Cocktail Treatment by GnRH-Antagonist, Letrozole, and Mifepristone for the Prevention of Ovarian Hyperstimulation Syndrome: A Prospective Randomized Trial

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Abstract

Ovarian hyperstimulation syndrome (OHSS) is a major complication of controlled ovarian hyperstimulation. This study is aimed to determine the efficacy of a cocktail style treatment by combining GnRH-antagonist, letrozole, and mifepristone on the prevention of ovarian hyperstimulation syndrome (OHSS) in high-risk women. This prospective, randomized controlled clinical trial included 170 women who identified as high risk of OHSS during the ovarian hyperstimulation and underwent cryopreservation of whole embryos. On the day of oocyte retrieval, the combination group (89 cases) received 0.25 mg Cetorelix for three days, 5 mg letrozole for five days, and 50 mg mifepristone for three days, the mifepristone group (80 cases) received 50 mg mifepristone for three days. 156 cases were included in final analysis. The combination group showed significantly decreased incidence of moderate and severe OHSS than mifepristone group (20.5% vs. 42.3%), with remarkably reduced serum estradiol level on hCG+3 and +5 day, decreased ovarian diameter, and shortened luteal phase. Oocyte retrieval number, levels of estradiol on hCG+0 and VEGF, and ovarian diameter on hCG+5 were associated with the severity of the symptoms. In Conclusion, the combination treatment effectively reduce the incidence of moderate/severe OHSS in high-risk women.

Clinical trial registration information:

Date of registration: 12-27-2017

Date of initial participant enrollment: 01-01-2018

Clinical trial identification number: ChiCTR-INR-17014174

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Introduction

Ovarian hyperstimulation syndrome (OHSS) is one of the most serious complications associated with controlled ovarian hyperstimulation (COH) in assisted reproductive technology (ART). Women who develop to OHSS experience different levels of gastrointestinal discomfort, liver and renal malfunction, edema, oliguria, ascites, and hydrothorax [1, 2]. In extreme cases, although rare, the patients suffer from life-threatening situations because OHSS can cause venous or arterial thromboembolic events, including stroke and loss of perfusion of an extremity [3, 4].

Modified COH protocols, such as low Gonadotropin dose, GnRH antagonist (GnRH-ant) protocol, GnRH agonist/hCG dual trigger, and coasting, has been widely used to minimize the incidence of OHSS in IVF cycles. The incidence of moderate and severe OHSS ranges from 0.2–2% of all COH cycles, our previous clinical study shows the incidence of severe OHSS is 1.2% (55/4735) in all COH cycles in our center [5]. However, moderate and severe OHSS has been calculated to occur in 15–45.8% in high-risk women, even

with above-mentioned modified protocols [5–7]. Therefore, secondary interventions after oocyte retrieval are needed to minimize the incidence and severity of OHSS in high-risk women.

OHSS can be classified as early-onset and late-onset based on the time of onset. Early-onset OHSS arises between 3–7 days following human chorionic gonadotropin (hCG) injection and relates to the exogenous hCG dosage; late-onset OHSS is related to endogenous hCG elevation due to pregnancy [8]. Early-onset cases are promptly mitigated with the arrival of the menses, but last longer if the patient becomes pregnant. On the other hand, late-onset cases caused by pregnancy are intended to be more severe and the symptoms might last for over a month, suggesting that ovarian corpus luteum may play an essential role in the progression of OHSS.

Indeed, hCG is the main hormone for maintaining the activity of corpus luteum [9], and the pro-luteolysis effect induced by GnRH-ant may decrease the production of vasoactive factors in the ovaries, resulting in the regression of severe OHSS [10]. Letrozole, an aromatase inhibitor, has been originally applied in the ovarian stimulation. Recently, letrozole treatment after oocyte retrieval shows a prevention effect on moderate and severe OHSS due to the reduction of serum E₂ level [6]. Progesterone is produced by corpus luteum after ovulation and essential for corpus luteum function, and progesterone withdrawn triggers corpus luteum regression and menstruation. Mifepristone (also termed as RU486), a progesterone receptor antagonist, is clinically used for pregnancy termination due to its luteolysis effect [11], but the application of mifepristone for OHSS prevention is limited in clinical studies. Some other adjuvant drugs are also used to prevent or minimize the severity of OHSS during ovarian stimulation or after oocyte retrieval, such as dopamine agonists and aspirin [12, 13]. However, hitherto it is hard to draw a conclusion that single protocol or drug can completely prevent OHSS. We theorize that a cocktail style treatment by combining GnRH-ant, letrozole, and mifepristone could effectively prevent OHSS in high-risk women. Here we design a prospective, randomized, clinical trial to determine whether this drug regimen decrease the incidence of OHSS in women who identified as high-risk status during the COH.

Materials & Methods

This is a prospective randomized controlled trial (Registration number: ChiCTR-INR-17014174) registered at the China Clinical Trials Registry (www.chictr.org.cn) on 2017-12-27 (indicated as Date of Registration on the Registration page), which was approved by Ethics Committee of Clinical Trials in Wuhan University Renmin Hospital (Reference number: 2018K-C001), the first participant was enrolled on 2018-01-01. All participants signed informed consent before the enrollment. Doctors who assess the patients' condition of OHSS were blinded to the grouping and the assignments throughout this trial.

Study participants

The participants were enrolled from the Center for Reproductive Medicine, Wuhan University Renmin Hospital from January 2018 to December 2018. Inclusion criteria: infertile women aged 20–38 underwent all embryo freezing during their first IVF/ICSI cycle, had at least one of the following high-risk criteria:

serum $E_2 \geq 4,000$ pg/ml on the day of hCG administration day (trigger day, hCG + 0); number of oocytes retrieval ≥ 18 ; ultrasonography proved ovarian hyperstimulation on the day of oocyte retrieval. Exclusion criteria: allergic to any of the three medicine; liver or kidney diseases; received any medicine affecting the permeability of blood vessel during IVF/ICSI cycle; coasting and any other interventions to prevent OHSS during ovulation stimulating; or possibility of pregnancy.

Sample size calculation: our preliminary experimental results showed that the incidence of moderate to severe OHSS was 50% (p_c) in mifepristone group and 26% (p_e) in combination group, respectively.

According to the following formula ^[10], $n=[(u_\alpha+u_\beta)^2(1+1/k)p(1-p)]/(p_e-p_c)^2$, $p=(p_e+k \times p_c)/(1+k)$, the sample size is calculated by comparing the two sample rates. Assume that the ratio of participants in two groups is 1:1, then $k=1$. To achieve 80% power and α 0.05 significant level (adopting two-tailed test, $u_\alpha=1.96$, $u_\beta=0.8417$), at least 69 participants should be included in each group. If the follow-up loss rate is 10%, a total of at least 152 participants would be required. 156 participants were included for final analysis in this study.

Protocols for controlled ovary hyperstimulation (COH)

The COH protocols were conducted according to the Guidelines in our center [5]. Briefly, in luteal GnRH agonist (GnRH-a) protocol, daily administration of GnRH-a (IPSEN PHARMA BTOTECH, France, 0.1 mg) at mid-luteal phase for at least 14 days before starting Gonadotropin stimulation. After 2–3 weeks, patients received ovarian stimulation with gonadotropin (Gn, Gonal-F, Merck-Serono, Aubonne, Switzerland) of 150–225 IU daily. The Gn dose was adjusted according to the hormonal level and the diameter of the oocytes. In follicular phase long GnRH-a protocol, the patients received a subcutaneous injection of 3.75 mg of a long-acting GnRH agonist (Diphereline, IPSEN PHARMA BIOTECH) on the 2nd day of menstruation. Patients were monitored to determine whether pituitary downregulation was achieved 30–40 days later, with $E_2 < 25$ pg/ml, $LH < 5$ U/L, and the diameter of the antral follicle < 5 mm, then received ovarian stimulation with gonadotropin as long luteal GnRH agonist protocol. In GnRH-ant protocol, Gn was used on the 2nd or 3rd day of menstruation, and 0.25 mg/d Cetrorelix was added on the 5th or 6th day of ovarian stimulation until hCG trigger day. 2,000–10,000 IU hCG (Lizhu Pharmaceutical Co., Ltd. China) with or without 0.2 mg GnRH agonist (Decapeptyl, Ferring Pharmaceuticals) was administered for triggering once at least 3 follicles reached to 18 mm or at least 2 follicles reached to 20 mm, the oocytes were retrieved and fertilized by IVF/ICSI 36–40 hours after triggering.

Grouping and medication regimen

The screening process was initiated at any time during the controlled ovarian stimulation, the participants were informed about the risk of OHSS, and all agreed to the cancellation of fresh embryo transfer and signed the inform consent. The “Serial number” and patient information was registered according to screening order. A specialized person who is not involved in the recruitment process was authorized to generate a “Random number” table by EXCEL Office Software based on the “Serial number”. The participants were randomly allocated into “Combination group” or “Mifepristone group” and received medication on the day of oocyte retrieval.

In mifepristone group, participants received 25 mg mifepristone (Beijing Famostat Pharmaceutical Technology Co, Ltd.) orally twice for 3 consecutive days. In combination group, participants received 0.25 mg Cetorelix (Merch Serono S.p.A, France) subcutaneously injection per day for 3 consecutive days, 5 mg Letrozole tablets (Jiangsu Hengrui Pharmaceutical Co, Ltd. China) orally per day for 5 consecutive days, and 25 mg mifepristone tablet orally twice for 3 consecutive days.

The classification of OHSS

Table 1 presented the classification of OHSS was based on clinical and laboratory features as previously described [6, 14]. Patients demonstrating any feature of severe or critical OHSS should be classified in that category. However, those only with ovarian enlargement without any clinical manifestations or abnormal laboratory indicators will be allocated to mild group.

Table 1
Classification of OHSS

Clinical features		Laboratory results
Mild	Abdominal bloating Mild abdominal pain Mild nausea/vomiting	Enlarged ovaries (7–8 cm)
Moderate	Moderate abdominal pain Nausea/vomiting	HCT > 41% WBC > 15 × 10 ⁹ /L Hypoproteinemia (ALB < 30g/L) Enlarged ovaries (8–12 cm)
Severe	Mild and moderate clinical feature Clinical ascites/hydrothorax Severe dyspnea Anuria/oliguria Weight gain (> 1 Kg/24 h)	HCT > 55% WBC > 25 × 10 ⁹ /L Creatinine clearance < 50 ml/min Serum creatinine > 115 μM/L Na ⁺ < 135 mmol/L; K ⁺ > mmol/L ALT > 80 U/L and/or AST > 70 U/L Enlarged ovaries (> 12 cm)
Critical	Thromboembolism Acute respiratory distress syndrome	Same as severe OHSS
WBC, White blood cell counts; HCT, Hematocrit; ALT, alanine transaminase; AST, aspartate aminotransferase.;		

Observational outcome measures

The main outcome measure was the incidence and severity of OHSS. The outcomes included no OHSS, mild, moderate, severe, and critical OHSS. The secondary outcomes included serum E₂, progesterone, and VEGF levels, ovarian size (maximum diameter), the duration of luteal phase after oocyte retrieval. Other outcome measures included the incidence of thoraco-abdominal puncture, blood concentration, abnormal liver and kidney function, and abnormal coagulation function, the total volume of pleural or peritoneal effusion, and etc.

Statistical analysis

Data analysis was processed by SPSS 22.0 statistical software (SPSS Inc, Chicago, IL). Metrological data were presented as means $\pm$ SEM. Independent sample *t* test was adopted when normal distribution and homogeneity of variance were met, otherwise Mann-Whitney U was performed. Rate was compared using the chi-square test, Fisher's Exact Test was applied when necessary. No critical OHSS was recorded in this study, we used four categories of OHSS (no OHSS, mild, moderate and severe OHSS) to serve as dependent variables, and ordinal logistic regression analysis was used to identified the factors that associated with the severity of OHSS. $P < 0.05$ is considered statistical significance.

Results

Participants

From January 2018 to December 2018, 170 women were screened for eligibility and assigned with Serial number, 169 women were consented to participate in this trial and randomly allocated into 2 groups to receive either combination (89 women) or mifepristone (80 women) treatment. 11 women were quitted from the combination group (7 were prescribed with traditional Chinese medicine and 4 withdrew the consent from this trial), and 2 women were quitted from mifepristone group (2 were prescribed with GnRH-ant and mifepristone simultaneously). Ultimately, 156 women (78 women in combination group and 78 in mifepristone group) were included in this trial, and the follow-up rate for primary outcome was 100% (Fig. 1).

Basal clinical characteristics between the two groups

The basal characteristics of participants between the combination and mifepristone groups were similar, with no significant differences in age, body mass index (BMI), antral follicle count (AFC), basal FSH, LH and E₂ levels on day 2 of the menstrual cycle, duration and causes of infertility, COH protocols, total dosage and duration of Gn, E₂ and progesterone (P) levels on hCG trigger day (hCG + 0), the number of oocytes retrieved and good-quality embryos ($P > 0.05$) (Table 2).

Table 2
Basal characteristics of the participants

	Combination (n = 78)	Mifepristone (n = 78)	P value
Age (years)	29.7 ± 3.6	29.6 ± 3.2	0.78
Duration of infertility (years)	3.9 ± 2.9	4.1 ± 3.2	0.73
BMI (kg/m ²)	22.0 ± 3.3	21.90 ± 3.7	0.56
AFC (n)	23.0 ± 6.5	23.0 ± 7.7	0.85
Basal FSH (IU/L)	6.6 ± 1.6	6.4 ± 1.6	0.40
Basal LH (IU/L)	6.0 ± 5.1	5.5 ± 4.0	0.51
Basal E ₂ (pg/ml)	47.1 ± 36.1	43.3 ± 18.8	0.86
Factors of infertility n (%)			
Tubal factor	33 (42.3)	29 (37.2)	0.51
Anovulatory	8 (10.3)	16 (20.5)	0.08
EMS ¹	2 (2.6)	5 (6.4)	0.44
Male factor	12 (15.4)	8 (10.3)	0.34
Multiple factors	18 (23.1)	14 (17.9)	0.43
Unexplained infertility	5 (6.4)	6 (7.7)	0.75
COH protocol, n (%)			
Luteal long GnRH-a protocol	34 (43.6)	28 (35.9)	0.33
GnRH-ant protocol	20 (25.6)	22 (28.2)	0.72
Follicular phase long protocol	24 (30.8)	28 (35.9)	0.50
Total Gn (IU)	1689.2 ± 641.7	1824.5 ± 809.7	0.63
Duration of Gn stimulating (days)	10.2 ± 2.1	10.4 ± 2.2	0.40
Number of retrieved oocytes (n)	26.6 ± 6.2	25.7 ± 4.3	0.29
Good-quality embryos (n)	11.5 ± 5.5	10.0 ± 4.8	0.09
¹ EMS: endometriosis;			

Incidence of OHSS in the two groups

After five days of treatment, the incidence of OHSS was comparable between the combination and mifepristone groups (80.5% vs. 84.6%, $P = 0.526$). However, the proportion of mild OHSS was significantly higher in the combination group than that in mifepristone group (60.3% vs. 55.1%, $P = 0.024$), and the incidence of moderate and severe OHSS was remarkably decreased in the combination group than mifepristone group (20.5% vs. 42.3%, $P = 0.003$) (Table 3). During the classification, patients with enlarged ovaries (diameter in 7–8 cm), but without any symptoms and abnormalities in laboratory tests, were allocated into mild group. The proportion of participants without any symptoms or discomforts was significantly higher in the combination group than that in mifepristone group (37.2% vs. 20.5%, $P = 0.033$). The incidence of moderate and severe OHSS showed no significant difference among luteal long GnRH-a, GnRH-ant, and follicular phase long GnRH-a protocol in both two groups (Table 4).

Table 3
Incidence of OHSS in two groups

	Combination (n = 78)	Mifepristone (n = 78)	<i>P</i> value
No OHSS (n, %)	15 (19.2)	12 (15.4)	0.53
Mild (n, %)	47 (60.3)	43 (55.1)	0.02
Moderate (n, %)	12 (15.4)	23 (29.5)	0.04
Severe (n, %)	4 (5.1)	10 (12.8)	0.09
Moderate and severe (n, %)	16 (20.5)	33 (42.3)	0.003
No symptoms (n, %)	29 (37.2)	16 (20.5)	0.03

Table 4
Incidence of moderate and severe OHSS in different COH protocols

	Combination	Mifepristone	<i>P</i> value
Luteal GnRH-a protocol	20.6 (7/34) ^a	42.9 (12/28) ^b	0.096
GnRH-ant protocol	15.0 (3/20) ^a	36.4 (8/22) ^b	0.116
Follicular phase GnRH-a protocol	25.0 (6/24) ^a	46.4 (13/28) ^b	0.110
^a : No significant difference among long GnRH-a, GnRH-ant, and ultra-long protocols in combination group.			
^b : No significant difference among long GnRH-a, GnRH-ant, and ultra-long protocols in mifepristone group.			

Serum E₂, P, and VEGF alteration in the two groups

The comparison of serum E_2 and P levels between the combination and mifepristone groups was shown in Fig. 2. There was no difference in serum E_2 level on hCG + 0 between the two groups. However, the serum E_2 level was dramatically reduced on hCG + 3 (110.9 ± 63.5 vs. 2857.2 ± 1111.4 , $P < 0.001$) and hCG + 5 (173.3 ± 132.6 vs. 3758.4 ± 2029.2 , $P < 0.001$) in the combination group than that in mifepristone group (Fig. 2A). The serum P4 levels on hCG + 0, + 3, and + 5 showed no statistical difference between the two groups (Fig. 2B).

VEGF has been identified as a key regulator in increased vascular permeability in the pathogenesis of OHSS [15]. As shown in Fig. 3A, the serum VEGF levels on hCG + 3 and + 5 were comparable between the combination group and mifepristone group. However, the VEGF level on hCG + 5 was significantly increased in patients who developed to OHSS, and the VEGF level was positively associated with the severity of OHSS (Fig. 3B).

Clinical and laboratory features comparison between the two groups

The clinical and laboratory features regarding to OHSS were summarized in Table 5. The ovarian diameter was significantly decreased in the combination group when compared with mifepristone group on hCG + 5 (8.7 ± 1.1 vs. 9.1 ± 1.5 , $P = 0.021$). The luteal phase (the duration between oocyte retrieval and next menstruation) was shorter in the combination group than that in mifepristone group (7.5 ± 2.6 vs. 9.6 ± 1.8 , $P < 0.001$), and the incidence of luteal phase < 5 days was increased in the combination group than mifepristone group (19.2% vs. 1.3%, $P < 0.001$). There was no statistical difference in ascites index (the total depth of ascites measured by ultrasound) and the incidence of ascites puncture and drainage between the two groups. The combination group showed decreases in white blood cell count (7.6 ± 2.0 vs. 9.6 ± 2.4 , $P < 0.001$) and the incidence of hemoconcentration (3.9% vs. 12.8%, $P = 0.043$) than mifepristone group on hCG + 3 day. The combination group also had decreased alanine transaminase (ALT, 59.6 ± 39.4 vs. 74.2 ± 41.9 , $P = 0.026$) level and increased albumin (ALB, 34.3 ± 3.5 vs. 32.4 ± 3.5 , $P = 0.001$) level than mifepristone group. The medical costs were similar between the two groups.

Table 5
Clinical and laboratory features between combination and mifepristone groups

	Combination (n = 78)	Mifepristone (n = 78)	P value
Ovarian diameter on hCG + 5 (cm)	8.7 ± 1.1	9.1 ± 1.5	0.021
Ascites index	7.1 ± 3.2	7.58 ± 4.04	0.460
Luteal phase (days)	7.5 ± 2.6	9.6 ± 1.8	< 0.001
Luteal phase < 5 days (n, %)	15 (19.2)	1 (1.3)	< 0.001
Puncture and drainage (n, %)	2 (2.6)	5 (6.4)	0.442
WBC on hCG + 3 (10 ⁹ /L)	7.6 ± 2.0	9.6 ± 2.4	< 0.001
HCT on hCG + 3 (%)	36.5 ± 3.5	36.8 ± 3.5	0.528
Hemoconcentration (n, %) ¹	3 (3.9)	10 (12.8)	0.043
D-D on hCG + 3	1.8 ± 2.8	2.1 ± 3.1	0.621
ALT on hCG + 5 (g/L)	59.6 ± 39.4	74.2 ± 41.9	0.026
AST on hCG + 5 (g/L)	62.8 ± 48.8	72.7 ± 42.9	0.183
ALB on hCG + 5 (g/L)	34.3 ± 3.5	32.4 ± 3.5	0.001
Medical costs (¥)	9616.8 ± 2815.2	9966.9 ± 4184.6	0.949
¹ Hemoconcentration: HCT > 41%.			

Ordinal multinomial logistic regression analysis

Ordinal multinomial logistic regression analysis was performed to predict the relationship between the incidence and severity of OHSS and patients' clinical features, in which the four categories of OHSS (no OHSS, mild, moderate, and severe OHSS) served as dependent variables, and patient's age, AFC, Gn duration and dosage, oocyte retrieved number, interventions (combination and mifepristone), serum E₂ on hCG + 0, and serum VEGF on hCG + 5 were served as independent variables. As shown in Table 6, the oocyte retrieved number (odds ratio 1.09, 95% confidence interval 1.00–1.19, *P* = 0.05), intervention (odds ratio 0.31, 95% confidence interval 0.15–0.63, *P* = 0.001), serum E₂ level on hCG + 0 (odds ratio 1.00, 95% confidence interval 1.00–1.00, *P* = 0.03), serum VEGF on hCG + 5 (odds ratio 1.03, 95% confidence interval 1.02–1.04, *P* < 0.001), and ovarian diameter on hCG + 5 (odds ratio 3.63, 95% confidence interval 2.35–5.60, *P* < 0.001) were associated with the severity of OHSS.

Table 6
Ordinal multinomial logistic regression analysis

Variables	OR ¹	95% CI	P value
Age	0.98	0.88–1.09	0.65
AFC	1.00	0.97–1.08	0.41
Duration of Gn	1.07	0.86–1.33	0.55
Gn dosage	1.00		0.20
Number of oocyte retrieved	1.09	1.00–1.19	0.05
Intervention			
Combination	0.31	0.15–0.63	0.001
Mifepristone	–	–	–
Serum E ₂ on hCG + 0	1.00	1.00 - 1.00	0.03
Serum VEGF on hCG + 5	1.03	1.02–1.04	< 0.001
Ovarian diameter on hCG + 5	3.63	2.35–5.60	< 0.001
¹ OR: odds ratio.			

Discussion

This is a prospective clinical randomized trial to investigate the efficacy of a cocktail style treatment by combining GnRH-ant, letrozole, and mifepristone on the prevention of OHSS in high-risk women compared with the monotherapy of mifepristone. Although the total incidence of OHSS is similar between the combination group and mifepristone group, the incidence of moderate and severe OHSS is remarkably decreased in the combination group (20.5% vs. 42.3%, $P = 0.003$), with significantly reductions in serum E₂ level, ovarian diameter, and luteal phase. We suggest that this drug regimen can be used in clinical to minimize the severity of OHSS in high-risk women.

OHSS is a potentially life-threatening complication in COH treatments, which results from the release of vasoactive substances from the multiple corpora lutea, such as estrogens and VEGF, due to the ovarian hyperstimulation after oocyte maturation trigger. The incidence of OHSS shows a gradual decrease as the incessant sculpture of ovarian stimulation protocols, however, certain patients are at high risk of OHSS, such as PCOS and high responsive patients [16]. The identification of patients susceptible to elicit a hyper-response to standard COH protocols would allow clinician to adjust their treatment, at a large extent, to minimize the severity of the symptoms. In clinical practice, all the symptoms, even with the severe OHSS symptoms, are vanished with the arrival of the menstruation. Therefore, medications that promote luteolysis are theoretically effective for the prevention or treatment of OHSS.

Recently, GnRH-ant administration during the luteal phase offers another therapeutic method for patients who developed to severe OHSS after oocyte retrieval. Treatment of 0.25 mg GnRH-ant for three days (from hCG + 5 to + 7) significantly accelerates patients' recovery from severe OHSS symptoms and shows similar pregnant outcomes with the control group [17]. Another study shows that 0.25 mg GnRH-ant administration from days 5 to 8 after oocyte retrieval for women who manifested early severe OHSS symptoms is associated with rapid regression of the syndrome and accelerated luteolysis [18]. Although these two studies suggest a valid therapeutic effect of GnRH-ant on severe OHSS, the effect of GnRH-ant on OHSS prevention during the luteal phase remains undefined. Letrozole manifests promptly E_2 reduction effect, two clinical trials show that letrozole treatment during the luteal phase effectively decrease the incidence of moderate and severe OHSS due to its luteolysis effect [6], and the incidence of OHSS is decreased as the increases in letrozole dose [19]. Progesterone is secreted by corpora lutea and functions to maintain the corpora lutea, and progesterone withdraw is the trigger for luteal regression and menses initiation. Mifepristone is a well identified progesterone receptor antagonist, which is clinically used for emergency contraception by inhibiting embryo implantation and early pregnancy termination. Although the clinical application of mifepristone in OHSS treatment is limited, animal models show that progesterone is a key regulator for increased vascular permeability in mouse uterine vessels, and mifepristone blocks the effect of progesterone on vascular permeability [20]. In hCG induced OHSS rat model, mifepristone could significantly reduce the ovarian enlargement and VEGF production [21, 22], suggesting the luteolysis effect of mifepristone is potentially effective for minimize the risk of OHSS.

We have previously used a rat model to determine the effect of GnRH-ant, letrozole, and mifepristone, alone or in combination, on the prevention of OHSS [23]. The results have shown that GnRH-ant, letrozole, and mifepristone are all beneficial to prevent the progression of OHSS through different luteolytic mechanisms, and the combination group shows enhanced synergistic effect on preventing the progression of OHSS. Therefore, we proposed this prospective, randomized study to evaluate the effect of combination of GnRH-ant, letrozole, and mifepristone on preventing OHSS in high-risk women. The combination group shows decreases in the proportion of moderate and severe OHSS than that in mifepristone group, the clinical and laboratory index comparison demonstrates that the combination treatment accelerates luteolysis process and improves the participants' hemoconcentration and liver function. These results suggest that the combination treatment by GnRH-ant, letrozole, and mifepristone is effectively to reduce the severity of OHSS in high-risk women.

GnRH-ant protocol has been recommended for PCOS women to reduce the incidence of OHSS. A meta analysis reported that GnRH-ant group does not reduce the incidence of severe OHSS, but significantly reduce the incidence of moderate and severe OHSS when compared with GnRH-a group [24]. Another study showed that GnRH-ant protocol significantly lower OHSS rates in general IVF patients and PCOS women. However, the ongoing pregnancy rate was significantly lower in GnRH-ant group than GnRH-a group in general IVF patients [25]. In China, luteal long GnRH-a and follicular phase long GnRH-a protocols are the preferred options for first IVF cycle patients in most IVF centers. In this study, tubal factor is the primary etiology in included participants, and the proportions of luteal GnRH-a, GnRH-ant,

and follicular phase GnRH-a protocols are not differed between combination and mifepristone groups. GnRH-ant protocol does not lower the incidence of moderate and severe OHSS in combination group or mifepristone group. These results suggest that COH protocols may not be associated with the severity of the symptoms.

In the present study, oocyte retrieval number, serum E_2 level on hCG + 0, VEGF level and ovarian diameter on hCG + 5 are associated with the severity of the symptoms. E_2 level has been served as a reliable predictor for identifying high-risk women of OHSS during the COH, and estrogens are shown to increase vascular permeability in uterine and ovarian circulation. However, some cases of OHSS are reported in women with low E_2 level and natural conceived pregnancy [26], and large doses of E_2 treatment could not reproduce OHSS in animal model, indicating that E_2 alone is not able to initiate OHSS. VEGF is strongly implicated as a vasoactive factor and plays important role in regulating angiogenesis and vascular permeability. VEGF is also involved in hCG-dependent ovarian angiogenesis, and increased VEGF mRNA and protein levels are detected in serum and peritoneal fluids in women with high risk of OHSS [27]. Cabergoline, a dopamine agonist with anti-VEGF activity, has been demonstrated to be an effective and safe drug for the prevention of OHSS [28]. E_2 itself does not exhibit direct vasoactive effects, but E_2 induces endometrial VEGF expression and stimulates microvessel endothelial cell proliferation and vascular expansion [29], suggesting that E_2 and VEGF are both essential for the initiation of OHSS.

Previous study shows letrozole treatment, although decrease the incidence of moderate and severe OHSS, could increase VEGF level on hCG + 7 [6], indicating that letrozole-induced E_2 reduction is not able to suppress VEGF expression. Follicular E_2 and VEGF levels are decreased in PCOS women received GnRH-ant protocol [30], and serum VEGF level is down-regulated by both of GnRHa and GnRH-ant, which may partially explain the decreases in OHSS in GnRHa trigger and GnRH-ant protocol [31]. Ordinal multinomial logistic regression analysis also shows serum E_2 level on hCG + 0 and VEGF level on hCG + 5 are associated with the severity of OHSS, therefore, targeting in reduction of serum E_2 and VEGF levels is theoretically for OHSS prevention. In the present study, serum E_2 level on hCG + 0 is comparable between the two groups. However, serum E_2 levels on hCG + 3 and + 5 are dramatically decreased in the combination group. Serum VEGF levels on hCG + 3 and + 5 are similar between the two groups, but the VEGF level on hCG + 5 is significantly higher in OHSS cases than that in non-OHSS women and positively related to the severity of OHSS.

OHSS is characterized as enlarged ovaries and the categories of OHSS is diagnosed partially based on the ovarian diameter. In this study, the ovarian diameter on hCG + 5 is significantly reduced in the combination group than that in mifepristone group, and logistic regression analysis shows that the ovarian diameter is associated with the severity of OHSS. The luteal phase in combination group is shorter than mifepristone group, and the proportion of women with < 5 days of luteal phase is higher in combination group. During the clinical observational frame after oocyte retrieval, the proportion of patients without any OHSS related symptoms is significantly increased in the combination group, and most of patients in combination group are recovered and back to normal life more quickly than

mifepristone group. These results suggest that the synergistic effect of GnRH-ant, letrozole and mifepristone could accelerate luteolysis effect and prevent the occurrence of moderate and severe OHSS.

Strengths and limitations

This is a prospective and randomized clinical trial, and the evaluation on participants' symptoms is blinded to the grouping. This study has a major limitation in control group design regarding the monotherapy of GnRH-ant or letrozole as a prospective randomized study. Our previous animal OHSS model showed that the combination group has a synergistic effect than monotherapy; Mifepristone has been used as the primary preventive therapy to accelerate luteolysis for high risk women in our center. However, the effect of mifepristone on OHSS prevention remains unknown. Therefore, the combination treatment was set as experiment group, and mifepristone was set as control group in this clinical trial.

Conclusions

The combination treatment by combining GnRH-ant, letrozole and mifepristone after oocyte retrieval is an effective drug regimen for reducing the severity of OHSS in high-risk women. This cocktail style treatment prevents moderate and severe OHSS syndrome may partially through targeting E_2 reduction and luteolysis.

Declarations

Not applicable.

Institutional Review Board Statement

This is a prospective randomized controlled trial (Registration number: ChiCTR-INR-17014174) registered at the China Clinical Trials Registry (www.chictr.org.cn) on 2017-12-27 (indicated as Date of Registration on the Registration page), which was approved by Ethics Committee of Clinical Trials in Wuhan University Renmin Hospital (Reference number: 2018K-C001), the first participant was enrolled on 2018-01-01.

Informed Consent Statement

All participants signed informed consent before the enrollment.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Authors' contribution

Conceptualization, Qingzhen Xie and Qianrong Qi, methodology, Yaqin Wang, software, Yi Xia, validation, Qianrong Qi, Yi Xia. and Jin Luo, formal analysis, Qianrong Qi, investigation, Yaqin Wang, resources, Qingzhen Xie, data curation, Yi Xia, writing—original draft preparation, Qianrong Qi, writing—review and editing, Qingzhen Xie, supervision, Yaqin Wang, project administration, Qingzhen Xie, funding acquisition, Qianrong Qi and Qingzhen Xie, All authors have read and agreed to the published version of the manuscript.

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Figures

Figure 1

Flow diagram for enrollment, randomization, follow-up, and analysis of study participants.

Figure 2

Alterations of serum E₂, P, and VEGF level between the combination and mifepristone groups. (A) Comparison of serum E₂ level on hCG+0, +3, and +5. (B) Comparison of serum P level on hCG+0, +3, and +5.

Figure 3

Comparison of serum VEGF level between the combination and mifepristone groups. (A) Comparison of serum VEGF level on hCG+3 and +5. (B) The relationship between serum VEGF level on hCG+3 and the severity of OHSS. (C) The relationship between serum VEGF level on hCG+5 and the severity of OHSS.