

Growth hormone treatment strategies in assisted reproduction for the poor responder patients

K. V. Ob'edkova^{1,2}, A. M. Gzgzryan^{1,2}, D. A. Niauri^{1,2},
L. Kh. Dzhemlikhanova^{1,2}, I. Yu. Kogan^{1,2}, I. O. Krikheli², I. D. Mekina²,
E. A. Lesik², E. M. Komarova², M. A. Mazilina^{1,2}

¹ St. Petersburg State University,

7–9, Universitetskaya nab., St. Petersburg, 199034, Russian Federation

² Department of Assisted Reproduction Technologies,

FSBI D. O. Ott Research Institute of Obstetrics, Gynecology and Reproductology,

3, Mendeleevskaya liniya, St. Petersburg, 199034, Russian Federation

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The goal of the study was to estimate the efficacy of growth hormone (GH) co-treatment to the antagonist protocol in IVF/ ICSI cycles in poor responders. A prospective observational study involved 75 patients. All patients underwent standard antagonist protocol with or without GH co-treatment. GH additional was given a daily subcutaneous injection of 1.33 mg (equivalent to 4 IU) of GH from day 1 of ovarian stimulation until the day of human chorionic gonadotropin (hCG). Concentrations of GH, insulin-like growth factor I (IGF-I) and IGF binding protein-3 (IGFBP-3) in serum and follicular fluid were analyzed. The GH co-treatment significantly lowered effective dose of gonadotropins, duration of stimulation, IGFBP-3 level in serum and follicular fluid day of oocytes retrieval. The number of oocytes recovered, metaphase II stage (MII) oocytes, 2 pronucleus (2 pn) zygote, good-quality transferred embryos were significantly higher in the GH+ group. Only patients GH+ group became pregnant. Positive correlation was found between IGF-I level in follicular fluid, dynamics of IGFBP-3 level changes during stimulation protocol and number of good-quality transferred embryos in the GH+ group. GH administration in IVF/ICSI cycles for poor responders raises ovarian sensitivity to the gonadotropin exogenous influence, this way, increasing number of high-quality embryos and the probability of pregnancy.

Keywords: growth hormone, poor ovarian response, in vitro fertilization (IVF), assisted reproductive technologies (ART).

Introduction

The prognosis for treatment by IVF is highly dependent upon ovarian response and the quality of oocytes retrieved, with both factors deciding the number of good quality embryos that will generated. Poor response to ovarian stimulation (POR) is not a rare event in assisted reproduction (ART); its reported frequency in IVF cycles varies from 9 to 30 % between investigators [1].

POR remains a problem in IVF cycles until today. Despite the use of different treatment strategies, clinical pregnancy rate is low in poor responders. It often leads to couples giving up the treatment or using oocyte donation. There are numerous strategies that have been proposed to improve the outcome in the poor responder women despite their limited successes. Meta-analysis by Kyrou et al. [2] suggested that, out of all of the recently proposed protocol alterations, GH addition significantly increased the IVF success rate.

In this research, we aimed to estimate the efficacy of GH co-treatment to the antagonist protocol in IVF/ ICSI cycles in poor responders.

Methods

A prospective observational study was performed at ART department of FSBSI D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation between September 2015 and January 2017.

The research included 75 women with POR. Poor responders were defined by the Bologna consensus criteria [1]. At least two of the following three features must be present:

1. The risk factors for POR are represented by maternal age ≥ 40 years and by all the known genetic or acquired conditions possibly linked to a reduced amount of resting follicles;
2. A POR is represented by a cycle cancelled (following the development of less than three growing follicles) or the collection of less than four oocytes in response to an ovarian stimulation protocol of at least 150 IU follicle-stimulating hormone (FSH) per day;
3. An abnormal ovarian reserve test (i.e. antral follicle count, 5–7 follicles or anti-Müllerian hormone (AMH), 0.5–1.1 ng/ml).

Two episodes of POR after maximal stimulation are sufficient to define a patient as poor responder in the absence of advanced maternal age or abnormal ovarian reserve test.

Exclusion criteria for the research were a day 3 FSH > 20 IU/l, body mass index (BMI) ≥ 35 kg/m² and severe male factor infertility. Written informed consent was obtained from all the couples before IVF treatment.

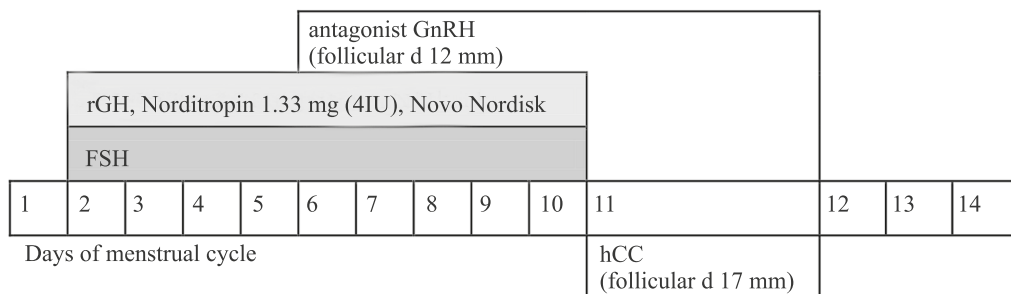
Study population was divided to two groups. 35 patients were allocated to GH co-treatment group (GH+ group), the other 40 were allocated to standard antagonist protocol without GH administration (GH- group).

All patients underwent gonadotropin releasing hormone (GnRH) antagonist conventional ovarian stimulation with recombinant or menopausal gonadotropins. GH co-treatment was given a daily subcutaneous injection of 1.33 mg (equivalent to 4 IU) of GH (Norditropin pen, Novo Nordisk, Denmark) from day 1 of ovarian stimulation until the day of hCG (Fig. 1).

Baseline serum concentrations of gonadotropins and ovarian hormones (estradiol (E2), prolactin (Prol), AMH, FSH and luteinizing hormone (LH)) were measured on day 3 of pretreatment cycle.

The serum GH, IGF-I and IGFBP-3 levels were analyzed on day 1 of ovarian stimulation and day of oocytes retrieval. Submitted markers were also determined in follicular fluid. Follicular fluid was obtained from during oocyte pick up; only samples not contaminated with blood and which didn't contain cumulus-oocyte-complexes were used for

Standard antagonist protocol with GH co-treatment



Standard antagonist protocol without GH co-treatment

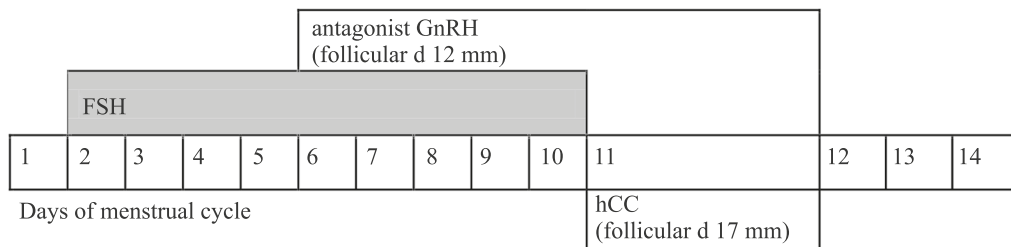


Fig. 1. Ovulation stimulation protocol

the analysis. GH, IGF-I and IGFBP-3 concentrations in serum and follicular fluid were measured by enzyme immunoassay (ELISA) (Mediagnost, Germany).

Statistical analysis was performed using the application package “STATISTICA v 10.0 for Windows” (Statsoft Inc., USA). Data were examined by non-parametric analysis. Median and quartiles (Me(LQ;UQ)) of distribution were determined for each continuous variable. Hormonal and cycle characteristics were compared by using the Mann-Whitney U test, the Wilcoxon test where appropriate. Non-parametric ANOVA was used to compare concentration of GH, IGF-I and IGFBP-3 in serum and follicular fluid. Association between two variables was estimated using Spearman correlation coefficient and gamma correlation coefficient. The significance level (p) was set at 0.05 for all statistical tests.

Results

Patients in the two groups did not differ significantly in age and gynecological background (Table 1). The baseline FSH level was significantly higher and the AMH level was significantly lower in the GH co-treatment cycle (Table 2). There were no significant differences in the serum GH, IGF-I and IGFBP-3 concentrations between the two groups (Fig. 2–4).

The GH concentration in follicular fluid and serum on the day of oocytes retrieval was significantly higher (Fig. 2) and IGFBP-3 level in this biological fluids was significantly lower (Fig. 4) in the GH+ group as compared with the GH- group. Moreover, IGF-I level in serum on the day of oocytes retrieval was significantly higher in the GH co-treatment cycle (Fig. 3).

Table 1. Structure of gynecological diseases

Characteristic		GH+ group, n = 35		GH- group, n = 40		X ²	p-value
		Abs.	%	Abs.	%		
Endometriosis	I st.	–	–	–	–	–	–
	II st.	5	14,29	5	12,5	1,418	>
	III st.	1	2,86	3	7,5	0,576	>
	IV st.	11	31,43	7	17,5	0,212	>
Adenomyosis		6	20	5	12,5	0,058	>
Myoma		8	22,86	5	12,5	0,768	>
Ovarian dysfunction		31	88,57	32	80	0,3605	>
Chronic salpingo-oophoritis		20	57,14	32	80	3,575	>

Table 2. Hormonal characteristics

Characteristic	GH+ group, n = 35			GH- group, n = 40			Mann – Whitney U-test	p-value
	Me	LQ	UQ	Me	LQ	UQ		
FSH, IU/l	12,5	9,2	14,9	7,15	5,81	8,295	188	0,000001
LH, IU/l	5,7	3,55	7,25	4,535	3,575	5,61	672,5	0,774115
AMH, ng/ml	0,32	0,21	0,5	0,75	0,655	0,85	648,5	0,588066
E2, pg/ml	138	103	187	142,6	100,25	181	95,5	0,254103
Prol, mME/ml	324	245,36	369,23	291,6	257,9	348,91	113,5	0,648383

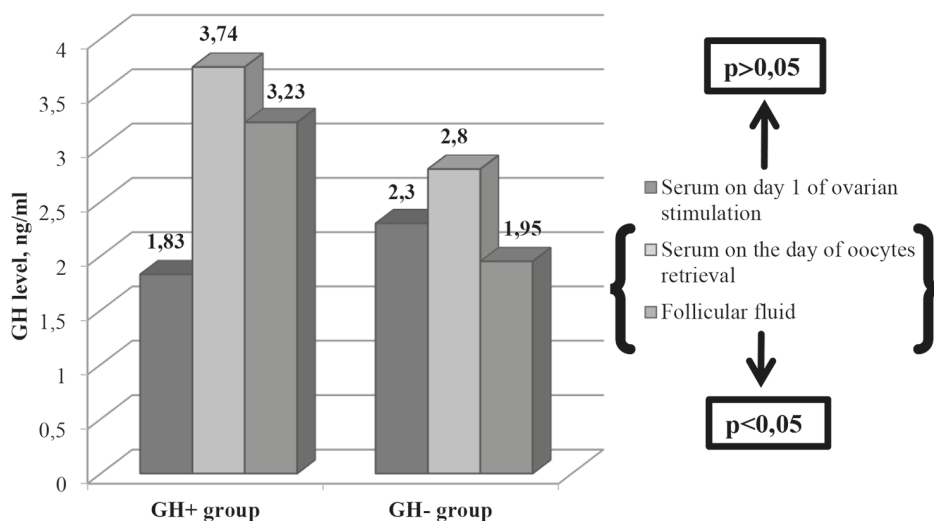


Fig. 2. Level GH in serum and follicular fluid

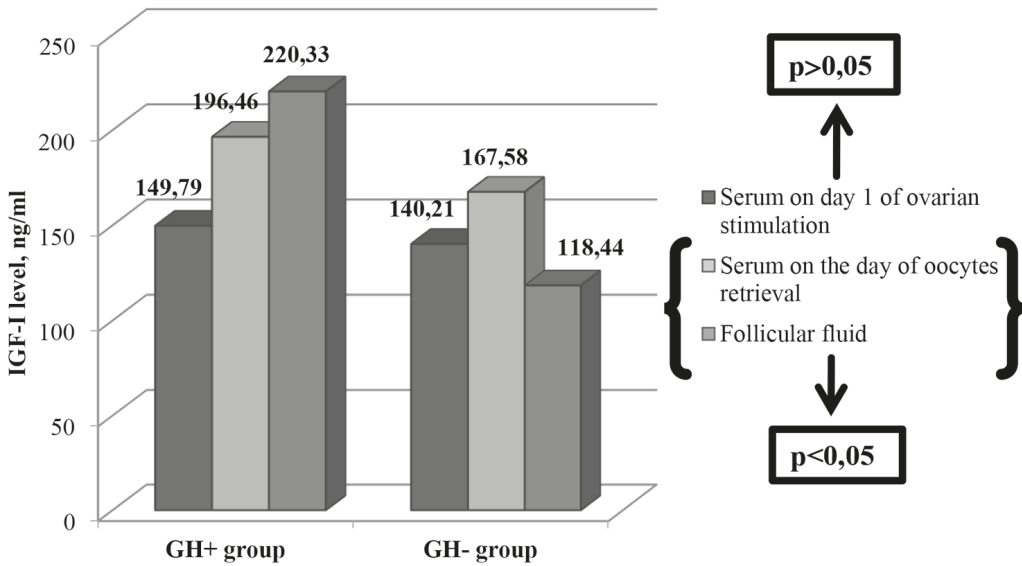


Fig. 3. Level IGF-I in serum and follicular fluid

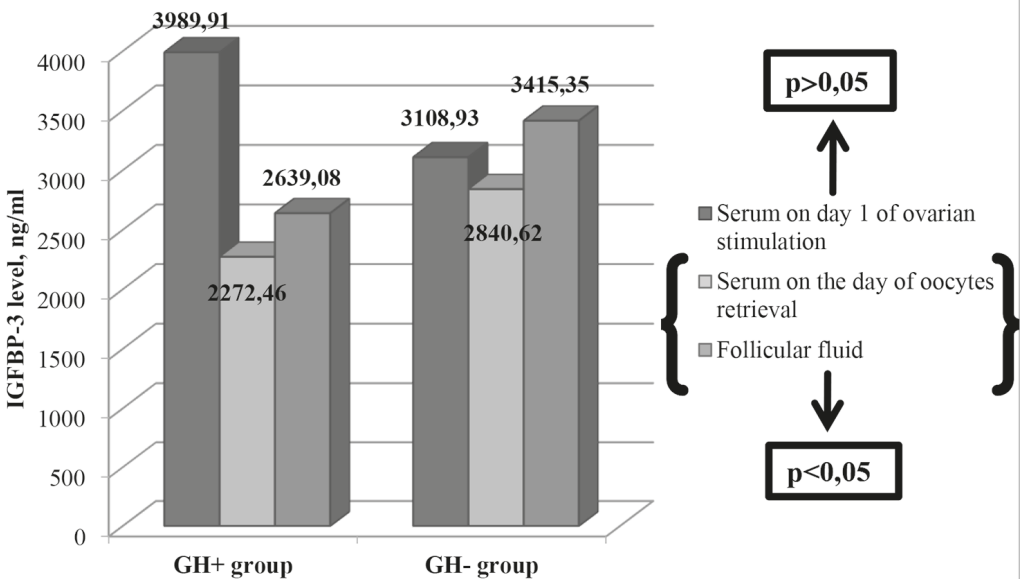


Fig. 4. Level IGFBP-3 in serum and follicular fluid

The serum GH concentration was significantly increased on the day of oocytes retrieval than on the first day of stimulation protocol ($W = 21$, $p = 0.015$) in the GH+ group. Additionally, IGFBP-3 level was significantly diminished in follicular fluid ($W = 24$, $p = 0.023$) and serum on the day of oocytes retrieval ($W = 10$, $p = 0.003$) than on the first day of stimulation protocol in the GH+ group.

Total dose of gonadotropins was not different between the two groups. However, effective dose of gonadotropins, duration of stimulation were significantly lower in the GH+

group as compared with the GH- group. The number of follicles on the day of hCG, oocytes retrieved, MII oocytes, 2 pn zygote, good-quality transferred embryos were significantly higher in the GH+ group as compared with the GH- group (Table 3).

Table 3. Cycle characteristics

Characteristic	GH+ group, n = 35			GH- group, n = 38			Mann-Whitney U-test	p-value
	Me	LQ	UQ	Me	LQ	UQ		
Starting dose of gonadotropins, IU	300	225	300	300	237,5	300	428,5	0,1987
Total dose of gonadotropins, IU	2250	1800	3000	2543,75	1850	3425	226	1,0
Effective dose of gonadotropins, IU	750	500	1325	1321,88	712,5	2100	578,5	0,008
Duration of stimulation, days	8	7	10	9	8	10	27,5	0,0004
No. of follicles on the day of hCG	4	3	7	3	2	4	209,5	0,045
No. of oocytes recovered	3	2	5	2	1	3	317	0,0005
No. of MII oocytes	4	3	6	1	0	2	292,5	0,043
No. of 2p zygote	2	1	4	1	0	2	195	0,0004
Day 3 from fertilization								
No. of transferred embryos	2	1	2	1	1	2	55,5	0,659
No. of good-quality transferred embryos	2	1	2	1	0	1	6	0,0004
Day 4 from fertilization								
No. of transferred embryos	2	2	2	2	1	2	3	0,7728
No. of good-quality transferred embryos	2	1	2	0	0	0	0	0,0489

Positive correlation was found between IGF-I level in follicular fluid and number of good-quality transferred embryos ($\gamma = 0.5$, $p = 0.048$); dynamics of IGFBP-3 level changes in follicular fluid and serum on the first day of stimulation protocol and number of oocytes retrieved ($\gamma = 0.53$, $p = 0.007$), number of fertilized oocytes ($R = 0.66$, $p = 0.005$), number of good-quality transferred embryos ($\gamma = 0.72$, $p = 0.004$) in the GH+ group.

Two patients in the GH+ group and seventeen patients in the GH- group didn't reach embryo transfer stage, but cycle cancellation rate was significantly higher in the GH-

group (Fisher's exact test 0.01; $p < 0.05$). The reasons for cancellation in the GH- group were absence of cumulus-oocyte-complexes retrieved in five patients, total fertilization failure in eleven patients, and no-cleavage in one patient.

Only patients GH+ group became pregnant; clinical pregnancy rate was 24.24%. Probability of pregnancy was significantly increased in the GH+ group as compared with the GH- group (OR (95 %CI) = 9.1 (1.034–80.093), $p < 0.05$).

No side effect was seen in any of the patients.

Discussion

Over 25 years ago, Owen et al. [3] concluded that GH co-treatment improves the ovarian response to ordinary ovarian stimulation protocols in sub-optimal responders. This conclusion was supported studies by Homburg et al. [4] demonstrating that GH administration raises ovarian sensitivity to the gonadotropins influence. GH mRNA and immunoreactivity are discovered in ovarian stromal and follicular tissue from human [5]. GH gene expression is initiated early in follicular development in humans, since GH mRNA and immunoreactivity were found in the oocyte cytoplasm and the granulosa cells of fetal primordial follicles [6]. In this research, effective dose of gonadotropins, duration of stimulation were significantly lower in the GH co-treatment. Furthermore, a study by Kucuk et al. [7] observed an improved ovarian response as well as increased ART success.

Despite numerous more recent studies, the GH co-treatment in IVF/ICSI cycles for poor responders remains controversial [8]. The problem is supposed to associate with the definition of a poor responder, underpowered statistical analysis, the pooling of patients with diverse risk factors such as age, and heterogeneous protocols of GH administration, ovarian stimulation, and luteal support [9]. However, most studies conclude that the outcomes improved by GH are generally those reflecting increased oocyte quality, such as the number of MII stage recovered, the fertilization rate [7], the number of embryos reaching the transfer stage [9], the pregnancy rate [10] and the rate of live births [11]. This research showed that GH co-treatment to the antagonist protocol in poor responders undergoing IVF/ICSI cycles significantly improved the number of oocytes collected, MII oocytes retrieved, 2 pn zygote and good-quality transferred embryos. Moreover, adjuvant GH therapy seems to enhance both the fertilization rate and the quality of the resulting embryos, as indicated by improved blastomere uniformity and cleavage rate and decreased apoptosis [12].

GH 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 [13; 14]. It was found, that follicular GH levels were positively correlated with IVF success [15], and follicles containing higher GH levels gave rise to the highest quality embryos [16]. In this study, positive correlation was found between IGF-I level in follicular fluid and number of good-quality transferred embryos; dynamics of IGFBP-3 level changes in follicular fluid and serum on the first day of stimulation protocol and number of oocytes retrieved, number of fertilized oocytes, number of good-quality transferred embryos in the GH+ group.

Most systematic reviews and meta-analyzes suggested that GH addition significantly increased clinical pregnancy rate and live birth rates [8; 9; 11]. In this study, probability of pregnancy was significantly increased in the GH+ group as compared with the GH- group.

Potential side-effects observed with GH treatment include increased fluid retention, resulting in edema, headaches, and/or joint pain, neoplasms, cerebrovascular events, and altered glucose metabolism [17]. However, GH co-treatment in IVF/ ICSI cycles is not associated with any adverse events except for slight edema. In this study, no side effect was seen in any of the patients.

In conclusion, GH administration in IVF/ICSI cycles for poor responders raises ovarian sensitivity to the gonadotropin exogenous influence, this way, increasing number of oocytes collected, MII oocytes retrieved, fertilization rate, number of high-quality embryos and the probability of pregnancy. The effectiveness of overcoming infertility by IVF in patients with POR in terms of adjuvant GH therapy depends on the dynamics of increasing IGF-I concentration and the dynamics of decreasing IGFBP-3 concentration in blood serum and follicular fluid during ovarian stimulation. Taking into consideration small sample of women examined, it is necessary to continue the study of GH use effectiveness and safety for the recommendation of its wide administration in clinical practice.

Declaration of interest

The authors report that they have no conflict of interest. No funding was required for this study.

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Authors' information:

Ksenia V. Ob'edkova — MD, PhD; obedkova_ks@mail.ru
Alexandr M. Gzgzyan — MD, PhD; agzgzyan@mail.ru
Dariko A. Niauri — MD, PhD, Professor; d.niauri@mail.ru
Liailia Kh. Dzhemlikhanova — MD, PhD; dzhemlikhanova_l@mail.ru
Igor Yu. Kogan — MD, PhD, Professor; ikogan@mail.ru
Inna O. Krikheli — MD, PhD; ikrekhily@gmail.com
Irina D. Mekina — PhD; ovr@ott.ru
Elena A. Lesik — PhD; ovr@ott.ru
Evgenia M. Komarova — PhD; ovr@ott.ru
Maria A. Mazilina — MSc; ovr@ott.ru