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Effects of Adjuvant Growth Hormone Therapy on Poor Ovarian Responders in Assisted Reproductive Technology

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ABSTRACT

Objective: *The present study aimed to evaluate the effect of adjuvant growth hormone (GH) therapy in antagonist protocol aiming to improve ovarian response and clinical outcomes of women with poor ovarian response.*

Materials and methods: *This clinical trial was a single-center study, controlled with equal randomization, which was carried out in Avicenna Infertility Clinic, Tehran, Iran. Totally, 118 patients were randomly allocated to either the intervention or the control group. The intervention group received GH and gonadotropin in gonadotropin-releasing hormone (GnRH) antagonist protocol (GH/GnRHant), while the control group received gonadotropin in GnRH antagonist protocol (GnRHant).*

Results: *The results revealed that the number of days of gonadotropin administration significantly decreased (p-value = 0.040) in the GH/GnRHant group compared to the GnRHant group. Also, our study findings showed that a number of top-quality day 3 embryos and clinical pregnancy rate were higher in the GH/GnRHant group (p-value = 0.007) compared to the GnRHant group (p-value = 0.036). However, there was no significant difference between the two groups in terms of number of received gonadotropin ampoules, number of retrieved MI and MII oocytes, chemical pregnancy rate, ongoing pregnancy rate and live birth rate.*

Conclusions: *These results suggest that adjuvant GH therapy in antagonist protocol in women with a history of poor ovarian response is effective to decrease the number of days of received gonadotropin ampoules and improve pregnancy rate.*

Keywords: assisted reproductive technology, ovarian stimulation, antagonist protocol, poor ovarian response, growth hormone.

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INTRODUCTION

Ovarian response is a main determinant of reproductive milestones and successful controlled ovarian stimulation (COS) for a clinical outcome in assisted reproductive technology (ART) cycles (1). Poor ovarian response (POR) is main challenge in ART, which leads to inadequate retrieval of mature oocytes, high cycle cancellation and low pregnancy rates after *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles (1). The estimated rate of POR among women experiencing ART is 9–24% (2). Due to the variety of risk factors, there is no widely accepted definition for POR. According to the BOLOGNA criteria, published by the European Society of Human Reproduction and Embryology (ESHRE) in 2011, POR is identified with at least two of the three following criteria: 1) advanced maternal age (≥40 years) or any other risk factor for POR; 2) a previously characterized POR cycle (≤three oocytes with a conventional stimulation protocol); and 3) an abnormal ovarian reserve test (antral follicle count (AFC) <5–7 follicles or anti Mullerian hormone (AMH) <0.5–1.1 ng/mL) (3). Various strategies and protocols have been developed to improve ovarian response in POR women. However, despite these efforts, there has been no valid treatment so far (4).

Growth hormone (GH) is a peptide hormone released primarily by the anterior part of the pituitary gland in a pulsatile manner, which is involved in metabolism, cell growth and development (5). The role of GH in female reproduction has gained renewed interest and has become a hot topic over the last decade (6, 7). The local GH production in the reproductive tissues exerts an important paracrine/intracrine effects, in addition to the pituitary production of GH (8). Also, growth hormone receptors (GHRs) are expressed in mammary glands, placenta, endometrial cells, oocytes, ovarian granulosa cells, theca cells and cumulus cells (9). Moreover, there are many *in vitro* and in vivo evidence that GH-related growth factors, including insulin-like growth factor 1 (IGF1), are stimulators of ovarian follicular development (10). IGF-I promotes either proliferation, or differentiation of granulosa cells, and plays an important role in the responsiveness of the ovary to follicule-stimulating hormone (FSH) action (10).

Evidence emerging from clinical practice suggests that GH administration during ovarian stimulation may improve oocyte quality, increase pregnancy rate, implantation rate and live birth rate (11, 12). It seems that GH administration can improve the success rate of (IVF/ICSI) cycles (13, 14), possibly through the positive effects on oocyte quality, as shown by a higher number of mature oocytes and embryos at the transfer stage in GH-treated individuals (15-17). Based on the current evidence, GH intervention has been suggested as a potential adjuvant therapeutic protocol in POR women. However, there is limited ovarian cellular information in women and great controversy still exists in the application of GH in ART. Although a number of cohort studies have claimed these benefits compared with prior treatment, several studies have reported unreliable results for GH in the IVF process (18, 19). In this regard, the present study aimed to evaluate the effect of adjuvant GH therapy in antagonist protocol in improving ovarian response and clinical outcomes among women with a history of POR. ❑

METHODS AND MATERIALS

Study design

The present study was a single-center controlled trial with equal randomization, which was carried out in Avicenna Infertility Clinic, Tehran, Iran. Patients were randomly allocated to either the intervention (GH/GnRHant) or the control (GnRHant) group. Randomization was performed using computer-generated simple random tables in a 1:1 ratio.

Study population

The study population included 118 patients with a history of POR undergoing ICSI-frozen embryo transfer (FET) cycles, which was carried out in Avicenna Infertility Clinic, Tehran, Iran between 2020 and 2021. Poor ovarian response was identified according to the BOLOGNA criteria (3). Failure to achieve pregnancy at previous ICSI cycle and body mass index (BMI) ≤30 kg/m^2 were deemed as inclusion criteria. Patients with known etiologies of implantation failure including immunological abnormalities, inflammatory conditions, hormonal or anatomical disorders,

endometriosis, and presence of space-occupying lesions, history of miscarriage or ectopic pregnancy, myomas, polyps, adhesions, previous pelvic surgeries were all excluded. In addition, participants with severe male factor of their spouses and chromosomal abnormalities were excluded.

COS protocol

Controlled ovarian stimulation was conducted using a GnRH antagonist protocol. Briefly, women received estradiol between the previous cycle day 21 to the treatment cycle days 2-3 and then underwent gonadotropin stimulation using follitropin α (Cinnal-f®, CinnaGen, Iran) in a dose of 75–300 IU/day beginning from day 2–3 of the menstrual cycle. Gonadotropin dose was selected based on several factors such as age and BMI, and it was adjusted, if necessary, after 5-6 days according to follicular development monitored by transvaginal ultrasonography. GnRH antagonist (0.25 mg/day; Cetrotide®, Merck Serono, Germany) commenced when the leading follicle reached the size of 14 mm in diameter and continued until the day of ovulation induction. Ovulation was triggered by the administration of human chorionic gonadotropin (hCG) in a dose of 10,000 IU (Choriomon®, IBSA, Switzerland) when two or more follicles were >17 mm in diameter.

Growth hormone co-treatment

Growth hormone co-treatment was given *via* a daily subcutaneous injection of 5 mg of GH (CinnaTropin®, CinnaGen, Iran) from day 21 of the previous cycle until the day of hCG administration.

Oocyte retrieval, denudation and fertilization

Ovum pick-up (OPU) was conducted transvaginally 36 hours after hCG injection. Cumulus cell-oocyte complexes (COCs) were retrieved and washed in MOPS-buffered medium (G-MOPS™ PLUS, Vitrolife Co., Sweden). Oocyte denudation was performed two hours after retrieval utilizing hyaluronidase (HYASE-10X™, Vitrolife Co., Sweden), followed by mechanical dissection. Intracytoplasmic sperm injection was conducted on all mature metaphase II oocytes 3-4 hours after OPU. Then, injected metaphase II oocytes were cultured in an embryo culture medium (SAGE 1-Step™, CooperSurgical Co., USA) until day 3. The embryo culture was performed in an incubator with humidified atmosphere and 6% CO_2.

On day 3, embryo quality was evaluated according to the previous literature (20). Briefly, the parameters including the number and symmetry of blastomeres, percent of fragmentation, presence of multinuclear blastomeres, and presence of intracytoplasmic and extracytoplasmic morphological abnormalities were assessed in embryos on day 3 of culture.

Top-quality day 3 embryos were determined as those with 8–10 symmetric blastomeres on day 3, <15% fragmentation, absence of multinucleation, absence of intracytoplasmic and extracytoplasmic abnormalities.

FET cycle

All participants undertook FET cycle and hormone replacement therapy (HRT) was done for preparation of endometrium as a standard protocol. In brief, estradiol valerate (Aburaihan Co., Tehran, Iran) was initiated in a dose of 6 mg/day orally from the second (or third) day of the menstrual cycle and it was continued up to 8 mg if the endometrial thickness did not reach at least seven millimeters. Progesterone suppository (Cyclogest; Actavis, England, UK) in a dose of 400 mg twice-daily was initiated when the endometrial thickness was more than seven millimeters. Single top-quality day 3 embryo transfers with embryo transfer catheter (Cook, USA) were performed under transabdominal ultrasound guidance, by an expert gynecologist with infertility fellowship, according to the American Society for Reproductive Medicine (ASRM) guidelines.

Clinical outcome assessment

Chemical pregnancy and clinical pregnancy rate were calculated by the number of positive serum β-hCG *per* number of ET after two weeks from the day of ET and the number of observed gestational sacs by ultrasonography *per* number of ET six weeks after ET, respectively. Ongoing pregnancy rate was calculated by number of pregnancies which had completed ≥20 weeks of gestation *per* number of ET. Every pregnancy was followed-up until the parturition. Live birth rate was calculated from the number of live births *per* number of ET.

Statistical analysis

The results were shown as mean±SD (standard deviation). Statistical analysis was carried out using the SPSS 21.0 statistical software package (SPSS Inc, Chicago, IL, USA). Student's t-test, exact test and Chi-square test were used for comparing the study groups. P <0.05 was considered significant. ❑

RESULTS

A total of 346 participants were assessed for eligibility to enter in this study, from which 197 patients fulfilled the inclusion criteria and were enrolled in this study. Eventually, 79 patients were left out for different causes and 118 couples accomplished the trial and their

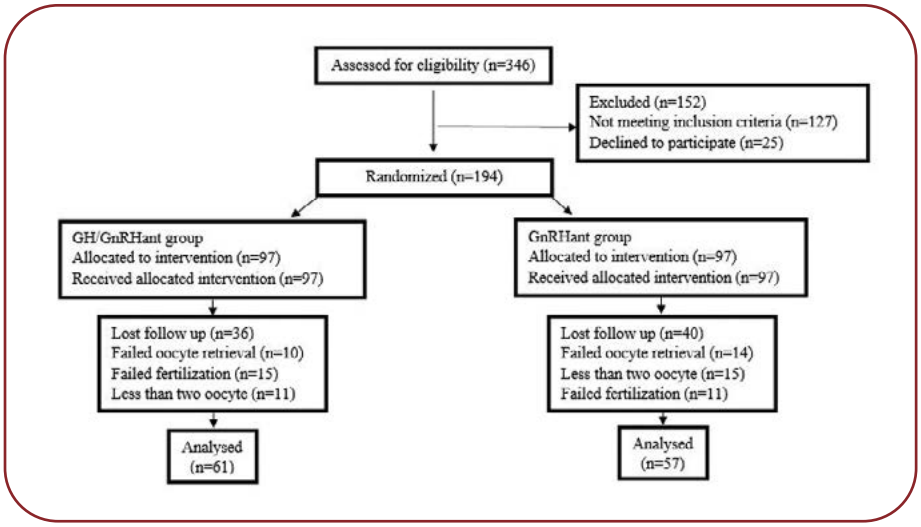

FIGURE 1. Consort flow diagram

TABLE 1. Demographic and clinical characteristics of the study groups

Variable	GH/GnRHant (N=61) mean±SD	GnRHant (N=57) mean±SD	P value
Number of patients	61	57	>0.999
Age (years)	36.47±4.28	38.09±3.67	0.069
BMI	24.52±2.60	24.65±2.65	0.797
FSH (Day 3) (mIU/mL)	13.05±2.97	13.95±3.42	0.130
AMH (ng/mL)	0.66±0.32	0.64±0.30	0.737
AFC	4.49±1.32	4.28±1.34	0.392
Gravidity	0.34±0.85	0.39±0.65	0.767
Parity	0.13±0.427	0.14±0.398	0.904
Gonadotropin ampoule (75 IU) (previous cycle)	51.20±14.68	53.41±19.93	0.491
Days of gonadotropin administration (previous cycle)	10.51±1.70	10.26±1.98	0.471
MI oocyte (previous cycle)	0.26±0.454	0.40±0.62	0.194
MII oocyte (previous cycle)	3.28±1.496	3.12±1.64	0.590
Day 3 embryo (previous cycle)	2.07±1.22	1.89±1.33	0.496
Sperm (count/mL)	$(39.44\pm4.25)\times10^6$	$(48.21\pm5.39)\times10^6$	0.201

AFC=antral follicle count; AMH=anti-Mullerian hormone; BMI=body mass index; FSH=follicle stimulating hormone; MI=metaphase-I; MII=metaphase-II.

Variable	GH/GnRHant group (n=61), mean±SD	GnRHant group (n=57), mean±SD	P value
Number of gonadotropin ampoule (75 IU)	50.25±16.38	53.46±16.93	0.297
Days of gonadotropin administration	9.82±1.44	11.04±4.33	0.040*
Number of MI oocyte	0.41±.64	0.37±0.59	0.716
Number of MII oocyte	3.75±1.72	3.19±1.64	0.073
Number of top-quality day 3 embryos	2.48±1.36	1.81±1.26	0.007**

MI: metaphase-I; MII: metaphase-II. *Significant. *P-value <0.05, **P-value <0.01.

TABLE 2. Ovarian stimulation characteristics and outcomes of the study groups

Variable	GH/GnRHant (n=61) (%)	GnRHant group (n=57) (%)	P value
Chemical pregnancy rate	16 (26.22)	7 (12.28)	0.066
Clinical pregnancy rate	14 (22.95)	5 (8.77)	0.036*
Ongoing pregnancy rate	11 (18.03)	5 (8.77)	0.142
Live birth rate	11 (18.03)	4 (7.01)	0.063

*Significant; *P-value <0.05

TABLE 3. Clinical outcome analysis of the study groups

data were analyzed (Figure 1). There was no significant difference between the two groups in mean age, BMI, serum level of day 3 FSH, serum level of AMH, AFC, gravidity, parity and sperm count of spouse, as well as number of gonadotropin ampoules (75 IU), days of gonadotropin administration, number of MI and MII oocytes and number of day 3 embryos at previous ICSI cycle. Table 1 summarizes the demographic and clinical characteristics of the study groups.

Ovarian stimulation characteristics and outcomes of the study groups

Table 2 shows the results of ovarian stimulation, including the number of gonadotropin ampules, days of gonadotropin administration, number of MI and MII oocytes and number of top-quality day 3 embryos at ICSI cycle of the present study. The results demonstrated that days of gonadotropin administration (p-value = 0. 0.040) significantly decreased in the GH/GnRHant group compared to the GnRHant group. Based on the obtained data, there was no significant difference in terms of number of gonadotropin ampules and number of MI and MII oocytes between the study groups. However, the number of top-quality day 3 embryos (p-value = 0.007) was significantly higher in the GH/GnRHant group compared to the GnRHant group.

Clinical outcomes in the studied groups

Overall comparison of clinical outcomes between the two study groups is presented in Table 3. The clinical pregnancy rate was significantly higher in the GH/GnRHant group compared to the GnRHant group (p-value = 0.036). However, there was no significant difference in terms of chemical pregnancy rate, ongoing pregnancy rate and live birth rate between the study groups. The chemical pregnancy rate was 26.22% (16/61) for the GH/GnRHant group and 12.28% (7/57) for the GnRHant group. The clinical pregnancy rate was 22.95% (14/61) for the GH/GnRHant group and 8.77% for (5/57) for the GnRHant group. Ongoing pregnancy rate was 18.03% (11/61) for the GH/GnRHant group and 8.77% (5/57) for the GnRHant group. Live birth rate was 18.03% (11/61) for the GH/GnRHant group and 4.01% (4/57) for the GnRHant group (Table 3). ❑

DISCUSSION

The present study showed that adjuvant GH therapy in antagonist protocol in women with a history of POR significantly decreased the number of days that gonadotropin was taken and significantly increased the number of top-quality day 3 embryos and pregnancy rate. However, there was no significant difference between the

two groups in terms of number of retrieved MII oocytes, chemical pregnancy rate, ongoing pregnancy rate and live birth rate.

Several approaches have been suggested to improve ovarian response in patients with POR; however, no consistent results have been reported so far. Increasing the daily gonadotrophin dose is the most used clinical approach for women with a history of POR (21, 22). Gonadotrophins can only support the cohort with follicle responsive to stimulation, but cannot significantly increased number of retrieved oocytes (4). On the other hand, reports have specified that a higher dose of gonadotropins resulted in an increased rate of aneuploidy in granulosa cells and embryos (23). Therefore, minimal ovarian stimulation is a fairly realistic approach for POR women compared to conventional high dose stimulation (21). However, considering the limited supply of oocytes, poor quality of embryos, and high occurrence of canceled cycles, minimal ovarian stimulation alone is not a reliable approach for POR patients. In this regard, adjuvant treatments such as GH have been claimed to be co-treatments of choice in COS protocols in these patients (24-26). Herein, our results demonstrated that adjuvant GH therapy in antagonist protocol in women with a history of POR significantly decreased the number of days of gonadotropin administration. In addition, GH therapy decreased the number of received gonadotropin ampoules; however, there was not a significant difference between the two groups. Our results are in agreement with those from studies in which adjuvant GH therapy was shown to lower the dose of gonadotropin and to shorten the ovarian stimulation time (15, 27). The administration of GH to patients with GH deficiency increase ovarian sensitivity to endogenous gonadotropins (28). The co-administration of gonadotropins and GH in COS protocol has been proposed to improve follicular growth in patients with hypogonadotropic hypogonadism (29). Together, our results as well as current evidence suggested that adjuvant GH therapy for COS protocols may play an important role in preventing side effects of gonadotropins by decreasing the number of received gonadotropin ampoules and the number of days that they were taken.

In the context of laboratory performance, the need for the retrieval of a large number of good quality oocytes through ovarian stimulation is an essential part of successful IVF treatment, since the number of oocytes and viable embryos are independent factors that increase clinical outcomes (21). Experimental studies showed that GH affected steroid production and gametogenesis through acting on the ovary (5). Human oocytes and cumulus cells have GHRs (30, 31). Therefore, oocytes can be directly influenced by the GH (30, 31). Also, GH may indirectly influence the ovarian function through IGF-I that is produced in ovarian granulosa cells (5). Binding of IGF-I with its receptor can activate the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway to regulate and stimulate normal follicular growth and development in cooperation with gonadotropins to increase the luteinizing hormone receptor level, consequently levitation the ovary sensitivity to the FSH (5, 24). GH is essential for optimal follicular maturation and survival as GH adding to *in vitro* maturation medium of immature and primordial follicles can promote survival, activation and development of preantral follicles of mice, sheep and goats (32–34). Growth hormone can also increase the mitochondria activity to directly improve the oocyte quality (35). Weall *et al* showed that GH could improve oocyte quality through the upregulation of its own receptors and increase of mitochondrial activity (35). Our findings are in line with previous studies demonstrating that adjuvant GH therapy in antagonist protocol among women with a history of POR significantly increased the number of top-quality day 3 embryos. However, there was no significant difference between the two groups in terms of number of retrieved MII oocytes. It was shown that higher concentrations of GH in follicular fluid were associated to good quality oocytes, rapid cleavage, good cleavage morphology, and high embryo implantation potential (36, 37). Therefore, it can be postulated that GH can improve oocyte quality and subsequently increase embryo development rate. However, in the present study, oocyte quality was not evaluated; therefore, further studies are required toconfirm these results.

The present study showed that GH significantly increased the pregnancy rate; however, there was no significant difference between the two groups in terms of chemical pregnancy, ongoing pregnancy rate and live birth rates. In a meta-analysis, Kolibianakis *et al* found that GH

addition significantly increased the clinical pregnancy rate and live birth rate in patients with a history of POR (38). However, in contrast with the present study, both Kucuk *et al* (15) and Eftekhar *et al* (6) showed no significant increase in pregnancy rate by addition of GH to ovarian stimulation protocol in POR patients, although the number of oocytes and embryos was significantly higher in the GH group. In spite of several studies, GH supplementation to the IVF procedure of women with a history of POR remains controversial (5). Some studies recommended that GH pretreatment could increase the ovarian response to gonadotropins, improve oocyte quality and consequently increase clinical outcomes (39-41). However, Some studies did not support the fact that GH was an effective adjuvant for IVF procedure because clinical outcomes were not increased even though some benefits might have been achieved through the use of GH (19, 42).

Several limitations should be considered when interpreting the results of the present study. Firstly, oocyte quality and fertilization rate were not evaluated in this study. Secondly, a possible dose-response effect of GH was not evaluated in this study. The meta-analysis performed by Liu *et al* showed the beneficial effect of GH on clinical pregnancy rate no matter how many doses of GH were used in ART (7). However, owing to the limited data, further studies to investigate the usage of GH in ICSI/FET cycles are needed. ❑

CONCLUSION

The present study showed that adjuvant GH therapy in antagonist protocol among women with a history of POR significantly decreased the number of days that gonadotropin was taken and significantly increased the number of top-quality day 3 embryos and pregnancy rate. Since some previous studies have suggested that improved clinical outcomes were associated with the administration of adjuvant GH therapy in POR women undergoing ICSI, while others have shown opposite results, further studies are needed to corroborate a therapeutic potential application of GH for improving clinical outcomes in these patients. ❑

Ethics approval and participants' consent: The present study was approved by the ethical committee of Avicenna Research Institute, Tehran, Iran. Written informed consent was obtained from all participants.

Trial registration: The present trial was registered at the Iranian Registry of Clinical Trials (IRCT20201003048912N2).

Availability of data and materials: Data that support the findings of this study are available from IVF center.

Conflicts of interests: none declared.

Financial support: none declared.

Authors' contributions: MA, AK, HH conceptualization; methodology; MA, SA, MB, HH collected, analyzed, and interpreted patients' data. HH performed the draft of the manuscript; AK, HH revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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