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Original Article

Effects of growth hormone supplementation in poor responders undergoing *in vitro* fertilization/intracytoplasmic sperm injection treatment

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Abstract: We aimed to evaluate the effects of growth hormone (GH) supplementation on *in vitro* fertilization (IVF) outcomes in patients with poor ovarian response (POR). The 107 patients in the study group received GH co-treated with a GnRH agonist long protocol undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). There were 118 patients without GH co-treatment in the control group. We found that GH supplementation significantly increased the number of retrieved oocytes and the number of matured and fertilized oocytes in women with POR, resulting in significant improvements in the number of available embryos, high-quality embryos, and implantation rate. However, the clinical pregnancy rate and live birth rate were not significantly different between the two groups. In younger POR patients, number of retrieved oocytes, fertilized oocytes, and high-quality embryos were significantly higher when co-treated with GH while significant increase in pre-hCG P_4 level and number of frozen embryo was found only in the older patients. In addition, GH co-treatment significantly increased the implantation rate and pregnancy rate only in older POR patients. GH supplementation in predicted poor responders undergoing IVF/ICSI cycles improves oocytes and embryo developmental potential, thus increasing successful implantation.

Keywords: *In vitro* fertilization, intracytoplasmic sperm injection, growth hormone, poor ovarian response

Introduction

Poor ovarian response (POR) presents a big challenge in the treatment of infertility. The incidence of poor response to ovarian stimulation in assisted reproductive technologies (ART) has been estimated to range from 9% to 24% [1-3] and has been associated with increasing female age, previous ovarian surgery, and body mass index (BMI) [4].

Recently, numerous interventions such as addition of pyridostigmine, L-arginine, testosterone, GH-releasing factor (GHRF), luteinizing hormone (LH), and growth hormone (GH) have been proposed for the management of POR [5]. All of the interventions aim at raising endogenous follicular insulin-like growth factor-1 (IGF-1) levels [6]. With increasing understanding of the GH/IGF-1 axis, which has been shown to improve folliculogenesis and endometrial receptivity, researchers have been focused on the effects of GH use in women undergoing ART treatment, especially in poor responders [7].

However, the effects of GH supplementation on patients with POR to controlled ovarian stimulation (COS) in ART treatment remain controversial [7]. A recent systematic review indicated that GH co-treatment increases the clinical pregnancy rate and live birth rate in poor responders in IVF/ICSI cycles [4], however, the latest meta-analysis did not show the impact of GH co-treatment on the probability of pregnancy in poor responders [8].

We postulated three reasons for the current controversy concerning GH use in poor responders in previous studies: (1) Sampling bias due to small number of patients studied; (2) Clinical variability in definition of POR; and (3) The age of poor responders, which influences the outcomes of ART. Therefore, in this study, we assessed the age-dependent effects of GH co-

treatment with a gonadotropin-releasing hormone (GnRH) agonist long protocol in poor ovarian responders.

Materials and methods

In our retrospective study, we analyzed the outcomes of 225 infertile Chinese women who visited Infertility Clinics at Women's Hospital, Zhejiang University, for IVF/ICSI from January 2010 to December 2014. According to Bologna consensus criteria [9], patients who were classified as poor ovarian responders should have at least two of the following risk factors: (1) Age ≥ 40 years, (2) Previous POR (≤ 3 retrieved oocytes and with subsequent three or less obtained embryos using GnRH agonist long protocol), (3) Antral follicle count (AFC) ≤ 7, and/or basal follicle stimulating hormone (FSH) ≥ 10.0 IU/L. Exclusion criteria were a day-3 FSH > 20 IU/L, or with diagnosis of polycystic ovary syndrome, endocrine or metabolic disorders, unilateral oophorectomy, and severe endometriosis. This study was approved by the Ethics Committee of Women's Hospital, Zhejiang University School of Medicine, China.

Starting on day 21 of the preceding cycle, 107 patients in the study group received daily injections of GnRHa (Decapeptyl or Diphereline; Ipsen, Paris, France) at a starting dose of 0.1 mg. The daily dose was decreased to 0.05 mg after the confirmation of pituitary downregulation and was maintained until the day of recombinant human Chorionic Gonadotropin (rhCG, Serono) administration. Pituitary downregulation was confirmed by an ultrasound scan showing an endometrial thickness < 5 mm and serum concentrations of estradiol (E_2) < 110 pmol/L and LH < 3 IU/L. Then, patients received gonadotrophins with recombinant FSH (Gonal-F; Serono, Abonne, Switzerland) at a starting dose of 225-450 IU per day for 4-5 days according to ovarian reserve function. From day 5-6 of stimulation, urinary human menopausal gonadotrophin (hMG, Lizhu Pharmaceutical Trading Co., China) was added based on patient's ovarian response. A daily subcutaneous injection of 5 IU growth hormone (recombinant human growth hormone, rhGH, China) was given with the starting use of gonadotropins until the day of hCG administration. Follicular development was monitored with vaginal ultrasound as well as the endocrine profile for two to four consecutive days. When two or more follicles > 17 mm in diameter were achieved, 6500 IU hCG was administered. Oocyte retrieval by ultrasound-guided transvaginal aspiration was performed 36 hours after hCG administration. The 118 patients in the control group received the same treatment except GH administration.

Semen samples were washed and prepared using the classical density gradient procedure [10]. IVF or ICSI was carried out according to seminal parameters and embryo culture was performed as previously described [11]. Embryo quality was evaluated and graded according to embryo viability with morphological predictors as previously described [12]. One to three embryos with the highest scores were chosen for embryo transfer (ET) on day 3 of embryo culture. The luteal phase was supported, beginning on the third day after hCG injection, with natural progesterone (Utrogestan®; Besins, France) administered via the vaginal route (90 mg/day) combined with oral Duphaston (Abbott Biologicals B.V., U.S.) (40 mg/day). The number of available embryos was defined as the sum of the number of embryos transferred and number of embryos frozen. Clinical pregnancy was defined as a positive pregnancy test 14 days after embryo transfer followed by a vaginal ultrasound 2 weeks later demonstrating gestational sacs and embryonic cardiac pulse.

Analysis was performed using SPSS version 16.0 for Microsoft Office (SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± SD of quantitative variables or percentage (%) of qualitative ones. Data between the two groups were analyzed by Student's *t*-test for quantitative variables and X^2 tests for qualitative ones. All tests were two-tailed and a *P*-value of < 0.05 was considered statistically significant.

Results

Patient baseline characteristics

The baseline characteristics of patients are shown in **Table 1**. There were no significant differences in age, BMI, infertility duration, number of previous POR cycles, day-3 FSH, LH or E_2 levels, or AFC between the two groups.

IVF/ICSI-ET outcomes in poor responders with GH co-treatment

The results of ovarian stimulation and oocyte quality are displayed in **Table 2**. In GH group,

Table 1. Baseline characteristics of patients with poor ovarian response

Variables	GH group n=107	Control group n=118	*P* value
Age (year)	36.24±5.29	36.97±5.04	0.290
BMI (kg/m^2)	21.69±5.19	21.31±5.24	0.586
Duration of infertility (year)	6.01±4.59	6.18±4.83	0.789
Previous POR cycles (n)	0.93±0.55	0.81±0.64	0.133
No. of AFC (n)	5.55±2.49	5.20±2.10	0.257
Day-3 FSH (IU/L)	9.70±3.24	10.30±2.94	0.148
Day-3 LH (IU/L)	4.26±1.96	4.27±2.49	0.997
Day-3 E_2 (pmol/L)	164.98±226.82	133.44±66.58	0.150

Table 2. Ovarian stimulation and oocyte quality in poor responders with or without GH co-treatment

Variables	GH group n=107	Control group n=118	*P* value
Total dose of gonadotropin (IU)	3604.51±1354.70	3685.93±1394.14	0.658
Stimulation period (day)	9.87±1.88	10.29±2.66	0.178
LH on hCG day (IU/L)	2.21±3.16	2.38±1.91	0.611
E_2 on hCG day (pmol/L)	6333.39±4622.49	5686.37±3159.25	0.218
P_4 on hCG day (nmol/L)	1.91±1.10	2.19±1.44	0.104
No. of retrieved oocytes (n)	6.52±4.09	5.20±2.27	0.003*
No. of fertilized oocytes (n)	4.74±3.82	3.54±2.04	0.003*
Oocyte retrieved rate (%)	98	96	0.394
Fertilization rate (%)	67	70	0.353
No. of 2PN oocytes (n)	4.02±3.17	3.14±1.65	0.008*
2PN fertilization rate (%)[a]	68	66	0.720
No. of MII oocytes (n)	4.28±3.08	3.44±2.46	0.022*
MII oocytes rate (%)	65.64	66.15	0.897
Non-fertilized oocytes (n)	0.84±1.34	1.00±1.28	0.364
Non-fertilized rate (%)	19	20	0.844

**P* value < 0.05 is considered statistically significant; [a]2PN fertilition rate=2PN number of fertilized oocytes/retrieved oocytes.

the 107 patients had more retrieved oocytes (*P*=0.003), metaphase II stage (MII) oocytes (*P*=0.022), and two pronuclei (2PN) oocytes (*P*=0.008) than those patients without GH co-treatment. The differences in the days of gonadotropin stimulation, the total doses of gonadotropin used, the mean E_2 value on the hCG day, and fertilization rate were not statistically significant between the two groups.

Embryo quality and pregnancy outcomes are shown in **Table 3**. The patients who received GH had significantly higher number of high-quality embryos (*P*=0.049), number of available embryos (*P*=0.028), and higher implantation rate (*P*=0.014) than those in the control group. These two groups did not differ significantly in number of cycles reaching ET, number of transferred embryos, clinical pregnancy rate, abortion rate, and live birth rate.

Age-dependent IVF/ICSI-ET outcomes in poor responders with GH co-treatment

Furthermore, we divided our patients into four subgroups based on patient age (**Table 4**). In the older groups (≥ 40 years), 44 patients who received GH had higher mean P_4 value on the hCG day, number of frozen embryos, implantation rate, and clinical pregnancy rate than those 54 patients who only received gonadotropin during IVF/ICSI-ET. Interestingly, 12 patients who received GH had cycles with frozen embryos, from which a total of 46 embryos were cryopreserved. In those patients who did not receive GH, 11 patients had cycles with cryopreserved embryos, from which a total of 19 embryos were cryopreserved. On the other hand, there were no statistically significant differences in the days of gonadotropin stimulation, the total doses of gonadotropin used, number of 2PN embryos, number of high-quality embryos, number of transferred embryos, and fertilization rate between the groups with/without GH co-treatment (**Table 4**).

In the younger group (< 40 years), 63 patients who received GH had higher mean E_2 value on the hCG day, number of retrieved oocytes, number of 2PN oocytes, and number of high-quality embryos than those in the 64 patients who did not receive GH during IVF/ICSI-ET. These two groups did not differ significantly in the days of gonadotropin stimulation, the total doses of

Table 3. Embryo quality and pregnancy outcomes in poor responders with or without GH co-treatment

Variables	GH group n=107	Control group n=118	*P* value
No. of cleavage embryos	3.95±3.22	3.05±1.65	0.008*
No. of high-quality embryos	2.17±2.27	1.67±1.46	0.049*
No. of transferred embryos	2.28±0.72	2.35±0.68	0.444
No. of frozen embryos	1.06±2.13	0.42±0.92	0.004*
No. of available embryos	3.21±2.39	2.64±1.37	0.028*
Rate for patients reached ET	94	94	0.917
Implantation rate (%)[a]	25	15	0.014*
Clinical pregnancy rate per ET (%)[b]	35.64 (36/101)	26.13 (29/111)	0.135
Abortion rate per ET(%)	55.56 (20/36)	44.83 (13/29)	0.398
Live birth rate per ET (n, %)	15.84 (16/101)	13.51 (15/111)	0.773

*P value < 0.05 is considered statistically significant; [a]Implantation rate=total number of implantation embryos/ total number of transplanted embryos; [b]Clinical pregnancy rate per ET=clinical pregnancy cycles/transplantation cycles.

gonadotropin used, number of transferred embryos, fertilization rate, implantation rate, and clinical pregnancy rate (**Table 4**).

Discussion

Our retrospective analysis demonstrated that GH supplementation increased the number of retrieved and fertilized oocytes in POR females. This resulted in significant improvements in the quality and number of available embryos, leading to successful implantations. On the other hand, the statistical difference was not identified in clinical pregnancy rate and live birth rate. If the age of the patients were taken into account, we found that the implantation rate and pregnancy rate were significantly increased in older POR females who received GH co-treatment during IVF/ICSI-ET while the differences were not displayed between the younger groups.

It has been shown that poor responders demonstrate lower intrafollicular IGF-1 concentrations [13], which is likely associated with the impaired ability of the follicles to respond to FSH. Exogenous GH regulates the effect of FSH on granulose cells via increasing the synthesis of IGF-1 and plays important roles in enhancing proliferation and aromatase activity of granulosa cells and inhibiting follicle apoptosis [4]. Several studies have demonstrated that GH as an adjuvant treatment improved the outcomes of IVF/ICSI cycles in poor responders, increased the number of retrieved oocytes or/and MII oocytes [14-16], and increased fertilization rate and more embryos available for transfer [14, 15]. Similarly, our data confirms previous findings and proves that GH improves implantation rate by increasing the quality of collected oocytes and embryos in POR patients.

In contrast to previous studies [14, 17], we did not find the positive effects of GH co-treatment on ovarian stimulation such as the duration of stimulation, the total doses of gonadotropin used, and mean E_2 value on the hCG day. One possible explanation is a shorter usage of GH with the starting use of gonadotropins in IVF/ICSI cycles, thus GH mainly affected oocyte competency. In a prospective randomized trial [14], Kucuk T et al. found a shorter mean duration of stimulation and decreased doses of FSH but the peak serum estradiol concentration achieved at the hCG day and number of 2PN embryos was significantly higher in women with GH co-treatment in the luteal phase of previous cycle than those without GH treatment. Combined with our results, we suggest that longer usage of GH from the previous luteal phase would enhance follicular recruitment and follicular response to FSH in the approaching ovarian stimulation cycle.

In addition to the proposed ability of GH to increase ovarian responsiveness, it has been reported that GH directly improves oocytes and embryos quality in IVF/ICSI-ET cycles. GH receptors at the mRNA transcript level are expressed in human oocytes and throughout the pre-implantation period of embryos [18]. Recent studies have revealed that GH has a specific stimulatory role in oocyte maturation via GH receptors to activate transcription [19]. Studies using bovine oocytes have shown that addition of GH to the culture medium accelerates cytoplasmic and/or nuclear maturation of cumulus enclosed oocytes which promotes early embryonic development following IVF [20, 21]. Moreover, GH modulates mitochondrial activity and/or calcium homeostasis [22] via

Table 4. Comparison of ovarian response and reproductive outcomes in poor responders based on age with or without GH co-treatment

Variables	Older group (≥ 40 years)			Younger group (< 40 years)		
	GH group n=44	Control group n=54	*P* value	GH group n=63	Control group n=64	*P* value
Total dose of Gn (IU)	3775.57±1317.33	3869.44±1465.46	0.739	3485.04±173.61	3531.09±165.34	0.848
Stimulation period (day)	9.41±2.17	9.91±2.17	0.261	10.19±0.20	10.61±0.37	0.328
E_2 on hCG day (pmol/L)	5048.95±3068.75	6231.14±3116.38	0.063	7230.47±666.80	5226.73±393.23	0.010*
P_4 on hCG day (nmol/L)	1.62±0.97	2.43±1.87	0.011*	2.11±0.15	2.00±0.12	0.525
No. of retrieved oocytes (n)	5.91±3.42	5.39±2.20	0.364	6.95±0.57	5.05±0.29	0.003*
No. of fertilized oocytes (n)	3.86±2.95	3.37±1.73	0.306	5.35±4.24	3.69±2.27	0.007*
Fertilization rate (%)	68	70	0.740	66	71	0.367
No. of 2PN oocytes (n)	3.70±2.99	3.17±1.59	0.257	4.24±0.42	3.11±0.21	0.017*
No. of high-quality embryos	1.75±1.93	1.61±1.38	0.679	2.46±0.31	1.72±0.19	0.043*
No.of transferred embryos	2.44±0.79	2.58±0.67	0.358	2.18±0.09	2.15±0.08	0.834
No. of frozen embryos	1.05±2.15	0.35±0.76	0.029*	1.06±0.27	0.48±0.13	0.054
Implantation rate (%)	19	3	0.001*	28	26	0.676
Clinical pregnancy rate per ET (%)	30.77 (12/39)	7.69 (4/52)	0.004*	38.71 (24/62)	42.37 (25/59)	0.685

**P* value < 0.05 is considered statistically significant.

upregulating its own receptors and improves oocyte quality [23]. It has been shown that higher levels of GH in follicular fluid are associated with rapidly cleaving embryo, good embryo morphology, and a high embryo implantation potential [24]. Here, we found that poor responders with GH administration achieved significantly more MII oocytes, 2PN oocytes, and high-quality embryos than those without GH co-treatment, consistent with previous studies [14, 19]. Supplementation of GH was associated with significant improvement in the ability of oocytes to form morphologically normal and competent embryos for implantation.

Supplementation of GH and day-2 transfer have been recommended to increase pregnancy rates in poor responders undergoing IVF [5]. Kolibirainakis et al. also demonstrated that GH co-treatment in poor responders with GnRH agonist and gonadotropins increases clinical pregnancy rate and live birth rate [4]. However, these effects were not observed in our patients. The results suggest that pregnancy rate is influenced by a variety of factors and that no single intervention could reliably increase clinical pregnancy rate in poor responders. Addition of GH appears to improve their prognosis but does not fully restore it to normal levels, as seen in normal responders. In other words, GH deficiency is only one part of their reproductive problem [19].

Furthermore, we found that the effects of GH co-treatment vary with patient age. Older women (≥ 40 years) have lower ovarian response and decreased oocyte quality. The oocytes which were retrieved from older women, particularly from women over 40 years of age, showed significant decrease in expression of GHRs and amount of functional mitochondria [6]. Younger poor responders (< 40 years) present with either premature reduction of ovarian reserve or elevated FSH threshold. In our study, GH co-treatment demonstrated positive effects in ovarian response and oocyte maturation in younger groups. But these beneficial effects did not improve implantation rate and pregnancy rate. In contrast, we found that GH co-treatment achieved significant improvement in implantation rate and clinical pregnancy rate in older POR patients. The clinical pregnancy rate for women aged ≥ 40 years was 12/39 in the GH group and 4/52 in those without GH co-treatment. The combined results showed a 30.77% pregnancy rate in older women treated with GH compared with 7.69% for their control counterparts. These results are consistent with the observations by Yovich and Stanger who reported clinical pregnancy rates of 12/49 in older women with GH treatment and 1/29 without GH treatment [19]. Tesarik et al. found that co-administration of GH in women over 40 years of age significantly improved the delivery rate from 4% to 22% in a very-poor-prognosis group of women with an average age of 42 years and a mean of almost three failed cycles [25]. Taken together, we suggest that the target population of GH co-treatment is older POR females [7]. However, this

suggestion needs further RCT study to confirm.

In conclusion, our study demonstrates that GH addition improves oocytes and embryos developmental potential and increases successful implantation in poor responders undergoing IVF/ICSI-ET cycles.

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Disclosure of conflict of interest

None.

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