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ASSISTED REPRODUCTION TECHNOLOGIES

Combination of cabergoline and embryo cryopreservation after GnRH agonist triggering prevents OHSS in patients with extremely high estradiol levels—a retrospective study

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Abstract
Purpose Embryo cryopreservation after triggering oocyte maturation with GnRH agonist (GnRHa) in GnRH antagonist protocols has been proposed to prevent ovarian hyperstimulation syndrome (OHSS). However, a small percentage of patients still developed severe OHSS. The purpose of the study was to investigate the efficacy of preventing OHSS in patients at very high risk when cabergoline was given in addition to elective cryopreservation after GnRHa triggering.
Methods This is a retrospective observational study. The patients were stimulated with GnRH antagonist protocol. When serum E_2 concentration was >6,000 pg/ml and there were more than 20 follicles ≥11 mm on the day of final oocyte maturation, GnRHa was used to trigger oocyte maturation. Cabergoline was given to augment the effect of preventing OHSS. The embryos were electively cryopreserved by vitrification and thawed in subsequent cycles. The primary outcome measure was the incidence of severe OHSS. The secondary outcome measure was the clinical pregnancy rate in the first frozen-thawed embryo transfer cycle.
Results One hundred and ten patients underwent 110 stimulated cycles were included for analysis. No patients developed moderate/severe OHSS. Mean E_2 concentration on the day of final oocyte maturation was 7,873 pg/ml, and an average of 22.7 oocytes was obtained from each patient. One hundred and ten thawing cycles were performed, resulting in 69 clinical pregnancies (62.7 %).
Conclusions Combining cabergoline and embryo cryopreservation after GnRHa triggering in GnRH antagonist protocol could prevent OHSS in patients at very high risk.

Keywords Cabergoline · Ovarian hyperstimulation syndrome · GnRH agonist triggering · Cryopreservation

Capsule Combination of cabergoline and embryo cryopreservation after GnRH agonist triggering prevents OHSS in patients with extremely high estradiol levels.

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Introduction

Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening complication of ovarian stimulation, characterized by ovarian enlargement and fluid sequestration from

intravascular volume to the third spaces. The incidence of OHSS increased dramatically after incorporation of GnRH agonist (GnRHa) into ovarian stimulation protocols. Although the etiology is not fully understood, studies have shown that human chorionic gonadotropin (hCG), by inducing the production of vascular endothelial growth factor (VEGF), is the key mediator of OHSS [49].

Administration of a bolus of GnRHa induces LH and FSH surges from pituitary and provides an alternative to hCG to trigger final oocyte maturation. Earlier studies have shown that GnRHa triggering can prevent OHSS [14, 33, 35]. Nonetheless, the popularity of GnRHa protocols limits its use because the pituitary gonadotrophe receptors are unresponsive to GnRHa after desensitization.

In GnRH antagonist protocols, GnRHa can be used as an alternative to hCG to trigger final oocyte maturation because the pituitary remains responsive. However, GnRHa triggering results in lower pregnancy and implantation rates due to defective luteal phase [21, 28, 36]. Elective cryopreservation of embryos or oocytes avoids the problem of defective luteal phase and is an alternative to prevent OHSS. This strategy has been reported with cryopreservation of oocytes [25], 2PN oocytes [19, 20, 23], and day-3 embryos [41] to effectively prevent OHSS in high-risk patients. However, in a later prospective multi-center study one out of 51 patients developed early-onset severe OHSS [22]. The patient had an estradiol (E_2) level of 13,041 pg/ml on the day of GnRHa triggering, and another 3 patients developed moderate OHSS. This suggested that the strategy could not completely prevent severe OHSS [22], especially in patients with extremely high serum E_2 level and multiple follicular development.

Cabergoline is a dopamine agonist that can antagonize VEGF effect on vascular permeability through VEGF receptor 2 dephophorylation [3]. Cabergoline has been reported to effectively prevent OHSS in high-risk patients. Although several studies showed that cabergoline prevented the occurrence of OHSS [7, 39], in one study 11.4 % of patients developed severe OHSS [4]. Even if cabergoline was combined with coasting, severe OHSS still occurred in patients with extremely high serum E_2 levels (>9,000 pg/ml) [31].

Most of the above-mentioned studies included patients fulfilled one or two of the following criteria (1) E_2 levels ≥3,500–4,000 pg/ml (2) more than 20 follicles ≥11 mm [19, 20, 23, 41]. High concentration of E_2 (>6,000 pg/ml) constitutes a risk factor and is a good indicator of OHSS [10]. In patients with E_2>6,000 pg/ml on the day of hCG administration, severe OHSS occurred in 38 % [5]. In the present study, we proposed a strategy of combining cabergoline and embryo cryopreservation after GnRHa triggering in GnRH antagonist protocol to prevent OHSS in patients at very high risk with E_2 levels >6,000 pg/ml and more than 20 follicles ≥11 mm. The prognosis of ART cycles in these patients with regards to OHSS was analyzed in this preliminary report.

Materials and methods

Study design

Institutional review board approval was obtained from the hospital. A retrospective analysis of the charts and ART database was performed. The primary outcome measure was the incidence of severe OHSS according to the classification of Golan et al. [16]. The secondary outcome measure was the clinical pregnancy rate in the first frozen-thawed embryo transfer (FET) cycle. Clinical pregnancy was defined as the presence of fetal heart activity on ultrasonography at 7 weeks of gestation.

Patients

This strategy for the patients at risk of OHSS was started since June 2008. All the participants of this study had to fulfill both of the two criteria (1) ovarian stimulation with GnRH antagonist protocol in patients undergoing IVF/ICSI; (2) serum E_2>6,000 pg/ml and more than 20 follicles ≥11 mm on the day of final oocyte maturation. Instead of hCG, GnRHa was used to trigger final oocyte maturation. Cabergoline was administered after oocyte retrieval to augment the effect of preventing OHSS. The embryos were electively cryopreserved by vitrification. No upper limit of ovarian response was defined to preclude the protocol. In other words, no patients were cancelled due to extremely high serum E2 levels or multiple follicle development. This preliminary report summarized our experience of 110 consecutive patients with 110 cycles using this treatment, concluding in June 2012.

IVF treatment protocol, embryo culture and assessment of OHSS

The GnRH antagonist protocol has been the primary stimulation protocol in our center since 2006, especially for patients undergoing their first IVF/ICSI cycles or potentially high responders. The standard gonadotropin starting dose is 150–187.5 IU/day recombinant FSH (rFSH, Gonal-f®; Merck Serono, Aubonne, Switzerland) for patients under 36 years old, 225 IU/day for patients between 36 and 38 years old, and a maximal dose of 300 IU/day for patients 39 years or older. For patients identified as potentially high responders, the usual starting dose was 75–112.5 IU/day. Ovarian stimulation started from cycle day 2 to day3. From day 5 of stimulation, the dose of rFSH was adjusted according to the follicular response, and 0.25 mg cetrorelix (Cetrotide; Serono, Frankfurt, Germany) was administered every day until the day of final oocyte maturation [38]. When more than 3 follicles had reached 17 mm, 0.5 mg buserelin acetate (Supremon; Sanofi-Aventis Deutschland Gmbh, Frankfurt, Germany) was

given subcutaneously to trigger final oocyte maturation in patients who fulfilled the criteria of this study. Oocyte retrieval was performed 36 h later. Cabergoline (Dostinex; Pfizer, New York, U.S.A.) at 0.5 mg per day was given after oocyte retrieval for 8 days. Fertilization was achieved by ICSI. Fertilization was assessed 16 to 18 h later by the appearance of 2 pronuclei (2PN). Each fertilized oocyte was transferred into a 20 μl droplet of Quinn's Advantage® Cleavage Medium (SAGE, Trumbull, CT, U.S.A.) in a tissue culture dish (35× 10 mm; Falcon, Becton Dickinson, Lincoln Park, NJ, USA) under mineral oil. The patients were assessed for signs and symptoms of OHSS on 3, 6, and 9 days after oocyte retrieval including history taking, physical examination, ultrasound scan, and blood tests for hematocrit, complete blood counts, urea nitrogen, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time (PT) and partial thrombin time (PTT). The severity of OHSS was according to the classification of Golan et al. [16].

Embryo vitrification

Vitrification of embryos was carried out on day 3 post oocyte retrieval using a modification of the protocols described by Mukaida et al. [42] and Desai et al. [12]. Dimethylsulphoxide (DMSO, Sigma; St. Louis, MO, U.S.A.) and ethylene glycol (EG, Sigma) were used as cryoprotectants. The base medium consists of Quinn's Advantage® Fertilization Medium with HEPES (SAGE; Trumbull, CT, U.S.A.) and 10 % (v/v) Quinn's Advantage® Serum Protein Substitute (SAGE). One to 2 embryos were vitrified in each cryoloop (Hampton Research, Laguna Niguel, CA, U.S.A.) after a two–step procedure to load the embryos at 37 °C. Initially, embryos were placed in the base medium containing 7.5 % (v/v) DMSO and 7.5 % (v/v) EG (cryoprotectant solution 1). After 2 min, the embryos were suspended in the base medium containing 15 % (v/v) DMSO, 15 % (v/v) EG, 10 mg/ml Ficoll 70 (Sigma) and 0.65 mol/l sucrose (cryoprotectant solution 2) for 30 s. The embryos were then washed quickly in solution 2 and transferred onto the filmy layer on the cryoloop. After loading embryos, the cryoloop was immediately plunged into liquid nitrogen. Using the stainless steel rod, the loop was sealed in a cryovial and stored in liquid nitrogen.

Thawing procedure

In a 4–well dish, 1 ml of base medium containing 0.33 mol/l sucrose, base medium containing 0.2 mol/l sucrose, and base medium only were warmed briefly in an incubator at 37 °C and then placed on the stage warmer of a dissecting microscope. With the cryovial submerged in liquid nitrogen, the loop containing the embryos was moved into the well containing 0.33 mol/l sucrose solution. After being warmed and diluted at 37 °C for 2 min, the embryos were transferred to the 0.2 mol/l sucrose solution. After an additional 3 min, embryos were washed and kept in the base medium for 5 min, and then transferred to Quinn's Advantage® Cleavage Medium (SAGE) for further culture until transfer.

Embryo replacement

When the patient had menstruation, norethindrone acetate (Primolut-Nor; Schering Gmbh, Weimar, Germany) 10 mg per day was given from cycle day 1 to day 7, and 1/4 amp leuprolide acetate depot (Leuplin depot; Takeda, Fujisawa, Japan) was administered on cycle day 5. Blood sample was obtained 10 days later to confirm pituitary desensitization. Endometrial preparation consisted of stepwise increasing doses of conjugated estrogens (Premarin; Wyeth, Newbridge, Ireland) as described previously [32, 51], followed by the addition of 90 mg vaginal progesterone (Crinone 8 %; Merck-Serono, Hertfordshire, U.K.) per day after 14 days of Premarin administration. ET was performed on the fourth day of progesterone administration. The frozen embryos were thawed in the morning of ET, and ET was performed in the afternoon. Hormone supplementation was continued in pregnant women until 10 weeks of gestation. Serum β-HCG level was measured 14 days after embryo transfer. Clinical pregnancy was defined as the presence of fetal cardiac activity on transvaginal ultrasonography at 7 weeks of gestation. Ongoing clinical pregnancy was defined as normal pregnancies ≥12 weeks of gestation. The implantation rate was calculated by dividing the number of fetal hearts on ultrasonography by the number of embryo transferred.

Results

This study included 110 patients undergoing a total of 110 cycles from June 2008 to June 2012. The mean age was 33.6 years. Among the patients, 83 (75 %) underwent their first IVF/ICSI cycles. The indications of treatment were ovulation factor (n=44, 40 %; 40 [90.9 %]) were PCOS), male factor (n=43, 39 %), tubal factor (n=21, 19 %), endometriosis (n=2, 2 %) and unexplained infertility (n=11, 13 %). All of them received first FET within 2 months after vitrification.

No patient developed moderate or severe OHSS. Table 1 summarizes the outcomes of ovarian stimulation. An average of 22.7 oocytes was retrieved from each patient. Mean serum E_2 level on the day of GnRHa triggering was 78 73 pg/ml. The hematocrit levels on luteal phase days 3, 6 and 9 were similar and all within normal range (data not shown), so only day 3 level was shown. A total of 1247 embryos were frozen from 1896 2PN-oocytes (65.7 %), or

Table 1 Clinical outcomes of ovarian stimulation

	Mean ± SD	Range
Age (years)	33.6±3.4	24–43
E_2 on day of GnRHa triggering (pg/ml)	7873±2642	6102–14375
No. of oocytes retrieved	22.7±6.3	12–38
No. of MII oocytes	18.5±5.4	5–31
MII rate (No. MII oocytes/No. oocytes retrieved, %)	83±16	31–100
No. of 2PN oocyte	17.2±5.7	5–29
Fertilization rate (No. of 2PN oocyte/No. of oocytes retrieved, %)	78±20	27–100
No. of embryos frozen	11.3±4.8	3–25
Hematocrit on luteal phase day 3 (%)	35.9±3.6	27–42

from 2493 retrieved oocytes (50.0 %). All patients had surplus embryos frozen, with an average of 11.3 embryos per patient.

The clinical outcomes of the first FET cycles were outlines in Table 2. All 110 cycles resulted in embryo transfer. Two hundred and twenty-eight out of 239 embryos survived freezing and thawing, with a survival rate of 95.3 %. The clinical pregnancy rate and ongoing pregnancy rate were 62.7 % and 56.3 %, respectively.

During the study period, 30 patients received second FET cycles. The clinical pregnancy rate and ongoing pregnancy rate were 56.6 % and 50.0 %, respectively.

Discussion

This preliminary study shows that cabergoline, plus GnRHa triggering and embryo cryopreservation, effectively prevent OHSS in patients at very high risk. The good MII rate, high fertilization rate and high pregnancy rate also indicate that the oocyte quality and embryo quality were not affected by this strategy. Most importantly, no patient developed moderate or severe OHSS. To the best of our knowledge, this report includes the largest cohort of patients with the strictest inclusion criteria.

Table 2 Clinical outcomes of first frozen embryo transfer cycle

Parameter	
No. of cycles[a]	110
Embryo survival rate (%)	97.1±9.2
No. of embryos thawed per patient	2.2±0.5
No. of embryos transferred per patient	2.1±0.4
Implantation rate	39.5 %
Clinical pregnancies (per transfer)	69/110 (62.7 %)
Ongoing pregnancies (per transfer)	62/110 (56.3 %)

Values are mean ± SD, number or number/total (%), unless otherwise stated

[a] All thawing cycles resulted in embryo transfer

Detection of patients at risk and administration of appropriate dose of gonadotropin is the first step of primary prevention of OHSS. Strategies of secondary prevention include canceling the cycle, embryo cryopreservation, reducing the dose of hCG, coasting, intravenous colloidal agents, and dopamine agonist such as cabergoline [1]. Among them, coasting is the most popular [11]. No strategy is OHSS-free except withholding hCG and cancelling the cycle.

Although coasting results in satisfactory pregnancy rate and low incidence of severe OHSS (<2 %) [37], it increases the duration of GnRHa injections and hormonal and ultrasound monitoring, thus increases cost and inconvenience. Besides, prolonged coasting (≥4 days) has been reported to reduce the implantation and pregnancy rates [37, 40, 50].

GnRH antagonist protocol was expected to decrease the incidence of severe OHSS. A recent meta-analysis showed that comparing to GnRHa protocols, there was a lower incidence of OHSS in the GnRH antagonist protocol [2]. However, GnRH antagonist protocol was not risk-free. The study found that with GnRH antagonist protocols, 2.65 % patients (84/3165) suffered from severe OHSS and 1.9 % patients (40/2096) needed cycle cancellation or coasting due to high risk of OHSS.

Triggering final oocyte maturation with GnRHa instead of hCG in GnRH antagonist protocol is a promising way of preventing OHSS [15, 21, 27]. However, GnRHa triggering is associated with lower pregnancy rates due to luteal phase deficiency [28, 36]. Luteal phase low-dose hCG supplementation [29, 30], dual trigger with GnRHa/low-dose hCG [24, 47, 48], or aggressive luteal support with estrogen and progesterone [15] have been proposed to rescue the luteal phase after GnRHa triggering. Dual GnRHa and low-dose hCG trigger still carries low risk of OHSS [46]. Similarly, luteal phase supplementation with low-dose hCG, a small percentages of patients still developed moderate or severe OHSS [8, 27, 44]. While aggressive luteal support with estrogen and i.m. progesterone has been reported to produce favorable implantation and pregnancy rates [15], high miscarriage rate was reported in another study [6]. Moreover, daily progesterone injections and

frequent hormonal monitoring also increased patient suffering and inconvenience.

GnRHa triggering in combination with a "freeze-all" strategy has been proposed to achieve an "OHSS-free" clinic [13, 22]. Cryopreservation has been reported to be associated with higher pregnancy rates comparing to fresh ET in a systemic review and meta-analysis [45], and there is a trend toward higher cumulative pregnancy rate with cryopreservation comparing to coasting [26]. In a retrospective analysis, Manzanares et al. reported elective slow freezing of day-3 embryos in 42 PCOS patients after GnRHa triggering, with a pregnancy rate 33 % in subsequent thawing cycles [41]. Another retrospective study using oocyte vitrification after GnRHa triggering in 96 high risk patients resulted in a clinical pregnancy rate of 45.8 % in subsequent thawing cycles [25]. Similarly, Griesinger et al. [19] cryopreserved 2PN oocytes by vitrification after GnRHa triggering in 40 patients at risk of OHSS in a prospective study, with a cumulated pregnancy rate in the thawing cycles of 35 %. No patients developed OHSS in the above three studies. However, the E_2 levels on the day of GnRHa triggering were not very high in those studies, i.e. 4518.5 (±2118.85) pg/ml [41] and 4016.3 (±1784.0) pg/ml [19], respectively. In another recent study comprising 51 patients with a mean E_2 level of 4248.7 pg/ml, 3 cases (5.9 %) of moderate OHSS and 1 case (2 %) of severe OHSS developed [22]. Although the case of severe OHSS was questioned to be a condition of subacute intraperitoneal hemorrhage after oocyte retrieval, it was reassured to be a case of early-onset severe OHSS [34]. These results suggest that GnRHa triggering and freeze-all strategy still carries some risk of OHSS [22], especially in patients with very high serum E_2 levels and multiple follicular development. E_2 concentration (>6,000 pg/ml) is an important marker to predict the development of OHSS [10], and 38 % of the patients with E_2>6,000 pg/ml developed severe OHSS [5]. A previous study showed that in a GnRH antagonist protocol, a threshold of >18 follicles and/or E_2>5,000 pg/ml yielded an 83 % sensitivity rate with a specificity of 84 % for severe OHSS cases [43]. The present study recruited patients with serum E_2>6,000 pg/ml and more than 20 follicles ≥11 mm on the day of final oocyte maturation. The mean E_2 level was 7,873 pg/ml and a mean of 22.7 oocytes were collected per patient, representing a very high-risk group. Even so, no any patients developed OHSS.

Recent studies showed that VEGF, by increasing vascular permeability, is the key mediator of the development of OHSS [49]. Expressions of VEGF and VEGF receptor 2 (VEGFR-2) mRNA begin to increase during ovarian stimulation by gonadotropin [18, 49], and peak at 48 h after hCG administration, resulting in increased vascular permeability [49]. Patients with high serum E_2 levels and multiple follicles have a large number of granulosa cells, and it's possible that the expressions of VEGF and VEGFR-2 mRNA have increased during gonadotropin stimulation. They may secret VEGF and develop severe OHSS even after GnRHa triggering. Moreover, although there was a significant reduction of VEGF concentration in the follicular fluid of women triggered with GnRHa instead of hCG, the plasma VEGF concentrations were similar [9]. These may explain why some patients still developed OHSS with the "GnRHa triggering plus cryopreservation" strategy. Cabergoline antagonizes VEGF effect on vascular permeability through VEGFR-2 dephophorylation [3, 17]. Thus addition of cabergoline to the strategy might augment its effectiveness in preventing OHSS. Further studies are needed to investigate the dose and duration of cabergoline treatment with this strategy.

The limitation of the study is its retrospective nature. It is impossible to discriminate the additional benefits of cabergoline in preventing OHSS in this study. However, this study represents the largest case series. No other adjunctive preventive procedures, such as coasting, were applied in this study. The inclusion criteria were very strict and no upper limit of ovarian response was defined to preclude the protocol. Nevertheless, it's still early to conclude that this strategy can eliminate OHSS and there is no need for luteal phase monitoring. An adequately-powered RCT and cost-effective analysis comparing this strategy with "GnRHa triggering plus freeze all" strategy is needed to prove the effectiveness of this strategy in preventing OHSS.

In conclusion, combining cabergoline and embryo cryopreservation after GnRHa triggering in GnRH antagonist protocol prevents OHSS even in patients at very high risk. We now offer GnRH antagonist protocol to most women undergoing their first IVF/ICSI cycles. If they are found to be at risk of OHSS during ovarian stimulation, this strategy is applied. Whether this strategy can eliminate OHSS requires more studies to verify.

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