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Comparative study between single versus dual trigger for poor responders in GnRH-antagonist ICSI cycles: A randomized controlled study

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Abstract

Objective: To investigate whether dual triggering of final oocyte maturation with a combination of gonadotropin-releasing hormone (GnRH) agonist and human chorionic gonadotropin (hCG) can improve the number of retrieved oocytes and clinical pregnancy rate in poor responders undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF-ICSI) cycles using a GnRH-antagonist protocol.

Methods: A randomized controlled trial included poor ovarian responders indicated for ICSI using a GnRH-antagonist protocol. They were divided equally into two groups: group I received 10 000 units of hCG plus 0.2 mg of triptorelin while group II received 10 000 units of hCG only for triggering of ovulation. The primary outcome parameter was the number of oocytes retrieved. Secondary outcomes included metaphase II oocytes number, cancellation rate, number of obtained embryos, chemical and clinical pregnancy rates.

Results: One hundred and sixty women were included in the study, with 80 women in each treatment group. Dual triggering was associated with higher number of retrieved oocytes (5.3 ± 1.9 vs 4.5 ± 2.4, *P*=0.014), metaphase II oocytes (3.8 ± 1.4 vs 3.1 ± 1.7, *P*=0.004), total and grade 1 embryos (2.7 ± 1.1 and 2.3 ± 1.0 vs 1.9 ± 1.2 and 1.1 ± 0.2, *P*=0.001 and 0.021 respectively), and transferred embryos (2.2 ± 0.9 vs 1.6 ± 0.9, *P*=0.043, and lower cancellation rate (7.5% vs 20%, *P*=0.037) compared with single triggering. There were significantly higher chemical (25% vs 11.3%, *P*=0.039) and clinical (22.5% vs 8.8%, *P*=0.028) pregnancy rates in women with dual triggering compared with those with single triggering.

Conclusion: Dual triggering is associated with better IVF outcome in poor responders compared with single trigger.

Clinical trial registration NCT04008966.

KEYWORDS

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

1 | INTRODUCTION

Controlled ovarian hyperstimulation (COH) is the initial essential step in in vitro fertilization (IVF) cycles as production of multiple follicles is the key to success in these cycles.[1] During COH, poor ovarian response is not an uncommon finding and occurs in 5% to 35% of subfertile women. It is associated with a low implantation rate and consequently low pregnancy and live birth rates.[2] Poor ovarian response is diagnosed after failure of the standard long gonadotropin-releasing hormone (GnRH) agonist protocol in at least one IVF cycle.[3]

Many protocols have been tried to improve IVF outcomes in poor responders (PORs). These trials include: pretreatment with letrozole orally, 2500 IU hCG subcutaneously, or AndroGel transdermally[4]; adding adjuvant treatment with luteinizing hormone (LH)[5], clomiphene citrate, and aromatase inhibitor[4]; increasing the starting gonadotropin dose[6]; using alternative protocols such as short flare-up, microdose flare-up,[7] GnRH antagonist,[8] or agonist stop protocol[9]; or using follicle-stimulating hormone (FSH) luteal phase support.[10]

In previous studies, there was an observed better response to COH after triggering with a GnRH agonist compared with classic hCG triggering. Nowadays a GnRH agonist can be used with hCG to trigger ovulation in women with abnormal follicular maturation either as dual or double trigger.[11] In PORs, dual and double triggering has been suggested to improve oocyte quality and follicular maturation.[12]

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.

The aim of our study was to investigate whether dual triggering of final oocyte maturation with a combination of a GnRH agonist and hCG can improve the number of retrieved oocytes and clinical pregnancy rate in PORs undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF-ICSI) cycles using a GnRH-antagonist protocol.

2 | MATERIAL AND METHODS

An open-label randomized controlled trial was conducted at the IVF Unit, Kasr Alainy Hospital, Cairo University between July 2016 and June 2019. The study was approved by Kasr Alainy ethical committee. 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 presence of 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).[13]

Inclusion criteria included women with a spontaneous normal menstrual cycle and a normal uterine cavity (evaluated by hysterosalpingography or hysteroscopy). Exclusion criteria included women with ovarian cysts, endometriosis, hydrosalpinx, and 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. 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. In a natural spontaneous cycle day 3 assessment of hormones (FSH, LH, and estradiol [E_2] using an Immulite system [Siemens Healthcare Diagnostics, United Kingdom] and AMH using an enzyme-linked immunosorbent assay kit [AMH ELISA; Ansh Labs, Webster, TX, USA] were carried out.[14]

During the cycle before stimulation, combined oral contraceptive pills were used by all participants (Gynera; Bayer Schering, Germany) between days 5 and 25 and 2 mg E_2 tablets were added between days 21 and 28 (white tablets of Cycloprogynova; Bayer Schering, Germany). At day 28 transvaginal ultrasound was done to exclude any follicle more than 10 mm.

All participants started with a combination of recombinant FSH 300 U (Gonal-f; Merck Serono, Darmstadt, Germany) and urinary gonadotropin 150 U (Menogon; Ferring, Saint-Prex, Switzerland) from the second day of the cycle and then the dose was adjusted according to the ovarian response evaluated by transvaginal ultrasound and serum E_2.[13]

From day 6 of the cycle, follow-up using transvaginal ultrasound was done either daily or on alternate days according to the ovarian response. Ultrasound follow-up reported the number and size of follicles in each ovary and the endometrial thickness and pattern. When the leading follicle reached 12 mm, the GnRH antagonist was started using Cetrotide (Merck Serono) 0.25 mg subcutaneously daily till the day of triggering. Triggering was done when at least three follicles were larger than 14 mm and at least one of them reached a mean diameter of 17 mm or more.[2]

At the day of triggering, women were randomized using an automated Web-based randomization system ensuring allocation concealment into two groups. Group I (single trigger group) included women who received triggering in the form of 10 000 IU of hCG (Choriomon; IBSA, Lugano, Switzerland) intramuscular injection. Group II (dual trigger group) included women who received triggering in the form of 10 000 IU of hCG (Choriomon; IBSA) intramuscular injection in addition to the GnRH agonist triptorelin (Decapeptyl; Ferring) 0.2 mg subcutaneously.

Ovum pickup was done 34 hours after triggering under the guidance of transvaginal ultrasound. ICSI was performed on all retrieved eggs (avoiding the lower fertilization rate that may be associated with conventional IVF). Assessment of fertilization was done 16–18 hours after the ICSI procedure. Grading of embryos was done on the second

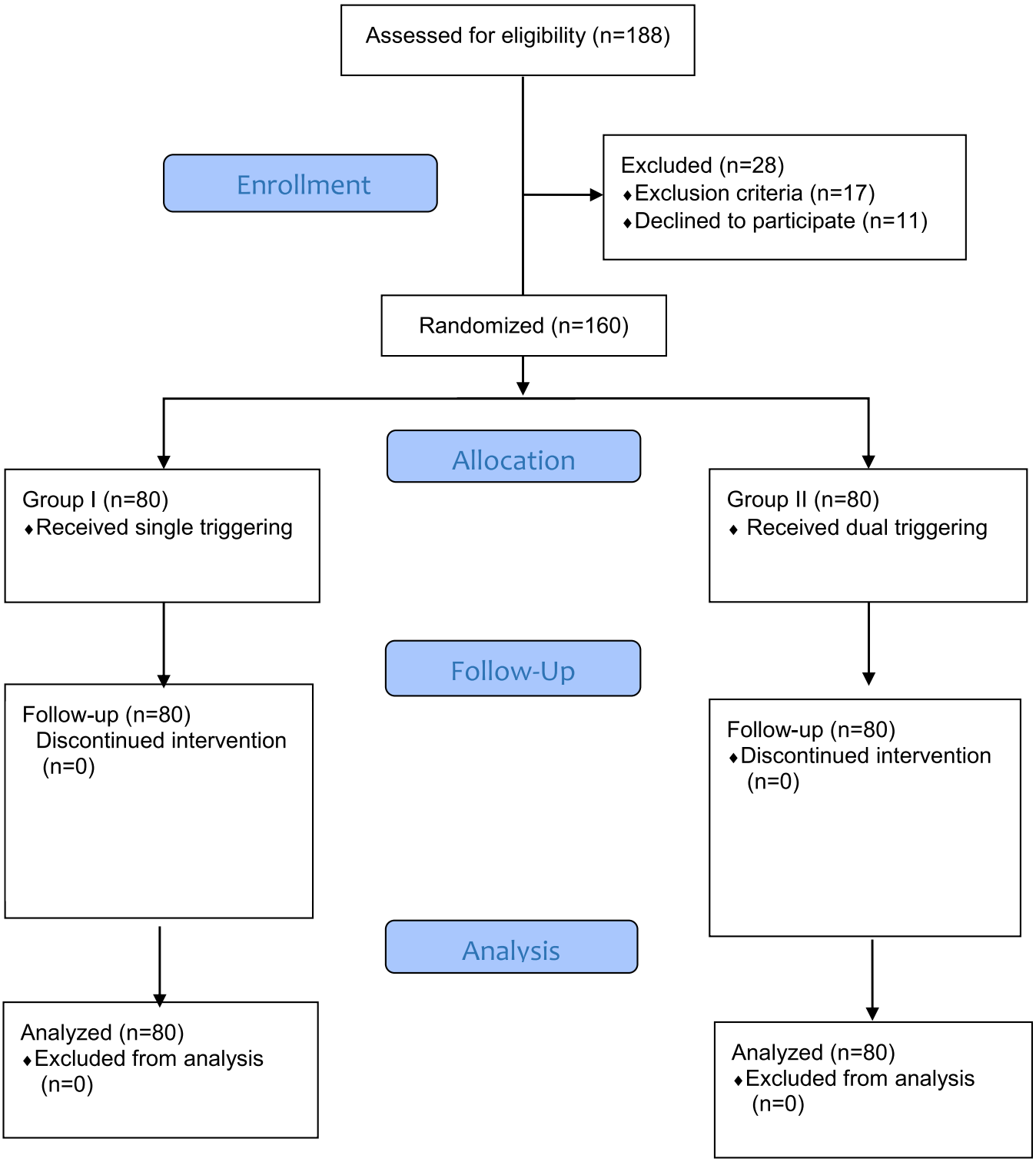

FIGURE 1 Flowchart of participants.

and third day after ICSI according to number of blastomeres, fragmentation degree, and multinucleation.[13] ISM1 culture medium (Origio Medicult media; CooperSurgical, Målov, Denmark) was used for oocyte collection and embryo culture. Transabdominal ultrasound-guided embryo transfer was done 3 days after oocyte retrieval using a Labotect semirigid catheter (Labotect; GmbH, Göttingen, Germany) by the same expert operator (AS).

Cycle cancellation was done if day 9 folliculometry revealed less than two mature follicles, no oocytes were retrieved, or if fertilization failed.[2]

Luteal phase support was started in all women on the day of oocyte retrieval and continued until the day of serum β-hCG assessment (done 14 days after embryo transfer) through administration of 400 mg of natural progesterone (Prontogest; AMSA, Rome,

Italy) twice daily per vagina. In women with positive serum β-hCG (>5 mIU/mL), transvaginal ultrasound evaluation was done 4 weeks after embryo transfer to confirm the presence and number of intrauterine gestational sacs.

The primary outcome parameter was the number of oocytes retrieved. Secondary outcomes included the number of metaphase II oocytes, cancellation rate, number of obtained embryos, and chemical and clinical pregnancy rates.

2.1 | Statistical analysis

Data were statistically described in terms of mean ± standard deviation or frequencies and percentages. Comparison between the study groups was done using the χ^2 test. Exact test was used instead when the expected frequency was less than five. Accuracy was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. A *P* value less than 0.05 was considered statistically significant. All statistical calculations were done using the computer program SPSS version 15 (SPSS Inc., Chicago, IL, USA) for Microsoft Windows.

2.2 | Sample size calculation

We planned a study of a continuous response variable from independent control and experimental subjects with one control per experimental subject. In a previous study,[15] the response within each subject group was normally distributed with standard deviation of 1.8. If the true difference between the experimental and control means is 0.8, we needed to study 80 experimental subjects and 80 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. Sample size calculation was done using PS Power and Sample Size Calculations software, version 3.0.11 for MS Windows (William D. Dupont and Walton D. Plummer) Vanderbilt University, Nashville, TN, USA).

3 | 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. A flow chart of the participants is shown in Figure 1.

No significant difference was found between the two study groups regarding basal hormonal profile, antral follicular count, the total dose of gonadotropin used for stimulation, duration of stimulation, the endometrial thickness, and E_2 level at the day of hCG triggering (Table 2).

Although the number of mature follicles was not significantly different between the two groups, dual triggering was associated with a higher number of retrieved oocytes and metaphase II oocytes, of total and grade 1 embryos, and of transferred embryos and a lower cancellation rate compared with single triggering (Table 2).

Table 3 shows there were significantly higher chemical and clinical pregnancy rates in women with dual triggering compared with those with single triggering. However, the implantation rate was not significantly different between the two groups.

4 | DISCUSSION

In the present study dual triggering with 10 000 IU of intramuscular hCG in addition to 0.2 mg subcutaneous GnRH agonist triptorelin was associated with better IVF outcome in PORs when compared with single trigger with hCG. Our study also found that dual trigger improved the number of retrieved oocytes and metaphase II oocytes, number of total and grade 1 embryos, and number of transferred embryos, and lowered the cancellation rate.

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.[16] However, its routine use as a single trigger was associated with lower implantation, ongoing, and live birth rates,[17] 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.[18] Dual triggering also improved ongoing pregnancy rates in normal responders[19] and was successful in women suffering from empty follicle syndrome.[20]

Our findings that the mean number of metaphase II oocytes and retrieved oocytes among women of the dual trigger group was

TABLE 1 Demographic data of the study population.

	Single trigger (n=80)	Dual trigger (n=80)	*P* value
Age (years)	38.9 ± 2.2	39.1 ± 2.5	0.547
BMI	26.9 ± 1.4	27.3 ± 1.8	0.199
Duration of infertility (years)	5.2 ± 2.9	5.7 ± 3.1	0.853
Type of infertility[a]			
Primary	58 (72.5%)	61 (76.3%)	0.727
Secondary	22 (27.5%)	19 (23.8%)	
Cause of infertility[a]			
Male	20 (25.0%)	21 (26.3%)	0.317
Tubal	26 (32.5%)	24 (30.0%)	
Ovarian dysfunction	22 (27.5%)	25 (31.3%)	
Unexplained	12 (15.0%)	10 (12.5%)	

Note: All results are presented as means ± SD.
Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); SD, standard deviation.
[a]Number (%).

TABLE 2 Cycle characteristics.

	Single trigger (n=80)	Dual trigger (n=80)	*P* value
Day 3 FSH (IU/L)	12.2 ± 1.6	12.3 ± 1.8	0.672
Day 3 LH (IU/L)	5.8 ± 1.2	6.1 ± 1.6	0.181
AMH (ng/mL)	0.9 ± 0.1	0.9 ± 0.1	0.852
D3 Estradiol (pg/mL)	92.4 ± 53	101 ± 61	0.342
Antral follicular count	4.5 ± 1.1	4.6 ± 0.9	0.399
Gonadotropin dose (IU)	4473 ± 652	4398 ± 661	0.620
Duration of stimulation (days)	13.1 ± 1.0	13.4 ± 1.2	0.316
E_2 at hCG triggering (pg/mL)	974 ± 421	1161.7 ± 482	0.196
Endometrial thickness at hCG injection mm	11.3 ± 1.0	11.0 ± 1.3	0.305
Mature follicle count	3.9 ± 1.3	4.4 ± 2.1	0.192
No. of retrieved oocytes	4.5 ± 2.4	5.3 ± 1.9	0.014
Metaphase II oocytes	3.1 ± 1.7	3.8 ± 1.4	0.005
Fertilization rate	59.8%	69.5%	0.013
Number of embryos	1.9 ± 1.2	2.7 ± 1.1	0.001
Number of grade 1 embryos	1.1 ± 0.2	2.3 ± 1.0	0.021
Number of embryos transferred	1.6 ± 0.9	2.2 ± 0.9	0.0001
Cancellation of OPU[a]	16/80 (20.0%)	6/80 (7.5%)	0.038

Note: All results are presented as means ± SD.
Abbreviations: AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; OPU, ovum pickup; SD, standard deviation.
[a]Number (%).

TABLE 3 Outcome parameters.

	Single trigger (n=80)	Dual trigger (n=80)	*P* value
Chemical pregnancy rate	9/80 (11.3%)	20/80 (25.0%)	0.039
Clinical pregnancy rate	7/80 (8.8%)	18/80 (22.5%)	0.028
Implantation rate	7/76 (9.2%)	13/148 (8.8%)	>0.99

Note:. All results are presented as number (percentage).

more than that of the single trigger group are supported by those of Seval and colleagues,[21] who 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.[22] Moreover, Lin et al.[23] demonstrated an increased number of mature and retrieved oocytes, and cryopreserved embryos in women who underwent dual triggering. Finally, double triggering improved IVF outcome in women with abnormal final follicular maturation despite a normal response to COH.[24]

In our study, the number of total embryos and grade 1 embryos was also significantly higher in women in the dual trigger group compared with those in the single trigger group. These findings are in keeping with those of Seval et al.[21] who demonstrated that the mean number of grade-A embryos was significantly higher in a dual trigger group than a single trigger group (1.6 ± 1.5 vs 1.1 ± 1.4, *P*=0.01), although the mean number of embryos obtained in their study differed from ours. This discrepancy may be due to differences in some of the criteria of the populations of the two studies.

The results of our study revealed that the fertilization rate among patients of the dual trigger group (69.5%) was significantly higher than that of the single trigger group (59.8%). In contrast, the implantation rate among the patients of the dual trigger group (8.8%) was not significantly different from that of the single trigger group (9.2%). Similarly, Erdem et al.[25] showed no significant difference between standard hCG trigger and dual trigger in terms of implantation (15.7% vs 18.3%).

In our study, the chemical and clinical pregnancy rates among patients of the dual trigger group were significantly higher than those of the single trigger group (25% and 22.5% vs 11.3% and 8.8%. Both Lin et al.[23] and Seval et al.,[21] also reported that the clinical pregnancy rate was significantly higher with dual triggering than with single triggering.

To the best of our knowledge, our study is the first randomized controlled trial with an adequate sample size to evaluate the effect of dual trigger in PORs undergoing IVF. However, our study has a major limitation, which is the inability to calculate the ongoing and live birth rate as many of our IVF patients are lost during the follow-up period.

In summary, our study showed that dual triggering of ovulation via hCG and a GnRH agonist in PORs undergoing GnRH-antagonist ICSI cycles improved the number of retrieved oocytes and metaphase II oocytes, number of embryos obtained, and number of embryos transferred. Also, dual trigger results in a higher pregnancy rate and implantation rate.

AUTHOR CONTRIBUTIONS

AMM: project development, manuscript writing; MAR: data collection, manuscript writing; AS: data analysis, manuscript writing; WS: data collection, manuscript writing; SE: data collection, manuscript writing; EAH: data analysis, manuscript revision; AE-M: statistical analysis, manuscript revision; AH: data collection, manuscript writing. All authors read and approved the manuscript.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

REFERENCES

1. Penzias AS. Improving results with assisted reproductive technologies: Individualized patient-tailored strategies for ovulation induction. *Reprod Biomed Online*. 2004;9:43–46.

2. Maged AM, Fahmy RM, Rashwan H, et al. Effect of body mass index on the outcome of IVF cycles among patients with poor ovarian response. *Int J Gynaecol Obstet*. 2019;144:161–166.
3. Ubaldi FM, Rienzi L, Ferrero S, et al. Management of poor responders in IVF. *Reprod Biomed Online*. 2005;10:235–246.
4. Schoolcraft W, Surrey E, Minjarez DA, Stevens JM, Gardner DK. Management of poor responders: Can outcomes be improved with novel GnRH antagonist/letrozole protocol? *Fertil Steril*. 2009;89:151–156.
5. Barrenetxea G, Agirregoikoa JA, Jimenez MR, de Larruzea AL, Ganzabal T, Carbonero K. Ovarian response and pregnancy outcome in poor responder women: A randomized controlled trial on the effect of LH supplementation on in vitro fertilization cycle. *Fertil Steril*. 2008;89:546–553.
6. Hofmann GE, Toner JP, Muasher SJ, Jones GS. High dose follicle stimulating hormone (FSH) ovarian stimulation in low responder patient for in vitro fertilization. *J In Vitro Fert Embryo Transf*. 1989;6:285–289.
7. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW. Clinical and endocrine effects of a micro dose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. *Fertil Steril*. 1998;69:419–424.
8. Craft I, Gorgy A, Hill J, Menon D, Podsiadly B. Will GnRH antagonists provide new hope for patients considered "difficult responders" to GnRH agonist protocols? *Hum Reprod*. 1999;14:2959–2962.
9. Garcia-Valesco JA, Isaza V, Requena A, et al. High dose of gonadotropins combined with stop versus non-stop protocol of GnRH analogue administration in low responders IVF patients: A prospective, randomized controlled trial. *Hum Reprod*. 2000;15:2292–2296.
10. Kucuk T, Sozen E. Luteal start of exogenous FSH in poor responder women. *J Assisst Reprod Genet*. 2007;24:635–638.
11. Orvieto R. Triggering final follicular maturation-hCG, GnRH-agonist or both, when and to whom? *J Ovarian Res*. 2015;8:60.
12. Orvieto R. A simplified universal approach to COH protocol for IVF: Ultrashort flare GnRH-agonist/GnRH-antagonist protocol with tailored mode and timing of final follicular maturation. *J Ovarian Res*. 2015;8:69.
13. Maged AM, Nada AM, Abohamila F, Hashem AT, Mostafa WA, Elzayat AR. Delayed start versus conventional GnRH antagonist protocol in poor responders pretreated with estradiol in luteal phase: A randomized controlled trial. *Reprod Sci*. 2015;22:1627–1631.
14. Motawi TMK, Rizk SM, Maurice NW, Maged AM, Raslan AN, Sawaf AH. The role of gene polymorphisms and AMH level in prediction of poor ovarian response in Egyptian women undergoing IVF procedure. *J Assist Reprod Genet*. 2017;34:1659–1666.
15. Haas J, Zilberberg E, Nahum R, et al. Does double trigger (GnRH-agonist + hCG) improve outcome in poor responders undergoing IVF-ET cycle? A pilot study. *Gynecol Endocrinol*. 2019;35:628–630.
16. Papanikolaou EG, Verpoest W, Fatemi H, Tarlatzis B, Devroey P, Tournaye H. A novel method of luteal supplementation with recombinant LH, when a GnRH-agonist is used instead of HCG for ovulation triggering: A randomized prospective proof of concept study. *Fertil Steril*. 2011;3:1174–1177.
17. Youssef MA, Van der Veen F, Al-Inany HG, Griesinger G, Mochtar MH, Aboulfoutouh I. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist assisted reproductive technology cycles. *Cochrane Database Syst Rev*. 2011;1:CD008046.
18. Griffin D, Benadiva C, Kummer N, Budinetz T, Nulsen J, Engmann L. Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders. *Fertil Steril*. 2012;97:1316–1320.
19. Schachter M, Friedler S, Ron-El R, Zimmerman AL, Strassburger D, Bern O. Can pregnancy rate be improved in gonadotropin-releasing hormone (GnRH) antagonist cycles by administering GnRH agonist before oocyte retrieval a prospective, randomized study. *Fertil Steril*. 2008;90:1087–1093.
20. Kasum M, Kurdija K, Orešković S, Čehić E, Pavičić-Baldani D, Škrgatić L. Combined ovulation triggering with GnRH agonist and hCG in IVF patients. *Gynecol Endocrinol*. 2016;32:861–865.
21. Seval MM, Özmen B, Atabekoğlu C, et al. Dual trigger with gonadotropin-releasing hormone agonist and recombinant human chorionic gonadotropin improves in vitro fertilization outcome in gonadotropin-releasing hormone antagonist cycles. *J Obstet Gynaecol Res*. 2016;42:1146–1151.
22. Haas J, Ophir L, Barzilay E, Yerushalmi GM, Yung Y, Kedem A. GnRH agonist vs hCG for triggering of ovulation-differential effects on gene expression in human granulosa cells. *PLoS One*. 2014;9:e90359.
23. Lin MH, Wu FS, Lee RK, Li SH, Lin SY, Hwu YM. Dual trigger with combination of gonadotropin-releasing hormone agonist and human chorionic gonadotropin significantly improves the live-birth rate for normal responders in GnRH-antagonist cycles. *Fertil Steril*. 2013;100:1296–1302.
24. Zilberberg E, Haas J, Dar S, et al. Co-administration of GnRH-agonist and hCG for final oocyte maturation in patients with low proportion of mature oocytes. *Gyn Endocrinol*. 2015;31:145–147.
25. Erdem A, Mutlu MF, Erdem M, Mutlu I; Department of Obstetrics & Gynecology, Gazi University Faculty of Medicine, Ankara, Turkey Department of Obstetrics & Gynecology, Koru Hospital, Ankara, Turkey, Novaart IVF Clinic, Ankara, Turkey. 7th World Congress on Ovulation Induction, Bolgna, Italy, 3–5 september 2015.