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Growth hormone co-treatment within a GnRH agonist long protocol improves implantation and pregnancy rates in patients undergoing IVF-ET

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Abstract
Objective The efficacy of growth hormone (GH) co-treatment within a GnRH agonist long regimen, in women with a normal ovarian response to controlled ovarian hyperstimulation (COH), for IVF was assessed.
Methods This retrospective clinical trial was performed in a private-assisted reproduction centre. The study involved 1114 patients who responded normally to high-dose gonadotropin treatment. The study group of 556 patients was given in a daily subcutaneous injection of 4.5 IU of GH co-treatment, starting from the initial day of gonadotropin treatment and lasting for 5 days. The control group of 558 patients received the same treatment protocol without the GH co-treatment. The participants were further divided into two subgroups: age ≥35 years and age <35 years. The primary endpoint of the study was IVF-ET outcomes.
Results The demographic characteristics did not significantly differ between the groups. The implantation rate (36.7 vs. 20.4 %, $P < 0.05$) and clinical pregnancy rate (57.3 vs. 30.1 %, $P < 0.05$) were significantly higher in the study group than in the control group. An analysis using a multivariate logistic regression model showed that GH was a significant factor for predicting pregnancy outcomes (OR 3.125, 95 % CI 2.441–4.000). Furthermore, for the ≥35-year-old group, the endometrial thickness was significantly greater (11.99 ± 2.21 vs. 11.62 ± 2.45, $P < 0.05$) in the study group than in the control group; in contrast, for the <35-year-old group, the high-quality embryo rate was significantly higher (71.7 vs. 68.3 %, $P < 0.05$) in the study group than in the control group.
Conclusion Our study showed that co-treatment with GH in a GnRH agonist long protocol in patients who responded normally while undergoing IVF-ET could increase the implantation and pregnancy rates.

Keywords Growth hormone (GH) · In vitro fertilization (IVF) · Agonist long protocol · Implantation rate · Pregnancy rate

Introduction

Growth hormone (GH) is a 191 amino acid, single chain polypeptide hormone that is produced, stored, and secreted by somatotroph cells in the anterior pituitary gland [1]. GH is involved in the processes of human growth [2] and metabolism, and it directly and indirectly participates in reproduction [3]. Since Homburg R first used GH to assist gonadotropin in ovarian stimulation in 1988 [4], researchers have gradually began to realize the value of GH as a treatment for IVF. Studies have indicated that GH is not only involved in sexual differentiation and pubertal maturation but is also related to gonadal steroidogenesis [5], gametogenesis [6, 7], and oocyte maturation [8, 9]. In addition to the above properties, GH can improve the receptivity of the endometrium during the proliferation and implantation phases in mice [10]. The prognosis for treatment by IVF is highly dependent on endometrial receptivity and the quality of the oocytes recovered, because both factors impact embryo implantation [11, 12].

Studies evaluating the benefits of co-treatment with GH during controlled ovarian stimulation for human-assisted

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reproduction treatment have reported controversial findings. A systematic review and meta-analysis indicated that GH can increase the pregnancy rate and live birth rate in patients who exhibit a poor response to controlled ovarian stimulation [13]; however, another study failed to confirm this effect [14]. In a small series of 12 patients, a better fertilization and pregnancy rate was reported with GH co-treatment than in the previous attempts without GH [15]. In contrast, a larger double-blind prospective study (21 patients in both the GH treatment and placebo arms) failed to show that GH had a significant effect on ovarian stimulation cycle characteristics and the number of oocytes collected, although the pregnancy rates were not evaluated [16].

It should be noted that both the positive and negative data concerning GH use in ovarian stimulation have been generated in small studies. Moreover, the target patient population was always poor ovarian responders, and no effect of GH has been noted in normal responders. Furthermore, the outcome measures did not always include the implantation rate and pregnancy rate, which are the most relevant parameters for assessing the success of assisted reproduction treatment. Thus, the effect of GH during ovulatory stimulation in IVF-ET requires further research.

In this study, we assessed the efficacy of GH co-stimulation within a GnRH agonist long protocol in normal responders to controlled ovarian hyperstimulation (COH) for IVF-ET cycles. The implantation rate and pregnancy rate were used as the main outcome measures.

Materials and methods

Patient selection

A single-centre retrospective study of 1114 infertile female Chinese patients examining the effect of the addition of recombinant GH to gonadotropins on the IVF outcome was performed at the Reproductive Medical Centre of the First Affiliated Hospital of Zhengzhou University from January 2013 to January 2015. The study was approved by the ethics committee. Couples were counselled about the treatment protocols, and written informed consent was obtained from all couples.

Patients were eligible if they met the following criteria: (1) age between 20 and 45 years; (2) the cause(s) of infertility could primarily be attributed to fallopian tube malfunction or male sterility; (3) FSH, LH, and oestradiol concentrations in the normal range during the early follicular phase; (4) normal uterine cavity with regular spontaneous menstrual cycles of 25–30 days; and (5) a body mass index (BMI) <25 kg/m^2.

The exclusion criteria were as follows: (1) recurrent spontaneous abortion; (2) serious pelvic adhesions or hydrosalpinx; (3) serious and unstable diseases, such as cerebrovascular, liver, and kidney disease; and (4) endocrine diseases (thyroid hyperfunction, diabetes, high lactation hyperprolactinemia, and adrenal cortex hyperfunction), polycystic ovary syndrome, endometriosis, uterine leiomyoma, and adenomyosis.

Stimulation protocol

All patients received a long protocol of pituitary downregulation with triptorelin (Decapeptyl, Ferring, Germany) starting on day 21 of the preceding cycle at a dose of 0.1 mg/day. The daily dose was decreased to 0.05 mg after confirmation of downregulation, and this reduced dose was maintained until the day hCG was administered. Pituitary downregulation was confirmed by an ultrasound scan showing an endometrial thickness <5 mm and/or serum concentrations of E2 <50 pg/ml and FSH <5 mIU/ml. Then, patients received gonadotropins with recombinant FSH (rFSH; Gonal-f,Merck Serono,Switzerland) at a starting dose dependent on ovarian response. The 1114 patients were divided into two groups as follows: those who received GH ($n = 556$, GH cycle) and those who did not ($n = 558$, non-GH cycle). Patients in the GH group received 4.5 IU recombinant human GH (rGH, Saizen; Kinsey) per day for 5 days, beginning on the initial day of FSH administration, and patients in the control group received a stimulation protocol with FSH only. Gonadotropins and GnRHa were administered until the criteria for triggering final follicular maturation (at least one follicle had reached a diameter larger than 18 mm) were reached.

Oocyte retrieval and fertilization

Ovulation was induced with 250 IU rhCG (Ovidrel, Merck, Serono, Switzerland) and 2000 IU hCG (HCG; lizhu, China). Oocytes were retrieved under vaginal ultrasonography guidance 36 h after hCG administration and then fertilized by conventional IVF in G-Fert Plus medium (Vitrolife, Sweden). After retrieval, granular cells and the corona radiata of the cumulus oophorous were removed; the maturity of the ova was evaluated, and ova were naturally fertilized. After fertilization, the zygote was incubated for 18 h in IVF nutrient solution at 37 °C with a 5 % CO_2 atmosphere. The fertilization status was observed at 24 h, and the nutrient solution was replaced. Embryos assessments were performed on day 3 after retrieval using the Peter score. Fresh embryos were transferred 3 or 5 days after oocyte retrieval if no contraindications were present.

Progesterone was started intramuscularly from the day of oocyte retrieval (40 mg per day). On the day of transplantation, the dose was increased to 60 mg per day, and 20 mg per day of oral dydrogesterone (Duphaston, Abbott, Netherlands) was added. After 7 days of transplantation, 2 mg per day of oral estradiol valerate tablets (Progynova, Bayer, France) was added. All doses were maintained constant until the first ultrasound evaluation, at which time, adjustments were made as necessary. Chemical pregnancy was defined as a serum hCG >0 IU/L at 14 and 18 days after embryo transfer. Clinical pregnancy was identified as the observation of foetal heart activity by trans-vaginal ultrasonography performed 5 weeks after embryo transfer followed by positive hCG at 14 days/18 days post transplantation [clinical pregnancy includes intrauterine pregnancy, ectopic pregnancy, bursal pregnancy, and uterine curettage (villi tissue is visible)].

Statistical analysis and method

Statistical data were analyzed using the SPSS17.0 statistical software. Data are represented by the mean and standard deviation ($X \pm s$). Numerical data were analyzed using a t test and the analysis of variance (ANOVA), and measurement data were analyzed using a Chi-square test. A logistic regression analysis was used to examine factors that predicted clinical pregnancy. A two-tailed P value <0.05 was considered statistically significant.

Results

Comparison of basic clinical data

Age, duration of infertility, BMI, day 3 serum FSH, LH, E2, and P levels, and the antral follicle count were not significantly different between the study group (with GH) and the control group (without GH) ($P > 0.05$) (Table 1).

Laboratory results and pregnancy outcome data between the study group and the control group

The estradiol-to-follicles index (EFI) on the day of hCG injection and oocyte retrieval, implantation rate, clinical pregnancy rate, and high-quality embryo rate in the study group were significantly higher than in the control group ($P < 0.05$). The differences in the duration of stimulation, gonadotropin dose, endometrial thickness on the embryo transfer (ET) day, and fertilization rate (2PN) were not significantly different ($P > 0.05$) (Table 2).

Multivariate logistic regression analysis for the prediction of clinical pregnancy

Table 3 shows the multivariate logistic regression analysis results. Factors included in the model were age, BMI, total number of transferable embryos, and the presence of GH. The presence of GH was a significant factor for predicting clinical pregnancy (OR 3.125, 95 % CI 2.441–4.000). Among the four parameters entered into the model, age (OR 0.949, 95 % CI 0.922–0.976) and the total number of transferable embryos (OR 1.453, 95 % CI 1.109–1.903) were independent significant factors. However, the BMI was not a significant factor for predicting clinical pregnancy in our study (Table 3).

Comparison of the laboratory results and pregnancy outcome between the study group and the control group in the two age groups

In the older group (35 years and older), the EFI on the day of hCG injection and oocyte retrieval, endometrial

Table 1 Comparison of the basic clinical data between the study group (with GH) and the control group ($X \pm s$)

	Study group (with GH) ($n = 556$)	Control group ($n = 558$)	P value
Age (years)	32.77 ± 4.28	31.56 ± 4.35	0.054
Duration of infertility (years)	4.02 ± 3.17	4.51 ± 2.89	0.088
Cause of infertility			
Primary	291 (52.33 %)	246 (44.08 %)	
Secondary	265 (47.47 %)	312 (55.92 %)	
BMI (kg/m^2)	22.43 ± 3.21	23.12 ± 3.05	0.348
Day 3 serum FSH (mIU/ml)	7.06 ± 1.72	6.83 ± 1.90	0.099
Day 3 serum LH (mIU/ml)	6.04 ± 2.59	6.23 ± 3.65	0.556
Day 3 serum E2 (mIU/ml)	45.37 ± 22.03	45.30 ± 20.18	0.955
Day 3 serum P (mIU/ml)	0.65 ± 0.43	0.61 ± 0.36	0.199
Antral follicles (n)	5.41 ± 2.63	5.66 ± 2.56	0.110

Table 2 Comparison of laboratory results and pregnancy outcomes between the study group (with GH) and the control group ($\overline{X} \pm s$)

	Study group (with GH) ($n = 556$)	Control group ($n = 558$)	P value
Duration of stimulation (days)	11.03 ± 1.42	10.90 ± 1.35	0.125
Gonadotropin dose (IU)	2158.70 ± 647.77	2087.66 ± 630.42	0.059
Endometrial thickness on ET day (mm)	12.18 ± 4.75	11.80 ± 4.85	0.179
EFI on the day of[a] hCG injection	511.56 ± 308.92	462.04 ± 193.31	0.001*
EFI on the day of oocyte retrieval (pg/ml)	271.13 ± 174.68	238.07 ± 119.49	0.000*
2PN Fertilization rate[b] (%)	66.2 (3550/5358)	65.0 (4464/6860)	0.171
High-quality embryo rate[c] (%)	72.1 (2526/3501)	68.8 (3037/4408)	0.002*
Implantation rate (%)[d]	37.6 (402/1069)	20.4 (212/1037)	0.000*
Clinical pregnancy rate[e] (%)	57.3 (319/556)	30.1 (168/558)	0.000*

* $P < 0.05$, the difference is statistically significant

[a] Estradiol-to-follicles index (EFI) = level of serum E2/retrieved oocytes

[b] 2PN fertility rate = 2PN number of fertilized oocytes/retrieved oocytes

[c] High-quality embryo rate = 2PN (I + II) embryo number/2PN cleavage embryo number

[d] Implantation rate = total number of implantation embryos/total number of transplanted embryos

[e] Clinical pregnancy rate = clinical pregnancy cycles/transplantation cycles

Table 3 Multivariate logistic regression analysis for the prediction of clinical pregnancy

	B	Standard error	P value	Exp(B) (95 % CI)
Presence of GH	1.139	0.126	0.000*	3.125 (2.441–4.000)
Age	−0.053	0.015	0.000*	0.949 (0.922–0.976)
BMI	−0.002	0.003	0.568	0.998 (0.993–1.004)
Total number of transferable embryos	0.374	0.138	0.007*	1.453 (1.109–1.903)

* $P < 0.05$, the difference is statistically significant

thickness on the day of ET, implantation rate, clinical pregnancy rate, and the high-quality embryo rate of the study group were significantly higher than those in the control group ($P < 0.05$). The duration of gonadotropin stimulation, gonadotropin dose, 2PN fertilization rate, rate of fertilization, and number of transferred embryos in the study group were not significantly different from those of the control group ($P > 0.05$).

In the younger group (less than 35 years old), the high-quality embryo rate, EFI on the day of hCG injection, number of oocytes retrieved, implantation rate, and clinical pregnancy rate in the study group were significantly higher than those in the control group ($P < 0.05$). The duration of gonadotropin stimulation, gonadotropin dose, 2PN fertilization rate, and number of transferred embryos in the study group were not significantly different from those of the control group ($P > 0.05$) (Table 4).

Discussion

The main observation of this study was a significant improvement in the implantation and pregnancy rates in normal responders treated with exogenous GH during ovarian stimulation in an IVF-ET programme. To the best of our knowledge, this is the first trial to begin GH along with gonadotropins in normal responders. In a recent review [17], only a few trials on this topic were reported, and the sample sizes were small. The number of patients in each study ranged from 14 to 61, whereas our study had 1114 patients. Our observations are consistent with the conclusions of this review that show adjuvant GH treatment in patients undergoing IVF treatment results in higher pregnancy rates.

In our study, the EFI achieved on the days when hCG was administered and on the day when oocytes were retrieved were higher in women co-stimulated with GH than in the GnRHa only group. The serum oestrogen concentrations are related to the number of follicles, and using the EFI can lead to more accurate hormone level assessments [18]. We may speculate that more estradiol was produced per follicle by midluteal GH administration in the GH co-treatment group. Our research is consistent with the conclusion of a study by Pereira G [19], who identified positive eGH-R immunostaining in cumulus cells, oocytes, and granulosa cells and found that the addition of eGH to the maturation medium increased the concentrations of testosterone and oestradiol [20]. Because higher

Table 4 Comparison of the laboratory results and pregnancy outcome between the study group and the control group for the two age groups ($\overline{X} \pm s$)

Classification	Older group (≥35 years old)			Younger group (<35 years old)		
	GH group ($n = 278$)	Control group ($n = 265$)	*P* value	GH group ($n = 278$)	Control group ($n = 293$)	*P* value
Duration of stimulation (days)	10.98 ± 1.48	10.78 ± 1.32	0.065	11.30 ± 1.67	11.04 ± 1.36	0.061
Gonadotropin dose (IU)	2414.10 ± 612.23	2374.81 ± 567.08	0.386	1768.80 ± 487.20	1710.10 ± 504.42	0.197
Endometrial thickness on the day of ET (mm)	11.99 ± 2.21	11.62 ± 2.45	0.038*	12.47 ± 7.03	12.04 ± 6.82	0.504
EFI on the day of hCG injection	525.75 ± 352.50	469.92 ± 193.93	0.010*	489.90 ± 225.84	451.67 ± 192.39	0.046*
EFI on the day of oocyte retrieval (pg/ml)	270.37 ± 164.20	242.04 ± 136.21	0.029*	271.89 ± 184.86	234.49 ± 102.15	0.003*
2PN fertilization rate (%)	66.3 (1654/2456)	64.9 (2036/3133)	0.069	65.3 (1896/2902)	65.1 (2428/3727)	0.876
Total no. of transferable embryos (*n*)	1.96 ± 0.52	1.92 ± 0.46	0.310	1.88 ± 0.39	1.80 ± 0.42	0.210
High-quality embryo rate (%)	72.6 (1185/1631)	69.5 (1367/1966)	0.042*	71.7 (1341/1870)	68.3 (1670/2442)	0.019*
Implantation rate (%)	34.0 (186/546)	16.6 (85/509)	0.000*	41.3 (216/523)	24.0 (127/528)	0.000*
Clinical pregnancy rate (%)	53.23 (148/278)	24.9 (66/265)	0.000*	61.5 (171/278)	34.1 (100/293)	0.000*

* $P < 0.05$, the difference is statistically significant

concentrations of estradiol in pre-ovulatory follicular fluid predict a higher pregnancy rate [21], this observation suggests that GH administration early in the recruitment phase appears to be a better method for normal responders.

In addition to the proposed ability of GH to stimulate gonadotropin secretion, we found that patients treated with GH had significantly more high-quality embryos than IVF-ET patients who were not treated with GH; however, the number of embryos transferred, and the improvement in the 2PN fertilization rate in the GH arm of the study did not reach statistical significance. These observations are inconsistent with the conclusions of studies by Folch et al. [22] and Moreira et al. [23], where co-stimulation with GH was reported to improve fertilization and preimplantation embryo development. Another study suggested that higher concentrations of oestrogen in follicular fluid can promote oocyte development and meiotic maturation by binding with oestrogen receptors that are specifically located on the oocyte surface [24]. In addition, experiments showed that GH plays an important role in preantral follicle growth and differentiation, and due to the IGF-I receptors on oocytes, GH might also promote secondary oocyte development by stimulating the formation of IGF-I and IGF-II. Furthermore, GH stimulates the development of small antral follicles to the gonadotropin-dependent stages in addition to the maturation of oocytes [25, 26]. These data indicate that GH directly impacts the ovum, which in turn affects embryo quality [27]. In addition, studies have shown that GHR is present in early embryos and that embryo development to the 2-cell stage, blastocyst, and hatched blastocyst stages can be improved with GH [28, 29]. In conclusion, GH may improve the implantation rate and clinical pregnancy rate by improving embryo quality.

Our patients treated with GH during IVF-ET had significantly greater endometrial thicknesses than patients who did not receive GH, especially in the ≥35-year-old group, suggesting that GH improved endometrial receptivity, which had a potential positive impact on endometrial adhesion, blastocyst endometrium communication, and embryo implantation. This possibility is supported by a meta-analysis that showed adding GH during IVF-ET in women with underdeveloped endometria (<6 mm thickness) significantly improved the morphology and thickness of the endometrium, leading to a significantly higher clinical pregnancy rate [30]. Research by Sbracia showed that the glandular cells of the human endometrium express GH in decidual tissue starting in the late luteal phase [31]. In addition, some studies indicate that injecting mice with GH increases the expression of endometrium-related factors, such as VEGF, EGF, and IGF-1 in the proliferating phase; meanwhile, factors, such as LIF, integrin$\alpha\gamma\beta$3, and MMP-9 increase significantly during the implantation stage [17, 32]. Research by Wolthers et al. shows that oestrogen needs the support and activation of IGF to promote the caryomitosis of endometrial cells and can also promote the proliferation of endometrial glands, blood vessels, and stroma. In addition, expanding the endometrial stroma increases endometrial thickness, which is needed to sustain early pregnancy [33]. In our study, improvements in the implantation and pregnancy rates were likely due to the increase in endometrial thickness.

The available data suggest that GH secretion is related to age. In post-adolescence, the secretion of GH decreases with age, which is why GH hyposecretion is observed in older patients. GH-insufficient states disrupt ovarian function and lead to reproductive difficulties [34]. In our study, GH-treated patients in the ≥35-year-old group had implantation and clinical pregnancy rates that were more than two times higher than those observed during IVF-ET cycles without GH. This result may indicate that adding GH is beneficial for older patients.

In the conclusion, our study shows that normal responders undergoing IVF-ET who are co-treatment with GH achieve higher fertilization rates, greater endometrial thickness, higher implantation rates, and higher clinical pregnancy rates, compared to women of the same status treated within a GnRHa long protocol. Further study is needed to determine the optimal dose, time, and duration of GH administration and to investigate the safety of GH on the patients and their offspring.

Compliance with ethical standards

Conflict of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "Growth hormone co-treatment within a GnRH agonist long protocol improves implantation and pregnancy rates in patients undergoing IVF-ET". We state that we have had full control of all primary data and that agree to allow the Journal to review our data if requested.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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