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# 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 $\geq 11$ 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 $\pm$ 0.90 weeks versus 36.88 $\pm$ 2.38 weeks) and neonatal weight (2392.73 $\pm$ 427.04 versus 2646.56 $\pm$ 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.

**STUDY FUNDING/COMPETING INTEREST(S):** No external funding was sought for this study and the authors have no conflict of interest to declare.

**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 $\geq$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/mm$^3$, 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-Systems GmbH, Germany) from Day 5 until the 7th week of gestation and 4500 anti-Xa IU (0.45 ml) tinzaparin sodium (Innohep; LEO Pharmacutica 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, $E_2$ 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 $E_2$ 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 diff$^{TM}$ 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)] [Ht (%) = RBC × 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 ± SD) was 66.1 ± 12.6 kg and the GnRH antagonist daily dose administered per kg (mean ± 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,

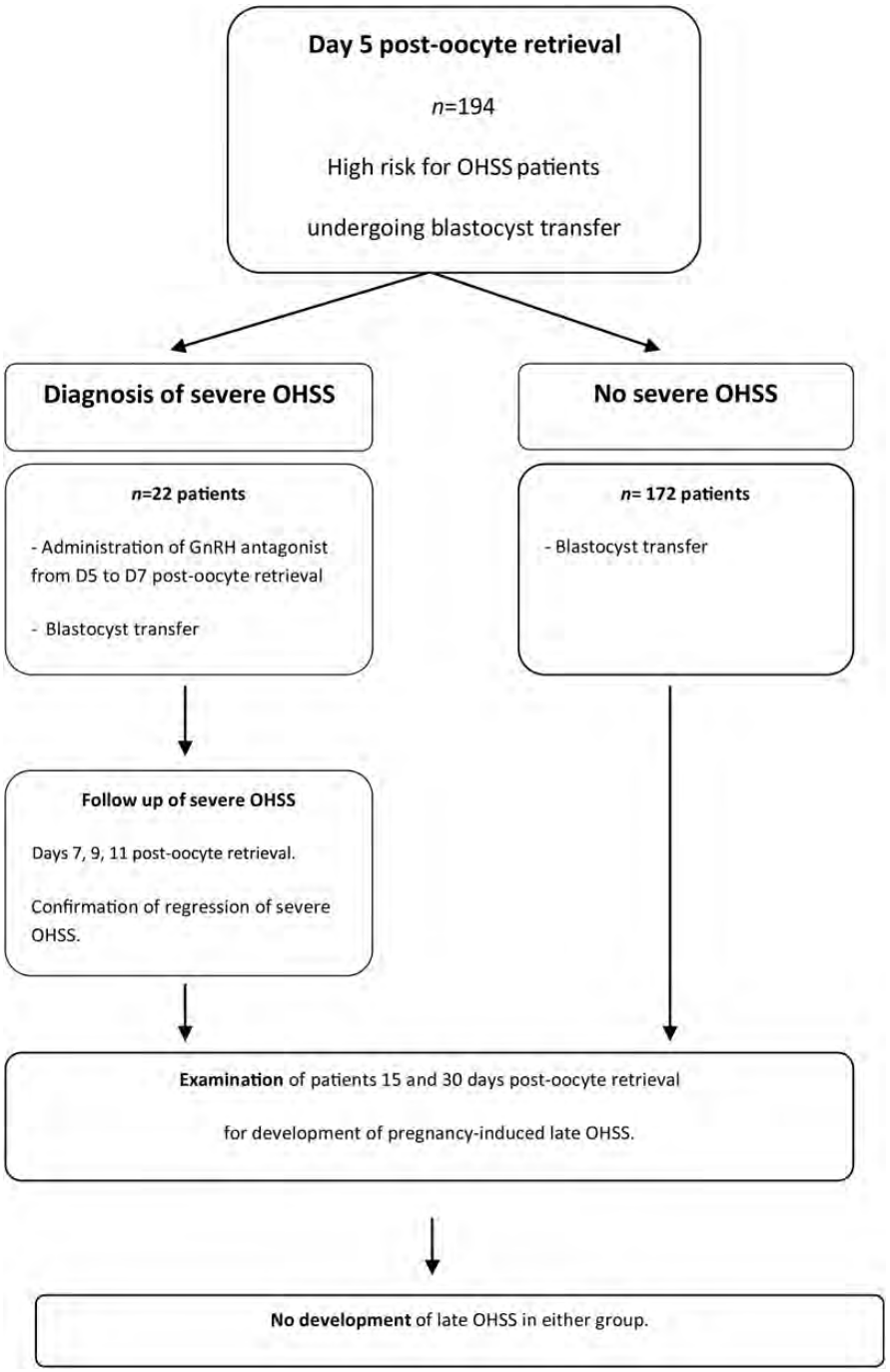


**Figure 1** Flow chart of the study design.

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 ± 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 |

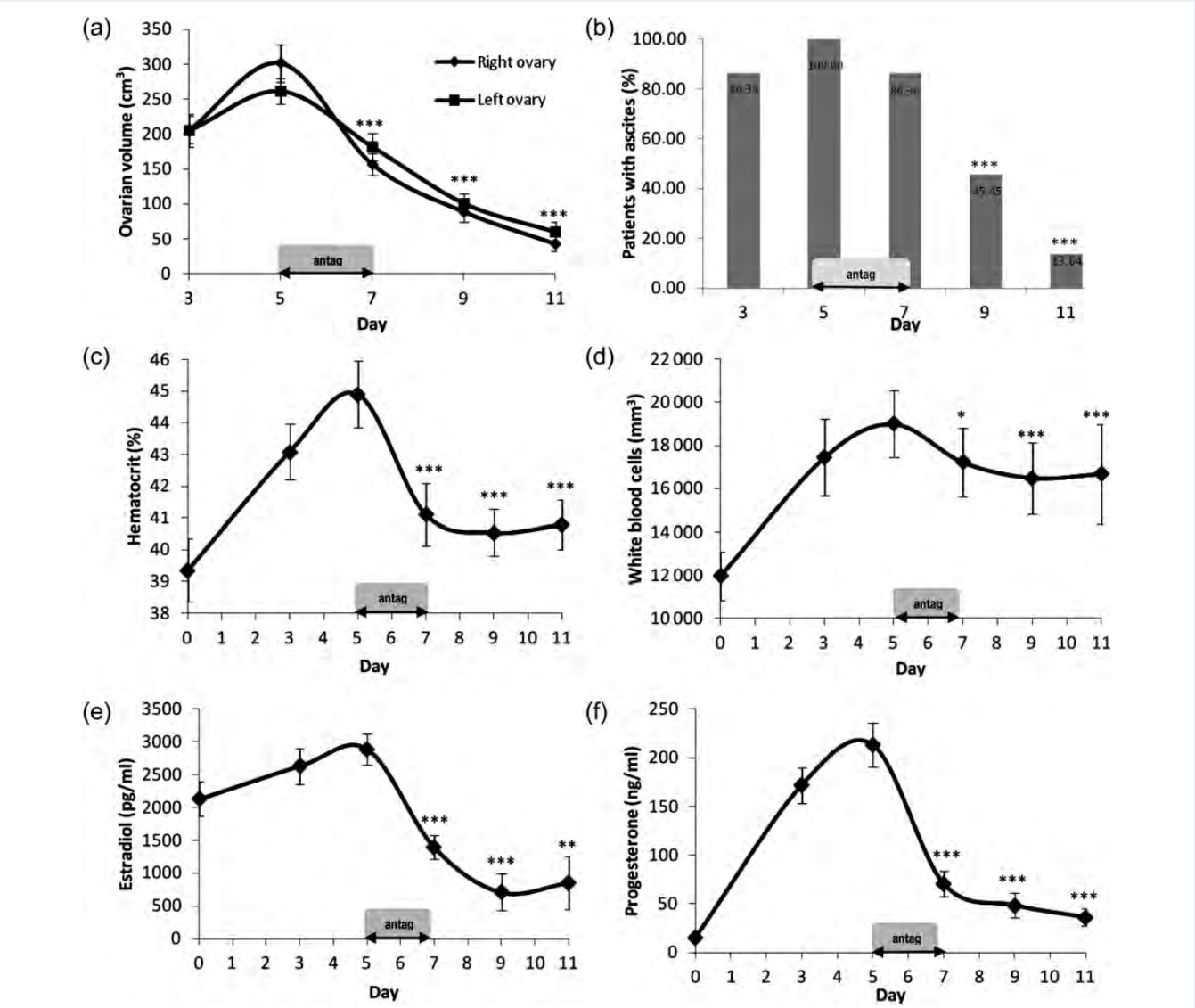


**Figure 2** Monitoring of (**a**) ovarian volume, (**b**) ascites, (**c**) hematocrit, (**d**) white blood cells, (**e**) estradiol and (**f**) progesterone in patients with severe early OHSS who were administered luteal GnRH antagonist. Oocyte retrieval was performed on Day 0. GnRH antagonist was administered 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 *et al.* (2011)<br>Kolibianakis *et al.* (2006)<br>Lainas *et al.* (2010)<br>Kurzawa *et al.* (2008)<br>Lainas *et al.* (2007a)<br>Bahceci *et al.* (2005)<br>Ragni *et al.* (2005)<br>Hwang *et al.* (2004) | Significantly lower incidence of OHSS<br>Similar pregnancy rates compared with long protocol<br>Possibility to replace hCG with GnRH agonist for triggering final oocyte maturation<br>Patient-friendly protocol<br>Basic component of OHSS-free clinic (Devroey *et al.*, 2011) | Slow acceptance of antagonist protocols compared with the long protocol (Fauser and Devroey, 2005) due to:<br>– Learning curve for use of antagonist protocols<br>– Unsubstantiated fear of decline in pregnancy rates<br>– Smaller flexibility in patient programming<br>– Long experience with the use of the long protocol worldwide<br>It was in 2011 when the latest Cochrane review by Al Inany *et al.* reversed the international skepticism against antagonist protocols |
| Secondary | GnRH agonist trigger with fresh transfer | Kolibianakis *et al.* (2005)<br>Humaidan *et al.* (2005b)<br>Orvieto *et al.* (2006) | Eliminates development of OHSS | Dramatically lower pregnancy rates<br>Increased early pregnancy loss compared with triggering with hCG<br>Can be used only in antagonist protocol |
| | GnRH agonist trigger and freeze-all | Griesinger *et al.* (2007a,b, 2010, 2011)<br>Manzanares *et al.* (2010)<br>Kolibianakis *et al.* (2012) | Eliminates development of OHSS | Cancellation of fresh embryo transfer and cryopreservation of all embryos<br>Requires highly efficient embryology laboratory optimal freezing protocols<br>Cancellation of embryo transfer may increase physical, psychological and financial burden of patients<br>Can be used only in antagonist protocol |
| | GnRH agonist trigger and luteal low-dose hCG | Humaidan *et al.* (2006)<br>Humaidan (2009)<br>Humaidan *et al.* (2010a)<br>Radesic and Tremellen (2011) | Eliminates development of OHSSRescues luteal phase and allows fresh transfer following GnRH agonist trigger | Majority of data available in normal responders<br>Limited evidence on inducing OHSS in high-risk patients<br>Can be used only in antagonist protocol<br>Limited data on the incidence of late OHSS 2 cases of late OHSS reported |
| | GnRH agonist trigger with aggressive luteal support | Babayof *et al.* (2006)<br>Engmann *et al.* (2006, 2008) | Eliminates development of OHSS<br>Rescues luteal phase and allows fresh transfer following GnRH agonist trigger<br>Similar pregnancy rates compared with triggering with hCG | Recommended when peak estradiol levels $\geq$4000 pg/ml<br>Can be used only in antagonist protocol<br>Limited number of studies available |
| | Dual trigger with GnRH agonist and low-dose hCG | Griffin *et al.* (2012)<br>Shapiro *et al.* (2008, 2011) | Higher implantation and pregnancy rates compared with agonist trigger Minimizes risk of severe OHSS | Can be used only in antagonist protocol<br>Limited data on the incidence of late OHSS 1 case of late OHSS reported |
| | Dopamine agonist | Alvarez *et al.* (2007a,b)<br>Busso *et al.* (2010) | Reduces incidence of moderate OHSS in high-risk patients<br>May be combined with fresh embryo transfer | Does not reduce incidence of severe OHSS<br>Poor tolerability of quanigolide at high-doses<br>Insufficient evidence on neonatal outcomes due to small number of neonates<br>Birth defects following quanigolide 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<br>Outpatient management<br>Significant improvement 2 days after antagonist administration<br>Effective for both agonist and antagonist protocols<br>Offers flexibility and allows the majority of high-risk patients 88.7% to proceed to embryo transfer if severe OHSS does not develop | Elective cryopreservation only in case of established severe early OHSS<br>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) | Allows fresh embryo transfer<br>Rapid regression of established severe early OHSS<br>High-pregnancy rates and birth of healthy offspring<br>Birthweight and gestational age similar to controls<br>No cases of late OHSS reported to date | Limited number of patients receiving the intervention and babies born<br>Should not be used in everyday clinical practice for all patients with severe early OHSS<br>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.

## References

Abdalla HI, Ah-Moye M, Brinsden P, Howe DL, Okonofua F, Craft I. The effect of the dose of human chorionic gonadotropin and the type of gonadotropin stimulation on oocyte recovery rates in an *in vitro* fertilization program. *Fertil Steril* 1987;**48**:958–963.

Abramov Y, Elchalal U, Schenker JG. Obstetric outcome of *in vitro* fertilized pregnancies complicated by severe ovarian hyperstimulation syndrome: a multicenter study. *Fertil Steril* 1998;**70**:1070–1076.

Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. *Hum Reprod* 2002;**17**:874–885.

Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception: a Cochrane review. *Reprod Biomed Online* 2007;**14**:640–649.

Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, Abou-Setta AM. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011;**5**:CD001750.

Alvarez C, Alonso-Muriel I, Garcia G, Crespo J, Bellver J, Simon C, Pellicer A. Implantation is apparently unaffected by the dopamine agonist Cabergoline when administered to prevent ovarian hyperstimulation syndrome in women undergoing assisted reproduction treatment: a pilot study. *Hum Reprod* 2007a;**22**:3210–3214.

Alvarez C, Marti-Bonmati L, Novella-Maestre E, Sanz R, Gomez R, Fernandez-Sanchez M, Simon C, Pellicer A. Dopamine agonist cabergoline reduces hemoconcentration and ascites in hyperstimulated women undergoing assisted reproduction. *J Clin Endocrinol Metab* 2007b;**92**:2931–2937.

Asimakopoulos B, Nikolettos N, Nehls B, Diedrich K, Al-Hasani S, Metzen E. Gonadotropin-releasing hormone antagonists do not influence the secretion of steroid hormones but affect the secretion of vascular endothelial growth factor from human granulosa luteinized cell cultures. *Fertil Steril* 2006;**86**:636–641.

Babayof R, Margalioth EJ, Huleihel M, Amash A, Zylber-Haran E, Gal M, Brooks B, Mimoni T, Eldar-Geva T. Serum inhibin A, VEGF and TNFalpha levels after triggering oocyte maturation with GnRH agonist compared with HCG in women with polycystic ovaries undergoing IVF treatment: a prospective randomized trial. *Hum Reprod* 2006; **21**:1260–1265.

Bahceci M, Ulug U, Ben-Shlomo I, Erden HF, Akman MA. Use of a GnRH antagonist in controlled ovarian hyperstimulation for assisted conception in women with polycystic ovary disease: a randomized, prospective, pilot study. *J Reprod Med* 2005;**50**:84–90.

Boerrigter PJ, de Bie JJ, Mannaerts BMJL, van Leeuwen BP, Passier-Timmermans DPJ. Obstetrical and neonatal outcome after controlled ovarian stimulation for IVF using the GnRH antagonist ganirelix. *Hum Reprod* 2002;**17**:2027–2034.

Bonduelle M, Liebaers I, Deketelaere V, Derde M-P, Camus M, Devroey P, Van Steirteghem A. Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). *Hum Reprod* 2002;**17**:671–694.

Bonduelle M, Oberye J, Mannaerts B, Devroey P. Large prospective, pregnancy and infant follow-up trial assures the health of 1000 fetuses conceived after treatment with the GnRH antagonist ganirelix during controlled ovarian stimulation. *Hum Reprod* 2010;**25**:1433–1440.

Bonilla-Musoles FM, Raga F, Castillo JC, Sanz M, Dolz M, Osborne N. High doses of GnRH antagonists are efficient in the management of severe ovarian hyperstimulation syndrome. *Clin Exp Obstet Gynecol* 2009;**36**:78–81.

Busso C, Fernandez-Sanchez M, Garcia-Velasco JA, Landeras J, Ballesteros A, Munoz E, Gonzalez S, Simon C, Arce J-C, Pellicer A. The non-ergot derived dopamine agonist quinagolide in prevention of early ovarian hyperstimulation syndrome in IVF patients: a randomized, double-blind, placebo-controlled trial. *Hum Reprod* 2010;**25**:995–1004.

Cheung LW, Wong AS. Gonadotropin-releasing hormone: GnRH receptor signaling in extrapituitary tissues. *FEBS J* 2008;**275**:5479–5495.

Choi J-H, Gilks CB, Auersperg N, Leung PCK. Immunolocalization of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and Type I GnRH receptor during follicular development in the human ovary. *J Clin Endocrinol Metab* 2006;**91**:4562–4570.

Das C, Talwar GP. Pregnancy-terminating action of a luteinizing hormone-releasing hormone agonist D-Ser(But)6desGly10ProEA in baboons. *Fertil Steril* 1983;**39**:218–223.

Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. *Hum Reprod* 2011;**26**:2593–2597.

DiLuigi AJ, Engmann L, Schmidt DW, Benadiva CA, Nulsen JC. A randomized trial of microdose leuprolide acetate protocol versus luteal phase ganirelix protocol in predicted poor responders. *Fertil Steril* 2011;**95**:2531–2533.

Eley RM. The effect of LHRH analogs on pregnancy in the baboon. *Contraception* 1987;**35**:389–393.

Engel JB, Riethmuller-Winzen H, Diedrich K. Extrapituitary effects of GnRH antagonists in assisted reproduction: a review. *Reprod Biomed Online* 2005;**10**:230–234.

Engmann L, Siano L, Schmidt D, Nulsen J, Maier D, Benadiva C. GnRH agonist to induce oocyte maturation during IVF in patients at high risk of OHSS. *Reprod Biomed Online* 2006;**13**:639–644.

Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing *in vitro* fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. *Fertil Steril* 2008;**89**:84–91.

Fabregues F, Iraola A, Casals G, Peralta S, Creus M, Balasch J. Evaluation of GnRH agonists and antagonists in tertiary prevention of OHSS. Clinical, neurohormonal and vasoactive effects in the luteal phase in high risk patients. *Hum Reprod* 2012;**27**:ii26–ii28.

Fanchin R, Castelo Branco A, Kadoch IJ, Hosny G, Bagirova M, Frydman R. Premenstrual administration of gonadotropin-releasing hormone antagonist coordinates early antral follicle sizes and sets up the basis for an innovative concept of controlled ovarian hyperstimulation. *Fertil Steril* 2004;**81**:1554–1559.

Fiedler K, Ezcurra D. Predicting and preventing ovarian hyperstimulation syndrome (OHSS): the need for individualized not standardized treatment. *Reprod Biol Endocrinol* 2012;**10**:32.

Fraser HM, Nestor JJ Jr, Vickery BH. Suppression of luteal function by a luteinizing hormone-releasing hormone antagonist during the early luteal phase in the stumptailed macaque monkey and the effects of subsequent administration of human chorionic gonadotropin. *Endocrinology* 1987;**121**:612–618.

Friden BE, Nilsson L. Gonadotrophin-releasing hormone-antagonist luteolysis during the preceding mid-luteal phase is a feasible protocol in ovarian hyperstimulation before *in vitro* fertilization. *Acta Obstet Gynecol Scand* 2005;**84**:812–816.

Garcia-Velasco JA, Kupesic S, Pellicer A, Bourgain C, Simon C, Mrazek M, Devroey P, Arce J-C. Follicular and endocrine profiles associated with different GnRH-antagonist regimens: a randomized controlled trial. *Reprod Biomed Online* 2012;**24**:153–162.

Golan A, Ron-el R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv* 1989;**44**:430–440.

Griesinger G. Ovarian hyperstimulation syndrome prevention strategies: use of gonadotropin-releasing hormone antagonists. *Semin Reprod Med* 2010;**28**:493–499.

Griesinger G, Kolibianakis EM, Papanikolaou EG, Diedrich K, Van Steirteghem A, Devroey P, Ejdrup Bredkjaer H, Humaidan P. Triggering of final oocyte maturation with gonadotropin-releasing hormone agonist or human chorionic gonadotropin. Live birth after frozen-thawed embryo replacement cycles. *Fertil Steril* 2007a;**88**:616–621.

Griesinger G, von Otte S, Schroer A, Ludwig AK, Diedrich K, Al-Hasani S, Schultze-Mosgau A. Elective cryopreservation of all pronuclear oocytes after GnRH agonist triggering of final oocyte maturation in patients at risk of developing OHSS: a prospective, observational proof-of-concept study. *Hum Reprod* 2007b;**22**:1348–1352.

Griesinger G, Berndt H, Schultz L, Depenbusch M, Schultze-Mosgau A. Cumulative live birth rates after GnRH-agonist triggering of final oocyte maturation in patients at risk of OHSS: a prospective, clinical cohort study. *Eur J Obstet Gynecol Reprod Biol* 2010;**149**:190–194.

Griesinger G, Schultz L, Bauer T, Broessner A, Frambach T, Kissler S. Ovarian hyperstimulation syndrome prevention by gonadotropin-releasing hormone agonist triggering of final oocyte maturation in a gonadotropin-releasing hormone antagonist protocol in combination with a 'freeze-all' strategy: a prospective multicentric study. *Fertil Steril* 2011;**95**:2029–2033.

Griffin D, Benadiva C, Kummer N, Budinetz T, Nulsen J, Engmann L. Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders. *Fertil Steril* 2012;**97**:1316–1320.

Humaidan P. Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose HCG: a pilot study. *Reprod Biomed Online* 2009;**18**:630–634.

Humaidan P, Bungum L, Bungum M, Hald F, Agerholm I, Blaabjerg J, Yding Andersen C, Lindenberg S. Reproductive outcome using a GnRH antagonist (cetrorelix) for luteolysis and follicular synchronization in poor responder IVF/ICSI patients treated with a flexible GnRH antagonist protocol. *Reprod Biomed Online* 2005a;**11**:679–684.

Humaidan P, Ejdrup Bredkjaer H, Bungum L, Bungum M, Grondahl ML, Westergaard L, Yding Andersen C. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study 10.1093/humrep/deh765. *Hum Reprod* 2005b;**20**:1213–1220.

Humaidan P, Bungum L, Bungum M, Andersen CY. Rescue of corpus luteum function with peri-ovulatory HCG supplementation in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with a GnRH agonist: a pilot study. *Reprod Biomed Online* 2006;**13**:173–178.

Humaidan P, Ejdrup Bredkjaer H, Westergaard LG, Yding Andersen C. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing

hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. *Fertil Steril* 2010a;**93**:847–854.

Humaidan P, Quartarolo J, Papanikolaou EG. Preventing ovarian hyperstimulation syndrome: guidance for the clinician. *Fertil Steril* 2010b;**94**:389–400.

Hwang JL, Seow KM, Lin YH, Huang LW, Hsieh BC, Tsai YL, Wu GJ, Huang SC, Chen CY, Chen PH *et al*. Ovarian stimulation by concomitant administration of cetrorelix acetate and HMG following Diane-35 pre-treatment for patients with polycystic ovary syndrome: a prospective randomized study. *Hum Reprod* 2004;**19**:1993–2000.

Kang IS, Kuehl TJ, Siler-Khodr TM. Effect of treatment with gonadotropin-releasing hormone analogues on pregnancy outcome in the baboon. *Fertil Steril* 1989;**52**:846–853.

Klemmt PAB, Liu F, Carver JG, Jones C, Brosi D, Adamson J, Mardon HJ, McVeigh E. Effects of gonadotrophin releasing hormone analogues on human endometrial stromal cells and embryo invasion *in vitro*. *Hum Reprod* 2009;**24**:2187–2192. 10.1093/humrep/dep181.

Kol S. Embryo implantation and GnRH antagonists: GnRH antagonists in ART: lower embryo implantation? *Hum Reprod* 2000;**15**:1881–1882.

Kol S. Luteolysis induced by a gonadotropin-releasing hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. *Fertil Steril* 2004;**81**:1–5.

Kolibianakis EM, Schultze-Mosgau A, Schroer A, van Steirteghem A, Devroey P, Diedrich K, Griesinger G. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. *Hum Reprod* 2005;**20**:2887–2892.

Kolibianakis EM, Collins J, Tarlatzis BC, Devroey P, Diedrich K, Griesinger G. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. *Hum Reprod Update* 2006;**12**:651–671.

Kolibianakis EM, Papanikolaou EG, Tournaye H, Camus M, Van Steirteghem AC, Devroey P. Triggering final oocyte maturation using different doses of human chorionic gonadotropin: a randomized pilot study in patients with polycystic ovary syndrome treated with gonadotropin-releasing hormone antagonists and recombinant follicle-stimulating hormone. *Fertil Steril* 2007;**88**:1382–1388.

Kolibianakis EM, Kyrou D, Venetis CA, Sfontouris I, Lainas TG, Tarlatzis BC. Triggering final oocyte maturation with GnRH agonist in patients with polycystic ovaries undergoing IVF. *Fertil Steril* 2012; **98**:S259.

Kurzawa R, Ciepiela P, Baczkowski T, Safranow K, Brelik P. Comparison of embryological and clinical outcome in GnRH antagonist vs. GnRH agonist protocols for *in vitro* fertilization in PCOS non-obese patients. A prospective randomized study. *J Assist Reprod Genet* 2008; **25**:365–374.

Lainas TG, Petsas GK, Zorzovilis IZ, Iliadis GS, Lainas GT, Cazlaris HE, Kolibianakis EM. Initiation of GnRH antagonist on Day 1 of stimulation as compared to the long agonist protocol in PCOS patients. A randomized controlled trial: effect on hormonal levels and follicular development. *Hum Reprod* 2007a;**22**:1540–1546.

Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT, Kolibianakis EM. Management of severe early ovarian hyperstimulation syndrome by re-initiation of GnRH antagonist. *Reprod Biomed Online* 2007b;**15**:408–412.

Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT, Alexopoulou E, Kolibianakis EM. Live births after management of severe OHSS by GnRH antagonist administration in the luteal phase. *Reprod Biomed Online* 2009a;**19**:789–795.

Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT, Iliadis GS, Kolibianakis EM. Management of severe OHSS using GnRH antagonist and blastocyst cryopreservation in PCOS patients treated with long protocol. *Reprod Biomed Online* 2009b;**18**:15–20.

Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT, Alexopoulou E, Kolibianakis EM. Flexible GnRH antagonist protocol versus GnRH agonist long protocol in patients with polycystic ovary syndrome treated for IVF: a prospective randomised controlled trial (RCT). *Hum Reprod* 2010;**25**:683–689.

Lainas G, Kolibianakis E, Sfontouris I, Zorzovilis I, Petsas G, Tarlatzi T, Tarlatzis B, Lainas T. Outpatient management of severe early OHSS by administration of GnRH antagonist in the luteal phase: an observational cohort study. *Reprod Biol Endocrinol* 2012;**10**:69.

Langman J. (ed.) *Medical Embryology*. Baltimore: Williams & Wilkins, 1981.

Lee HJ, Snegovskikh VV, Park JS, Foyouzi N, Han KT, Hodgson EJ, Guller S, Norwitz ER. Role of GnRH-GnRH receptor signaling at the maternal–fetal interface. *Fertil Steril* 2010;**94**:2680–2687.

Ludwig M, RiethmΓÓller-Winzen H, Felberbaum RE, Olivennes F, Albano C, Devroey P, Diedrich K. Health of 227 children born after controlled ovarian stimulation for *in vitro* fertilization using the luteinizing hormoneβ€"releasing hormone antagonist cetrorelix. *Fertil Steril* 2001;**75**:18–22.

Mais V, Kazer RR, Cetel NS, Rivier J, Vale W, Yen SS. The dependency of folliculogenesis and corpus luteum function on pulsatile gonadotropin secretion in cycling women using a gonadotropin-releasing hormone antagonist as a probe. *J Clin Endocrinol Metab* 1986;**62**:1250–1255.

Mannaerts B, Gordon K. Embryo implantation and GnRH antagonists: GnRH antagonists do not activate the GnRH receptor. *Hum Reprod* 2000;**15**:1882–1883.

Manzanares MA, GI$^{3}$mez-Palomares JL, Ricciarelli E, HernΓńdez ER. Triggering ovulation with gonadotropin-releasing hormone agonist in *in vitro* fertilization patients with polycystic ovaries does not cause ovarian hyperstimulation syndrome despite very high estradiol levels. *Fertil Steril* 2010;**93**:1215–1219.

Navot D, Bergh PA, Laufer N. Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. *Fertil Steril* 1992;**58**:249–261.

Orvieto R, Rabinson J, Meltzer S, Zohav E, Anteby E, Homburg R. Substituting HCG with GnRH agonist to trigger final follicular maturation—a retrospective comparison of three different ovarian stimulation protocols. *Reprod Biomed Online* 2006;**13**:198–201.

Papanikolaou EG, Tournaye H, Verpoest W, Camus M, Vernaeve V, Van Steirteghem A, Devroey P. Early and late ovarian hyperstimulation syndrome: early pregnancy outcome and profile. *Hum Reprod* 2005; **20**:636–641.

Papanikolaou EG, Humaidan P, Polyzos NP, Tarlatzis B. Identification of the high-risk patient for ovarian hyperstimulation syndrome. *Semin Reprod Med* 2010;**28**:458–462.

Papanikolaou E, Humaidan P, Polyzos N, Kalantaridou S, Kol S, Benadiva C, Tournaye H, Tarlatzis B. New algorithm for OHSS prevention. *Reprod Biol Endocrinol* 2011;**9**:147.

Pau E, Alonso-Muriel I, Gomez R, Novella E, Ruiz A, Garcia-Velasco JA, Simon C, Pellicer A. Plasma levels of soluble vascular endothelial growth factor receptor-1 may determine the onset of early and late ovarian hyperstimulation syndrome. *Hum Reprod* 2006; **21**:1453–1460.

Radesic B, Tremellen K. Oocyte maturation employing a GnRH agonist in combination with low-dose hCG luteal rescue minimizes the severity of ovarian hyperstimulation syndrome while maintaining excellent pregnancy rates. *Hum Reprod* 2011;**26**:3437–3442.

Ragni G, Vegetti W, Riccaboni A, Engl B, Brigante C, Crosignani PG. Comparison of GnRH agonists and antagonists in assisted reproduction cycles of patients at high risk of ovarian hyperstimulation syndrome. *Hum Reprod* 2005;**20**:2421–2455.

Raziel A, Friedler S, Schachter M, Strassburger D, Mordechai E, Ron-El R. Increased early pregnancy loss in IVF patients with severe ovarian hyperstimulation syndrome. *Hum Reprod* 2002;**17**:107–110.

Raziel A, Schachter M, Friedler S, Ron-El R. Outcome of IVF pregnancies following severe OHSS. *Reprod Biomed Online* 2009;**19**:61–65.

Rizk B, Aboulghar MA. Classification, pathophysiology and management of ovarian hyperstimulation syndrome. In: Brinsden P (ed.). *In-vitro Fertilization and Assisted Reproduction*. New York, London: The Parthenon Publishing Group, 1999;131–155.

Schenker J, Weinstein D. Ovarian hyperstimulation syndrome: a current survey. *Fertil Steril* 1978;**30**:255–268.

Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Thomas S. Gonadotropin-releasing hormone agonist combined with a reduced dose of human chorionic gonadotropin for final oocyte maturation in fresh autologous cycles of *in vitro* fertilization. *Fertil Steril* 2008; **90**:231–233.

Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Comparison of 'triggers' using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. *Fertil Steril* 2011;**95**:2715–2717.

Siler-Khodr TM, Khodr GS, Vickery BH, Nestor JJ Jr. Inhibition of hCG, alpha hCG and progesterone release from human placental tissue *in vitro* by a GnRH antagonist. *Life Sci* 1983;**32**:2741–2745.

Siler-Khodr TM, Kuehl TJ, Vickery BH. Effects of a gonadotropin-releasing hormone antagonist on hormonal levels in the pregnant baboon and on fetal outcome. *Fertil Steril* 1984;**41**:448–454.

Siler-Khodr TM, Khodr GS, Rhode J, Vickery BH, Nestor JJ Jr. Gestational age-related inhibition of placental hCG, alpha hCG and steroid hormone release *in vitro* by a GnRH antagonist. *Placenta* 1987;**8**:1–14.

Tavaniotou A, Devroey P. Luteal hormonal profile of oocyte donors stimulated with a GnRH antagonist compared with natural cycles. *Reprod Biomed Online* 2006;**13**:326–330.

The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;**19**:41–47.

Tug N, Uslu U, Cumbul A, Eyuboglu S, Cam C, Karateke A, Yilmaz B. Effects of the gonadotropin-releasing hormone antagonist cetrorelix in the early postimplantation period on rat pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2011;**155**:166–170.

Virolainen JV, Love RJ, Tast A, Peltoniemi OA. Effect of a gonadotrophin-releasing hormone antagonist on luteinising hormone secretion and early pregnancy in gilts. *Reprod Fertil Dev* 2003;**15**:451–9.

Yu B, Ruman J, Christman G. The role of peripheral gonadotropin-releasing hormone receptors in female reproduction. *Fertil Steril* 2011; **95**:465–473.