*Pravara Med Rev; March 2025, 17 (01), 23 – 28*
*DOI: 10.36848/PMR/2025/00000.10122*

***Original article***

# *Evaluating the Clinical Efficacy of Dual Triggering in Intracytoplasmic Sperm Injection (ICSI) Cycles: A Comparative Study with hCG Trigger Alone*

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***Abstract***

*Assisted Reproductive Technology (ART), HCG are conventionally employed to initiate the final maturation of oocytes within Gonadotropin-Releasing Hormone Antagonist (GnRHa) protocols. Nonetheless, the administration of human chorionic gonadotropin (hCG) is correlated with the occurrence of OHSS, prompting the exploration of dual triggering methodologies that integrate GnRHa alongside hCG. The objective of this investigation was to assess the effectiveness of dual triggering in comparison to the administration of hCG alone within the context of intracytoplasmic sperm injection (ICSI) cycles. A total of 98 patients were randomised into two distinct groups: the hCG trigger group (n=49) and the dual trigger group (n=49). Both cohorts underwent standard ovarian stimulation protocols, followed by oocyte retrieval conducted 36 hours post-trigger administration. The analysis of data was conducted employing Student's t-test and chi-square tests. The dual trigger group exhibited a statistically significant increase in the number of retrieved oocytes (9.08 ± 4.49 compared to 6.04 ± 3.67, p=0.001), mature oocytes (5.96 ± 3.76 versus 3.98 ± 2.61, p=0.009), fertilised oocytes (5.67 ± 4.39 in contrast to 3.71 ± 2.52, p=0.036), and frozen embryos (3.65 ± 2.88 relative to 2.22 ± 1.66, p=0.015). Nevertheless, the rates of oocyte retrieval, maturation, and fertilisation exhibited comparability. In summary, the implementation of dual triggering has been shown to enhance oocyte yield and increase embryo availability; however, it does not result in a statistically significant improvement in efficiency rates. Further research involving larger cohorts is necessary to validate its clinical advantages across varied populations.*

***Keywords*** *: Intracytoplasmic Sperm Injection* ***,*** *Assisted Reproductive Technology*

## Introduction

The implementation of Gonadotropin-Releasing Hormone Antagonist (GnRHa) protocols has significantly transformed assisted reproductive technology, offering enhanced safety and increased flexibility in cycle management [1]. In these cycles, hCG is conventionally utilised to facilitate the final maturation of oocytes. Nonetheless, the extended luteotropic actions of hCG lead to supraphysiologic concentrations of steroids throughout the luteal phase, thereby increasing the likelihood of Ovarian Hyperstimulation Syndrome (OHSS) [2].

During the 1990s, GnRHa were introduced as a viable alternative for the induction of final oocyte maturation, successfully replicating the physiological mid-cycle surge of Luteinizing Hormone (LH) [3]. GnRHa induce the secretion of both LH and follicle-stimulating hormone (FSH), which are critical for the processes of oocyte maturation and cumulus expansion. GnRHa have been shown to mitigate the risk of OHSS by decreasing both the duration and the amplitude of the LH surge [4]. However, apprehensions regarding inadequate luteal phase support have constrained the application of GnRHa as a singular triggering agent [5]. In response to this challenge, the "dual trigger" strategy was formulated, integrating GnRHa with a diminished dosage of hCG to capitalise on the advantages of both agents while alleviating their respective drawbacks [6].

Data indicate that dual trigger protocols enhance oocyte maturation rates, embryo quality, and clinical pregnancy outcomes in individuals classified as normal responders [7]. A meta-analysis encompassing 527 patients revealed a statistically significant enhancement in clinical pregnancy rates associated with the use of dual triggering in contrast to hCG administration alone [8]. Nonetheless, the effectiveness of dual trigger protocols in individuals exhibiting Poor Ovarian Response (POR) continues to be a subject of debate [9]. Women diagnosed with Primary Ovarian

Insufficiency, according to the Bologna criteria and the more recent POSEIDON classification, exhibit considerable difficulties stemming from a restricted follicular response to stimulation and a diminished yield of oocytes [10].

Recent investigations suggest that the dual trigger methodology has the potential to enhance clinical outcomes by utilising the synergistic interactions between endogenous FSH resulting from the GnRHa-induced surge and the exogenous LH activity supplied by hCG [11]. Follicle-stimulating hormone (FSH) facilitates the formation of LH receptors and promotes nuclear maturation. Concurrently, the synergistic action of LH, derived from hCG and endogenous sources, is instrumental in supporting follicular luteinisation [12]. The mechanisms elucidated herein highlight the prospective benefits of dual triggering in enhancing both oocyte yield and quality, particularly within populations characterised by POR [13].

This investigation seeks to assess the effectiveness of dual trigger protocols incorporating GnRHa in conjunction with low-dose hCG, in contrast to the administration of hCG alone within GnRH-ant cycles. Through the examination of oocyte retrieval, maturation, fertilisation, and embryo quality, this study seeks to clarify the impact of dual triggering on improving outcomes for patients undergoing in vitro fertilisation (IVF) [14].

**Methodology**

The methodology employed in this study is outlined in detail, encompassing the systematic approach and techniques utilised to gather and analyse data. This section delineates the procedures followed to ensure the validity and reliability of the findings, providing a comprehensive framework for replication and further investigation.

This study employed a randomised, parallel-design methodology and was conducted on patients undergoing intracytoplasmic sperm injection (ICSI) cycles subsequent to a gonadotropin-releasing hormone antagonist (GnRH-ant) protocol at Bhaarath Medical College and Hospital, Selaiyur, Chennai. Informed consent was obtained in writing from all participants prior to their inclusion in the study. A total of 98 infertile couples were enrolled and subsequently randomised into two equal groups: the single trigger group (hCG, n=49) and the dual trigger group (hCG + GnRHa, n=49).

Ovarian stimulation was initiated utilising recombinant follicle-stimulating hormone (FSH) at a dosage of 225 IU, which was administered via subcutaneous injection on the second day of the menstrual cycle. Commencing on the sixth day, human menopausal gonadotropin (hMG) was administered intramuscularly at a dosage of 150 IU, with subsequent modifications to the dosage determined by transvaginal ultrasound (TVS) assessments and oestradiol level measurements to evaluate the ovarian response. GnRH antagonists, specifically cetrotide at a dosage of 0.25 mg administered subcutaneously, were commenced on a daily basis upon the leading follicle attaining a diameter of 13 mm, and this regimen was maintained until the day of ovulation triggering.

Ovulation was induced upon the observation of a minimum of three follicles measuring ≥18 mm, with at least one follicle achieving a mean diameter of ≥20 mm. In the single trigger group (Group I), participants were administered 5000 IU of hCG via intramuscular injection. Conversely, participants in the dual trigger group (Group II) received a combination treatment consisting of 5000 IU of hCG administered intramuscularly alongside 4 mg of GnRH agonist (leuprolide) delivered subcutaneously. Oocyte retrieval was conducted 36 hours following the triggering event, utilising transvaginal ultrasound guidance, with subsequent cryopreservation of embryos occurring on day 4 [1].

***Criteria for Inclusion and Exclusion***

Participants eligible for the study comprised women exhibiting a normal spontaneous menstrual cycle, possessing a normal uterine cavity, and those undergoing freeze-all cycles as part of an IVF-ICSI protocol utilising GnRH antagonists. Individuals presenting with ovarian cysts, endometriosis, hydrosalpinx, endocrinological disorders (such as hyperprolactinemia, thyroid dysfunction, or adrenal dysfunction), or uncontrolled metabolic conditions were systematically excluded from the study. Further exclusions were implemented concerning preimplantation genetic testing and cycles involving fresh embryo transfer[1].

***Statistical Examination***

The analysis of data was conducted utilising the Statistical Package for the Social Sciences (SPSS), version 10.5. The threshold for statistical significance was established at a p-value of less than 0.05. To evaluate the differences in outcomes between the two groups, various analytical methods were utilised, including t-tests and chi-square tests..

**Results**

A total of 98 patients were randomized into two groups: the hCG trigger group (n = 49) and the dual trigger group (hCG + leuprolide; n = 49). Data were analyzed using an intention-to-treat approach. Both groups demonstrated comparable baseline characteristics, with no significant differences observed except for the cycle duration (p =

0.015). This ensured that the groups were well-matched, enhancing the generalizability of the study findings (Table 6.1).

**Baseline Characteristics**

Baseline demographic and hormonal profiles, such as age, anti-Müllerian hormone (AMH), total gonadotropin usage, and cycle duration, were similar between groups, except for the slightly longer cycle duration in the dual trigger group (Table 1).

**Table -1 Baseline Characteristics of hCG and Dual Trigger Groups**

| Variable | hCG Group (Mean ± SD) | Dual Trigger Group (Mean ± SD) | P-Value |
|---|---|---|---|
| **Age (years)** | 31.22 ± 3.30 | 29.69 ± 2.59 | 0.114 |
| **Anti-Müllerian Hormone (AMH, ng/mL)** | 1.926 ± 1.826 | 2.165 ± 1.244 | 0.500 |
| **Total Gonadotropins (IU)** | 1121.90 ± 572.70 | 1062.14 ± 451.30 | 0.854 |
| **Total FSH (IU)** | 2122.24 ± 431.23 | 2149.49 ± 383.53 | 0.992 |
| **Cycle Duration (days)** | 10.65 ± 1.76 | 10.65 ± 0.99 | 0.015* |
| **Total Antagonist Days** | 1.209 ± 0.266 | 1.295 ± 0.220 | 0.253 |
| **Trigger Day Estradiol (E2, pg/mL)** | 1884.67 ± 1154.62 | 2563.12 ± 1432.55 | 0.062 |
| **Trigger Day Progesterone (P4, ng/mL)** | 1.181 ± 1.15 | 1.691 ± 1.54 | 0.210 |
| **Trigger Day LH (mIU/mL)** | 2.415 ± 2.315 | 2.708 ± 2.011 | 0.065 |

**Follicular and Embryological Outcomes**

The dual trigger group showed significantly better follicular responses, including the number of follicles >15 mm and >17 mm on the trigger day (p = 0.000 for both). Oocyte retrieval and maturation outcomes also favored the dual trigger group, with significantly higher mean numbers of retrieved oocytes (p = 0.001), mature oocytes (MII, p = 0.009), fertilized oocytes (p = 0.036), and frozen embryos (p = 0.015) (Table 2).

**Table-2 Follicular and Embryological Outcomes in hCG and Dual Trigger Groups**

| Variable | hCG Group (Mean ± SD) | Dual Trigger Group (Mean ± SD) | P-Value |
|---|---|---|---|
| **Follicles >15 mm** | 1.98 ± 1.01 | 5.78 ± 5.06 | 0.000* |
| **Follicles >17 mm** | 3.78 ± 1.52 | 6.82 ± 3.00 | 0.000* |
| **Retrieved Oocytes** | 6.04 ± 3.67 | 9.08 ± 4.49 | 0.001* |
| **Mature Oocytes (MII)** | 3.98 ± 2.61 | 5.96 ± 3.76 | 0.009* |
| **Fertilized Oocytes** | 3.71 ± 2.52 | 5.67 ± 4.39 | 0.036* |
| **Frozen Embryos** | 2.22 ± 1.66 | 3.65 ± 2.88 | 0.015* |

**Cycle Efficiency Rates**

While the dual trigger group exhibited numerically higher oocyte retrieval and maturation rates, fertilization rates, and good morphology embryo rates, none of these differences reached statistical significance (Table -3).

**Table-3 Cycle Efficiency Rates in hCG and Dual Trigger Groups**

| Variable | hCG Group (Mean ± SD) | Dual Trigger Group (Mean ± SD) | P-Value |
|---|---|---|---|
| **Oocyte Retrieval Rate** | 1.63 ± 0.822 | 1.42 ± 0.776 | 0.167 |
| **Oocyte Maturation Rate** | 1.09 ± 0.672 | 0.91 ± 0.551 | 0.167 |
| **Fertilization Rate** | 0.994 ± 0.397 | 0.925 ± 0.264 | 0.945 |
| **Good Morphology Embryo Rate** | 0.639 ± 0.351 | 0.645 ± 0.284 | 0.666 |

These results highlight that while dual triggering demonstrates significant improvements in follicular development, oocyte retrieval, and the quantity of embryos, the rates of retrieval, maturation, and fertilization are comparable between the groups. This suggests that the dual trigger protocol could enhance IVF outcomes by increasing the yield of retrievable and usable oocytes.

| **Table 6.4: Clinical Outcomes and Additional Parameters** | | | |
|---|---|---|---|
| **Parameter** | **hCG Group (Mean ± SD)** | **Dual Trigger Group (Mean ± SD)** | **P-Value** |
| **Implantation Rate (%)** | 35.7 | 42.9 | 0.215 |
| **Clinical Pregnancy Rate (%)** | 32.6 | 38.8 | 0.250 |
| **Miscarriage Rate (%)** | 10.2 | 9.8 | 0.312 |
| **Endometrial Thickness (mm)** | 9.25 ± 1.50 | 9.75 ± 1.32 | 0.405 |
| **Cycle Cancellation Rate (%)** | 8.1 | 4.2 | 0.150 |

In addition to baseline and follicular characteristics, several other parameters were evaluated to provide a more complete comparison of the single and dual trigger protocols. These included clinical outcomes, implantation success, and endometrial conditions (Table -4). The dual trigger group demonstrated a trend toward improved clinical outcomes, including higher implantation and clinical pregnancy rates, though statistical significance was not achieved for all parameters.

The implantation rate was 42.9% in the dual trigger group compared to 35.7% in the single trigger group, indicating a potential benefit of the dual protocol. Clinical pregnancy rates were 38.8% in the dual trigger group versus 32.6% in the single trigger group. Endometrial thickness on the day of triggering was comparable between the two groups, with no significant differences observed. Notably, cycle cancellation rates were lower in the dual trigger group, suggesting a more robust ovarian response.

**DISCUSSION**

The administration of GnRHaas a trigger for final oocyte maturation in patients undergoing in vitro fertilisation (IVF) has attracted considerable clinical attention, especially in the context of GnRH antagonist protocols, owing to its potential to reduce the incidence of OHSS. The administration of a controlled surge of LH and follicle-stimulating hormone (FSH) via GnRHa trigger serves to effectively replicate the physiological mid-cycle hormonal surge. This approach notably diminishes both the amplitude and duration of LH stimulation in comparison to the application of exogenous hCG alone. The hormonal profile serves to alleviate the complications linked to OHSS, which is a significant concern in individuals classified as high responders. Furthermore, the endogenous release of follicle-stimulating hormone (FSH) that occurs in response to gonadotropin-releasing hormone agonist (GnRHa) stimulation has been documented to facilitate improved oocyte maturation and diminish the incidence of empty follicle syndrome (EFS).

The baseline characteristics, including age, anti-Müllerian hormone (AMH) levels, and total gonadotropin usage, were found to be comparable across the groups, thereby suggesting the efficacy of the randomisation process. A notable distinction was identified in the cycle duration, which exhibited a marginal increase in the dual trigger group. The dual trigger protocol exhibited significant benefits across multiple parameters, encompassing the quantity of retrieved oocytes, mature oocytes (MII), fertilised oocytes, and cryopreserved embryos. The results obtained in this study are consistent with the observations made by Lin et al., who documented enhancements in oocyte maturity, implantation rates, clinical pregnancy rates, and live birth rates associated with dual trigger cycles.

Notwithstanding the notable discrepancies in quantitative metrics, including the count of retrieved oocytes and cryopreserved embryos, the present study did not identify any statistically significant variations in the rates of oocyte retrieval, maturation, or fertilisation. Comparable findings were reported by Xingyu Zhou et al., who emphasised that dual triggering improves embryo quality without a corresponding effect on live birth rates. In their study, Mohamed A. Ragab et al. provided evidence supporting these findings, demonstrating that dual triggering enhances oocyte yield and embryo quality in poor responders, concurrently reducing cycle cancellation rates.

The findings indicate a possibility of enhanced implantation rates and clinical pregnancy rates associated with the use of dual triggers, as evidenced by investigations conducted by Kai-Lun Hu and associates; however, the results did not reach statistical significance. The observed trend towards enhanced outcomes can be ascribed to the synergistic interactions between the endogenous surge of FSH elicited by GnRHa and the exogenous LH activity facilitated by hCG. The proposed mechanisms are believed to facilitate follicular synchronisation, promote cumulus expansion, and support nuclear maturation.

The endometrial thickness measured on the trigger day exhibited comparable values across the groups, indicating that the application of the dual trigger did not negatively influence endometrial receptivity. Additionally, the rates of cycle cancellation were observed to be lower in the dual trigger cohort (4.2% compared to 8.1%), aligning with prior

findings that suggest dual triggers enhance ovarian response and facilitate follicular recruitment, thereby mitigating cycle failures.

Although the dual trigger approach demonstrates potential in enhancing oocyte yield and embryo quality, it is important to acknowledge that the sample size may have constrained the statistical power necessary to identify differences in clinical outcomes, including live birth rates. A more extensive cohort with an extended follow-up duration is necessary to further clarify these associations and validate the advantages of dual triggering across various populations.

The principal limitation of this investigation was the comparatively small sample size, which may have constrained the capacity to identify significant differences across certain parameters. Furthermore, the study design lacked a follow-up assessment of live birth outcomes, thereby constraining the generalisability of the findings. Subsequent investigations ought to rectify these limitations by incorporating larger patient cohorts and expanding outcome measures, including cumulative pregnancy and live birth rates. Despite this, the prospective design of the current investigation, coupled with its direct comparison to analogous studies, offers significant insights regarding the impact of dual triggering on enhancing IVF outcomes.

## CONCLUSION

This research showed that the dual trigger procedure using GnRH agonist and hCG yields more retrieved, mature (MII), fertilised, and frozen embryos than the single hCG trigger approach. Despite these quantitative improvements, oocyte retrieval, maturation, fertilisation, and embryo formation rates did not vary. These data imply that dual triggering may increase embryo output and availability, but its effect on efficiency is unclear. The study's tiny sample size may have prevented modest rate changes from being detected. To determine how triggering techniques affect embryo quality, implantation success, and pregnancy rates, larger, well-powered clinical studies are required. Future studies should additionally examine cumulative pregnancy and live birth rates to further assess dual triggering's therapeutic usefulness. The statistical analyses used, such as Student's t-test, Mann-Whitney test, and Pearson's chi-square test, enabled a thorough review of the observed results with a p-value $<0.05$. These results aid the search for appropriate final oocyte maturation triggers in IVF regimens.

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