Growth hormone co-treatment within a GnRH agonist long protocol in patients with poor ovarian response: a prospective, randomized, clinical trial

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Abstract

Objective The efficacy of growth hormone co-stimulation to long luteal GnRHa regimen in poor responders to COH for IVF was assessed.

Methods This prospective, randomized, clinical trial was performed in a private assisted reproduction center. The study involved 61 patients who responded poorly to high dose gonadotropin treatment in their first cycles in the same center. Study group of 31 patients were given growth hormone co-treatment, daily subcutaneous injection of 4 mg from day 21 of preceding cycle along with GnRHa, until the day of hCG. Control group of 30 patients received the same treatment protocol except the growth hormone co-treatment. Primary end-point of the study was the number of oocytes fertilized whereas the pregnancy rate was the secondary end-point.

Results Patients' demographic characteristics did not differ significantly between the two groups. 2PNs in growth hormone co-treatment group was significantly higher than the control group (4.4 ± 1.8 vs 1.5 ± 0.9 , $p < 0.001$). Although more pregnancies and more clinical pregnancies with fetal heart beat were achieved in growth hormone group (12/31), compared to the control group (6/30), the difference did not reach to statistical significance.

Conclusion Poor responder women undergoing repeated assisted reproduction treatment and co-stimulated with GH

achieve more oocytes, higher fertilization rate if growth hormone started in the luteal phase of previous cycle, as compared with women of the same status treated with GnRHa long protocol. The study was unable to show that clinical pregnancy rate was increased significantly.

Keywords Poor responder · Controlled ovarian hyperstimulation · Growth hormone · IVF

Introduction

Poor ovarian response to gonadotropin stimulation for IVF is not uncommon and is a predictor for low pregnancy rates. There is yet a lack of definition of this situation. The most common criteria by which poor response has been defined are either an elevated FSH on days 2 or 3 of the menstrual cycle, or previous cycle cancellation due to insufficient ovarian response. Other criteria include reduced antral follicle count, high gonadotropin requirement, reduced number of follicles, low peak E_2 level, or few oocytes at harvest [1].

There are numerous strategies that have been suggested to improve the outcome in the poor responder women despite their limited successes [2]. One other option is administration of growth hormone (GH) to potentiate the effect of exogenous gonadotropins [3]. Growth hormone is reported to modulate the action of FSH on granulosa cells by up-regulating the local synthesis of insulin-like growth factor-I. The IGF-I amplifies the effect of gonadotropin action at the level of both the granulosa and theca cell [4, 5]. Results from the published studies were conflicting regarding the effect of GH during controlled ovarian stimulation for IVF [6, 7]. A recent Cochrane Library review found a significant improvement in live birth rate in

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poor responders despite no effect in normal responders [8]. In a recent randomized controlled trial [9], it has been reported similar number of oocytes, embryos and pregnancies but improvement of delivery and live birth rates after ICSI in 50 women older than 40 years old by ovarian co-stimulation with growth hormone. The pre-conditioning of early antral follicles with the use of growth hormone was never reported before.

In this study, we assessed the efficacy of growth hormone co-stimulation to long luteal GnRHa regimen in poor responders to COH for IVF.

Material and method

Patient selection

This randomized prospective study was conducted at a private ART Center, Bursa, Turkey, between January 2005 and June 2007. The study was approved by the institutional board review of the center. The study involved 61 patients who responded poorly to high dose gonadotropin treatment in their first cycles in the same center. Exclusion criteria was a day 3 FSH > 20 IU/l. Allocation of the patients into the groups was done concurrently by disclosing the sealed envelopes which included computerized randomization assignments just before starting their repeat cycles; randomization was accomplished by the first author following the consent of the couples. Neither doctors nor patients were blinded to the treatment regimens. Thirty-one couples were allocated to GH co-treatment group (group A), the other 30 were allocated to only GnRHa long protocol group (group B) to serve as the control group. Signed informed consents were obtained. A power analysis was done on two outcomes: (1) an increase in the rate of the fertilized oocyte numbers in order to increase the number of transferred embryos, (2) increase in the pregnancy rate in the study group compared to the control group. Our previous results showed that a poor responder produces an average of two PN oocytes with a standard deviation of 1.5, therefore increasing this number at least twofold in the study group requires at least ten patients in each arm of the study with a power of 80% and a two-tailed alpha value of 0.05. The pregnancy rate in poor responders in our clinic is around 12%, a threefold percent increase in the pregnancy rate requires 40 patients in each arm of the study.

Patients in the group A received a long protocol of pituitary down-regulation with triptorelin (Decapeptyl; Ferring, Switzerland) started in the day 21 of preceding cycle at a dose of 0.1 mg/day. The dose was reduced to 0.05 mg/day on the day of the subsequent menstruation. This reduced daily dose was administered until the day hCG was given. Growth hormone co-treatment was given a

daily subcutaneous injection of 4 mg (equivalent to 12 IU) of GH (Norditropin pen, Novo Nordisk, Denmark) from day 21 of preceding cycle along with GnRHa, until the day of hCG. This dose of 4 mg (12 IU) of GH daily was reported to be a high dose in the literature [10]. The starting dose of gonadotropins was 450 IU of recombinant human FSH (Gonal-F, Serono, Switzerland), and this treatment was continued during the first 5 days of stimulation. From day 6 of stimulation the dose of gonadotropins was individualized accordingly until hCG day. When at least one follicle had reached a diameter of larger than 17 mm, ovulation was induced with 10,000 IU hCG (Profasi, Serono, Switzerland). Patients in group B received the same treatment protocol except the growth hormone co-treatment.

Oocyte retrieval and insemination

Oocyte retrieval was performed by ultrasound-guided follicle aspiration under general anesthesia. Oocyte–cumulus complexes were incubated in G-Fert Plus medium (Vitrolife, Sweden). The cumulus oophorus and the corona radiata were subsequently removed from the oocytes by a short incubation in hyaluronidase solution (Hyase, Vitrolife, Sweden) followed by a mechanical removal using glass pipettes (Humagen, The Netherlands). After this procedure oocyte maturity was assessed, and all metaphase II oocytes were inseminated by ICSI. ICSI was carried out with the usual technique and instruments (Narishige, Japan mounted on Olympus IX70, Japan).

After ICSI the oocytes were cultured at 37°C in IVF medium (G1 Plus, Vitrolife, Sweden) equilibrated with 5% CO₂ in air for around 18 h. Then, fertilization was assessed and fertilized oocytes were transferred into fresh medium of the same type for an additional 24 h. All incubations were performed in four-well culture dishes (Nunc, Denmark). Embryo transfer was done on the third day after ICSI.

Statistical analysis

The primary outcome of the study was the number of oocytes fertilized. The increase in the pregnancy rate was the secondary outcome. Differences between groups were assessed by using Fisher's exact test, Chi square test and Student's *t* test where appropriate. All analyses were performed using the SPSS 11.0 package (SPSS Inc., Chicago, USA; Fig. 1).

Results

Patients' demographic characteristics did not differ significantly between the two groups. Table 1 displays the age, body mass index, day 3 FSH and estradiol levels, and the

Fig. 1 Flow diagram

incidences of smoking and previous ovarian surgery among the patients.

The mean duration of ovarian stimulation cycles of the group A patients was 2 days shorter than those in the control group, although this did not reach to statistical significance. The total dose of FSH was significantly lower in the GH group (3187.1 ± 232.3 IU vs 4070.8 ± 598.2 IU, $p < 0.001$). Total cost of COH was significantly higher in

group A (4652.5 ± 229.1 vs 2272 ± 333.3 , $p < 0.001$). The peak value of serum estradiol achieved at hCG day was higher in the group A as compared with the group B (2095.8 ± 1619.2 vs 939.4 ± 394.5 , $p = 0.003$). The number of metaphase 2 oocytes recovered was significantly higher in GH group (6.5 ± 2.1 vs 3.2 ± 1.4 , $p < 0.001$). 2PNs in GH group was significantly higher than the control group (4.4 ± 1.8 vs 1.5 ± 0.9 , $p < 0.001$). Results are shown in Table 2. No side effect was seen in any of the patients.

All patients in group A reached embryo transfer stage, while eight patients in group B did not get an embryo transfer (100% vs 73.3%, $p = 0.0019$). The reason for cancellation was total fertilization failure in four patients, and no-cleavage in another four patients. Patients in the GH co-treatment group received significantly more embryos per transfer as compared with the control group (3.3 ± 1.2 vs 0.9 ± 0.7 , $p < 0.001$). Although more pregnancies and more clinical pregnancies with fetal heart activity were achieved in GH group (12 of 31), compared to the control group (6 of 30), the difference did not reach to statistical significance (Table 3).

Table 1 Demographic characteristics of the patients

Characteristic	Group A <i>n</i> =31	Group B <i>n</i> =30	<i>p</i> Value
Age (years)	35.8 ± 3.2	35.2 ± 2.5	NS
BMI (kg/m^2)	25.4 ± 2.5	24.3 ± 2.3	NS
Day 3 FSH (mean IU/l)	9.7 ± 1.2	9.8 ± 1.3	NS
Day 3 E_2 (mean pg/ml)	91.0 ± 9.0	88.8 ± 10.9	NS
Smoker (%)	11(51.3)	11(48.7)	NS
Previous ovarian surgery (%)	10(32.3)	12(46.2)	NS

Group A, growth hormone co-treatment group; group B, GnRHa long protocol group. Comparisons were made by using Student's *t* test.

Table 2 COH characteristics

Characteristic	Group A	Group B	<i>p</i> Value
Duration of stimulation (days)	10.5±1.0	12.5±1.4	NS
Total FSH (IU)	3187.1±232.3	4070.8±598.2	<0.001
COH cost (USD)	4652.5±229.1	2272±333.3	<0.001
E2 on hCG day (pg/ml)	2095.8±1619.2	939.4±394.5	0.003
M2 oocyte number	6.5±2.1	3.2±1.4	<0.001
2PN	4.4±1.8	1.5±0.9	<0.001

Group A, growth hormone co-treatment group; group B, GnRHa long protocol group. Comparisons were made by Student's *t* test, Fisher's exact test, and chi-square test where appropriate.

NS not significant

Discussion

Results of the current study differ significantly from those of many in the literature. To the best of our knowledge, this is the first trial employing a high dose GH commencing in late luteal phase of the preceding cycle along with GnRH agonist. In the Cochrane Review [8], GH co-treatment in previous poor responder patients produced better pregnancy rate comparing to placebo (OR 1.78). In all the studies included in the meta-analysis, GH co-treatment in various dosages was commenced simultaneously with COH. There was no significant difference among 4, 8 or 12 IU in any outcome measure. In our study, GH co-treatment was started in the late luteal phase simultaneously with the GnRH agonist, when the flare-up effect took place to increase the cohort of follicles recruitable in the approaching treatment cycle. Additionally, we employed a high dose among those reported in the literature. Despite the improvement in the pregnancy rate in the GH arm of the study, this difference did not reach statistical significance. A post-analysis power analysis showed that the sample size in this study must have been 110 in each group to validate these results.

The total dose of gonadotropins used and the number of metaphase II oocytes recovered were improved significantly in our study. These observations are in contrast to the conclusions of a Cochrane review compiling data of six studies [11]. Our results are in agreement with those of

European and Australian multicentre study [12] in which GH co-stimulation was shown cause a reduction of the gonadotropin dose and shortening of the ovarian stimulation time in hypogonadotropic hypogonadism patients. Moreover, further improving effects of GH co-treatment were seen in our study. This, again, might be due to high dosage and longer usage of GH.

In our trial, peak serum estradiol concentration achieved at hCG day was higher in women co-stimulated with GH as compared with GnRHa only group. We may speculate that there were more follicles in the cohort rescued by midluteal GH administration and additionally there was more estradiol produced per follicle in the GH co-treatment group. As higher concentrations of estradiol in pre-ovulatory follicular fluid predict a higher chance of pregnancy [13], GH administration early in the recruitment phase appears to be a better method to be employed in poor responders. Additional stimulating effect of GH on the production of estradiol by granulosa cells has previously been shown [14, 15]. Further appropriately designed studies are needed to determine whether effects of GH on follicular fluid estradiol concentration are the only mechanism involved in the improvement of oocyte number and better clinical pregnancy rates in poor responder women or whether other mechanisms also play a role.

It has been shown previously that the ability of human oocytes to form morphologically normal and implantation-competent embryos is related to the concentration of different hormones in follicular fluid [13]. Among the hormones studied, growth hormone (GH) showed the most consistent relationship with different parameters of embryo quality, and higher concentrations of GH in follicular fluid were associated with rapid cleavage, good cleaving embryo morphology and a high embryo implantation potential [16]. Additionally, considering the findings indicating a requirement for GH to support ovarian follicular development in rodents [17, 18] and the impact of GH insufficiency on ovarian function in women [19], the improvement in COH parameters and clinical pregnancy rate by GH co-stimulation demonstrated in the current study may be related to the

Table 3 Embryo and pregnancy outcomes

Outcome	Group A	Group B	<i>p</i> Value
Embryo transferred (mean)	3.3±1.2	0.9±0.7	<0.001
Number of transfers	31/31	22/30	0.0019
Pregnancy	12/31	6/30	NS
Implantation rate (%)	11.7	31.5	<0.05
Clinical pregnancy	10/31	5/30	NS

Group A, growth hormone co-treatment group; group B, GnRHa long protocol group. Comparisons were made by Fisher's exact test and chi-square, and Student's *t* test where appropriate.

NS not significant

increment of endogenous intrafollicular GH concentration during the whole course of oogenesis.

Even though a decline in oocyte number and quality are known to be the main cause of assisted reproduction treatment failure in poor responder females, the contribution of GH on decidual receptivity, similar to those suggested by a study using GH in cattle reproduction [20] cannot be excluded. In our study, it was found that implantation rate was not increased by growth hormone. Improvement of the pregnancy rate was mainly due to the increase in oocyte number.

The study has been scheduled to include 40 patients in each arm. But it was stopped earlier than planned, due to constitutional changes in the setting. Sufficient power was reached for the interpretation of the result on the number of oocytes fertilized; but the results on pregnancy should be interpreted with having this on mind.

In conclusion, this prospective randomized comparative trial shows that poor responder women undergoing repeated assisted reproduction treatment and co-stimulated with GH achieve more oocytes, higher fertilization rate if growth hormone started in the luteal phase of previous cycle, as compared with women of the same status treated with GnRHa long protocol. The study was unable to show that clinical pregnancy rate was increased significantly.

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