GnRH agonist with low-dose hCG (dual trigger) is associated with higher risk of severe ovarian hyperstimulation syndrome compared to GnRH agonist alone

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Abstract

Purpose The purpose of this study was to compare rates of ovarian hyperstimulation syndrome (OHSS) after using gonadotropin-releasing hormone agonists (GnRHa) alone and GnRHa in combination with low-dose human chorionic gonadotropin (hCG, dual trigger) for final oocyte maturation in women undergoing controlled ovarian hyperstimulation (COH).

Methods A retrospective cohort study was conducted at an academic center. Study population included 108 women who received GnRHa trigger and 66 women who received dual trigger (GnRHa + low-dose [1000 IU] hCG trigger). The main outcome measure was OHSS. Secondary outcomes included total oocyte yield and oocyte maturity.

Results The incidence of early OHSS was significantly higher after dual trigger than GnRHa trigger (8.6 vs 0 %). Moreover, four of the six patients that developed OHSS developed severe OHSS. Logistic modeling revealed that the combination of age, BMI, baseline AFC, and E2 >4000 pg/mL was predictive of OHSS with an area under the receiver operating characteristic curve of 0.84 and was superior to each factor alone. Adjusted analyses revealed that dual trigger was associated with a higher number of total oocytes (adjusted OR 1.27;

95 % confidence interval, 1.18, 1.38) and percentage of mature oocytes (AOR 1.10; 95 % confidence interval, 1.03, 1.17) obtained compared to GnRHa trigger alone.

Conclusions Dual trigger for final oocyte maturation using GnRHa and low-dose hCG is associated with a significantly increased risk of severe OHSS compared to GnRH alone. However, dual trigger may be associated with a modest increase in oocyte yield, both in terms of number and maturity.

Keywords Dual trigger · GnRHa trigger · Low-dose hCG · Oocyte maturity · OHSS

Capsule Dual trigger for final oocyte maturation using GnRHa and low-dose hCG is associated with a significantly increased risk of severe OHSS compared to GnRH alone.

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Introduction

Ovarian hyperstimulation syndrome (OHSS) complicates up to 10 % of all in vitro fertilization (IVF) cycles [1]. Moderate OHSS is characterized by ovarian enlargement, gastrointestinal symptoms, and fluid shifts and is of concern given the risk of progression to severe OHSS [2]. Severe OHSS occurs in 0.5–2.0 % of all IVF cycles; while rare, the consequences are potentially devastating, as death from severe OHSS can occur in 1 of every 400,000–500,000 superovulation cycles [3]. Early OHSS is classically a consequence of exogenous human chorionic gonadotropin (hCG) administration used to trigger oocyte maturation while late OHSS is induced by endogenous hCG from the early pregnancy and typically occurs more than 10 days after oocyte retrieval [4, 5]. Although risk factors for the development of OHSS have been identified— young age, low body weight, polycystic ovary syndrome (PCOS), prior history of OHSS, high or rapidly rising estradiol (E2) levels, and high number of follicles during stimulation—accurately predicting which individuals will develop OHSS remains difficult [6–10].

A number of strategies have been developed in an effort to reduce the development of OHSS, the most effective of which is use of a gonadotropin-releasing hormone agonist (GnRHa) to trigger final oocyte maturation [11–14]. Initial studies found that while the use of GnRHa trigger essentially eliminates the risk of OHSS, pregnancy rates were lower and miscarriage rates were higher compared to hCG trigger [15–18]. More recent studies suggest that addition of enhanced luteal support when using a GnRHa trigger results in comparable pregnancy, miscarriage, and live birth rates to protocols using an hCG trigger [11, 12, 16, 19]. Despite this success, concerns remain regarding the subgroups of patients who respond inadequately to a GnRHa trigger with decreased oocyte yield, in both quantity and quality, and subsequently failed IVF cycles. Although initially low-dose hCG (1000–2500 IU) was used after GnRHa trigger for enhanced luteal support [20–27], two studies described the co-administration of low-dose hCG (1000–2500 IU) at the time of GnRHa trigger for oocyte maturation (called dual trigger) [28, 29]. These studies did not find that dual trigger was associated with an increase in the number of oocytes retrieved and did not examine oocyte maturation rates.

A disadvantage of using low-dose hCG with GnRH trigger is the potential risk of OHSS. Both studies examining the use of a dual trigger reported low rates of OHSS; 1/102 patients developed mild early OHSS [29] and 1/182 patients developed severe late OHSS [28]. Few large studies have examined the risk of OHSS in patients receiving different doses of hCG alone; however, hCG doses as low as 3300 IU given 35 h prior to oocyte retrieval and 1500 IU given at the time of oocyte retrieval were found to be associated with concerning rates of moderate or severe early OHSS (15 and 22 %, respectively) [26, 30]. The objective of our study was to compare the impact of the final oocyte maturation method, GnRHa alone versus dual trigger (GnRHa plus low-dose hCG), on OHSS incidence as well as oocyte yield and maturity in women undergoing controlled ovarian hyperstimulation (COH) for IVF.

Material and methods

Study design and participants

A retrospective cohort study was performed at the University of Pennsylvania to examine the association between protocol used to trigger final oocyte maturation and the development of OHSS. All women who underwent COH and oocyte retrieval for the purposes of autologous IVF or embryo/oocyte banking where GnRHa or GnRHa with low-dose hCG (dual trigger) was used for the induction of oocyte maturation between January 1, 2008 (introduction of GnRH agonist trigger to our practice), and April 1, 2014 (time of data collection), were included for analysis. The antagonist protocol was selected if

the patient was considered a high responder based on prior treatment cycles, age, ovarian reserve testing, infertility diagnosis, and PCOS (as defined by Rotterdam criteria) [31]. In our practice, all high-responder patients are routinely triggered with either GnRHa alone or GnRHa and low-dose hCG at the discretion of the physician. The study protocol was approved by the Institutional Review Board at the University of Pennsylvania. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

Stimulation protocol

All patients underwent COH using a GnRH antagonist protocol. There were no age- or weight-based exclusion criteria. Determination of starting gonadotropin doses was made based on patient age, body mass index, infertility diagnosis, and ovarian reserve testing. Ovarian stimulation using recombinant Follistim (FSH; Merck & Co., Inc., Whitehouse Station, NJ, USA) or Gonal-F (EMD Serono, Inc., Rockland, MA, USA) was initiated by cycle day 2 after menses. Ganirelix acetate (Ganirelix acetate injection; Merck & Co., Inc., Whitehouse Station, NJ, USA) or Cetrotide (cetrotide acetate injection; EMD Serono, Rockland, MA, USA) was administered when serum E2 levels reached >300 pg/mL or when there was a 13–14-mm follicle. Highly purified human menopausal gonadotropin (Menopur; Ferring Pharmaceuticals, Parsippany, NJ, USA) supplementation was added with the introduction of the GnRH antagonist. Follicular growth, monitored by serial transvaginal ultrasonography, and serum E2 levels were used to titrate gonadotropin dosages. Leuprolide acetate (Lupron; Abbott Laboratories, Chicago, IL, USA) 80 U (4.0 mg) alone or in combination with 1000 IU hCG was administered subcutaneously when at least two ovarian follicles were ≥ 18 mm in mean diameter. The choice of either GnRHa trigger or dual trigger was based on physician preference. Although there were no formal criteria used by providers to choose dual trigger or GnRHa alone for oocyte maturation, GnRHa alone trigger was generally reserved for patients felt to be at highest risk for OHSS as estimated by young age, low/normal BMI, history of OHSS, and/or large number of follicles or high E2 on the day the trigger was administered. Oocyte donation was an exclusion criterion.

Serum E2, luteinizing hormone (LH), and progesterone (P) levels were measured on the morning of GnRHa administration and repeated 10–12 h after GnRHa administration along with progesterone measurements. A rise in serum LH and progesterone after a GnRHa trigger indicates that an endogenous flare has occurred which is required to initiate oocyte

maturation. As a result, post-trigger LH and P4 values of ≤ 15 mIU/mL and ≤ 3.5 ng/mL were used to counsel patients regarding potential risk of impaired induction of oocyte maturation [32]; however, additional doses of hCG or GnRHa were not given if these values were not obtained. Transvaginal ultrasound-guided oocyte retrieval was performed 35–36 h after trigger.

Luteal phase supplementation

Embryo transfer was performed on the third or fifth day post-retrieval. Luteal phase support started on the day after oocyte retrieval with daily intramuscular injection of 50 mg progesterone and three 0.1-mg transdermal patches of estradiol (Vivelle-Dot; Noven Pharmaceuticals Inc., Miami, FL, USA) changed every other day. Serum E2 and P levels were assessed weekly until the pregnancy test or up to 8 weeks gestation and to confirm the E2 > 200 pg/mL and the P levels > 20 ng/mL; if E2 and P levels were below these thresholds, estradiol patches and IM progesterone dosing were titrated to meet these parameters. Serum hCG was measured 10–14 days following embryo transfer, and a value above 5 IU/mL was considered to be a positive test.

Exposure and outcome variables

The primary outcome analyzed was incidence of OHSS. OHSS was assessed using the well-accepted classification system proposed by Golan et al. [33] and was compared between trigger groups. Specifically, diagnosis of mild OHSS required the presence of abdominal distension with or without nausea, vomiting, and/or diarrhea. Moderate OHSS was diagnosed when ultrasonographic ascites was present in addition to the features characterizing mild OHSS. Severe OHSS was diagnosed when in addition to features of moderate OHSS there was clinical evidence of ascites and/or hydrothorax or hemoconcentration, coagulation abnormalities, and/or diminished renal or liver function.

Notably, individuals were called daily for 1 week starting the day after administration of trigger and asked if they were experiencing abdominal distension causing notable discomfort, nausea, vomiting, and/or diarrhea. An affirmative response to any of these questions prompted an office evaluation by a physician where pelvic ultrasound was performed to assess for evidence of ascites and ovarian enlargement, and blood work was performed to detect evidence of hemoconcentration or liver and/or renal dysfunction.

Secondary outcomes included number of oocytes retrieved, percentage of mature oocytes retrieved, fertilization rate, clinical pregnancy rate, and spontaneous abortion rate. Percentage of mature oocytes was calculated by examining for evidence of first polar body extrusion and therefore was only assessed when oocytes were stripped of cumulus cells, which is

routinely done for intracytoplasmic sperm injection (ICSI) or oocyte vitrification. The fertilization rate was calculated by dividing the number of two pronuclear stage embryos by the number of total oocytes obtained for couples who had conventional insemination performed and by dividing the number of two pronuclear stage embryos by the number of total mature oocytes obtained for couples who had ICSI performed. Clinical pregnancy rate was calculated as the presence of fetal cardiac activity confirmed by transvaginal ultrasonography per embryo transfer (ET). Spontaneous abortion rate was defined as pregnancy loss after sonographic visualization of an intrauterine gestational sac per ET.

Sample size

A sample size calculation was performed a priori for the primary outcome of the study, the incidence of moderate–severe OHSS. Based on observations in prior studies of 12–15 % OHSS in high responders after receiving antagonist protocols [34] or a lower dose of hCG for oocyte maturation [30], we determined that at least 130 patients would be needed (65 in each arm) to have 80 % power to detect a 14 % difference in the incidence of OHSS between treatment arms (1 % incidence in the GnRHa trigger group vs 15 % in the dual trigger hCG group) with a type I error rate of 5 %. As there were known to be more patients who had received GnRHa trigger than dual trigger within this retrospective cohort, a power calculation accounting for unequal groups was performed using our sample size (108 in the GnRHa trigger group and 66 in the dual trigger) group and revealed 88 % power to detect a 14 % risk difference in moderate–severe early OHSS between the GnRHa trigger and dual trigger groups.

Statistical analysis

Baseline demographic and clinical characteristics were compared between trigger groups with Mann–Whitney *U* test for continuous variables and chi-square test for categorical variables. Univariable analysis was used to examine the association between trigger group and development of OHSS, total oocyte number, and oocyte maturity. Poisson regression and logistic regression models were used to assess for potential confounders. All clinically and statistically significant variables (type of trigger, age, antral follicle count (AFC), starting gonadotropin dose, maximum E2 level during stimulation, and diagnosis of PCOS) were initially included in the model. Backward elimination was performed to select the final clinical characteristics associated with OHSS, total oocyte number, and oocyte maturity in the model. Subsequently, logistic regression was used to develop predictive models for the development of OHSS. Model performance was examined using receiver operating characteristic curves and by comparing the area under the curve for each model iteration. The best

predictive model for the development of OHSS was selected according to the maximal area under the curve. Data analysis was conducted using STATA version 13.1 (StataCorp, College Station, TX, USA).

Results

Baseline characteristics

A total of 174 women underwent either an autologous IVF or an elective oocyte banking cycle in which a single GnRHa trigger or dual trigger (consisting of GnRHa and 1000 U low-dose hCG) was used for oocyte maturation and were therefore included in the analysis. Of these 174 cycles, 108 utilized a GnRHa trigger and 66 utilized a dual trigger for final oocyte maturation. Women were excluded if additional GnRHa or hCG was given after the time of initial trigger or in the rare case that alternative doses of hCG (i.e., 1500 or 2500) were used.

Baseline demographics of the groups are shown in Table 1. Women in the GnRHa trigger group were significantly older (32.4 vs 31.0 years, $p < 0.05$) and more likely to be nulliparous (47 vs 41 %, $p < 0.05$) than women in the dual trigger group were. In addition, patients in the GnRHa trigger group were more likely to have PCOS (41 vs 20 %, $p < 0.01$) and have a higher baseline AFC (23 vs 18, $p < 0.01$) than were those in the dual trigger group. Anti-Müllerian hormone values were available for 44 women in the GnRHa trigger group and 64 women in the dual trigger group and were not statistically different between the groups (3.9 vs 3.2 ng/mL, respectively, $p = 0.13$). Twenty-seven of the 66 women (41 %) in the dual trigger group were undergoing stimulation for oocyte or embryo banking compared to 5 of the 108 (5 %) in the GnRHa trigger group ($p < 0.01$).

Cycle characteristics

Cycle characteristics are shown in Table 2. Although the starting dose of gonadotropins and total days of stimulation were higher in the dual trigger group ($p < 0.01$), the total dose of gonadotropins used during stimulation and the number of pre-ovulatory follicles >15 mm on the day of trigger did not differ between groups. Total follicles on the day of trigger, maximum E2 levels, and E2 levels both pre- and post-trigger were higher in the GnRHa-only group ($p \leq 0.01$). LH and P4 levels post-trigger did not differ between the groups. The number of individuals with LH ≤ 15 mIU/mL and P4 ≤ 3.5 ng/mL did not differ between the groups.

Table 1 Demographics and baseline characteristics of study subjects

	GnRHa trigger (<i>n</i> = 108)	Dual trigger (<i>n</i> = 66)
Age (years)*	32.4 (30.3–35.1)	31.0 (29.0–34.1)
BMI (kg/m ²)	23 (21–27)	23 (20.7–25.3)
PCOS*	41 (38 %)	13 (20 %)
Male factor*	43 (40 %)	15 (23 %)
Oocyte/embryo banking*	5 (5 %)	27 (41 %)
Previous history of OHSS	6 (6 %)	4 (6 %)
Caucasian	69 (64 %)	46 (70 %)
Nulliparous*	47 (44 %)	41 (62 %)
Maximum day 3 FSH (mIU/mL)	6 (5–7)	6 (5–8)
Antral follicle count*	23 (16–31)	18 (11–25)

Data are median (interquartile range)

* $p < 0.05$

Ovarian hyperstimulation syndrome

The incidence of early OHSS was significantly higher after dual trigger than GnRHa trigger (8.6 vs 0 %, $p < 0.01$) (Table 2). Moreover, the majority of these patients (four of six) were diagnosed with severe OHSS. All of the patients that developed OHSS were less than 35 years of age and had a BMI ≤ 23 kg/m² (Table 3). Four of the women who developed OHSS had E2 levels on the day of trigger that exceeded 4000 pg/mL; two of the individuals with OHSS had E2 levels on the day of trigger < 3500 pg/mL. Only 50 % of the individuals in our study with OHSS had a baseline AFC > 20 . Patients that developed OHSS were not treated with dopamine receptor agonist or alternative therapies but did not have embryo transfer performed.

Univariate analysis-identified patient BMI was highly associated with OHSS (mean BMI 20 kg/m² [IQR 19.7–22.9] in the individuals that developed OHSS vs 23 kg/m² [IQR 21–26.7] in the individuals that did not develop OHSS, $p < 0.05$). Receiver operator characteristic curves were used to evaluate the ability of this factor and clinically relevant variables to predict development of OHSS alone and in combination. A logistic model that included age, BMI, baseline AFC, and E2 > 4000 pg/mL predicted OHSS with an area under the receiver operating characteristic curve of 0.84 and was superior to each factor alone.

IVF laboratory and pregnancy outcomes

IVF cycle outcomes are shown in Table 2. Dual trigger was associated with a higher number of oocytes obtained after adjusting for age, baseline AFC, and maximum E2 level (adjusted OR 1.27 [95 CI 1.18–1.38], $p < 0.01$). Of note, three patients did not have any oocytes retrieved after administration of GnRHa trigger. In all subjects, the post-trigger LH

Table 2 IVF cycle characteristics, endocrine parameters, and outcomes in women receiving GnRHa alone versus dual trigger

	GnRHa trigger (<i>n</i> = 108)	Dual trigger (<i>n</i> = 66)
Starting dose of gonadotropins (IU)*	150 (125–225)	225 (150–300)
Total dose of gonadotropins (IU)	2325 (1812–2931)	2512 (1800–3675)
Days of stimulation*	11 (9–12)	11 (10–12)
Maximum E2 (pg/mL)*	4360 (3517–5704)	3541 (2943–5014)
Follicles >15 mm day ⁻¹ of trigger	13 (10–16)	14 (12–16)
Total follicles day of trigger*	31 (25–35)	28 (22–34)
E2 levels on day of trigger (pg/mL)	3604 (2843–4536)	2993 (2597–4280)
LH levels on day of trigger (mIU/mL)	0.6 (0.3–1.4)	0.7 (0.3–1.4)
E2 levels post-trigger (pg/mL)	4329 (2295–5704)	3541 (2662–4913)
LH levels post-trigger (mIU/mL)*	52.2 (36–76)	56.5 (39–85)
P4 levels post-trigger (ng/mL) ^a	6.0 (4.5–9.0)	6.7 (5.1–9.5)
Total number oocytes retrieved	16.5 (11–21.5)	17.5 (12–24)
Oocyte maturity (%)**	70 (56–85)	82 (74–91)
No oocytes retrieved (%)	3 (2.8)	0
OHSS (%)**	0	6 (9)
Severe OHSS (%)**	0	4 (6)

Data are median (interquartile range)

p* < 0.05; *p* < 0.01^a Five individuals in the GnRHa trigger group did not have post-retrieval progesterone values available for analysis

level was >15 IU/mL and the post-trigger P4 level was >3 ng/mL. One woman had undergone a previous IVF cycle in which GnRHa trigger had also been used and the oocyte yield was appropriate.

Oocyte maturity was calculated for the subgroup of women for whom oocyte stripping was performed (i.e., those having

ICSI or oocyte cryopreservation). In the GnRHa group, 41 individuals had ICSI performed and 3 had oocyte banking. In the dual trigger group, 31 individuals had ICSI performed and 15 had oocyte banking. In this subset, oocyte maturity was higher after dual trigger than trigger with GnRHa alone (82 vs 70 %, *p* < 0.01). After adjusting for diagnosis of PCOS

Table 3 Characteristics of individuals who developed OHSS

	Age	BMI	AFC	Number of follicles day of trigger	E2 day of trigger	E2 post-trigger	Total oocytes retrieved	OHSS grade	Comments
1	31	21	6	23	1809	2449	26	Mild	Distension, nausea/vomiting
2	34	19	25	25	4448	4913	17	Moderate	Distension, ascites
3	33	21	24	40	4280	4277	15	Severe	Distension, ascites, hemoconcentration
4	32	19	9	22	4579	3840	18	Severe	Distension, ascites hemoconcentration, pleural effusion
5	31	23	13	22	5165	5190	20	Severe	Ascites, paracentesis
6	32	23	22	33	3315	3354	34	Severe	Ascites, hemoconcentration, pleural effusion, thoracentesis

and number of follicles >15 mm on the day of trigger, the percentage of mature oocytes obtained was higher after dual trigger than trigger with GnRHa alone (AOR 1.10 [95 CI 1.03–1.17], $p<0.01$). Similarly, when this analysis was restricted only to individuals having ICSI, oocyte maturity was higher after dual trigger than GnRHa alone (84 vs 71 %, $p<0.01$) (AOR 1.10 [95 CI 1.02–1.19], $p=0.01$). The three patients that did not have any oocytes retrieved after administration of GnRHa trigger were included in the calculation for total oocyte number but not oocyte maturity.

ICSI was used in a greater proportion of cycles in the dual trigger group than in the GnRHa trigger group (61 vs 40 %, $p<0.05$). The ICSI fertilization rate was significantly higher in the dual trigger group compared to GnRHa only (73 vs 50 %, $p<0.01$). The lower ICSI fertilization rate in the GnRHa trigger group may be related to the significantly higher proportion of patients with male factor in that group than in the dual trigger group (40 vs 23 %, $p<0.05$). There were no differences in the overall conventional fertilization rates between groups. In unadjusted analyses, the percentage of patients that had a blastocyst transfer was significantly higher in the dual trigger compared to the GnRHa trigger group (88 vs 45 %, $p<0.01$) and, as a result, analyses for pregnancy outcomes were limited to individuals that had blastocyst transfers. While there was a trend towards improved pregnancy rate, there were no statistically significant differences in the rates of clinical pregnancy (44 vs 63 %, $p=0.12$) or spontaneous miscarriage (7 vs 6 %, $p=0.90$) in the GnRHa trigger group compared to the dual trigger group.

Discussion

The results of this study demonstrate that dual trigger for final oocyte maturation using GnRHa and low-dose hCG (1000 IU) is associated with a significantly increased risk of severe OHSS. While the observed risk difference was less than that anticipated due to a lower incidence of OHSS in both groups, these results are of great importance clinically, as they demonstrate a higher incidence of OHSS in the dual trigger group than what has previously been reported [28, 29] and reiterate the challenges that remain with respect to prediction and risk reduction of OHSS in high responders.

The use of low-dose hCG and GnRHa to trigger oocyte maturation in normal/high responders has been reported in a few studies [28, 29, 35]. Although these studies were not powered to examine the risk of OHSS, Shapiro et al. reported very low rates of OHSS (<1 %) with a dual trigger in women at a relatively high risk for OHSS (mean serum E2 on the day of trigger was >4700 pg/mL and ≥ 27 follicles on the day of trigger) [28]. Griffin et al. limited the use of the dual

trigger to individuals with serum E2 <4000 pg/mL and reported the development of early mild OHSS in only 1/102 cycles. In our study, despite the patients in the dual trigger group having a lower risk profile for development of OHSS (fewer patients with PCOS, lower AFC, lower maximum E2 level and E2 level on the day of trigger, and lower number of follicles >15 mm on the day of trigger in the dual trigger group—Tables 1 and 2), dual trigger was associated with significantly elevated risk of development of OHSS, and most notably moderate or severe OHSS, than GnRHa trigger alone. Of note, none of the patients who developed OHSS had a diagnosis of PCOS. In support of our findings, other investigators have reported that administration of low doses of hCG prior to or at the time of oocyte retrieval is associated with even higher rates of OHSS (16–21 %) than were detected in our study [26, 27, 30, 36].

In early studies, the association between high levels of E2 and the occurrence of OHSS resulted in the hypothesis that E2 was the stimulating factor for the syndrome [37, 38]. Subsequent work demonstrated that OHSS does not occur if administration of hCG is withheld, despite high estradiol levels [39, 40]. Given that OHSS is characterized by increased vascular permeability, and hCG has no direct vasoactive properties [41], identifying the vasoactive substances mediating this relationship is key to understanding the pathophysiology of OHSS. Vascular endothelial growth factor (VEGF) has emerged as one of the primary substances responsible for the development of OHSS [42, 43]. Administration of hCG to rats stimulated with pregnant mare serum gonadotropin resulted in peak ovarian VEGF mRNA levels [41] and increased VEGF expression in granulosa lutein cells [44, 45]. As the endogenous LH surge induced by a GnRHa is shorter in amplitude and duration compared to the long-acting hCG trigger used in IVF [46, 47], it may result in a shorter period of secretion of vasoactive substances like VEGF. This mechanism likely explains the virtual elimination of OHSS after GnRH trigger [48–50].

Several studies have found that mean serum and/or follicular VEGF levels are higher in women who developed OHSS compared with those who did not [43, 51–53]. Other investigators did not detect differences in mean serum VEGF in women who developed OHSS after hCG trigger compared with those that did not experience OHSS [54] or in follicular levels of VEGF [55] in women triggered with GnRHa versus hCG. Chunderland et al. reported in a mouse OHSS model that granulosa cells secrete the anti-angiogenic factor pigment epithelium-derived factor (PEDF) and the PEDF/VEGF balance may play a more important role in OHSS than the VEGF level alone [56]. Moreover, in a follow-up study, these investigators

found that exposure of human granulosa cells to GnRHa in vitro induces an effect on PEDF/VEGF balance that is inverse of that seen with hCG [57]. These findings provide an explanation for the decreased risk of OHSS with GnRH alone. Although there are no studies examining the impact of varying doses of hCG on expression or secretion of VEGF, PEDF/VEGF ratio, or the balance of proangiogenic/anti-angiogenic factors, our study showed that a low dose of hCG (1000 IU) is sufficient to cause OHSS.

Although GnRHa eliminated the risk of OHSS in our study, it was associated with a lower number of oocytes retrieved and lower oocyte maturity than dual trigger. While higher starting doses of gonadotropin were used in the dual trigger group, the total number of follicles >15 mm present on the day of trigger administration was higher in the GnRHa group and there was no difference in the number of total follicles present on the day of trigger between groups. Additionally, the associations between greater oocyte yield and maturity and dual trigger remained significant after adjusting for number of follicles >15 mm on the day of trigger. These findings refute the argument that a higher starting dose in the dual trigger group resulted in increased follicular recruitment which could account for the higher number of oocytes recovered.

Some studies have found that GnRHa trigger was associated with an increase in the percentage of mature oocytes retrieved compared to hCG trigger alone [16, 58]. This is generally attributed to GnRHa's ability to cause the release of both endogenous LH and FSH, which mimics the natural cycle surge [16]. Addition of low-dose hCG to GnRHa trigger has been shown to improve luteal support; however, its impact on oocyte maturation has not been fully explored. While hCG has been commercially available since 1940, the lowest effective dose to achieve oocyte maturation has not been established. Studies in nonhuman primates have shown that hCG doses 3- to 10-fold lower than standard doses were sufficient to reinitiate meiosis and induce luteinization of granulosa cells [59]. However, one human study randomized patients undergoing IVF to three hCG dosages (2000, 5000, and 10,000 IU) and found that there was a significantly lower oocyte recovery with 2000 IU hCG (oocytes retrieved in 77 % of patients) compared with patients who received either 5000 IU hCG or 10,000 IU (oocytes retrieved in 95 and 98 % of patients, respectively) ($p < 0.01$) [60]. There is virtually no data examining oocyte maturity in dual trigger, but Humaidan et al. found that administration of 1500 IU hCG 12 h after GnRHa trigger was associated with a significantly higher number of oocytes retrieved [21].

This is the first report to find that use of a low-dose hCG administered at the time of GnRHa may be

associated with a modest increase in oocyte yield, both in terms of number and maturity. It is traditionally believed that LH and hCG are biologically equivalent since they both act via the same LH receptor; however, a study of cultured human granulosa cells found that while hCG was more potent on production of the intracellular signaling molecule cAMP, ERK and AKT activation was more potent and sustained by LH [61]. Given the importance in both of these pathways in granulosa cell/oocyte crosstalk and oocyte maturation, these findings could explain why dual trigger could result in a greater oocyte yield than GnRHa alone. However, given that these findings were drawn from less than 50 % of our original cohort, they must be interpreted with caution. Additionally, in this cohort there were significantly more patients with no underlying infertility diagnosis undergoing stimulation for fertility preservation in the dual trigger than in the GnRHa trigger group (33 vs 3 %, $p < 0.01$) which may impact the number and percentage of mature oocytes retrieved in this group. Assuming these findings are correct and can be repeated in other studies, dual trigger may be a useful tool to increase oocyte yield after COH and may be particularly valuable in patients who have a higher proportion of immature oocytes retrieved in previous cycles. Importantly, the improvement observed in the number and percentage of mature oocytes retrieved in this cohort came at the cost of increased rates of moderate and severe OHSS and therefore clinicians must carefully weigh these factors when selecting the most appropriate agent(s) used to trigger oocyte maturation.

A notable strength of this study was the assessment and verification of early OHSS. We confirmed the diagnosis both in individuals that reported symptoms concerning for OHSS with examination by a physician and in those that did not report symptoms of OHSS with daily phone calls from nurses. We chose to include individuals undergoing COH for the purpose of oocyte cryopreservation even though they are not at risk for late OHSS and do not contribute pregnancy data as (1) these were not our primary outcomes and (2) dual trigger is commonly used in these individuals both in our practice and in other centers and therefore is germane to clinical practice. This study is limited by its retrospective design, which could have led to selection and ascertainment bias. Of note, AMH levels were not available for approximately one half of the patients in the GnRHa trigger group which could lead to less precise estimates of required gonadotropin doses and could increase the risk of development of OHSS; however, this was not observed in our study as no cases of OHSS were observed in the GnRHa group. Although

an increase in oocyte yield is important, our study was not powered to determine the impact of the choice of trigger on pregnancy outcomes. Nonetheless, this study provides novel data to aid clinicians in determining the safest and most effective strategy to achieve final oocyte maturation after COH.

Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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