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REPRODUCTIVE MEDICINE

Efficacy of low-dose hCG in late follicular phase in controlled ovarian stimulation using GnRH agonist protocol

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Abstract
Objective Safe, simple and cost-effective protocol is an important goal in ART cycles. The aim of this prospective study was whether administration of low-dose hCG in late follicular phase can be used clinically to replace gonadotropin administration in GnRH long protocol.
Materials and methods 122 patients who were candidates for ART enrolled the study and randomly divided into two groups. The control group ($n = 62$) received standard long protocol and gonadotropin administration continued until the day of hCG injection (10,000 IU) for final follicular maturation. The study group ($n = 60$) received GnRH long protocol and when at least $\geq$6 follicles with mean diameter $\geq$12 mm were observed in both ovaries, hMG was displaced by 200 IU per day of hCG until final follicular maturation.
Results There were no significant differences in age, basal FSH, infertility duration and infertility etiology between two groups. There were no statistically significant differences between two groups regarding chemical pregnancy, clinical pregnancy, ongoing pregnancy, and abortion per cycle (50, 40, 40, and 20 % in study group vs. 45.2, 35.5, 35.5, and 21.4 % in control group, respectively). Mean dose of used gonadotropins was significantly higher in control group than that in the study group (2,524 $\pm$ 893 IU in control group and 1,439 $\pm$ 433 IU in study group) ($p = 0.000$).
Conclusion According to our data, we recommend the use of low-dose hCG in GnRH long protocol because of lower doses of used gonadotropins.

Keywords Pregnancy outcome · Low-dose hCG · Late follicular phase · GnRH long protocol · ART cycles

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Introduction

Controlled ovarian stimulation (COS) is an essential component of assisted reproduction technology (ART). A safe, simple and cost-effective protocol is an important goal in ART cycles [1]. A new protocol for COS on the basis of using low-dose human chorionoc gonadotropine (hCG) instead of follicle stimulating hormone (FSH) in late follicular phase was reported [2, 3].

During the menstrual cycle, increased FSH levels in early follicular phase stimulate growth and recruitment of small follicles, in late stage of follicle development, a decrease in FSH levels and a progressive elevation of luteinizing hormone (LH) are associated with selection of the dominant follicle. It seems that these events are dependent on the expression of LH receptor on the granulose cells of dominant follicle, thus making it sensitive to LH activity stimulation and less dependent to FSH [4, 5] .

In early follicular stage, FSH is key for follicle recruitment, LH may also play important roles in folliculogenesis and induction of ovulation [6].

The role of LH in the follicular phase and controlling the ovarian stimulation is controversial. The optimal level in ART treatment and drugs is still debated [5]. Moreover, recent evidences support a role for LH in embryo cleavage, blastocyst formation, better embryo quality and influence on uterine receptivity [5, 7, 8]. In the absence of continuing

FSH, LH activity in the form of low-dose hCG or recombinant LH is capable of stimulating follicular growth and maturation of large follicles while decreasing the growth of small follicles [4, 9].

Both hCG and recombinant LH (rLH) are beneficial for optimizing controlled ovarian hyperstimuation (COH). However, hCG has a longer half-life and more affinity for the LH or hCG receptors. So hCG is a better alternative for ovarian stimulation [1, 8, 9].

Gonadotropin releasing hormone (GnRH) agonists are now widely used in COH protocols; they cause suppression of pituitary gonadotropins and profound inhibition of LH that may lead to a suboptimal ovarian response [10].

Mild to late follicular phase FSH replacement by hCG in GnRH antagonist cycles has been reported. Blockeel et al. [1] showed that this approach leads to significant decreasing of cost whereas ART outcome in terms of retrieved oocyte and ongoing pregnancy rates remains comparable to the traditional regimen.

Van Horne et al. [9] in their study on recombinant follicle-stimulating hormone (rFSH) supplemented with low-dose hCG demonstrated that the low-dose hCG group had similar implantation and pregnancy rate but had significantly reduced rFSH requirement.

Most of the previous studies focused on the substitute of hCG in late follicular phase by FSH in antagonist protocol, but in this present study, we evaluated the effectiveness of a new ovarian stimulation protocol, which consists of using a low-dose hCG supplementation in conjunction with a GnRH agonist in the late follicular phase.

Materials and methods

This study was a prospective randomized clinical trial (with Project referral number in IRCT: IRCT201107286420N5) on 122 patients who were scheduled for ART between March 2009 and May 2011. The study was approved by ethics committee of research and clinical center for infertility, Yazd Shahid Sadoughi University of Medical Sciences, a written informed consent was obtained from all patients.

We studied 122 patients, all younger than 38 years old, with regular menstrual cycles (25–35 days), BMI <30 kg/m^2, normal uterus and ovaries in vaginal ultrasound and basal FSH <10 IU/L. Women with severe endometriosis, polycystic ovary syndrome (PCOS), history of pelvic surgery, azoospermia and more than two in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycle failures were excluded from the study. A computerized generated list was used for randomization.

Patients were divided into two groups (Fig. 1). All patients received a single injection of 3.75 mg of depot triptorelin (Diphereline S.R. 3.75 mg, Ferring, Germany) in mid-luteal phase of a spontaneous menstrual cycle and they menstruated before the initiation of gonadotropin treatment. Gonadotropin stimulation with intramuscular injection (IM) of 150–225 IU human menopausal gonadotropine (hMG) per day (Merional, IBSA, Lugano, Switzerland) was started from day 2 of cycle in both groups. Ovarian response was monitored by serial vaginal sonographies and evaluation of serum E2 levels. In group 1 (hCG group), the administration of hMG was discontinued when at least six follicles $\geq$12 mm were observed and E2 levels were higher than 600 pg/ml; hMG was displaced by 200 IU per day of hCG (pregnyl®, 500 organon, Oss, Netherlands) until final follicular maturation. A daily dose of 200 IU of hCG was administrated by diluting one 500 IU ampoule of hCG with 1 ml normal saline and injection of 0.4 ml of this solution. In group 2 (hMG group), patients received similar doses of hMG based on individual responses until the end of stimulation. In both groups, the ovulation trigger was done by IM injection of 10,000 IU of urinary hCG (pregnyl®, organon, Oss, Netherlands) when at least three follicles $\geq$18 mm were observed. Endometrial thickness and serum E2, P, and LH levels were measured on the day of hCG injection. Oocyte retrieval was performed 34–36 h after hCG injection and ICSI or conventional IVF was performed appropriately. Embryos were transferred using a labotect catheter (labotect, Gottingen, Germany) 2–3 days after oocyte retrieval. Luteal phase hormonal support consisted of daily IM injection of 100 mg progesterone in oil (progesterone, Aburaihan CO, Tehran, Iran), beginning on the day of oocyte retrieval and was continued until the observation of embryonic heart activity by ultrasonography. Chemical pregnancy was defined by positive beta-hCG 12 days after embryo transfer. Clinical pregnancy was identified as the presence of gestational sac with heart activity detected by ultrasonography, 3 weeks after positive beta hCG. Miscarriage was defined as loss of pregnancy before 20 weeks of gestation. Fertilization was identified by the observation of 2 pronuclei 18 h after ICSI. The implantation rate was defined as the number of gestational sacs per number of embryos transferred. Primary outcomes were total doses of gonadotropin and clinical pregnancy rate.

Statistical analysis

Statistical analysis was performed using the statistical package for social science version 15 for windows (SPSS inc, Chicago, IL, USA). Differences among variables were analyzed by Student's t test, Mann–Whitney, and Chi square tests. P value of less than 0.05 was considered statistically significant.

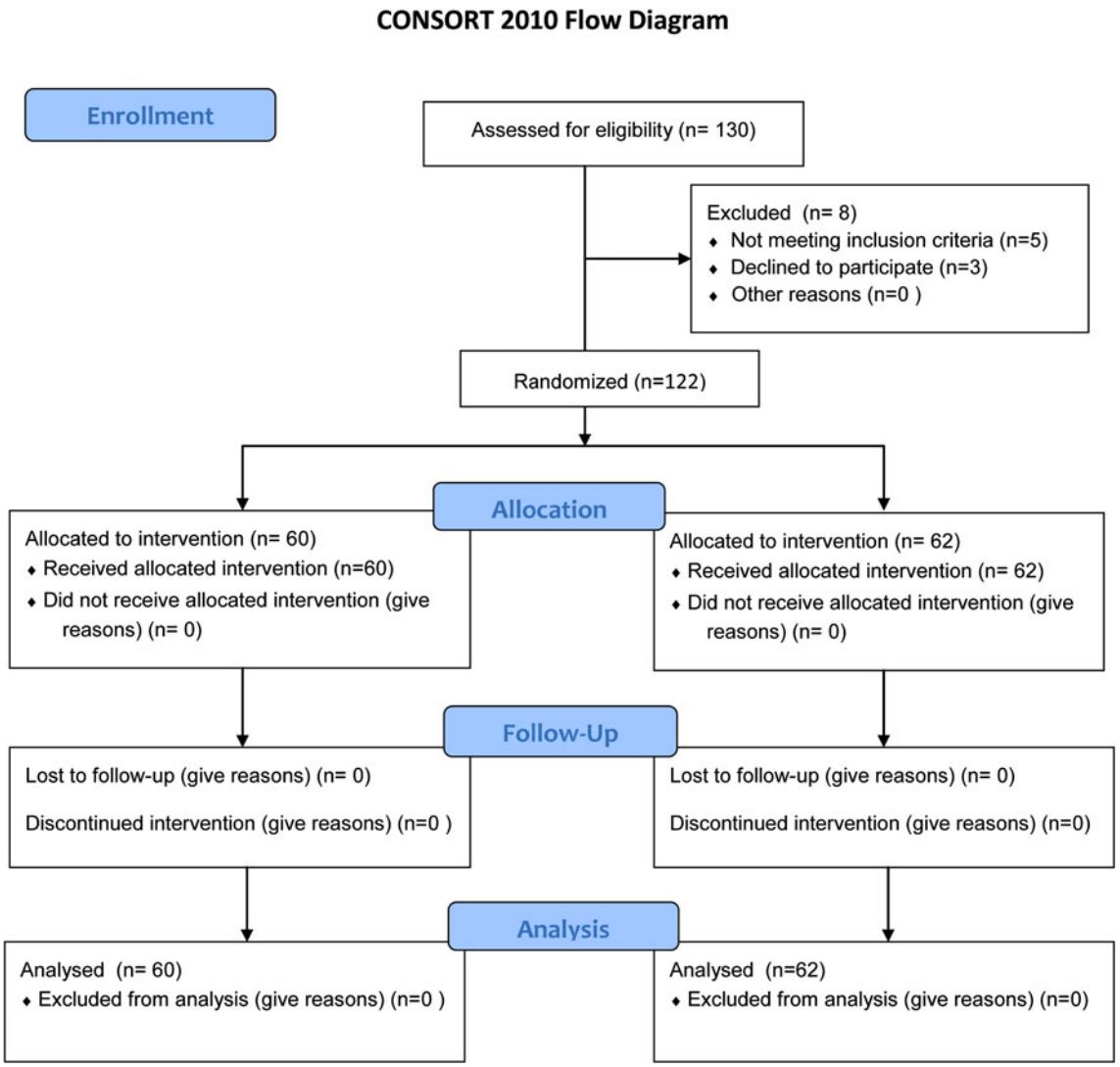

Fig. 1 CONSORT 2010 flow diagram

Results

One hundred and twenty-two couples were participated in this study and patients were divided into two groups: 60 patients were enrolled in hCG group and 62 patients in control (hMG) group. There were no significant differences between the groups, regarding demographic characteristics, as shown in Table 1.

Details referring to the stimulation are summarized in Table 2. The results of ovarian stimulation in two groups such as mean E2, LH and progesterone levels, endometrial thickness, retrieved oocytes and number of embryo obtained were statistically similar. The total dose of gonadotropins consumed was significantly lower in the hCG group (Table 2).

There were no statistical differences between two groups regarding fertilization and implantation rates. The clinical outcomes in terms of chemical pregnancy, clinical pregnancy and ongoing pregnancy per cycle were not statistically different between two groups (50, 40, and 40 % in study group vs. 45.2, 35.5, and 35.5 % in control group, respectively). The miscarriage rate was similar in both groups (20 vs. 21.4 %) (Table 3). None of the patients presented moderate or severe forms of the ovarian hyperstimulation syndrome (OHSS).

Discussion

The findings of our study demonstrated that ovarian stimulation started with hMG and followed by daily administration of 200 IU hCG in late follicular phase, showed similar ovarian responses and oocyte development, compared to cycles stimulated by hMG alone.

Table 1 Basic characteristics of patients in two groups

Variables	hCG group Mean (SD) ($n = 60$)	hMG group Mean (SD) ($n = 62$)	p value
Age (years)	28.6 ± 3.8	27.04 ± 4.0	0.265
Duration of infertility (years)	5.96 ± 3.1	6.09 ± 3.94	0.722
Basal FSH (IU/L)	5.67 ± 2.12	5.80 ± 1.54	0.325
BMI (kg/m^2)	23.3 ± 2.6	22.1 ± 2.7	0.261
Etiology of infertility			
Ovulatory, *n* (%)	7 (11.7 %)	9 (14.5 %)	0.396
Tubal, *n* (%)	6 (10 %)	5 (8.1 %)	
Mild endometriosis, *n* (%)	3 (5 %)	4 (6.5 %)	
Unexplained, *n* (%)	8 (13.3 %)	10 (16.1 %)	
Male, *n* (%)	26 (43.3 %)	31 (50 %)	
Mixed, *n* (%)	10 (16.6 %)	3 (4.8 %)	
Total, *n* (%)	60 (100 %)	62 (100 %)	

Table 2 Results of ovarian stimulation in two groups

Variables	hCG group Mean (SD) ($n = 60$)	hMG group Mean (SD) ($n = 62$)	p value
Total doses of used gonadotropins (IU)	1,439 ± 433	2,524 ± 893	0.0001
Duration of stimulation (days)	12.33 ± 1.91	12.54 ± 1.84	0.289
Endometrial thickness (mm)	9.93 ± 1.43	9.53 ± 1.18	0.194
E2 concentration (pg/ml)	2,190 ± 1,465	1,988 ± 1,508	0.453
LH (IU/L)	1.4 ± 1.4	2.1 ± 1.1	0.240
Progesterone (ng/ml)	1.1 ± 0.8	1.2 ± 0.4	0.541
No. of retrieved oocytes	10.15 ± 3.71	10.24 ± 3.65	0.891
No. of obtained embryos	6.16 ± 3.46	5.77 ± 2.99	0.504
No. of transferred embryos	1.93 ± 1.16	2.1 ± 1.49	0.342

Table 3 ART outcome in two groups

Variables	hCG group ($n = 60$)	hMG group ($n = 62$)	p value
Fertilization rate (%)	63.61	66.23	0.626
Implantation rate (%)	16.67	15.17	0.549
Chemical pregnancy rate, *n* (%)	30/60 (50 %)	28/62 (45.2 %)	0.717
Clinical pregnancy rate, *n* (%)	24/60 (40 %)	22/62 (35.5 %)	0.709
Ongoing pregnancy rate, *n* (%)	24/60 (40 %)	22/62 (35.5 %)	0.709
Miscarriage rate (%)	6/30 (20 %)	6/28 (21.4 %)	1.000
Multiple pregnancy rate, *n* (%)	2/24 (8.33 %)	2/22 (9.09 %)	1.000

Campbell et al. [11] demonstrated that LH not only plays an essential role in the ovulation, but also can exert nearly all physiologic actions of FSH on granulose cells. According to this information, the idea was introduced that LH can replace FSH in the late follicular phase of COH. LH in the form of low-dose hCG or rLH is able to stimulate the large follicles. The plasma half-life of hCG is longer than LH and it has a higher potency (approximately six to eight times that of LH) [4, 8, 12–15], so 200 IU of hCG is equal to 1,200 IU of LH [1].

Sullivan et al. [16] experienced this hypothesis by discontinuation of rFSH over the last 2 days of ovarian stimulation and substitution with rLH, they concluded that this protocol was as efficient as standard GnRH agonist protocol.

Our study showed that implantation and pregnancy rates, total oocyte retrieved and the number of obtained embryos were similar between patients stimulated by hMG alone and those stimulated by low-dose hCG in late follicular phase. But total dose of gonadotropins was significantly lower in hCG group.

Filicori et al. [4, 6] and Sullivan et al. [16] indicated that low dose of hCG may be used to substitute the LH activity in ovarian stimulation. Furthermore, they used 200 IU of

hCG instead of hMG in end stage of ovarian stimulation by GnRH agonist and concluded that gonadotropin consumption was reduced in hCG group. Whereas, ART outcome was comparable to conventional COH protocols [6]. In a similar design, Paulo Serafini et al. [2] in a large RCT showed that daily administration of low-dose hCG in late follicular phase resulted in a significant reduction of total dose of rFSH compared with standard antagonist protocol. Blockeel et al. [1] in their study concluded that effect of using 200 IU of hCG in late follicular phase of COH in antagonist protocol was similar in decrease of rFSH consumption, number of oocytes and ongoing pregnancy rates. Blockeel et al. [17] in another study evaluated gene expression profile in the endometrium of standard GnRH antagonist stimulation with 200 IU of hCG in late follicular phase; they reported no morphological differences between two groups. Administration of LH activity in the late follicular phase can selectively decrease the number of small follicles in end stage of stimulation and potentially decrease the risk of OHSS [4]. Kyono et al. [18] showed that administration of 200 IU/day of hCG in late follicular phase was associated with decreasing the chance of OHSS. Kosmas et al. [19] in a meta-analysis demonstrated that low dose of hCG improves pregnancy rates and lowers the frequency of OHSS in antagonist cycles.

None of these studies reported early increases of follicular phase progesterone secretion or premature LH surges in the hCG protocol [1, 2, 6, 8, 20].

In summary, according to our data, the administration of 200 IU of hCG in late follicular phase in GnRH agonist protocol leads to a lesser need of gonadotropins in patients undergoing ART but has no effect on ART outcomes. Future clinical trials should focus on the efficacy of this protocol for decreasing OHSS risk.

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Conflict of interest The authors have no conflicts of interest.

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