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Original Article

# The impact of HMG on follicular fluid hormone levels, embryo quality and IVF outcome

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**Abstract.** The objective of this study is to evaluate the effect of HMG on the follicular fluid hormone levels, embryo quality and IVF outcomes when added at different times of stimulation to rFSH in a long protocol. Seventy infertile women were randomized into three groups; group 1(n=23) was stimulated only with rFSH; whereas, HMG was added from the beginning (group 2, n=23) or when a 10 mm follicle developed (group 3, n=24). Follicular fluid hormone levels, day 3 embryo quality, implantation and clinical pregnancy rates were assessed. The number of grade 1 embryos, and estradiol/ progesterone ratio were significantly higher in group 2 compared to group 1. The numbers of grade 2 and 3 embryos, and day 3 embryos with less than 5 cells were significantly higher in group 1. Clinical pregnancy rate was significantly higher in group 2 than group 1. In conclusion, HMG supplementation to rFSH does not increase the number of oocytes retrieved, the number of day 3 embryos and day 3 embryos at 8-cell or more stages of development, fertilization and implantation rates. HMG supplementation from the beginning of rFSH stimulation result in improvement of clinical pregnancy rates and other indices of embryo quality such as embryo grading.

Key words: Gonadotropins, follicular fluid, embryo quality, IVF/ICSI outcome, FSH

## 1. Introduction

Two-cell, two-gonadotropin theory necessitates both FSH (follicle stimulating hormone) and LH (luteinizing hormone) for normal follicular development and steroidogenesis (1). It is well-documented that for normal folliculogenesis and steroidogenesis threshold levels of FSH and LH are mandatory and that follicular atresia results due to inappropriately timed and/or abundant levels of LH secretion (2). GnRH (gonadotropin releasing hormone) analogue-induced pituitary suppression with recombinant FSH (rFSH) for controlled ovarian hyperstimulation (COH) results in LH concentrations that are much lower than in normal cycles (3-5). As a result of these low LH concentrations androgen precursor production by thecal cells and subsequently ovarian estradiol biosynthesis is reduced and this unbalanced endocrine milieu may in turn affect the oocyte negatively (6,7). However, trials have suggested that these low LH levels are sufficient for folliculogenesis, so pure FSH can be used for COH without the need to add LH (4,5). Nonetheless, debate whether some patients would benefit from LH supplementation and if so about the timing of this supplementation still continues (5). There are clinical reports showing a beneficial effect of exogenous LH on the follicular response, especially on the quality, developmental competence and the implantation potential of the oocytes (8-10).

Follicular fluid reflects the metabolic and hormonal activities taking place in the microenvironment of the developing oocyte and is believed to predict the fertilization, embryo cleavage and pregnancy rates in IVF (11). Steroid hormone levels and ratios in follicular fluid differ for mature and immature oocytes and these hormonal levels can be used as a predictive marker for oocyte maturation and the quality of the future embryo (12,13). There are studies showing a more favorable hormonal milieu for the oocyte in the follicular fluid and a resultant

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better embryo quality and higher on-going pregnancy rates when human menopausal gonadotropin (HMG) is used for ovarian stimulation (14,15).

The objective of this study is to evaluate the effect of HMG on follicular fluid hormone levels, embryo quality and IVF outcomes when added at different times of stimulation to rFSH.

## 2. Materials and methods

All consecutive patients at IVF Unit, Marmara University Hospital, İstanbul, Turkey between November 2008 and February 2010 were assessed for eligibility. Each participant was informed about the aspects of the study and the risks, and gave an informed consent. Approval for the study from Marmara University Ethics Committee was obtained. The study was registered as an RCT with the number of ACTRN12611001133921. Seventy patients at ages 25-35 referred to our IVF clinic for mild male factor, tubal factor and unexplained infertility and who accepted to participate, were enrolled for the study Then they were randomly assigned to one of the three study groups.

Exclusion criteria included polycystic ovarian syndrome, poor responder patients (defined as having less than four follicles <15mm developed, or cycle cancellations in previous IVF attempts), previous fertilization failure (defined as fertilization rate less than 30% of the MII oocytes in a previous IVF cycle), any systemic, endocrine or metabolic illnesses, diminished ovarian reserve (defined as early follicular phase FSH>15 mIU/mL or AFC<5) and patients older than age 35, patients with a body mass index <18 and > 29 $kg/m^2$ and those with irregular menses (defined as menstrual period <21, >35 days). All patients underwent ovarian stimulation with long protocol using GnRH agonist leuprolide acetate (Lucrin;Abbot; Istanbul,Turkey) starting on the mid-luteal phase of the previous cycle. Group 1 was stimulated with only rFSH during the entire stimulation period whereas the others were also stimulated with HMG starting from the beginning of the stimulation (group 2) or when a follicle of ≥10mm is obtained (group 3). Ovulation was triggered with 10.000 IU chorionic gonadotropin (Pregnyl; Organon; İstanbul; Turkey) when a leading follicle diameter ≥17mm is reached and the oocytes were retrieved 36 hrs later. Follicular fluids were collected without flushing from the oocyte-containing follicles of 16mm and more in diameter, pooled and stored at $-20^0C$ until assayed for their hormone contents. Estradiol and progesterone levels in the aspirates of follicular fluids were determined by electrochemiluminescence immunoassay (Elecsys/ Cobas, Roche, USA). Intra- and inter-assay variations (CV%) for estradiol and progesterone levels were 2. 3 and 6. 2 % (n= 7), and 3.7 and 5.5 % (n=5), respectively. Quality of the day 3 embryos were assessed using the following criteria (16):

Grade 1 embryo (G1): no fragmentation, homogenous blastomeres

Grade 2 embryo (G2): < %20 fragmantation, equal or unequal blastomeres

Grade 3 embryo (G3): %20- 50 fragmantation, unequal blastomeres

Grade 4 embryo (G4): >%50 fragmantation unequal blastomeres

The groups were compared using ANOVA and multiple comparison posthoc tests where appropriate. Clinical pregnancy rates were compared with Fisher's exact test. $p<0.05$ is considered significant.

## 3. Results

Baseline characteristics of the patients were comparable among the groups (Table 1).

The number of oocytes retrieved, the number of MII oocytes, fertilization rates, the total number of embryos and embryos at 8 cell or more advanced stages of development on day 3 were comparable among the groups (Table 2).

Table 1. Baseline characteristics of the patients are shown

| | Group 1(n=23) | Group 2( n=23) | Group 3(n=24) | p |
|---|---|---|---|---|
| Mean age (year) | 28.96±2.72 | 29.70±3.18 | 30.71±2.72 | 0.120 |
| BMI ($kg/ m^2$ ) | 24.90±2.62 | 23.770±3.03 | 24.77±2.86 | 0.338 |
| Duration of infertility (years) | 5.13±2.45 | 5.4±2.16 | 6.3±2.54 | 0.205 |
| CD3 Antral follicle count | 11.60±3.17 | 10.82±3.20 | 11.00±4.19 | 0.737 |
| CD3 FSH (mIU/L) | 5.28±1.82 | 5.77±1.69 | 5.17±1.57 | 0.446 |
| CD3 Estradiol (pg/mL) | 38.15±14.05 | 36.22±16.85 | 34.76±16.75 | 0.766 |

BMI: body mass index; CD3: cycle day 3

However, patients stimulated with only rFSH (group 1) generated significantly higher number of day 3 embryos at or less than 5-cell stage compared to the patients in groups 2 and 3 (Table 2). Furthermore, the number of day 3 grade 1 embryos was significantly less, and the number of day 3 grade 2 and 3 embryos was significantly higher in group 1 compared to those in groups 2 and 3 (Table 2).

Implantation rate was comparable among the groups but clinical pregnancy rate was significantly higher in group 2 compared to group 1 (Table 2).

Follicular fluid estradiol and progesterone levels were similar among the groups (Table 3).

The estradiol/progesterone ratio was significantly higher in group 2 compared to those in groups 1 and 3 (Table 3).

## 4. Discussion

We designed this study to investigate the effect of HMG on follicular fluid hormone levels, embryo quality and IVF outcomes in a selected cohort of patients when it was added to rFSH at different times of ovarian stimulation. No significant differences were found among the groups regarding the number of oocytes retrieved, fertilization rates, and the number of day 3 embryos and day 3 embryos at 8-cell or more stages of development. However, when other indices of embryo quality were compared such as embryo grading; the number of grade 1 embryos was significantly higher and the number of grade 2 and 3 embryos were significantly lower in group 2 compared to group 1. Some studies reported no significant differences between recombinant FSH versus HMG in terms of the

Table 2. IVF parameters and outcomes are shown

| | Group 1 | Group 2 | Group3 | p |
|---|---|---|---|---|
| Mean FSH dose used (IU) | 1542.391±392.79 | 1514.56±508.04 | 1693.64± 516.55 | 0.385 |
| Mean HMG dose used (ampoul) | - | 11.7 ±4.83 | 6.45±3.03 | 0.000 |
| The number of oocytes retrieved | 10.65±5 | 11.30±6.0 | 11.62±4.65 | 0.819 |
| The number of MII oocytes | 8.91±4.17 | 8.00±4.59 | 8.75±4.05 | 0.743 |
| Fertilization rate | 70.8±15.5 | 72.8±16.3 | 74.4±13.5 | 0.712 |
| The number of day 3 embryos | 5.78±3.01 | 5.56±3.31 | 6.25±2.67 | 0.728 |
| The number of day 3 embryos at 8-cell stage and more | 2.65±1.52 | 3.73±2.3 | 3.33±1.97 | 0.172 |
| The number of day 3 embryos at ≤ 5 cell stage | 1.56±1.07 | 0.43±0.78 | 0.75±0.98 | 0.001 [a,b] |
| The number of day 3 grade 1 embryos | 2.13±1.54 | 4.56±2.48 | 4.29±2.29 | 0.001 [c,d] |
| The number of day 3 grade 2 embryos | 1.69±0.92 | 0.78±0.90 | 1.04±1.45 | 0.023 [e] |
| The number of day 3 grade 3 embryos | 1.95±1.49 | 0.21±0.51 | 0.91±1.01 | 0.001 [f,g] |
| The number of day 3 grade 1 embryos at 8-cell stage or more | 2.13±1.39 | 3.34±1.96 | 3.25±2.26 | 0.063 |
| The number of embryos transferred | 2.3±0.5 | 2.73±0.5 | 2.75±0.5 | 0.01 [h,i] |
| Clinical pregnancy rate | (6/23) 26.1% | (13/23) 56.5% | 10/24 41.6% | 0.05 [j] |
| Implantation rate | 15% | 32% | 20% | 0.13 |

a: Group 1 vs. Group 2 p<0.01 (multiple comparison posthoc test)
b: Group 1 vs. Group 3 p<0.01 (multiple comparison posthoc test)
c: Group 1 vs. Group 2 p<0.01 (multiple comparison posthoc test)
d: Group 1 vs. Group 3 p<0.01 (multiple comparison posthoc test)
e: Group 1 vs. Group 2 p<0.05 (multiple comparison posthoc test)
f: Group 1 vs. Group 2 p<0.001 (multiple comparison posthoc test)
g: Group 1 vs. Group 3 p<0.05 (multiple comparison posthoc test)
h: Group 1 vs. Group 2 p<0.05 (multiple comparison posthoc test)
i: Group 1 vs. Group 3 p<0.05 (multiple comparison posthoc test)
j: Group 1 vs. Group 2 p<0.05 (contingency table analysis)

Table 3. Follicular fluid estradiol and progesterone levels are shown

| | Group 1 | Group 2 | Group 3 | p |
|---|---|---|---|---|
| Estradiol (nmol/L) | 4146.43±1534.05 | 5573.02±4693.38 | 4429.00±2267.74 | 0.267 |
| Progesteron (nmol/L) | 42971±24139 | 30313±23355 | 37624±18063 | 0.154 |
| Estradiol/progesteron | 0.13±0.09 | 0.29±0.30 | 0.15±0.10 | 0.016 [a,b] |

a: Group 1 vs. Group 2, p<0.05 (multiple comparison posthoc test)
b: Group 2 vs. Group 3, p<0.05 (multiple comparison posthoc test)

number of mature oocytes, the number of treatment days, the embryo quality and clinical pregnancy rates (17-19). Additionally, some studies investigating the effects of recombinant LH supplementation to rFSH found no difference with respect to pregnancy rate (9,20). Recombinant LH supplementation starting on day 6 of FSH stimulation in down regulated women of advanced reproductive age showed that the oocyte yield, maturity and the number of fertilized oocytes were significantly higher in the group stimulated with rFSH alone; however, the number and quality of embryos, implantation and clinical pregnancy rates were similar (20). A study comparing the effectiveness of highly purified HMG with rFSH also indicated comparable fertilization, implantation and pregnancy rates between the groups (21).

On the other hand, a recent meta-analysis of seven randomized trials, found that HMG was associated with a pooled 4% increase in live birth rate when compared with rFSH in IVF-ICSI treatment following a long protocol (22). In accordance with this, we observed higher clinical pregnancy rates in patients stimulated with rFSH-HMG in comparison to those stimulated with only rFSH. Furthermore, clinical pregnancy rate was significantly higher in patients stimulated with rFSH and HMG from the beginning of the stimulation compared to those stimulated with only rFSH. Although clinical pregnacy rate was also higher in patients who were stimulated with HMG added to rFSH when a follicle of ≥10mm in diameter was observed, the difference could not reach statistical significance.

Recently a large retrospective cohort study demostrated that recombinant LH supplementation may improve pregnancy and live birth rates. As these results were associated with an improved fertilization and implantation rate, it has been suggested to reflect improvement in oocyte quality and/or uterine receptivity (23). Likewise, we observed statistically significant higher clinical pregnancy rates in patients with HMG supplementation from the beginning of rFSH stimulation; however, the fertilization and implantation rates were comparable among the groups. Improved embryo quality may at least in part explain higher clinical pregnancy rates in these patients. Indeed, a recently published study investigated the effect of HMG on ploidy of human cleavege state embryos. Results showed that despite similar numbers of chromosomally normal embryos in both groups, women undergoing HMG stimulation had significantly higher percentages of diploid embryos than did the FSH-stimulated patients suggesting the beneficial effect of LH –containing stimulation on diploidy rates in preimplantation embryos (18). In a study with a selected group of patients with >20% oocyte immaturity during stimulation with FSH alone, the addition of HMG in the second cycle also resulted in improved yield of mature oocytes and excellent morphologic grade embryos (5).

These observations may also explain higher IVF pregnancy rates in our patients stimulated with rFSH-HMG at early follicular phase compared to those stimulated with only rFSH. The number of grade 1 embryos were highest and the number of grade 3 embryos were lowest in the group stimulated with rFSH and HMG from the beginning and a significant difference was observed when compared with those stimulated with rFSH alone. We also aimed to compare hormone levels in the aspirates of follicles ≥16mm in rFSH versus rFSH-HMG regimens of ovarian stimulation and found that patients stimulated with rFSH-HMG had significantly higher estradiol/progesterone ratio than those stimulated with only rFSH. This finding could be a plausible explanation for higher embryo quality and clinical pregnancy rates in these patients. In a study investigating the relationship between oocyte morphology and ovarian follicle steroid hormone concentrations, an association between oocyte morphology and the ratios of estradiol/testesterone and estradiol/ progesterone but not with the absolute concentrations was observed. A higher estradiol/ testerone level was noted with large proportion of good quality oocytes (24). It has been suggested that reduced transforming growth factor–β1 by the LH in HMG, may result in increased androgen

production; and by increasing substrate for aromatization, estrogen levels may tend to be higher in HMG stimulation protocols compared with pure FSH stimulation ones (25). The direct inhibition of 17α-hydroxylase by transforming growth factor–β1 was noted to be responsible for limiting androgen production while enhancing progesterone production (26). Parallel with the literature, we found higher follicular fluid levels of estradiol in groups 2 and 3; and higher progesterone levels in group 1. It was shown that exogenous LH activity induces a higher estradiol/progesterone ratio in the follicular fluid endocrine profile consistent with our findings (14). The evaluation of discrepancy in the follicular fluid endocrine environment in different ovarian stimulation protocols, may play an important role in optimizing the intrafolliculer endocrine prolife and maintaining a better oocyte quality, thereby a better embryo quality and clinical outcome. In our study we suggest that not only HMG itself, but also the timing of LH addition in a stimulation protocol may play a critical role.

In conclusion rFSH co-stimulation with HMG may help to improve embryo quality and clinical pregnancy rates in selected cohort of patients. HMG supplementation from the beginning of stimulation may have more beneficial effects than its addition to the protocol when a follicle ≥10mm was observed. However these observations need to be corroborated by larger studies. The addition of HMG and the timing of its addition can be particularly important in increasing the chances of IVF.

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Where the work was done:

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The author Ozgur Oktem, participated in data analysis, manuscript drafting and critical discussion of this study.

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