

Pregnancy and neonatal outcomes following luteal GnRH antagonist administration in patients with severe early OHSS

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STUDY QUESTION: Do high-risk patients who develop severe early ovarian hyperstimulation syndrome (OHSS) and receive low-dose GnRH antagonist in the luteal phase have lower live birth rates compared with high-risk patients who do not develop severe early OHSS and do not receive GnRH antagonist in the luteal phase?

SUMMARY ANSWER: Low-dose luteal GnRH antagonist administration in women with severe early OHSS is associated with similar live birth rates to that of high-risk patients who do not develop severe early OHSS and do not receive GnRH antagonist in the luteal phase.

WHAT IS KNOWN ALREADY: It has been reported that luteal GnRH antagonist administration in patients with established severe early OHSS appears to prevent patient hospitalization and results in quick regression of the syndrome on an outpatient basis. However, the effect of such treatment on pregnancy outcome has been investigated in only a small number of animal studies.

STUDY DESIGN, SIZE, DURATION: This is a prospective cohort study of 192 IVF patients who were at high risk for OHSS and who did not wish to cancel embryo transfer and have all embryos cryopreserved. The study was conducted between January 2009 and December 2011 at Eugonia Assisted Reproduction Unit.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Patients were <40 years of age, with polycystic ovaries, at high risk for OHSS (defined by the presence of at least 20 follicles ≥ 11 mm on the day of triggering of final oocyte maturation) and not willing to cancel embryo transfer and cryopreserve all embryos, if severe early OHSS was diagnosed by Day 5 of embryo culture. Patients who were diagnosed with severe early OHSS on Day 5 post-oocyte retrieval were administered 0.25 mg of ganirelix for 3 days, from Day 5 until and including Day 7 (OHSS + antag group, $n = 22$). High-risk patients who did not develop the severe early OHSS did not receive GnRH antagonist in the luteal phase (control group, $n = 172$). All patients underwent embryo transfer on Day 5.

MAIN RESULTS AND THE ROLE OF CHANCE: Live birth rates (40.9 versus 43.6%), ongoing pregnancy rates (45.5 versus 48.8%), clinical pregnancy rates (50 versus 65.1%), positive hCG (72.7 versus 75%), duration of gestation (36.86 ± 0.90 weeks versus 36.88 ± 2.38 weeks) and neonatal weight (2392.73 ± 427.04 versus 2646.56 ± 655.74 g) were all similar in the OHSS + antag and control groups, respectively. The incidence of major congenital malformations was 2.9% (3/103) in children born in the control group compared with no cases (0/14) in children born following luteal GnRH antagonist administration. No stillbirths or intrauterine deaths, and no cases of pregnancy-induced late OHSS were recorded in either group. None of the 22 patients with severe early OHSS required hospitalization following luteal antagonist administration. Ovarian volume, ascites, hematocrit, white blood cell count, serum estradiol and progesterone decreased significantly ($P < 0.001$) by the end of the monitoring period (Day 11 post-oocyte retrieval), indicating rapid resolution of the severe OHSS.

LIMITATIONS, REASONS FOR CAUTION: This is a prospective cohort investigation with a very limited number of patients receiving the intervention and a larger number of control patients. Our findings suggest that low-dose luteal GnRH antagonist administration during the peri-implantation period may be safe, although larger studies with follow-up of the children born are required.

WIDER IMPLICATIONS OF THE FINDINGS: Our study suggests for the first time that low-dose luteal GnRH antagonist administration in women with severe early OHSS is associated with a favourable IVF outcome, comparable to control high-risk patients without severe OHSS and not receiving the intervention. Regarding the wider implications on the concept of an OHSS-free clinic, administration of GnRH antagonist in the luteal phase may present a tertiary management level in patients with established severe OHSS, along with the use of GnRH antagonist protocols for primary prevention and the replacement of hCG with GnRH agonist for triggering final oocyte maturation for secondary prevention. However, at present, fresh embryo transfer combined with antagonist administration should only be used with caution by experienced practitioners, after carefully deciding which patients can have a fresh transfer or embryo cryopreservation, until the current data are confirmed by larger trials.

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Key words: GnRH antagonist / OHSS / congenital malformations / pregnancy outcome / polycystic ovary syndrome

Introduction

The use of GnRH antagonists in the follicular phase for the prevention of premature LH surges has been extensively reviewed in the literature, with numerous clinical trials, Cochrane reviews and meta-analyses available regarding the analogue's safety, efficacy and optimization (Al-Inany and Aboulghar, 2002; Kolibianakis et al., 2006; Al-Inany et al., 2007, 2011).

However, limited data exist regarding the use of GnRH antagonists in the luteal phase. Luteal GnRH antagonist administration has been studied *in vivo* in a small number of animal studies focusing on hormonal changes and pregnancy outcome (Das and Talwar, 1983; Siler-Khodr et al., 1984; Eley, 1987; Fraser et al., 1987; Kang et al., 1989; Virolainen et al., 2003; Tug et al., 2011).

In humans, GnRH antagonist administration during the mid-luteal phase of a natural menstrual cycle is known to induce luteolysis by reducing pulsatile gonadotrophin stimulation, resulting in the rapid decline in serum estradiol and progesterone levels and the onset of menstrual bleeding (Mais et al., 1986).

Moreover, *in vitro* studies have shown that GnRH antagonists influence placental hormone release (Siler-Khodr et al., 1983, 1987), but not decidualization of endometrial cells (Klemmt et al., 2009).

In patients treated by IVF, the majority of relevant studies involve luteal GnRH antagonist administration in the preceding luteal phase prior to the onset of ovarian stimulation for the purpose of follicular synchronization or prevention of premature LH surges (Fanchin et al., 2004; Friden and Nilsson, 2005; Humaidan et al., 2005a; DiLuigi et al., 2011; Garcia-Velasco et al., 2012).

However, luteal phase GnRH antagonist administration has also been proposed for a different purpose, that of managing established severe early ovarian hyperstimulation syndrome (OHSS) (Lainas et al., 2007b, 2009a,b, 2012; Bonilla-Musoles et al., 2009). It has been reported that luteal GnRH antagonist administration in patients with established severe early OHSS appears to prevent patient hospitalization and results in quick regression of the syndrome on an outpatient basis (Lainas et al., 2007b, 2009b). This intervention appears to be effective in both agonist and antagonist-treated patients. In addition, there is some evidence to suggest that luteal GnRH antagonist is safe and efficient when administered concomitantly with embryo transfer in patients with severe early OHSS, leading to the birth of healthy offspring (Lainas et al., 2009a). However, the available

published data exist in the form of a small case series (Lainas et al., 2009a), which, although promising, requires further evaluation.

The aim of the present study was to investigate IVF and neonatal outcomes in patients with severe early OHSS, who received luteal GnRH antagonist administration, compared with a control group of high-risk patients, who did not develop severe OHSS and did not receive GnRH antagonist in the luteal phase.

Materials and Methods

Patient population and management

This prospective study included patients at high risk for OHSS, who underwent ovarian stimulation for IVF between January 2009 and December 2011 at Eugonia Assisted Reproduction Unit.

Patients were younger than 40 years of age, with polycystic ovaries (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004), at high risk for OHSS [defined by the presence of at least 20 follicles ≥ 11 mm on the day of triggering of final oocyte maturation (Papanikolaou et al., 2010)] and not willing to cancel embryo transfer and cryopreserve all embryos, even if severe early OHSS was diagnosed by Day 5 of embryo culture.

Blastocyst transfer allowed more extensive monitoring of high-risk patients in order to accurately diagnose the development of severe early OHSS, as previously proposed (Papanikolaou et al., 2011; Lainas et al., 2012).

Patients were allocated in two groups depending on the development or not of severe early OHSS. The control group included patients who did not develop severe OHSS. Patients diagnosed with severe early OHSS on Day 5 post-oocyte retrieval were presented with two options: (i) GnRH antagonist administration combined with embryo transfer cancellation and cryopreservation of all embryos (OHSS + cryopreservation group), which is the standard procedure followed for severe OHSS in our Unit (Lainas et al., 2009b); (ii) transfer of one or two blastocysts with concomitant initiation of GnRH antagonist administration and luteal phase support, in order to reduce the severity of the established OHSS, as well as to reduce the chance of pregnancy-induced late OHSS (OHSS + antag group).

The option for fresh embryo transfer was proposed after careful consideration of all the risks involved and after thorough discussion with the patients, explaining in detail all the reasons for caution regarding the method. We expressed our reservations about the development of late pregnancy-induced OHSS, the lack of solid bibliographic evidence due to

the novelty of the method apart from a small case series (Lainas *et al.*, 2009a) and the potential adverse effects of GnRH antagonist administration on pregnancy outcome.

The intervention was approved by the Centre's Institutional Review Board. Patients signed an informed consent form regarding risks of early and late OHSS, and risks of the intervention proposed. Institutional Review Board authorization and patient consents were also obtained for the additional monitoring of patients with severe early OHSS after embryo transfer.

Criteria for the diagnosis of severe OHSS

Severe OHSS was diagnosed using previously published criteria (Lainas *et al.*, 2012). Briefly, severe early OHSS was diagnosed in the presence of moderate/marked ascites and at least two of the following criteria: enlarged ovaries (>100 mm maximal diameter), haematocrit (Ht) >45%, white blood cell count (WBC) >15 000/mm³, hydrothorax, dyspnoea, oliguria or abnormal liver function tests, based on a modification of previous classification systems (Schenker and Weinstein, 1978; Golan *et al.*, 1989; Navot *et al.*, 1992; Rizk and Aboulghar, 1999; Pau *et al.*, 2006; Alvarez *et al.*, 2007b; Lainas *et al.*, 2010; Humaidan *et al.*, 2010b).

Ascites was classified according to the quantity of fluid accumulation in the peritoneal cavity (Table I), as already described (Lainas *et al.*, 2012), similar to previously published criteria (Pau *et al.*, 2006; Humaidan *et al.*, 2010b).

Description of the intervention

In patients with early severe OHSS, 0.25 mg of the GnRH antagonist ganirelix (Orgalutran, Organon, The Netherlands) was administered daily for 3 days, starting on the day of embryo transfer (Day 5) until and including Day 7 post-oocyte retrieval (Lainas *et al.*, 2007b, a,b).

In all patients included, luteal phase support was performed by administering micronized progesterone (600 mg) (Utrogestan, Laboratoires Besins International SA, France) from Day 3 post-oocyte retrieval until the 10th week of gestation, if pregnancy occurred. Additionally, patients with severe OHSS receiving the intervention were administered 17β-estradiol patches (Dermestil TTS-100, Lohmann Therapie-Systeme GmbH, Germany) from Day 5 until the 7th week of gestation and 4500 anti-Xa IU (0.45 ml) tinzaparin sodium (Innohep; LEO Pharmaceutica Products Hellas Ltd, Greece) for thromboprophylaxis, daily from Day 5 post-oocyte retrieval until resolution of the syndrome.

Table I Classification of ascites.

Grade	Description
No ascites	No presence of fluid
Low	Small amount of fluid, barely detectable by ultrasound in the pouch of Douglas
Moderate	Increased amount of fluid located in the small pelvis
Marked	Large amount of fluid reaching the level of the umbilicus
Massive	Significant accumulation of fluid reaching Morrison's pouch
Tense	Significant accumulation of fluid up to the level of the diaphragm with/without hydrothorax

The classification of ascites used in our Unit is similar to previously published criteria (Pau *et al.*, 2006; Humaidan *et al.*, 2010b) and distinguishes different levels of ascites, depending on the accumulation of ascetic fluid when the patient was at the anti-trendelenburg position.

Ovarian stimulation

Patients underwent ovarian stimulation for IVF/ICSI using either a long GnRH agonist down-regulation or a flexible GnRH antagonist protocol, as previously described (Lainas *et al.*, 2010).

All patients received oral contraceptive pills (Trigynera, Bayer Hellas, Greece) daily for 21 days, starting on Day 2 of spontaneous menses of the preceding cycle, after a blood test confirmed the presence of a baseline hormone profile.

The starting dose of rFSH was 150 IU/day for all patients. This dose was adjusted after Day 5 of stimulation, depending on the ovarian response, as assessed by E2 levels and ultrasound.

Triggering of final oocyte maturation and IVF

When at least three follicles of diameter ≥17 mm were present, final oocyte maturation was triggered by i.m. injection of 5000 IU hCG (Pregnyl; Organon, The Netherlands), as previously described (Abdalla *et al.*, 1987; Kolibianakis *et al.*, 2007). Transvaginal ultrasound-guided oocyte retrieval was performed 36 h later by double lumen needle aspiration. ICSI was performed only in cases with severe male factor or previous fertilization failure. Embryos were cultured in sequential media (Medicult/Origio, Denmark) for 5 days to the blastocyst stage.

All patients were examined again 15 and 30 days (in case of positive hCG test) after oocyte retrieval for the presence of late pregnancy-induced OHSS.

Follow-up of patients after the intervention

In patients with severe early OHSS who received the intervention, ultrasound assessment of ovarian size and ascetic fluid and measurement of serum estradiol, progesterone, Ht and WBC were performed on Days 5, 7, 9 and 11 post-oocyte retrieval. In addition, serum estradiol, progesterone, Ht and WBC were also evaluated on the day of oocyte retrieval (Day 0). Ovarian volume was calculated using the prolate ellipsoid formula $V = D1 \times D2 \times D3 \times 0.523$, where D1, D2 and D3 are the three maximal longitudinal, antero-posterior and transverse diameters, respectively.

Follow-up of children born

Congenital malformations, birthweight and gestational age of delivery were recorded for children born in the control and OHSS + antag groups.

Major congenital malformations were defined as congenital malformations that cause functional impairment or require surgical intervention, according to the definition proposed by Bonduelle *et al.* (2002, 2010). All remaining congenital malformations were defined as minor.

The data were collected from respective parents by a certified midwife. The patient's paediatrician was contacted in case of congenital malformations for further information regarding the type and severity of the malformation.

Ultrasound and laboratory assays

All ultrasound measurements were performed using a 7.5 or 6 or 5 MHz vaginal probe (Sonoline Adara, Siemens). FSH, LH, E₂ and progesterone levels were measured using an Immulite analyser and commercially available kits (DPC, Los Angeles, CA, USA). Analytical sensitivity were 0.1 mIU/ml for FSH, 0.1 mIU/ml for LH, 15 pg/ml for E₂ and 0.2 ng/ml for progesterone. Intra- and inter-assay precisions at the concentrations of most relevance to the current study (expressed as coefficients of variation) were 2.6 and 5.8% for FSH, 5.9 and 8.1% for LH, 6.3 and 6.4% for E₂ and 7.9 and 10% for progesterone. Ht and WBC count were determined by flow cytometry using Coulter A^C.T diffTM Analyzer (Coulter Corporation, Miami, FL, USA). Coefficient of variation, specifying

imprecision limits for white (WBC) and red blood cell count (RBC), was 3%. Ht was computed from the relative volume of erythrocytes [mean corpuscular volume (MCV)] [$\text{Ht} (\%) = \text{RBC} \times \text{MCV}/10$].

Outcome measures

The primary outcome was live birth rate per embryo transfer. Secondary outcomes included positive hCG rates, clinical and ongoing pregnancy rates (presence of gestational sac with fetal heart beat detection at 6–7 weeks and at 12 weeks of gestation, respectively), multiple pregnancy rates, as well as biochemical pregnancy rates (positive hCG not reaching clinical pregnancy) and clinical spontaneous abortion rates (clinical pregnancy not reaching ongoing pregnancy at 12 weeks). Major and minor congenital malformations, psychomotor problems, as well as birthweight and gestational age of delivery of children born were recorded in the two groups. In addition, progression or regression of severe OHSS was studied in terms of alterations in serum estradiol, progesterone, Ht, WBC count, ovarian volume and ascites following luteal GnRH antagonist administration in patients with established severe OHSS. The mean daily dose of GnRH antagonist per kg was also calculated in patients with severe OHSS receiving the intervention.

Statistical analysis

The outcome measures were subjected to Fisher's exact test or repeated measures ANOVA followed by post hoc pairwise comparisons with Bonferroni correction. The frequency distributions of the ascites levels were analysed using the Wilcoxon test. The level of significance was set at 0.05.

Results

A total of 194 patients at high risk for OHSS who underwent embryo transfer on Day 5 were included in the study. Of these, 22 patients were diagnosed with severe early OHSS on Day 5, while 172 patients did not develop severe early OHSS (control group).

All 22 patients with severe early OHSS wished to proceed to embryo transfer and luteal administration of GnRH antagonist (OHSS + antag group). No patient selected embryo transfer cancellation and cryopreservation of all embryos, because they had either experienced previous cancelled IVF cycles due to OHSS ($n = 8$) or did not wish to cancel the current cycle and compromise embryo viability following cryopreservation ($n = 13$). Thus, all 22 patients opted to proceed to embryo transfer using the new treatment approach proposed here (Fig. 1).

Baseline characteristics, ovarian stimulation and embryological data in the two groups compared are shown in Table II. Patients in both groups were at high risk for OHSS on the day of triggering final oocyte maturation (presence of at least 20 follicles > 11 mm at ultrasound scan) and had similar numbers of oocytes retrieved and embryos transferred, allowing valid comparisons between them.

Live birth rates (40.9 versus 43.6%) were similar in the OHSS + antag and control groups, respectively. In addition, positive hCG rates (72.7 versus 75.0%), clinical pregnancy rates (50.0 versus 65.1%), ongoing pregnancy rates (45.5 versus 48.8%) and multiple pregnancy rates (37.5 versus 44.2%) did not differ between the OHSS + antag and the control group (Table III). Biochemical pregnancy rates (31.3 versus 13.2%) and clinical spontaneous abortion rates (9.1 versus 24.1%) did not differ statistically, although the higher incidence of biochemical pregnancy in OHSS + antag patients

compared with control patients was close to statistical significance ($P = 0.07$) (Tables III and IV).

There were 9 deliveries with 14 live infants born (4 singleton and 5 twin deliveries) in the OHSS + antag group and 75 deliveries with 103 live infants born in the control group (47 singleton and 28 twin deliveries). No stillbirths or intrauterine deaths were recorded in either group.

The duration of gestation (36.9 ± 0.9 weeks versus 36.9 ± 2.4 weeks; $P = 0.9$) and neonatal weight (2392 ± 427 versus 2646 ± 656 g; $P = 0.22$) were similar in the OHSS + antag and control groups, respectively (Tables V and VI).

There were three cases of major congenital malformations in children born in the control group [3/103 (2.9%): one child with atrial/ventricular septal defect, one with hypospadias and one with aortic obstruction. No major congenital malformation were observed in children born in the OHSS + antag group [0/14 (0%)] (Tables V and VI).

In addition, four children in the control group needed treatment for speech disorders, compared with no children in the OHSS + antag group. No minor congenital abnormalities or psychomotor problems were observed in either group.

The high-risk patients who developed severe early OHSS received low-dose (0.25 mg) luteal GnRH antagonist administration for 3 days. Patient weight (mean \pm SD) was 66.1 ± 12.6 kg and the GnRH antagonist daily dose administered per kg (mean \pm SD) was 0.0039 ± 0.0008 mg/kg. Ovarian volume, ascites, serum estradiol, progesterone, Ht and WBC count reached their highest levels on the day of severe OHSS diagnosis (Day 5 post-oocyte retrieval). There was a rapid and statistically significant improvement of ultrasound and laboratory findings, beginning as early as 2 days after luteal administration of GnRH antagonist in all 22 patients. The decline in all parameters continued in a progressive manner until the end of the monitoring period (Fig. 2).

All 22 patients with established early severe OHSS were managed with luteal GnRH antagonist administration and frequent monitoring at an outpatient level. No patient required hospitalization, or received cortisone administration, or had paracentesis of ascetic fluid. No patient developed late OHSS in either group.

Discussion

The present study suggests, for the first time, that luteal GnRH antagonist administration concomitantly with embryo transfer in patients with established severe early OHSS does not compromise the chance of a successful pregnancy outcome. The data obtained suggest that high pregnancy and live birth rates are maintained in patients with severe early OHSS, who received low-dose GnRH antagonist, compared with those observed in the control group of high-risk patients, who did not develop severe OHSS and did not receive GnRH antagonist.

The incidence of biochemical pregnancy in the OHSS + antag group appeared higher, although not significantly so ($P = 0.07$). It has to be noted that, significantly higher biochemical pregnancy rates were previously shown in women with early OHSS compared with non-OHSS patients (Papanikolaou et al., 2005). In addition, there is evidence showing that severe OHSS is associated with increased spontaneous abortion rates and pregnancy complications (Abramov et al., 1998; Raziel et al., 2002, 2009). Therefore, it seems likely that this increase,

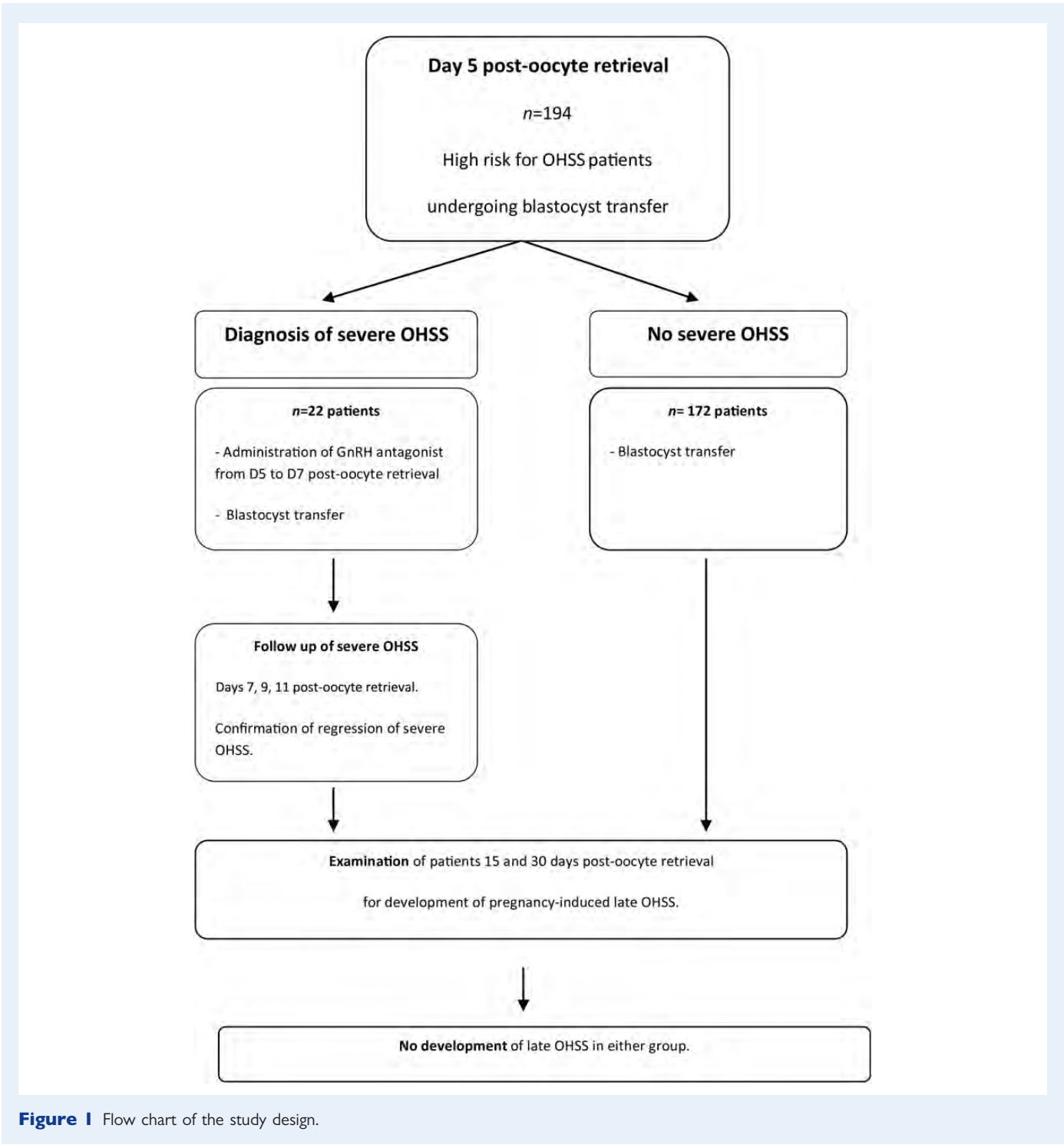


Figure 1 Flow chart of the study design.

albeit non-significant, in biochemical pregnancy rates in our study may be attributed to the presence of severe early OHSS. However, a possible deleterious effect of luteal administration of GnRH antagonist on extra-pituitary reproductive cells and organs, such as ovarian cells, oocyte, embryo and endometrium cannot be entirely excluded (Kol, 2000).

The administration of GnRH antagonist during the peri-implantation period may raise some concerns regarding potential adverse effects of the antagonist on embryo implantation, pregnancy establishment and

progression, as well as the health of the children born following the intervention. The current study suggests that pregnancy and live birth rates are similar between the two patient groups compared and do not seem to be affected by luteal administration of GnRH antagonist. All children born in the OHSS + antag group were healthy without any major or minor congenital abnormalities. In addition, the duration of gestation and neonatal weight were similar to those recorded in the control group. These observations are promising regarding the effect of luteal GnRH antagonist administration, although

Table II Baseline characteristics, ovarian stimulation and embryological data for the high-risk patients who did not develop severe OHSS or developed severe OHSS and were administered GnRH antagonist in the luteal phase.

	Severe OHSS and luteal GnRH antagonist administration (n = 22)	Control (no severe OHSS) (n = 172)	P
Baseline characteristics			
Age (year)	31.91 ± 4.12	32.12 ± 4.37	0.831
BMI (kg/m ²)	23.81 ± 4.12	23.98 ± 4.55	0.87
Duration of infertility (years)	3.73 ± 2.71	3.83 ± 4.39	0.92
Number of previous IVF attempts	0.86 ± 1.55	1.21 ± 1.94	0.422
Baseline FSH (IU/l)	5.63 ± 1.21	6.71 ± 1.68	0.007
Baseline LH (IU/l)	6.07 ± 2.58	5.61 ± 2.72	0.95
Baseline estradiol (pg/ml)	33.23 ± 20.42	33.41 ± 14.70	0.962
Baseline progesterone (ng/ml)	0.43 ± 0.19	0.52 ± 0.30	0.193
Ovarian stimulation			
Long protocol (n)	7	62	
Antagonist protocol (n)	15	110	
Duration of stimulation (days)	10.23 ± 0.97	10.92 ± 1.40	0.027
Total FSH (IU)	1575 ± 517.82	1858.16 ± 603.03	0.038
Number of follicles on day of hCG	30.36 ± 10.30	29.31 ± 3.85	0.353
Estradiol on day of hCG (pg/ml)	3894.76 ± 1845.32	3080.02 ± 1280.80	0.011
Progesterone on day of hCG (ng/ml)	1.06 ± 0.47	0.98 ± 0.43	0.446
Embryological data			
Number of oocytes retrieved	27.27 ± 7.36	24.77 ± 6.77	0.108
Mature oocytes (in ICSI patients)	14.33 ± 11.74	12.90 ± 8.76	0.498
Type of fertilization (IVF/ICSI/IVF + ICSI)	5/8/9	34/94/44	0.222
Number of 2PN	16.00 ± 8.29	15.61 ± 12.89	0.890
Number of embryos transferred*	2 (1–2)	2 (1–3)	0.802
Patients with cryopreservation of supernumerary blastocysts	15	104	0.643
Number of blastocysts cryopreserved per patient	7.33 ± 4.14	7.32 ± 4.24	0.993

Values are expressed as mean ± standard deviation (SD) except in * (number of embryos transferred) where they are expressed as medians (min–max).

P-values express the result of the independent samples t-test, except in * where the Mann–Whitney test was applied. P-values in bold depict statistical significance ($P < 0.05$).

Table III Pregnancy outcomes for the high-risk patients who either did not develop severe OHSS (control) or developed severe OHSS and were administered GnRH antagonist in the luteal phase (OHSS + antag).

	OHSS + antag (n = 22)	Control (n = 172)	P
Positive hCG test, n (%)	16 (72.7)	129 (75.0)	0.798
Clinical, n (%)	11 (50.0)	112 (65.1)	0.239
Ongoing, n (%)	10 (45.5)	84 (48.8)	0.834
Live birth, n (%)	9 (40.9)	75 (43.6)	1.000
Biochemical pregnancy	5/16 (31.3)	17/129 (13.2)	0.070
Clinical spontaneous abortion	1/11 (9.1)	27/112 (24.1)	0.453
Multiple pregnancy	6/16 (37.5)	57/129 (44.2)	0.790

the number of children born following the intervention is very small and therefore larger studies are necessary to verify the present findings.

The incidence of major congenital malformations in the control group of patients was 2.9%. Previous studies have reported an incidence ranging from 3.4 to 4.5% (Ludwig et al., 2001; Boerrigter et al., 2002), while the largest follow-up study to date comparing congenital malformations in ~2000 fetuses born after ovarian stimulation using GnRH antagonist and GnRH agonist protocols reported an incidence of 5 and 5.4% in antagonist and agonist protocols, respectively (Bonduelle et al., 2010).

Only a limited number of studies in animal species are available regarding post-implantation GnRH antagonist administration, and these show that the antagonist has a dose-dependent and gestational age-dependent effect on pregnancy progression and fetal outcome. In the baboon model, high doses of GnRH antagonists ranging from 3.6 to 100 mg were administered over 7 days during more advanced stages of early pregnancy (35–45 days of gestation). There was a dose-dependent increase in stillbirths following those high doses of

Table IV Pregnancy outcomes for the high-risk patients who underwent single (SET), double (DET) or triple (TET) embryo transfer in the GnRH + antag and control group.

	OHSS + antag (n = 22)		Control (n = 172)		
	SET (n = 2)	DET (n = 20)	SET (n = 18)	DET (n = 115)	TET (n = 39)
Positive hCG test, n (%)	2 (100)	14 (70)	8 (44.4)	93 (80.9)	28 (71.8)
Clinical pregnancy, n (%)	2 (100)	9 (45)	6 (33.3)	85 (73.9)	21 (53.8)
Ongoing pregnancy, n (%)	1 (50)	9 (45)	5 (27.8)	63 (54.8)	16 (41.0)
Live birth, n (%)	1 (50)	8 (40)	4 (22.2)	56 (48.7)	15 (38.5)
Biochemical pregnancy	0	5 (25)	2 (25)	8 (8.6)	7 (25)
Clinical spontaneous abortion	1 (50)	0	1 (12.5)	22 (23.7)	5 (17.9)
Singletons, n (%)	1 (100)	3 (37.5)	4 (100)	34 (60.7)	9 (60)
Twins, n (%)	0	5 (62.5)	0	22 (39.3)	6 (40)
Triplets, n (%)	0	0	0	0	0

Table V Neonatal outcomes of live born infants.

Category	OHSS + antag (n = 14 infants)	Control (n = 103 infants)	P
Major congenital malformations, n (%)	0 (0)	3 (2.91)	1.00
Atrial and ventricular septal defect, n (%)		1 (0.97)	
Hypospadias, n (%)		1 (0.97)	
Aortic obstruction, n (%)		1 (0.97)	
Stillbirths/intrauterine deaths, n (%)	0 (0)	0 (0)	1.00
Duration of gestation (weeks) (mean ± SD)	36.86 ± 0.90	36.88 ± 2.38	0.983
Neonatal weight (g) (mean ± SD)	2392.73 ± 427.04	2646.56 ± 655.74	0.221
Multiple births, n (%)	5/9 (55.6)	28/75 (37.3)	0.306

GnRH antagonist, suggesting that interference with GnRH activity during pregnancy may lead to placental insufficiency (Siler-Khodr *et al.*, 1984; Kang *et al.*, 1989). Similarly, a single dose of 0.1 mg/kg resulted in a 20% abortion rate in pregnant pigs (Virolainen *et al.*, 2003). However, a lower dose of 2 mg GnRH antagonist administered on Days 14–19 in pregnant baboons did not seem to have an adverse effect on pregnancy outcome (Eley, 1987). In rats, GnRH antagonist doses ranging from 0.015 to 0.15 mg/kg on Days 4 or 8 of gestation were associated with lower birthweight and altered histomorphometric characteristics in a dose-dependent manner (Tug *et al.*, 2011). It is clear that the doses administered in all the above animal studies are very high compared with the mean dose of 0.0039 mg/kg used in our study.

In humans, the effect of GnRH antagonist administration on pregnancy has been studied only *in vitro*. It was shown that GnRH antagonist did not influence the extent of decidualization of endometrial stromal cells, and no adverse effect was exerted on human blastocyst

invasion (Klemmt *et al.*, 2009). However, GnRH antagonists were shown to inhibit hormone release in mid-gestation human placental cell cultures by suppressing hCG, alpha-hCG, estrone, estradiol and progesterone secretion (Siler-Khodr *et al.*, 1983, 1987). This inhibition was found to be gestational age related, as there was significant hormone suppression in placental cultures from 13 to 15 weeks of gestations, but no effect was seen in cells from the earlier gestational ages of 6–15 weeks (Siler-Khodr *et al.*, 1987).

In addition, GnRH receptors are expressed in human trophoblasts (Lee *et al.*, 2010), indicating that GnRH antagonists may have a direct action on the embryo. However, it is reported that GnRH stimulates only hCG production by trophoblast cells without affecting the *in vitro* secretion of other cytokines by trophoblasts or decidua (Lee *et al.*, 2010). These findings are encouraging, providing additional evidence that exposure of the embryo to GnRH antagonists is unlikely to have an adverse effect on implantation and early placental development.

In our study GnRH antagonist was administered at a low dose (0.25 mg; 0.0039 mg/kg) for only 3 days, compared with the extremely high doses for prolonged periods used in the animal studies mentioned above. The low dose of 0.25 mg of ganirelix has an elimination half-life of only 13 h (Mannaerts and Gordon, 2000). Gastrulation in the human embryo, i.e. the formation of the three primary germ layers (ectoderm, endoderm and mesoderm) occurs in the third week post-fertilization, while the essential parts of the placenta are established and become functional by the fourth week post-fertilization (Langman, 1981). Therefore, following the last daily 0.25 mg dose on Day 7 post-oocyte retrieval (i.e. Day 6 post-fertilization) the GnRH antagonist should have been completely eliminated well before these critical events in early embryo development occur.

Regarding OHSS evolution, this study shows that successful outpatient management of severe OHSS with antagonist treatment in the luteal phase is feasible and is associated with rapid regression of the syndrome. The efficiency of luteal GnRH antagonist is supported by an increasing number of patients with severe OHSS who have been successfully treated using the intervention in previous reports (Lainas *et al.*, 2007b, 2009a,b, 2012), as well as in the present study. Therefore, it is possible to propose GnRH antagonist administration in the luteal phase as tertiary management level of OHSS, in addition to the use of GnRH antagonist protocols for primary

Table VI Neonatal outcomes in singletons and twins born in the OHSS + antag and control groups.

Category	Singletons			Twins		
	OHSS + antag	Control	P	OHSS + antag	Control	P
Major congenital malformations, n (%)	0	3 (2.91)	1	0	0	1.00
Atrial and ventricular septal defect, n		1				
Hypospadias, n		1				
Aortic obstruction, n		1				
Duration of gestation (weeks) (mean ± SD)	37 ± 1.0	36.86 ± 2.41	0.922	36.75 ± 0.96	36.83 ± 2.51	0.951
Neonatal weight (g) (mean ± SD)	2750 ± 183.85	2780.85 ± 686.88	0.951	2303.75 ± 458.44	2636.72 ± 681.94	0.201

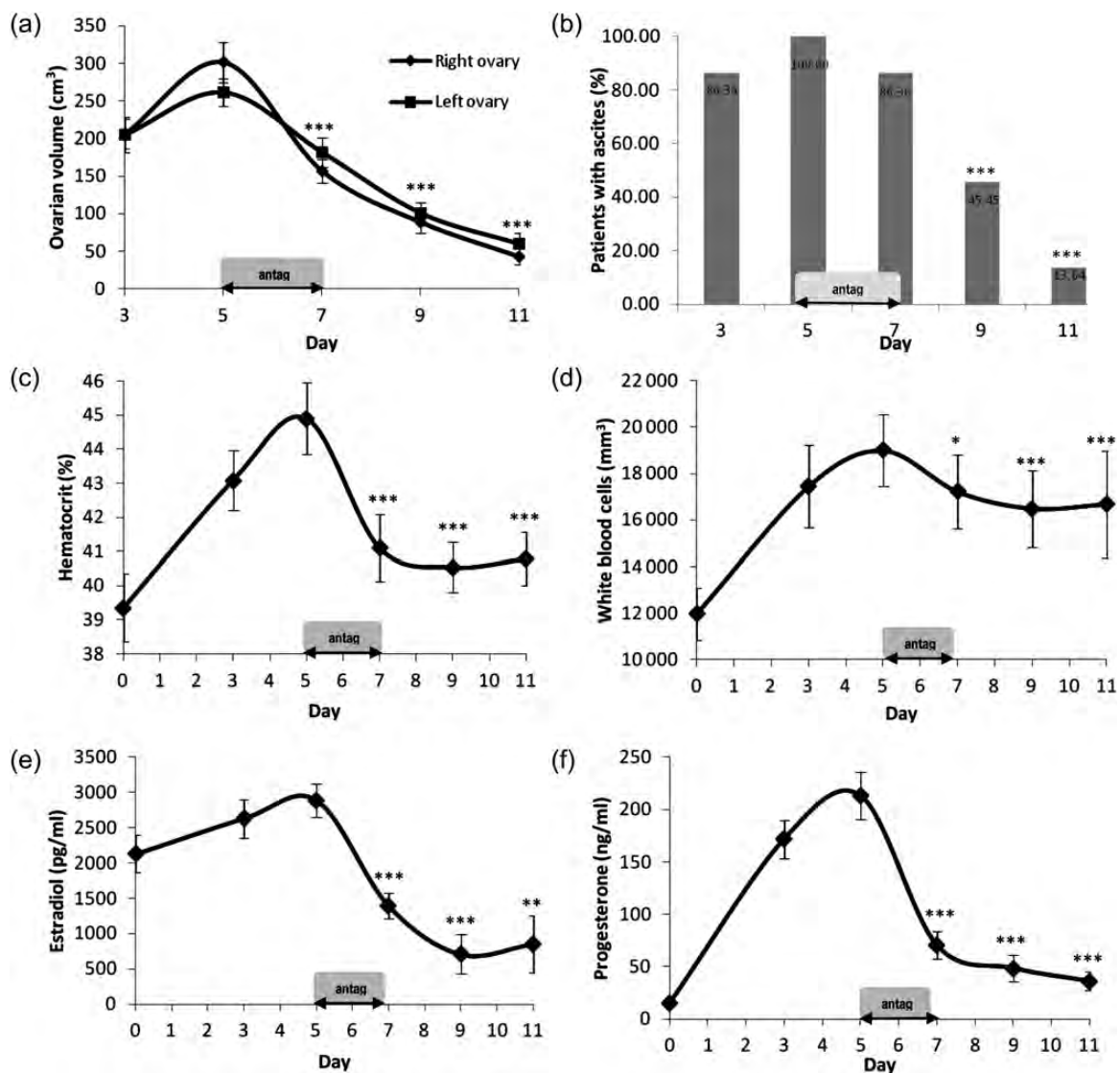


Figure 2 Monitoring of (a) ovarian volume, (b) ascites, (c) hematocrit, (d) white blood cells, (e) estradiol and (f) progesterone in patients with severe early OHSS who were administered luteal GnRH antagonist. Oocyte retrieval was performed on Day 0. GnRH antagonist was administered for 3 days, from Day 5 until and including Day 7 post-oocyte retrieval, as indicated by grey boxes on x-axis. Embryo transfer was performed on Day 5. Asterisks depict statistical significance compared with Day 5 (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Table VII New and promising strategies for the prevention of OHSS in high-risk patients.

Prevention level	Strategy	Studies	Advantages	Drawbacks
Primary	GnRH antagonist protocol	Al-Inany <i>et al.</i> (2011) Kolibianakis <i>et al.</i> (2006) Lainas <i>et al.</i> (2010) Kurzawa <i>et al.</i> (2008) Lainas <i>et al.</i> (2007a) Bahceci <i>et al.</i> (2005) Ragni <i>et al.</i> (2005) Hwang <i>et al.</i> (2004)	Significantly lower incidence of OHSS Similar pregnancy rates compared with long protocol Possibility to replace hCG with GnRH agonist for triggering final oocyte maturation Patient-friendly protocol Basic component of OHSS-free clinic (Devroey <i>et al.</i> , 2011)	Slow acceptance of antagonist protocols compared with the long protocol (Fauser and Devroey, 2005) due to: – Learning curve for use of antagonist protocols – Unsubstantiated fear of decline in pregnancy rates – Smaller flexibility in patient programming – Long experience with the use of the long protocol worldwide It was in 2011 when the latest Cochrane review by Al Inany <i>et al.</i> reversed the international skepticism against antagonist protocols
Secondary	GnRH agonist trigger with fresh transfer	Kolibianakis <i>et al.</i> (2005) Humaidan <i>et al.</i> (2005b) Orvieto <i>et al.</i> (2006)	Eliminates development of OHSS	Dramatically lower pregnancy rates Increased early pregnancy loss compared with triggering with hCG
	GnRH agonist trigger and freeze-all	Griesinger <i>et al.</i> (2007a,b, 2010, 2011) Manzanares <i>et al.</i> (2010) Kolibianakis <i>et al.</i> (2012)	Eliminates development of OHSS	Can be used only in antagonist protocol Cancellation of fresh embryo transfer and cryopreservation of all embryos Requires highly efficient embryology laboratory optimal freezing protocols
	GnRH agonist trigger and luteal low-dose hCG	Humaidan <i>et al.</i> (2006) Humaidan (2009) Humaidan <i>et al.</i> (2010a) Radesic and Tremellen (2011)	Eliminates development of OHSS Rescues luteal phase and allows fresh transfer following GnRH agonist trigger	Cancellation of embryo transfer may increase physical, psychological and financial burden of patients Can be used only in antagonist protocol Majority of data available in normal responders Limited evidence on inducing OHSS in high-risk patients Can be used only in antagonist protocol Limited data on the incidence of late OHSS 2 cases of late OHSS reported
	GnRH agonist trigger with aggressive luteal support	Babayof <i>et al.</i> (2006) Engmann <i>et al.</i> (2006, 2008)	Eliminates development of OHSS Rescues luteal phase and allows fresh transfer following GnRH agonist trigger	Recommended when peak estradiol levels ≥ 4000 pg/ml Can be used only in antagonist protocol Limited number of studies available
	Dual trigger with GnRH agonist and low-dose hCG	Griffin <i>et al.</i> (2012) Shapiro <i>et al.</i> (2008, 2011)	Similar pregnancy rates compared with triggering with hCG Higher implantation and pregnancy rates compared with agonist trigger Minimizes risk of severe OHSS	Can be used only in antagonist protocol Limited data on the incidence of late OHSS 1 case of late OHSS reported
	Dopamine agonist	Alvarez <i>et al.</i> (2007a,b) Busso <i>et al.</i> (2010)	Reduces incidence of moderate OHSS in high-risk patients May be combined with fresh embryo transfer	Does not reduce incidence of severe OHSS Poor tolerability of quaniolide at high-doses Insufficient evidence on neonatal outcomes due to small number of neonates Birth defects following quaniolide administration appear higher

Continued

Table VII Continued

Prevention level	Strategy	Studies	Advantages	Drawbacks
Tertiary	Luteal GnRH antagonist and freeze-all	Lainas et al. (2007b, 2009b, 2012)	Rapid regression of established severe early OHSS Outpatient management Significant improvement 2 days after antagonist administration Effective for both agonist and antagonist protocols Offers flexibility and allows the majority of high-risk patients 88.7% to proceed to embryo transfer if severe OHSS does not develop Allows fresh embryo transfer Rapid regression of established severe early OHSS High-pregnancy rates and birth of healthy offspring Birthweight and gestational age similar to controls No cases of late OHSS reported to date	Elective cryopreservation only in case of established severe early OHSS Cancellation of embryo transfer that may increase physical, psychological and financial burden of patients
	Luteal GnRH antagonist with fresh transfer	Lainas et al. (2009a, 2013, present study)		Limited number of patients receiving the intervention and babies born Should not be used in everyday clinical practice for all patients with severe early OHSS Should be used with caution by experienced practitioners

prevention, and the replacement of hCG with GnRH agonist for triggering final oocyte maturation for secondary prevention, as previously suggested (Griesinger, 2010) and outlined in Table VII.

It should be noted that, in our Unit, we routinely propose antagonist administration and total embryo cryopreservation, when other prevention methods have failed or have not been used leading to the development of severe OHSS. This method offers flexibility and minimizes unnecessary embryo transfer cancellations in the majority of high-risk patients who do not develop severe OHSS (88.7% of patients receiving low-dose hCG for triggering final oocyte maturation) as previously described (Lainas et al., 2012). The alternative, fresh embryo transfer combined with antagonist administration, should not be used in everyday clinical practice for the management of patients with severe early OHSS. This new intervention requires correct evaluation and grading of OHSS, and should be used with caution by experienced practitioners, after carefully deciding which patients can have fresh embryo transfer or cryopreservation, until the current data are supported by much larger trials with follow-up of the children born.

The concept of an OHSS free clinic begins with the choice of the proper protocol in high risk for OHSS patients (Fiedler and Ezcurra, 2012) at the level of primary prevention. An increasing amount of evidence suggests that the GnRH antagonist protocol should be the protocol of choice in these patients, as it has been consistently associated with significantly lower incidence of OHSS compared with the long protocol (Al-Inany and Aboulghar, 2002; Al-Inany et al., 2007, 2011; Lainas et al., 2010).

It may appear contradictory that some patients in the present study were treated with a long agonist protocol. However, the study period started in 2009, an era when the long protocol was still used in the majority of controlled ovarian stimulation protocols. It was only in May 2011 when the latest Cochrane review by Al-Inany et al. (2011) reversed the international skepticism against GnRH antagonist protocols. Also, the personal wish of patients/clinicians was taken into account; the worldwide use of GnRH antagonist protocols is currently estimated to be ~40% of analogue cycles. Moreover, our previous publication (Lainas et al., 2009b, 2012) showed that luteal GnRH antagonist administration is effective for the regression of severe OHSS in patients pre-treated not only with antagonist protocol, but also with a long agonist protocol.

In the present study, luteal GnRH antagonist administration was associated with rapid regression of severe early OHSS, shown by a significant decline in all ultrasound and laboratory parameters as early as 2 days following GnRH antagonist initiation, which continued until the end of the monitoring period for all 22 OHSS patients studied. No patients required hospitalization. A similar rapid regression of severe early OHSS following low-dose luteal GnRH antagonist administration has been previously reported (Lainas et al., 2007b, 2009a,b, 2012).

The absence of late OHSS in both patient groups, despite the elevated multiple pregnancy rates, may be related to the close monitoring of high-risk patients performed in the present study and the accurate diagnosis of early OHSS using our proposed strict classification system.

It was recently described that GnRH agonists and antagonists were administered in the luteal phase from the day of oocyte retrieval for a period of 7 days in order to prevent OHSS in high-risk patients (Fabregues et al., 2012). The incidence of moderate and severe OHSS in both study groups was similar to the controls and no patients were hospitalized, suggesting that luteal administration of GnRH analogues

does not reduce the incidence of OHSS. However, this study is only available as an abstract and includes a small number of patients, not offering specific OHSS incidence rates, classification criteria for OHSS, numbers of follicles and oocytes retrieved or other clinical parameters. Moreover, the study describes luteal GnRH analogue administration (starting on the day of oocyte retrieval) for the prevention of OHSS, and is therefore in a different context from our study, which describes luteal GnRH antagonist administration (starting on Day 5 post-oocyte retrieval) for the management/treatment of already established severe early OHSS.

It is hypothesized that GnRH antagonist administration intervenes in the pathophysiological pathway of OHSS by inducing luteolysis, as previously proposed (Lainas *et al.*, 2009a,b, 2012). Luteolysis results in the decline of secreted angiogenic ovarian factors associated with the OHSS, such as vascular endothelial growth factor (VEGF), and appears to be the key mechanism for OHSS regression (Kol, 2004).

There are a number of studies reporting luteolysis when GnRH antagonist is administered in the preceding luteal phase of IVF patients, prior to the onset of ovarian stimulation (Fanchin *et al.*, 2004; Friden and Nilsson, 2005; Humaidan *et al.*, 2005a; DiLuigi *et al.*, 2011; Garcia-Velasco *et al.*, 2012). However, the luteolytic effect of the GnRH antagonist in those cases was achieved mainly via a decrease in FSH and LH secretion. On the contrary, in our study it seems unlikely that the antagonist exerts a luteolytic effect by decreasing LH secretion, since LH concentrations are deeply suppressed in the luteal phase following ovarian stimulation, requiring luteal support (Tavaniotou and Devroey, 2006). It seems, instead, that the GnRH antagonist may have a direct action on the human ovary. This hypothesis is supported by the presence of GnRH receptors in the human ovary, among other extrapituitary tissues (Engel *et al.*, 2005; Choi *et al.*, 2006; Cheung and Wong, 2008; Yu *et al.*, 2011). Indeed, GnRH antagonists have been shown to inhibit the expression of VEGF, the primary factor responsible for OHSS development, in human granulosa–luteal cell cultures (Asimakopoulos *et al.*, 2006).

In conclusion, low-dose luteal GnRH antagonist administration in women with severe early OHSS was associated with a favourable IVF outcome, comparable to control high-risk patients without severe OHSS and not receiving the intervention. This protocol may be used with caution for the outpatient management of severe early OHSS without compromising pregnancy and live birth rates. In addition, the fact that all infants born following the intervention were healthy with no congenital abnormalities, similar neonatal weight and similar duration of gestation compared with controls, suggests that low-dose luteal GnRH antagonist administration during the peri-implantation period may be safe, although much larger studies with follow-up of the children born are required.

Authors' roles

G.T.L. participated in the study design, acquisition and analysis of data and writing of the manuscript. E.M.K. participated in the analysis and interpretation of data, writing and revision of the manuscript. I.A.S. participated in the acquisition, analysis and interpretation of data, writing and revision of the manuscript, and performed embryology work. I.Z.Z. and G.K.P. participated in the interpretation of data and performed clinical work. T.G.L. originally conceived and generally supervised the study, participated in study design, acquisition, analysis

and interpretation of data and the writing and revision of the manuscript, and performed clinical work. B.C.T. participated in the interpretation of data and revision of the manuscript and had overall supervision. All authors read and approved the final manuscript.

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Conflict of interest

None declared.

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