Original Article

Evaluation of Dual Trigger with Combination of Gonadotropin-Releasing Hormone Agonist and Human Chorionic Gonadotropin in İmproving Oocyte-Follicle Ratio in Normo-Responder Patients

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Received: 29-Oct-2019;
Revision: 06-Apr-2020;
Accepted: 23-Dec-2020;
Published: 14-Aug-2021

ABSTRACT

Objective: Our aim was to compare the efficacy of two triggering method one with dual triggering with gonadotropin-realising hormon (GnRH) agonist plus standard dosage human chorionic gonadotropin (hCG) and the other with hCG only for final oocyte maturation on oocyte/follicle ratio and pregnancy rates in normoresponders in GnRH antagonist cycles in invitro fertilization-intrastoplasmic sperm injection (IVF-ICSI).Material **Methods:** In this retrospective study, all patients underwent GnRH antagonist protocol. When at least ≥3 follicles reached ≥17 mm diameter, 116 patients received dual trigger with GnRH agonist plus hCG (1mg Leuprolide acetate plus 10.000 IU uhCG) and 178 patients received uhCG (10.000 IU u hCG) for final oocyte maturation. All follicles ≥10 mm diameter were aspirated. Number of oocytes and metaphase II oocytes retrieved per aspirated follicles, implantation rate, and clinical pregnancy rate per cycle was recorded. **Results:** There was no statistically significant difference in terms of metaphase II oocyte ratio per aspirated follicle, implantation rate and clinical pregnancy rate between the dual trigger group and hCG only group (45.7% vs. 51%; 35.4% vs.30.3% and 45%vs. 40% respectively). Oocyte/ follicle ratio was significantly higher in dual trigger group (68.2%vs 63.8% p=0,028). **Conclusions:** Dual triggering in normal responders with a GnRH-agonist and a standard dosage of hCG is superior to hCG only protocol in terms of oocyte/follicle ratio but does not improve metaphase II oocyte, implantation and clinical pregnancy rates in GnRH-antagonist cycles. Dual triggering method may be beneficial in patients with immature oocytes and emty follicle syndrome.

Keywords: *Dual trigger, in vitro fertilization, ovarian hyperstimulation, oocyte-follicle ratio*

Introduction

Controlled ovarian hyperstimulation (COH) combining with gonadotropin-realizing hormone (GnRH)-antagonist (GnRHant) prevents premature luteinization and reduces the incidence of severe ovarian hyperstimulation syndrome (OHSS).[1] In GnRH-antagonist in vitro fertilization (IVF) cycles, GnRH-agonist (GnRHa) triggering was introduced to eliminate the risk of OHSS.[2,3] But reduced implantation rate and higher abortion rate were observed with GnRH-agonist triggering.[4,5] After agonist triggering, intensive luteal-phase support with progesterone, estradiol or human chorionic gonadotropin (hCG) is required. For high responders after triggering with GnRHa adding one bolus of 1500 IU hCG 1 h after oocyte retrieval was developed to improve the outcome.[6,7]

Empty follicle syndrome (EFS) is defined as no retrieved oocytes after meticulous aspiration of follicles after ovarian

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_574_19

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How to cite this article: Gurbuz AS, Deveer R, Gode F. Evaluation of dual trigger with combination of gonadotropin-releasing hormone agonist and human chorionic gonadotropin in İmproving oocyte-follicle ratio in normo-responder patients. Niger J Clin Pract 2021;24:1159-63.

stimulation in IVF treatment. Stevenson and Lashen[8] classified it into two types as genuine and false types. In genuine type optimal hCG levels present on the day of oocyte retrieval. Whereas low hCG levels present in false type. An error in the administration or the bioavailability of triggering agent may be responsible for low hCG levels.[8,9]

Different triggering strategies have been developed to improve IVF outcome; however, varying degrees of success rates have been reported for normal responders.[4,6,10] Schachter *et al*. first described dual triggering with combination of hCG (5000 IU) and GnRH agonist (triptorelin 0.2 mg).[10] Our aim was to investigate the role of adding a single dose of GnRH agonist to the standard hCG dose in triggering GnRH antagonist cycles in terms of real EFS and mature oocyte recovery.

Materials and Methods

This retrospective analysis of medical records between November 2014 and March 2016 was performed for IVF-ICSI cycles with GnRH-antagonist protocol at Private Novafertil IVF Centre, Konya, Turkey. Local ethics committee approval was obtained (NEK. 16-4)

Participants

A total of 294 normoresponder women who received either dual trigger with GnRH agonist plus hCG or standard dose of hCG for final oocyte maturation were included. Normoresponder patients aged 20--40 with no systematic illnesses were included in the study. No patient had EFS history.

Exclusion criteria comprised of contraindications for the use of gonadotropins, severe male factor and severe endometriosis (grade 3 or higher), polycystic ovary or freeze-all patients, uterine or ovarian abnormalities and endocrinological abnormalities.

All patients underwent multidose GnRH-antagonist controlled ovarian stimulation (COH) protocol. Daily gonadotropin stimulation was started on the second or the third day of either a spontaneous or an induced menstrual cycle; the starting dose was determined according to age, body mass index, follicular phase serum follicle stimulating hormone (FSH) level, antral follicle count (AFC) and previous history of ovarian response if there had been a treatment. GnRH antagonist injections at a dose of 0.25 mg/day were started either on the sixth day of stimulation or when the leading follicle reached 14 mm. Gonadotropin dosage was adjusted according to ovarian response on day 5. Pelvic ultrasound and endocrine monitoring were performed thereafter. Injections were continued until ≥3 follicles reached ≥17 mm diameter.

Interventions

Throughout 2014–2016, the practice of dual trigger was disclosed to patients during treatment. The treatment protocol was applied based on patient preferences. Patient records were divided into two groups according to trigger method. Group 1 consisted of 116 patients who received dual trigger with GnRH agonist plus hCG (1 mg Leuprolide acetate; Lucrin, Abbott, plus 10.000 IU uhCG; Pregnyl, MSD, Turkey). Group 2 consisted of 178 patients who received u hCG (10.000 IU u hCG) for final oocyte maturation. All follicles ≥10 mm diameter were aspirated 35–36 h after the trigger. The retrieved cumulus oocyte complexes were counted, denuded, and the number of mature oocytes were assessed. The number of oocytes and metaphase II oocytes retrieved per aspirated follicles was recorded. Intracytoplasmic sperm injection was performed on all mature oocytes. Embryos were examined in terms of the number and regularity of blastomeres and the degree of embryonic fragmentation on the third day. In our clinic, it is applied as a basic principle to go to the transfer on the third day. Patients under 35 years of age were transferred one embryo in the first two trials; whereas those over 35 and patients with more than two IVF interventions were transferred two embryos according to Turkish laws.

Women received luteal phase support with vaginal micronized progesterone (Crinone 90 mg/day; Merck Serono) underwent fresh embryo transfer. A positive serum hCG on day 15 postoocyte retrieval was defined as positive pregnancy test and evidence of a gestational sac and fetal heart on ultrasound was defined as clinical pregnancy.

Primary outcome measures were m2 oocyte- aspirated follicule ratio, total oocyte number-aspired follicule ratio, empty follicule syndrome (efs), clinical pregnancy rate.

Secondary outcome measures were fertilization rate, number of cryropreserved embryos following transfer. There are issues regarding determining the live birth rates and cumulative birth rates due to the admittance of patients from different and distant regions of the country which resulted in lack of data.

Statistics

The statistical analysis was carried out using the statistical package for social sciences. Distribution of the groups was analyzed with one sample Kolmogrov–Smirnov test. All normally distributed data were compared using a students' two-tailed *t* test. Pwr package of R 4.02 software which was firstly developed in New Zealand was used for power analysis. Effect size and significance value were determined as 0.5 and 0.05, respectively. Group 1 and group 2 were evaluated as 116 and 178. Power of the study was found to be 0.98.

Table 1: Patient characteristics and ovarian stimulation outcomes between patients triggered with hCG and those triggered with GnRH agonist+hCG

	hCG group	Dual trigger group	*P*
Age (years)	30.3±6.0	31.6±5.6	0,185
Previous cycles	1.7±1.0	2.0±1.5	0,546
Length of stimulation (days)	10.6±2.9	10.2±1.9	0,254
Endometrial thickness (mm)	10.4±2.3	11.1±2.3	0,071
Total gonadotropin dose	2928±1125	3007±1073	0,580
Peak E2 levels (pmol/l)	2002±813	1790±717	0,586
Number of follicles of>14 mm on day of hCG administration	10.6±5.1	10.1±5	0,483
Number of aspirated follicles of>10 mm on day oocyte retrieval	16.5±9.7	15.7±8.9	0,654
Number of oocytes retrieved	10.2±5.2	10.4±4.2	0,883
Oocyte/aspirated follicle	0.6±0.2	0.7±0.2	0,028
MII oocytes/aspirated follicle	0.5±0.2	0.5±0.2	0,827

* Values are mean±SD (Standard deviation)

Table 2: Embryological parameters between patients triggered with hCG and those triggered with GnRH agonist+hCG.

	hCG Group	Dual trigger group	*P*
MII oocytes*	7.1±3.2	6.7±3.5	0.526
Number of 2PN embryos*	5.1±2.3	5.0±2.4	0.912
Cryopreserved embryos*	1.3±0.4	2.0±0.6	0.044
Number of embryos transferred*	1.5±0.6	1.6±0.5	0.333
Positive hCG rate	%53 (94)	%50 (58)	0.638
Clinical pregnancy rate	%40 (72)	%45 (52)	0.458

*Values are mean±SD (Standard deviation). MII: Metaphase 2, hCG: Human chorionic gonadotropin, GnRH: Gonadotropin releasing hormone, PN: pro-nucleus

Results

The mean age of patients was similar in both groups. No differences were observed between the groups in the length of stimulation, the total gonadotropin doses administered, peak estradiol, and progesterone levels and numbers of follicles >10 mm and >14 mm in diameter on day of trigger. The demographic and clinical characteristics of the IVF cycles in the two groups are shown in Table 1.

There was no statistically significant difference in terms of metaphase II oocyte ratio per aspirated follicle, implantation rate, and clinical pregnancy rate between the dual trigger group and hCG only group (45.7% vs. 51%; 35.4% vs. 30.3% and 45% vs. 40%, respectively). Oocyte/follicle ratio and cryopreserved embryos were significantly higher in dual trigger group (68.2% vs. 63.8% $P = 0.028$; 1.3 ± 0.4 vs. 2.0 ± 0,6 $P = 0.044$ respectively) [Table 2]. No EFS was encountered in either group.

Discussion

In our study we compared the two triggering methods, one with dual triggering with GnRH agonist plus 10000 IU hCG and the other with 10000 IU hCG only for normoresponders in GnRH antagonist cycles. We conducted this study in order to determine whether dual triggering prevents EFS and increases mature oocyte ratio.

After introduction of antagonist cycles, GnRHa triggering method gained attention which was first demonstrated by Gonen *et al*.[11] Its effect is more similar to that of natural cycle but its routine usage has not been accepted widely. There are some reported advantages and disadvantages on IVF outcome of agonist triggering. Reducing the risk of OHSS seems to be the most important feature. Increased number of metaphase II (MII) oocytes has also been reported[5,12,13] although other studies have not confirmed this.[14-17]

In the present study we found that dual trigger of GnRHa and a standard dose of HCG in antagonist cycles do not improve the number of metaphase II oocytes, implantation and clinical pregnancy rates. The only difference we found was the increased oocyte/follicle ratio and cryopreserved embryos in dual triggering group. A recent meta-analysis by Youssef *et al*. reported that agonist triggering was associated with lower pregnancy and live birth rates comparing to hCG.[18] For overcoming these undesired results dual triggering method was developed.[10] Lin *et al*. also found improved implantation, clinical pregnancy, and live-birth rates by dual triggering.[19] In their prospective randomized study,

Deccler *et al*. found no difference in the mean number of MII oocytes and pregnancy rates between two triggering groups.[20] Lin *et al*. did not report the number of follicles on the day of trigger or the number of aspirated follicles on the day of retrieval. So it is not clear if the increased number of oocytes or MII oocytes is due to dual triggering only.[19] Decleer *et al*. also did not indicate the number of follicles on day of trigger and the number of aspirated follicles on day of retrieval.[20] Recently, oocyte/follicle ratio was used to assess the efficacy of triggering agent by Madani *et al*.[21] We think that the best method to evaluate the efficacy of triggering agent is to assess the number of aspirated follicles of >10 mm on the day of oocyte retrieval and the rates of oocyte/aspirated follicle and MII oocytes/aspirated follicle.

In our clinic, all follicles >10 mm underwent aspiration due to the possibility of obtaining mature oocytes. The number of follicles >10 mm in hCG trigger group were more numerous despite being statistically insignificant. In spite of similar numbers of oocytes and MII oocytes recruited in both groups, it appears that oocytes per aspirated follicle in the hCG group were less. In the dual trigger group the aspiration of immature oocytes seemed to be easier. But, the dual triggering did not contribute to oocyte maturation more than hCG did.

In their previous studies Lin and Decler did not investigate MII oocyte/follicle ratio per aspirated follicle. Otherwise they would have noticed the parallelism between their results and ours.[19,20]

Griffin *et al*. reported a higher proportion of mature oocytes and higher fertilization rates with dual triggering.[22] In contrast we did not find any difference between fertilization rates in our study. We can conclude that dual triggering has beneficial effect on embryo quality due to more number of frozen embryos obtained. We obtained more frozen embryos in the dual trigger group due to the higher quality oocytes which can be explained by the near to natural LH peak. We observed no statistical significance regarding the difference of clinical pregnancy ratios. The number of cryopreserved embryos is the most significant advantage in dual trigger group in terms of cumulative pregnancy rate.

Dual triggering may be beneficial in order to prevent both genuine and false types of EFS. Castillo *et al*. also described a case of a successful pregnancy after the use of a dual trigger in a patient who had a history of repetitive immature oocytes and EFS.[23]

In our study, we did not encounter EFS in either group. Therefore it is not inferable using our data to conclude that dual triggering prevents EFS.

Weaknesses of our study, relatively small sample size, and retrospective design were the important limitations of our study. Due to large numbers of patients from other Turkish cities and abroad, and the missing data in patient files, we could not investigate live birth rates and cumulative birth rates to a high precision. If we could, then cryopreserved embryo count would have been more significant. The strong aspect is that in order to reveal triggering agent activity, we investigated previously unstudied mature oocyte/follicle ratio.

In conclusion dual trigger of GnRHa and standard dose of hCG for normoresponders in GnRH antagonist cycles does not make additional benefit in terms of metaphase II oocyte, implantation rate, and clinical pregnancy rate but may be beneficial in patients with immature oocytes, oocytes/follicle ratio, frozen embryo count, embryo quality, and EFS. Randomized controlled studies including cumulative birth rates and mature oocyte count per aspirated follicle are needed.

Acknowledgement

The authors thank all our research assistants for participating in the data collection. The authors also appreciate Omer Deveer, Zeynep Gurbuz, and Necati Ozcimen for providing some didactic support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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