

Low-Dose Human Chorionic Gonadotropin Adjunct to an Antagonist Protocol in Assisted Reproductive Technology: a Randomized Trial Study

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Abstract- The aim of this study was to evaluate the outcomes of adding low-dose hCG (human chorionic gonadotropin), as an LH active supplement, to a GnRH antagonist protocol in patients undergoing assisted reproduction techniques. In this parallel-group randomized clinical trial, 137 infertile female outpatients aged 20 - 39 years were randomized into two groups: hCG group and non-hCG group. All patients received r-FSH (150-300 IU) and then a GnRH-antagonist, Cetrorelix (0.25 mg/day). Concomitantly with Cetrorelix, patients in the hCG group received low-dose hCG (200 IU daily), but the patients in the non-hCG group did not. 10,000 IU Urinary hCG (10,000 IU) was injected to all patients, and ICSI was performed after oocyte retrieval. The primary outcome of this study was comparing the pregnancy rates between two study groups. Other differences between two groups such as serum estradiol concentration, fertilization rate, etc. were considered as secondary outcomes. A total of 130 patients completed this trial. No significant difference was detected between pregnancy rates of the two groups ($P=0.52$) as well as the fertilization, implantation and ongoing pregnancy rates ($P=0.11$, $P=0.75$ and $P=0.06$ respectively). The only significant difference between two groups was a higher concentration of estradiol in the hCG-treated patients ($P<0.05$). HCG-treated patients experienced a shorter treatment duration and a lower r-FSH required dose than the non-hCG group, but none of these differences were statistically significant ($P=0.19$ and $P=0.10$, respectively). The findings of the current study did not support advantages of adding low-dose hCG to GnRH antagonist plus r-FSH protocol in an unselected population of patients. Well-designed trials with a larger sample size for specific patients' subgroups are warranted.

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Introduction

Controlled ovarian stimulation plays a key role in reproductive medicine and achieving a successful pregnancy using assisted reproductive techniques (ARTs) (1,2). Over the past two decades, gonadotropin releasing hormone (GnRH) agonists and antagonists has attracted much more attention to be applied for controlling the ovarian stimulation (3-5). Many advantages make GnRH antagonists more favorable than GnRH agonists for use in patients undergoing ART. GnRH antagonists may have a shorter period and more suitable treatment plan. They decrease rate of ovarian hyperstimulation syndrome (OHSS) that sometimes occurs, as a result, of infertility medications (6-10). GnRH antagonists competitively block the GnRH

receptors which result in two fundamental consequences. First, luteinizing hormone (LH) secretion is profoundly decreased along with a lesser decline in the follicle stimulating hormone (FSH) concentrations. Second, premature ovulation is prevented because of inhibition of the premature LH surge. While the later effect of GnRH antagonists is useful, the former one may be undesirable because a minimum threshold of LH and FSH is required for steroidogenesis and follicular growth, respectively (8,11,12).

Using GnRH antagonists with recombinant FSH (r-FSH) cannot optimally improve the follicular growth and oocyte development. Similarly, deep suppression of LH in GnRH antagonist cycles does not have beneficial effects on the reproductive outcomes of ART (13-15). Some evidences suggest that using an LH activity

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supplement may be useful especially when GnRH antagonists protocol are applied for ovarian stimulation (16,17). Although LH activity has not proven to be advantageous yet, but it has been hypothesized that adding LH activity to antagonist cycles via administration of low-dose human chorionic gonadotropin (hCG) in late follicular phase may improve the pregnancy rates, reduce the duration of stimulation and decrease consumption of FSH (18,19). Serafini *et al.* reported that adding low-dose hCG to conventional antagonist protocols can result in the higher number of good quality embryos, the lower dose of r-FSH required for ovarian stimulation, and higher pregnancy rates (20). Several reports do not support this hypothesis. Cedrin *et al.*, could not found significant benefits for recombinant LH (r-LH) in supplementing the GnRH antagonist protocol for patients undergoing ARTs. In their study, patients who received r-LH had higher serum concentrations of estradiol but could not reach a higher number of oocytes and embryos or greater pregnancy rates (21).

According to these findings, the authors conducted a randomized clinical trial study to evaluate the efficacy of adding low-dose hCG, as an LH active supplement, in the late follicular phase in GnRH antagonists plus r-FSH protocol on the reproductive outcomes in patients undergoing in vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI) cycles.

Materials and Methods

Study design

This was a parallel-group randomized clinical trial. The study was conducted at the infertility specialty clinic in the women's outpatient clinic of Dr. Shariati Hospital (Tehran University of Medical Sciences, Tehran, Iran) from October 2012 to March 2013. The study was authorized by the Institutional Review Board (IRB) and approved by the ethics committee of the Tehran University of Medical Sciences (Reference number: 5739). The whole trial was performed in accordance with the Declaration of Helsinki and its subsequent revisions. Patients were completely informed about the study details. Written informed consent was obtained from participants before entering the study. This trial was registered in the Iranian Clinical Trials Registry (IRCT201211197165N2; www.irct.ir).

Participants

Female outpatients aged 20- 39 years who were infertile and had standard indications of applying ART

were eligible to participate in the study. Participants were recruited if they had body mass index (BMI) of 20-25 kg/m², a normal uterus and two functional ovaries, day 3 FSH concentration < 10 IU/L and estradiol concentration <60 pg/mL. Patients were excluded if they had a poor ovarian response in previous IVF/ICSI treatment, history of more than two unsuccessful IVF/ICSI attempts, or any endocrine disorders.

Interventions

A computer-generated code was used in order randomly to assign the eligible patients into two groups: hCG group and non-hCG group. For all of the patients in both study groups, 150-300 IU of r-FSH was administered subcutaneously on either day 2 or 3 of the menstrual cycle for ovarian stimulation that was continued with a full dose until two follicles reached 14-15 mm in size; then subcutaneous administration of a GnRH antagonist (Cetrorelix) was started with the dose of 0.25 mg/day and continued for 2-5 days. The dose of r-FSH was calculated individually based on patients' age, BMI, and ovarian responsiveness in previous ART cycles.

Patients in the hCG group received daily subcutaneous injections of low-dose hCG (200 IU daily) concomitant with Cetrorelix, but the patients in the non-hCG group did not. The drugs were continued until at least two follicles reached 18 mm in size and then. 10,000 IU of urinary hCG was injected intramuscularly for all patients, and oocyte retrieval was performed by transvaginal ultrasound-guided aspiration within 35–36 hours after hCG administration. ICSI was performed for all patients, and no more than three embryos were transferred three days after oocyte retrieval. Vaginal progesterone (Cyclogest 400 mg) was used to support the luteal phase. Patients received one Cyclogest (400 mg) on the day of oocyte retrieval followed by daily administration of two Cyclogest until 10th week of pregnancy. The only intervention that was different between two study arms was low-dose hCG.

Outcomes

The primary outcome of the current study was comparing the pregnancy rates between two study groups. In terms of pregnancy, β -hCG was checked in a blood sample about 14 days after embryo transfer and a β -hCG concentration >10 IU/L was considered as positive. Additionally, transvaginal ultrasounds were performed at five and seven weeks gestation to identify the gestational sac and fetal cardiac activity, respectively. Other differences between two groups

including serum peak estradiol concentration, required r-FSH dose, the total number of oocytes, number of mature oocytes, the total number of embryos, number of good quality embryos, endometrial thickness, fertilization rate, implantation rate, and ongoing pregnancy rate were considered as secondary outcomes. Endometrial thickness and implantation were determined by transvaginal ultrasound. A good-quality embryo was defined as the absence of multinucleated blastomeres, seven or more cells on day three, and $\leq 20\%$ anucleate fragments.

Side effects

Patients were encouraged to inform the research team about any unexpected symptom or probable side effect after entering the study. Side effects were recorded at each visit using a thorough checklist regarding the medications used in the study.

Sample size

Based on previous trials, considering the expected difference of pregnancy rates between two groups, and assuming a power of 80%, a two-sided significance level of 0.05, and attrition rate of 20%, a total sample size of 132 was calculated.

Statistical methods

IBM SPSS Statistics 20 (IBM Corporation) was used for data analysis. Categorical variables were described in a number (%) and continuous variables as mean \pm SD. Percentages or rates were compared by using Fisher's exact test or chi-square test. Continuous variables were analyzed by using Student's t-test. A two-sided significance level of <0.05 was considered statistically significant.

Results

A total of 178 patients were screened for the eligibility criteria and 137 patients were randomized into

two groups. Number of patients in the HCG group was 72. Five patients were dropped out (two severe OHSS, one poor ovarian response, and two fertilization failure). Number of patients in non HCG group was 65. Two patients were dropped out (one severe ohss, one fertilization failure). Finally, 130 patients (hCG group=67, non-hCG group=63) completed the trial, and their data were analyzed (Figure 1). No significant differences were determined between baseline characteristics of participants in two study groups (Table 1).

Outcomes

By the study endpoint, no significant difference was between two groups in the pregnancy rate ($P=0.52$) as our primary outcomes. Fertilization rate, implantation rate and ongoing pregnancy rate did not significantly differ between two groups as well ($P=0.11$, $P=0.75$ and $P=0.06$ respectively) (Table 2). Although the hCG-treated patients experienced a shorter treatment duration than the non-hCG group (11.88 vs. 12.19 days), but this difference was not statistically significant ($P=0.19$). Similarly, analysis of current study data did not show any significant difference between mean required doses of r-FSH for patients in the two study groups ($P=0.10$); however, the patients who received low-dose hCG required a slightly lower dose of r-FSH than the other group (1972.41 vs. 2199.87 IU).

Blood samples taken on the day of hCG injection showed significantly higher concentrations of estradiol in the hCG-treated patients than the non-hCG group ($P<0.05$). No significant difference was found between two study groups in the patients' endometrial thickness ($P=0.16$). Total number of retrieved oocytes and the number of mature oocytes were not significantly different between two groups ($P=0.90$ and $P=0.36$, respectively) as well as the total number of embryos ($P=0.83$) and the number of good quality embryos ($P=0.12$) (Table 3).

Table 1. Baseline characteristics of the patients

	GnRH antagonist + r-FSH + low-dose hCG (n=67)	GnRH antagonist + r-FSH (n=63)	P value
Age, years, mean \pm SD	29.72 \pm 3.58	28.90 \pm 4.34	0.59
Body mass index, kg/m ² , mean \pm SD	26.52 \pm 3.22	26.69 \pm 0.54	0.71
Duration of infertility, years, mean \pm SD	7.75 \pm 2.66	7.66 \pm 2.56	0.90

GnRG: gonadotropin releasing hormone; r-FSH: recombinant follicle stimulating hormone;
hCG: human chorionic gonadotropin

Table 2. Comparison of the cycle outcomes between two study groups

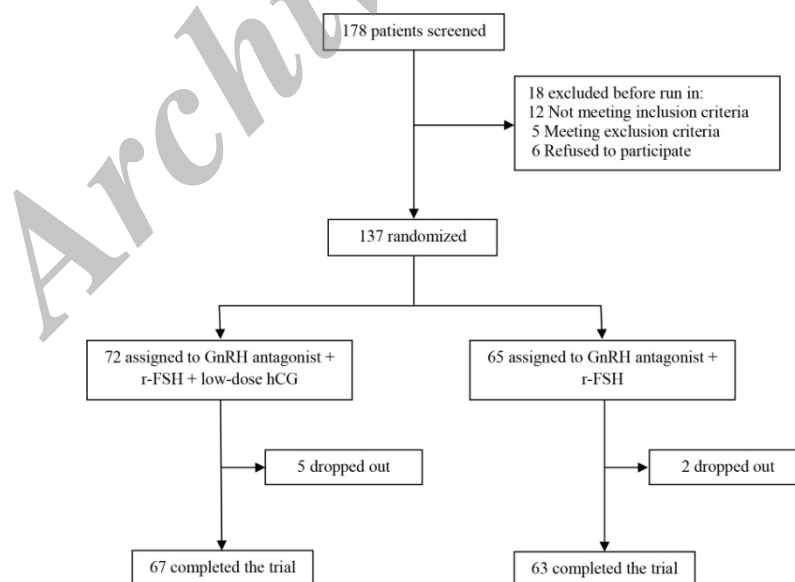
	GnRH antagonist + r-FSH + low-dose hCG (n=67)	GnRH antagonist + r-FSH (n=63)	P value
Pregnancy rate (%)	25	31	0.52
Fertilization rate (%)	69	75.6	0.11
Implantation rate (%)	29	32	0.75
Ongoing pregnancy rate (%)	17	25	0.06

GnRG: gonadotropin releasing hormone; r-FSH: recombinant follicle stimulating hormone; hCG: human chorionic gonadotropin

Table 3. Mean \pm SD of the reproductive measures in the two study groups

	GnRH antagonist + r-FSH + low-dose hCG (n=67)	GnRH antagonist + r-FSH (n=63)	P value
Treatment duration, days, mean \pm SD	11.8 \pm 1.40	12.19 \pm 1.31	0.19
Required r-FSH dose, IU, mean \pm SD	1972.41 \pm 678.43	2199.87 \pm 845.40	0.10
FSH level, IU/L, mean \pm SD	6.48 \pm 2.69	6.32 \pm 1.59	0.81
Estradiol level, pg/mL, mean \pm SD	2565.40 \pm 1758.60	1788.35 \pm 1298.02	<0.05
Endometrial thickness, mm, mean \pm SD	10.53 \pm 1.48	10.11 \pm 1.95	0.16
Total oocytes, n, mean \pm SD	11.61 \pm 5.47	11.50 \pm 4.38	0.90
Mature oocytes, n, mean \pm SD	8.64 \pm 4.42	8.01 \pm 3.373	0.36
Total embryos, n, mean \pm SD	5.87 \pm 3.51	6.06 \pm 3.57	0.83
Good quality embryos, n, mean \pm SD	4.03 \pm 2.20	4.95 \pm 2.21	0.12

GnRG: gonadotropin releasing hormone; r-FSH: recombinant follicle stimulating hormone; hCG: human chorionic gonadotropin; FSH: follicle stimulating hormone

**Figure 1. Flow diagram of the study**

Clinical complications and side effects

Most of the side effects in this study were mild and did not need any intervention. The most important complaint of participants was symptoms of OHSS, which was observed in four patients in the non-hCG group and six patients in the hCG group. No significant difference was between the two study groups in this regard ($P=0.74$).

Discussion

The results of the current study did not show significant beneficial effects for low-dose hCG when added to GnRH antagonist plus r-FSH protocol in patients treated for IVF/ICSI procedure. In contrast with authors hypothesis, patients who received low-dose hCG in this study did not show higher rates of pregnancy than the non-hCG group. Surprisingly, pregnancy rate that was considered as our primary outcome was even slightly lower in the hCG-treated patients. The only significant difference between two groups in this trial was serum peak concentrations of estradiol that was significantly higher in the hCG group. While this is a considerable finding, comparison of other fertility measures between two treatment strategies in this study raises doubts about the benefits of adding low-dose hCG to conventional antagonist protocols. Low-dose hCG was not able to positively influence the number of oocytes or embryos and could not improve the implantation or ongoing pregnancy rates. Inconsistent with results, some recent studies have reported a significant increase in the pregnancy rate and other fertility measures in patients receiving low-dose hCG during GnRH antagonists cycles (22-24). A number of these trials started hCG supplementation simultaneously with r-FSH (25-27) and our delay in the administration of low-dose hCG could be a probable reason for our different results (28). Diversities that exist between the biochemistry and bioactivity of miscellaneous available hCG products may be another explanation for such differences (29).

Patients who received low-dose hCG in this study had significantly higher concentrations of estradiol; a finding which is in line with previous studies (22,30,31). As the only difference between treatment regimens of two groups was hCG administration, these higher concentrations of estradiol can be justified directly by the effects of low-dose hCG. LH and hCG make the granulosa cells produce the precursors of estradiol synthesis. Previous studies have confirmed the positive

correlation of LH and hCG with increased peak concentrations of estradiol (32-34). It is reported that suppressed concentrations of LH negatively affect the reproductive outcomes and lead to decreased estradiol levels, retarded follicular growth, decreased pregnancy rates, and increased miscarriage rates (35-37). Some previous studies have found that patients with hypothalamic related amenorrhea may reach appropriate follicular and endometrial development after being treated with LH supplementation (38-41). Other studies show that some patients with normal gonadotropin concentrations achieve good results in IVF/ICSI cycles with FSH alone in both GnRH agonists and antagonists protocols (42-44). The probable reason is that baseline concentrations of LH in these patients are adequate for promoting steroidogenesis and oocyte maturity despite being suppressed by GnRH agonists and antagonists (45). However, no reliable prospective way is reported to find patients that would benefit from adding LH activity to their treatment plan prior to IVF/ICSI procedure (46).

Both LH and hCG consist of two subunits in their structure: α and β . The α -subunit is similar between two agents, but the glycosylation pattern of β -subunit of hCG makes it structurally different from LH leading to higher affinity of hCG for its receptors as well as a longer half-life (47-49). Binding to LH/hCG receptors and their activation results in the production of androgens and estrogen precursors in theca cells as well as accompanying with FSH in making optimal follicular development (50,51). Authors preferred to use low-dose hCG in this study for adding LH activity to the antagonist protocol because of its cost effectiveness and biologic activity at low doses (26). It is reported that administration of low-dose hCG shortens the treatment duration and decreases the patients' FSH need for controlled ovarian stimulation (25,52,53). The treatment duration was shorter, and the required r-FSH dose was lower in the hCG-treated patients in the current study, however, none of these differences reached a significant level compared with the patients who did not receive low-dose hCG. The authors did not insist on measuring serum LH concentrations after the start of GnRH antagonist in this study with the rational that immunoreactivity of LH does not necessarily reflect its bioactivity and may lead to misinterpretations (54,55). LH is released on a pulsatile fashion, and a single measurement of LH has little value for assessment of ovarian function (56,57).

A number of limitations need to be considered

regarding the present study. The sample size of this study was relatively small, and the participants were not selected to receive low-dose hCG based on their serum LH concentrations. Finally, the administration time and dosage of different drugs, especially hCG, in the protocol should be restudied. To evaluate the possible beneficial effects of low-dose hCG on IVF/ICSI cycles, especially in different subgroups of patients, further research is warranted through well-designed randomized trials. In conclusion, the findings of the current study do not support the advantages of adding low-dose hCG to GnRH antagonist plus r-FSH protocol in an unselected population of patients undergoing IVF/ICSI procedure.

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