Comparison of GnRH Agonist, hCG, and Dual Trigger for Final Oocyte Maturation in ICSI Cycle. A Case Control Study

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Abstract

BACKGROUND:

Gonadotrophin-releasing hormone (GnRH) agonist has been proposed as an alternative drug to human chorionic Gonadotropin (hCG) for triggering. The purpose of this study to analyze the effects of three types of trigger hCG, GnRH-a or combine of them (dual) on quality of oocyte and embryo and ovarian hyperstimulation syndrome (OHSS) in the ICSI cycle.

METHODS:

A prospective case control study was conducted on 320 women who referred to Milad, IVF Center, Mashhad, Iran, between May 2016 and June 2019. All patients underwent antagonist protocol and were classified according to the type of trigger in three groups, 118 patients in the GnRH-a group, 49 patients in the hCG group, and 153 in dual group. The oocytes were retrieved after 36 hours of injection of the trigger. The outcome measures were the number of metaphase I, metaphase II oocyte, Germinal vesicle (GV) oocytes, high-quality embryo and rate of OHSS.

RESULTS:

Three groups were not significantly different in terms of the proportion of retrieved oocytes, the number of embryos, M I oocyte, M II oocyte, and the number of GV oocytes. The quality of embryos between the three groups was difference significantly ($p < .05$). In comparison to dual and GnRH-a group trigger, women who received hCG group had a higher number of OHSS, and the number of severe OHSS in dual trigger was higher than GnRH-a vs and hCG groups.

CONCLUSIONS:

GnRH agonist alone and dual trigger (hCG, GnRH agonist) can be as effective as hCG trigger. GnRH agonist is preferable in high risk patient. Therefore, it should be used according to the patient's condition.

Background

During IVF process (in vitro fertilization,) following ovarian stimulation and the development of follicles by gonadotropins, the final maturation of oocytes is achieved utilizing an ovulation trigger (1). Over the past 2 decades, hCG (human chorionic Gonadotropin) has been administered as a conventional protocol to encourage final follicular maturation, due to its structural and biological similarities with endogenous luteinizing hormone (LH), which imitates the physiological LH surge for oocyte maturation (2)(3).

Nevertheless, the use of hCG is concomitant with the increase in the risk of developing ovarian hyperstimulation syndrome (OHSS) due to its long half-life in the luteal phase (4). In addition, it has been established that the Follicle-stimulating hormone (FSH) is needed to improve the quality of the oocyte and hCG lacks FSH in the middle of the cycle (5)(6). The presence of FSH in the oocyte maturation

process has been demonstrated in a large number of studies that the GnRH agonist rises the proportion of mature oocytes (7)(8). Contrary to hCG, the Gonadotropin releasing hormone agonists (GnRH-a) provides a more physiological trigger effect, presenting with a shorter half-life, which results in a gonadotropin surge that lasts 34 hours, hence there is often poor LH support for the corpora lutea after ovulation, so that intensive luteal support is required to ensure implantation and ongoing pregnancy (9) (10). Due to this reduced duration of luteotropic activity, the results greatly reduce the potential for OHSS (11).

In this regard, a number of researchers have reported a rate of mature oocytes and a number of appropriate quality embryos which were equivalent if not more by using GnRH-a trigger when compared to the hCG trigger (12).

Subsequent studies comparing clinical outcomes between GnRH-a and hCG triggers in oocyte donation cycles disclosed comparable efficacy, however, with a remarkably reduced rate of Ovarian hyperstimulation syndrome in GnRH agonist triggered cycles (13)(14). Moreover, there were no findings which suggested that there was a meaningful difference between implantation, pregnancy or miscarriage rates in GnRH agonist trigger versus hCG trigger (14).

An alternative proposed stimulation is a Dual trigger, which includes a combination of GnRH-a and a low dose of hCG (1000–1500 units). Studies have indicated that the effect of dual trigger in final oocyte maturation is similar to GnRH agonist trigger in autologous cycles (15). A systematic review by Oliveira et al, demonstrated that the presence of a dual trigger is an excellent alternative for final maturation in poor responders and normal responders however, there is no indication for using dual in high responders (16). Furthermore, Ding et al., 2017 and Chen et al., 2018 performed two systematic reviews and meta-analyses which concluded that the quantity of oocyte M II and mature oocyte was identical in both dual trigger and hCG instances in addition to the Dual Trigger which has also improved clinical pregnancy rates considerably (17)(18). In contrast ,Kai-Lun Hu1 et al, recently conducted the systematic review and meta-analysis of randomized trials and discovered that the dual trigger treatment enhanced the number of mature MII oocytes collected, number of oocytes retrieved, viable embryos and fertilized oocytes (19). These findings suggest that the GnRH-a, a component of dual trigger dual, is beneficial in maturing oocytes and boosting the chances of pregnancy, as well as reducing OHSS syndrome.

The aim of this study is comparison the number of retrieved oocytes, high qualified oocytes and OHSS rate between three groups: GnRH-a, dual hCG triggers and hCG triggers in antagonist IVF cycles.

Methods

Study design

A prospective case control study enrolled patients attending Mashhad university-affiliated Infertility and IVF center (Milad) between May 2016 and June 2019. 320 patients who were referred for treatment of infertility enrolled the study. The study was approved by our institutional review board (IRB) with number

approval code IR.MUMS.REC.1395.326 and all participants provided written informed consent before entry.

Study participants

The inclusion criteria for participating in the study were women age 18–40 years, body mass index (BMI, calculated as weight in kilograms divided by height in meters squared) < 30 kg/m and high risk for OHSS (having more than 20 follicles > 12mm on day of trigger administration).

Patients with a history of diabetes, hyperprolactinemia, asthma, hypertension, hemorrhagic patients, and history of heart disease were excluded. Also, patient dissatisfaction with continued treatment was considered as an exclusion criteria.

At the beginning of the study, women underwent complete evaluation such as clinical history, physical exam, laboratory test, transvaginal sonography, and profile hormone. If they met the inclusion criteria, entered the study.

Ovarian stimulation protocols

The participants underwent GnRH antagonist protocol ovarian stimulation for preparing IVF. At first, all patients investigated with Transvaginal ultrasound on the second day of menstrual cycle. If the endometrial thickness was less than 5 mm and the ovaries were quiet, ovarian stimulation would be started.

Stimulation protocol treatment was initiated on the third day of menses with the daily use of recombinant human follicle-stimulating hormone (FSH, CinnaGen, Tehran, Iran) 150–225 IU. The dose of gonadotropin individually adjusted according to reserve tests of ovary, BMI, age and response in prior cycles.

Once the leading follicle reached a size of 13 mm, co-treatment with GnRH antagonist 0.25 mg/day Cetrotide (Merck Serono, Mississauga, Canada) and highly purified human menopausal gonadotropin (Menotropin 75 IU, Darou Pakhsh Pharmaceutical Co, Tehran, Iran) were commenced. Follicle growth and hormone levels were serially monitored by ultrasound. The dose of the drug was changed based on response ovary. All ultrasound exams were performed by a one researcher using a Phillips affinity 70 device at the Milad infertility center.

When the dominant follicles reached an average diameter of 18–20 mm (the morning of the trigger day) the investigators recruited the patients for the study. After the participants provided a written informed consent, based on the physician's preference (36.9%), 118 patients in GnRH-a group, (15.3%) 49 patients in hCG group and finally (47.8%) 153 patients in dual group (GnRH-a and hCG) were assigned. In hCG group, patients were triggered for final follicular maturation with hCG (PDpreg 5000 IU; pooyesh Darou, Tehran, Iran), in the dual trigger group, patients were triggered with GnRHagonist (Decapeptyl 0.2 mg, Ferring, Germany) and low dose hCG 1500IU and in GnRHagonist group, used GnRHagonist (Decapeptyl 0.2 mg) alone to trigger. oocyte retrieval was performed via transvaginal US-guided needle (K-OPSD-1730-A-L; Cook Australia Pty Ltd., Brisbane, Australia) puncture 36 hours after injection of trigger.

Follicular fluids were observed under a microscope and then Cumulus-oocyte complexes (COCs) were washed in Cleave medium (Origio-Denmark) at 37 ° C with 26% CO and 5% O₂, two hours after collection of oocytes, the oocytes were denuded from their cumulus cell and were evaluated for their maturity under Polarized Light Microscopy (PLM)

Based on Oocyte morphological such as oocyte shape, size, characteristic of polar body, perivitelline space, zona pellucida, granulation and cytoplasmic parameters like refractile bodies, vacuoles, smooth endoplasmic reticulum, Oocytes are divided into three categories, M I, MII, GV. The best quality oocyte selected for intracytoplasmic sperm injection (ICSI). On the same day, semen samples were collected and examined to evaluate sperm parameters including concentration, morphology, and motility, according to the World Health Organization guidelines (WHO laboratory manual, 2010) (20). The semen was prepared with Density gradient centrifugation and injection into oocyte were done 2 hours after CCs removal. After 16-18 h, normal fertilization was confirmed with the presence of two pronuclei (2PN) and the extrusion of the second polar body. The zygotes were placed on the tip of the Cryotop (Kitazato Corporation, Tokyo, Japan) then they were plunged in liquid nitrogen.

Other results are recorded, including the absence of pronuclear, presence of one pronuclear, and degeneration.

Embryo quality was assessed on the third day of fertilization and evaluated according to Cummins et al. standard as grade 1 (excellent embryos with 8 blastomeres with cell regularity and size equality without necrosis and fragmentation, grade 2 (embryos 1–20% fragmentation), grade 3 (21–50% fragmentation) and grade 4 (fragmentation greater than 50%).(21)

Outcome measures

The primary outcome measure was the number of oocytes derived in every group and the number of M I, M II, and GV oocytes. The secondary outcome measure was the rate of fertilization and rate of embryos grade 1, 2, 3. Fertilization rate was calculated the ratio of oocyte injected divided by the number of embryos fertilized.

OHSS classification is based on the Humaidan et al. criteria (22). Mild OHSS was determined by the existence of pelvic discomfort, abdominal distension, and the presence of ascites in the Douglas pouch and enlarged ovaries in ultrasonography. Moderate OHSS was stated in the presence of pelvic discomfort, abdominal distension, ultrasonic evidence of fluid in Douglas pouch and around the uterus (major pelvis), Ovarian enlargement, and hemoconcentration (hematocrit > 45%).

Severe OHSS was defined in the presence of both objective criteria (fluid collection in the pelvic pouch and around intestinal loops, hematocrit > 45 percent, white blood cells > 15,000, urine outputs < 600 mL per 24 hours) and subjective criteria (pelvic discomfort, abdominal distension, severe dyspnea, and enlarged ovaries).

Statistical analysis

For continuous variables, the mean, standard deviation (SD) were calculated. Comparing continuous variables between groups was performed using the one way ANOVA, which was dependent on whether the data was normally distributed. For categorical variables, the Chi-squared test was used as the appropriate measure. ANCOVA analysis was used to compare the three groups by adjusting the effect of age and duration of infertility. Statistical analysis on all 524 patients was performed using the Statistical Package for Social Sciences (SPSS®) version 24. A p-value of ≤ 0.05 was considered statistically significant for all statistical tests.

Results

Patients' characteristics: In brief, 320 patients underwent IVF/ICSI cycles who referred to Milad Infertility Center.

The average age of participants that receiving GnRH-a was 29.56 ± 4.91 and in the hCG group was 31.18 ± 6.20 and dual trigger was 30.40 ± 5.31 . Mean age wasn't significantly different in the three groups ($P = .178$) but the average duration of infertility was significantly different in the three groups ($P = .044$). Body mass index were homogeneous in the three groups ($p > .05$). Additionally, there was no difference regarding serum FSH, LH between three groups. The ovarian stimulation characteristics before oocytes retrieval in three groups. There wasn't a statistically significant difference in the total gonadotropins doses used in stimulation in GnRH-a and hCG and dual trigger groups ($p = .098$). Otherwise, no differences regarding total days of gonadotrophins stimulation. In finally compares the fertilization characteristics between three groups. The majority of patients in the three groups had primary infertility and respectively, which showed that there were significant differences between them ($p < .05$) There were significant differences between the three groups in menses cycle characteristics and cause of infertility ($p < .05$). There was a statistically significant difference in the total Number of follicle in three groups, GnRH-a, hCG and dual trigger groups (30.76 ± 9.87 vs. 23.75 ± 6.45 , 26.81 ± 8.62 , $p < .01$) Tables 1.

Table 1
Demographic and clinical characteristics of the study participants in three groups

		GnRH-a N = 118	hCG N = 49	Dual trigger N = 153	
Age in year		29.56 ± 4.915	31.18 ± 6.20	30.40 ± 30.40	P = .178
BMI (kg/m2)		25.58 ± 3.591	26.26 ± 3.68	25.19 ± 3.98	P = .212
Duration of infertility(year)		6.96 ± 4.40	8.48 ± 4.67	6.74 ± 3.99	P = .044
Number of consumption drug		28.71 ± 11.61	32.61 ± 13.35	30.93 ± 10.64	P = .098
Total dose of gonadotrophins stimulation (IU/ml)		2153.84 ± 870.85	2445.91 ± 143.12	2320.27 ± 798.92	P = .098
Total days of gonadotrophins stimulation (days)		11.10 ± 1.94	10.85 ± 1.69	11.25 ± 2.20	P = .486
FSH (mIU/ml)		5.52 ± 2.06	5.90 ± 2.58	5.90 ± 2.10	P = .313
LH (mIU/ml)		8.10 ± 5.07	6.69 ± 4.60	6.93 ± 4.76	P = .095
Number of follicle		30.76 ± 9.87	23.75 ± 6.45	26.81 ± 8.62	.000*
Pattern of the cycle	Irregular	71(60.7)	32(65.3)	90(58.8)	P = .721
Infertility	Primary	92(78.6)	46(93.9)	131(86.2)	P = .035
Cause of infertility	Female	58(49.6)	14(29.2)	65(42.5)	P = .019
	Male	16(13.7)	14(29.2)	37(24.2)	
	Male and Female	22(18.8)	11(22.9)	38(24.8)	
	Unexpected	21(17.9)	9(18.8)	13(8.5)	
Data are presented as mean SD, or n (%).					
hCG; human chorionic gonadotrophin, BMI; body mass index Weight / (Height)2, FSH; follicle stimulating hormone, LH; luteinizing hormone					

Table II compares the number and quality of follicles, retrieved oocyte, mature and immature oocytes, between three groups. In comparison three groups, women who received dual trigger and GnRH-a had higher number of retrieved oocytes (17.86 ± 7.49 and 17.37 ± 6.70 vs. 15.43 ± 6.21 , $p = .115$) but these difference were not significant. Number embryo and values for metaphase I oocyte, metaphase II oocyte,

and the number of GV oocyte were not significantly different between the three groups. Also, total number of viable embryos was slightly higher dual trigger and GnRH-a groups Vs hCG group, but did not significant ($p = .715$).

Table 2 shows difference between three groups regarding the quality embryo percentile between three groups ($p < .05$). Also shows no difference between both groups regarding the chemical pregnancy in three groups ($p = .156$)

Table 2
Comparison of Embryological data in three groups.

		GnRH-a N = 118	hCG N = 49	Dual trigger N = 153	P value
Number of oocyte		17.86 ± 7.49	15.43 ± 6.21	17.37 ± 6.70	.115*
Number embryo		10.66 ± 6.55	9.86 ± 5.63	10.55 ± 5.43	.715*
Number of MI oocytes		.81 ± 2.02	.37 ± .85	.60 ± 3.82	.664*
Number of MII oocytes		14.74 ± 7.49	13.76 ± 5.68	15.02 ± 6.58	.505*
Gv oocyte		.72 ± 1.57	.51 ± 1.37	.65 ± 1.38	.696*
Fertilization rate		78.39%	72.42%	70.87%	P = .483*
quality embryo	Grade 1	37(33.0)	25(52.1)	71(47.7)	P = .017**
	Grade2	74(66.1)	22(45.8))	74(49.7)	
Chemical pregnancy rate		31(27.7)	7(14.9)	40(28.8)	P = .156**
Data are presented as mean _ SD, or n (%). * Statistical significant difference.					
* ANOVA					
** Pearson Chi-Square					

Table 3 compares the ovarian hyper stimulation syndrome (OHSS) between three groups. There were no OHSS in three groups. In comparison to dual trigger and GnRH-a, women who received hCG group had a higher number of OHSS (15.2 % VS 9.0% – 7.1%, $p = 0.506$), number of sever OHSS in dual trigger had higher vs GnRH-a and hCG groups (1.4 % vs. 0 %).

Table 3
Comparison of OHSS in three groups.

		GnRH-a N = 118	hCG N = 49	Dual trigger N = 153	P value
OHSS	mild	6(5.3)	5(10.9)	8(5.5)	P = .506
	moderate	2(1.8)	2(4.3)	3(2.1)	
	sever	0(0)	0	2(1.4)	
OHSS		8(7.1)	7(15.2)	14(9.0)	
Data are presented as n (%). * Statistical significant difference.					
* Pearson Chi-Square					

Discussion

This study identified that the mean number of metaphase II oocytes in dual trigger was higher than other groups and the mean number of GV oocytes was higher in GnRH-a groups. There was no statistical difference in terms of quality and number of the retrieved oocytes in three types of triggers in the GnRH antagonist protocol. Also, there was no difference the number of high-quality embryos between groups but the quality of embryo was higher in dual triggers.

The last two decades have seen a growing trend towards the use of GnRH agonist trigger to reduce the prevalence of ovarian hyper-stimulation syndrome (OHSS)(23)(24). Also, Use of GnRH-a for triggering imitates the natural cycle by induction of a mid-cycle FSH surge and improves the quality of oocytes.FSH is a crucial element to stimulate the expression of LH receptors in oocyte granulosa cells, resumption of oocyte meiosis, and cumulus expansion.(25)(26) Consequently, it would be more advantageous than an hCG trigger to increase proportion of metaphase II oocytes retrieved

Moreover, hassl et al evaluated the messenger RNA (mRNA) expression of reproduction-related genes in granulosa cells (GCs) in hCG triggered cycles with GnRH agonist and hCG (double trigger) triggered cycles and concluded that improving the quality of oocytes and embryos in patients receiving double triggers due to reduce expression of connexin43 and increase in expression of epiregulin and amphiregulin (27).

The effectiveness of dual trigger on number and quality of oocyte and embryo has been investigated in various studies. That focus on high responder(28)(29)(30)(15) poor responder,(31) and normal responder (32)(33)(34)(35). The proportion of mature oocytes in the dual trigger was significantly higher than the hCG trigger in some studies (35)(33). On the other hand, Zhou stated the identical number of collected oocytes in the two groups.(32)

Our study shows an increasing number of mature oocytes in the dual trigger but it was not significant. In spite of other studies, we compare three types of triggers, as a result, our finding may be due to the small

sample size in each group. However, our results are consistent with Alleyassin et al. and Decleer et al.(36) (32) In addition, our study demonstrated high-quality embryos are higher in dual trigger in comparison to hCG or GnRH-a alone. However, there was no difference in the number of high-quality embryos between groups. Similar to our result Shymaa S et al. reported a considerable increase in grade I embryo in the dual trigger group without any difference in terms of number of embryo cryopreservation in comparison to the hCG group (35) but, Hass .j et al. and Zue et al. revealed dual trigger improve both the quality and proportion of top embryo.(33)(32)

Our study also showed that risk of incidence of OHSS is highest in hCG trigger. Two patients in the dual trigger developed severe OHSS, Which is clinically important but overall, there was no significant difference in the incidence of OHSS between the three groups.

In a related study by shaprio the rate of OHSS in high responder after dual trigger very low less than 1 percent. Different from our study, the diagnosis of OHSS was based on the need to remove ascites fluid, which can be affected the result(28)

Giffen performed another study that, high responder patients with peak E < 4000 include the study. Dual trigger (hCG 1,000 IU as an adjuvant to GnRH-a) had the identical number of oocyte retrieval with GnRH-a trigger but the rate of OHSS was not increased (2.9%vs 0).(15)

Similar to our study, Narrio found dual trigger is associated with increased number and maturity of oocytes collected in comparison to GnRH agonist alone. But, they concluded dual triggers significantly increased the risk of early OHSS. (8.6 vs 0 %)(37).Their result almost is consistent with our finding that the risk of OHSS in dual trigger is 9 %

In contrary to previous findings, Saijiao Li reported GnRH-a 0.2mg and low-dose hCG 2,000 IU to compare conventional trigger (10000 IU of recombinant hCG) and low-dose hCG trigger (8000IU of recombinant hCG) prevent OHSS and have a more top quality embryo rate in high ovarian responder.(29)

Recent study have been conducted using three types of trigger GnRH-a,dual trigger and hCG in Donor cycles. In the end, they demonstrated the number of mature oocytes in GnRH-a and dual groups significantly increased in comparison to hCG and also the highest risk of OHSS goes to dual trigger in comparison to both others 8.5%vs 0.4% vs 0%.there was no case of OHSS in hCG trigger in study and The authors concluded that hCG could be used instead of GnRH-a in low-risk patients for OHSS especially where the GnRH-a is not effective. but in our study the rate of OHSS in hCG trigger very high 15.2% vs9.0% vs 7%.(30) In our study, as we expected, the OHSS level in the hCG group was higher, which is justifiable. The choice of trigger in our study was based on the clinician's preference. As a result, patient with a more number of follicles, GnRH-a alone was used but in the patient with the lowest risk for OHSS, HCG was used. These results are consistent with prior findings, which showed that the risk of OHSS in the hCG group was greater than in the GnRH-a group.

These conflicting consequences might be described by different methods that are employed. Such as inconsistency in the research community, the type and dose of drugs. In addition, the OHSS classification has been different in some studies. As Kai-Lun Hu, mentioned there is no agreement towards the best dose of hCG and GnRH agonist in the dual trigger (19).

A key strength of the present study was comparing three kinds of trigger together, However, these findings are limited by the use of a cross sectional design. Therefore, the type of trigger was based on physician preference. Another weakness of our study was that we did not examine the pregnancy outcome due to the freeze all embryo. It is recommended that the study be conducted as a clinical trial with a larger sample size.

Conclusions

The present study shows that three kinds of triggers have the same effect on oocyte but top embryo is more in dual trigger. In high risk patient, GnRH-a alone may be a preferable trigger to reduce OHSS.

Abbreviations

hCG: Human chorionic gonadotropin

ICSI: Intracytoplasmic sperm injection

GnRH-a: Gonadotropin-releasing hormone

IVF: In vitro fertilization

RR: Risk ratio

MD: Mean difference

CI: Confidence interval

SE: Standard error

M I: Metaphase1

M II: Metaphase2

GV: Germinal vesicle

OHSS: Ovarian hyperstimulation syndrome

LH: Luteinizing hormone

FSH: Follicle stimulating hormone

BMI: Body mass index

COCs: Cumulus-oocyte complexes

PLM:Polarized Light Microscopy

WHO: World Health Organization guidelines

2PN:2pronuclei

Declarations

- **Ethics approval and consent to participate**

The study was approved by our institutional review board (IRB) with number approval code IR.MUMS.REC.1395.326 and all participants provided written informed consent before entry the study.

- **Consent for publication**

Not applicable

- **Availability of data and materials**

The datasets generated for this study are available on request to the corresponding author

- **Competing interests**

There are no conflicts of interest

- **Funding**

No funding

- **Authors' contributions**

MMahmoudina, N jahanpak, MMahmoudinia, Data collection.T Sadeghi analysis the result .

M Mahmoudinia,T Sadeghi, Akhzaee: writingthe manuscript. M Mahmoudina, S A Mohammadi, M Mahmoudinia : design and implementation of the research.Nayereh Khadem supervise the research .All the author(s) revised and approved the final manuscript.

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