GnRH antagonist administered twice the day before hCG trigger combined with a step-down protocol may prevent OHSS in IVF/ICSI antagonist cycles at risk for OHSS without affecting the reproductive outcomes: a prospective randomized control trial

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Abstract

Purpose The purpose this study is to investigate whether a double antagonist dose (0.25 mg/12 h) administered the day before hCG trigger is effective in preventing ovarian hyperstimulation syndrome (OHSS) in GnRH antagonist IVF/intracytoplasmic sperm injection (ICSI) cycles at risk for OHSS.

Methods This is a prospective randomized control study, conducted from November 2012 to January 2016. A total of 194 patients undergoing a IVF/ICSI GnRH antagonist cycle that were at risk of OHSS and chose to proceed with embryo transfer and avoid cycle cancellation or embryo cryopreservation were allocated into two groups. The inclusion criteria consisted of a rapid rise of oestradiol ≥ 3500 pg/ml combined with ≥ 18 follicles > 11 mm in diameter without any mature follicle > 16 mm, in any day of stimulation. Overall, 97 patients (intervention group A) received a double dose of GnRH antagonist (0.25 mg/12 h) the day before hCG while 97 patients (control group B) did not. Recombinant FSH administration was tapered to 100 IU/24 h the day of the allocation in both groups.

Results Incidence of early-onset moderate/severe OHSS was significantly lower in intervention group A compared to control

group B (0 vs 12.37%, $P < 0.001$). Clinical pregnancy rate per cycle (50.52 vs 42.27%, $P = 0.249$) was not significantly different between the two groups. Oestradiol (3263.471 ± 1271.53 vs 5233 ± 1425.17 , $P < 0.001$), progesterone (0.93 ± 0.12 vs 1.29 ± 0.14 , $P < 0.001$) and luteinizing hormone (1.42 ± 0.31 vs 1.91 ± 0.33 , $P < 0.001$) were significantly lower in group A the day of the hCG triggering.

Conclusion The administration of a rescue double GnRH antagonist dose the day before hCG trigger may represent a safe alternative preventive strategy for early OHSS without affecting the reproductive outcomes.

Trial registration number ISRCTN02750360

Keywords GnRH antagonists · OHSS · Implantation · IVF-ICSI · Ovarian stimulation

Introduction

The use of GnRH antagonist protocol in IVF/intracytoplasmic sperm injection (ICSI) cycles has decreased the incidence of moderate and severe ovarian hyperstimulation syndrome (OHSS) [1, 2], but did not completely eliminate the risk [3]. While predicting the exact onset of OHSS is still debatable [4, 5], the combined criteria of E2 levels and number of follicles ≥ 11 mm have been currently used to commence the preventive measures [6]. In cases that transfer cancellation and cryopreservation of all embryos are not an option, GnRh agonist trigger with aggressive luteal support has been proposed to reduce the risk of OHSS in antagonist cycles [7]. Still, this strategy may adversely affect the reproductive outcome without completely eliminating the risk for OHSS [7–10].

Trial registration number: ISRCTN02750360

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Evidence from earlier studies implies an extrapituitary role for GnRH analogues and specifically a direct effect on the ovary due to the detection of surface GnRH receptors on granulosa cells of the human ovary [11–13]. GnRH analogues seem to exert a direct modulatory effect on ovarian function, regarding steroidogenesis, oocyte maturation and follicle rupture in animal granulosa-luteal cells in vitro [14]. Even more, GnRH antagonists have been shown to inhibit the expression of the cytokine VEGF in human granulosa-luteal cells in vitro [15].

It has been proposed that administration of the antagonist regimen twice a day (ganirelix 0.25 mg/12 h) in the late follicular phase, combined with a reduction in the daily dose of FSH for 1 to 3 days, might be enough to prevent OHSS in oocyte donation cycles [16]. In this protocol, mean serum oestradiol level dropped by 30% on the first day and remained relatively stable the second and the third day of the intervention.

The aim of this study was to investigate the incidence of the early and late OHSS and the reproductive outcomes in patients at risk for OHSS stimulated with the antagonist protocol, who received a double dose of the antagonist regimen (ganirelix 0.25 mg/12 h) the day before hCG trigger, in conjunction with a step down in daily dose of FSH to 100 IU.

Materials and methods

This is a prospective randomized controlled double-blinded trial performed at the IAKENTRO IVF Centre, Thessaloniki, Greece, from November 2012 to January 2016, after approval by the Institutional Review Board. The study included patients at high risk for OHSS who underwent ovarian stimulation for IVF using the sixth day fixed GnRH antagonist protocol. Using an allocation sequence generated from a computerized random number table, patients were allocated into intervention group A (taper FSH daily dose to 100 IU until hCG trigger and receive a double dose of GnRH antagonist the day before hCG trigger) and control group B (taper FSH daily dose to 100 IU until hCG trigger and receive a single dose as well as a placebo dose of GnRH antagonist the day before hCG trigger). Assignments were sealed in numbered opaque envelopes that were opened by the nurse on duty the day of the allocation. None of the medical staff was informed about the patient's group.

Patient population

Patients were nulliparous, entering ovarian stimulation for the first time, younger than 40 years of age, some of them with polycystic ovaries seen in ultrasound control but not diagnosed as polycystic ovary syndrome (PCOS) (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus Workshop

Group, [17]), at high risk for OHSS. Patients were considered at risk for developing moderate to severe OHSS when serum oestradiol ≥ 3500 pg/ml and 18 or more follicles ≥ 11 mm but without any mature follicle > 16 mm appearing at that time.

A total of 341 women were eligible to participate in the study. Overall, 194 IVF/ICSI women that fulfilled the inclusion criteria and signed the consent form to participate were included in the study. All patients were thoroughly informed about the risk of OHSS and that embryo transfer would be decided on day 5 of embryo culture at blastocyst stage, for allowing adequate time to diagnose early OHSS before embryo transfer [18]. In case that early OHSS occurred, transfer would be cancelled and cryopreservation of all embryos would follow [19]. In addition, GnRH antagonist regimens would be administered in luteal phase. The preventive options were explained in detail regarding methodology, reasons for being cautious and bibliographic references for the results and safety of each one. We expressed our concerns regarding the intervention with the administration of the double antagonist dose due to the novelty of the method and the lack of robust data, as there was only a single pilot study on donor cycles relative to the matter [16]. All cases entered the study only once, and an informed consent was obtained from all participants regarding the risks of the proposed intervention and also the occurrence of the early and late OHSS.

Ovarian stimulation protocol and IVF procedure

A standard fixed sixth day antagonist protocol (Orgalutran 0.25 mg, Organon) with 200 IU/day recombinant FSH (rFSH, Puregon Organon) starting on day 2 of the cycle was used for the ovarian stimulation. The initial dose was not adjusted for body mass index (BMI), ovarian reserve or previous response to stimulation as they entered stimulation for the first time. All women had an ultrasound control on day 2 of the cycle to detect any ovarian cyst. In the fifth and seventh days of the stimulation, ultrasound control of the growing follicles and blood measurement of the oestradiol levels were performed to evaluate ovarian response. Daily GnRH antagonist 0.25 mg (Orgalutran, NV Organon, The Netherlands) was started in the morning of day 6 of stimulation and continued up to the day before triggering ovulation in order to inhibit a premature luteinizing hormone (LH) surge. The daily dose of rFSH was kept steady to all patients to the level of 200 IU/day until at least two follicles with 15 mm of diameter appeared. Patients were included in the study when the inclusion criteria were met but were allocated to the groups the day before hCG trigger which was the day of the intervention.

Group A ($n=97$) continued their stimulation protocol by tapering the rFSH to 100 IU/day up to the final maturation, and an additional dose of GnRH antagonist 0.25 mg (0.25 mg/12 h per day) (Orgalutran, NV Organon, The Netherlands) was administered the day before hCG trigger. Final oocyte

maturation was achieved by administration of 10,000 IU of hCG (Pregnyl, NV Organon, The Netherlands) as soon as three or more follicles of ≥ 17 mm were present on ultrasound.

Group B ($n=97$) continued their stimulation protocol by tapering the rFSH to 100 IU/day up to the final maturation, and an additional placebo dose of GnRH antagonist was administered the day before hCG trigger. Final oocyte maturation was achieved by administration of 10,000 IU of hCG (Pregnyl, NV Organon, The Netherlands) as soon as three or more follicles of ≥ 17 mm were present on ultrasound.

Transvaginal oocyte aspiration was performed 36 h after hCG administration by ultrasound-guided follicle puncture and received anti-coagulant (Clexane 4000 anti-xa/24 h, Aventis) and cortisone (Prezolon 10 mg/24 h, Nycomed) treatment 6 h after the oocyte pickup (OPU). All women were examined at the maternal-foetal department by an independent OB/GYN clinically and by ultrasound 3 and 5 days post-OPU or earlier if any discomfort appeared, for signs of moderate or severe OHSS using clinical grading criteria and definitions as they were presented by Golan et al. and Grossman et al. [20, 21] (Tables 1 and 2).

If symptoms of moderate/severe OHSS were present, the embryo transfer was cancelled; all embryos were cryopreserved at the stage of blastocyst, and treatment with 0.25 mg of ganirelix was reinitiated [22] combined with paracentesis in cases with increased ascitic fluid [23]. Evaluation was performed by an independent examiner who was not informed regarding which women participated in each group (study or control). Patients in both groups, without signs of moderate/severe OHSS, had at least one embryo transferred on day 5 after OPU (blastocyst stage) of excellent to fair quality (higher than 2BB) based on classification of Gardner et al. (2000) [24]. Luteal phase support was obtained by daily intravaginal administration of 600 mg (200 mg $\times$ 3) micronized progesterone (Utrogestan, Faran, Greece), beginning immediately on the day of oocyte retrieval. A pregnancy test was performed 15 days after embryo transfer. A positive β -hCG test and a foetal heart beat seen by ultrasound at 7 weeks of gestation

Table 1 Classification of OHSS

Grade	Description
Mild G1	Abdominal distension and discomfort
G2	G1 distension plus nausea, vomiting and/or diarrhoea and ovarian enlargement of 5–12 cm
Moderate G3	Features of mild OHSS plus U/S evidence of ascites
Severe G4	Features of moderate OHSS plus clinical evidence of ascites and/or hydrothorax and breathing difficulty
G5	G4 disease plus a change in the blood volume, increased blood viscosity due to hemoconcentration, coagulation abnormalities and diminished renal perfusion and function

Table 2 Classification of ascites

Grade	Description
Absence	Absence of abdominal fluid
Low	Fluid barely detectable by U/S in the pouch of Douglas
Moderate	Increased amount of fluid in the small pelvis
Marked	Large amount of fluid reaching the umbilicus
Massive	Significant amount of fluid reaching the Morrison's pouch
Tense	Significant amount of fluid reaching the diaphragm with/without hydrothorax

were defined as a clinical pregnancy; otherwise, it was considered a biochemical pregnancy.

Outcome measures

The primary outcome of the study was early moderate/severe OHSS, late moderate/severe OHSS, early mild OHSS and late mild OHSS and clinical pregnancy rate. The secondary outcomes were E2, luteinizing hormone (LH) and progesterone (PR) levels.

Statistical analysis

Sample size was calculated for the incidence of early moderate/severe OHSS. Based on pilot results of unpublished data, incidence of early moderate/severe OHSS in the population of patients at risk for OHSS was approximately 11.5%. In order to detect a 10% difference in the incidence of moderate to severe OHSS between intervention and control group, we calculated that 188 patients (94 in each arm) are needed with statistical power of 80% when testing at a 5% level of statistical significance. Data analysis was conducted based on an intention-to-treat principle. Values are expressed as mean $\pm$ standard deviation (SD) or as proportions followed by the respective percentages accordingly. The independent samples *t* test was employed for the comparison of means of continuous variables (e.g. baseline characteristics), while the paired *t* test was used to assess the significance of the paired differences of continuous variables (e.g. evolution of E2, PR, LH). The chi-squared test was used for the comparison of proportions. Statistical significance was defined at $P \leq 0.05$. The Stata 11.2 software (StataCorp, College Station, TX) was used for data analysis. Power sample calculation was made with clincalc.com sample size free calculator.

Results

Data were prospectively collected for 194 IVF/ICSI patients being at risk of developing OHSS at the IAKENTRO IVF Centre between November 2012 and February 2016. A flow

chart of enrolment, inclusion, allocation and dropout cycles is shown in Fig. 1.

In all our patients, the inclusion criteria to the study groups were met from the seventh day of stimulation and onwards (Table 3).

Between the study groups, there was not a significant difference regarding the mean age of the patients, BMI, the total duration of rFSH stimulation, AFC, the average stimulation day that the patients entered the study, the average days of rFSH tapered to 100 IU received prior to reaching the hCG triggering criteria, the number of oocytes retrieved, the number of MII oocytes and the mean number of embryos transferred (Table 3).

Primary outcome measures

Incidence of OHSS

There was no cycle cancellation prior to hCG administration in both groups. In the intervention group A, two couples dropped out due to signs of mild early OHSS, while the rest proceed to embryo transfer as no cancellation was performed due to signs of moderate or severe early OHSS. In the control group B, four couples dropped out due to signs of mild early OHSS, while another 12 couples (12/97; 12.37%) developed signs of moderate or severe early OHSS on the first 3 days after OPU, and therefore, embryo transfer was cancelled. These patients were administered daily for the three following days a double dose of an antagonist regimen, while all their embryos that reached the blastocyst stage were vitrified. Couples in both groups developed signs of mild OHSS the day after hCG administration (6/97, 6.18% in group A vs 10/

97, 10.3% in group B) which disappeared soon after the oocyte retrieval with no further complications (Table 3). Late onset of moderate or severe OHSS was not developed in either study groups. However, mild late OHSS was developed in 2 out of 97 patients (2.06%) in group A and in 7 out of 97 cases (7.2%) in group B which resolved within a week without any further treatment.

Clinical pregnancy rates

The clinical pregnancy rate per cycle (50.52 vs 42.27%, $P = 0.249$) and the clinical pregnancy rate per transfer (50.52 vs 48.24%, $P = 0.759$), based on transvaginal scan findings at 7 weeks of gestation, were not proven significantly different between the intervention group A and the control group B, respectively (Table 3). Early pregnancy loss between groups A and B was not statistically different (16.33 vs 17.07%, $P = 0.925$).

Laboratory outcomes

Cleavage rate was 84.71% for group A vs 88.28% for group B ($P = 0.695$). No significant difference was also observed regarding blastocyst rate (total number of blastocyst formed out of the total number of fertilized oocytes) and top blastocyst rate (number of top-quality blastocysts formed per total number of fertilized oocytes) between two groups. Blastocyst rate was 63.42% for group A vs 65.56% for group B ($P = 0.311$), while top blastocyst rate was 44.13% for group A vs 45.22% for group B ($P = 0.772$).

Fig. 1 Flow diagram of the participants to the study

Table 3 Demographics and clinical outcomes for patients at risk of OHSS in intervention group A (double-dose ganirelix) and group B (single-dose ganirelix)

Parameter	Group A (<i>n</i> = 97)	Group B (<i>n</i> = 97)	<i>P</i> value
Age (mean ± SD)	28.9 ± 3.83	29.3 ± 3.62	0.456
BMI (mean ± SD)	24.3 ± 3.9	24.7 ± 4.8	0.525
AFC (mean ± SD)	15.7 ± 3.5	15.9 ± 3.2	0.854
Days of stimulation with rFSH	10.46 ± 1.23	10.17 ± 1.28	0.109
Days of stimulation with rFSH = 100 IU	2.13 ± 0.69	2.08 ± 0.71	0.620
No of oocytes retrieved	21.12 ± 8.37	20.83 ± 8.74	0.814
No. of MII oocytes	16.84 ± 7.12	15.32 ± 6.83	0.131
Endometrium the day of OPU	11.23 ± 2.26	11.44 ± 2.1	0.503
Severe early onset OHSS, <i>n</i> (%)	(0/97) 0.0	(12/97) 12.37	<0.001
Mild early onset OHSS, <i>n</i> (%)	(6/97) 6.18	(10/97) 10.3	0.297
Severe late-onset OHSS, <i>n</i> (%)	(0/97) 0.0	(0/97) 0.0	1.000
Mild late-onset OHSS, <i>n</i> (%)	(4/97) 2.06	(6/97) 7.2	0.088
Fertilization rate, <i>n</i> (%)	(1099/1630) 67.33	(964/1484) 64.87	0.147
Cleavage rate, <i>n</i> (%)	(931/1099) 84.71	(851/964) 88.28	0.695
Blastocyst rate, <i>n</i> (%)	(697/1099) 63.42	(632/964) 65.56	0.311
Top blastocyst rate, <i>n</i> (%)	(485/1099) 44.13	(436/964) 45.22	0.772
Embryos transferred (mean ± SD)	1.74 ± 0.44	1.62 ± 0.49	0.074
Implantation rate, <i>n</i> (%)	(57/166) 34.34	(48/138) 34.78	0.935
Pregnancy rate per cycle, <i>n</i> (%)	(49/97) 50.52	(41/97) 42.27	0.249
Pregnancy rate per transfer, <i>n</i> (%)	(49/95) 51.58	(41/81) 50.62	0.899
Early pregnancy loss, <i>n</i> (%)	(8/49) 16.33	(7/41) 17.07	0.925

Values are mean ± SD, unless otherwise stated

SD standard deviation, MII metaphase II, OHSS ovarian hyperstimulation syndrome, OPU oocyte pickup

Secondary outcome measures

The peak oestradiol concentration the day of inclusion to the study was not different between the group A and the group B (4657.35 ± 1154.57 vs 4378.47 ± 1203.83 , $P = 0.102$). The day of hCG trigger the intervention group A had significantly lower serum oestradiol levels compared to the control group B (3263.47 ± 1271.53 vs 5233.92 ± 1425.17 , $P < 0.001$). The mean serum oestradiol for the intervention group patients decreased by 29.93% the day after the additional antagonist dose was administered despite continuous treatment with exogenous

gonadotropins. A minimal rise of E2 levels of $\leq 15\%$ was observed in 12 out of 97 cases, 24 h after increasing the GnRH antagonist dose. The day of inclusion LH level was not significantly higher in group A compared to group B (2.03 ± 0.39 vs 1.95 ± 0.41 , $P = 0.165$), while the day of hCG triggering was significantly lower (1.42 ± 0.31 vs 1.91 ± 0.33 , $P < 0.001$). In addition, PR concentration the day of hCG triggering was significantly lower in the intervention group compared to the control group (0.93 ± 0.12 vs 1.29 ± 0.14 , $P < 0.001$) while at the day of inclusion, there was no difference (1.49 ± 0.17 vs 1.46 ± 0.14 , $P = 0.181$) (Table 4).

Table 4 E2, LH and PR levels during the ovarian stimulation cycles of the intervention group A (double-dose ganirelix) and of the control group B (single-dose ganirelix)

Parameter	Group A (<i>n</i> = 97)	Group B (<i>n</i> = 97)	<i>P</i> value
E2 the day of allocation (pg/ml)	4657.35 ± 1154.57	4378.77 ± 1203.83	0.102
E2 the day of hCG (pg/ml)	3263.47 ± 1271.53	5233.92 ± 1425.17	<0.001
<i>P</i> value	<0.001	<0.001	
LH the day of allocation (IU)	2.03 ± 0.39	1.95 ± 0.41	0.165
LH the day of hCG (IU)	1.42 ± 0.31	1.91 ± 0.33	<0.001
<i>P</i> value	<0.001	0.507	
PR the day of allocation (ng/ml)	1.49 ± 0.17	1.46 ± 0.14	0.181
PR the day of hCG (ng/ml)	0.93 ± 0.12	1.29 ± 0.14	<0.001
<i>P</i> value	<0.001	<0.001	

Values are mean ± SD, unless otherwise stated. E2 oestradiol, LH luteinizing hormone, PR progesterone, SD standard deviation

Discussion

The present study indicated that GnRH antagonist administered twice (0.25 mg/12 h) the day before hCG trigger in conjunction to tapering the rFSH dose seems effective to prevent severe early OHSS in GnRH antagonist IVF/ICSI cycles at risk for OHSS without compromising the reproductive outcomes. In addition, in similar patients stimulated with the single-dose antagonist protocol, tapering only the rFSH dose would result in developing early severe OHSS in about 12.37% of the cases.

There is no doubt that the use of GnRH antagonist protocol for ovarian stimulation combined with a GnRH agonist, instead of hCG, for final oocyte maturation as well as the freeze-all strategy seems to be, for the moment, the only safe approaches to eliminate the risk of OHSS [14, 25–27].

We have reported that in hyperresponding oocyte donation cycles stimulated with the fixed antagonist protocol, increasing the daily dose of GnRH antagonist (twice a day $\times$ 0.25 mg) before hCG triggering lowers the risk of developing OHSS while in parallel allows stimulation to continue for another 1 to 3 days with low doses of rFSH [16]. Further analysis of those oocyte donation cycles have shown that the mean oestradiol levels exhibited a sharp fall (30%) on the first day after doubling the administered dose of GnRH antagonist, remaining relatively stable (plateau) for the next 2 days [16]. Similar results regarding oestradiol levels in GnRH agonist protocols rescued by antagonists have been reported by Gustofson et al. [28], by Aboulghar et al. [29] and recently by Hill et al. [30]. Therefore, in the present study, in women at high risk for OHSS, we decided to administer an additional dose of antagonist (0.25 mg $\times$ 2 daily) just for a single day before hCG trigger combined with a tapering in the daily dose of rFSH. In this way, the follicles would not be deprived of the FSH or the LH during maturation and ovulation.

Although the exact physiologic mechanism by which the additional dose of GnRH antagonist suppresses oestradiol levels and prevents OHSS in hyperresponsive IVF/ICSI cycles remains to be clarified, some assumptions can be made. Acting on pituitary GnRH receptors may further restrain LH release in the serum, reducing androgen production and subsequently oestradiol secretion in the preovulatory follicles. In addition, the detection of surface GnRH receptors on granulosa cells of the human ovary [11–13, 31] implies a direct modulatory effect on ovarian function, regarding steroidogenesis, oocyte maturation and follicle rupture in animal granulosa-luteal cells in vitro [14, 32, 33].

It has been argued that lowering the rFSH dose alone would not be effective in reducing the oestradiol levels [34]. Our results are in favour of this opinion and have shown that simple reduction of the rFSH dose in hyperresponding IVF cycles (control group) could not have a significant effect neither in

lowering oestradiol levels nor in avoiding the OHSS since 12.37% of patients developed moderate or severe OHSS.

The cytokine VEGF expressed in human luteal cells plays a pivotal role in the pathogenesis and occurrence of OHSS, and it has been shown that the antagonists inhibit their release from the granulosa-luteal cells in vitro [15]. Obviously, the administration of the additional dose of GnRH antagonist could be a convenient explanation for the favourable OHSS occurrence of the intervention group in contrast to the control group.

These findings are consistent with earlier reports where luteal phase GnRH antagonist administration in patients, stimulated with either an agonist or an antagonist protocol, with established severe early OHSS seems to lead to quick regression of OHSS and avoidance of patient hospitalization [18, 35]. Ding et al. [36] reported that the withdrawal of GnRH agonist alone for 2 or 3 days in the late follicular phase in hyperresponsive agonist cycles markedly decreased the expression of CYP19A1, the production of oestradiol and VEGF mRNA concentration in granulosa cells, confirming the direct ovarian effect of GnRH agonist. Therefore, someone can assume that the administration of GnRH antagonist in the late follicular phase may directly block GnRH receptors on granulosa cells leading to a similar and maybe more profound effect on oestradiol and VEGF concentrations, as discontinuation of GnRH agonist does.

The role of oestradiol levels in OHSS and its predictive value has not been elucidated, but in the literature, the highly elevated or the acutely rising oestradiol levels during ovarian stimulation constitute a risk for OHSS while lowering serum E2 levels may reduce the likelihood of OHSS [37]. In all our patients, the oestradiol levels were decreased (mean oestradiol fall 32%) or reached a plateau 24 h after increasing the GnRH antagonist dose. Papanicolaou et al. [5] have reported that the levels of serum oestradiol have limited predictive value since the VEGF production could be associated with the number of follicles, but this is not necessarily so in the case of oestrogens because the metabolic pathway of their synthesis might be different. Morphometric and ultrastructural analyses of human granulosa cells have shown that in antagonist cycles, the percentage of granulosa cells with signs of functional activity were lower compared to the agonist-treated cycles [38]. Additionally, the lower oestradiol levels might be due to the protein kinase C activity, which has an inhibitory effect on gonadotropin-induced steroidogenesis and was found to be 2.5-fold higher in GnRH antagonist than in GnRH agonist-treated women [39]. Based on these data and our results, we consider that although the levels of serum oestradiol the day of hCG trigger may have lower predictive value compared to the follicle abundance, the oestradiol level variations during stimulation might reflect the functional activity of the granulosa cells and consequently the ability of them to produce VEGF.

Although the E2 decrease before hCG in IVF cycles was associated with a diminished likelihood of pregnancy [40], later works have shown that an iatrogenic decrease in E2 does not

appear to negatively impact IVF cycles [41, 42]. The sharp fall of the mean oestradiol concentration presented in our intervention group did not affect the implantation and pregnancy rates which were not significantly different compared to the control group where a decrease in oestradiol levels was not observed.

Recently, the role of the endogenous LH in GnRH antagonist protocols on the outcome has been debated. Dovey et al. [43] reported an 8% premature LH rise in GnRH antagonist cycles associated with decreased pregnancy rate, mainly observed in women with PCO phenotype at risk for OHSS [41]. On the other hand, it has been reported that there was no association between endogenous LH and pregnancy in GnRH antagonist protocols stimulated with either corifollitropin alfa or recombinant FSH [44, 45]. None of our patients in both groups had a premature LH rise, and after the double antagonist dose has been administered, LH level in the intervention group presented a further decrease.

It has been reported that a subtle increase in serum PR (above the level of 1.2 ng/ml) the day of hCG administration may adversely affect the pregnancy rate in IVF cycles [26, 46]. Additionally, this subtle increase was related to patients at high risk for OHSS and attributed to the excess number of follicles. In this study, we report that the double dose of GnRH antagonist has lowered the serum PR levels to <1 ng/ml the day of hCG administration while in the control group, the same day was statistically significantly higher. Obviously, a beneficial effect of lowering the PR levels in our study cannot be excluded.

High doses of GnRH antagonist may adversely affect implantation and pregnancy rates in a dose-dependent way without affecting the number and quality of oocytes and embryos [1, 47, 48]. These data have raised questions on the impact of GnRH antagonist on the endometrial receptivity. It has been postulated that high doses of GnRH antagonist may exert a direct effect on the endometrium and negatively affect the implantation [49, 50]. The implantation and pregnancy rates in our intervention group that was exposed to higher than normal GnRH antagonist dosage for a single day were not different compared to the control group.

The limitation of the study is that power calculation was based on retrospective analysis of our data regarding the incidence of OHSS from donation antagonist cycles before 2012 without any intervention, as well as on preliminary data from an earlier pilot study on donation cycles with a small sample size (72 cases) and lack of control group, which obviously can introduce bias. However, our study provides the first evidence that by doubling the GnRH antagonist dose the day before hCG trigger and at the same time by lowering but not coasting gonadotropin administration, in patients at high risk for OHSS, the oestradiol rise could be blocked, the serum LH and PR levels would be further reduced while a minimal follicular stimulation may continue without the risk of developing moderate or severe OHSS. Furthermore, the increased GnRH

antagonist dosage resulted in similar implantation and pregnancy rates compared to similar cycles stimulated with the classical GnRH antagonist dose protocol. As it is unlikely that all OHSS is preventable, this novel approach offers an alternative strategy for the management of ovarian hyperresponse in IVF/ICSI antagonist cycles. However, further randomized studies are necessary to confirm our results.

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