Growth hormone alleviates oxidative stress and improves IVF outcomes in Chinese women with poor ovarian response: A randomized controlled trial

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Abstract

Background Oxidative stress (OS), defined as an imbalance between reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) excessive production and antioxidant insufficient, has been suggested to be involved in the pathogenesis of poor ovarian response (POR). Growth hormone (GH) can function reduce OS in some types of cells. This study investigated whether GH can significantly improve OS and in vitro fertilization and embryo transfer (IVF-ET) outcomes in patients with POR.

Methods This study enrolled 105 and 58 patients with and without POR (controls), respectively, diagnosed according to the Bologna criteria, who underwent conventional IVF-ET. Patients with POR were randomly assigned to two groups: POR-GH group: pretreatment with GH 4IU/d on day 2 of the previous menstrual cycle before IVF till the trigger day; POR-C group: no pretreatment. The markers of OS in follicle fluid (FF),reactive oxygen species (ROS) levels in granulosa cells (GC), and IVF outcomes of the patients were compared between the three groups.

Result(s) The endometrial thickness on trigger day, number of cleaved embryos, higher quality embryos, the rates of implantation and clinical pregnancy were significantly increased in POR-GH group compared with POR-C group ($P<0.05$). Moreover, the FF malondialdehyde (MDA), total oxidant status (TOS), oxidative stress index (OSI) and ROS levels in GC were significantly higher,whereas superoxide dismutase (SOD) and total antioxidant capacity (TAC) were significantly lower in POR-C group compared with non-POR group ($P<0.05$). Furthermore, the FF TAC was significantly increased, whereas TOS, OSI and intracellular ROS levels were significantly reduced in POR-GH group compared with POR-C group ($P<0.05$).

Conclusion(s) Pretreatment with GH alleviated OS and improved oocyte quality and IVF outcomes in patients with POR.

Clinical Trial Registration: Chinese Clinical Trial Registry. ChiCTR1900021269. Registered 8 February 2019, http://www.chictr.org.cn/edit.aspx?pid=35837&htm=4.

Background

Poor ovarian response (POR) remains one of the significant challenges of in vitro fertilization and embryo transfer (IVF-ET). POR patients exhibit higher cycle cancellation rate, fewer oocytes and

cleaved embryos , lower pregnancy rate and higher miscarry rate than individuals without POR [1]. The incidence of POR ranges from 9 to 24% in IVF- ET [2], and which is increasing as women delaying childbirth. The IVF outcome of patients with POR is still disappointed despite using different stimulation protocols and multiple treatment courses [3].

Several factors including advanced female age, ovarian and pelvic surgeries, chemotherapy and radiotherapy are associated with POR[4]. The physiopathology of POR is complicated, including follicular loss by atresia/apoptosis, diminished expression of follicle-stimulating hormone receptor (FSHR), oocyte chromosomal defects and mitochondrial dysfunction [5,6]. Growing evidence suggests that oxidative injuries in ovarian aging is one of the important pathogenesis of POR [6,7]. Low to moderate levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) are involved in physiological processes including defense against infections, cellular signaling systems, and cell growth and differentiation[8]. Excessive ROS/RNS may damage innate antioxidant defense system, destruct proteins, DNA and lipids. Oxidative stress (OS) is defined as an imbalance between ROS/RNS excessive production, and/or decrease of antioxidant defense systems in pathologic conditions[9]. The abnormal morphology, dysfunction or low copy number of mtDNA in mitochondrial were displayed in oocytes of patients with POR. Mitochondrial dysfunction result in lower antioxidant production capacity, excessive ROS accumulation and DNA oxidative damage in oocytes, eventually contributing to poor embryo quality and IVF outcomes[10]. Therefore, treatment with antioxidants may be beneficial in patients with POR.

A meta-analysis reported that GH supplementation could significantly improve the clinical pregnancy rate and live birth rate of patients with POR [3]. The precise mechanism through which GH in patients with POR is not fully understood, and most researches focus on its direct or indirect function by growth hormone receptor (GHR) and insulin-like growth factor-I (IGF-I) [11]. Studies revealed that GH augmented the effect of gonadotropin on granulosa cells (GC) and theca cells via binding to GHR and increasing the synthesis of IGF-I, to improve follicle development and steroidogenesis[11,12]. Moreover, Weall et al. [13] reported that GH improved the expression of GHR, the mitochondrial function and fertilization rate of oocytes in older women. GH can function reduce OS in some types of

cells [14-16]. However, the ability of GH to improve OS in patients with POR has not been assessed in detail. It is very difficult to obtain human oocyte for study. Remarkably, the oocyte is supported and nourished by intimate cross-talk with its surrounding GCs[17]. Follicular fluid (FF) composition reflects the metabolic and hormonal processes occurring in the microenvironment of the maturing oocyte [18]. Therefore, FF and GCs can represent surrogate bioassays to study the biological processes involved in oocyte.

Based on the above evidences, this prospective, randomized, controlled study investigated the effects of GH on markers of OS in FF and GC, and IVF outcomes in patients with POR.

Materials And Methods

Ethics statements

This prospectively, randomized, open-label study was registered in the Chinese Clinical Trial Registry Center (Registration No. ChiCTR1900021269) and approved by the Medical Ethics Committee of Sichuan Provincial Hospital for Woman and Children. Signed informed consent was obtained from all participants. All procedures in this study complied with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration 1975 (2013 revision).

Sample size of calculation was based on the assumption that clinical pregnancy rate would increase threefold percent after GH pretreatment. The CPR in patients with POR in our center was around 13%, 48 patients were required in each group with α of 0.05 and a β error of 0.1 (power = 90%)

Study subjects

The patients with POR (age 33-43 years) diagnosed according to the Bologna criteria [1], who underwent IVF, were enrolled from Reproductive Medicine Center of Sichuan Provincial Hospital for Women and Children (between Feb. 2019 and Dec. 2019). The patients with POR were randomized 1:1 to either GH pretreatment (POR-GH group) or no (POR-C group) (using computer generated random numbers). The excluded criterion include: (1) hydrosalpinx, congenital uterine malformations and/or endometrial disease as tuberculosis, hyperplasia; (2) basal follicle stimulating hormone (bFSH) ≥15 IU/L; (3) systemic lupus erythematosus, sicca syndrome, polycystic ovarian syndrome; (4)

uncontrolled endocrinopathy as diabetes, hyperthyroidism, hypothyroidism, and hyperprolactinemia; (5) underwent IVF-ET treatment within three months; (6) intracytoplasmic sperm injection (ICSI) cycle because of male infertility; (7) supplementation with any antioxidants such as Vitamin E, Vitamin C, CoQ10, beta-carotene and selenium. The tubal factor infertile women (age 20-35 years) with normal ovarian reserve and regular menstrual cycle were recruited as non-POR controls during the same period, who underwent IVF-ET. The exclusion criteria for non-POR group were as the same as POR group.

Medical history of all participants was collected, such as regularity of menstrual cycles, duration of infertility, and history of treatment. Abnormal menstrual cycles included oligomenorrhoea, polymenorrhoea, irregular menstrual cycles, and amenorrhoea. Body weight index (BMI) was calculated by square of body height (M) divided by body weight (Kg). Antral follicle count (AFC) was performed by trans-vaginal ultrasound on day 2-3 of the menstruation or progestin induced withdraw bleeding.

Controlled Ovarian Stimulation (COS) and IVF

All patients underwent Controlled Ovarian Stimulation (COS) with GnRH antagonist protocol. Recombinant follicle stimulating hormone (rFSH) (Gonal-F; Merck-Serono KGaA., Darmstadt, Germany) was injected on day 2 of the menstrual cycle，and rFSH doses were adjusted according to follicle growth and serum hormone levels. In POR-GH group, 4 IU/d recombinant human growth hormone (Jinsai Pharmaceutical Co., Ltd., Changchun, Jilin, China) was injected subcutaneously on day 2 of the previous menstrual cycle before IVF till the trigger day (36-48 days). When a leading follicle reached 12 mm and/or serum E_2 levels reached 300 pg/mL, Ganirelix（Merck Sharp & Dohme co., Ltd, Hoddesdon, United Kingdom）was administered. When at least one follicle was above 18mm, recombinant human chorionic gonadotropin (hCG) (Ovitrelle®; Merck-Serono KGaA.) was administered as trigger. When there were no follicles with diameter ≥14 mm after 10 days of gonadotrophin injected or when peak E_2 level was below 300 pg/mL, the cycle was cancelled. Ultrasound-guided transvaginal oocyte retrieval was performed 36 hours later, and follicle flushing

was not performed. Once oocyte retrieval, only follicle fluid (FF) from follicles with a diameter ≥16mm were collected and immediately centrifuged at 700 g for 5 min in room temperature. The supernatant was stored at -80℃. The precipitates were suspended in 3ml PBS and gently layered in 3 ml 50% Lymphocyte separation medium Beijing Solarbio Science and Technology Corporation, Beijing, Solarbio Science and Technology Co., Ltd, China), then centrifuged at 700g for 10min to remove red blood cells and debris. GC layered at the interface of the gradient were washed twice with 5ml PBS (Nanjing KeyGen Biotech. Co., Ltd., Nanjing, Jiangsu, ChinaKeyGEN Bio TECH Co., Ltd., Jiangsu, China) and was immediately examined ROS using fluorescent microscope and Spectrophotometer. FF or GC from each patient were collected separately and considered as one sample. According to the criteria established by the Istanbul Consensus Workshop on Embryo Assessment, the cultured embryos on day 3 were assessed based on the number of blastomeres and the degree of fragmentation, and higher quality were categorized as grades A/B [19]. One or two embryos with the best morphological grade were selected for transfer. Luteal phase support with intramuscular injection of progesterone 60 mg/d commenced on oocyte retrieval day. Serum hCG was measured 12 days after ET and was considered positive for hCG≥5 IU/mL. The Clinical pregnancy was defined as demonstration of a gestational sac with an embryo showing cardiac activity. Early miscarriage was defined as loss of pregnancy before gestational week 12. The rates of implantation rate, clinical pregnancy and miscarriage were calculated. Ovarian hyperstimulation syndrome (OHSS) was diagnosed according to Navot D et al[20].

We suggest GH 4 IU/d pretreatment on day 2 of the previous menstrual cycle before IVF till the trigger day, because low physiological dose and longer treatment (from the antral follicle stage) might be more beneficial to follicular growth and development[2].

Measurement of endocrine and metabolic parameters

Plasma glucose was measured using the hexokinase method (Beijing Strong Biotechnologies, Inc., Beijing, China). Estradiol (E_2), progesterone (P), total testosterone (TT), luteinizing hormone (LH), FSH, and insulin levels were measured using the electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany). Serum level of anti-Mullerian hormone (AMH) was

measured using enzyme linked immunosorbent assay kit (Guangzhou Kangrun Biotech, Co., Ltd, Guangdong, China). Homeostasis model assessment (HOMA-IR) index was calculated as fasting glucose (mmol/l) × fasting insulin (mU/mL)/22.5 [21]. The intra- and inter-assay coefficients of above variation were less than 5% and 10%, respectively.

OS marker in FF assay procedures

FF malondialdehyde (MDA) concentrations (umol/L) were measured using micro-MDA detection kits (NanJing Jiancheng Bioengineering Institute Co. Ltd., Nanjing, Jiangsu, China) and ultraviolet spectrophotometry (Shanghai Meipuda instrument Co., Ltd., Shanghai, China) was performed at 532 nm. Superoxide dismutase (SOD) was determined using SOD kits (NanJing Jiancheng Bioengineering Institute Co. Ltd.) and spectrophotometry at 450nm. Total antioxidant capacity (TAC) (mmol Trolox Equiv./L) and TOS (umol H_2O_2 Equiv./L) were measured by the semi-automatic microplate colorimetric method using hydrogen peroxide (H_2O_2) or Trolox as a standard curve, respectively [22,23] . The oxidative stress index (OSI) expressed as the ratio of TOS to TAC. The detections were duplicated for each OS parameter. Serum samples from healthy volunteers were pooled for quality control. The intra- and inter-assay coefficients of above variations were less than 5% and 10%, respectively.

Detection of intracellular ROS levels

Using a dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe, ROS level in GC was detected by ROS assay Kit (Beyotime Biotechnology Co., Ltd., Shanghai, China), and green fluorescence was examined by fluorescent microscope (Olympus Corporation, Tokyo, Japan). The examination wavelength was 488 nm and the emission wavelength were 525 nm, respectively. The cell nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI) (NeoFroxx, Frankfurt, Germany). The value of intracellular ROS was measured by NanoDrop UV-Vis Spectrophotometers (Thermo Scientific, Massachusetts, USA), and fluorescence intensities shown as the ratio to the control group (non-POR group).

Statistical Analysis

All data were statistically analysed using SPSS 17.0 software (SPSS Inc., Chicago IL, USA). Continuous

variables were expressed as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to assess for normality of data distribution. Continuous variables with normal distribution were compared using Student-Newman-Keuls test, and Bonferroni's test was used as the post hoc test. Categorical data were compared using Chi-squared tests. Two-tailed *P* values less than 0.05 were considered as statistically significant.

Results

Clinical, basal endocrine and metabolic characteristics of the study population

The flow diagram of participants is shown in Figure 1. Among groups with POR, 11of them presented with abnormal menstrual cycles. Age, FSH/LH ratio and bE_2 were significantly higher, whereas AFC and AMH were significantly lower in POR-GH group and POR-C group, compared with non-POR group (P<0.05). The clinical, endocrine and metabolic characteristics did not significantly differ between POR-GH and POR-C groups (P>0.05). Table 1.

Table 1 The **clinical, basal endocrine and metabolic characteristics of study population**

	POR-GH (n=52)	POR-C (n=53	Non-POR (n
Age (years) [ab]	38.41±2.91	38.20±2.79	29.56±3.
Infertility duration (years)	4.57±3.14	4.39±3.22	3.47±2.2
Abnormal menstrual cycle (n)	6	5	0
BMI (kg/m^2)	22.09±1.73	22.62±2.73	22.19±2.(
FSH / LH ratio [ab]	2.74±1.57	2.53±1.68	1.38±0.7
E_2 (pg/mL) [ab]	75.98±34.05	72.34±26.87	40.62±11.
P (ng/mL)	0.64±0.39	0.54±0.35	0.55±0.2
TT (ng/mL)	0.37±0.19	0.41±0.20	0.43±0.1
AMH (ng/mL) [ab]	0.88±0.67	0.81±0.41	3.50±1.9
PRL (μIU/mL)	162.59±50.19	171.25±52.32	163.51±45
HOMA-IR	1.68±1.25	1.71±1.34	1.67±1.3
AFC [ab]	4.12±1.71	4.06±2.11	15.30±4.5

Note: Data are presented as mean±SD or number. BMI: body mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone; E_2:estradiol; P: progesterone; TT: total testosterone; AMH: anti-Mullerian hormone; PRL: prolactin; HOMA-IR: homeostatic model assessment of insulin resistance; AFC: antral follicle count.

[a] P<0.05 POR-GH group versus non-POR group.

[b] *P*<0.05 POR-C group versus non-POR group;

COS and IVF outcomes were improved in POR-GH group

In POR-GH group, 3 cycles canceled because of no oocytes retrieved or failed fertilization. In POR-C group, 8 cycles canceled because of absence of follicular growth, or failed oocytes retrieval or failed fertilization. In POR-GH group and POR-C group, the rFSH duration, E_2 levels on trigger day, the number of retrieval oocytes, MII oocytes, fertilized oocytes, cleaved embryos and higher quality embryos were significantly lower, whereas the rFSH doses were significantly higher than those in non-POR group ($P<0.05$). The endometrial thickness on trigger day, implantation rate and clinical pregnancy rate were significantly reduced, whereas cancel cycle rate was significantly increased in POR-C group, compared with non-POR group ($P<0.05$). The endometrial thickness (9.65±1.84 vs. 8.61±1.23), number of cleaved embryos (2.31±1.81 vs. 1.73±1.03) and high-quality embryos (1.26±0.65 vs. 0.72±0.56), implantation rate (28.21% vs. 9.72%) and clinical pregnancy rate (38.77% vs. 13.33%) were significantly increased in POR-GH group, compared with POR-C group ($P<0.05$). No side effects and moderate or severe OHSS were reported in study population. Table 2.

Table 2 Controlled ovarian stimulation, IVF outcomes and OS markers in FF

	POR-GH (n=52)	POR-C (n=53	Non-POR (
rFSH doses (IU) [a,b]	2491.46±996.47	2499.87±1345.16	1875.09±4
rFSH duration (d) [a,b]	8.93±2.57	8.97±4.11	10.65±1
E_2 on trigger day (pg/mL) [a,b]	985.13±348.44	887.85±372.21	1956.10±5
Endometrial thickness (mm) [b,c]	9.65±1.84	8.61±1.23	10.33±1
Oocytes retrieved [a,b]	3.71±2.50	3.24±2.56	12.51±6
MII oocytes [a,b]	3.18±1.78	2.87±1.89	9.95±4
Fertilized oocytes (2PN) [a,b]	2.36±1.86	2.02±1.21	7.04±4
Cleaved embryos [a,b,c]	2.31±1.81	1.73±1.03	5.38±3
Higher quality embryos [a,b,c]	1.26±0.65	0.72±0.56	3.34±2
No. ET	1.58±0.51	1.59±0.48	1.43±0
Cancel cycle rate (%) [b]	5.77% (3/52)	15.09% (8/53)	0/58
Implantation rate (%) [b,c]	28.21% (22/78)	9.72% (7/72)	43.37% (3
Clinical pregnancy rate (%) [b,c]	38.77% (19/49)	13.33% (6/45)	53.44% (3
Miscarriage rate (%)	21.05% (4/19)	33.33% (2/6)	6.45% (2
Multiple pregnant rate (%)	15.78% (3/19)	16.67% (1/6)	16.13% (
In FF			
MDA (nmol/mL) [a,b]	2.27±0.69	3.02±1.10	1.33±0
SOD (U/mgprot) [a,b]	12.99±2.32	11.21±1.71	14.76±1
TAC (mmol Trolox Equiv./L) [a,b,c]	0.59±0.13	0.42±0.16	0.71±0
TOS (μmol H_2O_2 Equiv./L) [a,b,c]	8.06±1.19	10.14±4.86	5.67±1
OSI [a,b,c]	16.49±8.19	23.87±10.13	8.16±1

Note: Data are presented as mean±SD or percentage (number). 2PN: Number of two pronuclear zygotes; ET: embryos transferred; FF: follicle fluid; MDA: malondialdehyde; SOD: Superoxide dismutase; TAC: total antioxidant capacity; TOS: total oxidant status; OSI: oxidative stress index. Chi-squared test was performed for comparing the rates of cancel cycle, implantation, clinical pregnancy, miscarriage and multiple pregnancy.

[a] $P<0.05$ POR-GH group versus non-POR group.

[b] $P<0.05$ POR-C group versus non-POR group;

[c] $P<0.05$ POR-GH group versus POR-C group;

The level of OS markers in FF of POR-GH group was decreased

In POR-GH group and POR-C group, values for MDA, TOS and OSI in FF were significantly higher, whereas SOD and TAC were significantly lower than those in non-POR group (P<0.05). The value of

TAC (0.59±0.13 vs. 0.42±0.16) was significantly increased, whereas the value of TOS (8.06±1.19 vs. 10.14±4.86) and OSI (16.49±8.19 vs. 23.87±10.13) were significantly decreased in POR-GH group compared with POR-C group (P<0.05). Table 2.

ROS accumulation in GC of POR-GH group was inhibited

The green fluorescent of ROS intensity in GCs was significantly higher in POR-GH group and POR-C group, compared with non-POR group. The fluorescent of ROS intensity (2.36±0.32 vs. 1.00±0.23) was significantly higher in POR-C group than that in non-POR group(P<0.05). ROS intensity (1.83±0.38 vs. 2.36±0.32) was significantly reduced in POR-GH group, compared with POR-C group. (P<0.05). Figure 1

Discussion

This study found the existence of OS state in FF and GC of patients with POR undergoing IVF. Meanwhile, we found that GH pretreatment lowered TOS and OSI in FF and intracellular ROS level, whereas increased TAC in FF. To the best of our knowledge, this is the first study to report that GH can alleviate OS in FF and GC of patients with POR, we also found that GH apparently improved the endometrial thickness on trigger day, number of cleaved embryos and embryo quality, implantation rate and clinical pregnancy rate. Alleviating OS may be one of the mechanisms that GH improve oocyte quality and IVF outcomes in POR patients.

We found the values of MDA, TOS and OSI in FF were higher, but SOD and TAC were lower in Chinese patients with POR similar with other ethics[6,24,25]. TAC represents the ability to eliminate free radicals. Oyawoye et al. reported[26] that the optimum value of TAC in FF was 0.68 mmol/l for fertilization and subsequent early zygote development, and the value of TAC was decreased when women aged > 37 years. Similarly, we found the value of TAC was 0.71±0.11 mmol Trolox Equiv./L in patients without POR and was decreased to 0.42±0.16 mmol Trolox Equiv./L in patients with POR. MDA is one of the end-products of the lipid peroxidation. TOS represents the antioxidant compounds, and OSI represents the relative level of OS. Interestingly, we found that GH significantly increased the value of TAC, decrease the values of TOS and OSI in FF, accompanying with more cleaved embryos

and higher quality embryos. FF is the important microenvironment for oocyte develop, therefore alleviate OS state in FF OS may improve oocyte quality[25,27]. Above results suggested that GH can improve oocyte quality via suppressing OS level in FF.

To further assess OS state in ovary, we examined the level of ROS in GSs. We found that intracellular ROS levels were significantly higher in patients with POR. Granulosa cells are steroidogenic cells tightly wrapped outside the oocytes. Their metabolic activity is based on mitochondrial and assumed to provide nutrients and maturation-enabling factors for oocyte[28]. Therefore, GCs play an important role in oocyte development, ovulation and fertilization, and ROS accumulation[29]. Low doses of ROS are important signal for oocyte maturation and ovulation. In normal physiological conditions, GCs protect oocytes from OS injury via antioxidant system, such as SOD and E_2 [30]. In patients with POR, antioxidant defense system is suppressed, and more abnormal mitochondrial formation and dysfunction are found in GC[30]. Furthermore, COS induces ROS production in GC due to actively metabolic and proliferation process [31]. In general , above factors trigger GC apoptosis and impair oocyte quality in patients with POR [32]. Here, we found that GH effectively decreased ROS levels in GC. Our result is consistent with the antioxidant effects of GH in other types of cells including vascular endothelial cells, myocardial cells and skeletal muscle cells [14-16] , suggesting an important mechanism that GH improves oocyte quality.

We found GH improves the number of cleaved embryos and embryo quality, accompanying decreased cancel cycle, but not affect the number of retrieved oocytes, MⅡ oocytes and fertilized oocytes. GH seems mainly improves the quality but not quantity of oocytes by alleviating OS and enhancing mitochondrial function [13]. However, other studies reported more overall and fertilized oocytes achieved by treatment with GH, but no difference in embryo quality [33]. The discrepancy results may be due to heterogeneity of GH protocol, different COS protocol and luteal support, or inconsistent POR definition in studies.

In this study, we found the endometrial thickness was thinner in patients with POR, and GH improved endometrial thickness. The uterus is also a site of both GH production and GH action[11]. The glandular cells of human endometrium and decidual tissue express GHR [34]. A series of studies have

confirmed that GH increases endometrial blood perfusion and the expression of receptivity related gene and cytokines to improve the endometrial thickness and receptivity[35,36]. Endometrial receptivity is an important factor for embryo implantation[36]. Therefore, the effects on endometrial is another mechanism of GH improves IVF outcomes of patients with POR.

Accompanying with above benefits, we found that GH improved implantation rate and clinical pregnancy rate in patients with POR. However, other study reported inconsistent results that GH could not improve clinical pregnancy rate[37]. We also found that the miscarriage rate was lower in patients with POR pretreated with GH, but without significant difference. However, Tesarik J et al. [33] reported that GH significantly decreased miscarriage rate. Above inhomogeneous results can be explained by the discrepancies of studies.

This study was not without limitations. the sample size was small and the average age of the patients was 38 years (approximately POSEIDON group 4), so the results should be analyzed and applied cautiously. Moreover, due to limitation of study period, we could not evaluate the effect of GH on the live birth rate, which is a more favorable indicator for IVF outcomes. On other hand，it is still controversial whether GH can improve live birth rate [2,38].

Conclusion

In conclusion, this study enriched the underlying mechanisms that GH improves IVF outcomes in patients with POR. We suggest 4IU/d GH pretreatment on day 2 of preceding cycle till the trigger day in patients with POR. Larger sample size and longer study period will be performed to confirm the effects of GH. The molecular mechanism underlying GH alleviated OS in patients with POR is still not fully revealed further basic research such as cellular experiments in vitro is needed to clarify the mechanisms.

Declarations

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Consent for publication: Not applicable.

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Competing interests: The authors declare that they have no conflict of interest.

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Authors' contributions: Yan Gong designed the study, measured the oxidative stress markers, and wrote the manuscript. Kun Zhang contributed to revise the article. Hao Tan and Sheng-fang Qin contributed to measure intracellular level of reactive oxygen species. Jia-jing Wei and Dong-sheng

Xiong participated in sample collection. All authors read and approved the final manuscript.

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Figures

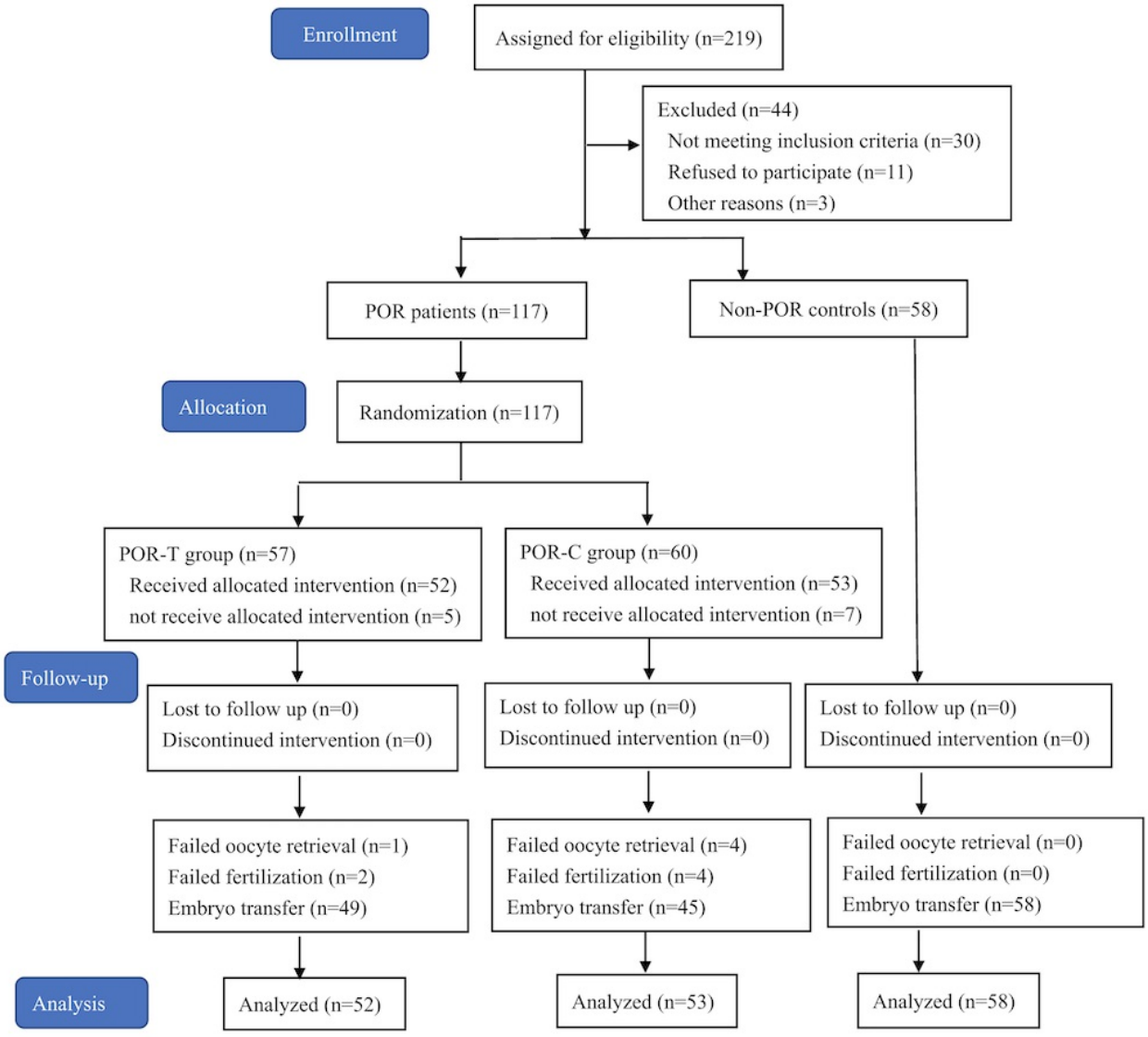
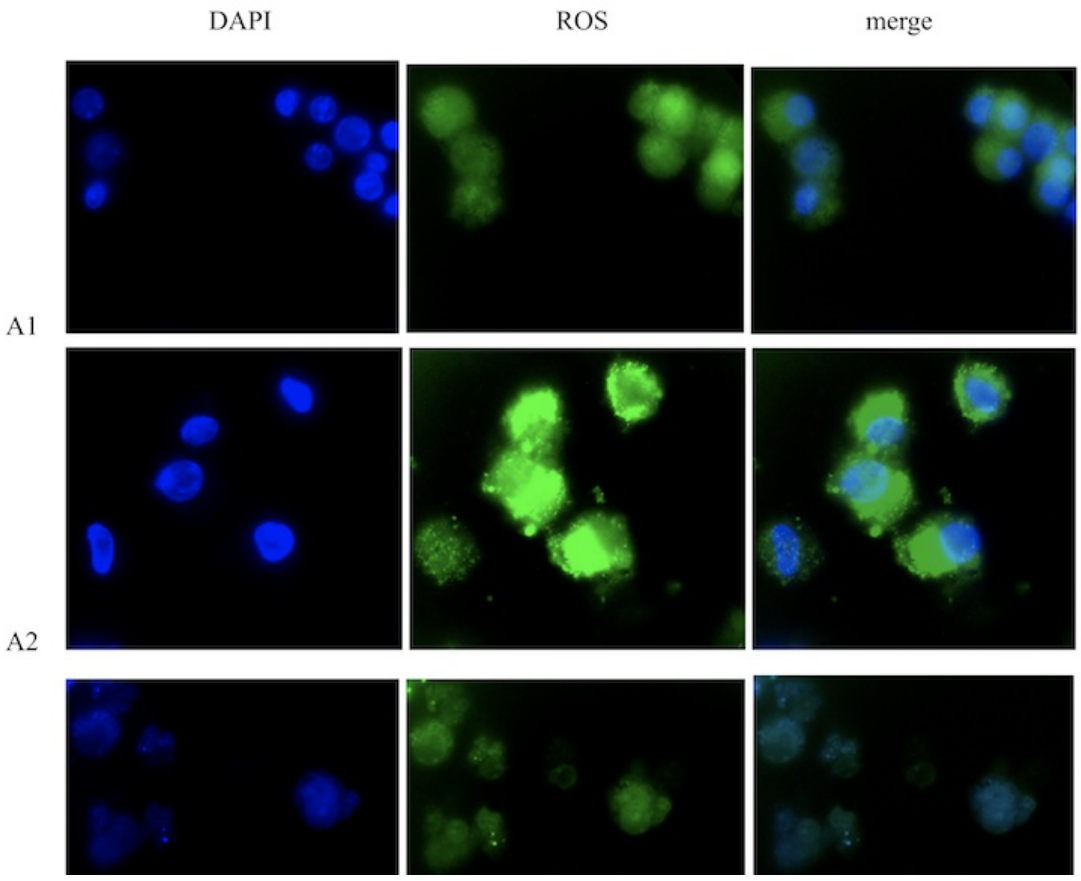


Figure 1

Flow diagram of this randomized controlled trial. Description: Progression from recruitment to completion.

A



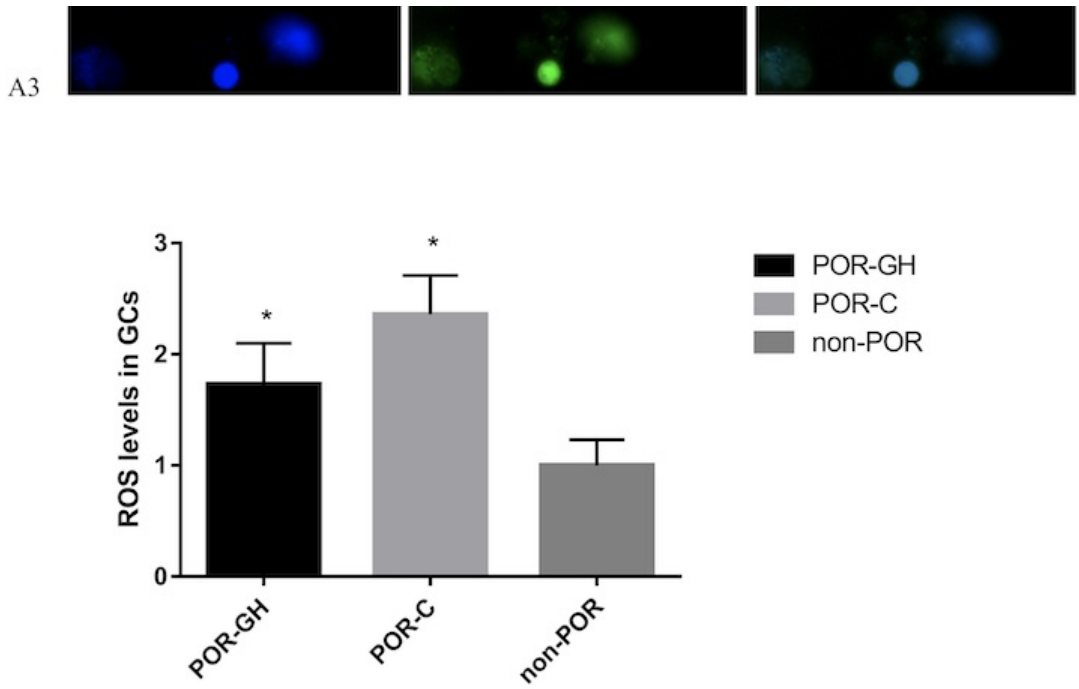


Figure 2

Fluorescence of ROS in GCs of the three groups. Description: A) The green fluorescence of ROS in GCs were observed under a fluorescence microscope. Cell nucleus was stained by DAPI. A1: POR-GH group, A2: POR-C group, A3: non-POR group. B) Measured by Spectrophotometers, the fluorescent of ROS intensity (2.36±0.32 vs. 1.00±0.23) was significantly higher in POR-C group than in non-POR group ($P<0.05$). GH significantly lowered ROS intensity in POR-GH group (1.83±0.38 vs. 2.36±0.32) ($P<0.05$).

Supplementary Files

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