Zeynep Kamil Med J 2022;53(4):183–188
DOI: 10.14744/zkmj.2022.37880

ORIGINAL ARTICLE

IVF/ICSI outcomes in women of age 40 years and older who underwent Dual Trigger: A retrospective cohort study

Sanai AKSOY
Sinem ERTAŞ

Department of Women's Health and IVF, VKV American Hospital, İstanbul, Türkiye

ORCID ID
SA : 0000-0003-4110-5290
SE : 0000-0002-1699-616X

ABSTRACT

Objective: Female age is the critical factor determining the success in assisted reproductive technology cycles. The objective of this study was to observe the impact of using dual trigger (gonadotropin-releasing hormone [GnRH] agonists and recombinant human chorionic gonadotropin (r-hCG)] for ovulation induction on *in vitro* fertilization (IVF)/Intracytoplasmic sperm injection outcomes of women with advanced age (40 or above).

Material and Methods: This retrospective cohort study was conducted on 408 patients aged 40 years or older. Only the first cycles of the patients were implicated in the study. Flexible GnRH antagonist protocol was used for ovarian stimulation in all patients. Ovulation was triggered when at least two follicles reached a size of 17 mm or more. The study group included 67 cycles that applied dual trigger. The control group consisted of 341 cases in which final oocyte maturation was triggered by hCG alone. In addition to the basic characteristics and cycle parameters of the cases, pregnancy and live birth rates (LBR) of both groups were compared.

Results: Statistically, there was no prominent difference in terms of average female age, duration of infertility, duration of stimulation, amount of gonadotrophin used, cycle cancellation rate, total oocyte rate, total MII oocyte rate, 2PN rate, fertilization rate, embryo utilization rate (usable embryo/2PN), and number of transferable embryos in dual trigger and hCG only groups. Oocyte maturation rate (Number of MII oocytes/total number of oocytes) was observed to be higher in study group. Clinical pregnancy rates and LBRs were the same in both groups.

Conclusion: In women aged 40 years and older, dual trigger did not show a significant increase in the oocyte maturation, clinical pregnancy, and LBRs according to hCG-only trigger patients.

Keywords: GnRH agonist, human chorionic gonadotropin, *in vitro* fertilization, infertility.

Cite this article as: Aksoy S, Ertaş S. IVF/ICSI outcomes in women of age 40 years and older who underwent Dual Trigger: A retrospective cohort study. Zeynep Kamil Med J 2022;53(4):183–188.

Received: June 16, 2022 **Accepted:** June 28, 2022 **Online:** November 07, 2022
Correspondence: Sinem ERTAŞ, MD. Amerikan Hastanesi, Kadın Hastalıkları, Dogum ve Tüp Bebek Kliniği, İstanbul, Türkiye.
Tel: +90 212 444 37 77 **e-mail:** drsinemertas@gmail.com

INTRODUCTION

Unlike the male, who produces sperm throughout his life, the woman is born with a certain number of follicles, estimated to be 1 million at birth and reduced to 300,000 by adolescence. Of these 300,000 follicles, only 300 will produce an egg during the reproductive years, while the others will end up in atresia.[1] This follicular atresia accelerates after the age of 35. Female fertility declines gradually between the ages of 26 and 34 and more rapidly after age 35.[1]

Observations made in natural cycles show that the pregnancy rate of women at the age of 20 is about 25% per cycle, which means the average conception time in this age group is about 4 months.[2] It is known that this rate gradually decreases and goes down to only 5% after the age of 40. The natural conception rate after the age of 42 is very low.[2] Advanced female age reduces not only the rate of natural conception but also the success rates of *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatments.[3] While the pregnancy rate is around 50% in women under the age of 35, this ratio goes down to 20% in patients aged between 40 and 42 years.[3] When the IVF patients treated in our clinic between 1996 and 2020 are examined, it is seen that the average age has increased from 32.1 to 36.[4] The percentage of patients over the age of forty tripled from 9% to 28.7%. Decreased ovarian reserve has become the major indication for IVF since 2015. During assisted reproductive treatments, ovarian reserve directly affects the response to the medication used in controlled ovarian stimulation. The number of oocytes remaining in reserve is related with the count of oocytes which will be retrieved in the IVF cycle. The larger the number of oocytes obtained during the IVF cycle, the greater the chances of pregnancy.[5] The increase in the percentage of older patients increases the need to obtain a large number of mature oocytes in such cases.

As women age, the maturation process of oocytes becomes impaired. Moreover, the rates of fertilization and implantation of the formed embryos also decrease. This is likely due to the derangements in the metabolic and energy systems of oocytes, as well as increased chromosomal abnormalities.

The luteinizing hormone (LH) peak that triggers ovulation is a crucial step for the resumption of the meiotic division and the acquisition of mature oocytes (metaphase II) with fertilization capacity. Human chorionic gonadotropin (hCG) is the reference molecule to trigger ovulation due to its LH-like activity and long elimination half-life. With the introduction of gonadotropin releasing hormone (GnRH) antagonist protocols in IVF, a wide space has been opened for the use of ovulation triggering with GnRH agonists. GnRH agonists have a much shorter elimination half-life than hCG. A single injection of agonists provides a surge of LH necessary to trigger ovulation. Triggering with GnRH agonists leads to an elevation in both LH and follicle-stimulating hormone (FSH), which is closer to the changes in the gonadotropin levels during physiological ovulation.[6] However, ovulation triggering with agonists alone causes pituitary down-regulation which, in turn, reduces the level of LH in the luteal phase. In this case, since LH levels cannot adequately support corpus luteum activity, serum progesterone values remain below the levels that will support pregnancy, which leads to an increase in miscarriage rates and a decrease in pregnancy rates. This fact limits the use of ovulation triggering with GnRH-agonists to the cycles in which all embryos will undergo cryopreservation.[6,7]

Several clinical studies, most of which were retrospective and were realized with a limited number of cases, showed that more mature oocytes were obtained if ovulation was triggered by combination of hCG with an GnRH analog in normal ovarian responders.[8–11] However, the available evidence is limited and conflicting.

In our study, we intended to compare laboratory and clinical outcomes in assisted reproductive technology in older age women who had ovulation triggering with either hCG or hCG plus GnRH analog (dual trigger).

MATERIAL AND METHODS

This study was conducted retrospectively on 408 patients of age 40 years and older who applied to the tertiary assisted reproduction technologies (ART) clinic between January 1, 2018, and December 31, 2020. Only the first treatment cycles of the cases were included in the study. The study group comprised 96 cases with dual trigger and the control group comprised of 341 cases who were triggered only with hCG.

The cycles of 114 patients (16 women in the study group and 98 women in the control group) were canceled owing to inadequate ovarian response, lack of oocytes, or fertilization failure.

For 51 dual trigger patients who were included in the study, 0.1 mg tryptorylene acetate (Gonapeptyl 0.1 mg/ml, Ferring Switzerland), and 250 mcg (6500 IU) rhCG (Ovitrel 250 micrograms/0.5 ml, Merck, Germany) were used as trigger injections. Only 500 mcg (13000 IU) rhCG (Ovitrel 250 micrograms/0.5 ml, Merck, Germany) was used to induce ovulation in the control group.

Ovarian stimulation was started on the 2nd or 3rd day of the menstrual cycle of women later on eliminating pathologies originating from the ovaries or the endometrium by transvaginal ultrasound (TVUS). The initial gonadotrophin dose was given from 150 IU/day to 450 IU/day (Meriofert, IBSA, Türkiye) depending on the woman's ovarian reserve and body mass index. On day 5 or 6 of the stimulation, the ovarian response was evaluated by TVUS. In cases where the follicle size was 12 mm or more, 25 mg of GnRH-a (Cetrotide, Merck, Germany) was added. When two or more follicles reached a size of 17 mm or more, ovulation triggering was performed, and oocyte retrieval was performed 35–37 h later. ICSI was performed in all cases. For luteal phase, 600 mg of vaginal micronized progesterone (Progestan, Koçak, Türkiye) and 25 mg of subcutaneous progesterone (Prolutex 25 mg, IBSA, Türkiye or Progestane dex 25 mg, Koçak, Türkiye) were applied. Luteal phase support was continued until 10th gestational week in cases where pregnancy was achieved.

Clinical pregnancy was defined as seeing fetal heartbeat by ultrasound in the 6th or 7th week of pregnancy. The maturity rate is the ratio of MII oocytes that underwent ICSI to the total number of oocytes collected. The fertilization rate is the ratio of the count of 2PN (pronucleus) embryos to the count of injected MII oocytes. Embryo utilization rate is defined as the ratio of the number of embryos used (transferred or frozen) per 2PN embryo number.

The study protocol was approved by the Ethics Committee of the Koç University (IRB no: 2022.224.IRB1.079).

Table 1: The distribution of pre-operative symptoms

	Mean (±2SD) (min–max)
Female age (years)	41.8±1.87 (40–47)
Duration of infertility (years)	4.4±4.4 (1–22)
Stimulation time (days)	10.4±2.4 (6–19)
Gonadotropin dosage (IU/day)	373.1±85.5 (150–450)
Total number of oocytes	5.7±3.5 (0–25)
Number of MII oocytes	4.3±3.0 (0–16)
Oocyte maturation rate (%)	77.4
Number of 2PN	3.4±2.6 (0–15)
Fertilization rate (%)	81.6
Number of usable embryos	2.2±1.4 (0–9)
Embryo utilization rate (%)	77.3

PN: Pronukleus; SD: Standard deviation; Min: Minimum; Max: Maximum.

Table 2: Cycle cancellation rates

Trigger type	Cancellation		Embryo transfer	
	n	%	n	%
hCG trigger (n=341)	98	28.7	243	71.33
Dual trigger (n=67)	16	23.9	51	76.1
Total	114	27.9	294	72.1

Fisher's exact test: p-value 0.460.

Table 3: Cancellation rates after follicle aspiration

Trigger type	Number of MII oocytes		At least one MII oocyte	
	n	%	n	%
hCG trigger	30	8.8	311	91.2
Dual trigger	8	11.9	59	88.1
Total	38	27.9	370	72.1

Fisher's exact test: p-value 0.489.

Statistical Analysis

Continuous variables were described by mean (±2 standard deviation) or median, while categorical variables were described by number and percentage. The control and study groups were compared in terms of continuous variables using the independent t test or Mann–Whitney U test according to their characteristics. The groups were compared using Fisher's exact test in terms of categorical variables. In the context of two-way hypothesis evaluation, $p<0.05$ was assumed statistically significant.

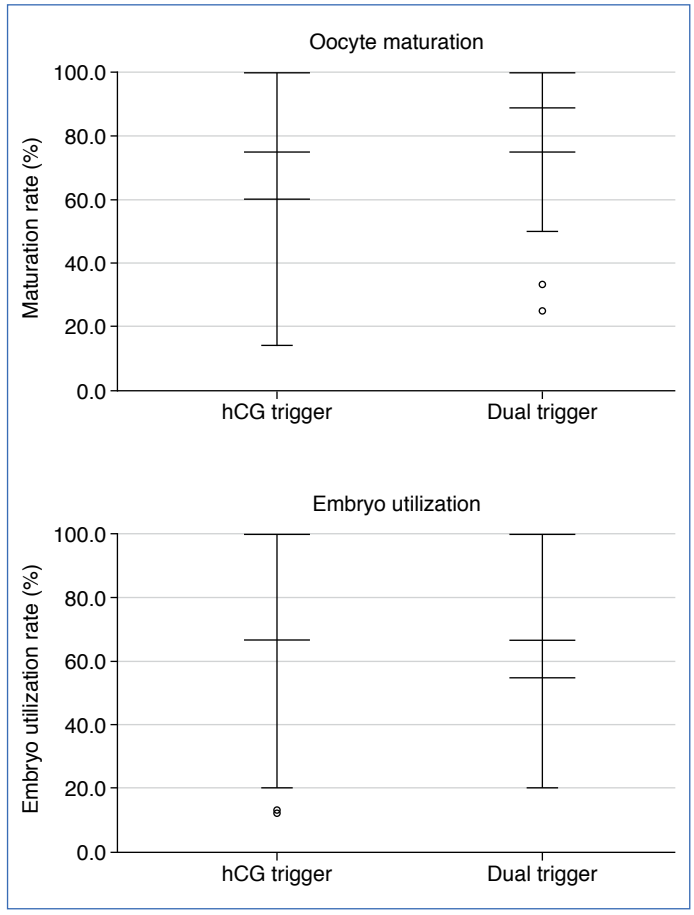

Figure 1: Box plot graphs for the comparison of oocyte maturation and embryo utilization rates in hCG trigger and dual trigger.

RESULTS

The descriptive characteristics of the patients participating in the study are shown in Table 1. There was no considerable difference between the cycle cancellation ratios between study and control groups (Table 2). Likewise, the cancellation rate due to failed oocyte retrieval or fertilization was similar same in both groups (Table 3).

The main characteristics of the 297 patients who were admitted to the study after cancellations are shown in Table 4. There was no difference in terms of basal characteristics between the groups. When the laboratory results of the two groups were compared, it was observed that all parameters were the same except for the maturation rate. The maturation rate was considerably higher in women who underwent dual trigger than in women who underwent classical triggering, but this did not appear to be reflected in the number of 2PN embryos obtained, fertilization rate, or number of usable embryos (Table 5 and Fig. 1). Clinical pregnancy and live birth rates (LBR) were similar between study and control groups (Table 6).

Table 4: Main characteristics of women in the dual trigger and hCG-only groups

	hCG trigger	Dual trigger	p	Mean difference	95% Confidence interval
Female age (years)	41.86±1.90	41.71±1.72	0.593	0.154	-0.413– -0.722
Duration of infertility (years)	4.35±4.49	4.93±4.00	0.438	-0.580	-2.050–0.890
Duration of stimulation (days)	10.45±2.51	9.98±1.70	0.223	0.473	-0.290–1.237
Gonadotropin dosage (IU/day)	370.72±84.20	384.78±91.66	0.310	-14.059	-41.265–13.147

Table 5: Comparison of the laboratory outcome parameters between study and control groups

	hCG trigger	Dual trigger	p	Mean difference	95% Confidence interval
Total oocytes	5.72±3.58	5.49±3.43	0.680	0.226	-0.581–1.302
Number of MII oocytes	4.25±3.05	4.49±3.82	0.600	-0.243	-1.115–0.669
Maturity rate (%)	75.95	84.04	0.018*	-8.085	-14.799– -1.372
Number of 2PN	3.33±2.62	3.65±2.33	0418	-0.322	-1.104–0.460
Fertilization rate (%)	81.29	83.40	0.511	-2.111	-8.418–4.196
Number of useable embryos	2.16±1.37	2.31±1.41	0.459	-0.157	-0.575–0.261
Embryo utilization rate (%)	78.03	73.93	0.296	4.097	-3.607–11.801
Number of embryos transferred	1.59	1.71	0.52	-0.154	-0.304–0.005

PN: Pronukleus.

DISCUSSION

In this study, dual trigger and hCG-only trigger for IVF patients of age 40 and older were compared. There were no considerable differences were found in terms of the total count of oocytes obtained, the count of metaphase II oocytes, the rate of fertilization or the number of usable embryos obtained. Although the oocyte maturation rate was higher in study group, clinical outcomes were similar between groups.

The impact of dual trigger compared to hCG trigger alone has been investigated in different studies that vary on a large scale in design, method and outcome parameters. In the literature, two randomized clinical trials (RCT) examined the impact of the dual trigger in normal responder patients. First, Decleer et al.[12] evaluated 120 women younger than 38 years of age who did not have endometriosis or polycystic ovarian syndrome. The average count of oocytes retrieved was alike between the dual trigger and hCG-only trigger groups.

Second, in another study, 192 normal responder women were randomized according to usage of dual trigger or hCG alone.[13] Average count of oocytes (10.85 vs. 9.35) and the count of embryos (6.86 vs. 5.34) were statistically higher in the study group. In this study, there were no remarkable differences between clinical pregnancy rate or implantation rates. Kim et al.[14] also compared two treatment choices (for each group 60 patients). In this study the count of oocytes retrieved, PN numbers and high-quality embryos were similar in both groups.[14] However, implantation rate, pregnancy rate and LBR were considerably lower in control than the study group.

Table 6: Clinical pregnancy and live birth rates

	hCG trigger (n=243)		Dual trigger (n=51)		p
	n	%	n	%	
Pregnancy rate	77	31.7	13	25.5	0.410
Live birth rate	39	16	9	17.6	0.835

This study showed that dual trigger for oocyte maturation also may be advantageous for endometrial receptivity and pregnancy ratios in GnRH antagonist cycles.[14]

Sloth et al.[15] compared 7 randomized controlled trials (RCTs) including 2474 women with low ovarian response who received dual trigger and hCG only trigger in a meta analyze. They deduced that dual trigger was better at improving oocyte maturation and pregnancy outcomes than hCG trigger only. However, they emphasize the need for more RCT to reach a definitive conclusion.

One of the last steps in the development of oocytes is its maturation. Oocyte maturation is described as the onset of meiosis division in the oocyte just before ovulation.[16,17] Oocytes located in the ovary stimulate follicular growth and development through gonadotropins, FSH, and LH, which then triggers the resumption of meiosis until metaphase II. The oocytes are then kept in meiotic

arrest again until fertilization when meiosis division is complete. Stimulation of oocyte maturation appears to involve a complex interaction of several important intracellular, paracrine, and structural factors, including sterols, steroids, growth factors, and cyclic adenosine monophosphate.[18]

The hypothesis that the rate of oocyte maturation will decrease due to the change in ovarian reserve with increasing female age could not be confirmed in the comparative study of 195 patients by Lee et al.[19] In this study, a higher maturation rate was not found in patients under 35 years of age. This is explained by the presence of a large number of antral follicles in young patients at different stages of maturation in the ovaries and the retrieval of small follicles also. The increase in the count of immature oocytes in women of age 40 and older is plausibly related to ovarian aging. The sensitivity of follicles to FSH decreases and the increase in serum FSH levels observed in these cases leads to a prominent increase in the count of immature oocytes. High exposure of follicles to FSH at the end of the luteal phase may play a critical role in the emergence of this condition.[20]

Mahajan et al.,[21] in a prospective randomized trial of 76 normal responder women, showed the similarity between the two groups in terms of oocyte count, mature oocyte count, fertilization rate, or count of usable embryos on day 3.

Zhou et al.[22] recently conducted a study reporting that the usage of dual trigger enhances the count of good quality embryos, but it is not associated with a higher count of oocytes collected, compared to triggering only with hCG or GnRHa.

On the other hand, there are many studies showings that dual triggering in patients who respond poorly, increases the count of oocytes retrieved, mature oocyte ratio, fertilization ratio, good quality embryo ratio, implantation ratio, pregnancy ratio, and live birth ratio.[23–26]

However, Zhang et al.[27] in their retrospective study of 1350 patients classified as poor responders (Bologna criteria), they observed an increase in the count and rate of mature oocytes with dual trigger, but they did not observe any difference between fertilization rate, transferable embryo rate, implantation ratio, and pregnancy ratio. Our study has also provided similar results.

Retrospective design of this study is the main limitation. The second limitation is the relatively small sample size. All studies in literature have emphasized the limitation of the number of patients as an important drawback. A high number of patient data is needed particularly to be able to evaluate clinical outcomes such as pregnancy and live birth ratios with more certainty. In our study also, when the alpha error rate is accepted as 0.05 in post-hoc power analysis, the rate of 76.7% obtained for maturation decreases to <20% in the evaluation of clinical outcomes. This reiterates the need for higher number of patients to be studied to determine the effect of dual trigger application on clinical outcomes.

CONCLUSION

Based on our study, dual trigger is associated with higher oocyte maturation ratio but does not cultivate clinical pregnancy and live birth ratios in women of age 40 and older. Available data on dual trigger show contradictory results in different women groups. Based on the currently available data, there is no solid evidence in favor of using dual trigger in older.

Statement

Ethics Committee Approval: The Koç University Clinical Research Ethics Committee granted approval for this study (date: 15.06.2022, number: 2022.224.IRB1.079).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – SA, SE; Design – SA; Supervision – SE; Resource – SA; Materials – SE; Data Collection and/or Processing – SE; Analysis and/or Interpretation – SA, SE; Literature Search – SA, SE; Writing – SA, SE; Critical Reviews – SE.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Strauss III JF, Williams CJ. Ovarian life cycle. Yen and Jaffe's reproductive endocrinology. Amsterdam: Elsevier; 2019. p. 167–205. e9.
2. Bull JR, Rowland SP, Scherwitzl EB, Scherwitzl R, Danielsson KG, Harper J. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. NPJ Digital Med 2019;2:1–8.
3. Dunson DB, Baird DD, Colombo B. Increased infertility with age in men and women. Obstet Gynecol 2004;103:51–6.
4. Yakin K, Urman B, Balaban B. Dynamic view of assisted reproduction in Türkiye from 1996 to 2020. Reproductive Biomedicine Online 2022;44:747–54.
5. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: An analysis of 400 135 treatment cycles. Hum Reprod 2011;26:1768–74.
6. Gonen Y, Balakier H, Powell W, Casper RF. Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for *in vitro* fertilization. J Clin Endocrinol Metab 1990;71:918–22.
7. Yding Andersen C, Vilbour Andersen K. Improving the luteal phase after ovarian stimulation: Reviewing new options. Reprod Biomed Online 2014;28:552–9.
8. Kolibianakis EM, Schultze-Mosgau A, Schroer A, van Steirteghem A, Devroey P, Diedrich K, et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum Reprod 2005;20:2887–92.
9. Humaidan P, Ejdrup Bredkjaer H, Westergaard LG, Yding Andersen C. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: A prospective, randomized, controlled study. Fertil Steril 2010;93:847–54.
10. Mizrachi Y, Horowitz E, Farhi J, Raziel A, Weissman A. Ovarian stimulation for freeze-all IVF cycles: A systematic review. Hum Reprod Update 2020;26:118–35.
11. Ben-Haroush A, Sapir O, Salman L, Altman E, Garor R, Margalit T, et al. Does 'Dual Trigger' increase oocyte maturation rate? J Obstet Gynaecol 2020;40:860–2.
12. Decleer W, Osmanagaoglu K, Seynhave B, Kolibianakis S, Tarlatzis B, Devroey P. Comparison of hCG triggering versus hCG in combination with a GnRH agonist: A prospective randomized controlled trial. Facts Views Vis Obgyn 2014;6:203–9.

13. Eftekhar M, Mojtahedi MF, Miraj S, Omid M. Final follicular maturation by administration of GnRH agonist plus HCG versus HCG in normal responders in ART cycles: An RCT. Int J Reprod Biomed 2017;15:429–34.
14. Kim CH, Ahn JW, You RM, Kim SH, Chae HD, Kang BM. Combined administration of gonadotropin-releasing hormone agonist with human chorionic gonadotropin for final oocyte maturation in GnRH antagonist cycles for *in vitro* fertilization. J Reprod Med 2014;59:63–8.
15. Sloth A, Kjølhede M, Sarmon KG, Knudsen UB. Effect of dual trigger on reproductive outcome in low responders: A systematic PRISMA review and meta-analysis. Gynecol Endocrinol 2022;38:213–21.
16. Albertini DF, Carabatsos MJ. Comparative aspects of meiotic cell cycle control in mammals. J Mol Med (Berl) 1998;76:795–9.
17. Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of ovarian follicle development. Reproduction 2001;121:647–53.
18. Jamnongjit M, Hammes SR. Oocyte maturation: The coming of age of a germ cell. Semin Reprod Med 2005;23:234–41.
19. Lee HJ, Jee BC, Suh CS, Kim SH, Moon SY. Oocyte maturity in relation to woman's age in *in vitro* fertilization cycles stimulated by single regimen. Yonsei Med J 2012;53:181–5.
20. Moore A, Arny M, Lynch K, Grow DR. Oocyte maturation arrest more common in younger patients undergoing IVF/ICSI. Fertility and Sterility 2007;88:S271.
21. Mahajan N, Sharma S, Arora PR, Gupta S, Rani K, Naidu P. Evaluation of dual trigger with gonadotropin-releasing hormone agonist and human chorionic gonadotropin in improving oocyte maturity rates: A prospective randomized study. J Hum Reprod Sci 2016;9:101–6.
22. Zhou C, Yang X, Wang Y, Xi J, Pan H, Wang M, et al. Ovulation triggering with hCG alone, GnRH agonist alone or in combination? A randomized controlled trial in advanced-age women undergoing IVF/ICSI cycles. Hum Reprod 2022:deac114.
23. Oliveira SA, Calsavara VF, Cortés GC. Final oocyte maturation in assisted reproduction with human chorionic gonadotropin and gonadotropin-releasing hormone agonist (Dual Trigger). JBRA Assist Reprod 2016;20:246–50.
24. Griffin D, Feinn R, Engmann L, Nulsen J, Budinetz T, Benadiva C. Dual trigger with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin to improve oocyte maturity rates. Fertil Steril 2014;102:405–9.
25. Zilberberg E, Haas J, Dar S, Kedem A, Machtinger R, Orvieto R. Co-administration of GnRH-agonist and hCG, for final oocyte maturation (double trigger), in patients with low proportion of mature oocytes. Gynecol Endocrinol 2015;31:145–7.
26. Schachter M, Friedler S, Ron-El R, Zimmerman AL, Strassburger D, Bern O, et al. 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;90:1087–93.
27. Zhang J, Wang Y, Mao X, Chen Q, Hong Q, Cai R, et al. Dual trigger of final oocyte maturation in poor ovarian responders undergoing IVF/ICSI cycles. Reprod Biomed Online 2017;35:701–7.