

Growth hormone alleviates oxidative stress and improves the IVF outcomes of poor ovarian responders: A randomized controlled trial

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Research

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Abstract

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

Methods: This study enrolled 105 patients with POR and 58 patients without POR (controls) who were diagnosed according to the Bologna criteria and underwent conventional IVF-ET. Poor ovarian responders were randomly assigned to two groups: the POR-GH group, which received pretreatment with GH 4 IU/d on day 2 of the previous menstrual cycle before IVF until the trigger day, and the POR-C group, which received no pretreatment. The OS markers in follicular fluid (FF), ROS levels in granulosa cells (GCs), and IVF outcomes of the groups were compared.

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

Conclusion(s): Pretreatment with GH alleviates OS and improves oocyte quality in and IVF outcomes of poor ovarian responders.

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) is a significant challenge in the application of in vitro fertilization and embryo transfer (IVF-ET). Poor ovarian responders exhibit a higher cycle cancellation rate, produce fewer oocytes and cleaved embryos, have a lower pregnancy rate and have a higher miscarriage rate than individuals without POR [1]. The incidence of POR in IVF-ET ranges from 9 to 24% and is increasing as women delay childbirth [2]. The IVF outcomes of poor ovarian responders are still low despite their use of 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 and includes follicular loss by atresia/apoptosis, diminished expression of follicle-stimulating hormone (FSH) receptor

(FSHR), oocyte chromosomal defects and mitochondrial dysfunction [5,6]. Accumulative evidence suggests that oxidative stress (OS) in ovarian ageing is an 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 defence against infections, cellular signalling systems, and cell growth and differentiation [8]. Excessive ROS/RNS may damage the innate antioxidant defence system and destroy proteins, DNA and lipids. OS is defined as an imbalance between excessive ROS/RNS production and a decrease in antioxidant defence systems in pathologic conditions [9].

Abnormal morphology, dysfunction and a low mitochondrial DNA (mtDNA) copy number have been observed in the oocytes of poor ovarian responders. Mitochondrial dysfunction results in a lower antioxidant production capacity, excessive ROS accumulation and DNA oxidative damage in oocytes, eventually contributing to poor embryo quality and IVF outcomes [10]. Therefore, antioxidant treatment may be beneficial for poor ovarian responders.

A meta-analysis reported that growth hormone (GH) supplementation could significantly improve the clinical pregnancy and live birth rates of poor ovarian responders [3]. The precise mechanism through which GH functions in poor ovarian responders is not fully understood, and most studies focus on its direct or indirect effects via growth hormone receptor (GHR) and insulin-like growth factor-I (IGF-I) [11]. Studies have revealed that GH augments the effects of gonadotropin on granulosa cells (GCs) and theca cells by 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 increased the expression of GHR, improved mitochondrial function and increased the fertilization rate of oocytes among older women. GH can reduce OS in some types of cells [14-16]. However, the ability of GH to improve OS in poor ovarian responders has not been assessed in detail. It is very difficult to obtain human oocytes for study. Remarkably, the oocyte is supported and nourished by intimate cross-talk with its surrounding GCs [17]. The composition of follicular fluid (FF) reflects the metabolic and hormonal processes that occur in the microenvironment of the maturing oocyte [18]. Therefore, FF and GCs can be used as surrogate bioassays to study the biological processes of the oocyte.

Based on the above evidence, this prospective, randomized, controlled study investigated the effects of GH on markers of OS in FF and GCs and the IVF outcomes of poor ovarian responders.

Materials And Methods

Ethics statements

This prospective, randomized, open-label study was registered in the Chinese Clinical Trial Registry Centre (Registration No. ChiCTR1900021269) and approved by the Medical Ethics Committee of Sichuan Provincial Hospital for Woman and Children. Signed informed consents were 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).

The sample size calculation was based on the assumption that the clinical pregnancy rate would increase threefold after GH pretreatment. The clinical pregnancy rate of the poor ovarian responders in our centre was approximately 13%, and 48 patients were required in each group, with an α of 0.05 and a β error of 0.1 (power = 90%)

Study subjects

The patients with POR (aged 33-43 years) diagnosed according to the Bologna criteria [1], who underwent IVF, were enrolled from the Reproductive Medicine Centre of Sichuan Provincial Hospital for Women and Children (between Feb. 2019 and Dec. 2019). The poor ovarian responders were randomized 1:1 to either GH pretreatment (the POR-GH group) or no GH pretreatment (the POR-C group) (using computer-generated random numbers). The exclusion criteria were as follows: (1) hydrosalpinx, congenital uterine malformations and/or endometrial disease such as tuberculosis or hyperplasia; (2) basal follicle-stimulating hormone (bFSH) level ≥ 15 IU/L; (3) systemic lupus erythematosus, sicca syndrome, or polycystic ovarian syndrome; (4) an uncontrolled endocrinopathy such as diabetes, hyperthyroidism, hypothyroidism, or hyperprolactinemia; (5) IVF-ET treatment within three months; (6) an intracytoplasmic sperm injection (ICSI) cycle due to male infertility; or (7) supplementation with any antioxidants such as vitamin E, vitamin C, CoQ10, beta-carotene or selenium. Women with tubal factor infertility (aged 20-35 years) with normal ovarian reserve and regular menstrual cycles who underwent IVF-ET were recruited as non-POR controls during the same period. The exclusion criteria for the non-POR group were the same as those for the POR group.

Data involving the medical history of each participant were collected and included the regularity of menstrual cycles, duration of infertility, and history of treatment. Abnormal menstrual cycles included oligomenorrhoea, polymenorrhoea, irregular menstrual cycles, and amenorrhoea. Body mass index (BMI) was calculated as body weight divided by the square of body height (kg/m^2). The antral follicle count (AFC) was determined by transvaginal ultrasound on days 2-3 of menstruation or progestin-induced bleeding withdrawal.

Controlled ovarian stimulation (COS) and IVF

All patients underwent controlled ovarian stimulation (COS) with the 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 the POR-GH group, 4 IU/d recombinant human growth hormone (Changchun GeneScience Pharmaceuticals Co., Ltd., Changchun, Jilin, China) was injected subcutaneously on day 2 of the previous menstrual cycle before IVF until 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 18 mm, recombinant human chorionic gonadotropin (hCG) (Ovitrelle®; Merck-Serono KGaA) was administered as the trigger. When there were no follicles with diameters ≥ 14 mm after 10 days of

gonadotrophin injection or when the peak E₂ 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. Upon oocyte retrieval, FF was collected only from follicles with a diameter ≥16 mm measured on the retrieval day. FF samples were immediately centrifuged at 700 g for 5 min at room temperature, and the supernatant was stored at -80°C. The precipitates were suspended in 3 ml PBS, gently layered in 3 ml 50% Lymphocyte separation medium (Beijing Solarbio Science and Technology Corporation, Beijing, Solarbio Science and Technology Co., Ltd, China) and then centrifuged at 700 g for 10 min to remove red blood cells and debris. GCs layered at the interface of the gradient were washed twice with 5 ml PBS (Nanjing KeyGen Biotech. Co., Ltd., Nanjing, Jiangsu, China KeyGEN Bio TECH Co., Ltd., Jiangsu, China) and immediately examined for ROS using fluorescence microscopy and spectrophotometry. FF or GCs from each patient were collected separately and considered to be 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 embryos were categorized as grade A/B [19]. One or two fresh embryos with the best morphological grade were selected for transfer. The remaining embryos were all cultured to the blastocyst stage to be frozen. Luteal phase support with intramuscular injection of progesterone 60 mg/d commenced on the oocyte retrieval day. Serum hCG was measured 12 days after ET, and hCG positivity was considered when hCG ≥ 5 IU/mL. 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 implantation rate was calculated as the observed number of gestational sacs divided by the number of transferred embryos. The clinical pregnancy rate was expressed as the number of clinical pregnancy cycles divided by the number of embryo transfer cycles. The miscarriage rate was expressed as the number of spontaneous pregnancy loss cycles before 12 weeks of gestation divided by the number of clinical pregnancy cycles. 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 until the trigger day because a 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). Oestradiol (E₂), progesterone (P), total testosterone (TT), luteinizing hormone (LH), FSH, and insulin levels were measured using the electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany). The serum level of anti-Mullerian hormone (AMH) was measured using an enzyme-linked immunosorbent assay kit (Guangzhou Kangrun Biotech, Co., Ltd, Guangdong, China). The 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 the above variation were less than 5% and 10%, respectively.

OS marker in FF assay procedures

FF malondialdehyde (MDA) concentrations ($\mu\text{mol/L}$) 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 450 nm. Total antioxidant capacity (TAC) (mmol Trolox Equiv./L) and TOS ($\mu\text{mol H}_2\text{O}_2$ Equiv./L) were measured by the semiautomatic microplate colorimetric method using hydrogen peroxide (H_2O_2) and Trolox, respectively, for the standard curve [22,23]. The oxidative stress index (OSI) was expressed as the ratio of TOS to TAC. The measurements were performed in duplicate for each OS parameter. Serum samples from healthy volunteers were pooled for quality control. The intra- and inter-assay coefficients of the above variations were less than 5% and 10%, respectively.

Detection of intracellular ROS levels

With a dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe, ROS levels in GC were detected by an ROS assay kit (Beyotime Biotechnology Co., Ltd., Shanghai, China). Briefly, GCs were resuspended in PBS and incubated with 10 $\mu\text{mol/L}$ DCFH-DA in the dark for 25 min (37°C) and then incubated together with 10 $\mu\text{g/mL}$ 4',6-diamidino-2-phenylindole (DAPI) (NeoFroxx, Frankfurt, Germany) for 5 min. After the cells were washed three times with PBS, GC suspensions were added to glass slides, and fluorescence was examined by fluorescence microscopy (Olympus Corporation, Tokyo, Japan). The examination wavelength was 488 nm, and the emission wavelength was 525 nm.

Similar with the above protocol but without DAPI, another part of GCs was used to measure the intracellular ROS level by NanoDrop UV-Vis spectrophotometry (Thermo Scientific, Massachusetts, USA). The fluorescence intensities are shown as the intensity of the POR group relative to that of 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 the mean \pm standard deviation (SD). The Kolmogorov–Smirnov test was used to assess the normality of the 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. A two-tailed $P \leq 0.05$ was considered statistically significant.

Results

Clinical, baseline endocrine and metabolic characteristics of the study population

The flow diagram of the participants is shown in Figure 1. Among poor ovarian responders, 38 of them had risk factors for POR (advanced age, abnormal menstrual cycles and ovarian surgery history), 44 of them had previous POR three months ago (≤ 3 oocytes with a conventional stimulation protocol), and 71 of them had an abnormal ovarian reserve test (ORT) (AFC $< 5-7$ and/or AMH $< 0.5-1.1$ ng/ml). Compared to those in the non-POR group, age, basal FSH, FSH/LH ratio and bE_2 were significantly higher, whereas AFC and AMH were significantly lower in the POR-GH group and POR-C group ($P < 0.05$). The clinical, endocrine and metabolic characteristics did not significantly differ between the POR-GH and POR-C groups ($P > 0.05$) (Table 1).

COS and IVF outcomes were improved in the POR-GH group

In the POR-GH group, 3 cycles were cancelled because no oocytes were retrieved or because fertilization failed. In the POR-C group, 8 cycles were cancelled because of the absence of follicular growth, failure of oocyte retrieval or failure of fertilization. Compared to those in the non-POR group, the rFSH duration, E_2 levels on trigger day, number of retrieved oocytes, number of MII oocytes, number of fertilized oocytes, number of cleaved embryos and number of higher quality embryos were significantly lower in the POR-GH group and POR-C group, but the rFSH doses were significantly higher ($P < 0.05$). The endometrial thickness on trigger day, implantation rate and clinical pregnancy rate were significantly reduced, whereas the cycle cancellation rate was significantly increased in the POR-C group compared with the 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), number of 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 the POR-GH group compared with the POR-C group ($P < 0.05$). No side effect or moderate or severe OHSS was reported in the study population (Table 2).

The levels of OS markers in the FF of the POR-GH group were decreased

Compared to those in the non-POR group, the levels of MDA, TOS and OSI in the FF were significantly higher in the POR-GH group and POR-C group, but the levels of SOD and TAC were significantly lower ($P < 0.05$). The TAC (0.59 ± 0.13 vs. 0.42 ± 0.16) was significantly increased, whereas TOS (8.06 ± 1.19 vs. 10.14 ± 4.86) and OSI (16.49 ± 8.19 vs. 23.87 ± 10.13) was significantly decreased in the POR-GH group compared with the POR-C group ($P < 0.05$) (Table 2).

ROS accumulation in GCs of the POR-GH group was inhibited

The green fluorescence intensity of ROS in GCs was significantly higher in the POR-GH group and POR-C group than in the non-POR group. Additionally, the fluorescence intensity of ROS (2.36 ± 0.32 vs. 1.00 ± 0.23) was significantly higher in the POR-C group than in the non-POR group ($P < 0.05$). Furthermore, ROS intensity (1.83 ± 0.38 vs. 2.36 ± 0.32) was significantly reduced in the POR-GH group compared with the POR-C group. ($P < 0.05$). The fluorescence intensity of ROS is expressed as the fold change relative to the control (Figure 2).

Discussion

This study found the existence of an OS state in the FF and GCs of poor ovarian responders undergoing IVF. GH pretreatment lowered TOS and OSI in FF and intracellular ROS levels but increased TAC in FF, suggesting that GH could alleviate OS in the FF and GCs of poor ovarian responders. We also found that GH improved the endometrial thickness on trigger day, increased the number of cleaved embryos, and improved embryo quality, the implantation rate and the clinical pregnancy rate. Alleviating OS may be one of the mechanisms by which GH improves oocyte quality in and the IVF outcomes of poor ovarian responders.

We found that the levels of MDA, TOS and OSI in FF were higher but that those of SOD and TAC were lower in poor ovarian responders, which was similar to other reports [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 that the TAC was decreased in women aged > 37 years. Similarly, we found that TAC was 0.71 ± 0.11 mmol Trolox Equiv./L in patients without POR and that it decreased to 0.42 ± 0.16 mmol Trolox Equiv./L in poor ovarian responders. MDA is one of the end-products of lipid peroxidation. TOS represents the antioxidant compounds, and OSI represents the relative level of OS. Interestingly, we found that GH significantly increased TAC and decreased the values for TOS and OSI in FF, with more cleaved embryos and higher quality embryos. FF plays a critical role in oocyte development; therefore, alleviating OS in FF may improve oocyte quality, which contributes to IVF outcomes [25,27]. The above results suggested that GH could improve oocyte quality, possibly by suppressing the OS level in FF.

To further assess the OS state in the ovary, we examined the level of ROS in GCs. We found that intracellular ROS levels were significantly higher in poor ovarian responders. GCs are steroidogenic cells that are tightly wrapped around the oocyte. Their metabolic activity is driven by mitochondria and is assumed to provide nutrients and maturation-enabling factors to oocytes [28]. Therefore, GCs play an important role in oocyte development, ovulation, fertilization, and ROS accumulation [29]. Low doses of ROS are important signals for oocyte maturation and ovulation. Under normal physiological conditions, GCs protect oocytes from OS injury via antioxidant systems, such as SOD and E_2 [30]. In poor ovarian responders, the antioxidant defence system is suppressed, and more abnormal mitochondrial formation and dysfunction are found in GCs [30]. Furthermore, COS induces ROS production in GCs due to active metabolic and proliferation processes [31]. In general, the above factors trigger GC apoptosis and impair oocyte quality in poor ovarian responders [32]. Here, we found that GH effectively decreased ROS levels in GCs. 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 a possible mechanism by which GH could improve oocyte quality.

We found that GH could increase the number of cleaved embryos and embryo quality, with a concomitant decrease in the cycle cancellation rate; however, GH may not affect the number of retrieved oocytes, MII oocytes or fertilized oocytes. GH may improve the quality but not quantity of oocytes by alleviating OS

and enhancing mitochondrial function [13]. However, other studies have reported that more overall and fertilized oocytes were achieved by GH treatment but that there was no difference in embryo quality [33]. These discrepant results may be due to heterogeneity of the GH protocol, different COS protocols and luteal support, or inconsistent POR definitions between studies.

In this study, we found that the endometrium was thinner in poor ovarian responders and that GH improved endometrial thickness. The uterus is also a site in which both GH production and GH actions are observed [11]. The glandular cells of the 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 genes and cytokines to improve endometrial thickness and receptivity [35,36]. Endometrial receptivity is an important factor for embryo implantation [36]. Therefore, the effects on endometrial tissue may be another mechanism by which GH affects the IVF outcome of poor ovarian responders.

In addition to the abovementioned benefits, we found that GH could improve the implantation and clinical pregnancy rates of poor ovarian responders. However, another study reported inconsistent results, namely, that GH could not improve the clinical pregnancy rate [37]. We also found that the miscarriage rate was lower in poor ovarian responders pretreated with GH, but the difference was not significant. However, Tesarik J et al. [33] reported that GH significantly decreased the miscarriage rate. These conflicting results can be explained by the discrepancies between the 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) [38]; thus, the results should be analysed and applied with caution. Moreover, due to limitations of the study period, we could not evaluate the effect of GH on the live birth rate, which is a more favourable indicator of IVF outcomes. However, whether GH can improve the live birth rate is still controversial [2,39].

Conclusion

In conclusion, this study enriched the underlying mechanisms by which GH could improve IVF outcomes in poor ovarian responders. We suggest 4 IU/d GH pretreatment on day 2 of the preceding cycle until the trigger day in poor ovarian responders. A larger sample size and longer study period will be implemented to confirm the effects of GH. The molecular mechanism underlying GH-alleviated OS in poor ovarian responders is still not fully characterized, and further research should be performed.

Declarations

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Ethics approval and consent to participate: Approval was obtained from the Medical Ethics Committee of Sichuan Provincial Hospital for Woman and Children. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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 analysing data and revising the article. Hao Tan and Sheng-fang Qin contributed to measuring the 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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References

1. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, Definition EwgoPOR. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod*. 2011, 26:1616-1624.
2. Kolibianakis EM, Venetis CA, Diedrich K, Tarlatzis BC, Griesinger G. Addition of growth hormone to gonadotrophins in ovarian stimulation of poor responders treated by in-vitro fertilization: a systematic review and meta-analysis. *Hum Reprod Update*. 2009, 15:613-622.
3. Jevc YB, Bhandari HM. Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis. *J Hum Reprod Sci*. 2016, 9:70-81.
4. Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. *Fertil Steril*. 2009, 91:749-766.
5. Eichenlaub-Ritter U. Oocyte ageing and its cellular basis. *Int J Dev Biol*. 2012, 56:841-852.
6. Tatone C, Amicarelli F, Carbone MC, Monteleone P, Caserta D, Marci R, et al. Cellular and molecular aspects of ovarian follicle ageing. *Hum Reprod Update*. 2008, 14:131-142.
7. Luddi A, Capaldo A, Focarelli R, Gori M, Morgante G, Piomboni P, et al. Antioxidants reduce oxidative stress in follicular fluid of aged women undergoing IVF. *Reprod Biol Endocrinol*. 2016, 14:57.
8. Murri M, Luque-Ramirez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. *Hum Reprod Update*. 2013, 19:268-288.
9. Aruoma OI, Grootveld M, Bahorun T. Free radicals in biology and medicine: from inflammation to biotechnology. *Biofactors*. 2006, 27:1-3.
10. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am J Physiol Regul Integr Comp Physiol*. 2007, 292:R18-36.
11. Hull KL, Harvey S. Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions. *Int J Endocrinol*. 2014, 2014:234014.
12. Alviggi C, Humaidan P, Howles CM, Tredway D, Hillier SG. Biological versus chronological ovarian age: implications for assisted reproductive technology. *Reprod Biol Endocrinol*. 2009, 7:101.
13. Weall BM, Al-Samerria S, Conceicao J, Yovich JL, Almahbobi G. A direct action for GH in improvement of oocyte quality in poor-responder patients. *Reproduction*. 2015, 149:147-154.
14. Suzuki K, Yanagi K, Shimizu M, Wakamatsu S, Niitani T, Hosonuma S, et al. Effect of growth hormone replacement therapy on plasma diacron-reactive oxygen metabolites and endothelial function in Japanese patients: The GREAT clinical study. *Endocr J*. 2018, 65:101-111.
15. Brioché T, Kireev RA, Cuesta S, Gratas-Delamarche A, Tresguerres JA, Gomez-Cabrera MC, et al. Growth hormone replacement therapy prevents sarcopenia by a dual mechanism: improvement of protein balance and of antioxidant defenses. *J Gerontol A Biol Sci Med Sci*. 2014, 69:1186-1198.

16. Kato Y, Iwase M, Ichihara S, Kanazawa H, Hashimoto K, Noda A, et al. Beneficial effects of growth hormone-releasing peptide on myocardial oxidative stress and left ventricular dysfunction in dilated cardiomyopathic hamsters. *Circ J*. 2010, 74:163-170.
17. Li R, Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. *Nat Rev Mol Cell Biol*. 2013, 14:141-152.
18. Barroso G, Barrionuevo M, Rao P, Graham L, Danforth D, Huey S, et al. Vascular endothelial growth factor, nitric oxide, and leptin follicular fluid levels correlate negatively with embryo quality in IVF patients. *Fertil Steril*. 1999, 72:1024-1026.
19. Alpha Scientists in Reproductive M, Embryology ESIGo. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod*. 2011, 26:1270-1283.
20. Navot D, Bergh PA, Laufer N. Reprint of: Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. *Fertil Steril*. 2019, 112:e209-e221.
21. Yang S, Wang Q, Huang W, Song Y, Feng G, Zhou L, et al. Are serum chemerin levels different between obese and non-obese polycystic ovary syndrome women? *Gynecol Endocrinol*. 2016, 32:38-41.
22. Zhang R, Liu H, Bai H, Zhang Y, Liu Q, Guan L, et al. Oxidative stress status in Chinese women with different clinical phenotypes of polycystic ovary syndrome. *Clin Endocrinol (Oxf)*. 2017, 86:88-96.
23. Zhou M, Liu XH, Liu QQ, Chen M, Bai H, Guan LB, et al. Lactonase Activity, Status, and Genetic Variations of Paraoxonase 1 in Women with Gestational Diabetes Mellitus. *J Diabetes Res*. 2020, 2020:3483427.
24. Fujimoto VY, Bloom MS, Huddleston HG, Shelley WB, Ocque AJ, Browne RW. Correlations of follicular fluid oxidative stress biomarkers and enzyme activities with embryo morphology parameters during in vitro fertilization. *Fertil Steril*. 2011, 96:1357-1361.
25. Siristatidis C, Vogiatzi P, Varounis C, Askoxylaki M, Chrelias C, Papantoniou N. The Effect of Reactive Oxygen Species on Embryo Quality in IVF. *In Vivo*. 2016, 30:149-153.
26. Oyawoye OA, Abdel-Gadir A, Garner A, Leonard AJ, Perrett C, Hardiman P. The interaction between follicular fluid total antioxidant capacity, infertility and early reproductive outcomes during in vitro fertilization. *Redox Rep*. 2009, 14:205-213.
27. Scalici E, Traver S, Molinari N, Mullet T, Monforte M, Vintejou E, et al. Cell-free DNA in human follicular fluid as a biomarker of embryo quality. *Hum Reprod*. 2014, 29:2661-2669.
28. Uyar A, Torrealday S, Seli E. Cumulus and granulosa cell markers of oocyte and embryo quality. *Fertil Steril*. 2013, 99:979-997.
29. Adashi EY. Endocrinology of the ovary. *Hum Reprod*. 1994, 9:815-827.
30. Carbone MC, Tatone C, Delle Monache S, Marci R, Caserta D, Colonna R, et al. Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent changes. *Mol Hum Reprod*. 2003, 9:639-643.
31. Aurrekoetxea I, Ruiz-Sanz JI, del Agua AR, Navarro R, Hernandez ML, Matorras R, et al. Serum oxidizability and antioxidant status in patients undergoing in vitro fertilization. *Fertil Steril*. 2010,

32. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. *Reprod Biol Endocrinol*. 2012, 10:49.
33. Tesarik J, Hazout A, Mendoza C. Improvement of delivery and live birth rates after ICSI in women aged >40 years by ovarian co-stimulation with growth hormone. *Hum Reprod*. 2005, 20:2536-2541.
34. Sharara FI, Nieman LK. Growth hormone receptor messenger ribonucleic acid expression in leiomyoma and surrounding myometrium. *Am J Obstet Gynecol*. 1995, 173:814-819.
35. Xue-Mei W, Hong J, Wen-Xiang Z, Yang L. The effects of growth hormone on clinical outcomes after frozen-thawed embryo transfer. *Int J Gynaecol Obstet*. 2016, 133:347-350.
36. Cui N, Li AM, Luo ZY, Zhao ZM, Xu YM, Zhang J, et al. Effects of growth hormone on pregnancy rates of patients with thin endometrium. *J Endocrinol Invest*. 2019, 42:27-35.
37. Eftekhar M, Aflatoonian A, Mohammadian F, Eftekhar T. Adjuvant growth hormone therapy in antagonist protocol in poor responders undergoing assisted reproductive technology. *Arch Gynecol Obstet*. 2013, 287:1017-1021.
38. Poseidon G, Alviggi C, Andersen CY, Buehler K, Conforti A, De Placido G, et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. *Fertil Steril*. 2016, 105:1452-1453.
39. Norman RJ, Alvino H, Hull LM, Mol BW, Hart RJ, Kelly TL, et al. Human growth hormone for poor responders: a randomized placebo-controlled trial provides no evidence for improved live birth rate. *Reprod Biomed Online*. 2019, 38:908-915.

Tables

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

| | POR-GH (n = 52) | POR-C (n = 53) | Non-POR (n = 58) |
|--|-----------------|----------------|------------------|
| Age (years) ^{ab} | 38.41 ± 2.91 | 38.20 ± 2.79 | 29.56 ± 3.12 |
| Infertility duration (years) | 4.57 ± 3.14 | 4.39 ± 3.22 | 3.47 ± 2.22 |
| Risk factors for POR cycle (n) ^{ab} | 20 | 18 | 0 |
| Abnormal ORT cycle (n) ^{ab} | 39 | 32 | 0 |
| Previous POR cycle (n) ^{ab} | 21 | 23 | 0 |
| BMI (kg/m ²) | 22.09 ± 1.73 | 22.62 ± 2.73 | 22.19 ± 2.67 |
| FSH (mIU/mL) ^{ab} | 11.49 ± 2.94 | 10.97 ± 2.75 | 5.89 ± 1.76 |
| LH (mIU/mL) | 4.59 ± 1.83 | 4.36 ± 1.57 | 4.29 ± 1.45 |
| FSH / LH ratio ^{ab} | 2.74 ± 1.57 | 2.53 ± 1.68 | 1.38 ± 0.75 |
| E ₂ (pg/mL) ^{ab} | 75.98 ± 34.05 | 72.34 ± 26.87 | 40.62 ± 11.19 |
| P (ng/mL) | 0.64 ± 0.39 | 0.54 ± 0.35 | 0.55 ± 0.20 |
| TT (ng/mL) | 0.37 ± 0.19 | 0.41 ± 0.20 | 0.43 ± 0.15 |
| AMH (ng/mL) ^{ab} | 0.88 ± 0.67 | 0.81 ± 0.41 | 3.50 ± 1.91 |
| PRL (μIU/mL) | 162.59 ± 50.19 | 171.25 ± 52.32 | 163.51 ± 45.79 |
| HOMA-IR | 1.68 ± 1.25 | 1.71 ± 1.34 | 1.67 ± 1.33 |
| AFC ^{ab} | 4.12 ± 1.71 | 4.06 ± 2.11 | 15.30 ± 4.56 |

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

| | POR-GH (n = 52) | POR-C (n = 53) | Non-POR (n = 58) |
|--|------------------|-------------------|------------------|
| rFSH doses (IU) ^{a,b} | 2491.46 ± 996.47 | 2499.87 ± 1345.16 | 1875.09 ± 467.39 |
| rFSH duration (d) ^{a,b} | 8.93 ± 2.57 | 8.97 ± 4.11 | 10.65 ± 1.65 |
| E ₂ on trigger day (pg/mL) ^{a,b} | 985.13 ± 348.44 | 887.85 ± 372.21 | 1956.10 ± 558.48 |
| Endometrial thickness (mm) ^{b,c} | 9.65 ± 1.84 | 8.61 ± 1.23 | 10.33 ± 1.93 |
| Oocytes retrieved ^{a,b} | 3.71 ± 2.50 | 3.24 ± 2.56 | 12.51 ± 6.81 |
| MII oocytes ^{a,b} | 3.18 ± 1.78 | 2.87 ± 1.89 | 9.95 ± 4.28 |
| Fertilized oocytes (2PN) ^{a,b} | 2.36 ± 1.86 | 2.02 ± 1.21 | 7.04 ± 4.29 |
| Cleaved embryos ^{a,b,c} | 2.31 ± 1.81 | 1.73 ± 1.03 | 5.38 ± 3.62 |
| Higher quality embryos ^{a,b,c} | 1.26 ± 0.65 | 0.72 ± 0.56 | 3.34 ± 2.78 |
| No. ET | 1.58 ± 0.51 | 1.59 ± 0.48 | 1.43 ± 0.50 |
| 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% (36/83) |
| Clinical pregnancy rate (%) ^{b,c} | 38.77% (19/49) | 13.33% (6/45) | 53.44% (31/58) |
| Miscarriage rate (%) | 21.05% (4/19) | 33.33% (2/6) | 6.45% (2/31) |
| Multiple pregnant rate (%) ^{In FF} | 15.78% (3/19) | 16.67% (1/6) | 16.13% (5/31) |
| MDA (nmol/mL) ^{a,b} | 2.27 ± 0.69 | 3.02 ± 1.10 | 1.33 ± 0.67 |
| SOD (U/mgprot) ^{a,b} | 12.99 ± 2.32 | 11.21 ± 1.71 | 14.76 ± 1.93 |
| TAC (mmol Trolox Equiv./L) ^{a,b,c} | 0.59 ± 0.13 | 0.42 ± 0.16 | 0.71 ± 0.11 |
| TOS (μmol H ₂ O ₂ Equiv./L) ^{a,b,c} | 8.06 ± 1.19 | 10.14 ± 4.86 | 5.67 ± 1.09 |
| OSI ^{a,b,c} | 16.49 ± 8.19 | 23.87 ± 10.13 | 8.16 ± 1.86 |

Note: Data are presented as the mean ± SD or percentage (number). 2PN: Number of two pronuclear zygotes; ET: embryos transferred; FF: follicular fluid; MDA: malondialdehyde; SOD: superoxide dismutase; TAC: total antioxidant capacity; TOS: total oxidant status; OSI: oxidative stress index. A chi-squared test was performed to compare the rates of cycle cancellation, 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.

Figures

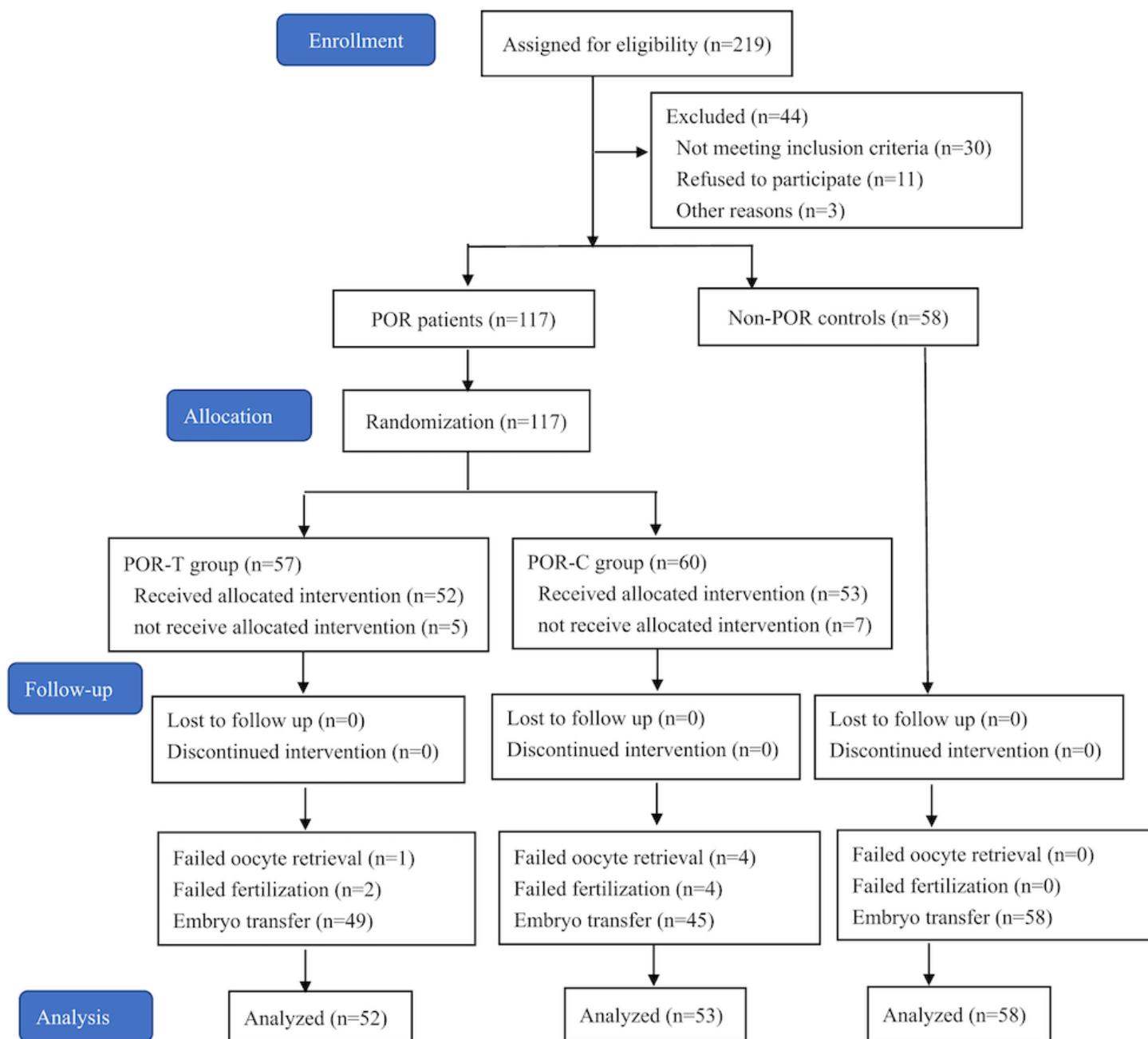
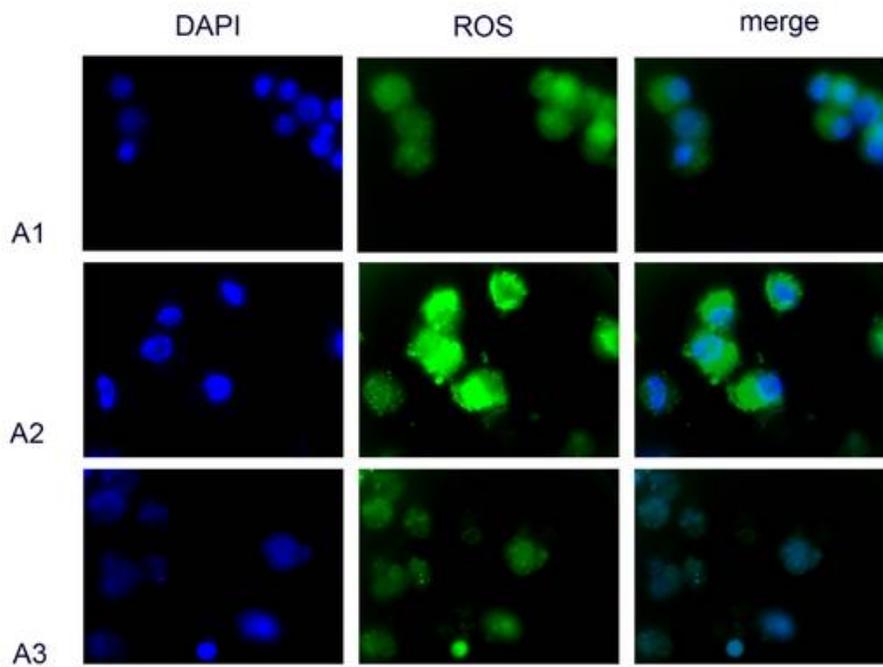


Figure 1

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

Figure 2

A



B

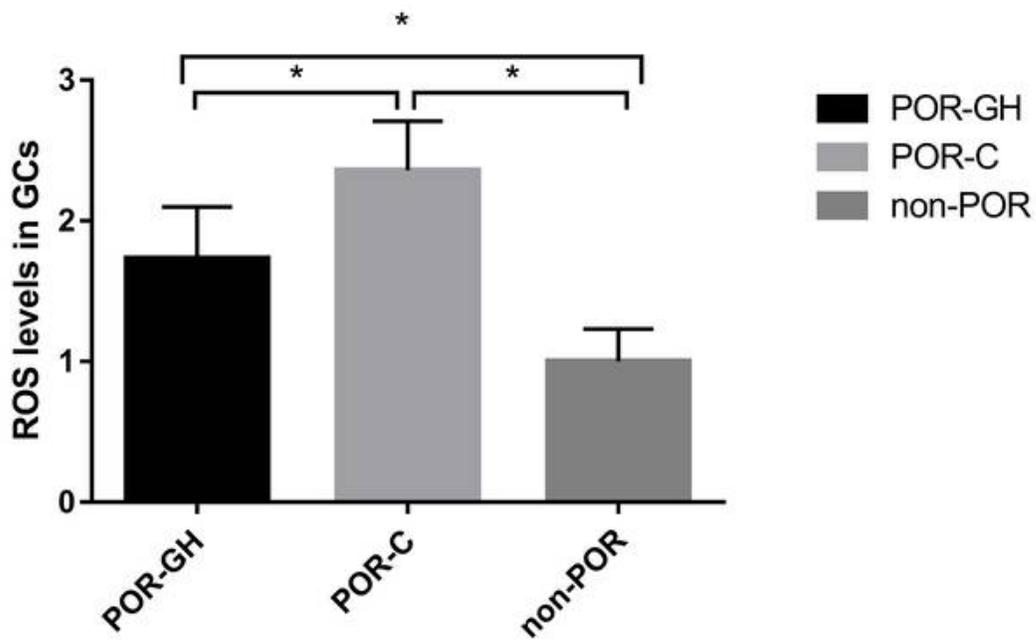


Figure 2

Fluorescence of ROS in GCs of the three groups. A The green fluorescence of ROS in GCs was observed under a fluorescence microscope. Cell nuclei were stained with DAPI. A1: POR-GH group, A2: POR-C group, A3: non-POR group. B The fluorescence intensity of ROS is expressed as the fold change relative to the control. Measured by spectrophotometry, the fluorescence intensity of ROS (2.36 ± 0.32 vs. 1.00 ± 0.23) was significantly higher in the POR-C group than in the non-POR group ($P < 0.05$). GH significantly lowered ROS intensity in the POR-GH group (1.83 ± 0.38 vs. 2.36 ± 0.32) ($P < 0.05$).

Supplementary Files

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