Gonadotrophin releasing hormone agonist with low dose human chorionic gonadotrophin co-triggers versus gonadotrophin releasing hormone agonist alone for reducing the risk of ovarian hyperstimulation in women with polycystic ovarian disease.

Ahmed Arafa  
*Assistant lecturer of obstetrics & gynaecology, Faculty of Medicine, Al-Azhar University, Egypt,*  
ahmedarafa700@gmail.com

Mofeed Mohamed  
*Obstetrics and gynecology Departments, Faculty of Medicine, Al-Azhar University, Egypt,*  
arafaa826@yahoo.com

Ahmed Aboulserour  
*Obstetrics and gynecology Departments, Faculty of Medicine, Al-Azhar University, Egypt,*  
ahmedaboulserour@yahoo.com

Mohamed Gebreel  
*Obstetrics and Gynecology Department, Al-Azhar University, Cairo, Egypt,*  
drgebreel@hotmail.com

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Gonadotrophin Releasing Hormone Agonist With Low Dose Human Chorionic Gonadotrophin Co-Triggers Versus Gonadotrophin Releasing Hormone Agonist Alone for Reducing the Risk of Ovarian Hyperstimulation in Women with Polycystic Ovarian Disease

Ahmed Elsayed Mahmoud Mohamed Arafa 1, 2*, Mohamed Mohamed Gebril 1 MD, Mofeed Fawzy Mohamed 1 MD and Ahmed Gamal Eldin Abou El Serour 1 MD

*Corresponding Author:
Ahmed Elsayed Mahmoud Mohamed Arafa
ahmedarafa700@gmail.com

ABSTRACT

Background: “Dual triggering” for final oocyte maturation using a combination of a gonadotropin-releasing hormone agonist (GnRHa) and human chorionic gonadotropin (hCG) can improve clinical outcomes in high responders during in vitro fertilization–intracytoplasmic sperm injection (IVF–ICSI) GnRH-antagonist cycles.

Aim of the work: To compare gonadotrophin releasing hormone agonist with low dose human chorionic gonadotrophin co-triggers versus gonadotrophin releasing hormone agonist alone for reducing the threat of severe ovarian hyperstimulation in women suffering polycystic ovarian disease correlated with outcomes.

Patients and methods: There were 120 infertile women who joined the ART department at the International Islamic Center for Population Studies and Research (IICPSR) Al-Azhar University hospitals who took part in this randomised control study.

Results: The difference in the number of oocytes in M1 between the groups was statistically important (p= 0.0147). As well, there was a statistically significant difference between the two groups regarding number of oocytes in M2 (p= 0.0140).

Conclusion: The use of Gonadotrophin releasing hormone agonist with low dose human chorionic gonadotrophin co-triggers was not significantly prevent the risk of mild and moderate form ovarian hyperstimulation in women suffering from polycystic ovarian disease in comparison with gonadotrophin releasing hormone agonist alone with better outcomes for dual triggering. Also, both protocols were not record any cases of severe form.

Keywords: Dual trigger; human chorionic gonadotropin; luteal phase support; oocyte maturation.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) usually applied for curing infertility attributable to tubal factor, important infertility affects up to one in seven couples all over the world. Proportion of these couples may be able to ultimately conceive, but for the majority conception is unlikely without some form of medical intervention.1

Infertility can be caused by a variety of factors such as gamete quality, congenital anatomical defects, and surgical problems. Infertility can also be caused by inadequate uterine blood flow, according to previous findings.2

Despite recent advancement in ovarian stimulation protocols in IVF and embryo transfer procedures, the pregnancy rate has remained stagnant, with cycle pregnancy rates of no more than 40% and implantation rates per embryo transplanted being disappointingly low (15 percent ).3

Ovarian hyper stimulation syndrome (OHSS) affects women who have recently received gonadotropin stimulation to achieve ovulation or have been treated with assisted reproductive technologies like in vitro fertilization.4

Early OHSS is generally mild to moderate, and it appears three to seven days after hCG is given. Late OHSS is frequently severe and develops twelve to seventeen days after starting hCG therapy. Because pregnant hCG worsens the condition, the early form
is caused by an exogenously injected hormone, whereas the late variety is caused by an implanting or implanted pregnancy.

OHSS is marked by ovarian enlargement and a shift of fluid from the intravascular compartment to the extravascular space. This occurs in response to the vasoactive substances that are secreted following human chorionic gonadotropin (hCG) trigger used prior to the egg retrieval. OHSS is graded based on the severity of the subjective symptoms as well as the clinical or laboratory findings. Mild OHSS requires no treatment, but supportive therapy is needed for the more severe cases. 5

The Aim was to compare gonadotrophin releasing hormone agonist with low dose human chorionic gonadotrophin co-triggers versus gonadotrophin releasing hormone agonist alone for decreasing the threat of severe form of ovarian hyperstimulation in women suffering polycystic ovarian illness.

**PATIENTS AND METHODS**

This randomized control study designed compare (GnRHa) with low dose human chorionic gonadotrophin co-triggers versus Gonadotrophin releasing hormone agonist alone for reducing the risk of severe ovarian hyperstimulation in women with polycystic ovarian disease under antagonist protocols in vitro-fertilization (IVF) / intracytoplasmic sperm injection (ICSI) cycles.

142 infertile women joining the ART department in the International Islamic Center for Population Studies and Research (IICPSR), Al-Azhar University Hospitals and private IVF centers. 22 patients were missed during the study, only 120 completed the study, all of them were randomized and allocated in two groups: Dual group and GnRHa alone group. Each group with 60 patients.

Diagnosis of the couples was confirmed by basic infertility work up and investigations.

**The inclusion criteria:** Age of patients from 18 to 42 years, estradiol level more than 400 pg/ml and >10 follicles in each ovary on day of triggering, women with documented polycystic ovarian disease according to Rotterdam criteria (PCOM, clinical or biochemical hyperandrogenism and anovulation) and normal uterine cavity (confirmed by ultrasound and/or Hysterosalpingography (HSG), and/or Hysteroscopy to exclude any anomalies).

**The exclusion criteria:** The patients above 42 years old, uterine anomalies such as bicornuate uterus, rudimentary horn or uterine septum, patients with drug hypersensitivity to GnRH analogue and percutaneous Epididymal Sperm Aspiration (PESA) or Testicular Sperm Extraction (TESE)

All participants in this study exposed to complete history taking, general examination, abdominal examination as well as vaginal examination. All included couples patients counselled about the purpose and details of the programme. Full data collected from the eligible patients including detailed personal and menstrual history also general, abdominal and vaginal examinations as well as transvaginal ultrasound on 2nd day of the cycle.

Examination were done for each studied patient using SIEMENS® ultrasound SONOLINE® sienna 8500 MT 7.5 MHz vaginal angel 120° and 3.5-5.5 MHz abdominal, to exclude the presence of ovarian masses, uterine myomas or endometrial polyp and counting the antral follicle count and using other ultrasonic machine in centre.

**Hormonal Profiles Determination:** On day three of the cycles preceding ovarian stimulation, blood sample (10 cc) was collected through vein puncture at early morning, samples were allowed to clot at room temperature for at least one hour. All samples were centrifuged, supernatant serum separated within 2 hours after withdrawal and stored at −20 °C until assay of basal hormones.

Serum FSH, LH, E2 and prolactin measured in sample collected with radioimmunoassay (RIA) using Gamma counter immunoassay analyzer.

**Ovarian stimulation by Flexible antagonist protocols:** All patients under Controlled Ovarian Hyperstimulation (COH) with: Flexible antagonist protocol started when the largest follicle reach 14 mm with dose 250ug. (Cetrotide) with FSH / HMG Stimulation giving daily till triggering of ovulation. All patients randomized either to triggering of ovulation with GnRH agonist alone or activating of ovulation with GnRH agonist and low dose HCG 1500 iu.

**Ultrasound:** Transvaginal 2D color Doppler ultrasound done at the day of hCG injection to measure endometrial thickness, pattern, volume, RI, PI. All patients were asked to sit in a waiting area for at least twenty minutes before being scanned in order to reduce the possibility of any negative effects of physical activity on uterine blood flow. The examination was carried out with the patient in the lithotomy position, and colour Doppler Ultrasonography was used in conjunction with a 5-MHz trans-vaginal probe to get images. For this investigation, all scans were done by the same operator in order to avoid interobserver variability.

**Oocyte preparation:** Oocyte retrieval done 34-36 hours after hCG injection by trans-vaginal ultrasound-guided needle aspiration under general anesthesia. Follicular fluid was aspirated into sterile tubes.

**Sperm preparation:** The World Health Organization's guidelines were followed while analyzing the sperm. A discontinuous Percoll gradient will be used to treat the sperm sample. A mini-Percoll gradient was utilized to generate motile spermatozoa in cases of severe oligoasthenozoospermia. 6

**ICSI procedure:** The ICSI procedure performed with very fine instruments under a microscope after the granulosa cells have been stripped away from the oocyte with enzymes, the oocyte is held in place by a holding pipet. The other pipet which is much smaller and sharper is used to pick up a single sperm. The smaller pipet is then brought into proper position and then inserted through the zona pellucida and into the cytoplasm of the oocyte where the sperm is injected. 3
Embryo grading: Approximately 18 hours after ICSI procedure, the oocytes checked for signs of fertilization (two pronuclei or two distinct polar bodies).

Embryo transfer: 2 to 5 days after oocyte retrieval. Up to 4 Grades A embryos per patient will be transferred according to the age of the patient, the indication for IVF, the count of previous attempts, and the number and quality of embryos available for transfer. All the embryo transfers were performed by senior physicians using soft ET catheter (Labotect) without ultrasonography guidance (Except in difficult embryo transfer).

Detection, diagnosis and management of OHSS

Outcomes: pregnancy rate, ongoing pregnancy rate and cycle cancellation and aspiration of ascetic fluid, ICU admission, morbidity and mortality.

Ethical approval: Approval of Departmental and Ethical Committees were obtained from quality education assurance unit, Faculty of Medicine, Al-Azhar University Egypt.

Statistical analysis: the gathered data will be tallied and statistically examined by SPSS program (Statistical Package for Social Sciences) software version 26.0, Microsoft Excel 2016 and MedCalC program software version 19.1. If the P value is less than 0.05, the result is significant; otherwise, it is non-significant. The p-value is a statistical indicator of the likelihood that a study's findings might have happened by chance.

RESULTS

Table 1: Comparison between the two groups concerning age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean± SD</th>
<th>Median</th>
<th>Range</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
<td>Dual group (No. = 60)</td>
<td>GnRha alone group (No. = 60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38-&lt;40 years</td>
<td>38.23± 2.47</td>
<td>39.0</td>
<td>38.0 – 42.0</td>
<td>30</td>
<td>50.0%</td>
<td>28</td>
<td>46.7%</td>
<td>ZMWU= 0.5508</td>
<td>0.5828</td>
</tr>
<tr>
<td>≥40 years</td>
<td>38.47± 2.30</td>
<td>38.0 – 43.0</td>
<td></td>
<td>30</td>
<td>50.0%</td>
<td>32</td>
<td>53.3%</td>
<td>X²= 0.033</td>
<td>0.588</td>
</tr>
</tbody>
</table>

Table 2: Comparison between the two groups as per duration of infertility

<table>
<thead>
<tr>
<th>Duration of infertility (years)</th>
<th>Mean± SD</th>
<th>Median</th>
<th>Range</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual group (No. = 60)</td>
<td>5.55± 2.04</td>
<td>2.0 – 10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T= 0.757</td>
<td>0.451</td>
</tr>
<tr>
<td>GnRha alone group (No. = 60)</td>
<td>5.82± 1.82</td>
<td>2.0 - 12.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Comparison among the two groups as per Antral follicle count, AMH and E2

<table>
<thead>
<tr>
<th>Antral follicle count (AFC)</th>
<th>Mean± SD</th>
<th>Median</th>
<th>Range</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual group (No. = 60)</td>
<td>20.46± 4.58</td>
<td>11.0 – 29.0</td>
<td>4.40± 1.22</td>
<td>1.90 – 7.30</td>
<td>4762.61± 1183.89</td>
<td>2997.0 – 8948.0</td>
<td>T= 1.075</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>GnRha alone group (No. = 60)</td>
<td>19.59± 4.28</td>
<td>8.0 - 27.0</td>
<td>4.68± 1.46</td>
<td>2.30 - 9.70</td>
<td>4857.97± 1084.82</td>
<td>2101.0 - 7445.0</td>
<td>T= 1.126</td>
<td>0.262</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AMH</th>
<th>Mean± SD</th>
<th>Median</th>
<th>Range</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual group (No. = 60)</td>
<td>4.40± 1.22</td>
<td>4.40± 1.22</td>
<td>4.40± 1.22</td>
<td>4.40± 1.22</td>
<td>4.40± 1.22</td>
<td>4.40± 1.22</td>
<td>T= 1.126</td>
<td>0.262</td>
<td></td>
</tr>
<tr>
<td>GnRha alone group (No. = 60)</td>
<td>4.68± 1.46</td>
<td>4.68± 1.46</td>
<td>4.68± 1.46</td>
<td>4.68± 1.46</td>
<td>4.68± 1.46</td>
<td>4.68± 1.46</td>
<td>T= 1.126</td>
<td>0.262</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E2</th>
<th>Mean± SD</th>
<th>Median</th>
<th>Range</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual group (No. = 60)</td>
<td>4762.61± 1183.89</td>
<td>4762.61± 1183.89</td>
<td>4762.61± 1183.89</td>
<td>4762.61± 1183.89</td>
<td>4762.61± 1183.89</td>
<td>4762.61± 1183.89</td>
<td>T= 0.460</td>
<td>0.646</td>
<td></td>
</tr>
<tr>
<td>GnRha alone group (No. = 60)</td>
<td>4857.97± 1084.82</td>
<td>4857.97± 1084.82</td>
<td>4857.97± 1084.82</td>
<td>4857.97± 1084.82</td>
<td>4857.97± 1084.82</td>
<td>4857.97± 1084.82</td>
<td>T= 0.460</td>
<td>0.646</td>
<td></td>
</tr>
</tbody>
</table>

p≤0.05 is thought to be statically important, p≤0.01 is considered highly significantly important, SD= standard deviation, *Student T test

Table 1: Comparison between the two groups concerning age

The age in dual group ranged from 38 to 42 years with mean ±SD was 38.23± 2.47 years while in GnRha alone group the age ranged from 38 to 43 years with mean ±SD was 38.47± 2.30 years with no statistical important change (p=0.5828) between the two groups. Likewise, when it came to age groups, no significant variation existed among the two groups (p=0.588).

Table 2: Comparison between the two groups as per duration of infertility

The period of infertility in dual group ranged from 2 to 10 years with mean ±SD was 5.55± 2.04 years while in GnRha alone group the it ranged from 2 to 12 years with mean ±SD was 5.82± 1.82 years with no statistical substantial distinction between the two groups (p=0.451).

Table 3: Comparison among the two groups as per Antral follicle count, AMH and E2

In terms of Antral follicle count, there was no significantly wide variation groups (p= 0.285). In terms of AMH, there were no notable differences among 2 groups. (p= 0.262). As well, there was no important variation among the two groups concerning E2 (p= 0.646).
A retrospective cohort study by Lin et al., that there was no
rate in women with embryos

Number of M2 oocyte (M1)

Mean ± SD

Range

Mean ± SD

Range

Mean ± SD

Range

Mean ± SD

Range

Mean ± SD

Range

Total

Dual group (No. = 60)

GnRha alone group (No. = 60)

Test value

P-value

Number of oocytes

2.84± 2.17

14.86± 4.80

11.07± 3.24

2.45± 0.50

0.0 – 9.0

6.0 – 26.0

3.0 – 18.0

2.0 – 3.0

3.94± 2.61

12.87± 3.83

10.14± 2.80

2.43± 0.53

1.0 – 11.0

2.0 – 20.0

4.0 – 16.0

2.0 – 4.0

2.504

0.014

2.507

0.014

1.682

0.095

0.188

0.851

p≤0.05 is thought to be statically important, p≤0.01 is considered high statistically substantial,
SD = standard deviation, * Mann-Whitney test

Table 4: Comparison between the two groups as per ICSI characteristics

There was variation with a statistical significance among the two groups concerning count of oocytes in M1 (p=0.014). As well, there was notably change among the two groups concerning count of oocytes in M2 (p=0.014). In regarding of the quantity of embryos frozen and transferred, there was no significantly substantial distinction among the two groups (p=0.095 & 0.851 respectively). Table (4)

<table>
<thead>
<tr>
<th></th>
<th>Dual group</th>
<th>GnRha alone group</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(No. = 60)</td>
<td>(No. = 60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHSS</td>
<td>No</td>
<td>46</td>
<td>53</td>
<td>X² = 2.83</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>12</td>
<td>6</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>1</td>
<td>100.0%</td>
</tr>
<tr>
<td>ICU Admission</td>
<td>No</td>
<td>60</td>
<td>60</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>Negative</td>
<td>59</td>
<td>47</td>
<td>X² = 1.768</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>11</td>
<td>13</td>
<td>26.7%</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>Negative</td>
<td>58</td>
<td>60</td>
<td>X² = 2.034</td>
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<tr>
<td></td>
<td>Positive</td>
<td>2</td>
<td>0</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

p≤0.05 is thought to be statically important p≤0.01 is considered high statistically significant,
SD = standard deviation, OHSS: Ovarian hyperstimulation syndrome* Chi-Square Test ♦Fischer Exact test

Table 5: Comparison between the two groups as per outcome

There was no important change in prevalence of OHSS in dual group compared to GnRha alone group (p=0.243). In terms of ICU admission, no statistically substantial distinction between the two groups was not found. As well, In terms of clinical pregnancy, there was no significantly change between the two groups. (p= 0.184) and multiple pregnancy (p= 0.496), Table (5)

**DISCUSSION**

The response of ovarian follicles to controlled ovarian hyper-stimulation (COH) with gonadotropins varies greatly across patients as well as from cycle to cycle within the same patient. Patients with a rising responder status have an exaggerated reaction to gonadotropin injection, in addition to a higher risk of ovarian hyper-stimulation syndrome (OHSS). The study’s main goal was to compare (GnRHa) with a little dosage (hCG) co-triggers against (GnRHa alone for minimizing the risk of severe ovarian hyperstimulation in women with polycystic ovarian disease.

Regarding the Demographic characteristics among the studied groups, we found that the mean (range) age was 38.23± 2.47 (33–42) years while in GnRha alone group was 38.47± 2.30 (34–43) years with no notably important change (p=0.923) among the two groups. Likewise, there was no substantial distinction regarding age groups (p=0.692). The mean (range) BMI in dual group was 35.27± 1.56 (22.8–30) Kg/m2 while in GnRha alone group was 25.22± 1.77 (22–30) Kg/m2 with no notably significant change (p=0.723) among the two groups. Likewise, there was no notably important change between the two groups regarding BMI classification (p=0.845). Lin et al., did a retrospective case control research to examine if a dual trigger of (GnRHa) and (hCG) might improve the live birth rate in women with ovarian reserve depletion. They decided to enroll 427 women in the dual-trigger-group and 130 women in the control group (hCG trigger group), no statistically significant differences in age among the two groups was found.

In earlier Retrospective cohort study by Lin et al., they enrolled 191 women in the dual-trigger group and 187 control group (hCG trigger group), there was no notably important change between the two groups concerning age and BMI. Regarding clinical characteristics among the studied groups, we found that the mean duration of infertility in dual group was 5.54± 2.0 (2-12) years while in GnRha alone group was 5.82± 1.96 (2-12) years with no statistically important change between the two groups (p=0.480). Also, we found that there was no statistically important change among the two groups concerning Antral follicle count, AMH and E2 (p> 0.05),
Lin et al., confirmed our findings, stating that there was no statistically important variation among the two groups regarding Antral follicle count and AMH (p > 0.05).

In agreement with our study Elgindy et al., revealed that mean time of infertility in dual group was 5.2 ± 2.6 years while in HCG alone group was 4.8 ± 2.6 years with no notably vital change among the two groups. They also found that there was no notably important change between the two groups concerning Antral follicle count, AMH (p > 0.05).

Also, Seval et al., revealed that there was no statically important variation between the two groups regarding E2 in the day of stimulation (p > 0.05).

Comparison between the two groups regarding ICSI characteristics revealed that there was no statistically important change among the two groups concerning No. of oocytes in M1, number of oocytes in M2 and No. of embryo (p > 0.05).

Our results were not confirmed by the study by Lin et al., as they reported that there was no statistically notable change among the two groups about No. of oocytes Retrieved, No. of MII oocytes Retrieved and No. of embryo transferred (p > 0.05).

While in contrast the earlier study Lin et al., revealed that there was no notably important change among the two groups regarding No. of embryo obtained and No. of embryo transferred (p > 0.05) but there was a statistically significant difference between the two groups regarding No. of oocytes Retrieved, No. of MII oocytes Retrieved (p < 0.01).

Elgindy et al., published a research that validated our findings, stating that no important alteration in number of embryos among the two groups (p more than 0.05).

While Seval et al., found a statistically distinction among the two groups concerning count of MII oocytes extracted, there was no statistically important change in terms of count of oocytes retrieved, count of embryos acquired, or Number of embryos transferred.

Regarding outcome between the two groups, we found that there was no statistically important rise prevalence of OHSS in dual group compared to GnRHa alone group (p=0.234), also there were no recorded cases of severe form of OHSS. There was no statistically significant change between the two groups regarding ICU admission. As well, there was no change among the two groups regarding clinical pregnancy (p= 0.415) and multiple pregnancy (p= 0.125).

In contrast with our results the study by Lin et al., as they reported that there was a significantly variation between the two groups regarding Fertilization rate, Clinical pregnancy rate per cycle, Live birth rate per cycle and abortion rate.

Also, the study by Lin et al., agrees with our findings revealed that there was no statistically important change among the two groups regarding OHSS and there was statistically significant difference between the two group’s Clinical pregnancy rate per embryo transfer and Live birth rate per embryo transfer.

Our results were in disagreement the findings by Elgindy et al., as they reported that in terms of Considerable OHSS, there was a significantly change among the two groups. (p less than 0.05), but in Elgindy study two group were GnRHa group and HCG group not dual trigger, there was notable change among the two groups Clinical pregnancy rate per embryo transfer. While there was no statistically important change among the two groups regarding Mild and moderate OHSS (p > 0.05)

In addition, the study by Castillo et al., as they concluded that GnRHa trigger plays an important role beyond OHSS prevention.

In contrast with our results the study by Mutlu et al., reported that Fertilization rates, implantation rates, clinical pregnancy rate per embryo transfer and live birth rate per embryo transfer were also greatly increased in the dual trigger group as compared to the hCG trigger group. The utilize of dual trigger with a Gonadotrophin releasing hormone agonist and a standard dosage of human chorionic gonadotrophin could advance clinical pregnancy percent and live birth levels in reduced ovarian responders go through GnRH antagonist IVF/ICSI cycles.

CONCLUSION

The use of Gonadotrophin releasing hormone agonist with low dose human chorionic gonadotrophin co-triggers was significantly prevent the risk of severe form not mild and moderate OHSS in women with polycystic ovarian disease in comparison with gonadotrophin releasing hormone agonist alone with better outcome.

Conflict of interest : none

REFERENCES


