Original Article

Dual trigger with gonadotropin-releasing hormone agonist and recombinant human chorionic gonadotropin improves the outcome of intrauterine insemination

Binarwan Halim, PhD, Hilma Putri Lubis, MD

Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Running title: Dual trigger improves the outcome of IUI

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Corresponding author: Hilma Putri Lubis, MD
Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Kampus Padang Bulan, Medan 20155, Indonesia.
Email: hilmaputrilubis@gmail.com
https://orcid.org/
Abstract

**Objective**
The objective of this study was to evaluate the effectiveness of dual trigger, which is a combination of gonadotropin-releasing agonist (GnRH-a) and recombinant human chorionic gonadotropin (hCG) in the final oocyte maturation, in the outcome of intrauterine insemination (IUI).

**Methods**
This retrospective observational study was conducted from January 2016 to October 2018 and involved 639 IUI cycles at the Halim Fertility Center, Indonesia. Controlled ovarian stimulation was performed during IUI cycles. The ovulation triggers were divided into two groups: group I received a combination of GnRH-a and recombinant hCG as a dual trigger, and group II received only recombinant hCG as a single trigger. The baseline characteristics, cycle parameters, and IUI outcomes of both groups were compared.

**Results**
Our study included a total of 639 IUI cycles, 334 were in the dual trigger group and 305 in the single trigger group. The clinical pregnancy rates were significantly higher in the dual trigger group than in the single trigger group \( (P<0.001) \). Based on the multivariate analysis, the dual trigger increased the clinical pregnancy rate by 2.524 times than that by the single trigger.

**Conclusion**
Our data showed that the dual trigger combination of GnRH-a and recombinant hCG significantly improves the outcome of intrauterine insemination.

**Keywords:** IUI, Dual trigger, Single trigger, Outcome
Introduction
Intrauterine insemination (IUI) is the first treatment option for unexplained infertility after the failure of expectant treatment and before in vitro fertilization (IVF) [1]. It has been accepted as a more economical method than other assisted reproductive techniques. IUI is indicated for the treatment of unexplained infertility, mild male factor, cervical factor, anovulation, and minimal to mild endometriosis [1,2]. Success rates of IUI range between 10% and 20% per treatment [1,3]. Various methods are performed to increase the success rates of IUI, including ovarian stimulation, sperm preparation, endometrial preparation, ovulation trigger, increasing progesterone level, and monitoring of follicles [1]. However, some factors that influence the success rate of IUI outcomes include the quality of oocytes and the appropriate timing of IUI.

Several studies have found that ovulation triggers affect the oocyte quality and the appropriate timing of IUI [1,4-6]. Ovulation is assumed to occur 36-38 hours after human chorionic gonadotropin [hCG] administration because of biochemical similarities between hCG and luteinizing hormone (LH) in ovulation induction. Andersen et al. [7] stated that the interval between hCG administration and ovulation was 38.3 hours (range, 34-46 hours). The number of patients in whom follicular rupture occurred more than 38.3 hours after hCG administration was 11 patients (34.3%) [7]. As an alternative to hCG, the concept of a “dual trigger” that combines a bolus of gonadotropin-releasing agonist (GnRH-a) with hCG at the time of triggering has been investigated for IVF [8-15]. GnRH-a triggers have some benefits, such as prevention of ovarian hyperstimulation syndrome (OHSS), induction of follicle-stimulating hormone (FSH) surge, and individualized luteal phase support based on ovarian response to stimulation. FSH surge increases oocyte nuclear maturation, resumption of meiosis, and expansion of cumulus [16-19]. Zhang et al. [12] reported that the oocyte retrieval rate and the oocyte maturation rate in poor ovarian responders significantly increased in the dual trigger groups in a study of 1,389 cycles. Fabris et al. [10] observed that dual triggers increased the number of mature oocytes and oocyte maturation rates in women with a high immature oocyte rate in a previous cycle in a study of 163 ovarian stimulation cycles. Lin et al. [8] observed that the number of mature oocytes, implantation rates, clinical pregnancies, and live birth rates significantly increased in the dual trigger groups of normal responders in a study of 378 cycles of IVF. From the studies mentioned, an increased number of MII oocytes in dual trigger maturation might be due to the more appropriate timing of oocyte retrieval and the combined effect of endogenous LH surge from GnRH trigger and exogenous LH effect from hCG [8,10,12].

Similar to previous studies on dual triggers in IVF, we aimed to evaluate the outcome of the dual trigger in the IUI procedure. The objective of this study was to evaluate the effectiveness of the dual trigger, which is combined of GnRH-a and recombinant hCG for final oocyte maturation in the outcome of IUI.

Material and methods
1. Study design and participants

This retrospective observational study was conducted at the Halim Fertility Center IVF Center between January 2016 and October 2018. Couples seeking infertility treatment were eligible if they fulfilled one of the following inclusion criteria: female aged <40 years, unexplained infertility, bilaterally patent tubes, mild male factor with total motile sperm count (TMSC) 10-20x10⁶/mL, FSH level <10 mIU/mL, and anovulation. Exclusion criteria were pre-existing medical conditions, female aged >40 years, endometriosis cyst, adenomyosis, congenital uterine anomalies, severe male infertility with TMSC≤5x10⁶, spontaneous ovulation, and poor ovarian response. We used single trigger and dual trigger in this study based on the period of time. The single trigger was used for final oocyte maturation from January 2016 to May 2017, and the dual trigger was used from June 2017 to October 2018 (Fig. 1).

Fig. 1. Schematic study of single trigger and dual trigger in IUI. IUI, intrauterine insemination; IVF, in vitro fertilization.

2. IUI protocol
We stimulated our patients with gonadotropins on day 2 or 3 of the cycle using 75 IU of recombinant FSH (Gonal-F, Merck KGaA, Germany). Ovarian stimulation was monitored using ultrasound to measure the follicle diameter and endometrial growth from day 8 of the cycle until the follicle reached a diameter of ≥18 mm. When the leading follicle reached 18 mm in diameter, we observed the final oocyte maturation. In the single trigger group, it was triggered by only recombinant hCG (Ovidrel 250 μg; Merck KGaA, Darmstadt, Germany) and in the dual trigger group, ovulation was triggered with GnRH-a (Lucrin 1 mg; AbbVie, COMP,Australia) and recombinant hCG (Ovidrel 250 μg, Merck KgaA) at the same time. The IUI procedure was performed only once, 36 hours after ovulation triggering.

3. Semen preparation and insemination

Semen samples were collected at the Halim fertility center after 3 to 5 days of sexual abstinence. Fresh sperm was used for the IUI. The swim-up protocol was used for all the samples. Insemination was performed 36 hours after triggering with either single or dual trigger.

4. Luteal phase support

We supported the luteal phase with oral micronized progesterone 300 mg for 14 days. The biochemical pregnancy rate was observed after 14 days using a urinary pregnancy test. Clinical pregnancy was defined by the presence of fetal cardiac activity and an intrauterine gestational sac confirmed by transvaginal ultrasound 2 weeks after a positive urinary pregnancy test.

5. Statistical analysis

We used the Kolmogorov-Smirnov test to assess the normality of the sample quantitative variables. The data were non-parametric, and baseline comparisons were made using the Mann-Whitney U test. Mann-Whitney and chi-squared tests were used to analyze demographic characteristics. The Mann-Whitney U test was also used to analyze endometrial thickness, the number of basal antral follicles, and the number of follicles >17 mm. Furthermore, we used the chi-squared test to analyze clinical pregnancy rates, multiple pregnancies, and OHSS rates between the two groups. A multivariate logistic regression analysis was used to evaluate factors related to the outcome of IUI. Analyses were performed using the Statistical Package for Social Sciences version 20.0 (SPSS, Chicago, IL, USA). Statistical significance was set at \( P<0.05 \).

Results

From January 2016 to October 2018, 639 IUI cycles were included in this study. Table 1 presents the characteristics of participants: the median age of participants in the dual trigger group was 32 years (range, 21-40 years), and there was no significant difference in the ages of women between the single trigger group and the dual trigger group (\( P=0.945 \)). The median duration of infertility in the dual trigger group was 4 years (range, 2-13 years), and there was a significant difference in the duration of infertility between the two groups (\( P=0.048 \)). In this study, the etiology of infertility in single and dual trigger groups was mild male factor, but there was no significant difference between them (\( P=0.841 \)). The median basal FSH level in the dual trigger group was 6.5 with no significant difference in basal FSH levels between the two groups (\( P=0.411 \)). The median AMH in the dual trigger was 2.4 with no significant difference in the AMH level between the two groups (\( P=0.340 \)). The median total amount of
rFSH in the dual trigger group was similar to that of the single trigger group, and there was no significant difference in the total amount of rFSH between the two groups ($P=0.873$). The median sperm concentration, motility, and morphology were similar between the 2 groups with no significant difference ($P>0.05$). The median TMSC in the dual trigger group was 12.6 and there was no significant difference in the number of TMSCs between the single trigger group and the dual trigger group ($P=0.545$).

**Table 1.** The participants’ demographic data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Single trigger group (n=305)</th>
<th>Dual trigger group (n=334)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age (yr)</td>
<td>32 (22-40)</td>
<td>32 (21-40)</td>
<td>0.945</td>
</tr>
<tr>
<td>Infertility duration (yr)</td>
<td>4 (1-10)</td>
<td>4 (2-13)</td>
<td>0.048</td>
</tr>
<tr>
<td>Etiology of Infertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild male factor (%)</td>
<td>111 (36.6)</td>
<td>126 (37.7)</td>
<td>0.841</td>
</tr>
<tr>
<td>Female factor (%)</td>
<td>88 (29)</td>
<td>90 (26.9)</td>
<td></td>
</tr>
<tr>
<td>Combined (%)</td>
<td>106 (34.3)</td>
<td>118 (35.3)</td>
<td></td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>6.3 (3.2-9.7)</td>
<td>6.5 (3.6-9.7)</td>
<td>0.411</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>2.7 (1.1-5.2)</td>
<td>2.4 (1.1-5.2)</td>
<td>0.340</td>
</tr>
<tr>
<td>Total amount of rFSH (IU)</td>
<td>900 (750-975)</td>
<td>900 (750-975)</td>
<td>0.873</td>
</tr>
<tr>
<td>Sperm concentration (10^6/mL)</td>
<td>17.2 (14.2-20.9)</td>
<td>17.3 (14.2-20.9)</td>
<td>0.488</td>
</tr>
<tr>
<td>Sperm motility (A+B%)</td>
<td>35 (29-46)</td>
<td>35 (29-46)</td>
<td>0.847</td>
</tr>
<tr>
<td>Sperm morphology (%)</td>
<td>13.9 (8.9-18.9)</td>
<td>12.6 (7.9-17.2)</td>
<td>0.545</td>
</tr>
<tr>
<td>TMSC 10^6/ejaculate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FSH, follicle-stimulating hormone; AMH, EXPLANATION; rFSH, EXPLANATION; TMSC, total motile sperm count.

As shown in Table 2, the median endometrial thickness in the dual trigger group was similar to that in the single trigger group (range, 5-16 mm) with no significant difference in endometrial thickness between the two groups ($P=0.337$). The median number of basal antral follicles in the dual trigger group was 7 follicles (range, 1-34 follicles), but there was no significant difference in basal antral follicle count between the 2 groups ($P=0.587$). The median number of follicles >17 mm in the single and dual trigger groups was 2 follicles (range, 1-3 follicles), and there was no significant difference in the number of follicles >17 mm between the two groups ($P=0.974$).

**Table 2.** The stimulation cycle parameters

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Dual trigger group (n=334)</th>
<th>$P$-value</th>
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</thead>
<tbody>
<tr>
<td>Endometrial thickness (mm)</td>
<td>9 (5-19)</td>
<td>9 (5-16)</td>
<td>0.337</td>
</tr>
<tr>
<td>Number of basal antral follicles</td>
<td>7 (1-41)</td>
<td>7 (1-34)</td>
<td>0.587</td>
</tr>
<tr>
<td>Number of follicles &gt;17 mm</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>0.974</td>
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**Table 3.** Outcome of the intrauterine insemination procedure

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<tr>
<td>TMSC 10^6/ejaculate</td>
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<td></td>
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In Table 3, we observe that the clinical pregnancy rate was higher in the dual trigger group than in the single trigger group (28.7% vs 14.1%), and there was a significant difference between the two groups ($P<0.001$). Although the multiple pregnancy rates were higher in the dual trigger group than in the single trigger group (7.3% vs 7%), there was no significant difference between the groups ($P=0.627$). Furthermore, we found that the OHSS rate was not significantly higher in the dual trigger group than in the single trigger group (2.7% vs. 2.6%), but there was no significant difference between the two groups ($P=0.576$). Most of OHSS in the dual trigger group was in the mild form.

As shown in Table 4, after the seventh selection of variables related to the outcome of IUI, which consisted of female age, infertility duration, etiology of infertility, FSH and AMH levels, total amount of rFSH, sperm concentration, sperm motility, sperm morphology, TMSC, endometrial thickness, number of basal antral follicles, number of follicles >17 mm, and dual trigger, there were some factors that were related to the outcome of IUI: female age, endometrial thickness, and dual trigger. Based on the RR in this study, it can be concluded that the majority of the successful outcomes of IUI was influenced by a dual trigger with an RR value of 2.524 (95% CI, 1.681-3.790), which means that dual trigger could increase the clinical pregnancy rate by 2.524 times than that by single trigger.

### Table 4. Multivariate analysis of factors related to the outcome of IUI

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>P-value</th>
<th>RR</th>
<th>95% CI for RR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Female age</td>
<td>-0.069</td>
<td>0.006</td>
<td>0.933</td>
<td>0.888-0.980</td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>0.126</td>
<td>0.009</td>
<td>1.134</td>
<td>1.032-1.246</td>
</tr>
<tr>
<td>Dual trigger</td>
<td>0.926</td>
<td>0.000</td>
<td>2.524</td>
<td>1.681-3.790</td>
</tr>
<tr>
<td>Constant</td>
<td>-0.801</td>
<td>0.409</td>
<td>0.449</td>
<td></td>
</tr>
</tbody>
</table>

IUI, intrauterine insemination; B, **EXPLANATION**; RR, **EXPLANATION**; CI, **EXPLANATION**.

Discussion

In this study, we found that the clinical pregnancy rate was significantly higher in the dual trigger group than in the single trigger group (14.1% vs. 28.7%; $P<0.001$). Based on the multivariate analysis, the dual trigger could increase the clinical pregnancy rate by 2.524 times than that by single trigger. This result was similar to that of other studies on IVF.

Final oocyte maturation with human chorionic gonadotropin (hCG) is commonly performed in patients undergoing IUI. However, in assisted reproductive cycles, hCG triggers
are associated with a higher risk of developing OHSS and premature LH surge. Other than HCG, GnRH-a has been used as an alternative to induce ovulation in the last two decades. GnRH agonists induce the endogenous surge of LH and FSH by their flare-up effect, which is helpful in triggering ovulation and decreasing the risk of OHSS [1-3]. Ovulation triggers with hCG or GnRH are widely used in the IUI cycle.

Ding N et al. [15] observed that implantation and pregnancy rates were higher in the dual trigger group than in the hCG-only trigger group. In addition, Chen et al. [14] reported that the dual trigger group had significantly higher clinical pregnancy rates than those of the hCG-only group. The dual trigger of final oocyte maturation with GnRH-a and hCG improves the outcomes of IVF in patients with normal responders, poor ovarian responders, high proportion of immature oocytes, and high responders [8,9,12,13,20-22]. These studies reveal that the final maturation of oocyte with dual trigger improves the number of mature oocytes (MII) and oocyte quality, which is reflected by the higher number of good quality embryos, thus improving the clinical pregnancy rate. HCG has traditionally been used as the gold standard for triggering oocyte maturation in controlled ovarian stimulation cycles [1-3]. GnRH-a is one of the triggers of final oocyte maturation that is used as an alternative to hCG to prevent OHSS in PCOS patients [16-19]. GnRH-a can stimulate FSH surge and LH surge. FSH surge has a beneficial effect on oocyte maturation and causes cumulus expansion of the oocyte [16-20,22].

This study also showed that in addition to increasing the number of oocyte maturation and oocyte quality, the purpose of triggering ovulation with the dual trigger in IUI is also related to the appropriate timing of IUI. The timing of IUI should be as close as possible to the time of ovulation to increase the success rate of IUI. Early studies reported discrepancies in the time of ovulation after an hCG trigger. Ovulation was expected 36 hours after hCG administration. Andersen et al. [7] found that follicular rupture occurred more than 38.3 hours after hCG administration in 34.3% of patients. AboulGheit et al. [23] found that the clinical pregnancy rate per cycle was higher in the group in which ovulation occurred 48 hours after hCG administration than that in the 34-hours group and 24-hours group (19% vs. 14.6% vs. 9.5%, respectively). The above study observed that partial ovulation occurred at 36 hours after hCG-only trigger and some ovulation occurred after more than 36 hours. The final maturation oocyte with the dual trigger is expected to be even closer to the time of ovulation at 36 hours so that IUI could be performed closer to the time of ovulation (36 hours).

The main limitation of this study was its retrospective nature, as data sampling was not well-controlled. Data recruitment was based on a period because we modified the ovulation trigger from a single trigger to a dual trigger after approximately 1 year, and then we evaluated the outcome of IUI between the single and dual trigger groups. Further prospective studies are needed to validate the results of this study and avoid bias. A prospective study should be performed in a randomized controlled trial using a homogeneous sample, for instance, in an unexplained infertility group.

To the best of our knowledge, this is the first study to evaluate the outcome of the dual trigger on ovulation triggers in IUI. In conclusion, ovulation with dual triggers significantly improve the outcome of IUI. Further well-designed prospective studies are required to validate these results.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval
This study was approved by the Health Research Ethical Committee of Stella Maris Women’s and Children’s Hospital on October 20, 2018 (reference number :1496-1/Dir/RSIA).SM/X/2018).

Patient consent

In this study, informed consent was obtained from all the participants.

Funding information

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