Original Article

Comparison of ART Outcomes between Two COH Protocols: Gonal-F versus Gonal-F Plus HMG

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Abstract

Background: The purpose of this prospective, randomized study was to compare ovarian response as well as oocytes, embryo yields and pregnancy rates in women who underwent ovulation induction for intracytoplasmic sperm injection (ICSI) with recombinant human FSH (rFSH) alone or in combination with human menopausal gonadotropin (HMG).
Materials and Methods: Out of 300 patients in assisted reproductive technique (ART) cycles who underwent down regulation with GnRH analogue in a long protocol, 64 patients received 150 IU/d r FSH until day six when they were randomly allocated into two study groups: group A, who received rFSH alone (n=32) and group B, (n= 32) who received rFSH and HMG.
Results: The total number of ampoules of rFSH, the numbers of oocytes retrieved, embryos and serum concentrations of luteinizing hormone (LH) on the day of hCG administration were similar in both treatment groups. However, the numbers of follicles ≥15mm, serum concentrations of progesterone and estradiol on the day of hCG administration were significantly higher in group B when compared to group A. Although the number of high quality embryos (grades A and B) were significantly better in group B, the number of pregnancies and live birth rates were similar in both groups.
Conclusion: The study shows that the addition of LH to rFSH in pituitary – suppressed women undergoing ART improves some parameters of ovarian response, but doesn't improve overall pregnancy rates.

Keywords: Recombinant, FSH, HMG, Pregnancy Rate, Ovulation Induction

Introduction

Assisted reproductive techniques (ART), most commonly *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), have evolved greatly during the past two decades both in the technical and medical aspects, such as controlled ovarian hyperstimulation (COH).
The role of follicle stimulating hormone (FSH) and luteinizing hormone (LH)in ovarian follicular growth and maturation have been known for a long time. Today, it is clear that LH plays a basic role in the final stages of follicle maturity. In response to FSH, the antral follicle reaches a stage in which granulosa cells become sensitive to LH stimulation and LH is capable of influencing both the theca and granulosa cells. This effect depends predominantly on the concentration of LH. Moreover, as LH receptors appear, the dependence of follicular growth and maturation on FSH decreases (1, 2). Therefore LH may also play a fundamental role in the final maturation of oocytes in ICSI cycles. On the other hand, if follicles are affected by high improper LH concentrations an adverse effect may be seen (3, 4).
Studies worldwide have used recombinant LH in order to show its effect on follicular maturation. However since recombinant LH is not currently available in Iran, this study is based on the addition of human menopausal gonadotropin (HMG), with equal content of 75 IU of LH and FSH from the seventh day of ovulation stimulation in order to show the effects of LH on patients treated with human FSH in cycles down-regulated with a GnRH agonist in the long protocol.

Materials and Methods

Patient selection

This controlled, double-blind randomized trial was conducted from June 2006-June 2007 at the Infertil-

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ity Department of Vali-e-Asr Hospital as a gynecology resident thesis after being approved by the medical university research committee. From among 300 patients who were in their ART cycles, 64 were chosen and after obtaining written consent they were allocated to one of two groups by simple random sampling, using a random numbers table. Patients, ages 20-35 years with a body mass index (BMI) range of 18-30 Kg/m^2 were included if they had no underlying medical illnesses and no contraindications for pregnancy. The couples had normal karyotypes with the primary cause of their infertility as either tubal or male factor. Patients diagnosed with polycystic ovarian syndrome (PCOS) and those with FSH levels higher than 12 IU/L were also excluded.
Data collection was done via questionnaires completed by clinic staff and laboratory analyses.

Study design

For all patients, baseline FSH and LH values were measured in their previous cycles. All patients underwent pituitary down regulation receiving a once daily subcutaneous dose of 0.2cc Buserelin (Suprefact, Hoechst, AG-Germany), a short-acting GnRH analog from the 21^{st} day of their cycles with oral contraceptive pills (OCP) pretreatment. After ceasing OCP and at least 12 days of pituitary desensitization, all patients received recombinant FSH (Gonal-F, Serono, Switzerland) at a fixed dose of 150 IU/d for the first six days. Thereafter, they were randomly allocated into two groups of 32 patients each.
Group A continued the given dose of treatment if they had 2-3 follicles ≥ 10 mm. On alternating days, patients underwent sonography until they had at least two follicles ≥ 18 mm and at least two other follicles with a diameter > 16 mm when they received 10000 IU hCG. If their response was insufficient, on the seventh day they received 1-2 additional ampoules (75-150 IU) of Gonal-F.
Group B received the same treatment as group A until day seven, when instead of 1-2 ampoules of Gonal-F, they were administered one Gonal-F and one HMG (Merional, IBSA Switzerland). If the response was insufficient, patients received an additional 1-2 ampoules of HMG until at least 2 follicles ≥ 18 mm were observed.
After treatment completion serum progesterone, estradiol and LH levels were measured followed by an intramuscular injection of 10000 IU of hCG. Oocyte pickup was performed 34 to 36 hours following hCG administration. Oocyte maturation was assessed with the criteria described by Veeck (5). After the ICSI procedure, embryos were scored according to the morphologic appearance of their blastomers blastomeres and fragmentation (6).
Embryo transfer was performed on day three of ovum pickup with no more than 3 embryos being transferred per patient. In all patients, the luteal phase was supported by Cyclogest (Actover, Alpharma, England) a vaginal progesterone at a dose of 400mg/Bid, which started from the day of oocyte retrieval. In cases where chemical pregnancy was detected two weeks following embryo transfer, clinical pregnancy was confirmed with ultrasound examination six weeks thereafter.

Statistical analysis

Results were expressed as mean ± standard deviation. Student's t test was used to evaluate the differences between both groups. Logistic regression model was used to assess the simultaneous effect of variables on ovary response. P-value <0.05 was considered statistically significant. Data were analyzed using SPSS software version 15.

Results

Out of 64 patients in this study who responded to ovulation stimulation, 32 received rFSH alone and 32 received a combination of rFSH and HMG. In total, two patients in each group were excluded from the study and the remainder received ovum pickup and embryo transfer. Both groups had similar demographics and basic characteristics (Table 1).
Table 2 display a comparision of variables between the two groups.

Table 1: Demographic and basic characteristics of patients

Characteristics	Group A rFSH (n=30)	Group B rFSH + HMG (n=30)	p-value
Age (years)	27.66 ± 4.3	28.6 ± 3.97	0.387
BMI (kg/m^2)	24.32 ± 3	23.31 ± 4.2	0.415
Length of infertility (years)	5.75 ± 3.20	6.43 ± 3.40	0.426
Basic LH (mIU/ml)	1.37 ± 0.28	1.30 ± 0.27	0.366
Basic FSH (mIU/ml)	5.70 ± 1.47	4.92 ± 1.69	0.065
Basic Estradiol (pg/ml)	27.56 ± 9.26	29.03 ± 8.77	0.531

Note: Numbers are Mean ± SD

Table 2: Results of stimulation in both groups

Variable	Group A rFSH (n=30)	Group B rFSH + HMG (n=30)	p-value
Estradiol (day of hCG) (pg/ml)	502.2 ± 129.3	522 ± 196.5	0.64
Follicles 15mm (No.)	9.1 ± 3.6	10 ± 4.1	0.3
Oocytes (No.)	7 ± 3.2	8.3 ± 3.6	0.15
Gonal-F ampoules used (No.)	20.6 ± 6.2	20.5 ± 4.8	0.92
Total number of embryos	4.2 ± 1.9	5.2 ± 2.1	0.08
Endometrial thickness(mm) (day of hCG)	9.53 ± 1.9	10 ± 1.2	0.21
Chemical pregnancy (No.)	6	6	1
Clinical pregnancy (No.)	6	6	1
Live birth (No.)	6	6	1
Progesterone (day of hCG) (ng/ml)	0.72 ± 0.27	1.7 ± 0.28	< 0.001
LH (day of hCG) (mIU/ml)	1.45 ± 0.28	1.7 ± 0.29	0.001
Metaphase II oocyte (No.)	5.36 ± 2.57	6.76 ± 2.95	0.05
Grade A embryos (No.)	2.46 ± 1	3.6 ± 1.52	0.001
Grade B embryos (No.)	0.6 ± 0.6	2 ± 1.1	< 0.001

Note: Numbers are Mean ± SD

In order to assess the simultaneous effect of the variables on ovarian response, a logistic regression model was used. According to the results, on the day of hCG administration, a significant difference between both groups in the serum levels of progesterone ($p<0.001$) and estradiol ($p= 0.037$), the number of follicles 15mm ($p= 0.040$) and number of grade B embryos ($p= 0.003$) existed.

Discussion

The results of this study are in favor of using an exogenous LH supplementation during COH in ART cycles which can be either in the form of rLH or the LH component in HMG. In the present study, patients in group B of the treatment protocol (those who received HMG supplement to provide LH) were superior to group A regarding the number of metaphase II oocytes, levels of LH, progesterone and estradiol on the day of hCG administration in addition to the numbers of grade A and B embryos. However both groups were similar in pregnancy rates and the rate of live births. These findings are close to the results of a systematic review performed by Mochtar et al. (7).

In their study on eleven trials involving 2396 women who had used a GnRH agonist, there was no evidence of a statistical difference in the live birth rate reported in two trials (OR=1.51, 95% CI=(0.79 - 2.87)) and no evidence of a statistical difference in clinical pregnancy rates reported in seven trials (OR=1.15, 95% CI=(0.91 - 1.45)).

Successful ART cycles depend both on ovarian stimulation and pituitary suppression in cycles treated by GnRH agonists. GnRH agonists do not cause complete elimination of LH. In order to have the maximum estradiol response, in most cases enought LH levels to occupy less than 1% of LH receptor, can be beneficial. Hence, the residual levels of LH (1-10 IU/d) seems to be able to produce maximum theca cell stimulation (8).

A study on oocyte donors has shown that in patients with LH level of less than 1 IU/d due to profound pituitary suppression, adding LH to treatment protocol in the form of HMG could improve the oocytes in terms of both quality and quantity (9). In this study however, Tesarik et al. implemented long acting GnRH which could cause a longer and stronger suppression of the pituitary whereas in the present study we used the daily short acting subcutaneous form.

De Placido et al. in their study have shown that the immune reactive LH level is not related to a possible need for LH in the process of a follicular response to ovulation induction. Although there is a relation between immune reactive and bioactive LH, the differences are also notable (10). In the present study, patients were homogenous in their baseline LH levels, nevertheless they responded differently to exogenous LH.

Several other studies conducted in recent years have indicated the positive impact of adding LH in ovarian folliculogenesis. In a study, using a dose of 150 IU of rLH , O'Dea et al. were able to produce a serum LH concentration of 1.2 IU/d, lower concentrations

of which resulted in decreased success rate of IVF. They also showed that LH has a threshold window (11) and it exerts its beneficial effects in higher levels compared to those which induce atresia in smaller follicles (12).
In another study conducted by researchers at Serono, Swiss company in 1998, a dose of 75 IU/d of rLH was implemented. They observed that on the day of hCG administration, an improvement in follicular maturation as well as increased estradiol and progesterone levels were seen in most cases (13). In our study the hormonal profile showed comparable results.
Nowadays, a number of new protocols including LH have been proposed. There is no doubt about the positive impact of LH on ART cycles, both by improving the quality of oocytes and its positive impact on endometrial receptivity due to higher levels of estrogen (14). Most studies have recommended using LH in poor responders (15) but many others are suggesting it should be also included in protocols for normoresponders (16).
rLH is still not available in many countries. Therefore according to the present work, instead of rLH, starting HMG from the seventh day of stimulation can be a suitable substitute. Finally despite the present knowledge about LH effects, it remains to be elucidated which patients would benefit the most from this practice and therefore more studies are needed to show the importance of LH in ovarian response.

Conclusion

Based on our findings, adding LH either as rLH or HMG to rFSH in women under pituitary suppression during their ART cycles would most probably result in improvement of a number of ovarian response parameters; however it does not have considerable impact on pregnancy rates.

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References

1. Hillier SG. Gonadotropic control of ovarian follicular growth and development. Mol Cell Endocrinol. 2001; 179(1-2): 39-46.
2. Zeleznik AJ. Follicle selection in primates: Many are called but few are chosen. Biol Reprod. 2001; 65(3): 655-659.
3. Shoham Z. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. Fertil Steril. 2002; 77(6): 1170-1177.
4. Daya S. Gonadotropin releasing hormone agonist protocols for pituitary desensitization in in vitro fertilization and gamete intrafallopian transfer cycles. Cochrane Database Syst Rev. 2000; 2(2): CD001299.
5. Veeck L. Oocyte assessment and biological performance. Annals of the New York Academy of Sciences. 1988; 541: 259-274.
6. Veeck L. An atlas of human gametes and conception. London: Parthenon; 1999.
7. Mochtar MH, Van der Veen, Ziech M, van Wely M. Recombinant Luteinizing Hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. Cochrane Database Syst Rev. 2007; 18(2): CD005070.
8. Chappel SC, Howles C. Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. Hum Reprod. 1991; 6(9): 1206-1212.
9. Tesarik J, Mendoza C. Effects of exogenous LH administration during ovarian stimulation of pituitary down-regulated young oocyte donors on oocyte yield and developmental competence. Hum Reprod. 2002; 17(12): 3129-3137.
10. De Placido G, Alviggi C, Mollo A, Strina I, Varricchio MT, Molis M. Recombinant follicle stimulating hormone is effective in poor responders to highly purified follicle stimulating hormone. Hum Reprod. 2000; 15(1): 17-20. Reprod2000 Jan;15(1):17-20.
11. O'Dea LSL. Recombinant LH in support of recombinant FSH in female hypogonadotropic hypogonadism--evidence of threshold effect. Fertil Steril. 2000; 74 Suppl: S36-S.
12. Lahoud R, Jefout M, Tyler J, Ryan J, Driscoll G. A relative reduction in mid-follicular LH concentrations during GnRH agonist IVF/ICSI cycles leads to lower live birth rates. Hum Reprod. 2006; 21(10): 2645.
13. Group TERHLS. Recombinant Human Luteinizing Hormone (LH) to Support Recombinant Human Follicle-Stimulating Hormone (FSH)-Induced Follicular Development in LH- and FSH-Deficient Anovulatory Women: A Dose-Finding Study. J Clin Endocrinol Metab. 1998; 83(5): 1507-1514.
14. De Placido G, Alviggi C, Perino A, Strina I, Lisi F, Fasolino A, et al. Italian Collaborative Group on Recombinant Human Luteinizing Hormone. Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. Hum Reprod. 2005; 20(2): 390-396.
15. Chung K, Krey L, Katz J, Noyes N. Evaluating the role of exogenous luteinizing hormone in poor responders undergoing in vitro fertilization with gonadotropin-releasing hormone antagonists. Fertil Steril. 2005; 84(2): 313-318.
16. Alviggi C, Clarizia R, Mollo A, Ranieri A, De Placido G. Who needs LH in ovarian stimulation? Reprod biomed online. 2006; 12(5): 599-607.