The effect of growth hormone on low-prognosis patients: a retrospective study based on POSEIDON criteria

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Abstract

Objective: To investigate the effect of growth hormone on low-prognosis patients who were diagnosed by POSEIDON criteria.

Methods: Poor ovarian reserve patients were included if their AMH <1.2ng/ml, and according to POSEIDON criteria they were further divided into Group 3 and Group 4. Mild stimulation protocol was adopted in all groups. Growth hormone was administered in GH group. Further study on non-first cycles was conducted. Linear regression and logistic regression analysis were carried out to control the confounding factors.

Results: A total of 574 cycles (155 in GH group, 419 in Control group) were analyzed. For all the poor ovarian reserve patients, there were no significant differences between GH and control group, in aspects of HMG dosage, duration of HMG, the number of oocytes retrieved, the number of 2PN, the number of embryos, transferrable embryos ,good-quality embryos, clinical pregnancy rate, miscarriage rate and clinical live birth rate. In stratified analysis, the variables of G4 were not significantly different, and in G3, duration of HMG (8.11±1.86vs. 8.80±1.98, P 0.05) was significantly different. Further study on non-first cycle patients, a total of 274 cycles (122 in GH group, 152 in Control group) were analyzed, there were no differences between GH group and control group, in aspects of HMG dosage, duration of HMG, the number of oocytes retrieved, the number of 2PN, the number of embryos, transferrable embryos ,good-quality embryos, clinical pregnancy rate, miscarriage rate and clinical live birth rate. In stratified analysis, the variables of G3' were not significantly different. In G4', duration of HMG (8.74±2.31 vs. 7.90±2.56, P 0.05) was significantly different, the number of oocytes retrieved(8.74±2.31 vs. 7.90±2.56, P 0.05), clinical pregnancy rate(22.97% vs. 8.45%, P 0.05), and clinical live birth rate(14.86% vs. 4.23%, P 0.05) were significantly different between GH and control groups. The outcomes of linear regression and logistic regression analysis were approximately consistent with that of Chi square test.

Conclusions: GH co-treatment with the mild stimulation protocol in poor ovarian reserve patients who reached or were older than 35 years old, and failed in at least one previous cycle, could significantly increase the number of oocytes retrieved, clinical pregnancy rate and live birth rate.

Introduction

According to WHO, 10%-25% reproductive aged couples are suffering from infertility, while assisted reproductive technology (ART) has made great progress[1].Among the infertile population, there's a subpopulation which often respond poorly to controlled ovarian stimulation termed as poor ovarian responders (POR), they usually get diminished ovarian reserve, fewer oocytes retrieved, poor embryos, low pregnancy rate, and eventually reduced live birth rate, in assisted reproduction, they are termed as 'poor ovarian responders'. The incidence of POR during ovarian stimulation has been reported to range from 5.6 to 35.1% [2-6]. Although the concept has been brought up over 30 years, there's no definite definition until 2011, ESHRE set up 'Bologna criteria' to standardize the definition of 'poor ovarian response', which suggested that classification of a poor responder requires two of the following features: (i) advanced maternal age (≥40 years) or other risk factors for poor ovarian response;(ii) a previous poor ovarian response (≤3 oocytes with a conventional stimulation protocol);(iii) an abnormal ovarian response test (antral follicle count 5-7 or anti-Mullerian hormone 0.5-1.1 ng/ml [3.6-7.9 nmol/l])[7].

Thereafter, 'Bologna criteria' is widely used in numerous studies, as the application of 'Bologna criteria' in different clinical researches, the limitations are more and more obvious. The three main shortcomings of the criteria are: 1) it classified heterogeneous patients who have different prognosis into the same group, and this may lead to the situation that different subgroups use the same stimulation protocol resulting different clinical outcomes[8]; 2) the range to predict POR is wide and vague [9]; 3) 'other risk factors for poor ovarian response' is an ambiguous standard without definite meanings[10]. Therefore, in 2016 Alviggi et al. attempted to establish a new classification by proposing the Poseidon criteria and changing the concept of POR to 'low prognosis ', dividing patients into four groups with different degrees of low prognosis. The criteria are based on ovarian low response heterogeneity and are used to classify patients into different prognostic categories to better reflect the reproductive potential of these patients [11]. The POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) criteria suggest the following four groups with different degrees of low prognosis: (1) Group 1: Patients <35 years of age with good ovarian reserve (AFC ≥5, AMH ≥1.2ng/ml) who present with an unexpectedly poor or suboptimal ovarian response. The group was further divided into: Group 1a, with less than 4 eggs; Group 1b, with 4 to 9 eggs.

(2) Group 2: Patients ≥35 years of age with good ovarian reserve (AFC ≥5, AMH ≥1.2ng/ml) but with an unexpectedly poor or suboptimal ovarian response. The group was further divided into: Group 1a, with less than 4 eggs obtained; Group 1b, with 4 to 9 eggs. (3) Group 3: Patients <35 years of age with low ovarian reserve (AFC <5, AMH <1.2ng/ml). (4) Group 4: Patients ≥35 years old with low ovarian reserve (AFC <5, AMH <1.2ng/ml).

In recent years, 'POSEIDON criteria' and the concept 'low-prognosis' have been adopted and further studied by many researchers in their studies on people who were classified as POR before by 'Bologna criteria'. Due to the stratification of different subgroup patients, the outcomes are more convincible and consistent [12-14].

There are diverse stimulation protocols proposed to improve clinical outcome in these women, in POR population, GHRH-a download protocol and antagonist protocol are suggested in many literatures[15]. However, mild stimulation protocol has been few mentioned, which has some superiority over POR population, such as shorter duration and dosage of Gn, low cost and comparable effect compared with conventional stimulation protocols [14].

Besides stimulation protocols, researchers have also been focusing on different adjuvant agents to improve the quality of oocytes. Abundant adjuvant agents are used currently, the most frequently proposed adjuvant therapies include growth hormone, dehydroepiandrosterone and coenzyme 10. GH is the one mostly attract reproductive clinicians to investigate its effect on poor responders. The true effects of the mentioned adjuvant agents are debatable [2, 16-18]. GH is a 191-amino acid, single-chain polypeptide that is synthesized, stored and secreted by the somatotroph cells within the lateral wings of the anterior pituitary gland[19]. It plays an important role in female reproductive system, different experiments either on human or animals reveal that GH binds to its receptor on theca cells, granular cells and oocytes, acting directly or indirectly through IGF-1to influence the development of follicles in various stage, so there're physiological evidences that GH may refine the quality of oocytes and the ovarian response[20, 21]. Although there is no consensus on the timing or the

duration or the dosage or the frequency should GH be added, there's a trend that GH need to be added in the previous cycle of the Gn cycle in a relatively low dosage[22].

Materials and methods

Patients

A retrospective analysis was performed on poor ovarian responders who received mild stimulation protocol in the Reproductive Medicine Center of Wuxi Maternal and Child Health Hospital in the period from May 2018 to November 2021. The Ethics Committee of Wuxi Maternal and Child Health Hospital gave a positive approval for this retrospective study. Informed consent was obtained from all participants included in the study.

Poor ovarian reserve patients were included if their AMH <1.2ng/ml, and according to POSEIDON criteria they were further divided into Group 3 and Group 4 which were: Group 3: Patients <35 years of age with low ovarian reserve (AFC <5, AMH <1.2ng/ml), Group 4: Patients ≥35 years old with low ovarian reserve (AFC <5, AMH <1.2ng/ml). The exclusion criteria were as follows: (1) abnormalities of uterine cavity or untreated hydrosalpinx; (2) a history of malignant or border line tumors; (3)systemic or endocrine or metabolic disorders such as cardiovascular and hepatic and kidney diseases and diabetes mellitus; (4) abnormal karyotypes; and (5) azoospermia or severe oligospermia. The patients were divided into two groups, GH group and control group. We performed a stratified analysis by age to get a further comprehension of the effect of GH on clinical results. All patients were divided into POSEIDON group 3 (G3, age<35 years old) or group 4 (G4, age≥35 years old),

Stimulation protocol

Both groups adopted mild stimulation protocol: ovarian stimulation started with a daily dose of 50 mg of Clomiphene citrate (Fertilan; Codal Synto Ltd, Cyprus) and 150 IU-225IU HMG (Menotropins for injection; Maanshan Fengyuan Pharmaceutical company, China) according to BMI and ovarian function from Day 3 of menstrual cycle, when dominant follicles reached 18mm in diameter, hCG (Chorionic Gonadotropin for injection; Livzon Pharmaceutical Group Inc., China) was injected for trigger. Transvaginal ultrasound guided oocyte retrieval was done 36h later.

In GH group, 4 IU of GH (Haizhiyuan;Sinobioway Hygene, Zhongshan, China) was administered from the preceding menstrual cycle on day 15 every other day, from the first day of the stimulation cycle, GH was injected subcutaneously every day until ovum pick-up (OPU). In control group, patients received mild stimulation without GH treatment.

Embryo assessment

The cleavage stage embryos were graded by the international grading system: grade1, blastomeres are almost even with no particle cytoplasm, fragmentation rate is <5%; grade 2, blastomeres are slightly uneven with cytoplasm contained some particles, fragmentation rate is between 5 and 10%; grade 3, blastomeres are obviously uneven with obvious particles in cytoplasm, fragmentation rate is between 10 and 20%; grade 4,blastomeres are severely uneven with severe particles in cytoplasm and the fragmentation rate is more than 20%[23]. Blastocysts were graded by Gardner's system[24], according to the extent to which the volume of the embryo is occupied by the blastocoele, the blastocyst stage is graded for 1-6 stages, which are early blastocyst, blastocyst, full blastocyst, expanded blastocyst, hatching blastocyst and hatched blastocyst; inner cell mass is graded for A, tightly packed, many cells, B loosely grouped, several cells, C very few cells; trophectoderm is graded for A Many cells forming a tightly knit epithelium, B Few cells; C Very few cells forming a loose epithelium. Cleavage stage embryos of grade 1-3 are considered as transferrable embryos, one or two transferrable embryos of cleavage stage were cryopreserved on day 3, the rest were cultured sequentially until day 5 or 6, if they formed blastocysts then all be cryopreserved.

Embryo transfer

The endometrium was prepared by hormonal therapy, if the thickness reached 8mm, the one or two embryos would be transferred 3 or 5 days later. Pregnancy was confirmed by serum hCG concentration 12 days after ET in all patients. When the test results were positive, ultrasound evaluations were performed about 4 weeks after transfer. The clinical pregnancy was defined as a gestational sac with fetal heart beat.

Statistical analysis and method

All statistical analyses were conducted using STATA version 16.0 (Stata Corp, College Station, Texas, USA). For quantitative variables, mean and SD values were calculated, while for qualitative ones, frequencies and percentages were calculated. Differences between quantitative variables were assessed by t tests, and qualitative ones by Chi square test. A two-tailed P value < 0.05 was considered statistically significant. Linear regression analysis and logistic regression analysis were used to control the confounders that had significant differences. A coefficientβand odd ratio (OR) with a 95% confidence interval (CI) at P < 0.05 was computed to assess the strength of the association.

Results

A total of 574 cycles (155 in GH group, 419 in Control group) were available for analysis. Basal characteristics between the two groups were listed in table 1. In terms of age (35.88±5.76vs. 34.10±4.99, P

0.05), basal FSH (10.41±4.06vs. 9.59±3.51, P 0.05), AFC (4.42±2.15vs. 4.87±2.13, P 0.05), there were significant differences between two groups, the rest such as BMI, duration of infertility, AMH, basal LH and basal E2 were not different significantly. In stratified analysis, the variables of G4(100 in GH group, 172 in Control group) were not significantly different, and in G3(55 in GH group, 247 in Control group), age (29.20±2.77vs. 30.72±2.71, P 0.05) was significantly different. The above mentioned significant differences, we would use linear regression and logistic regression to control them and analyzed the effect of GH.

As to the cycle characteristics and clinical outcomes(table 2), there were no differences between GH and control group, in aspects of HMG dosage, duration of HMG, the number of oocytes retrieved, the number of 2PN, the number of embryos, transferrable embryos ,good-quality embryos, clinical pregnancy rate, miscarriage rate and clinical live birth rate. In stratified analysis, the variables of G4 were not significantly different, and in G3, duration of HMG (8.11±1.86vs. 8.80±1.98, P 0.05) was significantly different, which means for POR patients who were younger than 35 years old, GH may reduce the stimulation time.

Linear regression and logistic regression were played to clarify the real effect of GH on patients (table 3). In group all and G4, there were no significant differences between GH group and control group in HMG dosage, duration of HMG, the number of oocytes retrieved, the number of 2PN, the number of embryos, transferrable embryos ,good-quality embryos, clinical pregnancy rate, miscarriage rate and live birth rate. Only in G3, duration of HMG(β=-0.71, 95%CI(-1.28,0.13), P 0.05) was significantly reduced after GH addition. The above results show that for POR patients who were younger than 35 years old, GH adjuvant may reduce the stimulation time, while GH adjuvant may not bring beneficial effects in other aspects such as good-quality embryos, clinical pregnancy rate and live birth rate etc.

Table 1 Basal characteristics of different groups of patients.

	all			G3			G4		
	GH(n=155)	Control(n=419)	P	GH(n=55)	control(n=247)	P	GH(n=100)	control(n=172)	P
Age(years)	35.88±5.76	34.10±4.99	0*	29.20±2.77	30.72±2.71	0*	39.54±3.05	38.96±3.12	0.139
BMI(kg/m^2)	22.97±2.90	22.85±3.21	0.685	22.54±2.95	22.44±3.39	0.844	23.20±2.85	23.43±2.85	0.527
Duration of infertilty(years)	4.29±3.73	4.38±3.45	0.795	4.04±2.38	4.01±2.54	0.938	4.43±4.31	4.91±4.45	0.39
AMH(ng/ml)	0.66±0.30	0.71±0.30	0.066	0.78±0.26	0.74±0.29	0.345	0.59±0.31	0.67±0.30	0.047*
FSH(IU/L)	10.41±4.06	9.59±3.51	0.018*	9.13±3.02	9.25±3.34	0.807	11.11±4.38	10.09±3.69	0.041*
LH(IU/L)	3.94±1.82	3.73±1.62	0.179	3.69±1.61	3.57±1.74	0.606	4.08±1.92	3.97±1.42	0.606
E2(pg/ml)	42.89±17.91	41.96±17.64	0.578	48.03±18.75	42.16±18.42	0.034	40.06±16.86	41.68±16.50	0.441
AFC(n)	4.42±2.15	4.87±2.13	0.027*	4.96±2.25	5.11±2.16	0.645	4.12±2.05	4.51±2.05	0.13

Table 2 Cycle characteristics and clinical outcomes of different groups of patients.

	all			G3			G4		
	GH(n=155)	control(n=419)	P	GH(n=55)	control(n=247)	P	GH(n=100)	control(n=172)	P
Duration of HMG(days)	8.53±2.06	8.70±2.13	0.399	8.11±1.86	8.80±1.98	0.02*	8.76±2.14	8.56±2.33	0
Dosage of HMG(IU)	1682.90±619.71	1701.61±602.60	0.743	1579.09±506.91	1682.59±555.67	0.206	1740.00±669.29	1728.92±664.97	0
Oocytes retrieved(n)	2.97±2.54	3.40±2.59	0.077	3.35±2.70	3.89±2.79	0.562	2.60±2.38	2.70±2.08	0
2PN(n)	2.05±1.91	2.25±2.00	0.284	2.62±2.09	2.52±2.14	0.753	1.74±1.74	1.87±1.71	0
embryos(n)	2.03±1.94	2.27±2.04	0.194	2.65±2.20	2.55±2.20	0.761	1.68±1.69	1.87±1.71	0
Transferrable embryos(n)	1.38±1.51	1.62±1.66	0.124	1.53±1.71	1.78±1.76	0.346	1.30±1.39	1.39±1.49	0
Good-quality embryos(n)	1.09±1.44	1.28±1.44	0.153	1.24±1.71	1.35±1.50	0.614	1.01±1.28	1.19±1.34	0
Clinical pregnancy(%)	38(24.52%)	121(28.88%)	0.3	18(32.73%)	90(36.44%)	0.604	20(20.00%)	31(18.02%)	0
Miscarriage(%)	9(5.81%)	24(5.73%)	0.971	1(1.82%)	13(5.26%)	0.272	8(8.00%)	11(6.40%)	0
Live birth(%)	29(18.71%)	97(23.15%)	0.254	17(30.91%)	77(31.17%)	0.969	12(12.00%)	20(11.63%)	0

Table 3 Linear regression and logistic regression analysis.

group		β/OR	95%CI	P
all	Duration of HMG	-0.21	(-0.59-0.18)	0.304
	Dosage of HMG	-39.6	(-143.48-64.28)	0.454
	Oocytes retrieved	-0.01	(-0.43-0.42)	0.977
	2PN	0.07	(-0.27-0.41)	0.697
	embryos	0.02	(-0.33-0.37)	0.919
	Transferrable embryos	-0.08	(-0.37-0.21)	0.588
	Good-quality embryos	-0.09	(-0.35-0.17)	0.48
	Clinical pregnancy	1.02	(0.65-1.60)	0.947
	Miscarriage	0.88	(0.39-2.00)	0.763
	Live birth	1.02	(0.62-1.69)	0.946
G3	Duration of HMG	-0.71	(-1.28-0.13)	0.016*
	Dosage of HMG	-119.88	(-270.31-30.55)	0.118
	Oocytes retrieved	-0.19	(-0.96-0.59)	0.634
	2PN	0.27	(-0.33-0.87)	0.376
	embryos	0.26	(-0.37-0.89)	0.413
	Transferrable embryos	-0.15	(-0.66-0.36)	0.572
	Good-quality embryos	-0.05	(-0.50-1.97)	0.986
	Clinical pregnancy	1.5	(0.61-3.71)	0.381
	Miscarriage	0.63	(0.07-5.60)	0.678
	Live birth	1.05	(0.53-2.11)	0.884
G4	Duration of HMG	0.13	(-0.43-0.68)	0.66
	Dosage of HMG	11.56	(-142.30-165.42)	0.882
	Oocytes retrieved	0.19	(-0.29-0.66)	0.44
	2PN	0.05	(-0.35-0.46)	0.803
	embryos	-0.14	(-0.42-0.39)	0.944
	Transferrable embryos	0.05	(-0.30-0.40)	0.782
	Good-quality embryos	-0.06	(-0.39-0.26)	0.693
	Clinical pregnancy	1.65	(0.83-3.30)	0.153
	Miscarriage	1.42	(0.53-3.78)	0.484
	Live birth	1.84	(0.76-4.50)	0.178

Given that in our reproductive medicine center, we don't add GH as a regular treatment principle in the first cycle, if the first cycle failed, we consider to add GH in the next cycle. So we exclude those first cycles of each group to reanalyze the effect of GH on non-first cycles.

A total of 274 cycles (122 in GH group, 152 in Control group) were analyzed. Basal characteristics between the two groups of non-first cycles were listed in table 4. There were no significant differences between two groups in age, BMI, duration of infertility, AMH, basal FSH, basal LH and basal E2. In stratified analysis, the variables of G4'(74 in GH group,71 in Control group) were not significantly different, and in G3'(48 in GH group, 81 in Control group), age (29.17±2.82 vs. 30.79±2.77, P 0.05) was significantly different.

Table 5 shows the cycle characteristics and clinical outcomes of non-first cycle patients, there were no differences between GH group and control group, in aspects of HMG dosage, duration of HMG, the number of oocytes retrieved, the number of 2PN, the number of embryos, transferrable embryos ,good-quality embryos, clinical pregnancy rate, miscarriage rate and clinical live birth rate. In stratified analysis, the variables of G3' were not significantly different. In G4', duration of HMG (8.74±2.31 vs. 7.90±2.56, P 0.05) was significantly different, which means for non-first cycle POR patients who reached or were older than 35 years old, GH addition may not be beneficial, nevertheless, the number of oocytes retrieved(8.74±2.31 vs. 7.90±2.56, P 0.05), clinical pregnancy rate(22.97% vs. 8.45%, P 0.05), and clinical live birth rate(14.86% vs. 4.23%, P 0.05) were significantly different between GH and control groups, all these results indicate that GH may remarkably improve the clinical outcomes of the non-first cycle POR patients who reached or were older than 35 years old.

Linear regression and logistic regression analysis were also performed in non-first cycles (table 6). In group all', the number of oocytes retrieved(β=0.59, 95%CI[0.10, 1.08], P 0.05), the number of 2PN(β=0.51, 95%CI[0.11, 0.91], P 0.05), live birth rate(β=2.08, 95%CI[1.02, 4.25], P 0.05) were significantly improved

in GH group. In G3', the cycle characteristics and clinical outcomes were comparable between GH and control group. In group G4', the number of oocytes retrieved(β=0.91, 95%CI[0.28, 1.53], P 0.05), the number of 2PN(β=0.56, 95%CI[0.05, 1.07], P 0.05), the number of transferrable embryos(β=0.42, 95%CI[0.00, 0.84], P 0.05) , clinical pregnancy rate(OR=3.06 95%CI[1.02, 9.17], P 0.05) and live birth rate(OR=5.26, 95%CI[1.13, 24.49], P 0.05) were significantly improved in GH group. The above results show that for POR patients who were failed in previous cycles, GH may increase the number of oocytes retrieved, 2PNs and live birth rate, for younger ones, GH may have no significant effects on the clinical outcomes, for older ones, GH obviously increase the number of oocytes retrieved, 2PNs, transferrable embryos and clinical pregnancy rate and live birth rate.

Table 4 Basal characteristics of non-first cycle patients.

	All			<35			≥35		
	GH(n=122)	control(n=152)	P	GH(n=48)	control(n=81)	P	GH(n=74)	control(n=71)	P
Age(years)	35.35±5.78	35.01±5.46	0.619	29.17±2.82	30.79±2.77	0.002*	39.36±2.95	39.83±3.35	0.375
BMI(kg/m^2)	23.24±2.97	23.00±3.01	0.513	22.79±2.99	22.83±2.28	0.944	23.53±2.95	23.20±2.67	0.477
Duration of infertilty(years)	4.61±3.78	5.34±3.86	0.117	4.40±2.30	4.85±2.72	0.339	4.74±4.50	5.90±4.80	0.138
AMH(ng/ml)	0.68±0.30	0.64±0.28	0.269	0.79±0.25	0.66±0.30	0.010*	0.60±0.30	0.61±0.26	0.795
FSH(IU/L)	10.20±3.96	9.87±3.88	0.487	9.20±3.14	9.25±3.40	0.948	10.84±4.32	10.57±3.65	0.686
LH(IU/L)	3.78±1.66	3.84±1.86	0.781	3.78±1.65	3.66±2.14	0.735	3.78±1.68	4.05±1.47	0.34
E2(pg/ml)	43.42±17.00	43.73±20.59	0.892	48.64±18.70	44.61±22.33	0.296	40.04±14.98	42.75±18.51	0.334
AFC(n)	4.51±2.14	4.68±2.22	0.508	5.08±2.26	4.94±2.17	0.718	4.14±1.99	4.39±2.25	0.462

Table 5 Cycle characteristics of non-first cycle patients.

	All'			G3'			G4'		
	GH(n=122)	control(n=152)	P	GH(n=48)	control(n=81)	P	GH(n=74)	control(n=71)	P
Duration of HMG(days)	8.48±2.19	8.30±2.34	0.517	8.06±1.96	8.64±2.08	0.12	8.74±2.31	7.90±2.56	0.039*
Dosage of HMG(IU)	1730.53±655.41	1669.24±662.13	0.445	1625.00±524.96	1727.78±614.52	0.335	1798.99±722.85	1602.47±711.09	0.101
Oocytes retrieved(n)	3.16±2.71	2.66±2.11	0.093	3.69±2.80	3.28±2.35	0.382	2.81±2.61	1.96±1.53	0.018*
2PN(n)	2.22±2.03	1.82±1.70	0.073	2.67±2.18	2.16±1.90	0.169	1.93±1.88	1.42±1.34	0.063
embryos(n)	2.15±2.04	1.88±1.87	0.262	2.60±2.27	2.30±2.16	0.444	1.85±1.83	1.41±1.34	0.099
Transferrable embryos(n)	1.47±1.62	1.26±1.28	0.236	1.55±1.78	1.47±1.39	0.767	1.42±1.52	1.03±1.10	0.079
Good-quality embryos(n)	1.14±1.57	0.96±1.00	0.253	1.27±1.81	1.06±1.03	0.403	1.05±1.39	0.85±0.97	0.298
Clinical pregnancy(%)	33(27.05%)	29(19.08%)	0.117	16(33.33%)	23(28.40%)	0.555	17(22.97%)	6(8.45%)	0.017*
Miscarriage(%)	6(4.92%)	8(5.26%)	0.897	0(0.00%)	5(6.17%)	0.079	6(8.11%)	3(4.23%)	0.333
Live birth(%)	26(21.31%)	20(13.16%)	0.073	15(31.25%)	17(20.99%)	0.192	11(14.86%)	3(4.23%)	0.030*

Table 6 Linear regression and logistic regression analysis of non-first cycle patients.

group		β/OR	95%CI	P
All’	Duration of HMG	0.08	(-0.46-0.61)	0.782
	Dosage of HMG	27.86	(-119.36-175.07)	0.71
	Oocytes retrieved	0.59	(0.1-1.08)	0.018*
	2PN	0.51	(0.11-0.91)	0.012*
	embryos	0.35	(-0.09-0.78)	0.118
	Transferrable embryos	0.27	(-0.06-0.6)	0.106
	Good-quality embryos	0.21	(-0.09-0.51)	0.173
	Clinical pregnancy	1.79	(0.97-3.31)	0.062
	Miscarriage	0.84	(0.27-2.61)	0.756
	Live birth	2.08	(1.02-4.25)	0.044*
G3’	Duration of HMG	-0.71	(-1.49-0.07)	0.073
	Dosage of HMG	-113.42	(-322.93-96.1)	0.286
	Oocytes retrieved	0.31	(-0.52-1.13)	0.462
	2PN	0.6	(-0.06-1.26)	0.076
	embryos	0.42	(-0.35-1.19)	0.281
	Transferrable embryos	0.09	(-0.48-0.66)	0.751
	Good-quality embryos	0.18	(-0.34-0.7)	0.489
	Clinical pregnancy	1.5	(0.61-3.71)	0.381
	Miscarriage	/	/	/
	Live birth	1.69	(0.65-4.35)	0.28
G4’	Duration of HMG	0.74	(-0.05-1.53)	0.066
	Dosage of HMG	150.1	(-71.12-371.32)	0.182
	Oocytes retrieved	0.91	(0.28-1.53)	0.005*
	2PN	0.56	(0.05-1.07)	0.033*
	embryos	0.48	(-0.03-0.99)	0.065
	Transferrable embryos	0.42	(0-0.84)	0.049*
	Good-quality embryos	0.24	(-0.15-0.63)	0.235
	Clinical pregnancy	3.06	(1.02-9.17)	0.046*
	Miscarriage	1.47	(0.31-6.98)	0.625
	Live birth	5.26	(1.13-24.49)	0.034*

Discussion

In this retrospective study, we firstly analyze all patients whose AMH less than 1.2ng/ml, GH seemed had no significant effect on these groups, then according to POSEIDON criteria we stratified them by age into two groups which were POSEIDON Group 3 and POSEIDON Group 4, and the results showed that only in POSEIDON Group 3, GH adjuvant may reduce the stimulation time, while it had no beneficial effects in other aspects such as good-quality embryos, clinical outcomes. Taking the actual situation of our own reproductive center into consideration, for the patients who attended the first cycle, we usually did not add GH, that is, we routinely add GH to those ones who failed at least one cycle, so we took a further step to analyze non-first cycle patients to investigate the effect of GH on those who did not success in the first cycle. The conclusion is GH has a significant effect on those who did not success in the first cycle, the stratified analysis by age showed that for non-first cycle POR patients who were younger than 35 years old, GH may not bring positive effects in every respect, neither reduce HMG dosage nor increase live birth rate. Nonetheless, for those who reached or were older than 35 years old, even though GH didn’t shorten duration of HMG, it significantly increased the number of oocytes retrieved, clinical pregnancy rate, clinical live birth rate. The outcomes of linear regression and logistic regression analysis were approximately consistent with that of Chi square test.

In previous researches or systematic review and meta-analysis, for poor ovarian responders, whether classified by Bologna criteria or Poseidon criteria, GH has been proven to be beneficial to the pregnancy outcomes[20, 21, 25]. In these studies or meta-analysis, GH supplementation gets more oocytes retrieved, good-quality embryos, and significantly improve the live birth rate and clinical pregnancy rate, while shorten the duration of stimulation and dosage of gonadotropin, which are consistent with the results of our study. GH acts through different ways, for one aspect, GH up-regulate the local synthesis of insulin-like growth factor-I, which amplifies the effect of gonadotropin action at the level of both granulosa and theca cells, increase the receptor density for granulosal FSHR, BMPR1B, LHR, and GHR, which may improve the maturation process of luteinization in older patients with reduced ovarian reserve[26]. For the second aspect, compared to younger women, the older ones with poor prognosis have a feature of growth hormone deficiency, GH supplementation can reduce IGFBP-3/IGF-1 ratio to a normal range[19], for the third aspect, GH improved embryo quality and implantation rate and alleviated oxidative stress in follicle fluid by influencing Nrf2/Keap1 expression[27].

However, there still are debates about the effect of GH. Some researches or meta-analysis come to a different conclusion. A Cochrane review included 16 RCTs (1352 women), shows that in normal responders, GH has uncertain effect on live birth rates and number of oocytes retrieved, while it slightly increases the number of oocytes retrieved and pregnancy rates in poor responders, while there is an uncertain effect on live birth rates in POR group[18]. And in another research, GH did not improve the clinical pregnancy rates, live birth rates or cumulative live birth rate in POR[17]. Another RCT says It was not possible to demonstrate an increase in live birth rate from the addition of growth hormone in women with a previous poor ovarian response to IVF[16].

In 2020 ESHRE guideline: ovarian stimulation for IVF/ICSI, For predicted poor responders, GnRH antagonists and GnRH agonists are equally recommended[15]. Mild stimulation wasn’t mentioned in this guideline, nonetheless, in clinical practice, for PORs, we mostly apply mild stimulation protocol. In spite of the fact that most POR patients were treated by mild stimulation protocols, the researches of GH application in POR groups using this protocol are few, a retrospective study in 2018 similar to ours got the conclusion that GH treatment in mild stimulation protocol for poor responders could significantly improve good-quality embryo rate, and improve the clinical outcomes, which was in accordance with our conclusion[14]. The difference between the two studies is that, we used HMG as the main stimulation drug while they used gonadotropin. Taking the cost into consideration, maybe our protocol will more cost-efficient to POR patients.

The major limitation of this study is that it's a retrospective study and in G3 study group the sample size is small, yet for PORs who older than 35 years old the sample size is relatively appropriate, and there are some significant baseline differences in some groups, we use regression analysis to control these confounding factors. For all that, the results still provide important information for clinical doctors to make effective therapeutic strategies for POR patients.

In conclusion, our study indicated that GH co-treatment with the mild stimulation protocol in poor responders who reached or were older than 35 years old, and failed in at least one previous cycle, could significantly increase the number of oocytes retrieved, clinical pregnancy rate and live birth rate. Meanwhile, in comparison with the conventional protocols, such as GnRH antagonist and GnRH agonist protocols, mild stimulation protocol is economically more advantageous and avoids extra economic burden for POR patients. So we can sum up that if POR patients experienced one failure cycle, the next cycle GH adjuvant therapy is suggested to improve the clinical outcomes. However, well-designed, multicenter, prospective high-quality RCTs with adequate sample size are still needed to reach more valid consensus.

Declarations

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.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

QPL : Project designing , partial data analysis and manuscript writing. YZ and FX collected the data. HZ and YFG were responsible for statistical analysis.

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Availability of data and materials

Please contact author for data requests.

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