Archives of Gynecology and Obstetrics (2019) 300:223–233
https://doi.org/10.1007/s00404-019-05163-3



# The effect of luteal GnRH antagonist on moderate and severe early ovarian hyperstimulation syndrome during in vitro fertilization treatment: a prospective cohort study

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Received: 18 December 2018 / Accepted: 12 April 2019 / Published online: 19 April 2019


## Abstract

**Purpose** Ovarian hyperstimulation syndrome (OHSS) is a serious complication of assisted reproductive technology (ART) treatment. However, there are limited data regarding the ability of the luteal GnRH antagonist cetrorelix to reduce the incidence of moderate and severe OHSS, and the mechanism remains unclear. Thus, we designed a study to assess the effectiveness of cetrorelix to prevent early moderate and severe OHSS in high-risk patients undergoing controlled ovarian stimulation for IVF/ICSI.

**Methods** In this prospective cohort study, 105 patients with high-risk OHSS undergoing cryopreservation of all embryos were divided into two groups according to their personal choice. The cetrorelix group ($n=65$) received 0.25 mg of cetrorelix by subcutaneous injection daily, from days 3 to 5 post-oocyte retrieval (POR); the control group ($n=40$) received no drug. The primary outcome measures were the incidence and severity of early moderate and severe OHSS. Secondary measures included serum estradiol levels, ovarian volume, ascites volume, hematocrit values, and WBC count on days 3, 6, and 9 POR. VEGF and EGR-1 levels were assessed, and binary logistic regression analysis was applied to predict associations between clinical variables and OHSS.

**Results** Ninety-six patients were examined. The incidence of moderate and severe OHSS was significantly lower in the cetrorelix group than in the control group (18.03% and 37.14%, respectively; $P=0.037$). Serum estradiol ($P=0.013$), white blood cell count ($P=0.031$), ascites volume ($P=0.036$), EGR-1 ($P=0.025$), and VEGF levels ($P=0.015$) were significantly higher in the control group on day 6 POR than on day 3 POR, while no increase was observed between day 3 POR and day 6 POR in the cetrorelix group, indicating a faster regression of OHSS symptoms. Cetrorelix intervention was associated with the incidence and severity of OHSS (OR 0.29, 95% CI 0.11–0.78, $P=0.014$).

**Conclusion** Cetrorelix effectively reduces the incidence of early moderate and severe OHSS in high-risk women and decreases serum VEGF levels.

**Keywords** GnRH antagonist · Dopamine agonist · OHSS · VEGF · IVF

## Introduction

Ovarian hyperstimulation syndrome (OHSS) is a serious complication of assisted reproductive technology (ART) treatment [1]. Early OHSS is defined as onset of the syndrome during the first 9 days after human chorionic gonadotropin (hCG) administration, and late OHSS is defined as onset from 10 days after hCG administration [2]. The disease severity of OHSS has been classified as mild, moderate, severe, and critical by Golan and colleagues [3] according to clinical manifestations and laboratory findings. After gonadotropin stimulation for IVF, the reported incidence of moderate OHSS is 3% to 6%; the incidence of severe OHSS is 0.1–2%. However, the incidence

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of moderate and severe OHSS can reach 20.0% for high-risk women [4, 5].

The pathophysiology of OHSS has not yet been completely elucidated. Increased vascular permeability causing loss of fluid into the third space (abdominal and pleural cavities) is the central feature of clinically significant OHSS, which triggers events that result in associated symptoms (such as abdominal pain and distension) [5]. Supportive evidence is provided by studies, showing that serum vascular endothelial growth factor (VEGF) levels correlate with OHSS severity [6]. By affecting angiogenesis through VEGF, other vasoactive substances and transcription factors, including insulin-like growth factor 1 (IGF-I), early growth response protein 1 (EGR-1), and interleukins (ILs), may also be directly or indirectly involved in the pathogenesis of OHSS [7–9]. Overall, understanding the pathophysiology of this condition may aid in identifying measures to prevent its development and to treat associated symptoms.

Numerous OHSS prevention methods have been described, including cycle cancellation or coasting, cryopreservation of embryos rather than immediate fresh embryo transfer, GnRH agonist (GnRHa) triggering, letrozole, cabergoline, and the gonadotropin-releasing hormone (GnRH) antagonist protocol [10–13]. To date, however, there is no consensus regarding the most favorable strategy for preventing OHSS, and no approach has thus far led to the eradication of this complication.

Increasing evidence suggests that the administration of the luteal GnRH antagonist cetrorelix in women with established severe early OHSS leads to rapid regression of the syndrome at an outpatient level [14, 15]. Direct actions of GnRH antagonist on the ovary may lead to a decline in serum VEGF levels, as well as declines in estradiol and progesterone levels and ovarian volume, suggesting a luteolytic effect, as recently proposed [16]. Nonetheless, data regarding cetrorelix administration in preventing moderate and severe OHSS are still limited, and the mechanisms of this drug in OHSS prevention and treatment remain unclear.

Here, we report a prospective cohort study to investigate whether treatment with the luteal GnRH antagonist cetrorelix after oocyte retrieval is effective in preventing moderate and severe OHSS development in women at high risk for the condition. In addition, the current study aimed to assess changes in symptoms, ultrasound findings and laboratory findings, including estradiol, ascites, and serum VEGF, following cetrorelix administration in women at high risk for OHSS.

## Methods

### Patient population and management

This was a prospective observational cohort study of IVF patients at high risk for OHSS conducted at the Reproductive Medicine Center of Peking University First Hospital between April 2016 and August 2017. This study was approved by the institutional ethics review board of Peking University First Hospital. Signed informed consent was obtained from all patients included in this study. For inclusion, patients had at least one of these high-risk criteria: number of oocytes retrieved $\geq 15$; estradiol level $\geq 5000$ pg/mL on the day of hCG administration; clinically or ultrasonography-proven OHSS on the day of oocyte retrieval (performed 36 h after hCG triggering), such as obvious ultrasonographic evidence of ascites [17]. The patients were informed of the risk and symptoms of OHSS, and all patients agreed to forego fresh embryo transfer and to instead cryopreserve all embryos. Patients were excluded if other preventive measures for managing OHSS had been applied if they had discontinued intervention or if they could not complete the follow-up process.

### Classification of OHSS symptoms

The severity of OHSS was evaluated according to clinical manifestations and laboratory findings, as previously reported [18, 19]. A brief description of the classification of OHSS severity is shown in Table 1. The four categories of OHSS are mild, moderate, severe, and critical. Ascites and pleural effusion were monitored by ultrasound and recorded for all patients on day 3, day 6, and day 9 post-oocyte retrieval. Biochemical values such as hematocrit, white blood cell (WBC) count, creatinine, liver enzymes, serum potassium, and sodium were also measured. Liver dysfunction was defined as elevated liver enzymes, including alanine aminotransferase $> 40$ IU/L or aspartate transaminase $> 35$ IU/L. An elevated WBC count was defined as $> 15 \times 10^9$/L. Severe hemoconcentration was defined as hematocrit $> 55\%$. Evidence of ascites included ultrasonographic detection of ascites or a clinical indication of ascites.

### Study design and intervention

A flexible GnRH antagonist protocol or a short GnRH agonist suppressive protocol (Daphiline Beaufour, IPSEN, 0.1 mg) was used for controlled ovarian stimulation, as previously described by Lanias et al. [15]. Gonadotropins (Gonal-F; Merck Serono, Geneva, Switzerland) were administered at variable starting doses (112.5–150 IU) according to the individual's age, BMI, and/or ovarian responsiveness in previous cycles. The dose was adjusted after day 5 of stimulation according to follicular development, as assessed by ultrasound and serum estradiol levels every 2 or 3 days. Final oocyte maturation was triggered by the administration of 250 mg of recombinant hCG when ultrasound indicated that at least 3 follicles reached a diameter $\geq 18$ mm. Transvaginal ultrasound-guided oocyte retrieval was performed 36 h after hCG triggering by single-lumen needle aspiration.

**Table 1** Classification of OHSS symptoms Adapted from Navot et al. [18] and Practice Committee of the American Society for Reproductive Medicine (Prevention and treatment of moderate and severe OHSS. Fertil Steril 2016)

| OHSS stage | Clinical feature | Laboratory feature |
|---|---|---|
| Mild | Abdominal distension/discomfort | No important alterations |
| | Mild nausea/vomiting | |
| | Mild dyspnea | |
| | Diarrhea | |
| | Enlarged ovaries | |
| Moderate | Mild features | Hemoconcentration (Hct > 41%) |
| | Ultrasonographic evidence of ascites | Elevated WBC ( > $15*10^9$/L) |
| Severe | Mild and moderate features Clinical evidence of ascites Hydrothorax | Severe hemoconcentration (Hct > 55%) |
| | Severe dyspnea | WBC > $25*10^9$/L |
| | Oliguria/anuria | CrCl < 50 mL/min |
| | Intractable nausea/vomiting | Cr > 1.6 mg/dL |
| | Low blood/central venous pressure | |
| | Pleural effusion | |
| | Rapid weight gain ( > 1kgin24h) Syncope | Na + < 135 mmol/L |
| | Severe abdominal pain | K + > 5.0 mmol/L |
| | Venous thrombosis | Elevated liver enzymes |
| Critical | Anuria/acute renal failure | Worsening of findings |
| | Arrhythmia | |
| | Thromboembolism | |
| | Pericardial effusion | |
| | Massive hydrothorax | |
| | Arterial thrombosis | |
| | Adult respiratory distress syndrome | |
| | Sepsis | |

*Hct* hematocrit, *WBC* white blood cell, *CrCl* creatinine clearance, *Cr* creatinine, *Na+* sodium, *K+* potassium

ICSI was performed only in cases with severe male factor infertility or previous fertilization failure. Embryos were cultured in G1/G2 medium (Vitrolife, Denmark) for up to 6 days. No luteal support was given.

Patients with high-risk OHSS undergoing cryopreservation of all embryos after oocyte retrieval were divided into two groups according to their personal choice. The cetrorelix group received 0.25 mg of the GnRH antagonist cetrorelix (Merck Serono, Germany) by subcutaneous injection daily, from day 3 post-oocyte retrieval (POR, day 5 after hCG triggering) until and including day 5 POR (day 7 after hCG triggering) for 3 days. The control group received no drug, but underwent clinical observation.

## Follow-up of patients and laboratory assays

The patients were clinically followed, with phone calls to encourage patient follow-up with the researchers. Patient baseline characteristics, including age, body mass index (BMI), antral follicle count (AFC), baseline follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) levels, were collected from medical records. Baseline hormone levels were obtained on the third day of the menstrual cycle, at 1–3 months before the treatment cycle. The existence and severity of OHSS were evaluated during each follow-up visit, and the percentage of patients who experienced moderate and severe OHSS was determined. On day 3 (day 5 after hCG triggering) and day 6 (day 8 after hCG triggering) POR, blood samples were collected from the ulnar vein of each patient. The follow-up procedure for patients with high-risk OHSS included an evaluation of E2, hematocrit, WBC count, VEGF, and EGR-1 and an ultrasound assessment of ovarian size and ascitic fluid on days 3 and 6 POR. Ovarian size and ascitic fluid volume were calculated using the prolate ellipsoid formula, as follows: $V = D_1 \times D_2 \times D_3 \times 0.523$, where $D_1$, $D_2$, and $D_3$ are the three-maximal longitudinal, antero-posterior, and transverse diameters, respectively.

The estradiol level was additionally quantitated by chemiluminescence immunoassay (UniCel DXI 800, Beckman Coulter Inc, California, USA). Assay sensitivity was < 10 pg/mL for E2, and the intraassay and interassay coefficients of variation were < 5% and < 6%, respectively. Hematocrit and WBC count were determined by nucleic acid fluorescence

staining plus flow cytometry (XN-3000, SYSMEX, Kobe, Inc). The coefficient of variation, specifying the imprecision limits for WBC and red blood cell (RBC) counts, was 3%. Hematocrit was computed from the relative volume of erythrocytes [Ht(%) = RBC × MCV/10]. Serum VEGF and EGR-1 levels were measured using enzyme-linked immunosorbent assay kits (Ray Biotech, Inc, Norcross, GA, USA; Cusabio, Wuhan, Hubei). The sensitivity of the assay was 10 pg/ml. Intra- and interassay coefficients of variation for serum VEGF and EGR-1 were 8% and 10%, and 10% and 12%, respectively. No cross-reactivity against human cytokine standards was found.

### Outcome measures

The primary outcome measure was the incidence and severity of early moderate and severe OHSS. Secondary outcome measures included evaluations of clinical changes in serum E2 level, WBC count, hematocrit value, ovarian volume, and ascites volume, which reflected the progression or regression of early OHSS on day 3 POR (day 5 after hCG triggering) and day 6 POR (day 8 after hCG triggering). Moreover, laboratory changes, including VEGF and EGR-1 levels, were assessed following GnRH antagonist administration in the luteal phase.

### Statistical analysis

Data analyses were performed using SPSS 22 package program (SPSS Inc, Chicago, IL, USA). Categorical data are presented as the number and percentage. Continuous variables are provided as the mean ± standard deviation (SD); Student's *t* test was applied if the variables presented normal distributions. To compare grade and qualitative variables, nonparametric tests (Mann–Whitney *U*, Chi-square, and Fisher's exact tests) were used as indicated. Binary logistic regression analysis (two categories of OHSS served as dependent variables) was employed for predicting associations between clinical variables and moderate and severe OHSS after cetrorelix treatment. All analyses of significance were twosided, and a value of $P < 0.05$ was considered statistically significant.

## Results

For the cohort of 105 high-risk patients included in the present study, luteal administration of GnRH agonist was performed in 65 (cetrorelix group), and clinical observation alone was performed in 40 (control group). Nine patients were excluded from the study for different reasons, and ultimately, 61 patients in the cetrorelix group and 35 patients in the control group finished the study (Fig. 1). The follow-up rate was 91.43%.

Baseline characteristics, ovarian stimulation, and embryological data for the two groups are shown in Table 2, with no statistically significant difference in age, BMI, duration of infertility, baseline FSH, LH and E2 levels, duration of stimulation, total Gn, E2, or progesterone on the day of hCG administration. AFC was significantly reduced in the control group ($P = 0.001$), and a significantly smaller number of follicles on the day of hCG administration were observed in the control group than in the cetrorelix group ($P = 0.004$).

The primary outcomes are provided in Table 3. The incidence of moderate and severe OHSS was significantly lower in the cetrorelix group than in the control group (18.03% and 37.14%, respectively; $P = 0.037$). The frequencies of clinical and laboratory dysfunctions related to OHSS in the groups are shown in Table 3. The frequency of elevated WBC count, liver dysfunction, severe hemoconcentration, and hospital admission and the average duration of hospitalization were reduced in the cetrorelix group compared with the control group, but no significant difference was observed.

The secondary outcomes during the monitoring period in the groups are presented in Fig. 2. There was no statistically significant difference in serum E2, the WBC count, hematocrit, ascites, ovarian volume or EGR-1 on day 3 POR (day 5 after hCG triggering) between the cetrorelix group and the control group. In the control group, there was a significant increase in serum E2 ($P = 0.013$), WBC count ($P = 0.031$), and ascites ($P = 0.036$) on day 6 POR (day 8 after hCG triggering) compared with day 3 POR. However, after administration of cetrorelix, no significant increase on day 6 POR was observed, indicating a faster regression of OHSS symptoms. With regard to laboratory changes, there was a significant tendency for an increase in serum VEGF during the course from day 3 POR to day 6 POR in the control group ($P = 0.015$) and a significant increase in serum EGR-1 ($P = 0.025$). In the cetrorelix group, no significant increase or decline in VEGF or EGR-1 from day 3 POR to day 6 POR was observed.

Table 4 shows the prediction of associations between clinical variables and OHSS after cetrorelix treatment, as based on binary logistic regression analysis in which two categories of OHSS served as dependent variables. The occurrence of no and mild OHSS was determined as 0, whereas the occurrence of moderate and severe OHSS was determined as 1. After adjusting for age, baseline FSH, LH, and E2, total gonadotropin dosage, and E2 on the day of hCG administration and the number of follicles on the day of hCG administration, only cetrorelix intervention was associated with the incidence and severity of OHSS (OR 0.29, 95% CI 0.11–0.78, $P = 0.014$), indicating a protective effect against the incidence of moderate and severe OHSS.

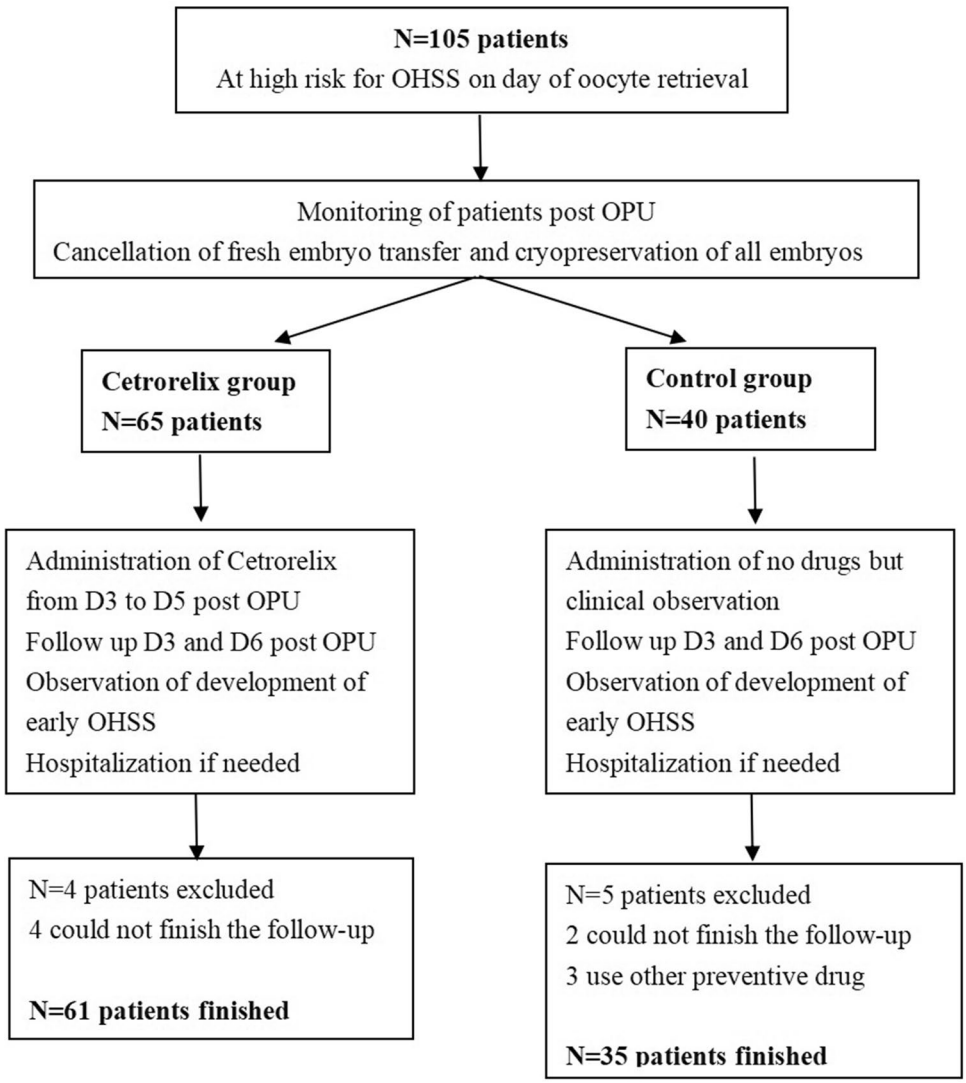


**Fig. 1** Flow chart of the study design

A total of 1211 in vitro fertilization retrieval cycles occurred in the time frame of the study at our reproductive center. The differences between our study group and other patients treated during this period who did not have high-risk factors for OHSS are shown in Table 5. Regarding baseline characteristics, ovarian stimulation and embryological data, patients at high risk for early OHSS had a younger age ($P=0.000$), lower BMI ($P=0.000$), higher AFC level ($P=0.000$), higher percentage of male factor ($P=0.001$), lower FSH ($P=0.000$) and higher LH ($P=0.001$) at the baseline, lower total Gn dose ($P=0.000$), greater number of follicles ($P=0.000$) and higher E2 levels ($P=0.000$) on the day of hCG administration, higher number of oocytes retrieved ($P=0.000$), greater number of mature oocytes ($P=0.000$), higher number of transplantable embryos ($P=0.000$), and more high-quality embryos ($P=0.000$) compared to patients who did not have high-risk factors for OHSS (Table 5).

## Discussion

The present study investigates the preventive effect of GnRH antagonist cetrorelix administration during the luteal phase on decreasing moderate and severe early OHSS in high-risk women. The results demonstrate that the administration of cetrorelix is effective at decreasing the incidence of moderate and severe early OHSS in women with high-risk OHSS syndrome with cryopreservation of all embryos POR. In addition, this prospective cohort study demonstrates that luteal administration of cetrorelix is associated with a non-significant increase in serum VEGF levels, ascites, estradiol, and WBC count after administration compared with the control group, which reflects the natural course of the syndrome.

Previous evidence indicates that there are some patients who are at a much higher risk for early OHSS, and identifying these patients who are at high risk is essential to preventing and lowering the incidence of OHSS. The results of the

**Table 2** Baseline characteristics, ovarian stimulation, and embryological data of the study groups

| | Cetrorelix ($n=61$) | Control ($n=35$) | $P$ value |
|---|---|---|---|
| Baseline characteristics | | | |
| Age (years) | 31.34 ± 3.77 | 31.66 ± 3.42 | 0.687 |
| BMI (kg/m$^2$) | 21.42 ± 2.44 | 21.30 ± 2.79 | 0.824 |
| Duration of infertility (years) | 3.37 ± 2.33 | 3.49 ± 1.99 | 0.804 |
| AFC | 16.72 ± 2.79 | 13.17 ± 5.65 | **0.001** |
| Baseline FSH (IU/L) | 6.94 ± 1.43 | 6.91 ± 1.49 | 0.934 |
| Baseline LH (IU/L) | 4.96 ± 1.96 | 5.16 ± 1.96 | 0.616 |
| Baseline E2 (pg/ml) | 48.30 ± 18.67 | 46.94 ± 19.58 | 0.738 |
| Ovarian stimulation | | | |
| Duration of stimulation (days) | 10.82 ± 1.32 | 11.03 ± 1.38 | 0.466 |
| Total Gn (IU) | 2318.25 ± 861.75 | 2367.75 ± 798.75 | 0.781 |
| E2 on day of hCG (pg/ml) | 7352.9 ± 2761.28 | 7102.37 ± 1923.66 | 0.636 |
| Progesterone on day of hCG (ng/ml) | 1.40 ± 0.60 | 1.58 ± 0.86 | 0.222 |
| Number of follicles on day of hCG | 21.53 ± 7.08 | 18.09 ± 4.43 | **0.004** |
| Embryological data | | | |
| Number of oocytes retrieved | 16.15 ± 6.70 | 13.66 ± 5.74 | 0.068 |
| Type of fertilization (IVF/ICSI) | 23/38 | 20/15 | 0.065 |
| Mature oocytes (in ICSI patients) | 13.30 ± 5.66 | 11.09 ± 5.43 | 0.065 |
| Number of 2PN | 10.41 ± 4.85 | 8.69 ± 4.68 | 0.093 |
| Number of transplantable embryos | 10.16 ± 4.90 | 8.94 ± 4.93 | 0.244 |
| Number of high quality embryos | 4.23 ± 3.40 | 4.49 ± 2.76 | 0.705 |
| Number of embryos cryopreserved | 6.54 ± 2.85 | 6.31 ± 1.95 | 0.677 |

Values are expressed as the mean ± standard deviation (SD) unless otherwise stated. $P<0.5$ depicts statistical significance

*BMI* body mass index, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *Gn* gonadotropin, *hCG* human chorionic gonadotropin

**Table 3** Primary outcomes

| | Cetrorelix ($n=61$) | Control ($n=35$) | $P$ value |
|---|---|---|---|
| No OHSS (%) | 7 (11.48) | 4 (11.43) | 0.099 |
| Mild OHSS (%) | 43 (70.49) | 18 (51.43) | |
| Moderate OHSS (%) | 10 (16.39) | 11 (31.43) | |
| Severe OHSS (%) | 1 (1.64) | 2 (5.71) | |
| Critical OHSS (%) | 0 (0) | 0 (0) | |
| Moderate and severe OHSS (%) | 11 (18.03) | 13 (37.14) | **0.037** |
| Elevated WBC (%) | 10 (16.39) | 11 (31.43) | 0.086 |
| Hydrothorax (%) | 0 (0) | 0 (0) | – |
| Liver dysfunction (%) | 1 (1.64) | 2 (5.71) | 0.300 |
| Severe hemoconcentration (%) | 0 (0) | 0 (0) | |
| Hospital admission | 4 (6.58) | 7 (20.00) | 0.073 |
| Average duration of hospitalization | 5.75 ± 0.96 | 8.57 ± 1.90 | 0.332 |

Values are presented as $n$, $n$ (percentage), or the mean ± standard deviation (SD). Dashes indicate no $P$ value. $P$ values in bold depict statistical significance (Mann–Whitney $U$, chi-square $P<0.05$)

largest study to evaluate risk factors for OHSS showed that among 214,219 ART cycles, younger age, tubal factor, and unexplained infertility were all associated with an increased risk of OHSS [20]. Moreover, in a prospective analysis of 1012 first ART cycles, the risk of OHSS increased from 2.2% in women with AFC < 24 to 8.6% with AFC ≥ 24 [21]. In addition, analysis of 256,381 cycles demonstrated that retrieval of > 15 oocytes significantly increases the risk of OHSS without improving live-birth rates in fresh cycles [22]. Other researchers have reported a dramatic increase

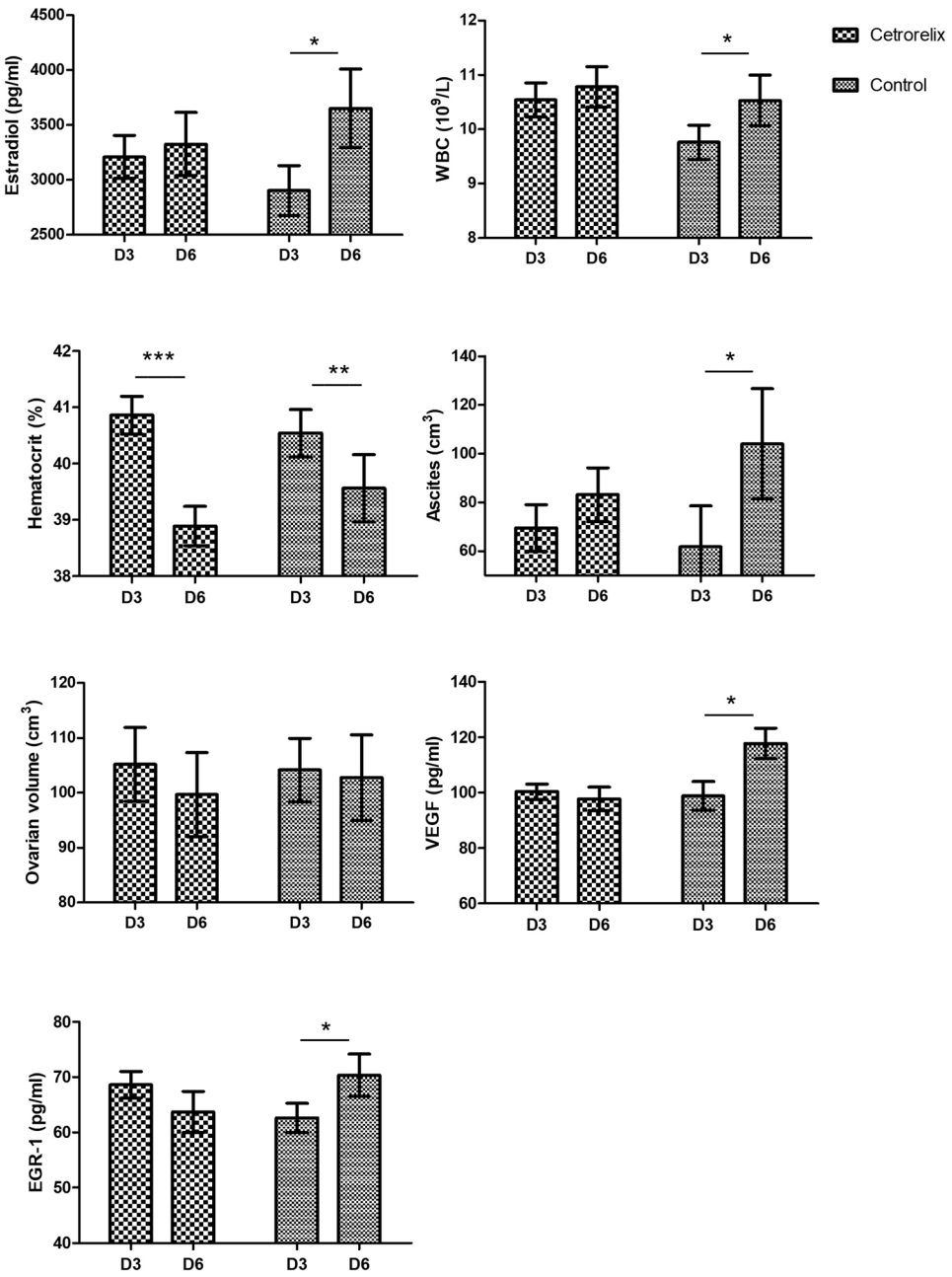


**Fig. 2** Secondary outcomes: concentrations of E2, WBC, hematocrit, ascites, ovarian volume, VEGF, EGR-1, and IGF-I during the monitoring period in the different groups. D3, day 3 post-oocyte retrieval; D6, day 6 post-oocyte retrieval; E2, estradiol; WBC, white blood cell; VEGF, vascular endothelial growth factor; EGR-1, early growth response protein 1; IGF-I, insulin-like growth factor 1. Asterisks depict statistically significant differences compared to day 3 (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

in the moderate–severe OHSS rate for ≥ 15 oocyte group (6.9%) when compared to a 10–14 oocyte group (0.8%), though the cumulative LBR only increased 5.8% (from 83.4 to 89.2%) [23]. In studies investigating the association between serum estradiol concentration and OHSS, the mean estradiol value in patients with OHSS was > 3500 pg/ml [24, 19]. In our study, we found younger age, lower BMI, higher AFC, male factor, and peak estradiol levels on the day of triggering final oocyte maturation, multifollicular development, and a large number of oocytes retrieved to be associated with an increased risk of OHSS, consistent with the previous studies. However, cutoff points require further validation. Overall, AFC > 15, development of ≥ 20 follicles, estradiol value ≥ 7000 pg/ml, and ≥ 15 oocytes retrieved appear to be highly associated with a high risk of OHSS in our study.

Early OHSS is defined as onset of the syndrome occurring during the first 9 days after hCG administration (7 days POR), and OHSS symptoms may begin as soon as 24 h after hCG administration, but become most severe approximately 5–7 days after POR, which is usually associated with increased vascular permeability [2]. Many studies have demonstrated that VEGF serum levels are associated with the probability of developing OHSS and with its clinical

**Table 4** Binary logistic regression analysis prediction of the association with clinical variables and OHSS after cetrorelix treatment

| Variables | OR | OR (95% CI) | *P* value |
|---|---|---|---|
| Group | | | |
| Cetrorelix | 0.29 | 0.11–0.78 | **0.014** |
| Age | 0.99 | 0.89–1.10 | 0.818 |
| Baseline FSH | 1.12 | 0.85–1.47 | 0.413 |
| Baseline LH | 1.00 | 0.83–1.20 | 0.976 |
| Baseline E2 | 1.01 | 0.99–1.03 | 0.441 |
| Total Gn | 0.99 | 0.95–1.03 | 0.728 |
| E2 on day of hCG | 1.00 | 1.00–1.00 | 0.782 |
| Number of follicles on day of hCG | 1.02 | 0.95–1.10 | 0.576 |
| Number of oocytes retrieved | 1.01 | 0.93–1.08 | 0.893 |

Two categories of OHSS served as dependent variables: the occurrence of no and mild OHSS was determined as 0, and the occurrence of moderate and severe OHSS was determined as 1

*OR* odds ratio, *CI* confidence interval, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *Gn* gonadotropin, *hCG* human chorionic gonadotropin *OHSS* ovarian hyperstimulation syndrome

Dashes indicate no *P* value. *P* values in bold depict statistical significance

symptoms. It has also been reported that VEGF levels are significantly higher in women who develop OHSS than in those who do not and that VEGF persists at high levels without declining for at least 10–14 days POR if no intervention is administered [25]. This observation was reported not only for women who developed severe early OHSS, but also for patient groups with milder forms of the syndrome [26]. In our study, the serum VEGF level of the control group showed an increasing tendency during the course of the study, from day 3 POR to day 6 POR, indicating the severity of vascular permeability in high-risk OHSS patients. The serum VEGF level on day 6 in the control group was increased significantly compared with that on day 3 POR, which is consistent with the findings of the previous research [25].

With respect to the pathophysiological mechanism of OHSS, considering the general consensus that VEGF plays a key role in the increase in angiogenesis and vascular hyperpermeability through its interaction with VEGF receptor-2 (VEGFR-2), many approaches have been proposed with the aim of targeting VEGF synthesis, bioavailability, or downstream signaling in an attempt to prevent or delay the development of OHSS [19]. To date, the use of ovarian stimulation protocols with GnRH antagonists instead of GnRH agonists is well established as a primary means of preventing OHSS. However, evidence supporting the use of the GnRH antagonist cetrorelix in the luteal phase as a tertiary preventive measure to induce rapid regression of the early onset of OHSS syndrome is still limited. A recent retrospective study compared pregnancy outcomes between a luteal cetrorelix administration group and control group, concluding that luteal-phase GnRH antagonist administration did not influence the chance of pregnancy [27]. Lainas et al. [16] also reported a statistically significant decline in serum VEGF on days 7–11 POR compared to day 5 POR, with GnRH antagonist administration initiated on day 5 and terminated on day 8 POR for 4 days; this indicates that the GnRH antagonist may have direct actions on the human ovary, which may lead to a decline in serum VEGF levels, as indicated by the presence and function of ovarian GnRH receptors. GnRH antagonists have been shown to inhibit expression of VEGF in human granulosa luteal cell cultures without influencing secretion of steroid hormones, supporting the hypothesis of direct actions of GnRH antagonist on the ovary [28]. Nonetheless, in the absence of a control group in that study, the resolution of the syndrome as part of its natural course cannot be firmly excluded. In our study, the incidence of moderate and severe OHSS was significantly lower in the cetrorelix group than in the control group. Compared with day 3 POR, no significant increase of serum E2, WBC count and ascites on day 6 POR was observed after administration of cetrorelix, indicating rapid regression of OHSS symptoms. As early OHSS symptoms usually become most severe approximately 5–7 days POR, we advanced the starting date of GnRH antagonist administration to day 3 POR to improve prevention, which was different from a previous study that started on day 5 POR [16], and the serum VEGF level was not significantly increased on day 6 POR after cetrorelix administration compared with day 3 POR. This result indicates that the intervention effect of GnRH antagonist administration for only 3 days in our present study was as good as the 4-day treatment described in the previous study mentioned above, with both leading to rapid regression of OHSS symptoms by suppressing VEGF secretion.

The only contradictory observation in this study was that no obvious mean ovarian volume enlargement was found in either group. Interestingly, mean ovarian enlargement was found to be greater in a mild compared to a severe group in another prospective observational study by Nagraj et al. [13]. Furthermore, the frequency of elevated WBCs, liver dysfunction, severe hemoconcentration, hospital admission, and average duration of hospitalization were reduced in the cetrorelix group, but with no statistical significance. Possible reasons might be the small sample size and deviation caused by the subjective operation of clinicians. Thus, large-scale clinical randomized trials are needed in the future.

The development of OHSS following ovarian stimulation with gonadotropins is mainly associated with hCG administration, as the syndrome rarely develops if hCG is withheld [10]. hCG upregulates VEGF expression in granulosa cells during OHSS [29], and dopamine may block VEGF and

**Table 5** Comparison of baseline characteristics, ovarian stimulation, and embryological data of the study participants included and OHSS non high-risk patients

| | OHSS High-risk group ($n=96$) | OHSS non High-risk group ($n=1115$) | $P$ value |
|---|---|---|---|
| Baseline characteristics | | | |
| Age (years) | 31.45 ± 3.63 | 33.61 ± 4.91 | **0.000** |
| BMI (kg/m$^2$) | 21.38 ± 2.56 | 23.09 ± 3.53 | **0.000** |
| Duration of infertility (years) | 3.40 ± 2.20 | 3.69 ± 2.96 | 0.231 |
| AFC | 15.42 ± 4.40 | 9.77 ± 5.01 | **0.000** |
| Cause of infertility ($n$, %) | | | |
| Tubal factor | 30 (31.25) | 350 (31.39) | 0.977 |
| Endometriosis | 11 (11.46) | 108 (9.69) | 0.576 |
| Male factor | 50 (52.08) | 397 (35.61) | **0.001** |
| Unexplained infertility | 1 (1.04) | 0 (–) | – |
| Multiple factors | 11 (11.46) | 88 (7.89) | 0.221 |
| Baseline FSH (IU/L) | 6.93 ± 1.45 | 8.21 ± 2.65 | **0.000** |
| Baseline LH(IU/L) | 5.03 ± 1.96 | 4.29 ± 2.11 | **0.001** |
| Baseline E2 (pg/ml) | 47.83 ± 18.91 | 45.39 ± 22.81 | 0.307 |
| Ovarian stimulation | | | |
| Duration of stimulation (days) | 10.89 ± 1.34 | 10.50 ± 2.26 | 0.091 |
| Total Gn (IU) | 2335.50 ± 835.21 | 2913.17 ± 1006.61 | **0.000** |
| E2 on day of hCG (pg/ml) | 7261.56 ± 2480.86 | 2465.21 ± 1732.27 | **0.000** |
| Progesterone on day of hCG (ng/ml) | 1.47 ± 0.71 | 1.02 ± 0.85 | **0.000** |
| Number of follicles on day of hCG | 20.28 ± 6.44 | 10.30 ± 5.64 | **0.000** |
| Embryological data | | | |
| Number of oocytes retrieved | 15.24 ± 6.45 | 6.49 ± 3.55 | **0.000** |
| Type of fertilization (IVF/ICSI) | 43/53 | 613/502 | 0.055 |
| Mature oocytes (in ICSI patients) | 12.49 ± 5.65 | 5.20 ± 3.24 | **0.000** |
| Number of 2PN | 9.78 ± 4.83 | 4.11 ± 2.86 | **0.000** |
| Number of transplantable embryos | 9.87 ± 4.88 | 4.22 ± 2.92 | **0.000** |
| Number of high quality embryos | 4.32 ± 3.17 | 1.88 ± 1.85 | **0.000** |
| Number of embryos cryopreserved | 6.45 ± 2.55 | 2.55 ± 2.07 | **0.000** |

Values are expressed as the mean ± standard deviation (SD) unless otherwise stated. $P<0.5$ depicts statistical significance

BMI, body mass index; AFC, antral follicle count; PCOS, polycystic ovarian syndrome; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; Gn, gonadotropin; hCG, human chorionic gonadotropin

IL-8-induced endothelial permeability by inhibiting common VEGFR-2-dependent signals [30]. EGR-1 plays an important role in regulating angiogenesis, and studies have shown that EGR-1 influences mouse embryo implantation by affecting expression of VEGF [31] and that EGR-1 possesses a strong inhibitory capacity against the angiogenic activity of VEGF via VEGFR-2 in vivo [32]. However, the signaling pathway through which hCG mediates VEGF secretion remains unclear. In our present study, similar to VEGF, a significant increase in the serum EGR-1 level was evident in the control group on day 6 POR compared with day 3 POR, indicating the possible role of EGR-1 in the mechanism of OHSS progression. In addition, no significant increase or decline in VEGF and EGR-1 from day 3 POR to day 6 POR was observed in the cetrorelix group, showing that cetrorelix may block VEGF signaling via VEGFR-2 by interacting with EGR-1. The possible role of IGF-I in the mechanism of OHSS prevention needs further study.

A major limitation of this prospective study is that the study was non-randomized. At present, only one study has compared the therapeutic effects of cetrorelix and cabergoline in early OHSS patients [33], showing that cetrorelix was more effective than cabergoline, with a significantly lower percentage of hospitalizations and incidence of moderate and severe OHSS. Moreover, comparison between GnRH antagonist and other drugs, such as letrozole and dopamine agonists, in the prevention of moderate and severe OHSS should be considered. In addition, our sample size was small, and further large clinical randomized trials are needed to consider the risks of the GnRH antagonist cetrorelix, to compare the effects of GnRH antagonist with other possible prevention drugs, and to investigate the mechanism of

OHSS progression and the potential mechanism of cetrorelix in early moderate and severe OHSS prevention.

## Conclusion

According to our results, luteal administration of the GnRH antagonist cetrorelix is effective in reducing the incidence and alleviating the symptoms of early moderate and severe OHSS in high-risk women. The possible mechanism of cetrorelix might occur via direct actions on the ovary, which may lead to a decline in serum VEGF levels. The present data provide further support for the administration of the GnRH antagonist cetrorelix during the luteal phase as outpatient management for moderate and severe OHSS. A further large randomized clinical investigation is still needed.

**Author contributions** CZ: study design, data management and analysis, and manuscript writing/editing. JS: project development. AMJ: data analysis. PLW and XL: data collection and management. QX: study design, project development, and manuscript editing. All authors read and approved the final manuscript.

**Funding** This work was supported by the National Key R&D Program of China (2017YFC1001200).

### Compliance with ethical standards

**Conflict of Interest** The authors declare that they have no competing interests.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethics review board of Peking University First Hospital and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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