Efficacy of low dose hCG on oocyte maturity for ovarian stimulation in poor responder women undergoing intracytoplasmic sperm injection cycle: a randomized controlled trial

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Abstract

Purpose To investigate the effect of late follicular administration of low dose hCG on oocyte maturity in *poor responding women* undergoing intracytoplasmic sperm injection (ICSI).

Materials and methods This prospective randomized pilot trial was performed on 73 *poor responders* undergoing ICSI, in Reproductive Biomedicine Research Center, Royan Institute, Tehran, Iran. All eligible patients underwent a GnRH-a long protocol and were randomly allocated into three study groups for ovarian stimulation: group A received recombinant FSH alone, group B received recombinant FSH supplemented by 100 IU hCG. Group C received recombinant FSH supplemented by 200 IU hCG. The main endpoint was the number of metaphase II oocytes retrieved.

Results Of 78 poor responding patients entered to this study, 73 women were considered eligible for enrolment. Of these,

26 women were allocated to receive only recombinant FSH, 24 patients allocated to receive recombinant FSH and 100 IU hCG and 23 patients were assigned to receive recombinant FSH and 200 IU hCG. Number of oocytes retrieved were significantly higher in group B compared to group A (6.5 ± 3.3 versus 4.0 ± 2.3 ; $P=.03$). Other cycle and clinical outcomes were comparable between three groups.

Conclusions The present study demonstrated that adding 100 IU hCG to rFSH in a GnRH agonist cycle in poor responders improve response to stimulation whereas the number of metaphase II oocytes remains comparable between groups. The existence of a possible trend toward higher mature oocytes and lower total dosage rFSH in patients received 100 or 200 IU hCG is probably due to the small sample size that means further large clinical trials in a more homogenous population is required (clinical trial registration number; NCT01509833).

Capsule Adding 100 IU hCG to rFSH in a GnRH agonist cycle in poor responders improve response to stimulation

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Keywords Low dose hCG · IVF · ICSI · Oocyte maturity · Poor responder

Introduction

Poor response to controlled ovarian hyperstimulation is one of the greatest challenges in assisted reproduction which may occur in 9–24 % of the women undergoing IVF [1, 2]. Although no definite cause has been shown, various factors such as advanced age [3], history of previous ovarian surgery [3], serious endometriosis [4] and pelvic infections [5] could be attributed to a poor response to COH.

Poor responders to ovarian hyperstimulation are strongly resistant to therapeutic interventions and despite the application

of various strategies, there is still a high rate of cycle cancellation and pregnancy failure [2, 6, 7].

It is clear that, both LH and FSH have a prominent role in normal ovulatory actions of a natural cycle [8] and low endogenous LH concentrations in the late follicular phase of an IVF cycle might lead to poor pregnancy outcomes [9, 10].

The role of exogenous LH in hypogonadotrophic hypogonadism patients has been shown in many trials [11–14] whereas the efficacy of this stimulation protocol in other populations such as poor responders is somewhat undetermined. A recent study reported that a certain group of infertile women such as older women might benefit from LH supplementation [15].

Low dose hCG can mimic the LH actions on follicular growth and oocyte maturation and longer half life and greater affinity to LH/hCG receptors, make it a more effective alternative for ovulation induction protocols [16].

Fillicori and colleagues were the initiators who assessed the effect of low dose hCG on ovarian response and achieved favorable outcomes with supplementation of 50 IU hCG [17]. Thereafter, many researchers noted that adding LH activity, through administration of low-dose hCG in the late follicular phase resulted in follicles development and oocytes maturation, avoidance of premature luteinization, improved clinical pregnancies, lowering the rFSH requirements and reducing COH costs [18–21]. Conversely, some other authors failed to confirm these findings [22–25].

However, the role of hCG supplementation during COH is still a matter of debate and more studies on poor responding women is needed. Thus, the objective of this trial was to investigate whether LH activity in the form of low dose hCG would improve the quality of oocytes in women who respond poorly to COH protocols.

Materials and methods

Study design

This prospective, parallel, randomized, single center, controlled trial was performed on 73 poor responders undergoing ICSI treatment in Reproductive Biomedicine Research Center, Royan Institute, Tehran, Iran, from October 2009 to March 2011. This study was registered in the Clinical Trial Website (www.clinicaltrials.gov, number NCT01509833) and was approved by the institutional review board and Ethics Committee of royan institute. All participants were informed regarding the purpose of the study and signed the written informed consent.

Since we chose the mean number of mature oocytes as the primary end point, all patients underwent ICSI cycle which the cumulus oophorus is removed for maturity assessment.

Patients

The inclusion criteria were listed as per here under:

- 1- Poor responders to ovarian stimulation according to the existence of at least two of the following criteria:

Advanced maternal age (37 to 43 years), antral follicles count <5, prior history of poor response to controlled ovarian hyperstimulation (peak E₂ <500 pg/ml and/or ≤3 oocytes retrieved)

- 2- Indication for ICSI treatment, second or third cycle
- 3- Body mass index (BMI) ≤30 kg/m²
- 4- The presence of two functional ovaries and no previous ovarian surgery
- 5- The presence of normal uterine cavity and 2 normal tubes based on recent hysterosalpingographic or hystroscopic evaluation
- 6- Basal (day 2 or 3) serum FSH levels ≤13 IU/L
- 7- Normal semen analysis [26]
- 8- No history or signs of endometriosis
- 9- No untreated endocrinologic disease

Randomization and stimulation regimens

The treatment protocol was illustrated in Table 1. All of the patients underwent a standard long protocol using GnRH-a (Superfact, Aventis, Frankfurt, Germany) at a daily dose of 0.5 mg subcutaneous commencing on the day 17–19 of the natural menstrual cycle as a pre-treatment. Once pituitary desensitization was confirmed (endometrial thickness <5 mm and serum estradiol level <50 pg/ml), the GnRH-a dose was reduced by one-half (0.2 mg) and ovarian stimulation was initiated.

In all study patients, ovarian stimulation started with a fix dose of 300 IU r-FSH (gonal-F, Merck Serono, Switzerland) and was continued for the first 5 days of the stimulation cycle. On day 6, all women were randomly allocated into three study groups by a permuted block randomization method. The permuted block was generated by the statistician and applied by a midwife in clinic. The block size was considered equal to six. Group A (control group) continued with recombinant FSH alone, whereas Groups B and C received rFSH supplemented by daily administration of 100 IU and 200 IU hCG (Pregnyl, Organon, Netherland) respectively in the late follicular phase when the follicle size reached to 14mm diameter. In three groups, the first ultrasound scan was performed on day 6 to monitor response and the dose of rFSH adjusted according to the ovarian response and continued until the day of ovulatory hCG administration.

When at least two follicles were greater than 18 mm, 10,000 IU urinary hCG (Choriomon, IBSA, Lugano,

Table 1 stimulation protocols in groups A, B and C

Stimulation protocols		Day												
													hCG day	ovum pickup
Day 17 of previous cycle		2	3	4	5	6	7	8	9	10	11	12	13	14
Group A														
GnRH-a	0.5(mg)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
rFSH(IU)		300	300	300	300	300	Following days flexible base on stimulation response							
hCG									0	0	0			
Group B														
GnRH-a	0.5(mg)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
rFSH(IU)		300	300	300	300	300	Following days flexible base on stimulation response							
hCG	(from size 14mm of follicle)								100	100	100			
Group C														
GnRH - a	0.5(mg)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
rFSH(IU)		300	300	300	300	300	Following days flexible base on stimulation response							
hCG	(from size 14mm of follicle)								200	200	200			

Switzerland) was administered and oocyte retrieval was performed 34–36 h later. Immediately prior to micro-manipulation for the ICSI procedure, the cumulus corona cells were removed and each oocyte was examined under an inverted high-resolution microscope for assessment of maturity. The oocyte maturity assessed according to published criteria [27] and categorize as the followings:

1- Metaphase II oocyte (mature oocyte) were defined by the absence of the germinal vesicle and the presence of first polar body and a light color cytoplasm with homogeneous granularity. 2- Metaphase I oocyte (MI, nearly mature), characterized by absence of the germinal vesicle (GV) and first polar body, and a light color cytoplasm with homogeneous granularity. 3- Prophase I (immature oocyte); defined as oocytes with germinal vesicle and coarse granular cytoplasm.

ICSI was performed and after 44–72 h, embryos were scored according to the quality criteria as previously stated [28]. Embryo transfer routinely performed on 2nd or 3rd day after oocyte retrieval and depends on the patient characteristics (age, history of poor response

to COH and number and quality of available embryos) up to 4 embryos per patient were transferred at most. Luteal phase was supported by 400 mg twice a day of progesterone (Cyclogest; Actavis, United Kingdom) vaginally from the day after oocyte retrieval.

Outcome measures

Primary outcome measures included the number of mature oocytes retrieved (MII oocytes). Secondary outcome measures were duration and total dosage of rFSH consumption, cancellation rate, the number of oocytes retrieved, the number and quality of generated embryos, the number of transferred embryos, implantation rate, chemical pregnancy rate, clinical pregnancy rate, and live birth rate.

Cycle cancellation was defined when fewer than two growing follicles of an appropriate growth pattern were noted. The implantation rate was considered as the number of gestational sacs with fetal heart rate, divided by the number of embryos transferred. Live birth rate was the number of deliveries that resulted in at least one live born baby per embryos transfers.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 18.0. For comparison of quantitative variables with normal distribution between three groups one way analysis of variance (ANOVA) was applied, whereas for abnormal distributed data the kruskal-wallis test was used. Qualitative variables were analyzed by Chi-square test. A *P*-value of $<.05$ was considered statistically significant. The results are shown as mean $\pm$ SD or number and percentage in tables.

Results

Of 78 *poor responding patients* entered to this study, 73 women were eligible for enrolment. 26 women were allocated to receive only recombinant FSH, 24 patients allocated to receive rFSH and 100 IU hCG and 23 patients were assigned to receive rFSH and 200 IU hCG (Fig. 1).

Baseline characteristics of patients in three study groups have been shown in Table 2. No statistically significant differences were observed in age, body mass index, duration of infertility, levels of FSH, LH, E₂ day 3 between three groups.

Table 3 shows cycle outcomes in three study groups. Duration and total amount of FSH consumption, E₂ level

on the day of hCG administration, endometrial thickness on the day of hCG administration were not significantly different between groups.

No cycle cancellation was observed in all three groups during the treatment. Number of retrieved oocytes were significantly higher in group B (hCG 100) compared to group A (6.5 ± 3.3 vs 4.0 ± 2.3 ; $P=0.03$).

Number of MII oocytes and immature oocytes (Metaphase I and Germinal vesicle), Number of generated embryos, excellent quality embryos, good quality embryos and transferred embryos were not significantly different between groups.

3 patients (11.5 %) in group A, 3 patients (12.5 %) in group B and 4 patients (17.4 %) in group C didn't reach to embryo transfer phase.

Table 4 shows the clinical outcomes of the treatment cycles of three groups.

All three groups were comparable regarding the implantation rate, chemical and clinical pregnancy rates and live birth rate.

Discussion

According to the two cell two gonadotropin theory, both endogenous FSH and LH are needed for normal follicular

Fig. 1 Flow diagram of participated women in the trial

Table 2 Baseline characteristics of patients in group A (rFSH), group B (rFSH + rhCG100IU) and group C (rFSH + rhCG200IU)

Variable	Group A no hCG <i>n</i> =26	Group B hCG100 <i>n</i> =24	Group C hCG200 <i>n</i> =23	<i>P</i> -value
Age (years)	39.2±1.9	39±1.9	38.9±1.9	.79 ^a
BMI(kg/m ²)	25.1±1.7	26.3±2.0	25.6±1.4	.17 ^a
Infertility duration(years)	11.7±6.7	13.8±5.6	13.3±6.7	.47
No. of previous IVF attempts				
One	19(73.1)	11(45.8)	15(65.2)	.12
Two	7(26.9)	13(54.2)	8(34.8)	
Day3 FSH(IU/L)	9.0±2.2	7.4±2.9	7.9±2.7	.08
Day3 LH(IU/L)	4.7±2.0	4.4±2.0	5.0±2.6	.51
Day3 E2(pg/ml)	24.4±6.6	22.1±6.6	22.6±8.0	.91

Values are expressed as mean ± standard deviation or number (percentage)

^a Kruskal-wallis test were used unless otherwise specified

growth and steroidogenesis [2]. In a normal menstrual cycle the endogenous LH is necessary to produce theca cell androgens which are subsequently transformed into estrogens in granulosa cells under the action of FSH, and exert positive effects on oocyte and embryo maturation [8, 29, 30].

Although physiologic actions of LH in a natural menstrual cycle are well established, the role of exogenous LH in COH, oocytes maturity and embryos development has not still been determined. It is reported that Granulosa cells (GC) of larger follicles (≥10-mm diameter) become responsive to exogenous LH through the expression of FSH induced LH/hCG receptors and estrogens [16, 21]. Hence, LH plays a critical role in the final stages of folliculogenesis and

low response to FSH during ovarian stimulation might be related to endogenous LH deficiencies which are associated with unfavorable results in GnRH-a cycles [9, 31, 32].

LH and hCG bind to the same receptor and have the same natural function but as stated before, hCG is a more potent alternative in ovarian stimulation protocols [16]. Drakakis et al, found better results in early hCG administration than rLH for ovarian stimulation in women with previous IVF failure [33].

Filicori et al confirmed that low dose hCG can improve the ovarian response and folliculogenesis in women with hypogonadotropic hypogonadism [21] and further demonstrated this ovarian stimulation approach would be associated with favorable outcomes in terms of reduced number of small

Table 3 Cycle outcomes in group A (rFSH), group B (rFSH + rhCG100IU) and group C (rFSH + rhCG200IU)

Variable	Group A no hCG <i>n</i> =26	Group B hCG100 <i>n</i> =24	Group C hCG200 <i>n</i> =23	<i>P</i> -value
Total dose of rFSH used(IU)	3657.7±917.1	3165.6±512.5	3335.9±790.1	.07
Days of rFSH stimulation	10.5±1.6	9.9±2.1	10.1±1.4	.48
Days of rhCG stimulation	—	2.2±0.8	2.0±0.9	—
E2 on hCG administration (pg/ml)	458.1±567.4	497.7±576.7	425.6±408.7	.23 ^b
Endometrial thickness on the day of hCG administration(mm)	8.8±1.1	9.3±1.6	9.2±1.5	.46
No. of oocytes retrieved	4.0±2.3 ^a	6.5±3.3	5.8±4.3	.02 ^b
Oocyte maturity				
No. of MII oocytes	3.4±1.7	5.2±2.1	5.2±4.4	.16 ^b
No. of MI oocytes	.1±.4	.5±.8	.2±.5	.07 ^b
No. of germinal vesicle oocytes	.3±.7	.6±.9	.1±.5	.05 ^b
No. of atretic oocytes	.1±.4	.1±.4	.1±.4	.8 ^b
No. of generated embryos	2.3±1.6	3.5±2.5	3.6±3.6	.24 ^b
Excellent quality embryos	.6±.9	.9±.9	.7±1.3	.23 ^b
Good quality embryos	1.1±1	1.9±2.3	1.4±1.7	.88 ^b
No. of embryos transferred	2.0±1.2	2.5±1.3	2.1±1.4	.47 ^b

Values are expressed as mean ± standard deviation

^a Group A versus Group B, *P*=.03

^b Kruskal wallis test were used unless otherwise specified

Table 4 Clinical outcomes in group A(rFSH), group B (rFSH + rhCG100IU) and group C(rFSH + rhCG200IU)

Variable	Group A no hCG <i>n</i> =26	Group B hCG100 <i>n</i> =24	Group C hCG200 <i>n</i> =23	<i>P</i> -value
Implantation rate (%)	5.6(3/54)	6.7(4/60)	14.0(7/50)	.24
Chemical pregnancy rate (per ET; %)	17.4(4/23)	19.0(4/21)	26.3(5/19)	.75
Clinical pregnancy rate (per ET; %)	13 (3/23)	19.0(4/21)	26.3(5/19)	.55
Live birth rate (per ET; %)	13 (3/23)	14.3(3/21)	21.1(4/19)	.75

Data are expressed as n (%)

follicles, higher competent oocytes, higher pregnancy rate and reduced chances of OHSS [34, 35]. A number of authors reported that older patients and women with inadequate response to FSH get the most benefit from LH supplementation [15, 36–39].

The present pilot study demonstrated that in a standard long protocol in poor responders, adding late follicular hCG to rFSH in a dose of 100 IU is associated with higher total number of retrieved oocytes than control group which received rFSH alone.

An earlier study found no significant greater number of mature oocytes in patients treated with only rFSH compared to the women who received LH activity in the form of LH or hCG supplementation [22]. This trend was inversely detected by our study which higher number of mature oocytes was seen in poor responders who received 100 or 200 IU hCG compared to the rFSH only group, although the differences among the groups were not statistically significant. Despite this finding, the implantation rate remained nearly low. It is noteworthy that the low implantation rate in all three groups of this study which has been resulted to poor pregnancy outcomes could be related to the low quality of oocytes in these advanced reproductive aged patients compared to the normal responders.

Our study showed no significant differences between three groups in terms of implantation rate, chemical pregnancy rate and clinical pregnancy rate.

Some studies noted [22, 23] that exogenous LH/hCG administration has no further benefit in poor responders to ovarian stimulation. However, several other studies have shown better outcomes with additional LH in poor responders when GnRH-a short or long protocols were applied [40–42] whereas in the study of Bjercke et al and Hompes et al [43, 44] in normal responders, the clinical outcomes were similar between rFSH and hMG groups. Some other trials demonstrated favorable treatment outcomes in an antagonist protocol in patients who received low dose hCG [18, 45].

Our study showed no differences regarding the live birth rate between three study groups. A similar result was reported in a meta analysis on seven randomized clinical trials which rLH added to rFSH in GnRH agonist protocols [46]. Although all patients in this study were selected from a

poor respond population, no cycle cancellation was noted in three groups during the treatment cycle.

According to the findings of the current study, a trend toward lower consumption of recombinant FSH was seen in patients who received hCG low dose (100 and 200 IU) compared to the patients who received FSH alone, even though no significant differences were found between three groups. A comparable trend which didn't achieve statistical significance, was also noted in a similar trial in poor responders [22]. Some other previous studies reported a significant decrease in total dosage and duration of rFSH treatment in administration of rFSH supplemented or substituted by low dose hCG in a GnRH antagonist protocol [18, 19, 35, 47].

Study limitations

This trial was performed as a pilot study to calculate the definitive sample size of the future study. Hence, the limitation of this study was the small sample size in each group and the non significant outcomes in terms of mature oocytes, implantation and pregnancy outcomes and total dose of rFSH consumption are more likely to be statistically significant with higher sample size.

Sample size calculations using PASS 2008 [“Hintze, J. (2008). PASS 2008. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com.”] revealed that for achievement of significant differences with a 0.05 significance level and power of 80 %, at least a sample size of 40 patients in each group would be required.

One of the most common problems in poor responder studies is the insufficient number of patients which prolong duration of the study. On the other hand, before introduction of the new ESHRE definition [48], there was no universal definition for poor responders which make difficulties in interpretation and comparison of the results with the previous reports. However, apart from patient selection, different sample size, retrospective or prospective study designs, different stimulation protocols applied (agonist or antagonist) and administration of LH regimen in the form of recombinant LH, HMG or hCG in different doses and methods in many IVF centers lead to such discrepancies between studies.

Conclusion

The present study demonstrated that adding 100 IU hCG to rFSH in a GnRH agonist cycle in poor responders improve response to stimulation whereas the number of metaphase II oocytes remains comparable between groups. The existence of a possible trend toward higher mature oocytes and lower total dosage rFSH in patients received 100 or 200 IU hCG is probably due to the small numbers which needs further large clinical trials in a more homogenous population.

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