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Original Article

Efficacy of growth hormone supplementation with ultrashort GnRH antagonist in IVF/ICSI for poor responders; randomized controlled trial

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ABSTRACT

Objectives: To compare the ICSI-ET outcomes in poor responders who underwent ovarian stimulation by the ultrashort GnRH antagonist protocol with or without adjuvant GH injection.
Material and methods: This randomized controlled study was conducted at Al-Azhar University from December-2018 to June-2019 upon 156 participants. All patients received the same preparations. After randomization, in the study group, women have received GH 4 IU/day subcutaneous injection from the second day of the cycle stopped one day before ovum pickup. While in the control group, women have received subcutaneous saline in the same dosing as in the study group. After intervention, all procedures were the same in both groups. The main outcome measure was the clinical pregnancy rate. Statistical analysis was based on the intention-to-treat population.
Results: Both groups were comparable with regard their baseline characteristics (p-values > 0.05). Ovulation characteristics were comparable (p-values > 0.05). The level of E2 is significantly (p-value $= 0.003$) higher in the GH group. The oocyte retrieved number was significantly (p-value < 0.001) higher in the GH group 4.94 ± 1.77 than in the control group 3.74 ± 1.82. The mean number of MII oocytes was significantly (p-value < 0.001) higher in the GH group 3.3 ± 1.36 than in the control group 2.29 ± 1.24. Fertilization characteristics, implantation rate, pregnancy rate were comparable (p-values > 0.05).
Conclusion: Despite the fact that this study showed no significant increase in the clinical and chemical pregnancy rates by the addition of GH to the ultrashort antagonist protocol in poor responders, the number of retrieved oocytes was significantly higher in the GH group.
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Introduction

Despite the various advancements in artificial reproductive technology (ART), the clinical pregnancy and the live-birth rates remain at approximately 30–40% [1,2].

The controlled ovarian stimulation (COS) protocols for in-vitro fertilization (IVF) are continually under revision in an endeavor to reduce hormone (gonadotrophin) requirement, enhance follicular recruitment, and fundamentally to improve the live-birth rates [3].

Some of these protocols have considered the use of the growth hormone (GH) which is synthetically produced using recombinant Deoxyribo Nucleic Acid (DNA) technology and is licensed to be used in the human population. Currently, there is no consensus as to the route, dose, or timing of GH administration in IVF protocols [4].

Until now, to the best of the available knowledge, no research studied the impact of adding GH to the ultrashort gonadotrophin-releasing hormone (GnRH) antagonist protocol in terms of ovulation, fertilization, implantation, and pregnancy rate. Thus, the

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rationale intended for this study was to compare the clinical pregnancy rate in poor responder women who underwent COS by the ultrashort GnRH antagonist protocol with or without the addition of GH.

Methods

This parallel-randomized controlled double-blinded, single-center study was conducted at Al-Azhar University Assisted Reproductive Technology Unit to assess the effectiveness of adjuvant GH during COS by ultrashort GnRH antagonist protocol, in women with poor ovarian response undergoing intracytoplasmic sperm injection (ICSI) procedures during the period from December-2018 to June-2019.

This study was conformed to the principles of the Declaration of Helsinki and was following the Medical Research Involving Human Subjects Act. The ethical review committee approved the study. The purpose of this study was clearly explained to all women before their enrollment, and an informed consent form was signed by all who were enrolled.

We invited all infertile women with a poor ovarian response that were eligible for participation in this study. For inclusion in the study, all of the following criteria were to be fulfilled: age 25–38 years, IVF previous poor responders with at least two failed cycles with < five oocytes, abnormal ovarian reserve testing (ORT) e.g. anti-mullarian hormone (AMH) < 1, patients with unexplained infertility, normal hormonal profile (FSH, LH, PRL), normal ovarian ultrasound, normal pelvic ultrasound, women that were willing to do intracytoplasmic sperm injection – embryo transfer (ICSI-ET) [5].

Exclusion criteria included: women with a known medical disease (e.g. severe hypertension or hepatic disease), history of altered karyotype in one or both partners, history of chronic, autoimmune or metabolic diseases, and the presence of endocrinopathies or male factor infertility.

Randomization and blinding

For the allocation of the participants, a computer-generated list of random numbers was used. Block randomization with a block size of four was used with a 1:1 ratio of the GH group and the control group. The allocation was done using the sealed envelope technique. The study was a double-blinded study, as the patient did not know which groups she is assigned for, and the assessor was blinded.

Procedures

After randomization, in all patients of both study groups, COS by the ultrashort GnRH antagonist protocol was started at day 2–3 of the menstrual cycle. Transvaginal ultrasound was made; COS was started only if no follicle ≥10 mm in diameter was observed and the estradiol level was <50 pg/mL. COS was performed using ultrashort GnRH antagonist protocol with an injection of 0.1 mg SC GnRH daily, triptorelin acetate (Decapeptyl, Ferring Pharmaceutical GmbH – Germany) or Leuprolide (Lupron, Takeda Pharmaceutical, Japan) for pituitary flare followed by down-regulation and endogenous gonadotropin depletion, which was continued for three consecutive days. HMG (Merional, IBSA, Switzerland) at 450 IU per day started from day 2 of the cycle [6].

However, in all patients of the study group, women received GH (Somatropin, Sedico, Egypt) 4 IU/day administered subcutaneously from the second day of the cycle and stopped one day before ovum pickup. In all patients in the control group, women have received subcutaneous saline (as a placebo) in the same dosing and timing as in the study group.

After the intervention, in all patients of both groups, transvaginal ultrasound was done starting from day 6 of COS for assessment of follicular development and assessment of endometrial thickness. Also, serial E2 measurement was scheduled to start on day 6 of COS repeating every other day. The GnRH antagonist (Cetrorelix, Serono Laboratories, Aubonne, Switzerland) at a dose of 0.25 mg SC per day was started on day 6 of COS. Final follicular maturation was triggered when the leading follicle >18 mm in diameter, using recombinant human chorionic gonadotropin (Choriomon, IBSA, IBSA, Switzerland) 10,000 IU, single injection. After 34–36 h, oocytes retrieval was done. Follicular fluid was aspirated into sterile tubes.

After denudation, the oocytes were assessed for maturity and quality, using an inverted (Olympus 1x71) microscope with Hoffman optics, hot stage, and automatic manipulators Narishige. Maturation stages were recorded as prophase I, metaphase I (MI), metaphase II (MII) and post-mature [7].

Semen was applied to the swim-up technique and centrifuged at 1800 rpm for 10 min. The injection procedure was carried out using holding pipettes and injection needle. ICSI was performed on MII oocyte. After 17 h, assessment for normal fertilization was done. Two pronuclei (PN) are considered as normal fertilization [8].

Embryos that are cleaved were identified and embryos grading were done according to the equality of blastomeric size and the proportion of nucleate fragments. Then, best embryos were transferred to the uterus in 30 μL of Global medium containing 10% HSA using ET catheter 48–72 h after oocyte retrieval [9].

Luteal phase support was given to the patient for 14 days, using micronized progesterone 600 mg/day, and; then, beta hCG titter was done for the detection of pregnancy which was confirmed by transvaginal ultrasound examination after 10–15 days of gestation [10].

Statistical considerations

The primary outcome measure was the clinical pregnancy, defined as the presence of at least one fetus with a heartbeat. The secondary outcomes were: E2 levels at hCG day, the number of collected and MII oocytes; the number of G1 embryos, the number of embryos transferred, the implantation rate, the chemical pregnancy rate, multiple pregnancies, the endometrial thickness when at least one follicle ≥17 mm is observed.

The sample size was calculated using EpiInfo version 7.0, setting the power at 80% and the two-sided confidence level at 95%. Data from the Cochrane systematic review conducted by Duffy et al. showed that the overall combined pregnancy rates were 31.7% and 12.2% in poor responders who received GH and placebo, respectively. A minimal sample size of 70 women in each group was needed. To count for any dropout, 158 women were enrolled [11].

The statistical analysis was made on the intent-to-treat (ITT) population. All statistical tests were made using a significance level of 95%. A p-value <0.05 was considered statistically significant. SPSS software (Statistical Package for the Social Sciences, version 20.0, SSPS Inc., Chicago, IL, USA) was used. Data were presented as (mean ± SD) for continuous variables and as frequency & percent for categorical variables. Comparisons between groups were made using the Chi-square test for categorical variables and the independent t-test for the continuous variables.

Results

A total of 176 women were invited to participate. Eight refused to participate, and 12 were excluded before randomization, leaving 156 participants for randomization with 78 assigned to each group (Fig. 1).

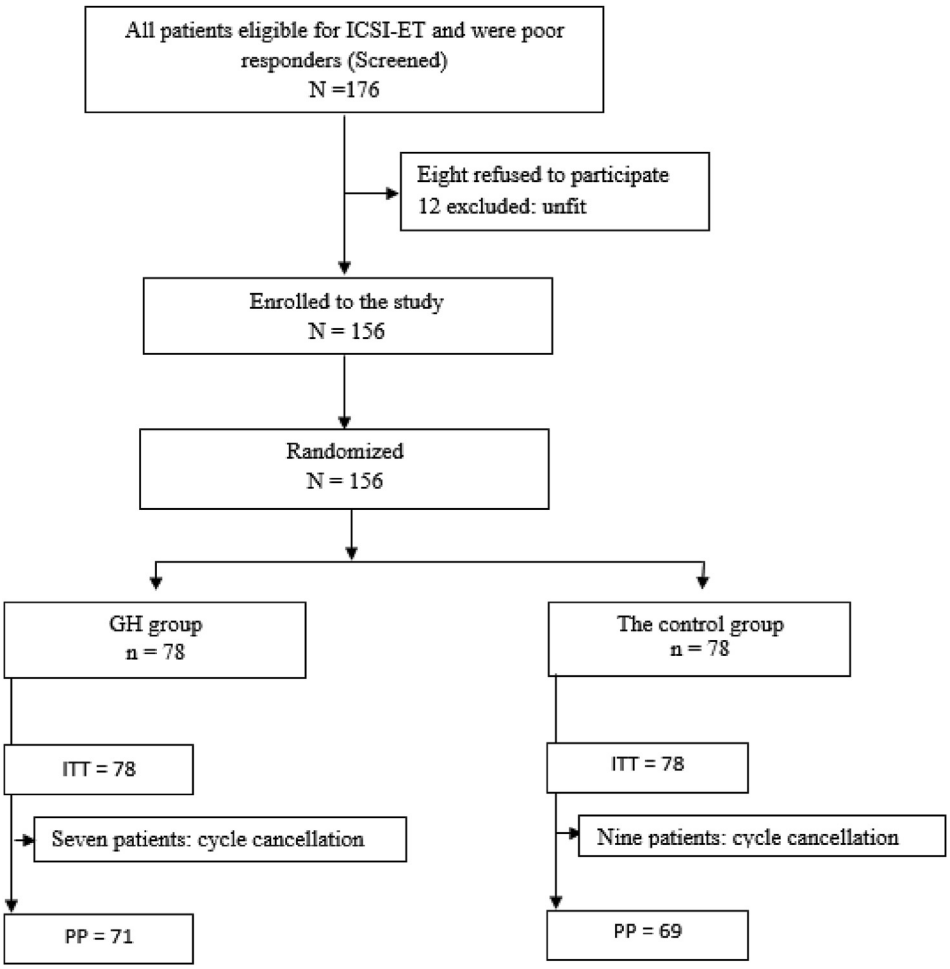

Fig. 1. CONSORT diagram.

Both groups were comparable with regard to their baseline characteristics. There was no statistically significant difference (p-values > 0.05) between the two groups regarding the age, BMI, the duration of infertility, and the number of previous cycles with a poor response (Table 1).

In both groups, the antral follicle count (AFC) is comparable (p-value = 0.782) between groups; it was 5.73 ± 1.82 & 5.81 ± 1.78 for the GH group and the control group, respectively. Both groups were comparable with regard to the AMH (p-value = 0.151). It was 0.72 ± 0.09 ng/mL and 0.69 ± 0.16 ng/mL for the study group & the control group, respectively.

The number of ovarian stimulation days was not significantly different between both groups (p-value = 0.520), it was 12.62 ± 1.05 and 12.52 ± 1.08 days for the study group & the control group, respectively. The cycle cancellation rate was 7 (8.97%) in the GH group versus 9 (11.54%) in the control group (p-value = 0.774).

Both groups were comparable with regard to the endometrial thickness (p-value = 0.236). However, the level of E2 on the same day is significantly (p-value = 0.003) higher in the GH group 929.94 ± 306.02 versus the control group 777.97 ± 319.81 pn/mL. The oocyte retrieved number was significantly (p-value < 0.001) higher in the GH group 4.94 ± 1.77 than in the control group 3.74 ± 1.82. Furthermore, the mean number of MII oocytes was significantly (p-value < 0.001) higher in the GH group 3.3 ± 1.36 than in the control group 2.29 ± 1.24 (Table 2).

The mean number of embryos developed per patient was 2.32 ± 1.01 in the GH group and 2.11 ± 1.12 in the control group (p-value = 0.221). The mean number of the good embryo grade (G1)

Table 1
Baseline characteristics, AFC and hormonal profile.

	GH $N = 78$	Control $N = 78$	p value
Age in years, mean ± SD	34.27 ± 2.41	34.74 ± 1.98	0.185
BMI (kg/m^2), mean ± SD	24.39 ± 1.52	25.06 ± 3.47	0.120
Duration of infertility in years, mean ± SD	6.62 ± 2.13	6.35 ± 2.01	0.417
Number previous cycles with poor response, mean ± SD	2.5 ± 0.18	2.56 ± 0.28	0.113
AFC, mean ± SD	5.73 ± 1.82	5.81 ± 1.78	0.782
AMH (ng/mL), mean ± SD	0.72 ± 0.09	0.69 ± 0.16	0.151

Table 2
Ovarian induction, ICSI parameters.

	GH $N = 78$	Control $N = 78$	p value
Number of stimulation days, mean ± SD	12.62 ± 1.05	12.51 ± 1.08	0.520
E2 (pg/mL), mean ± SD	929.94 ± 306.02	777.97 ± 319.81	**0.003**
Endometrial thickness at day of HCG mm, mean ± SD	10.27 ± 1.79	9.99 ± 1.06	0.236
Cycle cancellation, *n* (%)	7 (8.97%)	9 (11.54%)	0.770
Ovulation characteristics			
Oocyte retrieved number, mean ± SD	4.94 ± 1.77	3.74 ± 1.82	<0.001
M II, mean ± SD	3.3 ± 1.36	2.29 ± 1.24	<0.001
Fertilization characteristics			
Number of embryos fertilized, mean ± SD	2.32 ± 1.01	2.11 ± 1.12	0.221
G1 embryos, mean ± SD	1.82 ± 0.68	1.68 ± 0.71	0.210
Number of transferred embryos, mean ± SD	1.73 ± 0.72	1.58 ± 0.69	0.186
Total number of embryos transferred: *n*	123	109	
Single embryo transfer (SET)	30	37	0.397
Double embryo transfer (DET)	30	24	
Triple embryo transfer (TET)	11	8	

was 1.82 ± 0.68 in the GH group and 1.68 ± 0.71 in the control group (p-value = 0.210). The mean number of embryos transferred per patient was 1.73 ± 0.72 in the GH group and 1.58 ± 0.69 in the control group (p-value = 0.186).

The number of embryos transferred was comparable between groups (p-value = 0.397). In the GH group a total number of 123 embryos were transferred; single embryo (SET) in 30, double embryos (DET) in 30, and triple embryos (TET) in 11 patients. On the other hand, in the control group a total number of 109 embryos was transferred; single embryo (SET) in 37, double embryos (DET) in 24, and triple embryos (TET) in 8 patients (Table 2).

The implantation rate was comparable (p-value = 0.11) between groups. It was 78.05% in the GH study group versus 67.87% in the control group. The chemical pregnancy rate was insignificantly (p-value = 0.367) higher in the GH group than in the control group. It was 30.77% in the GH and 23.08% in the control group (Table 3).

The clinical pregnancy rate was insignificantly (p-value = 0.519) higher in the study group than in the control group. It was 19.23% in the GH group and 14.10% in the control group. Twin pregnancy was seen in one case (out of 15) in the GH group and one case (out of 11) of the control group (Table 3).

Discussion

To the best of our knowledge, this study is considered the first one to assess the impact of adding GH to the ultrashort GnRH antagonist protocol in terms of ovulation, fertilization, implantation, and pregnancy rate. Therefore, in our discussion, the comparison will be made against the nearest COS protocol, which is the antagonist protocol.

Bassiouny et al. (2016), in their study and in accordance with our study, found that the number of retrieved oocytes was significantly higher in the GH group. In addition, in discordance to our study, they found that the number of fertilized, as well as the number of transferred embryos, were significantly higher in the GH group. Moreover and similar to the results of our study, they found no statistically reliable difference when comparing the pregnancy rates. However, the study was different from ours not only the COS protocol but also in the dosage of GH (8 IU/d) [12].

Table 3
Implantation rate and pregnancy rate.

	GH $N = 78$	Control $N = 78$	p value
Total number of embryos transferred	123	109	
Implantation rate/ET, *n* (%)	96 (78.05%)	74 (67.89%)	0.110
Chemical pregnancy rate, *n* (%)	24 (30.77%)	18 (23.08%)	0.367
Clinical pregnancy rate, *n* (%)	15 (19.23%)	11(14.10%)	0.519
Number of fetuses, *n*	16	12	
Singleton	14	10	0.887
Twins	1	1	

Another study conducted by Eftekhar et al. (2013), concluded that the addition of GH to the antagonist protocol increased the number of retrieved oocytes as in our study and increased the number of obtained embryos in discordance with our study. On the other hand, there were no statistically reliable differences when comparing the implantation rate or the pregnancy rate. In addition, the number of stimulation days, as well as the cycle cancellation rate, were comparable between groups in this study. Hence, the usage of GH does not affect the number of stimulation days nor the cycle cancellation rate. However, this study was different from ours not only the controlled ovarian stimulation protocol but also in the timing of GH administration [6].

A higher preovulatory level of E2 in the follicular fluid leads to a better likelihood of pregnancy. As one of the physiological actions of GH, it makes the addition of GH a promising method in poor responders [13]. The results of our study demonstrated that the mean serum level of E2 on HCG day was significantly higher in the study group than in the control group, which can be attributed to the higher number of recruited follicles generating E2. This finding is in agreement with the results showed by Bassiouny et al. (2016); however, it is in disagreement with Eftekhar et al. (2013) [6,12].

The critical roles played by GH in ovarian function, steroidogenesis, follicles' development, and oocyte maturation had been advocated by both animal and human research studies. The current study showed that the number of retrieved oocytes was significantly higher in the GH group. Also, several studies demonstrated an increased number of oocytes retrieved [6,12,14].

In addition, the results of the current trial showed a significantly higher number of MII oocytes collected in the GH group. That is in agreement with Bassiouny et al. (2016) [12]. However, Eftekhar et al. (2013) study showed no significant difference between groups as regards the number of MII oocytes [6].

The results of the current study showed an insignificant higher number of embryos developed per patient, the number of the excellent embryo grade (G1), and the number of embryos transferred in the GH group than in the control group. These higher fertilization rates and more embryos available for transfer were also reported by other research studies [6,12].

Several meta-analyses and systematic reviews studied the impact of adding GH for different ovarian stimulation protocols in the improvement of the IVF/ICSI outcomes in poor responders.

One of them, Kolibianakis et al. (2009) advocated that the administration of GH might lead to more patients reaching the stage of embryo transfer and hence have the chance of pregnancy. Conversely, this was not evidenced by the results of the current study. The variability between studies concerning the COS protocol can jeopardize the results of the meta-analysis. Likewise, GH doses varied, ranging from 4 IU daily to 24 IU administered on alternate days [15].

A recent meta-analysis, Li et al. (2017), included eleven studies, concluded that the addition of GH could significantly improve the clinical pregnancy rate. Furthermore, the GH addition time may affect the pregnancy outcome. However, the included studies were highly heterogeneous; eligibility criteria were variables; the used COS protocols were not the same; GH dose and time were not consistent [16].

One strength of this current study is its randomized nature and with enough sample size. However, one limitation is that the live-birth rate was not reported because the follow-up of patients was not possible since they were from locations far from the hospital. That, of course, added another limitation regarding the assessment of the long-term safety of GH on the mothers and their children. Also, the study used a low-dose of GH that may jeopardize the effect; however, one reason for this dose was to avoid any adverse effects due to the higher doses.

In conclusion, this study showed no significant increase in the clinical and chemical pregnancy rates by the addition of GH to the ultrashort antagonist protocol in poor responders. However, the number of retrieved oocytes was significantly higher in the GH group.

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Declaration of competing interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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