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Article

Comparing two protocols for final oocyte maturation in poor responders undergoing GNRH-antagonist ICSI cycles: a randomized controlled trial

Elsayed Ahmed Elsayed[1], Mohamed Salah Abd Rabbo[1], Mervat Sheikh El-Arab Elsedeek[1], Sherif Salah Elsayed Gaafar[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Alexandria University

***Correspondence:** Elsayed Ahmed Elsayed, Department of Obstetrics and Gynecology, Faculty of Medicine, Alexandria University**; Email address:** elsayed.ahmed549@gmail.com

Abstract. *Background:* Poor ovarian responders (POR) include a significant proportion of women referred for IVF treatments (ranging from 9 to 24 %), most of whom are in late reproductive age. Attempts to improve IVF cycle outcomes for poor responders. Final oocyte maturation trigger is one of the most important key success factors in assisted reproductive technologies (ARTs). The authors in this study sought to investigate the role of daual trigger in final oocyte maturation in poor responders undergoing GnRH-antagonist ICSI cycles. *Methods:* A prospective randomized controlled trial conducted on 160 poor ovarian responders indicated for ICSI using a GnRH-antagonist protocol. They were randomized to either group A or group B.Group A received 10000 IU of hCG (Choriomon5000 IU; IBSA) given intramuscularly while group B received 10000 IU of hCG (Choriomon5000 IU; IBSA) intramuscular injection in addition to the GnRH agonist triptorelin 0.2 mg (Decapeptyl 0.1 mg; Ferring) subcutaneously for triggering of ovulation. The primary outcome parameter was the number of metaphase II oocytes retrieved. Secondary outcomes included the total number of oocytes, ratio between number of follicles seen on day of trigger and number of oocytes retrieved, maturity index, fertilization rate. *Results:* Dual trigger was associated with higher number of fertilized oocytes (3.3±1.81 vs. 3.92±1.90, p=0.039) , fertilization rate (80.82±23.04 vs 91.86±15.62, p=0.002) and maturity index (81.5% vs 86.9%, p=0.043). There were no significant differences in terms of number of number of oocytes retrieved, FOI % and number of metaphase 2 oocytes. *Conclusion:* Dual trigger was associated with better oocyte competence in poor responders compared with single trigger.

Keywords: Dual trigger; GnRH-antagonist protocol; ICSI; Poor responders

Introduction

Poor ovarian responders (POR) include a significant proportion of women referred for IVF treatments (ranging from 9 to 24 %), most of whom are in late reproductive age.(1, 2) According to the "Bologna criteria", patients are classified as POR based on three conditions: if two or more of the following features are present: 1) advanced maternal age (>40 years); 2) a previous poor ovarian response (cycles cancelled or <3 oocytes with a conventional protocol); 3) an abnormal ovarian reserve test (antral follicle count 5-7 follicles or anti-Mullerian hormone 0.5-1.1 ng/ ml). Two of these criteria are required for a POR diagnosis.

In addition, two cycles with POR after maximal stimulation are sufficient to classify a patient as a poor responder even in the absence of other criteria mentioned.(3)

In fact the live birth rate in the entire POR category is poor (about 6 % per cycle).(4, 5) however patients <40 years have a significantly better prognosis compared to older patients, mainly due to better oocyte quality.(6)

Attempts to improve IVF cycle outcomes for poor responders included modifying the steps of ovarian stimulation protocols, such as different luteal phase pretreatments, increasing ovarian stimulation doses, as well as addition of various supplements. So far, most of the modifications had limited success, therefore, optimal protocol for poor responders has remained elusive.(7)

ESHRE in 2019 stated GnRH antagonists and GnRH agonists are equally recommended for predicted low responders.(8)

Final oocyte maturation trigger is one of the most important key success factors in assisted reproductive technologies (ARTs). Oocyte maturation refers to a release of meiotic arrest that allows oocytes to advance from prophase I to metaphase II of meiosis. LH surge by dismantling the gap junctions between granulosa cells and oocyte inhibits the flow of maturation inhibitory factors into ooplasm and causes drop in concentration of cAMP.

Decreased concentration of cAMP in turn increases concentration of Ca and maturation-promoting factor (MPF), which are essential for the resumption of meiosis in oocyte and disruption of oocyte-cumulus complex triggering follicular rupture and ovulation about 36 h the LH surge.(9)

Until now, administering 5000IU to 10,000IU of hCG 34–36h prior to oocyte retrieval remained the standard protocol for the induction of final oocyte maturation in IVF cycles worldwide. Traditionally, human chorionic gonadotropin (hCG) has been the trigger of choice for oocyte maturation due to its molecular and biological similarity with LH.(10)

Gonadotropin-releasing hormone (GnRH) agonists were first suggested for final oocyte maturation by Gonen et al. in 1990, as it is able to trigger endogenous release of both FSH and LH.(11) With a shorter mean duration of LH surge of about 34 hours, it is similar to the natural cycle duration of 48 hours,(12) effectively reducing

the incidence of OHSS in high responders.(13, 14) However, some problems surfaced with the substitution of GnRH-agonists as trigger. The risk of empty follicle syndrome was reported to be increased following isolated GnRH-agonist trigger due to a suboptimal LH surge,(15) in addition, increased early pregnancy loss and decreased rates of ongoing pregnancy were noted by multiple studies.(16, 17) As such, the idea of a dual trigger was developed.(18) Indeed, the hCG component of dual trigger could serve as a rescue trigger in case of poor response to GnRH-agonist, which occurs in about 2.71% of a study population.(19)

In combining GnRH-agonist and hCG for the final oocyte maturation, we get the benefits of both. HCG administration alone also does not produce FSH activity, while GnRH-agonist releases an endogenous FSH and LH surge, resulting in a more physiologic response.

In addition, another proposed advantage with dual trigger is potential enhancement of endometrial receptivity by the GnRH-a component. Significant elevation of both isoforms of human GnRH mRNA expression have been detected in the secretory phase of the human menstrual cycle,(20-22) indicating the possible role of these hormones in regulation of endometrial receptivity.(20, 23) Specifically, in vitro studies with human extra-villous cytotrophoblasts and decidual stroma cells have demonstrated the ability of GnRH to activate urokinase type plasminogen activator, a key component in decidualization and trophoblast invasion.(24, 25) Therefore, inclusion of GnRH-a as part of luteal support regimen has been explored as a mean to improve the implantation rate.

Since its development, multiple investigations have shown the benefits of using a dual trigger for final oocyte maturation in normal responders,(16, 26) including an improvement in total number of retrieved oocytes, MII oocytes, rates of embryo implantation, clinical pregnancy, and live birth rates.(27) Evidence from available meta-analysis in 2018 involving four studies including 527 patients found a significantly improved clinical pregnancy rate following dual trigger.(28) However, for poor ovarian responders (PORs), the situation is less clear cut.

ESHRE in 2019 stated that dual triggering is not recommended in normal ovarian responders. However, there was no clear recommendation regarding PORs, giving rise to the need to perform a well-designed randomized controlled trial for the evaluation of dual triggering in PORs.(29, 30)

The aim of the study is to compare the oocyte yield, oocyte quality and the fertilization rate between dual trigger treatment (combination of gonadotrophin-releasing hormone (GnRH) agonist and human chorionic gonadotrophin) and human chorionic gonadotrophin alone in PORs undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF-ICSI) cycles using a GnRH-antagonist protocol.

Material and methods

A prospective open-label randomized controlled trial was conducted at EL-Shatby Main University Hospital, Alexandria university and private ART centers between october 2021 and December 2022. The study was approved by Alexandris University Ethical Review Board. An informed written consent was signed by all participants after explanation of the aim, benefits, and risks of the study. All participants also gave their consent for publication. The participants were POR candidates for ICSI. Poor ovarian response was defined according to Bologna criteria with the presence of at least two of the following three criteria: (1) advanced female age (40 years or older) or presenceof other risk factors for poor response; (2) poor response in a previous cycle with production of three or less oocytes after stimulation with a conventional stimulation protocol; and (3) low ovarian reserve test (antral follicle count of five to seven follicles or anti-Müllerian hormone [AMH] levels of 0.5–1.1 ng/mL). (3)

Inclusion criteria included women with a spontaneous normal menstrual cycle, a normal uterine cavity (evaluated by hysterosalpingography or hysteroscopy), Body mass index (BMI) <35, Age less than 45, AMH ≤1.1 ng/ ml and AFC ≤7 follicles. Exclusion criteria included women with ovarian cysts, endometriosis, communicating hydrosalpinx, those with endocrinologic disorders such as hyperprolactinemia, thyroid or adrenal disorders, Couples with an azoospermic male partner and those with severe uncontrolled medical or metabolic disorders were also excluded.

Before enrollment in the study, All participants were evaluated through full history with special concerns about age, duration and cause of infertility, full examination, and basal transvaginal ultrasound assessment to ensure adherence to strict inclusion and exclusion criteria. Then women were randomized using computer-based randomization (Random Digit Software).

All included women underwent a fixed GnRH antagonist protocol of COH. COS was initiated on the second day of the menstrual cycle by administration of (FSH and HMG) in a total dose of 300 IU daily for 5days. Follow up visit was done after 5 days to assess the degree of elevation of serum E2, the thickness and pattern of the endometrium and the size and number of the growing follicles.

GnRH antagonist; cetrorelix acetate 0.25 mg (cetrotide; Merck Serono) was given daily starting on stimulation day 6- regardless of the size of the dominant follicles by subcutaneous route "fixed antagonist protocol" to suppress endogenous luteinizing hormone.

Follow up was done repeatedly every two days with ultrasonography and E2 analysis and the doses of FSH and HMG were adjusted according to the individual response of each patient till the leading follicles reach 18 mm or more in size, then serum progesterone level was tested and the trigger was given.

Cycle cancellation was did if folliculometry on day 8 revealed no growing follicles, serum estradiol level less than 150 pg/mL on the day of hCG administration, no oocytes were retrieved, or if fertilization failed.

At the day of triggering, number of follicles and the number of oocytes expected to be retrieved were documented and women were given the trigger according to the randomization done at the enrollment.

Two main groups will be created depending on the trigger protocol used:

Group A: 80 subjects were triggered by 10000 IU of hCG (Choriomon5000 IU; IBSA) given intramuscularly.

Group B: 80 subjects were triggered by 10000 IU of hCG (Choriomon5000 IU; IBSA) intramuscular injection in addition to the GnRH agonist triptorelin 0.2 mg (Decapeptyl 0.1 mg; Ferring) subcutaneously.

Oocyte retrieval was performed by ultrasound-guided vaginal follicle aspiration under a strictly aseptic technique 36 hours after giving the trigger.

Luteal phase support was started in all women on the day of oocyte retrieval and continued until the day of serum β-hCG assessment (14 days after embryo transfer) through administration of 400 mg of natural progesterone (Prontogest 400 mg pessary; Marcyrl) twice daily per vagina and 100 mg intramuscular progesterone(Prontogest 100mg amp; Marcyrl) once daily. Serum progesterone was measured on day 6 of transfer. Beta human chorionic gonadotropin (β-hCG) was measured 14days after embryo transfer for evaluating chemical pregnancy. Clinical pregnancy was confirmed by transvaginal ultrasound at two weeks later. Then, luteal support was continued through administration of 400 mg of natural progesterone (Prontogest 400 mg pessary; Marcyrl) twice daily per vagina until the 10th weeks of gestation.

The primary outcome parameter was the number of metaphase II oocytes retrieved. Secondary outcomes included the total number of oocytes, ratio between number of follicles seen on day of trigger and number of oocytes retrieved, maturity index, fertilization rate.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

The used tests were: **1. Chi-square test:** For categorical variables, to compare between different groups; **2. Fisher's Exact or Monte Carlo correction:** Correction for chi-square when more than 20% of the cells have expected count less than 5; **3. Student t-test:** For normally distributed quantitative variables, to

compare between two studied groups; **4. Mann Whitney test:** For not normally distributed quantitative variables, to compare between two studied groups.

Sample size calculation

Sample size was estimated at Medical Research Institute, Department of Biomedical Informatics & Medical Statistics using PASS Version 20 Program. Based on a recently published study, it was proven that Dual triggering is associated with better IVF outcome in poor responders compared with single trigger by about 17%.Thus, the minimal hypothesized sample size of 160 eligible female patients is needed to assess the effect of dual triggering of final oocyte maturation with a combination of a GnRH agonist and hCG on the number and the quality of retrieved oocytes and the effect on clinical pregnancy rate; taking into consideration 95% confidence level and 80% power using Chi Square-test.

Results

One hundred and sixty women were included in the study, with 80 women in the single trigger group and 80 women in the dual trigger group. 15 cycles excluded in the hCG-only group and 4 cycles were excluded in the dual-trigger group due to no response, no retrieved oocytes, no methaphase 2 oocytes or no fertilization. A flow chart of the participants is shown in (Figure 1).

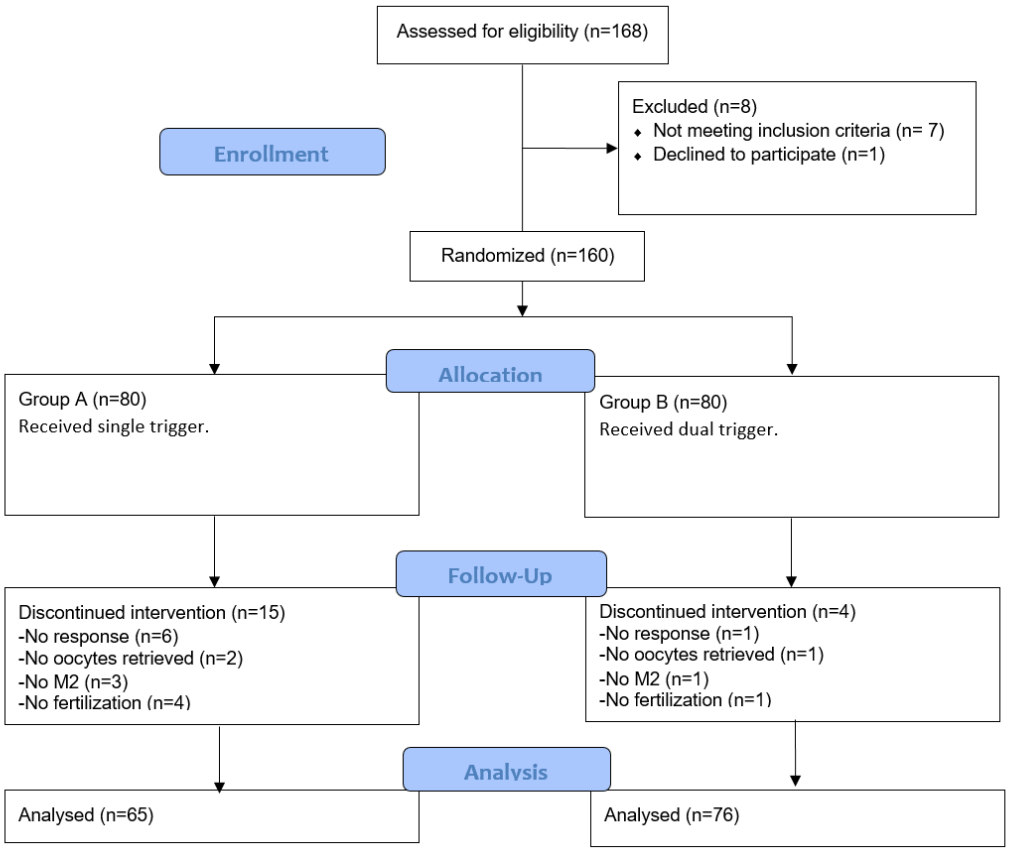

Figure (1): A flow chart of the participants.

Table (1): Comparison between the two studied groups according to demographic data and baseline characteristics

	Group A (n = 80)	Group B (n = 80)	Test of Sig.	p
Age (/years)				
Min. – Max.	23.0 – 44.0	22.0 – 43.0	t= 1.197	0.233
Mean ± SD.	34.18 ± 4.87	33.21 ± 5.29		
Median (IQR)	34.0 (30.5 – 38.0)	34.0 (29.5 – 37.0)		
BMI (kg/m²)				
Min. – Max.	19.0 – 33.0	20.0 – 34.0	t= 1.525	0.129
Mean ± SD.	26.33 ± 3.28	27.11 ± 3.20		
Median (IQR)	26.0 (24.0 – 28.5)	27.5 (25.0 – 30.0)		
Type of Infertility				
1ry	69 (86.3%)	71 (88.8%)	χ^2= 0.229	0.633
2ry	11 (13.8%)	9 (11.3%)		
Duration of Infertility				
Min. – Max.	1.0 – 20.0	1.0 – 17.0	U= 2749.5	0.121
Mean ± SD.	6.21 ± 4.0	5.44 ± 3.95		
Median (IQR)	5.0 (3.0 – 9.0)	4.0 (2.0 – 7.50)		
TSH				
Min. – Max.	0.33 – 4.10	0.23 – 4.0	t= 1.384	0.168
Mean ± SD.	1.91 ± 0.80	2.10 ± 0.87		
Median (IQR)	1.90 (1.30 – 2.30)	2.10 (1.35 – 2.70)		
PRL				
Min. – Max.	2.50 – 50.0	2.70 – 54.0	U= 2725.50	0.105
Mean ± SD.	15.87 ± 8.10	17.55 ± 8.13		
Median (IQR)	14.0 (10.8 – 18.9)	16.35 (12.0 – 22.0)		
LH				
Min. – Max.	1.0 – 13.60	1.0 – 8.0	U= 2948.50	0.384
Mean ± SD.	4.48 ± 2.34	3.99 ± 1.69		
Median (IQR)	4.0 (3.0 – 5.0)	4.0 (3.0 – 5.0)		

AMH				
Min. – Max.	0.20 – 1.10	0.10 – 1.10	U= 3163.0	0.899
Mean ± SD.	0.71 ± 0.30	0.72 ± 0.25		
Median (IQR)	0.76 (0.40 – 1.0)	0.70 (0.50 – 0.92)		
AFC				
Min. – Max.	3.0 – 7.0	2.0 – 7.0	U= 2726.0	0.077
Mean ± SD.	6.06 ± 1.04	6.31 ± 1.01		
Median (IQR)	6.0 (5.0 – 7.0)	7.0 (6.0 – 7.0)		

Comparisons between the populations of two groups revealed no difference in patient age, body mass index, infertility duration, primary or secondary infertility, TSH, Prolactin, basal luteinizing hormone (LH), AFC and AMH (Table 1).

Cycle characteristics between the two groups are presented in (Table 2). There were significant differences in terms of stimulation duration(10.73 ± 1.79 vs 11.55 ± 1.86, p = 0.006) and total gonadotropin dose (6427.5 ± 1089.3 vs 6930 ± 1117, p = 0.006).

There were no significant differences in terms of number of follicles at trigger, FORT% and serum estradiol on day of trigger.

Table (2): Comparison between the two studied groups according to cycle characteristics.

	Group A	**Group B**	**Test of Sig.**	**P**
Number of days	**(n = 80)**	**(n = 80)**		
Min. – Max.	7.0 – 14.0	7.0 – 15.0	U= 2403.0*	0.006*
Mean ± SD.	10.73 ± 1.79	11.55 ± 1.86		
Median (IQR)	11.0 (9.0 – 12.0)	12.0 (10.0 – 13.0)		
Daily dose	**(n = 80)**	**(n = 80)**		
600	80 (100.0%)	80 (100.0%)	–	–
Total dosage	**(n = 80)**	**(n = 80)**		
Min. – Max.	3600.0 – 8400.0	4200.0 – 9000.0	U= 2402.50*	0.006*
Mean ± SD.	6427.5 ± 1089.3	6930.0 ± 1117.0		
Median (IQR)	6600.0 (5400.0 – 7200.0)	7200.0 (6000.0 – 7800.0)		
No. follicles at trigger	**(n = 80)**	**(n = 80)**		
Min. – Max.	0.0 – 7.0	0.0 – 7.0	U= 2661.5	0.057
Mean ± SD.	5.25 ± 1.93	5.81 ± 1.42		
Median (IQR)	6.0 (5.0 – 7.0)	6.0 (5.0 – 7.0)		
Non-Responder (Cancelled) [0]	6 (7.5%)	1 (1.3%)	χ^2= 3.735	FEp= 0.117
Responder	74 (92.5%)	79 (98.8%)		

FORT (%)	**(n = 74)#**	**(n = 79)#**		
Min. – Max.	0.0 – 100.0	0.0 – 100.0	U= 3124.5	0.765
Mean ± SD.	86.41 ± 27.12	90.94 ± 16.54		
Median (IQR)	100.0 (85.7 – 100.0)	100.0 (85.7 – 100.0)		
E2 day of trigger	**(n = 74)#**	**(n = 79)#**		
Min. – Max.	270.0 – 3105.0	352.0 – 1768.0	U= 2672.50	0.360
Mean ± SD.	1059.6 ± 493.6	1104.8 ± 382.4		
Median (IQR)	967.5 (675.0 – 1425.0)	1183.0 (820.0 – 1399.0)		

IQR: **Inter quartile range;** SD: **Standard deviation; U: Mann Whitney test;** χ^2: **Chi square test; FE: Fisher Exact** p: p value for comparing between the two studied groups; *: Statistically significant at $p \leq 0.05$; #: Cancelled cases were excluded

Cycle outcomes between the two groups are presented in (Table 3). There were no significant differences in terms of number of number of oocytes retrieved, FOI % and number of metaphase 2 oocytes.

Table (3): Comparison between the two studied groups according to cycle outcomes

	Group A	**Group B**	**Test of Sig.**	**P**
No. oocytes retrieved	**(n = 74)#**	**(n = 79)#**		
Min. – Max.	0.0 – 7.0	0.0 – 7.0	U= 2820.50	0.704
Mean ± SD.	4.74 ± 1.84	4.84 ± 1.91		
Median (IQR)	5.0 (4.0 – 6.0)	5.0 (3.0 – 6.50)		
Not ovulated	72 (90.0%)	78 (97.5%)	χ^2= 0.410	^{FE}p= 0.610
Ovulated before OPU[0]	2 (2.5%)	1 (1.3%)		
FOI (%)	**(n = 72)$**	**(n = 78)$**		
Min. – Max.	20.0 – 100.0	20.0 – 100.0	U= 2701.0	0.681
Mean ± SD.	78.64 ± 20.83	75.72 ± 23.96		
Median (IQR)	83.33 (71.4 - 100)	84.52 (60.0 – 100)		
M2 No.	**(n = 72)@**	**(n = 78)@**		
Min. – Max.	0.0 – 7.0	0.0 – 7.0	U= 2586.50	0.398
Mean ± SD.	3.97 ± 1.71	4.26 ± 1.94		
Median (IQR)	4.0 (3.0 – 5.0)	4.0 (3.0 – 6.0)		
No M2 [0]	3 (4.2%)	1 (1.3%)	χ^2=1.200	^{FE}p=0.351
Maturity Index (%)	**(n = 69)♦**	**(n = 77)♦**		
	286/351 (81.5%)	332/382 (86.9%)	χ^2=4.077*	0.043*
No. Fertilized oocytes	**(n = 69)♦**	**(n = 77)♦**		
Min. – Max.	0.0 – 7.0	0.0 – 7.0	U= 2135.5*	0.039*
Mean ± SD.	3.28 ± 1.81	3.92 ± 1.90		
Median (IQR)	3.0 (2.0 – 4.0)	4.0 (3.0 – 5.0)		
No Fertilization	4 (5.8%)	1 (1.3%)		

Fertilization	**65 (94.2%)**	**76 (98.7%)**	χ^2= 2.226	^{FE}p= 0.189
Fertilization rate (%)	**(n = 65)**	**(n = 76)**		
Min. – Max.	25.0 – 100.0	33.0 – 100.0	U= 1821.0*	0.002*
Mean ± SD.	80.82 ± 23.04	91.68 ± 15.62		
Median (IQR)	100.0 (67.0 - 100)	100.0 (84.5 - 100)		

IQR: **Inter quartile range;** SD: **Standard deviation; U: Mann Whitney test;** χ^2: **Chi square test; FE: Fisher Exact;** p: p value for comparing between the two studied groups; #: Cancelled cases were excluded; $: Cancelled and Ovulated before OPU cases were excluded; @: Cancelled and Ovulated before OPU cases were excluded; ♦: For M2 cases; *: Statistically significant at p ≤ 0.05; **FOI (%) = No. oocytes retrieved / AFC**

There were significant differences in terms of number of fertilized oocytes(3.3±1.81 vs. 3.92±1.90, *p*=0.039) and fertilization rate (80.82±23.04 vs 91.86±15.62, *p*=0.002) were significantly higher in the dual-trigger group compared with the hCG-only group.

Maturity index per group was statistically significant higher in the dual trigger group (81.5% vs 86.9%, *p*=0.043) (Figure 2).

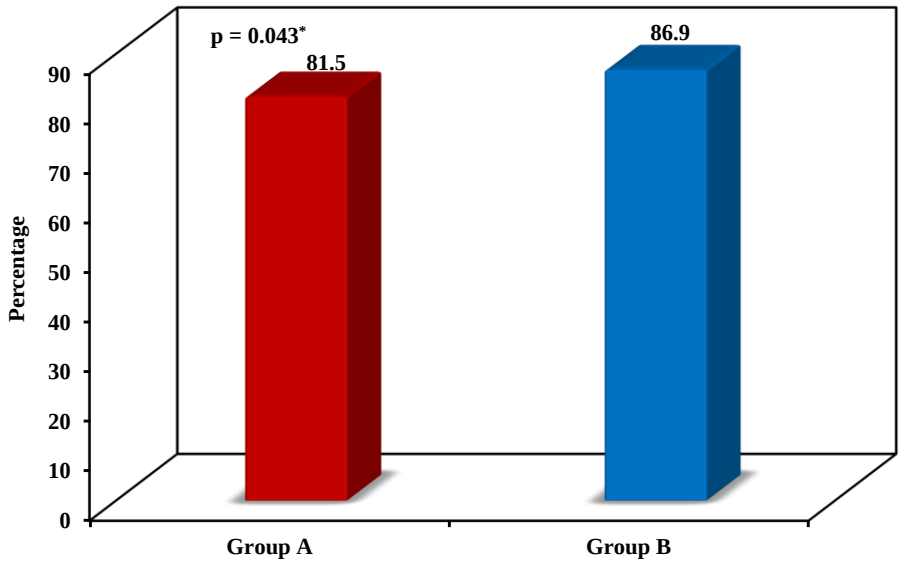

Figure (2): Comparison between the two studied groups according to maturity index.

Discussion

The use of a GnRH agonist to trigger final oocyte maturation was first offered more than 20 years ago by Gonen and colleagues but it did not gain popularity until the introduction of a GnRH-antagonist protocol in IVF to decrease the risk of ovarian hyperstimulation syndrome.(31) However, its routine use as a single trigger was associated with lower implantation, ongoing, and live birth rates,(32) effects that were linked to an inadequate luteal phase and poor endometrial receptivity.

As in our study, several trials have also studied the use of GnRH agonists in dual trigger protocols and in high responders demonstrated better live-birth and ongoing pregnancy rates and lower risk of ovarian hyperstimulation syndrome.(33) Dual triggering also improved ongoing pregnancy rates in normal responders,(34) and was successful in women suffering from empty follicle syndrome.(35)

One of the advantages of triggering with GnRH-a is the simultaneous induction of a mid-cycle FSH surge that is similar to the hormonal events in a natural ovulatory cycle. Animal studies have confirmed the importance of FSH in up regulating of luteinizing hormone (LH) receptor sites formations in granulosa cells.(36, 37) The expression of LH receptors is essential for preparing the maturing follicle for the pre-ovulatory LH surge and the luteinzation of granulosa cells. FSH also has a key role in promoting the resumption of oocyte meiosis,(38, 39) and the expansion of cumulus cells, (40, 41) all of which are critical steps in the oocyte maturation process. Therefore, one of the proposed benefits of GnRH-a triggering is the increased rate of mature oocytes retrieved.

The results of our study revealed that the mean number of metaphase II oocytes (3.97±1.71 vs 4.26±1.94, p=0.398) and retrieved oocytes (4.74±1.84 vs 4.84±1.91, p=0.704) among women of the dual trigger group was more than that of the single trigger group but not significant. However, maturity index per group was statistically significant higher in the dual trigger group (81.5% vs 86.9%, p=0.043).

Seval et al.,(42) found a significantly higher number of metaphase II and retrieved oocytes among women with dual trigger compared with those with single trigger. Similarly, Haas and colleagues demonstrated a significantly higher number of retrieved oocytes in PORs who received double triggering. However, their study was not a randomized controlled one.(43) Moreover, Lin et al.(27) demonstrated an increased number of mature and retrieved oocytes in normal responder women who underwent dual triggering. Finally, double triggering improved IVF outcome in women with abnormal final follicular maturation despite a normal response to COH.(44)

However, our study and Lin et al.,(45) cannot find statistically significant difference in the number of retrieved oocytes and number of metaphase 2 oocytes since low oocyte yield was anticipated for this specific study population, and a statistically significant difference would have been difficult to observe with the present sample size. But, Seval et al.,(42) and Lin et al.,(27) included population with high oocyte yield.

The results of our study revealed that the fertilization rate among patients of the dual trigger group (91.68%) was significantly higher than that of the single trigger group (80.82%).The number of frozen embryo among patients of the dual trigger group(1.10±1.38) was significantly higher than that of the single trigger group(0.43±1.02) Lin et al. confirmed in a recent retrospective cohort study involving 427 GnRH antagonist IVF cycles with fresh embryo transfer that dual triggering

significantly increases fertilization rate (73.1% vs. 58.6%, p = 0.015) in women with diminished ovarian reserve, compared to hCG-alone trigger.(45)

Higher rates of oocyte fertilization showed in the present study could also be viewed as an enhancement of oocyte competence from dual triggering. In a related study, the authors simulated an artificial mid-cycle FSH surge by adding a single bolus of FSH (450 IU) to the hCG as trigger.(46) Compared to the control group triggered by hCG and placebo, the study group triggered by FSH and hCG had significantly improved oocyte competence, as demonstrated by greater oocyte recovery and fertilization rate. In another report, a case of repetitive immature oocytes and empty follicle syndrome was also successfully treated with dual triggering, resulting in a singleton live birth at term.(47)

Furthermore, in a pilot study by Haas et al.,(48) the differential messenger RNA (mRNA) expression of reproduction-related genes in the oocyte granulosa cells (GCs) of patients triggered with hCG were compared to the same cohorts triggered with GnRH-a plus hCG (dual trigger) in the subsequent IVF cycles. The authors found that higher levels of amphiregulin and epiregulin were expressed in the GCs after dual triggering. Amphiregulin and epiregulin are ligands of the epidermal growth factor (EGF) receptors, and both have been indicated to participate important roles in cumulus expansion(49, 50),oocytes maturation(50),and meiosis resumption.(49) Since both amphiregulin and epiregulin expressions are up-regulated directly in the presence of FSH and LH, the surges of these two hormone induced by the GnRH-a trigger may be one of the mechanisms responsible for the improved fertilization observed in the present study.

In conclusion, the results from the present study demonstrated that in GnRH antagonist down-regulated IVF-ICSI cycles, dual triggering the final oocyte maturation with GnRH-a and standard dose of hCG could significantly improve the maturity index as well as rate of fertilization in women of diminished ovarian reserve. The strength of our study is that all clinical decisions and oocyte pick-ups were performed by the same physician, leading to less variability in performance and the prospective design of the study making it less susceptible to selection bias. The limitation of the present study is its small sample size and not calculating the live birth rate. However, large-scale randomized controlled studies are needed to confirm these findings.

Conflicting interests

None declared

Funding

None declared

Ethical approval

The study was approved by Alexandris University Ethical Review Board (Approval Number: 0201543 Phone: 01223374415 Ext: +2 Email: ghanemmaha63@gmail.com Address: Faculty of Medicine Alexandria University,17 Champollion street, Elmessalah,Alexandria,Egypt)

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