Dual triggering for final oocyte maturation compared to single triggering in GnRH antagonist (IVF-ICSI) protocols (Randomized Controlled Trial)

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Abstract

Background: Infertility is one of the major medical problems in the world which has led to continuous research and advances in the field of assisted reproductive technology (ART),

Aim of the work: to investigate whether co-administration of GnRH-a and hCG for final oocyte maturation (dual trigger) would improve number of oocytes retrieved& its quality and eventually IVF/ICSI clinical outcomes compared to single triggers in women with normal ovarian response undergoing (IVF/ICSI) technique using GnRH antagonist protocol of stimulation.

Patients and Methods: A total 120 patients were included in this study, randomized and divided into two groups: Group (1): The study group; included 60 patients who received the dual trigger. Group (2): The control group; who included 60 patients, age matched, who received the hCG trigger alone. All participants were subjected to proper history taking, complete general, abdominal and pelvic examination, and full investigations to confirm criteria of the study. All participants were subjected to controlled ovarian hyper stimulation protocol according to GnRH antagonist protocol starting on day 2-3 of the menstrual cycle with a starting daily administration of FSH, human menopausal gonadotropin hMG, or highly purified hMG, or highly purified FSH or with recombinant FSH (r.FSH) intramuscularly for 4–5 days, and continued until the day of final oocyte maturation injection.

Results: the current study showed statistically significant difference with p-value <0.05 between study groups as regards to the number of retrieved oocytes (cases: 11.42±4.2 vs. control: 9.8±4.9), number of MII oocyte retrieved (dual trigger: 6.2±2.7 vs. single trigger: 4.6±3.1), and number of fertilized oocyte (dual trigger: 4.03±2.2 vs. single trigger: 3.05±2.5) with higher mean among dual trigger group. In the current study also the dual-trigger group demonstrated a significantly higher percentage as regards to biochemical pregnancy rate (cases: 68.3%vs. 33.3% among controls), clinical pregnancy rate (cas-

es: 58.3% vs. 31.7% among controls) and implantation rate (cases: 41.3% vs. 21.4% among controls) with a statistically significant difference with p-value <0.05 between study groups. Both groups showed no statistically significant difference as regards to the mean number of embryos transferred (1.9±1.01 in cases vs. 1.7±1.2 in control) and number of frozen embryos (1.33±1.08 in cases vs. 1.1±1.4 in control), or as regards to complications; whether ET cancellation or incidence of sever OHSS.

Conclusion: In conclusion, in terms of the number of mature retrieved oocytes, implantation rate and clinical pregnancy rate in normal responders undergoing IVF/ICSI using antagonist protocols, a dual-trigger approach with a GnRH agonist and the standard dosage of hCG was found to be significantly superior to an hCG trigger alone.

Key words: Dual triggering, oocyte maturation, single triggering, GnRH antagonist.

Introduction

Infertility is one of the major medical problems in the world which has led to continuous research and advances in the field of assisted reproductive technology **(ART)** [1].

Controlled ovarian hyperstimulation **(COH)** is a fundamental step of in vitro fertilization **(IVF)** that has been in practice since the 1970s [2].

Over the past two decades, gonadotropin-releasing hormone **(GnRH)** antagonist protocols have been proposed as a safer and efficacious way for ovarian stimulation [3].

GnRH antagonist protocols have several advantages over the long agonist, including the rapid decrease in luteinizing hormone **(LH)** and follicle-stimulating hormone **(FSH)** levels without flare-up effect, decreased number of days of stimulation and the amount of gonadotropin administered, (4) and statistically significant reduction of ovarian hyperstimulation syndrome **(OHSS)** [5,6].

Since the pioneering days of in vitro fertilization **(IVF)**, human chorionic gonadotropin **(hCG)** has been used as a surrogate for the natural mid-cycle luteinizing hormone **(LH)** surge [7].

The administration of hCG results in sustained luteotrophic effect and supraphysiological levels of estradiol and progesterone; the sustained luteotrophic effect may contribute to the development of ovarian hyperstimulation syndrome **(OHSS)** [8].

More than 30 years ago, Nakano et al., described that it was possible to trigger an endogenous LH surge sufficient for induction of ovulation with a single injection of a gonadotropin-releasing hormone agonist **(GnRH-a)**. Unfortunately, this finding was soon underestimated, as GnRH-a rapidly became the first line treatment to prevent premature luteinization, which precluded the use of GnRH-a to induce final follicular maturation [9].

When the third generation GnRH antagonist was introduced into the market for the use in ovarian stimulation protocols during the 1990's,it became possible to trigger final oocyte maturation and ovulation with a single bolus of a GnRH-a as an alternative to hCG [10].

Though some studies have suggested an increase in the percentage of mature oocytes retrieved when triggered with GnRH-a compared with hCG (11), it has been found that triggering ovulation with GnRH agonist leads to a suboptimal luteal phase [12].

"Dual trigger" was first defined as the concept of a combination of GnRH agonist and a low-dose hCG in GnRH antagonist cycles for triggering final oocyte maturation and prevention of Ovarian Hyperstimulation syndrome **(OHSS)**, [13].

Lin et al. conducted a retrospective study, consisted of normal responders undergoing IVF with GnRH antagonist protocol and showed significant improvement in total number of retrieved oocytes and number of

mature **(MII)** oocytes, also rates of embryo implantation, clinical pregnancy, ongoing pregnancy and live birth when dual trigger regimen was used [14].

Lu et al. also presented a retrospective data analysis of medical records where final oocyte maturation was triggered using a GnRH-a alone **(Decapeptyl 0.1–0.2 mg)** or in combination with hCG **(1,000, 2,000, or 5,000 IU)**, and concluded that using a dual trigger with a low dose of hCG **(1,000 IU)** as an adjuvant to GnRH-a to induce final oocyte maturation significantly improved the oocytes retrieval rate of suboptimal responders [15].

Aim of the Work

The objective of the present study is to compare between single trigger with standard dose of hCG alone and dual triggering with the combination of GnRH agonist and hCG in **IVF/ICSI** cycles in improving the number of oocytes retrieved and oocyte quality.

Patients and methods

The current study was designed as a prospective, case-control, randomized study, from February 2020 to April 2021, conducted at ART unit of Ain Shams University, maternity hospital. One hundred and twenty participants **(120)** were assigned into the study and prepared to undergo IVF/ICSI trial using GnRH antagonist protocol of controlled ovarian hyper-stimulation.

After getting approval of Research Ethics Committee **(REC)**, Ob/Gyn department, Faculty of Medicine, Ain Shams University and written consent obtained from all participants with restrict confidentiality of the data after explanation of the purpose of the study, the participants were divided by random allocation computer program into two groups: **Group (A)**: The study group; who included **60** patients who received the dual trigger and **Group (B)**: The control group; who included **60** patients, age matched, who received the hCG trigger alone.

The study aimed to compare between single trigger with standard dose of hCG alone and dual triggering with the combination of GnRH agonist and hCG in IVF/ICSI cycles in improving the number of oocytes retrieved and oocyte quality.

Study Participants

The study was conducted on infertile women attending Ain Shams University assisted reproductive technology unit; fulfilling the inclusion criteria and investigations eligible for IVF/ICSI and to confirm criteria of the study.

Recruitment was done at day of trigger. At our hospital during the COVID-19 pandemic, all patients have obligatory PCR testing for COVID-19 before any operative intervention, and who had +ve results; operative interventions were cancelled.

To be noted, we started our study with 120 patients, **4** of them were dropped out from the study **(2** in the study group; after the day of OPU and **2** in the control group; one before ET and **the other** before the day of OPU) due to their +ve PCR results.

The study included patients with ages between **20** and **35** years old, undergoing **IVF/ICSI** trial using **GnRH** antagonist protocol of controlled ovarian hyper-stimulation, with expected normal ovarian response, which is defined as: Antral follicle count **(AFC)** between **3-8** for each ovary, Serum anti-mullerian hormone **(AMH)** level of **1.0-4.0 ng/mL** on cycle day **3** and Serum estradiol **(E2)** level on the day of triggering between **500-4000 pg/mL**).

While patients with body mass index, **BMI≤18 or ≥35 kg/m²**, undergoing **IVF/ICSI** trial using GnRH agonist or minimal stimulation protocols, occult ovarian failure which is defined as day-3 follicle stimulating hormone **(FSH)** concentration of **≥10 IU/L**

or serum anti-mullerian hormone **(AMH)** level of ≤ 1.0 ng/mL, either poor response to controlled ovarian hyper-stimulation **(COH)**, which is defined as a serum estradiol **(E2)** level less than **500 pg/mL** on the day of triggering or as the number of retrieved oocytes≤3, or high ovarian response, defined as an E2 level greater than **4,000 pg/mL** on the day of triggering or as the number of retrieved oocytes ≥20, presence of endocrine disorders as (diabetes mellitus, hyper-prolactinemia, thyroid dysfunction, congenital adrenal hyperplasia, Cushing syndrome, or polycystic ovary syndrome) or presence of uterine anomaly confirmed by either hystero-salpingography or hysteroscopy were excluded from the study.

Ovarian Stimulation Protocol

All patients began controlled ovarian hyper-stimulation on day 2-3 of the menstrual cycle with a starting daily administration of human menopausal gonadotropin hMG **(Menogon 75IU, Ferring Pharmaceutical,Ltd, Germany)**, or highly purified hMG **(Menopur 75IU, Ferring Pharmaceutical, Ltd, UK, or Merional 150IU, IBSA Pharmaceutical, Switzerland)**, or highly purified FSH **(Fostimon 150IU, IBSA Pharmaceutical, Switzerland)** or with recombinant FSH rFSH **(Gonapure 150IU, Mina Pharm pharmaceuticals, Egypt)** intramuscularly for 4–5 days, and continued until the day of final oocyte maturation injection.

The starting dosage was determined according to patient age, **AFC, BMI**, serum **FSH** on day 2–3, and previous ovarian response to COH. The dose was adjusted on the basis of serum estradiol and follicular growth, and monitored by serial trans-vaginal ultrasound.

After at least one follicle had reached 14 mm in diameter, or on reaching the number of ten follicles, patients also began subcutaneous injection of GnRH antagonist, cetrorelix **(Cetrotide; Merck Serono, S.P.A-Italy)** at a dosage of 0.25 mg per day along with the HMG/FSH. GnRH antagonist administration was continued until the trigger day for final oocyte maturation.

When at least two leading follicles reached 18 mm in diameter, final oocyte maturation was triggered in (recruitment point): Group A, by single dose of hCG **5,000 IU (Choriomon, IBSA Pharmaceutical, Switzerland)** plus 0.2 mg of triptorelin acetate **(Decapeptyl, Ferring Pharmaceuticals, Germany)**. Group B, by standard dose of hCG 10,000 IU **(Choriomon, IBSA Pharmaceutical, Switzerland)** alone.

These dose adjustments were planned to achieve the induction of an endogenous LH surge that would coincide with the LH-like effect of the standard hCG administration 34–36 hours before oocyte retrieval.

Serum LH, E2 and progesterone levels were assessed the day after trigger to ensure adequate LH surge response and hCG absorption. Oocyte retrieval was done under general anesthetic using a starting pressure of 180 mmHg. Oocyte retrieval and embryos transfer procedures were performed only by the senior supervisor. All embryo transfers were performed 72 hours after oocyte retrieval. The remaining viable embryos were cultured to the blastocyst stage and were cryopreserved.

Luteal Phase Support and Confirmation of Pregnancy

The luteal phase support included daily vaginal supplementation of progesterone 400mg **(Cyclogest, Actavis pharmaceutical, UK)** starting on the day of oocyte retrieval.

Serum β-hCG was measured 14 days after embryo transfer, and a value above 5 IU/mL considered being a positive pregnancy. The luteal support was continued until the 10th week of gestation after the establishment of luteal-placental shift for all positive pregnancies.

Outcome Variables

The study's main outcome variable was the Implantation rate, defined as the number of gestational sacs on ultrasound at 6 weeks divided by total number of embryos transferred x 100.

Other analyzed variables included the oocyte number and stage of maturity, the fertilization rate defined as the percentage of transformation of micro injected oocyte into two pronuclie, the clinical pregnancy rate, the incidence of sever OHSS, and embryo transfer cancellation rate.

Clinical pregnancy was defined as viable pregnancy when there is evidence of gestational sac with fetal heart beat by trans-vaginal ultrasound between the 5th to 6th weeks of gestation.

Embryo transfer cancellation, defined as discontinuation of embryo transfer due to fertilization failure or embryonic cleavage arrest.

Statistical Analysis

Data collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis performed using the Statistical Package of Social Science (SPSS) software version 22 in windows 7 (SPSS Inc., Chicago, IL, USA). Simple descriptive analysis in the form of numbers and percentages of qualitative data, and arithmetic means as central tendency measurement, standard deviations as a measure of dispersion of quantitative parametric data. Quantitative data included in the study first tested for normality by One-Sample Kolmogorov-Smirnov test in each study group then inferential statistic tests selected.

- For quantitative parametric data:

- **Independent samples t-test** was used to compare quantitative measures between two independent groups

- For quantitative non parametric data

The Mann-Whitney test used to compare two independent groups.

- For qualitative data

- **Chi square test** used to compare between two of more than two qualitative groups.
- **The P-value< 0.05** was considered as statistical significant.

Results

Table (1): Comparison of demographic characteristics differences of study groups.

Variables	Cases (N=60)		Control (N=60)		P-value	Sig.
	Mean	SD	Mean	SD		
Age (years)	30.35	4.4	29.6	3.8	0.32	NS
BMI (kg/m^2)	21.88	2.2	22.58	2.4	0.08	NS

The table illustrates that there is no statistically significant difference with p-value >0.05 between both study groups as regards age and BMI which indicates proper matching between groups.

Table (2): Comparison of infertility characters in different study groups:

Variables	Cases (N=60)		Control (N=60)		P-value	Sig.
	No.	%	No.	%		
Type of infertility						
1 ry	25	41.7%	35	58.3%	0.1	NS
2 ry	35	58.3%	25	41.7%		
Cause of infertility						
Male	21	35%	28	46.7%	0.4	NS
Female	17	28.3%	18	30%		
Mixed	4	6.7%	2	3.3%		
Unexplained	18	30%	12	3.3%		
Infertility duration						
Mean ± SD	3.45 ± 1.9		4.26 ± 2.6		0.1	NS

The table illustrates that there is no statistically significant difference with p-value >0.05 between both study groups as regards infertility characters (type, cause, and infertility duration), which indicates proper matching between groups.

Table (3): Comparison of hormonal profile and AFC in different study groups:

Variables	Cases (N=60)	Control (N=60)	P-value	Sig.
	mean ± SD	mean ± SD		
AFC	10.73 ± 3.57	10.77 ± 3.57	0.5	NS
Baseline FSH	6.67 ± 2.1	6.93 ± 2.37	0.35	NS
Baseline LH	4.58 ±2.03	4.78 ± 2.3	0.61	NS
Baseline E2	57.4 ± 13.2	52.6 ± 17.2	0.08	NS
Baseline TSH	2.18 ± 0.96	2.09 ± 0.89	0.54	NS
Baseline Prolactine	12.76 ± 4.9	12.22 ± 5.4	0.56	NS
AMH	1.94 ± 0.63	2.11 ± 0.85	0.63	NS

The table illustrates that there is no statistically significant difference with p-value >0.05 as regards hormonal profile (FSH, LH, E2, TSH, prolactin, and AMH level) and AFC.

Table (4): Comparison of type and dose of Gonadotropins injection in different study groups:

Type of injection	Cases (N=60)		Control (N=60)		P-value	Sig.
	No.	%	No.	%		
HMG	8	13.3%	6	10%	0.68	NS
Highly purified HMG	16	26.7%	15	25%		
Highly purified FSH	17	28.3%	23	38.3%		
Recombinant FSH	19	31.7%	16	26.7%		
Dose of injection						
Mean ± SD	49.7 ± 9.4		49.3 ± 17.9		0.9	NS

The table illustrates that there is no statistically significant difference with p-value >0.05 between study groups as regards type and dose of Gonadotropins injection used.

Table (5): Comparison of intervention outcomes in different study groups:

Variables	Cases (N=60)	Control (N=60)	P-value	Sig.
	mean ± SD	mean ± SD		
Number of oocyte retrieved	11.42 ± 4.2	9.8 ± 4.9	0.01	S
Number of MII oocyte retrieved	6.2 ± 2.7	4.6 ± 3.1	0.002	HS
Number of fertilized oocyte	4.03 ± 2.2	3.05 ± 2.5	0.01	S
Number of embryos transferred	1.9 ± 1.01	1.7 ± 1.2	0.48	NS
Number of cryopreserved embryos	1.33 ± 1.08	1.1 ± 1.4	0.08	NS

The table illustrates that there is a statistically significant difference with p-value <0.05 between study groups as regards number oocyte retrieved (dual trigger: 11.42±4.2 vs. single trigger: 9.8±4.9), Number of MII oocyte retrieved (dual trigger: 6.2±2.7 vs. single trigger: 4.6±3.1) and Number of fertilized oocyte (dual trigger: 4.03±2.2 vs. single trigger: 3.05±2.5) with higher mean among dual trigger group.

On the other hand, there is no statistically significant difference with p-value >0.05 as regards other variables (number of embryos transferred and number of cryopreserved embryos) between dual trigger and single trigger groups.

Table (6): Comparison of outcomes in different study groups:

Outcomes		Cases (N=60)		Control (N=60)		P-value	Sig.
		No.	%	No.	%		
Biochemical pregnancy							
Positive		41	68.3%	20	33.3%	0.001	HS
Negative		12	20%	27	45%		
Complication		7	11.7%	13	21.7%		
Clinical pregnancy							
Abortion rate (%)		6	14.6% (6/41)	1	5% (1/20)	0.4	NS
Pregnancy	Single	22	58.3% (35/60)	15	31.7% (19/60)	0.003	HS
	Twin	13		4			
Implantation rate							
Implantation rate		41.3% (48/116)		21.4% (23/107)		0.02	S

The table illustrates that there is a statistically significant difference with p-value <0.05 between study groups as regards biochemical pregnancy rate (cases: **68.3%vs. 33.3%** among controls), clinical pregnancy rate (cases: **58.3% vs. 31.7%** among controls), and implantation rate (cases: **41.3% vs. 21.4%** among controls), with higher percentage among the dual trigger group of study.

Table (7): Comparison of complications in different study groups:

Variables	Cases (N=60)		Control (N=60)		P-value	Sig.
	No.	%	No.	%		
ET cancellation	7	11.7% (7/60)	12	20% (12/60)	0.2	NS
Sever OHSS	0	0%	1	1.7% (1/60)	0.3	NS

The table illustrates that there is no statistically significant difference with p-value >0.05 between study groups as type of complications.

Discussion

Numbers of retrospective cohort studies [16,17,18], and few numbers of randomized controlled studies [19,20] have investigated whether dual triggering of final oocyte maturation with a gonadotropin-releasing hormone agonist (GnRH-a) and standard dose of human chorionic gonadotropin (hCG) can improve clinical outcomes for normal ovarian, subnormal and poor responders in GnRH antagonist cycles, however it's still debated between researchers who found significant improvement of the outcomes of IVF/ICSI cycles and those who didn't find a significant improvement [21,22].

A total 120 patients were recruited in the current study, randomized and divided into two groups; each group consisted of 60 patients, where the study group received the dual trigger, and the control group received the standard dose hCG trigger alone for final oocyte maturation, in a trial of improving number of oocytes retrieved& its quality and eventually IVF/ICSI clinical outcomes compared to single triggers in women with normal ovarian response undergoing (IVF/ICSI) technique.

In the current study, the baseline characteristics and demographics (mean age& BMI), infertility characters (type, cause & duration of infertility), hormonal profile, AFC, and type and dose of gonadotropins injection where all incomparable between study groups.

As regards to the interventional main outcome, there was a statistically significant difference with p-value <0.05 between study groups as regards number oocyte retrieved (dual trigger: 11.42±4.2 vs. single trigger: 9.8±4.9), number of MII oocyte retrieved (dual trigger: 6.2±2.7 vs. single trigger: 4.6±3.1) and number of fertilized oocyte (dual trigger: 4.03±2.2 vs. single trigger: 3.05±2.5) with higher mean among dual trigger group.

As regards to the outcomes of biochemical pregnancy, implantation and clinical pregnancy rates, there was a statistically significant difference with p-value <0.05 between study groups as regards biochemical pregnancy rate (cases: 68.3%vs. 33.3% among controls), clinical pregnancy rate (cases: 58.3% vs. 31.7% among controls), and implantation rate (cases: 41.3% vs. 21.4% among controls), with higher percentage among the dual trigger group of study.

In the present study, the baseline characteristics and demographics showed no statistically significant difference between the study and control groups (Table 1), with p-value>0.05 regarding mean age (y) (30.35 ± 4.4 vs. 29.6 ± 3.8 respectively), and body mass index (BMI) (kg/m^2) (21.88 ± 2.2 vs. 22.58 ± 2.4 respectively); which agreed with all of the studies done before like Lin et al., (2013), where mean age (y) (34.81 ± 3.70 vs. 34.68 ± 3.44 respectively), and body mass index (BMI) (kg/m2) (22.2 ± 5.4 vs. 22.0 ± 3.1 respectively) were comparable between the study and control groups respectively.

Zhou et al. [18] conducted a study comparing dual trigger with combination of GnRH agonist and hCG versus hCG alone trigger for oocyte maturation in normal ovarian responders; where there was no statistically significant difference between the study groups as regards infertility characters in terms of the percentage of the cause of infertility whether male factor (cases; 9.8% vs. control; 5.9%), female factor (cases;63.4% vs. control;50.5%), mixed (cases; 12.5% vs. control; 25.7%) or unexplained infertility (cases;5.4% vs. control; 2.0%), or infertility duration; with mean between the study group and control was (4.55± 3.23 vs. 5.92± 4.34 respectively).

In the current study, there was no statistically significant difference with p-value >0.05 between both study groups as regards to infertility characters (Table 2) in terms of [type of infertility; where the percentage between the study and control groups was (41.7% vs. 58.3% respectively) in primary type and (58.3% vs. 41.7% respectively) in secondary

type, cause of infertility; with percentage related to male factor (35% in cases vs. 46.7% in controls), female factor (28.3% in cases vs. 30% in controls), mixed (6.7% in cases vs. 3.3% in controls) or unexplained infertility with percentage of (30% in cases vs. 20% in controls), or infertility duration; with mean (cases; 3.45± 1.9 vs. control; 4.26± 2.6)], which indicated proper matching between groups.

To exclude any hormonal disturbance factor that may affect the purpose of the study, it was essential to study the hormonal profile of both study groups with special emphasis on FSH, LH, Estradiol, TSH, AMH and Prolactin levels which all showed no statistically significant differences between study groups (Table 3); with p-value >0.05, where the mean FSH among cases was (6.67±2.1), while in control group was (6.93±2.37). Mean LH among cases was (4.58±2.03), while in control group was (4.78±2.3). Mean TSH among cases was (2.18±0.96), while in control group was (2.09±0.89). Mean Estradiol among cases was (57.4±13.2), while in control group was (52.6±17.2). Mean AMH among cases was (1.94±0.63), while in control group was (2.11±0.85), and mean Prolactin among cases was (12.76±4.9), while it was (12.22±5.4) in control group.

Also, there was no statistically significant difference with p-value >0.05 between study groups as regards mean antral follicle count (AFC) (Table 3) (10.73±3.57 in study group vs. 10.77±3.57 in control group) or COH variables (Table 4) such as: type of gonadotropins used for injection, where percentage of HMG was (13.3% in cases vs. 10% in control), percentage of Highly purified HMG was (26.7% in cases vs. 25% in control), Highly purified FSH was (28.3% in cases vs. 38.3% in control) and Recombinant FSH was (31.7% in cases vs. 26.7% in control), or mean total dose of gonadotropins used (49.7±9.4 in study group vs. 49.3±17.9 in control group), which all actually agreed with previous studies of Lin et al., Griffin et al., and Zhou et al.

In a previous prospective randomized study [19], 221 normal responder patients were randomized either to receive hCG or dual trigger for final oocyte maturation. There was no statistical difference between the study and control groups as regards to the number of oocytes retrieved (9.9±7.8 vs. 7.9±11.1 respectively). However; the results in our present study showed statistically significant difference with p-value <0.05 between study groups (Table 5) as regards to the number of retrieved oocytes (cases: 11.42±4.2 vs. control: 9.8±4.9).

In the current study, the number of MII retrieved oocytes (cases: 6.2 ± 2.7 vs. control: 4.6 ± 3.1), and number of fertilized oocytes (cases: 4.03 ± 2.2 vs. control: 3.05 ± 2.5) showed statistical significant difference with p-value <0.05 with higher mean in the dual group which came in agreement with Hass et al. (2020), who conducted a prospective, randomized, double-blinded clinical trial on 155 normal responder patients either to receive hCG or dual trigger for final oocyte maturation where there was statistical difference between the study and control groups as regards to the number of MII retrieved oocytes (cases:10.3 vs. controls:8.6, p-value=0.009), and number of 2 pronuclie (cases:7.8 vs. control: 6.3,p-value=0.007) with higher significance among the dual trigger group.

On the other hand, there was no statistically significant difference with p-value >0.05 as regards other variables; the mean number of embryos transferred (1.9 ± 1.01 in cases vs. 1.7± 1.2 in control) and number of cryopreserved embryos (1.33±1.08 in cases vs. 1.1 ± 1.4 in control) between dual trigger and single trigger groups.

In terms of the main present study outcomes, the dual-trigger group demonstrated a significantly higher percentage as regards to biochemical pregnancy rate (cases: 68.3% vs. 33.3% among controls), clinical pregnancy rate (cases: 58.3% vs. 31.7% among controls), and implantation rate (cases: 41.3% vs. 21.4% among controls) with a statistical-

ly significant difference with p-value <0.05 between study groups (Table 6). The difference in abortion rate between the two groups was not statistically significant.

These results actually came in agreement with Hass et al. (2020) study, where their results showed statistically significant improvement in the implantation rate (22.8% vs. 43.7%), and the clinical pregnancy rate (37.3% vs. 56.8%) with significantly higher percentages in the dual trigger group.

Conversely, Şükür et al., [22] conducted a retrospective cohort study in a total 214 normal re¬sponders who underwent ICSI trial following a cycle down-regulated by a GnRH antagonist protocol. The biochemical pregnancy rate (33.9 in cases vs. 36.5% in control), and clinical pregnancy rate (33.9% in cases vs. 30.6% in control) were similar among both study groups.

Also Eser et al., [21] conducted a case-control study of a total 109 ICSI cycles "in poor responders" where a dual trigger was used for final oocyte maturation compared with hCG trigger, where they reported no statistically significant difference between ICSI outcomes as regards to biochemical pregnancy rate (in cases 16% vs. 12.1% in control), Clinical pregnancy rate (4% in cases vs. 12.1% in control), and implantation rate (3.2% in cases vs. 9.3% in control).

In our present study, (Table 7) there was no statistically significant difference with p-value >0.05 between study groups as regards to complications. One out of 13 patients of the control group had sever OHSS, but she did not require hospitalization, and none occurred in the dual trigger group. Nine patients of the control group out of the 60 participants had their ET cycle cancelled due to failed fertilization, one had ET cycle cancelled due to oocyte degeneration and 2 had ET cycle cancelled due to +ve. COVID-19 PCR, where 7 patients in the study group out of the 60 had their ET cycle cancelled, 3 out of 7 due to arrest of cleavage, 2 due to failed fertilization and 2 had ET cycle cancelled due to +ve. COVID-19 PCR.

One of the weaknesses of the present study is that we did not include a third arm of patients who were triggered with GnRH agonist alone. If we had added the third arm, we would have been able to test whether it was the administration of GnRH agonist or the co-administration of GnRH agonist and hCG that improved the outcome as demonstrated in the study.

Conclusion

In conclusion, in terms of the number of mature retrieved oocytes, implantation rate and clinical pregnancy rate in normal responders undergoing IVF/ICSI using antagonist protocols, a dual-trigger approach with a GnRH agonist and the standard dosage of hCG was found to be significantly superior to an hCG trigger alone.

The results we presented here are another proof-of-concept that suggests a possible paradigm shift in ovulation triggering agents in GnRH antagonist cycles.

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