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The effect of low dose human chorionic gonadotropin on follicular response and oocyte maturation in PCOS patients undergoing IVF cycles: a randomized clinical trial of efficacy and safety

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Abstract

Purpose To compare the efficacy of two regimens of low dose human chorionic gonadotropin (hCG) on follicular response and oocyte maturation in women with polycystic ovarian syndrome (PCOS).

Methods Ninety women with PCOS who underwent assisted reproduction were eligible for this controlled, prospective, randomized study. Our trial was performed at Royan Institute Reproductive Research Center over a 24-month period. Ovarian stimulation in all groups was initiated with recombinant FSH, 150 IU daily. The dose and duration of FSH treatment were adjusted by monitoring follicular development with ultrasound and estradiol levels. Patients were randomized using a block randomization technique which assigned them to three groups: group A (control group) continued r-FSH until oocyte retrieval. In group B, r-FSH was reduced to 75 IU once the lead follicle reached 14 mm in mean diameter and low dose hCG (100 IU/day) was initiated. In group C, r-FSH was discontinued and low dose hCG (200 IU/day) was begun when the lead follicle reached 14 mm in mean diameter. The main outcome measure was follicular response and oocyte maturation.

Results As compared to the FSH only group, groups which were given low dose hCG had lower gonadotropin consumption and fewer immature oocytes than the control group. No women in the low dose hCG groups developed severe ovarian hyper-stimulation syndrome. Fertilization, implantation and pregnancy rates were similar in the three groups.

Conclusions A combination of FSH and low dose hCG improved oocyte maturity and preserved outcomes with improved safety and lowered cost.

Keywords Low dose hCG · Polycystic ovarian syndrome · Follicular response · Oocyte maturation

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Introduction

Women with polycystic ovary syndrome (PCOS) have more FSH-sensitive antral follicles in their cohort compared with eumenorrheic women [1]. Therefore, they often have multi-follicular development during ovarian stimulation and they are at risk for ovarian hyper-stimulation syndrome (OHSS) or multiple pregnancy.

Controversy exists as to the best approach for ovarian stimulation with IVF treatments in PCOS patients. The goal is to obtain good follicular response with sufficient mature oocytes while avoiding the risk of OHSS. One approach [2–6] adds low dose human chorionic gonadotropin (hCG) in the late follicular phase. Theoretically, substituting LH for FSH in the late follicular phase would permit the more mature follicles to continue to develop while the less mature follicles would undergo atresia due to insufficient FSH stimulation [7]. It is because the more

mature follicles have acquired the capacity to respond to LH while the less mature ones have not.

Low dose hCG has a longer half-life and lower cost compared with recombinant FSH or LH. In addition, full development of large follicles [2, 4, 6, 8], adequate ovarian hormonal levels [4, 9], oocyte maturation [9], avoidance of premature LH surge [4, 9], and increased pregnancy rates have been demonstrated by the addition of low dose hCG in late folliculogenesis [9].

We undertook this study because there is no general agreement about the ideal amount of exogenous LH or low dose hCG needed for ovarian stimulation particularly in PCOS patients. We hypothesized that the substitution of hCG for r-FSH during controlled ovarian stimulation in infertile women with PCOS would reduce the rates of immature oocytes and OHSS while yielding comparable fertility outcomes, since follicles in women with PCOS, as with follicles in eumenorrheic women, become LH responsive as they mature.

The objective of this prospective randomized clinical trial was to evaluate the effectiveness of two regimens of low dose hCG on follicular response and oocyte maturation in PCOS women undergoing IVF cycles and compare them with r-FSH alone.

Materials and methods

This controlled, prospective, randomized clinical trial was performed at Royan Institute Reproductive Research Center over a 24-month period from January 2006 to December 2008. Ninety PCOS patients who met all of the following inclusion criteria were randomly assigned into one of three treatment groups (Fig. 1). Participants attended clinic visits at the time of randomization (third day baseline), on the day of commencement of gonadotropin stimulation, every other day from the day of ovarian stimulation until the day of pre-ovulatory hCG administration, on the day of ovulation triggering and were subsequently followed until clinical pregnancy.

Eligibility criteria for inclusion were

1. PCOS diagnosis by Rotterdam criteria [10] of which a minimum of the following two criteria were used for approving the PCOS diagnosis: oligo- or anovulation, clinical or biochemical hyperandrogenism, polycystic ovaries;
2. normal uterine cavity and patent tubes by hysterosalpingogram, laparoscopy or hysteroscopy;
3. normal semen analysis according to WHO criteria [11].

Patients with previous IVF or ICSI cycles or patients who received gonadotropins for ovarian stimulation during the three previous months were also not eligible for study participation.

The trial was performed in accordance with the Declaration of Helsinki and subsequent revisions and approved by the Ethics Committee at Royan Institute Research Center. Written informed consents were obtained before entering into the study.

Based on 0.8 power to detect a significant difference ($p = 0.05$, two-sided), 30 patients were required for each study group.

Patients were randomized by block randomization technique which assigned them to three groups: group A (rFSH alone, 150 IU daily), group B (rFSH + 100 IU hCG), and group C (rFSH + 200 IU hCG) (Fig. 2). The blocks were generated by using a permuted block design. The block lengths were 6. Patients remained on the same allocation throughout the duration of the study. A computerized random number sequence was generated by the statistician. Each possible permuted block was assigned a number. Using each number in the random number sequence in turn selected the next block, determining the next six participant allocations. Numbers in the random number sequence greater than the number of permuted block combinations were not used to select blocks. The physician responsible for patient visits allocated the next available number upon entry into the trial (in the PCOS clinic or the ultrasound department).

Outcome assessors including laboratory technicians and data analysts were blinded to group assignment.

Stimulation protocol for all the patients was according to the standard long protocol [12]. Gonadotropin stimulation commenced 14 days following subcutaneous GnRH agonist injection with recombinant FSH (Gonal F, Serono, Switzerland), 150 IU daily. The dose and duration of FSH treatment were adjusted by monitoring follicular development with ultrasound and estradiol levels. The maximum FSH dose was 225 IU/day. The goal of ovarian stimulation was to achieve an average of two ovarian follicles with a mean diameter of ≥17 mm on the day of hCG administration.

In group B, ovarian priming with r-FSH was reduced to 75 IU once the lead follicle reached 14 mm in mean diameter and low dose hCG (100 IU/day) was administered and continued until at least 2–3 follicles with a mean diameter of ≥17 mm were achieved.

In group C, ovarian stimulation with r-FSH was discontinued and low dose hCG (200 IU/day) was administered when the lead follicle reached 14 mm in mean diameter and continued until at least 2–3 follicles with a mean diameter of ≥17 mm were achieved.

For all women in all groups, if the follicle mean diameter failed to grow sufficiently after 2 weeks of ovarian stimulation, monitoring was stopped and the cycle was

Fig. 1 Flow diagram of patients invited to participate in the research, number of subjects who declined to participate, randomization process, and number of patients in each group that began ovarian stimulation
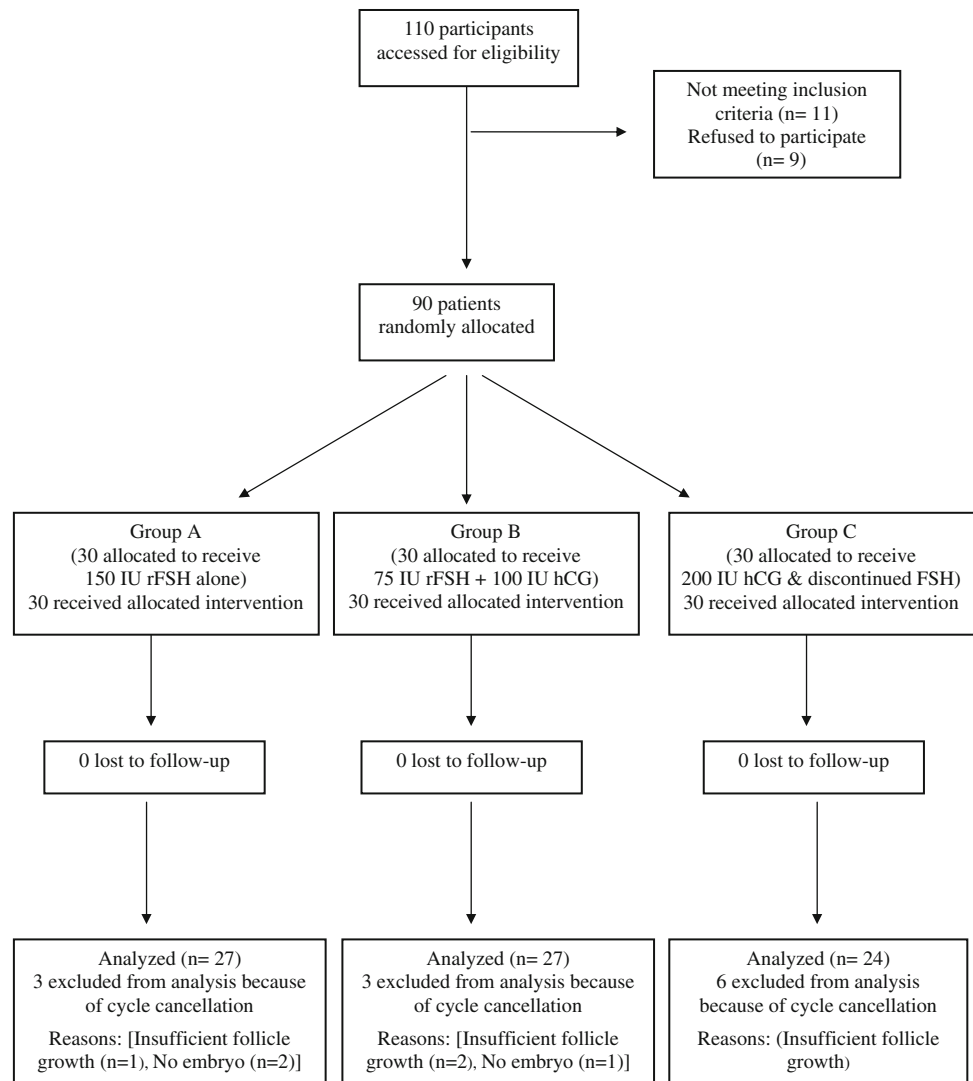
canceled. Furthermore, patients who had no embryos for transfer did not continue their treatments.

In all groups, gonadotropin stimulation was continued until 2–3 follicles with sizes ≥17 mm were achieved. Then, 10,000 IU of hCG (Choriomon, IBSA, Switzerland) was administered and oocyte retrieval was performed 34–36 h later. All oocyte retrievals were performed by a skilled gynecologist through follicles with a size more than 12 mm.

After ICSI procedure, normal fertilization was confirmed when two distinct pronuclei were present, 16–18 h later. The cleaved embryos with good quality were transferred 2–3 days later (grade A, B, or AB, with 4–6 blastomeres on day 2, or 6–8 blastomeres on day 3). Embryo quality was assessed according to morphology, cleavage stage, and fragmentation rate [13]. Good quality spare embryos were frozen.

Luteal-phase support was provided with vaginal progesterone (Aburaihan Co., Tehran, Iran), 400 mg twice a day until the day of β-HCG assay. If patients had a positive β-HCG, then progesterone was continued until 10 weeks of gestation.

The primary endpoint was the mean for immature oocytes in PCOS patients. In the current study, we tested the hypothesis that low dose hCG supplementation in the late follicular phase can decrease less mature follicles in addition to less mature oocytes in PCOS women. Medication consumption, OHSS, clinical pregnancy and multiple pregnancy rates were the secondary outcomes.

In our ICSI cycles, cumulus-enclosed oocytes were treated with 0.1% hyaluronidase, and the cumulus cells were mechanically removed by pipetting. Oocyte maturation stage and morphology were assessed under an inverted microscope at 400× magnification. Oocytes were classified as follows: (a) metaphase II oocyte (mature oocyte) which was characterized by the presence of the first polar body and a light colored ooplasm with fine homogeneous granularity; (b) metaphase I oocyte, characterized by the
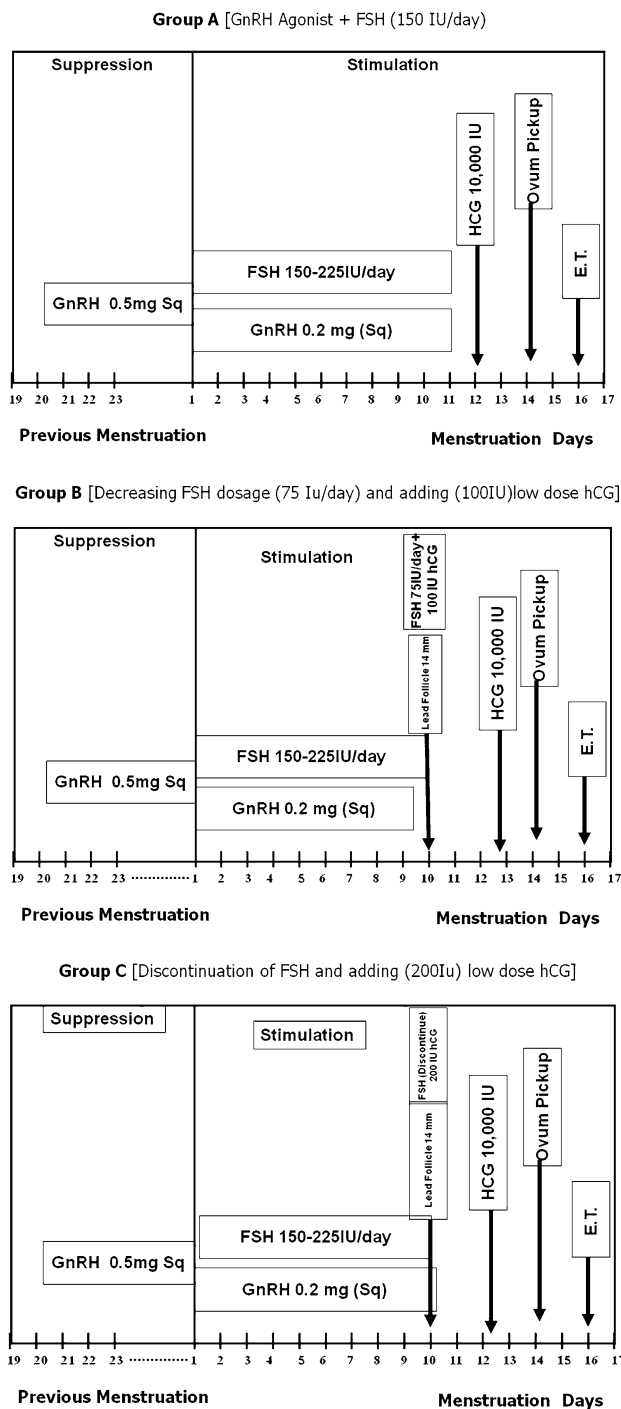
Fig. 2 Treatment plans in the three study groups

performed for all patients who presented with abdominal pain. Patients with respiratory distress or clinical abdominal ascites with hemoconcentration (hematocrit > 15) were considered to have severe OHSS [15].

The fertilization rate was defined as the ratio of the number of embryos formed relative to the number of oocytes injected. The implantation rate was defined as the number of gestational sacs visualized by transvaginal pelvic ultrasound relative to the number of embryos transferred. Clinical pregnancy was defined as a positive pregnancy test followed by the presence of a gestational sac visualized by transvaginal ultrasound 4 weeks after transfer. The pregnancy rate was calculated by dividing the number of clinical pregnancies detected by the number of transfers performed (clinical pregnancy/transfer).

Monitoring and hormone assays

All patients were initially monitored by vaginal ultrasound (Aloka, α10, Japan) on the third day of menses and on the day of commencement of gonadotropin stimulation. From the day of ovarian stimulation until the day of pre-ovulatory hCG administration, ultrasound assessments were repeated at least every 1–2 days for all patients.

Day 3 serum levels of FSH, LH, estradiol (E2), progesterone (P) and testosterone (T) were determined in all patients (first sample). Blood samples were also obtained on the day of ovulation triggering and pre-ovulatory hCG injection (second sample).

Statistical analysis was performed using the Statistical Packages for Social Sciences version 13.0 (SPSS Inc., IL). Normality of distribution of continuous variables was assessed with the Kolmogorov–Smirnov test. Between-group differences of normally distributed continuous variables were assessed with parametric statistics, analysis of variance (one-way ANOVA), whereas non-parametric statistics (Kruskal–Wallis H test) were used when the data were not normally distributed. We adjusted for multiple comparisons using Bonferroni and Tukey's procedures. The Kruskal–Wallis non-parametric test, followed by the Mann–Whitney test when there was a significant difference, was used for data that were not normally distributed.

Significant differences were evaluated by the Chi-square test to compare the non-continuous variables. The data were expressed as mean $\pm$ standard error of mean (SE) unless otherwise specified. Statistical significancy was set at p values < 0.05.

absence of both the germinal vesicle and the first polar body, round and even in form; and (c) prophase I oocyte which was characterized by its distinct germinal vesicle and refractile nucleolus, irregular shape, darkened center and coarse granular ooplasm. Oocytes were classified according to Veeck [14].

A detailed history and physical evaluation, along with pelvic ultrasound and laboratory analysis for OHSS, was

Results

The baseline patient characteristics for the three treatment groups are shown in Table 1. There were no statistically

Table 1 Comparison of baseline characteristics of PCOS patients in the three treatment groups

	Group A (rFSH), $n = 27$	Group B (rFSH + 100 IU hCG), $n = 27$	Group C (rFSH + 200 IU hCG), $n = 24$	p value
Age (years)	29.5 ± 0.64	28.5 ± 0.74	29.4 ± 0.81	0.549[a]
BMI (kg/m^2)	27.5 ± 0.81	27.8 ± 0.99	27.7 ± 0.89	0.974
Infertility duration (years)	9.2 ± 0.78	8.3 ± 0.88	7 ± 0.76	0.175[a]
Day 3 FSH (IU/l)	5.11 ± 0.52	5.6 ± 0.33	6.6 ± 0.77	0.152
Day 3 LH (IU/l)	5.6 ± 0.85*	10.3 ± 1.64*	6.7 ± 1.06	0.047
Day 3 E2 (pg/ml)	43.07 ± 5.77	77.2 ± 26.11	73.8 ± 14.89	0.201
Day 3 P (ng/ml)	0.44 ± 0.10	0.26 ± 0.04	0.29 ± 0.05	0.408
Day 3 T (ng/ml)	0.53 ± 0.04	0.53 ± 0.05	0.66 ± 0.09	0.541

Values are mean ± SE

* Significant statistical differences between two groups

[a] One-way ANOVA test. The other variables were assessed by Kruskal–Wallis test

significant differences in age, body mass index (BMI), duration of infertility, or basal hormonal assessments, except for LH levels.

Hormonal measurements were repeated on the day of ovulation triggering (Table 2). Our results showed higher serum FSH levels in group B (rFSH + 100 IU hCG) compared with group C (rFSH + 200 IU hCG; $p = 0.012$). Testosterone level was significantly higher in group C (rFSH + 200 IU hCG) compared to group A ($p = 0.004$).

The clinical results of three treatments are shown in Table 3. The total number of immature oocytes was significantly higher in group A than group B ($p = 0.044$). The total dosage of rFSH used was also significantly higher in the control group (group A) than the low dose hCG groups ($p < 0.001$). However, there were no statistically significant differences in stimulation day, total number of MII oocytes retrieved, total number of oocytes retrieved and number of embryos transferred. The number of large (diameter > 14 mm), and medium (diameter = 10–14 mm) follicles were also similar among groups on the day of hCG administration for ovulation induction.

The fertilization, implantation, pregnancy and multiple pregnancy rates were comparable among groups. Four patients of group A showed severe OHSS; however, there was no severe OHSS in the other groups (groups B and C; $p = 0.019$; Table 4).

Discussion

The advantages of using 100 or 200 IU hCG in normal ovulatory patients were pioneered almost a decade ago by Filicori et al. [2–4, 16]. Several studies also demonstrated the benefits of using LH in controlled ovarian stimulation, especially in the final stages of follicular maturation [17–21]. Low dose hCG, like LH, selectively binds to LH receptors and has the same actions as LH itself. In the present study, we assessed the effect of two low dose hCG regimens on folliculogenesis and cycle outcome in PCOS patients.

The baseline characteristics, except for LH levels, were similar among PCOS patients in the three groups. All patients used oral contraceptive pills 1 month before ovarian stimulation for modulating the baseline endocrine measures such as FSH and LH levels. Therefore, the LH levels were similar at the time of gonadotropin initiation despite the different LH levels at the baseline assessment.

Our results showed that replacement of rFSH with low dose hCG (100 or 200 IU, daily) stimulated follicle growth and estradiol production to levels comparable with those of patients who continued to receive rFSH alone. Although group B (100 IU hCG) showed higher serum FSH levels than group C (200 IU hCG), the similar E2 level and number of large pre-ovulatory follicles could be the reasons for similar folliculogenesis in low dose hCG groups. In other words, the combination of FSH and low dose hCG (100 IU) has similar folliculogenesis when compared with low dose hCG alone (200 IU).

Nevertheless, we found lower immature oocytes in group B patients who received a combination of FSH and low dose hCG compared with those of patients who continued to receive rFSH alone. The granulosa cell communication in cumulus oophorus is critical to maintain the oocyte in the dictyate stage of meiosis until ovulation [22]. The disruption of this communication could be responsible for abnormal oocyte maturation. It seems that adding low dose hCG in the late follicular phase to the FSH regimen has a positive impact on oocyte maturation.

In the present study, we designed stopping or lowering FSH administration in the study groups to evaluate the efficacy of two low dose hCG protocols on follicle growth. Our findings illustrated statistically significant reduction in

Table 2 Hormonal levels of PCOS patients on hCG day (the day of ovulation triggering) in the three treatment groups

	Group A (rFSH), $n = 27$	Group B (rFSH + 100 IU hCG), $n = 27$	Group C (rFSH + 200 IU hCG), $n = 24$	p value[a]
FSH (IU/l)	5.32 ± 0.56	7.3 ± 1.17*	3.8 ± 0.66*	0.012
LH (IU/l)	0.7 ± 0.14	1.3 ± 0.27	1.02 ± 0.22	0.122
E2 (pg/ml)	1501.8 ± 159.9	1664.6 ± 211.4	1903.8 ± 315.5	0.937
P (ng/ml)	0.42 ± 0.05	0.56 ± 0.11	1.13 ± 0.48	0.907
T (ng/ml)	0.58 ± 0.04*	0.8 ± 0.07	0.97 ± 0.12*	0.037

Values are mean ± SE

* Significant statistical differences between two groups

[a] Kruskal–Wallis test

Table 3 Clinical characteristics of PCOS patients in the three treatment groups

	Group A (rFSH), $n = 27$	Group B (rFSH + 100 IU hCG), $n = 27$	Group C (rFSH + 200 IU hCG), $n = 24$	p value
Stimulation duration (days)	11.3 ± 0.43	10.9 ± 0.36	11.3 ± 0.48	0.791[a]
Total rFSH consumption[b]	20.6 ± 1.04	17.04 ± 0.83	15.7 ± 1.24	<0.001
MII oocytes retrieved	5.9 ± 0.73	8.5 ± 0.98	6.6 ± 1.07	0.134
Immature oocytes	7.8 ± 1.37*	3.7 ± 0.85*	4.8 ± 1.11	0.044
Total number of oocytes retrieved	13.7 ± 1.66	12.3 ± 1.38	11.5 ± 1.71	0.574
No. of embryos formed	8.4 ± 1.22	6 ± 0.7	6.5 ± 1.2	0.313
No. of embryos transferred	2.8 ± 0.16	2.6 ± 0.12	2.2 ± 0.2	0.081
No. of embryos cryopreserved	3.1 ± 0.98	1.07 ± 0.53	1.7 ± 0.8	0.162[a]
No. of medium growing follicles on hCG day	7.6 ± 1.68	7.6 ± 1.16	5.62 ± 1.31	0.209
No. of large growing follicles on hCG day	14 ± 1.64	13.7 ± 1.68	12.4 ± 1.3	0.782
Endometrial thickness on hCG day (mm)	10.04 ± 0.4	9.6 ± 0.51	9.4 ± 0.35	0.597

Data are expressed as mean ± SE, otherwise it has mentioned

* Significant statistical differences between two groups

[a] One-way ANOVA test. The other variables were assessed by Kruskal–Wallis test

[b] Significant statistical differences between groups A and B, and group A and group C

Table 4 Cycle outcomes of PCOS patients in the three treatment groups

	Group A (rFSH), $n = 27$	Group B (rFSH + 100 IU hCG), $n = 27$	Group C (rFSH + 200 IU hCG), $n = 24$	p value[a]
Fertilization rate, n (%)	202/346 (58.4)	155/287 (54)	145/250 (58)	0.494
Implantation rate, n (%)	24/75 (32)	16/69 (23.2)	14/53 (26.4)	0.487
Clinical pregnancy rate/ET, n (%)	14/27 (51.9)	13/27 (48.1)	13/24 (54.2)	0.910
Multiple pregnancy rates, n (%)	4/27 (14.8)	2/27 (7.4)	1/24 (4.2)	0.389
Severe OHSS, n (%)	4/27 (14.8)	0/27 (0)	0/24 (0)	0.019*

* Significant statistical difference

[a] Chi-square test

the total dose of recombinant FSH which was required for ovarian stimulation in low dose hCG treatment groups (groups B and C), when compared to group A. Although this was our protocol, however, patients benefited from lower FSH consumption which leads to lowered cost and

increased safety for them. Previous studies also observed a significant decline in the FSH consumption in COH with concomitant administration of LH or hCG [3, 23, 24]. The addition of LH has been reported to heighten ovarian response to FSH [16, 25, 26]. Since a high dose of FSH has

been associated with severe OHSS and multiple gestations, the lower FSH exposure reduced adverse outcomes [27]. Thus, a combination of FSH and low dose hCG preserved outcomes with improved safety and lowered cost.

The number of MII oocytes generated by the three ovarian stimulation protocols was similar in this study (Table 3). This finding was similar to Filicori et al. [3] who reported a similar number of mature oocytes that developed in both the conventional protocol and low dose hCG regimen. Our results also showed comparable fertilization rates among the three groups of patients. Similar pregnancy rates in PCOS women who received low dose hCG in the late proliferative phase and those treated with standard GnRH agonist protocol also gives credibility to this alternate approach for PCOS patients undergoing IVF cycles.

PCOS patients are at increased risk for OHSS due to a high cohort of antral follicles. Our results showed no severe OHSS in the two regimens of low dose hCG. This is similar to those reported by other researchers [2, 28] who found no OHSS with the use of low dose hCG. Kyono et al. [23] have also shown a lower incidence of OHSS in the low dose hCG group. It seems that low dose hCG or LH results in lower vasopressin and less vascular endothelial growth factor (VEGF) produced by granulosa cells in superovulated patients [29, 30]. The lower concentration of VEGF minimizes the increase in vascular permeability that leads to OHSS [28]. However, since we did not measure this factor in our study, additional investigation is needed. Patients with higher numbers of small or intermediate follicles are predisposed to the development of OHSS. These patients need high doses of FSH for follicular growth. By lowering or stopping FSH administration, the small or intermediate follicles did not grow and go to atresia. Therefore, although there were no significant differences between follicle sizes among the three groups, lowering or stopping FSH administration in the low dose hCG groups leaded to low OHSS in these patients.

HCG has a longer half-life and thus is at least six times more potent than LH [30]. There is a concern for premature follicular luteinization with the low dose hCG administration. However, we could not find any pre-ovulatory progesterone levels of 2.0 ng/ml or greater in any of the groups.

We found a higher pre-ovulatory serum level of T in patients who received 200 IU low-dose hCG (group C) compared with group A. This could have been related to the effect of hCG upon theca cell production of androgens. Significant correlation between the amount of LH activity administered and serum testosterone levels has previously been demonstrated [31]. The increase in androgen levels in the low dose hCG groups may also be due to decreasing FSH concentration and inability of the small follicles to aromatize androgens to estrogen.

In conclusion, daily low dose hCG supplementation in the late follicular phase (100 or 200 IU/day) can improve folliculogenesis and oocyte maturation in PCOS women. In addition, low dose hCG is less expensive compared with recombinant FSH. Therefore, this regimen can be more cost-effective than the FSH alone regimen.

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