Growth hormone co-treatment in IVF/ICSI cycles in poor responders

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ABSTRACT
To estimate the efficacy of growth hormone (GH) co-treatment within an antagonist protocol in IVF/ICSI cycles in poor responders. A prospective observational study involving 50 patients underwent a standard antagonist protocol with or without GH co-treatment. GH was administered by a daily subcutaneous injection of 1.33 mg (equivalent to 4 IU) starting from day 1 of ovarian stimulation until the day of 10,000 IU human chorionic gonadotropin (hCG) triggering. Concentrations of GH, insulin-like growth factor I (IGF-I) and IGF binding protein-3 (IGFBP-3) in serum and follicular fluid were the subject matter of analysis. The GH co-treatment significantly lowered the effective dose of gonadotropins, duration of stimulation, IGFBP-3 level in serum and follicular fluid on the day of oocyte retrieval. The total number of oocytes as well as the number of metaphase II stage (MII) oocytes, two pronucleus (2 pn) zygotes, good-quality transferred embryos was significantly higher in the GH+ group. Pregnancy was achieved in patients GH+ group only. Positive correlation was found between IGF-I level in follicular fluid, dynamics of IGFBP-3 level changes during stimulation protocol and the 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, increasing number of high-quality embryos and the probability of pregnancy.

Introduction
The prognosis for treatment by IVF is highly dependent upon ovarian response and the quality of oocytes retrieved, with both factors determining the number of generated good-quality embryos. 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% in different studies [1].

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

The present study was aimed at estimating the efficacy of GH co-treatment within an antagonist protocol in IVF/ICSI cycles in poor responders.

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

The study included 50 women with POR. Poor responders were defined according to the Bologna consensus criteria [1].

Exclusion criteria for the study were the day 3 follicle-stimulating hormone (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 into two groups. 25 patients were allocated to GH co-treatment group (GH+ group), while the other 25 – to standard antagonist protocol without GH administration (GH– group).

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

Baseline serum concentrations of gonadotropins and ovarian hormones (estradiol (E2), prolactin (Prol), for anti-Müllerian hormone (AMH), FSH and luteinizing hormone (LH)) were measured on day 1 of stimulation protocol. The total number of oocytes as well as the number of metaphase II stage (MII) oocytes, two pronucleus (2 pn) zygotes, good-quality transferred embryos was significantly higher in the GH+ group. Pregnancy was achieved in patients GH+ group only. Positive correlation was found between IGF-I level in follicular fluid, dynamics of IGFBP-3 level changes during stimulation protocol and the 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, increasing number of high-quality embryos and the probability of pregnancy.
Table 1. Hormonal characteristics.

| Characteristic                      | GH + group (n = 25) | GH-group (n = 25) | Mann–Whitney |
|------------------------------------|--------------------|-------------------|--------------|
|                                   | Me | LQ   | UQ   | Me | LQ   | UQ   | U-test | p value |
| Level in serum on day 1 of ovarian stimulation |     |       |      |     |       |      |        |         |
| FSH, IU/l                          | 11.57 | 8.38 | 15.03 | 6.9 | 6.2 | 8.57 | 81.0000001 |         |
| LH, IU/l                           | 3.82 | 3.14 | 5.21 | 4.3 | 3.45 | 5.34 | 301.83 |         |
| AMH, ng/ml                         | 0.5 | 0.3 | 0.79 | 0.73 | 0.5 | 0.87 | 137.5 | .0005 |
| E2, pg/ml                          | 152 | 112 | 190 | 134.5 | 98 | 184 | 306.91 |         |
| Prol, mME/ml                       | 326.33 | 245 | 375 | 275.6 | 234.7 | 335 | 257.25 | .29 |
| GH, ng/ml                          | 1.83 | 1.15 | 2.42 | 2.15 | 1.74 | 2.78 | 86.55 | .449 |
| IGF-I                              | 149.79 | 105.79 | 197.36 | 137.39 | 112.65 | 154.44 | 78.5 | .268 |
| IGFBP-3                            | 4080.08 | 3221.38 | 4719.53 | 2938.4 | 2442.3 | 3901.3 | 62.0068 |         |

Level in serum on the day of oocytes retrieval

| GH, ng/ml                          | 3.74 | 2.73 | 5.99 | 2.81 | 1.93 | 3.6 | 63 | .075 |
| IGF-I, ng/ml                       | 146.86 | 123.62 | 203.66 | 164.65 | 115.86 | 202.18 | 102 | .948 |
| IGFBP-3, ng/ml                     | 2398.03 | 2064.07 | 2857.61 | 2917.54 | 2566.24 | 4618.71 | 58 | .045 |

Level in follicular fluid

| GH, ng/ml                          | 3.24 | 2.06 | 4.34 | 1.77 | 1.55 | 2.43 | 47.5 | .012 |
| IGF-I, ng/ml                       | 174.83 | 122.66 | 221.62 | 118.76 | 107.54 | 187.95 | 69.5 | .132 |
| IGFBP-3, ng/ml                     | 2994.64 | 2313.27 | 3409.73 | 3421.06 | 3251.44 | 4283.06 | 54 | .028 |

Table 2. Cycle characteristics.

| Characteristic                      | GH + group (n = 25) | GH-group (n = 25) | Mann–Whitney |
|------------------------------------|--------------------|-------------------|--------------|
|                                   | Me | LQ   | UQ   | Me | LQ   | UQ   | U-test | p value |
| Starting dose of gonadotropins, IU | 300 | 250 | 300 | 300 | 300 | 300 | 252 | .247 |
| Total dose of gonadotropins, IU    | 2650 | 2100 | 3000 | 2750 | 2500 | 3600 | 226 | .095 |
| Effective dose of gonadotropins, IU | 750 | 533.3 | 1312.5 | 1375 | 862.5 | 2962.5 | 160 | .008 |
| Duration of stimulation, days      | 8 | 7 | 10 | 9 | 8 | 10 | 208 | .043 |
| No. of follicles on the day of hCG | 4 | 3 | 7 | 3 | 2 | 4 | 209.5 | .045 |
| No. of oocytes recovered           | 3 | 2 | 6 | 2 | 1 | 3 | 197.5 | .025 |
| No. of MII oocytes                 | 2 | 1 | 6 | 1 | 0 | 2 | 15 | .007 |
| No. of 2p zygote                   | 2 | 1 | 3 | 1 | 1 | 2 | 156 | .018 |
| day 3 from fertilization           |     |       |      |     |       |      |        |         |
| No. of transferred embryos         | 2 | 1 | 2 | 1 | 1 | 1.5 | 32 | .238 |
| No. of good-quality transferred embryos | 1.5 | 1 | 2 | 0 | 0 | 1 | 17 | .016 |
| day 4 from fertilization           |     |       |      |     |       |      |        |         |
| No. of transferred embryos         | 1 | 1 | 2 | 1 | 1 | 1 | 8 | .476 |
| No. of good-quality transferred embryos | 1 | 1 | 1 | 0 | 0 | 0 | 2 | .038 |

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. The association of two variables was estimated using Spearman correlation coefficient and gamma correlation coefficient. The significance level (p) was set at .05 for all statistical tests.

Results

Patients in the two groups did not differ significantly in age and gynecological background. The baseline FSH level was significantly higher while the AMH level – significantly lower in the GH co-treatment cycle. No significant difference was detected in the serum GH, IGF-I and IGFBP-3 concentrations in both study groups (Table 1).

The serum GH concentration significantly increased on the day of oocytes retrieval compared to that of day 1 of stimulation ($W = 2, \ p = .015$) in the GH+ group. Additionally, IGFBP-3 level significantly decreased in follicular fluid ($W = 24, \ p = .023$) and serum on the day of oocytes retrieval ($W = 10, \ p = .003$) compared to day 1 of stimulation in the GH+ group (Table 1).

Total dose of gonadotropins did not differ between the two groups. However, the effective dose of gonadotropins and duration of stimulation were significantly lower in the GH+ group as compared to the GH– group. The number of follicles on the day of hCG, oocytes retrieved, MII oocytes, 2 pN zygote, good-quality transferred embryos was significantly higher in the GH+ group compared to the GH– group (Table 2).

Positive correlation was found between IGF-I level in follicular fluid and the number of good-quality transferred embryos ($g = 0.5, \ p = .048$) as well as between the dynamics of IGFBP-3 level changes in follicular fluid and serum on day 1 of stimulation and the number of oocytes retrieved ($g = 0.53, \ p = .007$), the number of fertilized oocytes ($R = .66, \ p = .005$) and that of good-quality transferred embryos ($g = 0.72, \ p = .004$) in the GH+ group.

Two patients in the GH+ group and 10 patients in the GH– group did not reach embryo transfer. The cycle cancelation rate was significantly higher in the GH– group (Fisher’s exact test 0.01; $p < .05$). The reasons for cancelation in the GH– group were: absence of cumulus-oocyte-complexes retrieved in two patients, total fertilization failure in seven patients, and embryo cleavage arrest in one patient.

Pregnancy was achieved in patients GH+ group only. The clinical pregnancy rate was 21.74%. Probability of pregnancy significantly increased in the GH+ group in comparison with the GH– group (OR (95%CI) = 9.1 (1.034–80.093), $p < .05$).
No side effect was observed in any of the patients.

Discussion

Over 25 years ago, Owen et al. [3] concluded that GH co-treatment improved the ovarian response to ordinary ovarian stimulation protocols in suboptimal responders. This conclusion was supported by studies of Homburg et al. [4] demonstrating that GH administration raises ovarian sensitivity to the gonadotropins influence. GH mRNA and immunoreactivity are discovered in human ovarian stroma and follicles [5]. GH gene expression first was detected in human growing follicles, and afterwards GH mRNA and immunoreactivity were found in the oocytecyto-plasm and the granulosa cells of fetal primordial follicles [6]. In this study effective dose of gonadotropins, duration of stimulation were significantly lower in the GH co-treatment. Furthermore, Kucuk et al. [7] observed an improved ovarian response as well as increased ART success. Despite numerous more recent studies, the applicability of GH co-treatment in IVF/ICSI cycles for poor responders remains controversial [8]. The problem is supposed to be bound with the definition of a poor responder, underpowered statistical analysis, the pooling of patients with diverse additional risk factors such as age and heterogeneous protocols of GH administration, ovarian stimulation and luteal support [9]. However, most studies show that GH treatment usually increases 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]. Another study showed that GH co-treatment within an 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, cleavage rate and decreased apoptosis [12].

GH is reported to modulate the activity 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 granulosa and theca cells [13,14]. It was found that follicular GH levels positively correlated with IVF success [15], and follicles containing higher GH levels gave rise to the highest quality embryos [16]. In our study, positive correlation was found between IGFI-I level in follicular fluid and the number of good-quality transferred embryos; also between dynamics of IGFBP-3 level changes in follicular fluid and serum on day 1 of stimulation and the number of oocytes retrieved, the number of fertilized oocytes and that of good-quality embryos in the GH + group.

Most systematic reviews and meta-analyses suggested that GH addition significantly increased both clinical pregnancy rate and live birth rates [8,9,11]. In our study, probability of pregnancy significantly increased in 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. No side effect was seen in any of the patients.

In conclusion, GH administration in IVF/ICSI cycles for poor responders improves ovarian sensitivity to the exogenous gonadotropin activity The GH co-treatment increases the number of oocytes collected, MII oocytes retrieved, fertilization rate, number of high-quality embryos and the probability of pregnancy. Taking into consideration the small size of study group, it seems necessary to continue the study of GH use effectiveness and its safety for further recommendation to be applied in clinical practice.

Disclosure statement

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

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