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Original Article

# Effects of growth hormone plus gonadotropins on controlled ovarian stimulation in infertile women of advanced age, poor responders, and previous in vitro fertilization failure patients

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ABSTRACT

*Objective:* To investigate the effects of growth hormone (GH) cotreatment in ovarian stimulation in infertile women of advanced age, poor responders, and patients with one or more previous IVF treatment failures.

*Materials and methods:* We conducted a retrospective observational study of 436 patients undergoing GH cotreatment in ovarian stimulation. The first arm included 134 infertile women of advanced age. The second arm included 236 patients with one or more IVF previous treatment failures, and the third arm included 66 younger poor responders. Main outcome measures were the number of oocytes and embryos, quality of embryos, and implantation and pregnancy rates.

*Results:* In infertile women of advanced age, GH plus ovarian stimulation yielded no statistical differences in the numbers of oocytes and embryos, quality of embryo, and rates of implantation and pregnancy. In the second arm, the mature oocyte number (8.2 vs. 6.8), implantation rate (16.1% vs. 0%), and pregnancy rate (33.9% vs. 0%) in the GH cotreatment group differed significantly from those in the control group; the rate of good-quality embryos in the GH cotreatment group improved from 35.5% ± 31.1%–41.4% ± 30.6% in this arm. Similar results were observed in the third arm; in this arm, the clinical pregnancy rate was 30.3% in the GH cotreatment group and 6.1% in the control group.

*Conclusion:* No significant differences were observed in infertile women of advanced age, which may be due to the low GH dose. The GH adjuvant therapy for patients with one or more previous IVF treatment failures and for poor responders significantly improved the oocyte and embryo numbers as well as implantation and pregnancy rates.



## Introduction

Human infertility, typically defined as the failure of conception after at least 1 year of unprotected intercourse (six months if the woman is over age 35), is a common problem that has affected 1 in 6 couples over the past decade [1]. One method for treating infertility is in vitro fertilization (IVF) and embryo transfer. IVF involves the administration of hormones to stimulate ovarian function to increase follicular growth, thus facilitating the development of more oocytes and increasing the chances of pregnancy.

Nevertheless, the chances of live birth after assisted reproductive treatment decrease with increasing female age and markedly decrease after the age of 40 years [2,3]. Moreover, a poor ovarian response to gonadotropin stimulation for IVF is not uncommon and

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is a predictor of low pregnancy rates, the incidence of which has been estimated to be 9%–29% [4,5], representing a marked therapeutic challenge in controlled ovarian hyperstimulation. Furthermore, in the group of "normal responders," in whom ovarian stimulation is expected to yield acceptable results, repeated IVF failures has always been a source of distress.

Therefore, IVF protocols are always critically examined mainly to improve live birth rates but also to reduce the requirements of hormones (gonadotrophin) and optimize the recruitment of follicular cells. Despite their limited success, many strategies have been suggested to promote the outcomes in patients with infertility [6]. Some protocols have considered the role of growth hormone (GH) in IVF to potentiate the effects of exogenous gonadotropins [7,8].

GH transduces multiple signals. It uses the signal transducer and activator of transcription or cyclic AMP response element-binding, mitogen-activated protein kinase pathway [9,10]. GH and insulin-like growth factor I (IGF-I) play an important role in ovarian folliculogenesis [11]. In mice lacking the GH receptor and GH-binding protein, follicular development is significantly reduced in response to gonadotrophin stimulation [12]. In addition, several meta-analyses showed addition of GH to gonadotropins of poor responders significantly increased pregnancy rate and live birth rate [13–16]. GH is reported to modulate the action of follicle-stimulating hormone on granulosa cells by upregulating the local synthesis of IGF-I. IGF-I amplifies the effect of gonadotropin action at the level of both granulosa and theca cells [17,18]. GH may also increase the intraovarian production of IGF-I [19,20], which is considered important for ovarian function [21,22], follicular cell stimulation, estrogen production, and oocyte maturation [20]. Furthermore, GH may improve nuclear and cytoplasmic maturation, acting independently of IGF-I, in mice, cows, and monkeys [23–26]. Growth hormone (GH) also facilitates the complete maturation of naked oocytes in humans [27,28]. The GH receptor is present in cumulus cells and in the oocytes of all the aforementioned species as well as in humans [29].

Nevertheless, results regarding the effects of GH on controlled ovarian stimulation for IVF are inconsistent in the literature [30,31]. In a recently updated Cochrane review of all randomized controlled trials using GH for ovarian stimulation, GH showed no effects in normal responders, but it significantly improved live birth and pregnancy rates in poor responders. The exact subgroup of poor responders who would benefit from GH augmentation must be identified [14]. Moreover, women older than 40 years undergoing assisted reproductive treatment and costimulation with GH were reported to achieve more ongoing pregnancies and experience less pregnancy wastage, resulting in more deliveries and live births [32].

In this study, we investigated the usefulness of GH costimulation in 3 arms of patients, namely infertile women of advanced age, patients with one or more previous IVF treatment failures, and poor responders.

## Materials and methods

This retrospective, observational study was conducted in the reproductive center of Lee Women's Hospital from January 2005 to December 2009 and was approved by the Institutional Review Board of Chung Shan Medical University.

The women participating in this study followed a long IVF protocol as described previously [33]. In brief, the protocol began with daily subcutaneous injections of leuprolide acetate (Lupron; Takeda Pharmaceutics, Germany) 0.5 mg on Day 21 of the prestimulation cycle. Gonadotrophin (Gonal-F, 225 IU/day; Serono, Bari, Italy) was administered subcutaneously on cycle Days 3–7. The does was then adjusted according to the ovarian response to stimulate follicular development. The resulting ovarian response was monitored by transvaginal ultrasound. When two or more follicles reached a maximum diameter of 18 mm, 10,000 IU human chorionic gonadotropin (hCG; Profasi; Serono) was administered. Transvaginal oocyte retrieval was performed 32–34 h after the hCG injection. Fertilization was performed by conventional insemination or by intracytoplasmic sperm injection (ICSI), depending on the semen parameters. Fresh ET was performed with the replacement of at most two blastocysts with the best quality.

The first arm of this study included 98 infertile women of advanced age (age: 40–44 years) treated with 3 international units (IU) of GHs from cycle day 3, when exogenous gonadotrophin was started to the day of human chorionic gonadotropin (hCG) injection for final follicular maturation. The control group of 36 advanced age patients received the same treatment protocol except for the GH cotreatment.

The second arm of this study included 118 patients younger than 38 years and who had failed IVF at least once at the same center. The mean previous IVF treatment failures for study and control groups were $2.0 \pm 0.6$ and 0. The third arm of this study involved 33 patients younger than <38 years who responded poorly (oocyte number $\leq 5$ and embryo number $\leq 3$) to gonadotropin treatment in their first cycles at the same center. The second and third arms of patients treated with 2 IU of GH from day 3 of exogenous gonadotrophin administration until the day following hCG injection. Oocyte and embryo numbers, embryo quality, and implantation and pregnancy rates were determined for cycles completed using gonadotropin-releasing hormone (GnRH) and GH protocols. Implantation rate was defined as the number of gestational sacs observed divided by the number of embryos transferred. Pregnancy rate was defined as the number of clinical pregnancies expressed per embryo transfer cycles.

The differences between GH and Control groups were analyzed using Student's or paired t test. Statistical analysis was performed using the Statistical Program for Social Sciences, version 15.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

## Results

The baseline characteristics of the of the cotreatment and control group patients did not differ significantly. Table 1 shows the baseline data and ovarian stimulation characteristics of the patients. Table 2 lists the embryo and pregnancy outcome measures.

In infertile women of advanced age, GH plus ovarian stimulation revealed no statistical differences in oocyte and embryo numbers, embryo quality, and implantation and pregnancy rates.

In the second arm of the study, the number of oocytes in metaphase 2 ($8.2 \pm 5.0$ vs. $6.8 \pm 3.4$; $p < 0.05$), rate of implantation ($16.1\% \pm 31.0\%$ vs. 0%; $p < 0.01$), and rate of clinical pregnancy (33.9% vs. 0%; $p < 0.01$) showed significant differences in the GH cotreatment group compared with the control group. Although the difference in the rate of good-quality embryos was nonsignificant, it improved from $35.5\% \pm 31.1\%$–$41.4\% \pm 30.6\%$.

Similar results were observed in the third arm of the study. The number of mature oocytes recovered ($5.5 \pm 3.7$ vs. $3.2 \pm 0.9$; $p < 0.05$), rate of implantation ($16.7\% \pm 31.0\%$ vs. $1.6\% \pm 8.8\%$; $p < 0.01$), and rate of clinical pregnancy (30.3% vs. 6.1%, $p < 0.01$) were significantly higher in the GH cotreatment group than in the control group. The rate of good-quality embryos was higher in the GH cotreatment group than in the control group ($47.2\% \pm 36.2\%$ vs. $31.0\% \pm 38.3\%$); however, no significant differences were observed in this study arm. In addition, patients in the GH cotreatment group received significantly more embryos per transfer than did those in

**Table 1**
Baseline and ovarian stimulation characteristics of patients.

| Arm | First | | Second | | Third | |
|---|---|---|---|---|---|---|
| Group | GH | Control | GH | Control | GH | Control |
| No. of cycles | 98 | 36 | 118 | 118 | 33 | 33 |
| Age | 41.2 ± 1.1 | 41.5 ± 1.4 | 32.3 ± 3.4 | 32.0 ± 3.4 | 33.9 ± 2.3 | 33.6 ± 2.5 |
| BMI | 22.4 ± 2.4 | 22.5 ± 2.3 | 21.3 ± 2.6 | 21.2 ± 2.5 | 21.5 ± 2.7 | 21.5 ± 2.7 |
| Day 3 FSH | 4.6 ± 2.4 | 4.2 ± 2.5 | 6.3 ± 2.7 | 6.1 ± 2.3 | 8.8 ± 4.2 | 8.5 ± 4.1 |
| LH | 1.3 ± 1.0 | 1.3 ± 1.5 | 0.8 ± 0.4 | 0.9 ± 0.5 | 0.7 ± 0.4 | 1.0 ± 0.7 |
| E2 | 958.6 ± 660.3 | 1046.2 ± 474.0 | 1432.9 ± 940.8 | 1532.6 ± 1014.4 | 1234.3 ± 829.3 | 748.3 ± 390.5 |
| OPU No. | 5.4 ± 2.7 | 5.7 ± 2.1 | 10.5 ± 7.1[a] | 8.7 ± 4.0[a] | 6.9 ± 5.1[c] | 4.1 ± 0.9[c] |
| MII No. | 4.2 ± 2.3 | 4.7 ± 2.7 | 8.2 ± 5.0[b] | 6.8 ± 3.4[b] | 5.5 ± 3.7[d] | 3.2 ± 0.9[d] |

First arm: Infertile women of advanced age (40–44 years).
Second arm: Patients with one or more previous IVF treatment failures (age: <38 years).
Third arm: Patients with poor responses to at least one previous IVF cycle (age: <38 years).
a, b, c, and d indicate paired t test; $p < 0.05$.
OPU No.: Number of retrieved oocytes.
MII No.: Number of mature oocytes in metaphase 2.

**Table 2**
Embryo and pregnancy outcome measures.

| Arm | First | | Second | | Third | | Total control (<38 years) 2005.1–2009.12 |
|---|---|---|---|---|---|---|---|
| Group | GH | Control | GH | Control | GH | Control | |
| No of cycles | 98 | 36 | 118 | 118 | 33 | 33 | |
| FR (%) | 81.3 ± 21.3 | 82.4 ± 16.2 | 76.4 ± 21.6 | 75.0 ± 24.0 | 77.8 ± 20.1 | 75.8 ± 26.1 | |
| D2 good (%) | 60.5 ± 33.1 | 55.2 ± 32.8 | 46.3 ± 31.5 | 40.1 ± 32.4 | 53.2 ± 33.6 | 41.2 ± 38.1 | |
| D3 good (%) | 48.4 ± 33.2 | 51.5 ± 33.9 | 41.4 ± 30.6 | 35.5 ± 31.1 | 47.2 ± 36.2 | 31.0 ± 38.3 | |
| IR (%) | 14.1 ± 22.9 | 12.7 ± 18.4 | 16.1 ± 31.0[a] | 0 | 16.7 ± 31.0[c] | 1.6 ± 8.8[c] | |
| Clinical PR (%) | 33.7% (33/98) | 36.1% (13/36) | 33.9% (44/118)[b] | 0 | 30.3% (10/33)[d] | 6.1% (2/33)[d] | 50.5% (1236/2445) |

a, b, c, and d indicate paired t test; $p < 0.01$.
FR: Fertilization rate.
IR: Implantation rate.
Clinical PR: Clinical pregnancy rate.
D2 good (%): Day 2 good embryo rate.
D3 good (%): Day 3 good embryo rate.

the control group in the third arm of the study (4.0 ± 3.2 vs. 2.0 ± 0.9; $p < 0.05$; data not shown in the tables).

## Discussion

As an important treatment procedure in assisted reproductive technology, ovarian stimulation aims to develop and mature multiple oocytes to improve the chances of conception through IVF. Ovarian stimulation is traditionally performed with gonadotropins or clomiphene, whereas many adjuvant therapies have been used to improve the yield and results of the modality. These therapies include the addition of GH or GH-releasing factor, pyridostigmine, oral L-arginine, transdermal testosterone, and letrozole [34].

A Cochrane review of GH supplementation reported improved pregnancy rates in poor responders [14]. In women with no history of poor responses to IVF stimulation protocols, the routine use of GH as an adjuvant was not associated with improved live birth rate (odds ratio [OR]: 1.32, 95% confidence interval [CI]: 0.40–4.43) and pregnancy rate (OR: 1.78, 95% CI: 0.49–6.50). However, in the patients with a history of poor responses to IVF stimulation, the OR for live birth (5.39, 95% CI: 1.89–15.35) favored GH administration. Similar results were observed for the pregnancy rate (OR: 3.28, 95% CI: 1.74–6.20).

In the third arm of our study, the GH cotreatment group comprised 33 young women (<38 years) with poor responses to at least one previous IVF cycle (defined as oocyte number $\leq 5$ and embryo number $\leq 3$). The ovarian responses, namely the numbers of ovum pickup and recovered oocytes in metaphase 2 and embryos transferred, showed significant improvement. Significant differences were also observed in implantation and clinical pregnancy rates. The clinical pregnancy rate was in concordance with the conclusion of a Cochrane review of 7 studies [14]. Thus, we might infer that GH administration resulted in more follicles in the examined cohort and higher numbers of mature oocytes and embryos transferred. Additionally, more estradiol was produced per follicle in the GH cotreatment group.

The ability of human oocytes to form morphologically normal and implantation competent embryos is reportedly associated with the concentration of different hormones in follicular fluid [35]. Among these hormones, GH played an important role in embryo quality, and higher concentrations of GH in follicular fluid were related to rapid cleavage, good cleavage morphology, and high embryo implantation potential [36]. In our study, the fertilization and good-quality embryo rates were higher, but nonsignificantly, in the GH cotreatment group compared with the control group.

In the second arm of our study, the GH cotreatment group included 118 young women (<38 years) with one or more previous IVF treatment failures. The ovarian responses, including the numbers of ovum pickup and recovered oocytes in metaphase 2, showed significant improvement. Moreover, significant differences were observed in implantation and clinical pregnancy rates. Unexpectedly, all 118 women in the control group could not achieve pregnancy.

In the first arm of our study, the GH cotreatment group included 98 infertile women of advanced age. The ovarian responses, including the numbers of ovum pickup and recovered oocytes in

metaphase 2, as well as embryo and pregnancy outcomes, including number of good-quality embryos, number of embryos transferred, and clinical pregnancy rate, in the GH cotreatment group were not significantly different from those in the control group. In a recent randomized controlled study [32] of 50 women older than 40 years who were undergoing ovarian costimulation with GH, the numbers of oocytes, embryos, and pregnancies were similar. However, the clinical pregnancy rate (26% vs. 6%) and delivery rate (22% vs. 4%) were higher in the GH cotreatment group than in the control group. In that trial, the author used a higher daily dose of GH (8 IU). The nonsignificance of the differences in this study may be a result of the smaller GH dose (3 IU).

The administration of GH to patients with GH deficiency (GHD) has been proven successful in increasing ovarian sensitivity to endogenous gonadotropins [37]. The coadministration of GH and gonadotropins for ovarian stimulation has been suggested to improve follicular growth, and to some extent the pregnancy rate, in patients with hypogonadotropic hypogonadism [38]. The addition of GH to gonadotropin therapy for such patients reduced the gonadotropin dose required to achieve ovulation [37]. Women with isolated GHD, hypogonadotropic hypogonadism, or panhypopituitarism have been reported to have smaller uterine dimensions than do healthy controls. GH might have independent or estrogen-mediated effects on uterine size, which may contribute to its therapeutic effect [39]. A Cochrane review [14] reported no differences in outcome measures and adverse events after the routine use of adjuvant GH in IVF protocols. However, the administration of GH to poor responders has been reported to significantly improve live birth rates.

In conclusion, many adjuvant GH therapies have been reported to yield inconsistent responses to GH adjuvant therapy. Our study revealed that in poor responders and patients with one or more previous IVF treatment failures, the GH adjuvant therapy significantly improved the numbers of oocytes and embryos and rates of implantation and pregnancy. However, in infertile women of advanced age, GH administration was not associated with improved clinical pregnancy rates. In addition, good patient selection has been reported to improve the effects of GH cotreatment for female infertility. Therefore, GH cotreatment is effective in appropriately selected cases; however, further research is warranted to improve patient selection as well to devise some effective and efficient GH treatment protocols.

## Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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