P = 0.2588)
Table 1: Baseline characteristics of the patients in study group

| Variable                  | Dual trigger | hCG | P   |
|---------------------------|--------------|-----|-----|
| Number of patients (n)    | 38           | 38  |     |
| Age (years)               | 32.4±4.5     | 33.1±4.1 | 0.4878 |
| BMI (kg/m²)               | 25.8±3.9     | 24.2±3.2 | 0.0532 |
| FSH (mIU/ml)              | 7.7±3.0      | 7.2±2.5 | 0.5055 |
| LH (mIU/ml)               | 5.3±3.1      | 4.8±2.8 | 0.4715 |
| AMH (ng/ml)               | 2.3±1.3      | 2.0±1.0 | 0.3184 |

Values are expressed as mean±SD. SD=Standard deviation, BMI=Body mass index.

FSH=Follicle stimulating hormone, hCG=Human chorionic gonadotropin, LH=Luteinizing hormone, AMH=Antimullerian hormone

Table 2: Stimulation characteristics and outcome variables in the study group

| Variable                      | Dual trigger | hCG | P   |
|-------------------------------|--------------|-----|-----|
| Total days of stimulation (days) | 10.0±1.0     | 10.3±1.4 | 0.1897 |
| Total dose of gonadotropins (IU/ml) | 2851.6±573.0 | 2879.6±809.9 | 0.8626 |
| Oocytes retrieved (n)         | 10.0±5.6     | 8.7±5.0 | 0.2816 |
| MII oocytes (n)               | 8.4±5.0      | 7.2±4.0 | 0.2588 |
| 2PN zygotes (n)               | 5.9±4.2      | 5.6±3.3 | 0.7390 |
| Usable embryos (D3)           | 4.0±3.0      | 4.0±2.4 | 0.8991 |

Table 3: Hormonal data in the study group on the day of triggering final oocyte maturation

| Variable (mean±SD) | Dual trigger | hCG | P   |
|--------------------|--------------|-----|-----|
| E₂ (pg/ml)         | 2121.9±985.3 | 1717.1±1051.7 | 0.0876 |
| P (pg/ml)          | 1.0±0.5       | 0.9±0.5 | 0.3041 |

The results of this study found no significant differences in the number of oocytes retrieved between Group 1 receiving hCG and Group 2 receiving the dual trigger. Differences in fertilization rate and usable embryos on day 3 were also insignificant between the two groups. Our study results emphasize that there is no significant benefit associated with the use of a dual trigger in comparison to conventionally used hCG for increasing the number of mature oocytes and usable day 3 embryos in in vitro fertilization (IVF). Various studies in past have been conducted to compare the efficacy of hCG with GnRHa in triggering ovulation for IVF with varying results.

Kolibianakis et al. conducted a randomized study on 106 patients to compare the efficacy of hCG with GnRHa in triggering oocyte maturation. The study showed no significant differences in the proportion of MII oocytes, fertilization rates, or the number and quality of embryos transferred between the two groups. The study was suspended due to significantly lower ongoing pregnancy in the GnRHa group (odds ratio 0.11; 95% confidence interval 0.02–0.52).[11]

Humaidan et al. (2005) also compared the efficacy of GnRHa with hCG in triggering ovulation in 121 patients who were given 0.5 mg GnRHa (n = 55) or 10,000 IU of hCG (n = 67). They reported significantly more number of oocytes retrieved in GnRHa group. However, their study also revealed lower implantation and clinical pregnancy rate and a higher rate of early pregnancy loss with the use of GnRHa trigger.[20]

The study by Engmann et al. which...
It has been suggested that the greater oocyte maturity reported with GnRHa might be related to the more rapid increase in serum LH after agonist trigger when compared with the rise of serum hCG level after 10,000 IU IM injection of hCG,[27] the concurrent FSH surge, or possibly both.[28] An increase in the number of mature oocytes recovered would theoretically improve IVF results by providing more embryos.[29]

Research has shown improved oocytes maturation rates if retrieved 38 h after the hCG bolus administration.[30,31] The improved oocyte maturation rates in the study by Griffin et al. might have been the result of increased trigger/oocyte retrieval time. This speculates that the use of dual trigger could not be indisputably associated with improved oocyte maturation. It appears that the use of hCG alone is helpful in achieving the same number of metaphase oocytes as compared to its combined use with GnRHa. Our study showed contrasting results to the study by Griffin et al. The dose of hCG in the dual trigger in Griffin’s study varied from 5000 IU to 10,000 IU, the hCG group underwent agonist down-regulation whereas the dual trigger group was given an antagonist protocol. In our study, the dose of hCG in dual trigger was fixed to 5000 IU. The increased dosage of hCG might have favored the increase in oocyte maturation rate in the study by Griffin et al.[14]

A study by Lin et al. assessed the effectiveness of dual trigger as compared to conventional hCG trigger in improving live birth, clinical pregnancy, and implantation rates. This was probably due to a significantly higher number of patients with at least one embryo of excellent quality in patients of the dual trigger group. However, in this study, oocyte maturation rates were not measured.[15] These study findings were not in agreement with the study by Griffin et al.[14]

AMH level is known to decline with age in women and is recognized as a crucial marker for the poor response to ovarian hyperstimulation in IVF.[32] Lukaszuk et al., in his study, found that AMH <1.4 ng/ml is associated with less success rate of live birth in women undergoing

Table 4: Baseline characteristics of the patients in subgroup analysis

| Variable (mean±SD) | Dual trigger (AMH <1.4) | hCG (AMH <1.4) | P     |
|-------------------|-------------------------|----------------|-------|
| Number of patients (n) | 14 | 11 | 0.3758 |
| Age (years) | 34.3±4.8 | 35.9±3.9 | 0.6697 |
| BMI (kg/m²) | 27.3±3.6 | 25.5±2.1 | 0.1601 |
| FSH (mIU/ml) | 9.4±3.1 | 8.4±3.9 | 0.4927 |
| LH (mIU/ml) | 4.6±2.1 | 4.2±2.7 | 0.6941 |
| AMH (ng/ml) | 1.0±0.3 | 0.8±0.2 | 0.1241 |

Values are expressed as mean±SD. SD=Standard deviation, BMI=Body mass index, FSH= Follicle stimulating hormone, hCG=Human chorionic gonadotropin, LH=Luteinizing hormone, AMH=Antimullerian hormone.

Table 5: Stimulation characteristics and outcome variables in the sub-group analysis

| Variable (mean±SD) | Dual trigger (AMH <1.4) | hCG (AMH <1.4) | P     |
|-------------------|-------------------------|----------------|-------|
| Total days of stimulation (days) | 9.6±1.0 | 10.0±1.5 | 0.4003 |
| Total dose of gonadotropins (IU/ml) | 2914.3±511.9 | 2986.4±834.8 | 0.7929 |
| Oocytes retrieved (n) | 6.5±5.2 | 4.2±2.9 | 0.2009 |
| MI oocytes (n) | 5.5±5.1 | 3.7±2.6 | 0.3045 |
| 2PN zygotes (n) | 3.4±3.6 | 2.8±2.0 | 0.6167 |
| Usable embryos (D3) | 2.6±2.7 | 2.5±1.8 | 0.9783 |

hCG=Human chorionic gonadotropin, SD=Standard deviation, AMH=Antimullerian hormone.

Table 6: Hormonal data in the sub-group on the day of triggering final oocyte maturation

| Variable (mean±SD) | Dual trigger (AMH <1.4) | hCG (AMH <1.4) | P     |
|-------------------|-------------------------|----------------|-------|
| E₂ (pg/ml) | 1663.8±944.3 | 1027.2±1002.5 | 0.1170 |
| P (pg/ml) | 0.9±0.6 | 0.8±0.4 | 0.6697 |

E₂=Estrogen, P-Progesterone.
We conducted a subgroup analysis of women with AMH <1.4 ng/ml who were triggered with hCG and dual trigger. We found no significant difference in the number of usable embryos in both the groups.

CONCLUSION

There is no significant difference in the outcomes in terms of the number of mature oocytes, fertilization rate, and number of usable embryos by day 3 on using either dual trigger or hCG. The use of any kind of trigger can be based on the type of patient, for example, for a patient who is at risk of OHSS and needs to go with a fresh embryo transfer, dual trigger can be opted for as it uses a lower dose of hCG. However, future studies and research are required to confirm our findings and improve our understanding.

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Conflicts of interest

There are no conflicts of interest.

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