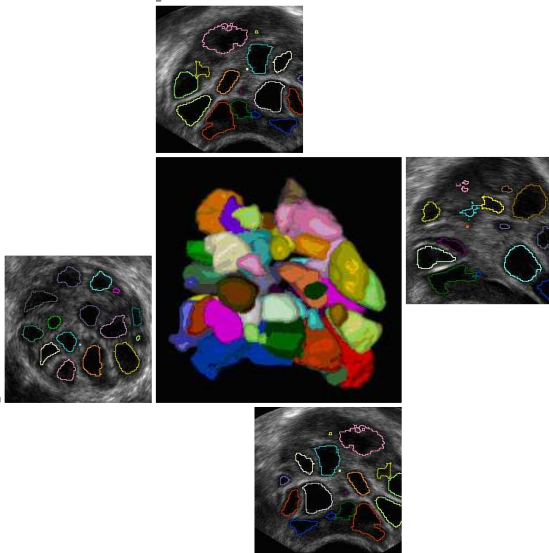


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Universitat Autònoma de Barcelona

# **GNRH ANTAGONISTS IN OOCYTE DONOR CYCLES: THE KEY TO SAFE, SIMPLE AND EFFICIENT STIMULATION PROTOCOLS**



**DOCTORAL THESIS**  
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Gynaecology and Paediatrics  
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**Ph.D. course (2007-2010)**

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*Bellaterra 2010*



# **GNRH ANTAGONISTS IN OOCYTE DONOR CYCLES: THE KEY TO SAFE, SIMPLE AND EFFICIENT STIMULATION PROTOCOLS**

Doctoral thesis presented by

**DR. DANIEL BODRI**

in order to obtain a

**Ph.D. degree**

in the doctoral programme of the  
Department of Gynaecology and Paediatrics  
and Preventive Medicine of the  
Autonomous University of Barcelona

**Dr. Daniel BODRI**

*Bellaterra, 2010*



## **Acknowledgments**

To my family (*Ildikó, Lotti and Cherry*)

To Dr. Juan José Guillén



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# 1. INTRODUCTION

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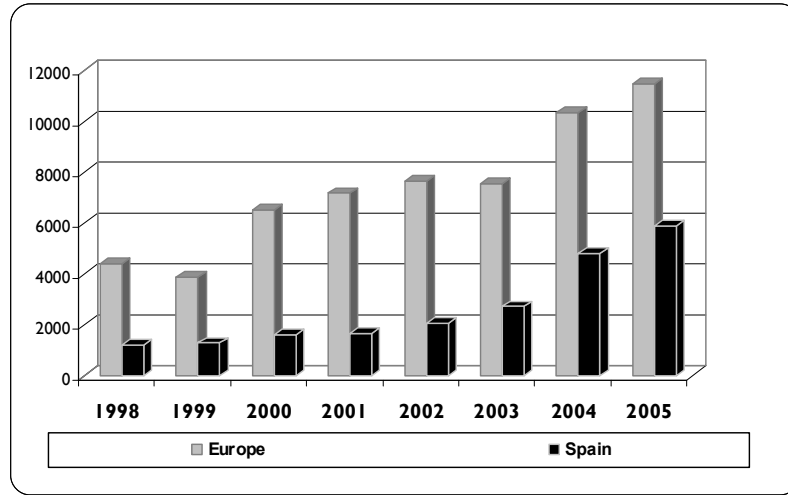
## **1.1. Oocyte donation and its worldwide evolution**

Oocyte donation was first described in 1984 (Lutjen et al. 1984) when an ongoing pregnancy with a birth of a healthy child was obtained in a patient with primary ovarian failure. During the next 25 year-period the indications of this treatment option widened considerably; it was successfully used in patients with primary ovarian failure, after repeated IVF failures, with previous low response, reduced ovarian reserve, for the prevention of genetic diseases or in patients with recurrent miscarriages. Currently oocyte donation is expected to give the highest pregnancy rates among all available assisted reproduction techniques (ART).

Its widened application brought about a continuous increase in the number of oocyte donation cycles all over the world. Official ART registries give an insight into the yearly evolution of oocyte donation cycles. The registry of the European Society of Human Reproduction and Embryology (ESHRE) reports data on oocyte donation since 1998. During an eight-year period a 2.6-fold increase was observed (Figure 1). This increase was of course not uniform in all European countries and is mainly due to the increasing activity of countries where gamete donation is legally permitted.

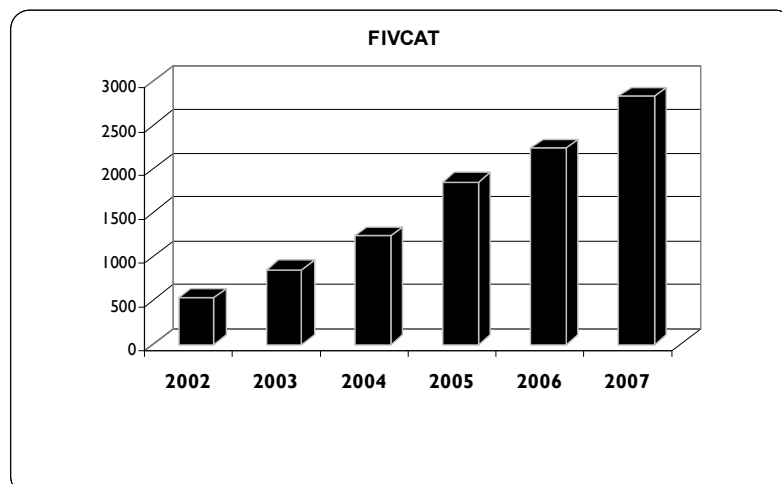
Particularly Spain contributed in large part to this spectacular growth which is due to the fact that gamete donation is encouraged by a favourable legal background and socioeconomic circumstances. In fact currently Spain is Europe's leader in egg donation; in 2005 more than half of the cycles were performed in this country. This phenomenon is also related to the very heterogeneous European legislation which constrains a large number of patients to choose cross-border reproductive care.

**Figure 1.** The number of oocyte donation cycles in Europe and Spain between 1998-2005



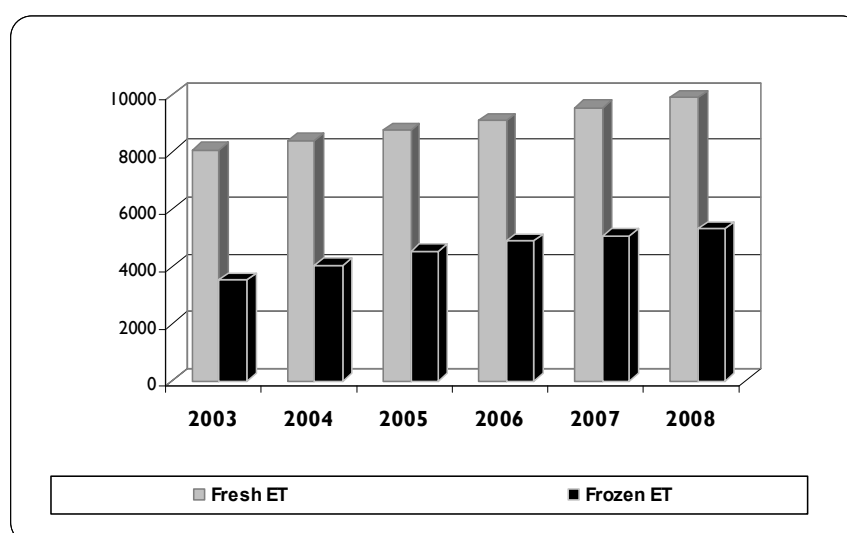
Moreover the number of cycles is underreported in Spain because there is no obligatory national registry with a complete coverage with the exception of the FIVCAT registry in Catalonia. The voluntary registry of the Spanish Fertility Society (SEF) which reports data to the ESHRE registry currently does not have a complete coverage (60% of centres participated in 2007). Data obtained from the FIVCAT.NET registry showed a much steeper (more than 4-fold) increase of oocyte donation cycles during the six-year study period compared to overall European or US data.

**Figure 2.** Oocyte donor cycles performed in Catalonia between 2002-2007



A somewhat similar picture is emerging if one examines the SART (Society for Assisted Reproduction Technology) registry from the United States. According to data available during the 2003-2008 period the increase was slower than in Europe but still reached 22.3% and 50.6% in fresh and frozen embryo transfers, respectively. This difference might be probably explained by the huge differences in the practice of assisted reproduction and gamete donation in these two regions of the world.

**Figure 3.** The number of embryo transfers involving donor oocytes in the United States between 2003-2008



## 1.2. Ovarian stimulation protocols

Although the first successful in-vitro fertilization (IVF) treatment was carried out in a spontaneous natural cycle (Steptoe and Edwards 1978) later ovarian stimulation became an inherent part of IVF treatment. In non down-regulated gonadotropin only stimulation protocols unwanted LH surges caused cycle cancellations in approximately 20% of the cases (Loumaye 1990). With the

isolation of the natural GnRH decapeptide, the synthesis and subsequent utilization of GnRH analogues cycle cancellations were dramatically reduced.

Although the use of GnRH agonist suppression increased greatly the efficiency of stimulation protocols used in IVF it also had several shortcomings. GnRH agonists act through irreversible binding to pituitary GnRH receptors which cause an initial FSH and LH surge (flare-up effect) and afterwards internalization and down-regulation of the agonist-receptor complex. The initial flare-up effect could cause formation of ovarian cysts whereas the deep suppression achieved after prolonged agonist treatment could cause debilitating menopausal side-effects and increases the total dose of required gonadotropins. Furthermore the prolonged suppression also mandates intensive luteal phase support after oocyte retrieval.

Beside the most frequently used long luteal-phase protocol other protocol modifications (such as short or ultra-short) were also introduced however they were found to be less efficient compared to the classical long protocol (Daya 2000). The development of side-effect free third generation GnRH antagonists however allowed for a much more physiologic suppression of LH surges.

### **1.3. Experience with GnRH antagonist based protocols in IVF patients**

GnRH antagonists were developed during the nineties and were introduced in the clinical practice in Europe from 1999. Their mechanism of action is related to the fact that the GnRH antagonists achieve an immediate, dose-dependent but reversible competitive inhibition of the pituitary GnRH receptors. In fact the GnRH antagonist has a 20 times higher affinity to the receptor compared to native GnRH. In early dose-finding studies (Diedrich et al. 1994; Albano et al. 1997) it was



established that a daily 0.25 mg dose was adequate to prevent premature LH surges during ovarian stimulation.

The main advantage of the GnRH antagonist lies in its immediate action which permits starting administration only when a real risk of LH surges exists usually from day 5-6 of stimulation. Consequently no long pre-treatment is necessary as with GnRH agonists and oestrogen withdrawal symptoms are rare. Furthermore lower doses of gonadotropins are needed and the duration of stimulation was shown to be significantly lower. After co-treatment with antagonists the number of developing intermediate-size follicles is lower which might contribute to the observed lower rate of OHSS compared to agonist protocols. Importantly the pituitary also remains responsive to exogenous hormonal signals which allow the use of a GnRH agonist for the induction of final oocyte maturation (Itskovitz-Eldor et al. 2000). This strategy is especially useful in avoiding or even eliminating moderate/severe OHSS.

Initial phase III studies (Albano *et al.* 2000; Felberbaum *et al.* 2000; Olivennes *et al.* 2000) performed in the early 2000s showed that the use of both clinically available antagonists (cetorelix and ganirelix) compared favourably with the classical long agonist protocol. Nonetheless an early meta-analysis (Al-Inany and Aboulghar 2001) summarizing these previously mentioned trials highlighted a significant lower chance of clinical pregnancy with the new antagonist protocol. This fact which was intensively debated afterwards (Huirne et al. 2007) and had a negative effect on the uptake and acceptance of antagonist protocols by clinicians worldwide. In fact a German registry based study published in 2005 (Griesinger et al. 2005) confirmed that GnRH antagonists were used as a second choice treatment in older patients with a higher cycle rank. More recently updated meta-analyses (Al-Inany et al. 2006; Kolibianakis et al. 2006) performed in the general IVF

population showed a more optimistic picture suggesting that if there is a real difference between GnRH analogues it is of little clinical importance. A recent opinion paper (Devroey et al. 2009) has suggested that benefits of the GnRH antagonists in terms of patient convenience and reducing risk and burden of ovarian stimulation outweigh the possibility of somewhat lower live birth rates.

The application of GnRH antagonists could be particularly interesting in specific patient groups such as low responders, PCOS patients or high responders. A meta-analysis (Griesinger et al. 2006) of 8 RCTs that compared the use of antagonists versus agonists (short and long protocols) in poor responders has found no significant difference in clinical outcomes but suggested a higher number of retrieved oocytes with the antagonist compared to the long agonist protocol. In PCOS patients although the number of analyzed available trials was lower the possible non-outcome benefits of antagonists (shorter stimulation duration, lower gonadotropin consumption and OHSS incidence) could lead to their preferential use in this patient population. In GnRH antagonist protocols cycle scheduling can be an important issue because cycle initiation is related to the onset of the patient's spontaneous menstrual cycle. This problem however can be circumvented by oral contraceptive pill pre-treatment. A recent systematic review (Griesinger et al. 2008) that analyzed available evidence consisting of 4 randomized clinical trials has found that OAC pre-treatment does not negatively affect ongoing pregnancy rates in GnRH antagonist cycles even if the duration of stimulation and gonadotropin consumption was increased.

One of the clinically available antagonist preparations also exists in an intermediate depot form which has permitted the development of a single-dose (SD) GnRH antagonist protocol (Olivennes et al. 1998). With this SD protocol a single dose (3 mg) of cetrorelix is administered on stimulation day 7-8 and it

remains effective in preventing LH surges for at least 96 hours. Therefore in the majority of cycles a single antagonist injection is sufficient which contributes to patient commodity and increased compliance. Although the currently available two antagonist molecules were never compared directly in a randomized clinical trial evidence from phase III trials and meta-analyses suggests equal clinical efficiency and safety profile.

The development and refinement of GnRH antagonist protocols is still ongoing and further improvement and tailoring is still needed (Devroey et al. 2009). GnRH antagonists also provide the base for developing innovative stimulation protocols. Recently minimal stimulation (Baart et al. 2007) and natural cycle IVF protocols (Pelinck et al. 2002) have gained increased interest. In both of these approaches exogenous gonadotropin exposure is reduced or completely eliminated to maximally reduce interference with the natural follicle selection and improve subsequent oocyte quality and endometrial receptivity. However given that the rate of cycle cancellations due to premature LH surges is still high the application of GnRH antagonists remains a cornerstone of these protocols.



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## **2. OBJECTIVES**

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1. To **compare efficiency** of GnRH antagonist protocols in comparison with GnRH agonist-based protocols in the context of oocyte donation by means of a systematic review and meta-analysis (Article N° 1).
2. To illustrate that GnRH antagonist protocols substantially **increase the safety** of ovarian stimulation for oocyte donors by reducing or even eliminating the incidence of moderate/severe OHSS (Article N° 2).





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## **3. MATERIALS AND METHODS**

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### 3.1. Materials and methods of objective n° 1

Published article: GnRH agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: a systematic review and meta-analysis (*Fertility and sterility*, 2010 Aug 3.)

Exhaustive literature searches were performed in electronic databases, register of clinical trials, meeting proceedings for relevant studies covering a period until December 2009. Studies were selected if the target population was women undergoing oocyte donation IVF treatment and the interventions were GnRH agonist versus GnRH antagonist based protocols for COH. The primary outcome was recipient ongoing pregnancy rate per randomized donor. Secondary outcome measures were number of retrieved oocytes, duration of stimulation, total gonadotropin consumption and OHSS incidence per randomized oocyte donor. Studies were selected in a two-stage process by two reviewers and any disagreements about inclusion were resolved by consensus after consultation with a third reviewer.

From each study, binary data were extracted in 2x2 tables and the results were pooled and expressed as risk ratio with 95% confidence intervals using fixed effects models if significant heterogeneity was absent. Weighed mean difference (WMD) was calculated for continuous variables using means and standard deviations (SD) from individual studies. Heterogeneity of treatment effects was evaluated graphically using forest plot and statistically using chi-squared heterogeneity test and the  $I^2$  statistic. Exploration of clinical heterogeneity was conducted using variation in features of the study population, intervention and study quality. (For more details see Materials and methods section of Article N° 1).

### **3.2. Materials and methods of objective n° 2**

Published article: Early OHSS is completely prevented by GnRH agonist triggering in high risk oocyte donor cycles: a prospective, luteal-phase, follow-up study (*Fertility and sterility, 2010;93:2418-20.*)

The observational study period was between April and September 2008. Institutional Review Board approval was obtained for the present study and included donors were consented to follow-up. For the purpose of the present study those donors who were at increased OHSS risk were scheduled 3-6 days after their oocyte retrieval for a single follow-up examination. In all high risk cases triggering was exclusively performed with 0.2 mg of triptorelin s.c. and hCG was carefully avoided for inducing final oocyte maturation. On consultation a physical examination, a vaginal and abdominal ultrasound scan was performed by a physician together with a blood analysis (blood count including leukocytes and hematocrit, liver and renal function). (For more details see Materials and methods section of Article N° 2).

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## **4. RESULTS**

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## 4.1.

To **compare efficiency** of GnRH antagonist protocols in comparison with GnRH agonist-based protocols in the context of oocyte donation by means of a systematic review and meta-analysis (*Article N° 1*).

***4.1.1. Published article: GnRH agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: a systematic review and meta-analysis (Fertility and Sterility, 2010)***





# Gonadotropin-releasing hormone agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: a systematic review and meta-analysis

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**Objective:** To compare GnRH agonists and antagonists in oocyte-donation IVF treatment cycles by a systematic review and meta-analysis of trials.

**Design:** Systematic review and meta-analysis of randomized clinical trials (RCT). Systematic literature searches were conducted, and all randomized trials that compared GnRH agonists with antagonists in oocyte-donation IVF treatment cycles were included. Study selection, quality appraisal, and data extractions were performed independently and in duplicate.

**Setting:** Tertiary fertility center.

**Patient(s):** A total of 1,024 oocyte donors treated in eight RCTs.

**Intervention(s):** Comparison of GnRH agonists versus antagonists in oocyte-donation IVF treatment.

**Main Outcome Measure(s):** Ongoing pregnancy, oocytes retrieved, duration of stimulation, gonadotropin consumption, and ovarian hyperstimulation syndrome incidence (OHSS) per randomized oocyte donor.

**Result(s):** Meta-analysis of these studies showed no significant difference in ongoing pregnancy rate between the GnRH agonists and antagonists (risk ratio [RR] 1.15, 95% confidence interval [CI] 0.97 to 1.36). The duration of stimulation was significantly lower with the GnRH antagonist protocol (weighed mean difference [WMD]  $-0.90$  days, 95% CI  $-1.61$  to  $-0.20$ ). No significant differences were observed in the number of oocytes retrieved (WMD  $-0.60$ , 95% CI  $-2.26$  to  $+1.07$ ), gonadotropin consumption (WMD  $-264$  IU, 95% CI  $-682$  to  $+154$ ), or OHSS incidence (RR 0.62, 95% CI 0.18 to 2.15).

**Conclusion(s):** No significant differences were observed in ongoing pregnancy rate or the number of retrieved oocytes after donor stimulation with GnRH agonist or antagonist protocols. (Fertil Steril® 2010; ■: ■-■. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Oocyte donation, GnRH antagonist, GnRH agonist, COH, meta-analysis, in vitro fertilization

Gonadotropin-releasing hormone antagonists have appeared on the market since the early 1990s, and over the years have proved to be an effective alternative to GnRH agonists to prevent LH surges during controlled ovarian hyperstimulation (COH) in IVF patients. GnRH antagonists represent a more physiologic mechanism of LH suppression and provided the basis for the development of innovative stimulation protocols (1). They also proved to be beneficial in specific patient groups, such as women with polycystic ovary syndrome (2) and poor responders (2, 3), and in minimal-stimulation or natural-cycle IVF treatment protocols (4).

Although an initial meta-analysis (5) evaluating the influence of the type of GnRH analogue used on cycle outcome in IVF patients found that several outcomes (shorter duration of stimulation, reduced gonadotropin consumption, and reduced ovarian hyperstimulation syndrome incidence [OHSS] incidence) were clearly in favor of the GnRH antagonists, drawbacks were highlighted. Most importantly, a significant reduction in pregnancy rates was observed, which

had a negative influence on the acceptance and diffusion of GnRH antagonists. Subsequent updated meta-analyses (6, 7) were performed in the general IVF population and had excluded trials that included oocyte donors.

GnRH antagonist protocols were also rapidly applied in the context of oocyte donation, where they provided benefits of treatment simplification and donor convenience. Moreover, several recent retrospective studies (8–10) and randomized clinical trials (RCTs) (11–13) performed in oocyte donors showed a significantly reduced OHSS incidence when a GnRH agonist was used for inducing final oocyte maturation instead of hCG. Because triggering of ovulation with GnRH agonists is only feasible in GnRH antagonist-based protocols, the choice of the most adequate GnRH analogue for donor stimulation protocols is a clinically important question. To date, no meta-analysis existed evaluating the effectiveness of different GnRH analogues in oocyte donation, with individual studies yielding inconsistent and conflicting results. Therefore we conducted a systematic review and meta-analysis of studies comparing the GnRH agonist- versus antagonist-based protocols for COH in the context of oocyte-donation IVF treatment.

## MATERIALS AND METHODS

Literature searches were performed via Medline, Embase, Scopus, the Cochrane Library, and the National Research Register for relevant studies.

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D.B. was the first author in one of the trials included in this review.

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The search also included Institute for Scientific Information conference proceedings as well as databases for registration of ongoing and archived controlled trials (International Standard Randomised Controlled Trial Number and Metaregister of Controlled Trials). Additionally, references of retrieved articles and meeting proceedings of the American Society for Reproductive Medicine (2001–2009) and the European Society for Human Reproduction and Embryology (2001–2009) were hand searched. The literature search covered the period through December 2009. A combination of Medical Subject Headings and text words were used to generate two subsets of citations, one including studies involving GnRH analogues (“gonadotropin releasing hormone/GnRH analogue/agonist/antagonist/inhibitor”) and the other studies related to oocyte donation (“oocyte/egg donor/donation”). These two subsets were combined using “AND” to generate a set of citations relevant to the research question. We also made enquiries about unpublished studies from researchers investigating in this field. No language restrictions were placed in any of the searches. Institutional Review Board approval was not required for the present study, owing to its retrospective nature including already published studies and the fact that data were managed in a way which excluded the subjects’ identification.

### Study Selection

Studies were selected if the target population was women undergoing oocyte-donation IVF treatment and the interventions were GnRH agonist– versus antagonist–based protocols for COH. The primary outcome was recipient ongoing pregnancy rate per randomized donor. Secondary outcome measures were number of retrieved oocytes, duration of stimulation, total gonadotropin consumption, and OHSS incidence per randomized oocyte donor. Studies were selected in a two-stage process. First, two reviewers (D.B. and S.K.S.) scrutinized the titles and abstracts from the electronic searches independently, and full manuscripts of all citations that definitely or possibly met the predefined selection criteria were obtained. Second, final inclusion or exclusion decisions were made on examination of the full manuscripts. Assessment of the manuscripts was performed independently by two reviewers (D.B. and S.K.S.), and any disagreement about inclusion were resolved by consensus after consultation with a third reviewer (A.C.).

### Data Extraction

The selected studies were assessed for methodologic quality by using the components of study design that are related to internal validity (Center for Reviews and Dissemination, 2001). The information on the method of randomization, allocation concealment, blinding, intention-to-treat analysis, and follow-up rates was sought by examining the full text articles and by contacting the authors if clarification was needed. The following data were also recorded from each of the studies: demographic (author, type of study, country of origin, period of enrollment), procedural (number of patients included, inclusion and exclusion criteria for patients populations, type of COH protocol, type of gonadotropin administered, criteria for triggering final oocyte maturation, the type and dose of triggering agent, type of fertilization, type of protocol used for recipient preparation) and outcome data (ongoing pregnancy rate, number of retrieved oocytes, duration of stimulation, gonadotropin consumption, incidence of OHSS in oocyte donors).

### Statistical Analysis

From each study, binary data were extracted in  $2 \times 2$  tables and the results were pooled and expressed as risk ratio (RR) with 95% confidence interval (CI) by using fixed-effects models if significant heterogeneity was absent. Ongoing pregnancies were meta-analyzed for studies in which one donor donated eggs to one recipient only; the reason for this approach is that studies that contain multiple recipients from one donor have multiple nonindependent outcome data which invalidate the assumptions made in standard meta-analytical approaches. Weighed mean difference (WMD) was calculated for continuous variables using means and standard deviations from individual studies. Heterogeneity of treatment effects was evaluated graphically using forest plot and statistically using chi-square heterogeneity test and the  $I^2$  statistic. Exploration of clinical heterogeneity was conducted using variation in features of the study population, intervention, and study quality. All statis-

tical analysis was performed with RevMan 5.0 software (14). To assess for publication bias, a funnel plot analysis was performed (15).

## RESULTS

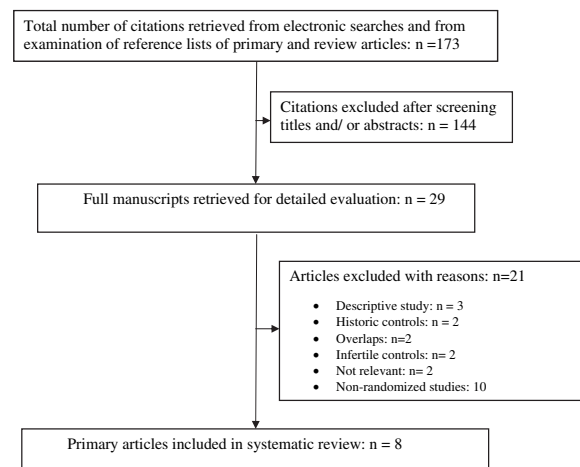
The process of literature identification and selection is summarized in Figure 1. Out of the 173 citations identified, 29 were selected during the initial screening, and on examination of manuscripts, eight studies (16–23) including a total of 1,024 randomized oocyte donors satisfied the selection criteria for the review. The quality of the included trials was generally good, showing evidence of adequate randomization method and allocation concealment in six of the eight studies. The study populations uniformly included young (18–35 years) oocyte donors with regular menstrual cycles, normal body mass index (BMI; 18–29 kg/m<sup>2</sup>), and basal hormonal values (FSH <10 UI/mL). In seven RCTs the GnRH agonist long regimen was compared with the GnRH antagonist regimen, whereas in one trial it was compared with the short flare-up GnRH agonist regimen. Three different analogues (leuprorelin, triptorelin, buserelin) were used in the agonist arm, and both available GnRH antagonists (ganirelix and cetrotide) were used in the antagonist arm. Recombinant FSH was used for ovarian stimulation in the majority of trials (7/8). The treatment characteristics of the included studies are presented in Table 1. Funnel plot analysis for the outcome of ongoing pregnancy showed that publication and related biases were unlikely.

### Primary Outcome: Ongoing Pregnancy Rate

Six of the eight studies reported recipient ongoing pregnancy rate as an outcome. However, the donor recipient ration was 1:1 in only five of the eight studies (Table 1). Pooling of results from those five studies showed no significant difference in recipient ongoing pregnancy rate per randomized donor after COH with GnRH agonist– or antagonist–based protocols: RR 1.15 (95% CI 0.97 to 1.36;  $P=.12$ ; heterogeneity  $P=.80$ ;  $I^2$  0%, fixed effects model; Fig. 2).

## FIGURE 1

Study selection process for systematic review of GnRH agonists versus antagonists in oocyte-donation cycles.



Bodri. GnRH analogues in oocyte donor cycles. *Fertil Steril* 2010.

**TABLE 1****Characteristics of randomized controlled trials comparing GnRH agonists versus antagonists in oocyte donation cycles.**

Study	Donors (allocation), recipients	Hormonal pretreatment	GnRH agonist protocol/gonadotropin type	GnRH antagonist protocol/gonadotropin type	Criteria for triggering final oocyte maturation	Triggering final oocyte maturation	Type of fertilization	Donor-recipient ratio	Recipient preparation
Shamma et al., 2003 (17)	29 donors (17 agonist, 12 antagonist), 62 recipients	OCP	Depot Leuprorelin 3.75 mg (midluteal phase)/rFSH/3-5 ampoules/day + 1 ampoule hMG (if baseline LH <0.5)	Ganirelix by two follicles 12-14 mm (0.25 mg/d)/rFSH 3-5 ampoules + 1 ampoule hMG (if baseline LH <0.5)	At least two follicles 18-20 mm	10,000 hCG	ICSI	1:2-3	Oral E <sub>2</sub> 6 mg/d, E <sub>2</sub> patch 0.1 mg/d, P IM 100 mg/d
Lan, 2004 (20)	222 donors (124 agonist, 98 antagonist), 222 recipients	OCP (Marvelon)	Buserelin 0.5 mg/d and reduced to 0.2 mg/d after down-regulation (midluteal phase)/Follitropin- $\beta$ 100-300 IU/day	Ganirelix by a follicle > 14 mm (0.25 mg/d)/Follitropin- $\beta$ 100-300 IU	At least two follicles $\geq$ 17 mm	5,000 hCG	IVF/ICSI	1:1	Oral E <sub>2</sub> valerate 4mg/d, micronized vaginal P 600 mg/d
Prapas et al., 2005 (23)	98 donors participating with 148 cycles (75 agonist, 73 antagonist), 148 recipients	Oral contraceptive (Gynofen)	Triptorelin 0.1 mg/d (midluteal phase)/Follitropin- $\beta$ 300 IU/d	Ganirelix fixed on day 8 (0.25 mg/d)/Follitropin- $\beta$ 300 IU	At least three follicles $\geq$ 17 mm	10,000 hCG	ICSI	1:1	Oral E <sub>2</sub> valerate 6 mg/d, micronized vaginal P 600 mg/d
Simón et al., 2005 (16)	42 donors (14 agonist, 14 standard-dose antagonist, and 14 high-dose antagonist), 51 recipients	Not mentioned	Buserelin 0.6 mg/d (21st-24th cycle day)/Follitropin- $\beta$ 150 IU/d	Ganirelix fixed on day 6 (0.25 mg/d for the standard-dose group and 2.0 mg/d for the high-dose group)/Follitropin- $\beta$ 150 IU	At least three follicles $\geq$ 17 mm	10,000 hCG	IVF/ICSI	1:1.6	Oral E <sub>2</sub> valerate 6mg/d, micronized vaginal P 800mg/d
Shamma et al., 2006 (18)	58 donors (27 agonist, 31 antagonist), 107 recipients	OCP	Depot Lupron 0.125 mg (luteal phase)/rFSH 3-5 ampoules/d	Cetrorelix by a follicle > 14 mm (0.25 mg/d)/rFSH 3-5 ampoules	At least four follicles 18-20 mm	250 $\mu$ g r-hCG	ICSI	1:1.8	Oral E <sub>2</sub> 6 mg/day, E <sub>2</sub> patch 0.1 mg/d, P IM 100 mg/d
Bodri et al., 2006 (19)	118 donors (60 agonist, 58 antagonist), 166 recipients	None	Triptorelin 0.1 mg/d (cycle day 2)/Follitropin- $\alpha$ 187.5 IU/d	Cetrorelix fixed on day 6 (0.25 mg/d)/Follitropin- $\alpha$ 225 IU	At least three follicles $\geq$ 18 mm	250 $\mu$ g r-hCG	ICSI	1:1.5 overall, 66 had 1:1 donor-recipient ratio	Oral E <sub>2</sub> valerate 6mg/d, micronized vaginal P 800mg/d
Martínez et al., 2008 (21)	323 donors (160 agonist, 163 antagonist), 273 recipients	Vaginal contraceptive (Nuvaring)	Depot Leuprorelin 3.75 mg (cycle day 20-22)/hMG 2-3 ampoules/d	Ganirelix fixed on day 6 (0.25 mg/d)/Follitropin- $\beta$ 150-200 IU	At least three follicles $\geq$ 20 mm	10,000 hCG	IVF/ICSI	1:1	Oral E <sub>2</sub> valerate 6mg/d, micronized vaginal P 600mg/d

*Bodri. GnRH analogues in oocyte donor cycles. Fertil Steril 2010.*

TABLE 1

Continued.

Study	Donors (allocation), recipients	Hormonal pretreatment	GnRH agonist protocol/gonadotropin type	GnRH antagonist protocol/gonadotropin type	Criteria for triggering final oocyte maturation	Triggering final oocyte maturation	Type of fertilization	Donor-recipient ratio	Recipient preparation
Martinez et al., 2008 (22)	84 donors (40 agonist, 44 antagonist), 61 recipients	OCP in the GnRH antagonist group (Microdiol)	0.5 ampoule depot Leuprorelin 3.75 mg (cycle day 20–22)/hMG/Follitropin- $\beta$ 150–200 IU/d	Cetrorelix by a follicle > 14 mm (3 mg + 0.25 mg/d after 4 days)/Follitropin- $\beta$ 150–200 IU	At least three follicles $\geq$ 20 mm	10,000 hCG in the agonist group/hCG or GnRH agonist in the antagonist group	IVF/ICSI	1:1	Oral E <sub>2</sub> valerate 6mg/d, micronized vaginal P 600mg/d

Note: ICSI = intracytoplasmic sperm injection; IM = intramuscular; IVF = in vitro fertilization; OCP = oral contraceptive pill; rFSH = recombinant FSH.

Bodri. GnRH analogues in oocyte donor cycles. *Fertil Steril* 2010.

## Secondary Outcomes

**Oocytes retrieved** Meta-analysis of seven of the eight studies that reported the number of oocytes retrieved as an outcome showed no significant difference in the number of retrieved oocytes after COH with the GnRH agonist or antagonist protocols: WMD  $-0.60$  (95% CI:  $-2.26$  to  $1.07$ ;  $P=.48$ ; heterogeneity  $P=.002$ ;  $I^2$  71%, random effects model; Fig. 3).

**Duration of stimulation** Five of the eight studies reported the duration of stimulation with gonadotropins as an outcome. Meta-analysis of those five studies showed that the duration of stimulation was significantly lower after COH with the GnRH antagonist protocol compared with the agonist protocol: WMD  $-0.90$  days (95% CI  $-1.61$  to  $-0.20$  days;  $P=.001$ ; heterogeneity  $P<.00001$ ;  $I^2$  91%, random effects model).

**Gonadotropin consumption** Data from one study (19) where different gonadotropin starting doses were used were excluded from analysis. Meta-analysis of the remaining four studies that reported data on gonadotropin consumption showed no significant difference after COH with the GnRH agonist versus antagonist protocols: WMD  $-264$  IU (95% CI  $-682$  to  $154$  IU;  $P=.22$ ; heterogeneity  $P<.00001$ ;  $I^2$  95%, random effects model.)

**OHSS incidence** Meta-analysis of four of the eight studies that reported data on OHSS incidence showed no significant difference after COH with the GnRH agonist versus antagonist protocols: RR 0.61 (95% CI 0.18 to 2.15;  $P=.45$ ; heterogeneity  $P=.45$ ;  $I^2$  0%, fixed effects model).

## DISCUSSION

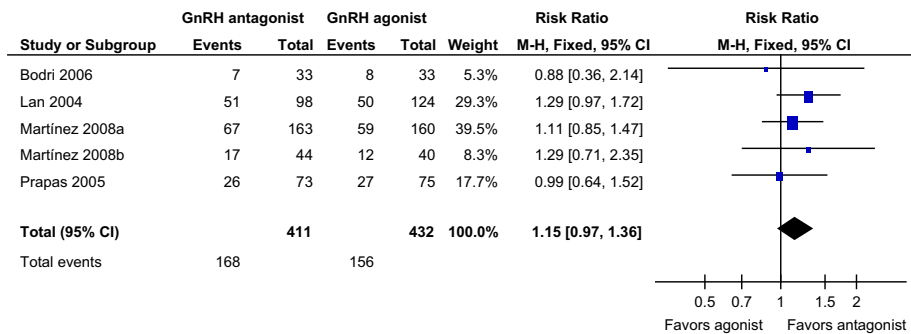
The present systematic review and meta-analysis analyzed data from eight RCTs on the use of GnRH agonists versus antagonists in COH of oocyte donors. The findings suggest that there are no statistically significant differences in ovarian response or recipient ongoing pregnancy rates with the use of either GnRH agonist or antagonist protocols.

Comparing our findings with other meta-analyses performed in general IVF population (6, 7), a similar trend was observed in the duration of stimulation and gonadotropin consumption. On the other hand, the difference in the number of retrieved oocytes was less important (0.60 vs. 1.07–1.19 fewer oocytes with antagonist protocols) and ongoing pregnancy rates were not negatively affected (RR 1.16 vs. odds ratio 0.82–0.86). This could be related to the smaller number of trials included, but it could also suggest real differences between donor and nondonor IVF. The oocyte donation model allows studying oocyte quality and embryo implantation potential separately from possible endometrial effects of different stimulation protocols. In this context, it is interesting to notice that pregnancy rates do not seem to be negatively affected after oocyte donation compared with IVF patients, which might suggest a negative endometrial effect related to the GnRH antagonist.

The incidence of OHSS was not significantly different between the two treatment groups, but this might be related to the fact that the aggregated sample size was too small to detect any significant difference. Furthermore it must be pointed out that hCG was used exclusively for inducing the final oocyte maturation in all trials except in the most recent one where GnRH agonist was partially used in the antagonist arm. It has been suggested that the choice of the triggering agent is directly related to the reduction in OHSS incidence in the antagonist arm (24). Recent trials (8–10) demonstrated extensively the elimination of the syndrome in

FIGURE 2

Risk ratio for recipient ongoing pregnancy rate per randomized donor (studies with 1:1 donor-recipient ratio). Heterogeneity:  $\chi^2 = 1.64$ ;  $df = 4$  ( $P = .80$ );  $I^2 = 0$ . Test for overall effect:  $Z = 1.57$  ( $P = .12$ ).



Bodri. GnRH analogues in oocyte donor cycles. *Fertil Steril* 2010.

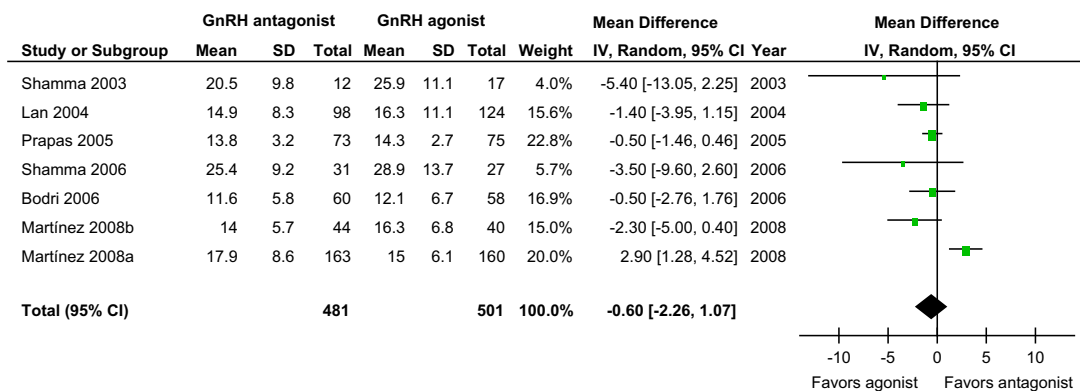
oocyte donors after GnRH agonist triggering. Moreover recent RCTs (11–13) also showed similar outcome in terms of oocyte maturation, fertilization rates, and recipient pregnancy rates after substituting hCG with a GnRH agonist for inducing final oocyte maturation in GnRH antagonist protocols. Therefore, owing to their increased safety potential, GnRH antagonist-based protocols coupled with GnRH agonist triggering could be advocated as a first choice treatment regimen for donor stimulation (25, 26).

In the RCTs included and analyzed in the present review, recipients were not formally randomized, owing to the fact that a thorough donor-recipient phenotype matching—which is an inherent part of all oocyte donation programs—prevents random donor attribution. Nonetheless, it is unlikely that this would introduce significant bias, because the matching procedure is not influenced by the type of stimulation protocol received by the donor. This is supported by the fact that in the majority of the included trials, recipients were similar regarding baseline characteristics such as age, BMI, and indication for oocyte donation.

In this review, it was demonstrated that donor and recipient outcome were not significantly different according to the type of GnRH analogue used in donor stimulation. The validity of this finding depends on the methodologic rigor of the review and the component primary studies. A prospective protocol was used, and a concerted effort was made to find all of the evidence. Two independent reviewers assessed study quality and extracted data, and the agreement between the two reviewers was high. One major problem usually encountered when synthesizing the evidence in meta-analysis is clinical and methodologic heterogeneity between the studies. In our review, the methodologic quality of the included RCTs was good and recipient follow-up complete. The donor population was also uniform, owing to generally accepted criteria for inclusion in an oocyte-donation program. Some clinical heterogeneity (type of GnRH analogues used) existed between treatment protocols used for donor stimulation, but owing to the limited number of studies identified, it was not feasible to perform a subgroup analysis. It is also important

FIGURE 3

Weighted mean difference for the number of retrieved oocytes. Heterogeneity:  $\tau^2 = 2.91$ ;  $\chi^2 = 20.49$ ;  $df = 6$  ( $P = .002$ );  $I^2 = 71\%$ . Test for overall effect:  $Z = 0.70$  ( $P = .48$ ).



Bodri. GnRH analogues in oocyte donor cycles. *Fertil Steril* 2010.



to highlight that this review was only sufficiently powered to detect an ~9% difference in recipient ongoing pregnancy rates between groups.

In conclusion, currently available evidence suggests a similar effectiveness of GnRH antagonists and agonists in the context of oocyte donation. Owing to its increased safety potential, the GnRH an-

tagonist protocol combined with GnRH agonist triggering could be advocated as the treatment of first choice for oocyte donors.

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## 4.2.

To illustrate that the above-mentioned innovative stimulation protocols substantially **increase the safety** of ovarian stimulation for oocyte donors by reducing or even eliminating the incidence of moderate/severe OHSS (*Article N° 2*).

***4.2.1. Published article: Early OHSS is completely prevented by GnRH agonist triggering in high risk oocyte donor cycles: a prospective, luteal-phase, follow-up study (Fertility and sterility, 2010)***





# Early ovarian hyperstimulation syndrome is completely prevented by gonadotropin releasing-hormone agonist triggering in high-risk oocyte donor cycles: a prospective, luteal-phase follow-up study

In this prospective, follow-up study of 102 high-risk oocyte donors in their luteal phase, we found a complete absence of ovarian hyperstimulation syndrome (no signs of hemoconcentration or ascites) after the donors were triggered with a gonadotropin releasing-hormone (GnRH) agonist. Due to its powerful preventive effect, the GnRH antagonist protocol combined with a GnRH agonist trigger should preferentially be used in egg donors; in conjunction with an effective luteal support or embryo cryopreservation, the protocol could also be applied to high-risk in vitro fertilization patients. (*Fertil Steril*® 2010;93:2418–20. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** GnRH antagonist, GnRH agonist triggering, OHSS, oocyte donation

From the early 1990s until recently, several studies have confirmed that after triggering women with gonadotropin releasing-hormone (GnRH) agonist the incidence of moderate/severe early-onset ovarian hyperstimulation (OHSS) is significantly reduced, making this strategy potentially valuable for use in high-risk in vitro fertilization (IVF) patients or oocyte donors. Two randomized controlled trials with high-risk IVF patients (1, 2) confirmed the absence of moderate/severe OHSS and implantation rates seemed not to be affected by the use of vigorous luteal support. In the context of oocyte donation, the reduction in OHSS incidence was demonstrated both by retrospective series (3–5) as well as randomized clinical trials (6, 7). Our group has published the largest experience (4, 7) to date with GnRH-agonist triggered oocyte donor cycles. This strategy contributed in large part to the overall safety of our egg donation program, which is predominantly based on the use of the GnRH antagonist stimulation protocol (8).

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On the other hand, a small number of reports have described OHSS cases despite the fact that final oocyte maturation was induced by the GnRH agonist (9, 10). These cases occurred almost exclusively during conception cycles, which means that late-onset cases could be completely avoided only by combining GnRH-agonist triggering with an effective embryo cryopreservation program. In a proof-of-concept study of 20 high-risk IVF patients, Griesinger et al. (11) described an approach that focused on cumulative pregnancy rates and OHSS incidence. This study also presented data on luteal-phase follow-up evaluations of GnRH-agonist triggered IVF patients and found no signs of moderate/severe OHSS.

The exact degree of OHSS prevention and incidence of milder symptoms yet to be evaluated on a larger sample of high-risk patients. Therefore, to gather more information on the safety and side effects of GnRH-agonist triggering, we conducted a prospective study that evaluated clinical symptoms, and biochemical and ultrasound signs of OHSS in high-response oocyte donor cycles.

## MATERIALS AND METHODS

The observational study period was between April and September 2008. Institutional review board approval was obtained and included donors consented to follow-up evaluations. Gonadotropin stimulation started from day 2 of the menstrual cycle with recombinant follicle-stimulating hormone (FSH) or purified human menopausal gonadotropin (hMG). The GnRH antagonist (Cetrotide, 0.25 mg/day) was introduced according to a multiple-dose, flexible protocol. In all high-risk cases, triggering was exclusively performed with 0.2 mg of subcutaneous triptorelin, and hCG was carefully avoided for inducing final oocyte maturation. Preventive interventions (i.e., coasting, or intravenous albumin administration) were not applied after GnRH-agonist triggering.

For the purpose of our study, donors who were at increased OHSS risk were scheduled 3 to 6 days after their oocyte retrieval for a single follow-up examination. Every patient was included only once in the study. Increased OHSS risk was defined as

≥20 follicles ≥10 mm on the last ultrasound scan and/or a final E<sub>2</sub> level of ≥4000 pg/mL, and/or ≥20 retrieved cumulus-oocyte complexes (COCs). At consultation, a physical examination and a vaginal and abdominal ultrasound scan were performed by a physician together with a blood analysis (blood count including leukocytes and hematocrit, liver, and renal function). We classified OHSS according to the criteria described by Navot et al. (12) and Aboulghar and Mansour (13) where ultrasound signs of moderate OHSS were defined as ovarian enlargement (5–12 cm) and evidence of ascites. It was considered to be “severe” when moderate OHSS was accompanied by severe hemoconcentration (Hct >45%), hyperleucocytosis, and kidney or liver dysfunction. We defined ascites as free liquid in the abdominal cavity detected by abdominal ultrasound, or by the presence of notable periovarian and periuterine liquid detected by vaginal ultrasound. The quantity of free liquid in the Douglas pouch was measured as described by Álvarez et al. (14). According to their observations, an average 3.5 ± 2.8 cm<sup>2</sup> fluid pocket was a routine finding in donors without OHSS risk, and only >9 cm<sup>2</sup> fluid pockets were considered as a significant amount of free pelvic fluid.

## RESULTS

A total of 102 donors were prospectively enrolled in the study according to the inclusion criteria. Data regarding ovarian stimulation and luteal-phase follow-up evaluations are summarized in Table 1. No cases of moderate/severe OHSS were observed in the study group. Seven patients (6.8%) reported pain and moderate abdominal distension at the follow-up examination (without any concomitant biochemical or ultrasound sign of OHSS), but these symptoms completely disappeared a few days later at the onset of menses. No cases of ascites were detected with abdominal ultrasound. The mean area of fluid pocket in the Douglas pouch was 3.1 ± 3.8 cm<sup>2</sup>. In eight patients, a fluid pocket >9 cm<sup>2</sup> was detected but without any clinical symptoms or hemoconcentration. The mean ovarian diameter was 49.7 ± 10.7 mm and 46.9 ± 9 mm for the right and the left ovary, respectively.

Luteal phase hematocrit values (37.7 ± 2.8) were statistically significantly lower than the basal values (39.7 ± 2.5) recorded in the same patient before ovarian stimulation ( $P < .0001$ ). A transient, mild elevation (range: 28–104 IU/mL for ALAT and 39–60 IU/mL for ASAT) of hepatic enzymes was found in six patients (5.8%). Luteal leukocyte count and creatinine values were in normal ranges. The length of the unsupported luteal phase was 5.6 days.

## DISCUSSION

Our observational study demonstrated that GnRH-agonist triggering completely prevented the development of early-onset OHSS (absence of hemoconcentration or accumulation of ascites) in high-risk oocyte donors.

In addition to clinical assessment, we also evaluated the objective ultrasound and biochemical signs of OHSS. Hemoconcentration is a particularly sensitive marker of the degree of OHSS severity (15). It is remarkable that mean luteal hematocrit values were significantly different (2 points lower) from basal values recorded in the same patient before ovarian stimulation, thus excluding any OHSS-related hemoconcentration. On the other hand, in our series in a small proportion of donors we detected a transient mild elevation of hepatic enzymes without accompanying hemoconcentration, ascites, or any other clinical sign of OHSS. It could be hypothesized that these alterations were related more to the ovarian stimulation itself (possibly mediated by elevated estradiol levels) than to OHSS (16).

In our series, the ultrasound OHSS markers found after a high ovarian response (Douglas fluid pocket or ovarian size) were almost identical to those found in patients without any OHSS risk (average ovarian diameter <5 cm and Douglas fluid pocket <3.5 cm<sup>2</sup>). Although in a few patients (7.8%) the size of Douglas pouch fluid pocket was above the level (9 cm<sup>2</sup>) that determined significant pelvic fluid, these patients did not present clinical symptoms or hemoconcentration. In our opinion, these cases cannot be classified

**TABLE 1**

Donor cycles and luteal phase follow-up evaluation.

	Mean ± standard deviation	Range
Mean donor age (y)	25.9 ± 4.4	18–35
Starting FSH dose (IU)	203.0 ± 35.0	112.5–300
Days of stimulation	10.1 ± 1.3	7–15
Total FSH (IU) used	1983.0 ± 476.0	1012.5–3375
Final estradiol level (pg/mL)	3337.0 ± 1825.0	143–8474
Number of follicles ≥10 mm	25.1 ± 6.2	13–42
Retrieved oocytes (COC)	19.8 ± 7.2	5–40
Basal hematocrit	39.7 ± 2.5 <sup>a</sup>	34.1–45.7
Luteal phase hematocrit	37.7 ± 2.8 <sup>a</sup>	26.5–46.4
Luteal leukocytes	7880.0 ± 1980.0	4240–13580
Serum ALAT (IU/mL)	20.0 ± 7.0	7–104
Serum ASAT (IU/mL)	16.0 ± 8.0	9–60
Serum creatinine	0.8 ± 0.1	0.6–1.1
Diameter, right ovary (mm)	49.7 ± 10.9	29.5–79
Diameter, left ovary (mm)	48.9 ± 9.0	28–71
Douglas pouch fluid pocket (cm <sup>2</sup> )	3.1 ± 3.8	0–19.6

<sup>a</sup> Independent *t*-test ( $P = .0001$ ).

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as moderate OHSS and are probably related to some degree of intraperitoneal bleeding that occurred after oocyte retrieval.

Recently, several pilot studies investigated the luteal-phase administration of different drugs (cabergoline, letrozole, or GnRH agonist/antagonists) as possible methods of OHSS prevention (14, 17, 18). Similarly to our study, they examined high-risk oocyte donors but with a smaller number of patients. Cabergoline, which is one of the most investigated new drugs, reduced the occurrence of early OHSS but was not sufficient to completely eliminate it. Álvarez et al. (14) observed the occurrence of moderate/severe OHSS in 31.4% of his experimental group (compared with 62.5% in the high-risk control group). Moreover, Douglas fluid pocket measurements in the cabergoline-treated group of Álvarez et al. (14) were significantly higher than in our series. Therefore, our findings suggest that compared with other pharmaceutical interventions GnRH-agonist triggering is currently the most effective way of OHSS prevention.

This argument strongly supports the preferential use of a GnRH antagonist protocol coupled with GnRH-agonist triggering in the stimulation of oocyte donors. In addition to increased donor safety, the clinical management of oocyte donor cycles is also profoundly influenced. Cycle monitoring becomes much easier (reducing or even eliminating the need for hormonal assays), and interventions

for OHSS prevention (coasting or cycle cancellation) are no longer necessary (5). It must be pointed out, however, that in a few patients (6% to 7%) some milder symptoms are still present and this is probably related to oocyte retrieval and/or a high ovarian response. Therefore, to minimize donor discomfort and to also avoid possible alterations in egg quality related to a high response, excessive stimulation of oocyte donors is still not recommended. It could be argued that one of the main limitations of our observational study is the absence of a control group triggered with human chorionic gonadotropin (hCG). Nonetheless, as pointed out by Kol and Solt (19), currently with the available body of evidence supporting the powerful preventive effect of GnRH-agonist triggering, any prospective study that exposes the control group to a hCG-mediated high OHSS risk could be considered unethical.

Our findings suggest that GnRH agonist triggering is highly efficient in preventing clinically significant early OHSS in oocyte donation cycles. Due to this fact, this stimulation protocol should become the treatment of first choice for egg donors; in conjunction with an effective luteal support or embryo cryopreservation, it could also be applied to high-risk IVF patients.

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## **5. DISCUSSION**

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### 5.1. GnRH antagonists in oocyte donation cycles

Since their introduction in the clinical practice GnRH antagonists were also rapidly applied to stimulation of oocyte donors. In the setting of oocyte donation several studies have been published concerning the use of GnRH antagonists for the stimulation of oocyte donors (Sauer et al. 1997; Ricciarelli et al. 2003; Thong et al. 2003; Prapas et al. 2005; Bodri et al. 2006; Erb and Wakim 2008). Available randomized clinical trials (Martinez et al.; Prapas et al. 2005; Bodri et al. 2006; Martinez et al. 2008) on the use GnRH antagonist protocols in oocyte donors have suggested a comparable ovarian response and recipient outcome with GnRH agonist-based stimulation protocols.

One of the first RCTs published as a full publication (Prapas et al. 2005) included a total of 148 donor cycles randomly attributed to a GnRH long agonist or GnRH antagonist protocol. No significant differences were observed in clinical pregnancy (39.72% versus 41.33%) and implantation rates (23.9% versus 25.4%) in recipients which were the primary outcome measures of the study. Martínez and co-workers performed two other RCTs (Martinez et al.; Martinez et al. 2008) in oocyte donors. The first larger study included 323 donors who were randomized to receive ovarian stimulation with a long agonist (leuprorelin+hMG) or antagonist protocol (ganirelix+recFSH). Although ongoing pregnancy rates per embryo transferred were in favour of the agonist group due to a higher cancellation rate pregnancy rates per randomized turned in favour of the antagonist arm (36.8% versus 41.1%). Similarly a the second more recent smaller pilot study (Martinez et al.) that used the single-dose protocol in the antagonist arm has found a higher pregnancy rate in favour of the antagonist protocol (38.6% versus 30%). Apart from well known advantages which contribute to donor commodity (shorter duration of stimulation and decreased gonadotropin consumption) the application

of GnRH antagonists has also provided the possibility of substituting hCG with a GnRH agonist as the triggering agent for final oocyte maturation.

## **5.2. Comparing the efficiency of GnRH analogues in oocyte donation cycles**

### ***5.2.1. Comparison between a GnRH antagonist and a GnRH agonist flare-up protocol in egg donors: a randomized clinical trial***

A randomized clinical trial, approved by the local Ethics committee, was performed between August 2004 and May 2005 in a private fertility centre. Inclusion criteria were as follows: the oocyte donors were between 18 and 30 years old had a BMI less than 30 kg/m<sup>2</sup>, regular menstrual cycles (26-36 days) and normal basal FSH and LH levels. Donors with a previous history of low response to ovarian stimulation or with polycystic ovaries were excluded. One hundred eighteen oocyte donors that fulfilled eligibility criteria and gave their informed consent were prospectively randomized to receive COH with a GnRH antagonist or short GnRH agonist protocol. A total of 113 donors randomly received COH using either GnRH antagonist or GnRH agonist. In the antagonist protocol the ovarian stimulation began with 225 IU of recombinant FSH (Gonal-F, Serono, Madrid, Spain) from day 2 of the menstrual cycle, and the GnRH antagonist (Cetrotide, Serono, Madrid, Spain) (0.25 mg/day) was introduced on the sixth day of the stimulation according to a multiple-dose, fixed protocol. The agonist stimulation protocol consisted of the administration of the GnRH agonist (Decapeptyl, Ipsen Pharma, Barcelona, Spain) (0.1 mg/day) from day 2 of the menstrual cycle followed by 187.5 IU recombinant FSH from day 4 of the cycle.



The primary endpoint was the mean number of mature oocytes retrieved per started donor cycle. Secondary endpoints were the mean number of cumulus-oocyte complexes (COC) retrieved, the mean proportion of mature oocytes, pregnancy and implantation rates in recipients. Oocytes were distributed to 166 recipients. All recipients were <50 years old. All recipients who had ovarian function were down regulated with the administration of a long-acting GnRH agonist (Decapeptyl, i.m. 3.75 mg, Ipsen Pharma, Barcelona, Spain) on the 21<sup>st</sup> day of their cycle. Thereafter, oral oestradiol valerate (Progynova, Schering, Spain) was used in a constant dose regime for endometrial preparation.

In each treatment group 93% of the started cycles resulted in oocyte donation. In twenty-seven antagonist (50%) and in twenty-three agonist (45%) cycles oocytes could be donated to more than one recipient. One donor presented a moderate ovarian hyperstimulation syndrome (OHSS) in the antagonist group. Oocytes were distributed to 166 recipients; 87 in the antagonist group and 79 in the agonist group. Embryo transfer was performed on day 2 in 53 out of the 86 recipients (62%) in the antagonist group, and in 50 out of the 76 recipients (66%) in the agonist group. In the rest of the recipients reaching embryo transfer, the procedure was performed on day 3. There was no significant difference observed in the antagonist and agonist groups, in the mean number ( $\pm$ SD) of COC ( $11.6\pm 5.8$  versus  $12.1\pm 6.7$ ), and mature oocytes retrieved ( $8.4\pm 4.4$  versus  $8.9\pm 5.3$ ) per started donor cycle (including cancelled cycles where no oocytes were retrieved). Similar proportion of mature oocytes ( $70.8\pm 23.8$  % versus  $75.7\pm 14$  %), and similar fertilization rates ( $65.5\pm 22.8$  % versus  $67.2\pm 20.8$  %) after ICSI were observed. The mean number of cleavage-state ( $3.01\pm 1.34$  versus  $3.38\pm 1.46$ ) and transferred embryos ( $1.92\pm 0.38$  versus  $1.92\pm 0.39$ ), as well as the quality of transferred embryos were almost identical. The clinical (40.2% versus 45.6%) and ongoing pregnancy (32.2% versus 37.9%) rates expressed per attributed recipient

(including recipients without transfer for fertilization failure or bad-quality embryos), as well as per started donor cycle (including cancelled donor cycles) were comparable in both study groups. There was also no significant difference observed in implantation (26.1% versus 30.1%) or miscarriage rates between the two groups.

This was the first study on the comparing two short COH protocols among oocyte donors; the short GnRH agonist versus the antagonist protocol. This study suggests that ovarian response; embryo development, pregnancy and implantation rates are comparable among short GnRH agonist and antagonist protocols in oocyte donation programs. The only difference found between the two study groups was the significantly higher serum oestradiol levels in the agonist group. In conclusion, this study shows that in oocyte donation cycles both the short GnRH agonist and antagonist protocols appear to be similar in ovarian response and embryo quality and comparable in terms of recipients' pregnancy and implantation rates. The GnRH antagonist protocol could be the protocol of choice for ovarian stimulation in oocyte donation cycles, especially if the risk of OHSS could be reduced by the triggering of ovulation with a GnRH agonist.

### ***5.2.2. GnRH agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: a systematic review and meta-analysis***

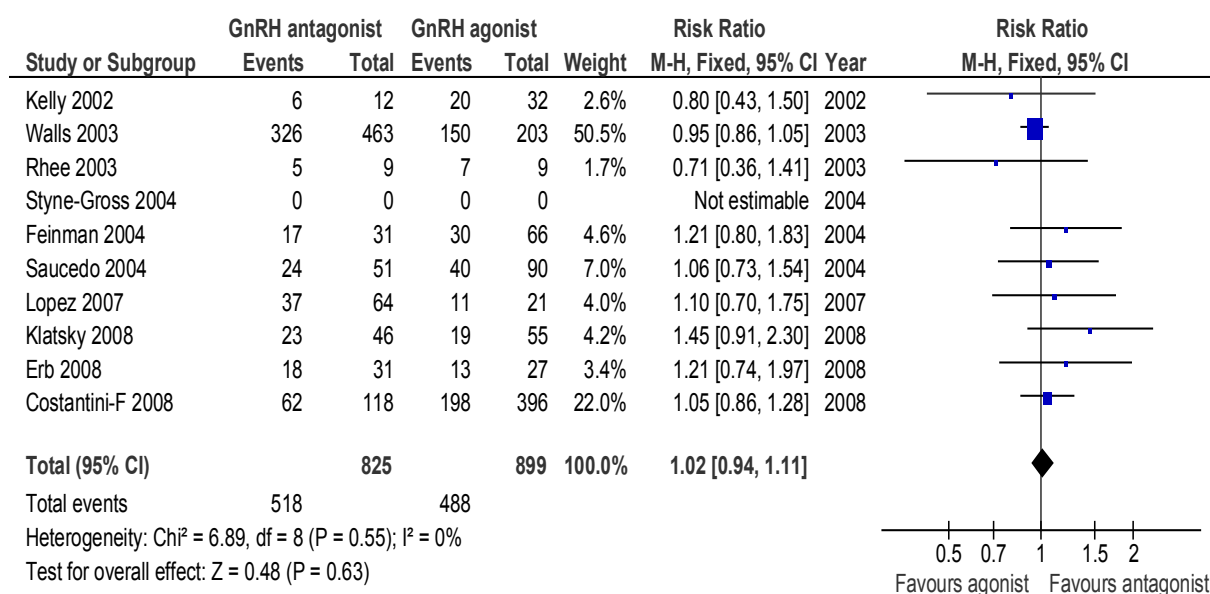
Since the introduction of GnRH antagonists in the clinical practice several randomized clinical trials were performed comparing this newer protocol with the classical long agonist protocol which was the established gold standard stimulation protocol used in the general IVF population. In an early meta-analysis including five trials (Al-Inany and Aboulghar 2001) a significantly lower chance of clinical pregnancy was observed in the antagonist arm (OR: 0.79, 95%CI: 0.63-0.99). This

finding had a significant impact on the acceptance and dissemination of GnRH antagonist protocols worldwide. Later an updated meta-analysis including 22 trials (Kolibianakis et al. 2006) concluded that the chance of live birth is not influenced (OR: 0.86, 95%CI: 0.72-1.02) by the type of GnRH analogue used for pituitary suppression. However the evident non-outcome advantages of GnRH antagonists (shorter duration of stimulation, lower gonadotropin consumption, reduced OHSS incidence) clearly outweigh a possible decrease of approximately 5% in live birth rates.

The efficacy of GnRH agonists versus GnRH antagonists in the context of oocyte donation cycles was compared in a recent systematic review and meta-analysis (Article N° 2) included in the present thesis. Although the number of included trials was relatively limited (5 full publications and 3 abstracts) a significant total number of oocyte donors was included in the above-mentioned trials. The methodological quality of included trials was overall good characterized by adequate randomization methods and allocation concealment. Clinical heterogeneity in the donor and recipient population was low whereas some heterogeneity was present in the different stimulation protocols used. The main findings of this systematic review were that no significant differences were observed in the number of retrieved oocytes (WMD: -0.60 oocytes, 95%CI: -2.26 to +1.07) and recipient ongoing pregnancy rates (RR: 1.15, 95%CI: 0.97 to 1.36) per randomized donor. The fact that recipient pregnancy rates were comparable and not influenced by the type of analogue used is further supported by the analysis of controlled retrospective studies (RR: 1.02, 95CI%: 0.94 to 1.11). In fact a total of 9 retrospective studies (mainly abstract form publications) were found in the literature including a total of 1724 recipients. As pointed out in the meta-analysis it is of particular interest that compared to conclusions of meta-analyses performed in the general IVF population recipient pregnancy rates in oocyte

donation cycles seemed to be not worse and even showed a trend to somewhat higher rates. This could be caused by a real difference between non-donor and donor IVF and could suggest a negative effect endometrial effect in non-donor antagonist IVF cycles.

**Figure 4.** Retrospective controlled studies: ongoing pregnancy per transferred recipient



The above findings are largely reassuring and encourage the use of GnRH antagonists in the context oocyte donation for donor stimulation. Beside other non-outcome benefits such as shorter duration of stimulation (WMD: -0.90 days, 95%CI: -1.61 to -0.20) and lower gonadotropin consumption (WMD: -264 IU, 95%CI: -682 to +154) the main implication of the application in oocyte donation cycles is the possibility of inducing the final oocyte maturation with a GnRH agonist instead of hCG.

### **5.3. Single-dose GnRH antagonist protocol in oocyte donation cycles**

One of the ways reducing the burden of treatment caused by multiple daily injections is the development of depot form drugs. Apart from a daily 0.25 mg injection the GnRH agonist cetrotide also exists in an intermediate depot form (3 mg) which has been shown effective in suppressing LH surges during at least 96 hours after its administration (Olivennes et al. 1998). In the general IVF population the single-dose (SD) antagonist protocol was evaluated in several trials comparing it with the long agonist protocol (Olivennes et al. 2000; Roulier et al. 2003; Sauer et al. 2004). In a few trials the two different GnRH antagonist protocols (SD versus MD) were also compared (Ng and Ho 2001; Olivennes et al. 2003; Lee et al. 2005; Wilcox et al. 2005).

In the context of oocyte donation SD antagonist protocol was only used in two small scale studies (Martinez et al.; Erb and Wakim 2008). To our knowledge there are no reports published in the literature comparing the SD and the MD antagonist protocols in egg donation cycles. In a retrospective cohort study included the present thesis we have found that recipient pregnancy rates were not significantly different whereas ovarian response was somewhat higher with SD protocol. This was probably related to the fact that in this non-randomized trial the SD protocol was systematically applied to donors with a higher initial response. Although this inclusion bias diminishes the validity of our findings we could extrapolate that the SD protocol is at least as efficient as the MD protocol in oocyte donors. Furthermore the fact that fewer injections were needed with the SD protocol could increase donor satisfaction and also improve patient compliance.

***5.3.1. Single- versus multiple-dose GnRH antagonist stimulation protocol in oocyte donor cycles: findings of large, retrospective cohort study (unpublished manuscript)***

To our knowledge there are no reports published in the literature comparing the SD and the MD antagonist protocols in egg donation cycles. Therefore the aim of our retrospective review was to compare a large cohort of single-dose GnRH antagonist stimulated cycles with the multiple-dose regimen and to search for opportunities further to improve the SD protocol. All consecutive oocyte donation cycles that reached oocyte retrieval performed between July 2007 and March 2009 were analyzed retrospectively. Donors were stimulated with recombinant FSH (Gonal-F, Serono, Madrid, Spain) or purified hMG (Menopur, Ferring, Madrid, Spain) from day 2 of their menstrual cycle with an average starting dose of  $211 \pm 37$  IU per day. The first control (ultrasonography and serum estradiol) was normally performed after 5 days of stimulation and the daily gonadotrophin dose was adjusted individually according to the ovarian response. In the MD group the GnRH antagonist (Cetrotide, Serono, Madrid, Spain) was introduced according to a flexible multiple-dose protocol (0.25 mg/day) when a leading follicle of 14 mm and/or an estradiol level of 400 pg/ml were present. In the SD group the GnRH antagonist was introduced according to the same flexible criteria. A single-dose of GnRH antagonist 3 mg was injected at the clinic. When needed, further daily doses of GnRH antagonist (0.25 mg) were administered 96 hours after the first single-dose injection until and including the day of triggering. The choice between the two stimulation regimens was decided by the physician who performed the first control. Due the absence of previous experience no specific clinic policy existed on the application of the SD protocol however it was preferentially used in cycles with a somewhat higher initial response as detected at the first control. The last dose of the daily GnRH antagonist was injected  $\leq 24$  hours before triggering. Triggering

was performed when at least three follicles of  $\geq 18$  mm were present, either with 250  $\mu$ gr. recombinant human choriogonadotrophin (rhCG) (Ovitrelle, Serono, Madrid, Spain) or with 0.2 mg of triptorelin s.c. (Decapeptyl, Ipsen Pharma, Barcelona, Spain). The triggering agent was chosen by the physician who performed the last control taking into account the total number of follicles and/or the final serum estradiol level. Donors at high OHSS risk received the GnRH agonist exclusively. The oocyte retrieval was scheduled 36 hours after triggering. The primary outcome measures were the duration of stimulation, total FSH dose and the number of GnRH antagonist injections, final E2 levels and follicular count, the number of retrieved oocytes (COC and MII) and the fertilization rate. Secondary outcome measures were clinical and ongoing pregnancy per embryo transfer and the miscarriage rate in recipients.

A total of 2101 stimulation cycles performed on 1003 donors were analyzed retrospectively. Six-hundred and four cycles (28.7%) and 1497 cycles (71.3%) were stimulated with the SD and MD protocol, respectively. The SD protocol was preferentially used in cycles with a somewhat higher initial response as detected at the first control. The duration of ovarian stimulation and the total FSH dose was slightly lower in the SD group, whereas the final estradiol level and follicular count at the last ultrasound were significantly higher in the SD group. The number of retrieved oocytes (COC and mature) was significantly higher in the SD group (a crude difference of 2.71 COCs). There was no difference in the proportion of mature oocytes or fertilization rates between the two groups. In order to control for confounding variables (such as the initial higher response and the type of gonadotropin used) the number of retrieved oocytes (outcome of interest) was adjusted for confounding factors in a multiple regression analysis. This showed that the effect of the type of GnRH antagonist protocol was less important (1.15 COCs in favour of the SD protocol) than the initially observed crude difference in

the number of retrieved oocytes. Due to the depot GnRH antagonist formulation used in the SD protocol fewer injections were administered compared to the MD regimen (2.3 versus 5.2). In the SD group the majority of the donors (71%) needed further daily GnRH antagonist injections (ranging from 1 to 7 vials). In these cycles the follicular count was also higher and more oocytes were retrieved ( $p=0.02$ ). The proportion of cycles with a single antagonist injection has increased from 24 to 49% by the delayed the initiation of the GnRH antagonist. Mature oocytes from the SD and MD donor group were attributed to 1104 and 2314 recipients, respectively. Embryo transfer ensued in 1074 and 2264 recipients. Embryo transfer was cancelled due to low-quality embryos, fertilization failure or other reasons in 30 (2.6%) and 50 (2.1%) patients, respectively ( $p=0.33$ ). No significant differences were observed in the clinical (49.8% versus 48.5%,  $p=0.67$ ) and ongoing (41.3% versus 40.3%,  $p=0.71$ ) pregnancy rates per embryo transfer between the two recipient groups. Miscarriage rates (17% versus 16.6%,  $p=0.86$ ) were also comparable.

In our large comparative cohort study we have found a statistically significant difference in ovarian response in favour of the single-dose GnRH antagonist protocol. This difference which is probably related to an unwanted bias in applying the SD protocol to donors with a higher initial response was clinically less important after adjusting for confounding variables in a multivariate analysis. No difference was detected in fertilization rates and subsequent recipient pregnancy rates according to the type of antagonist protocol used. In our study in the majority of the cycles the GnRH antagonist was introduced on the 6<sup>th</sup> day of stimulation. Mainly due to this fact most donors needed further daily antagonist injections and therefore in the present study the “simplifying” effect of the SD protocol was less pronounced. The significant reduction of the number of GnRH antagonist injections (preferably to only one) is of great interest in oocyte donor



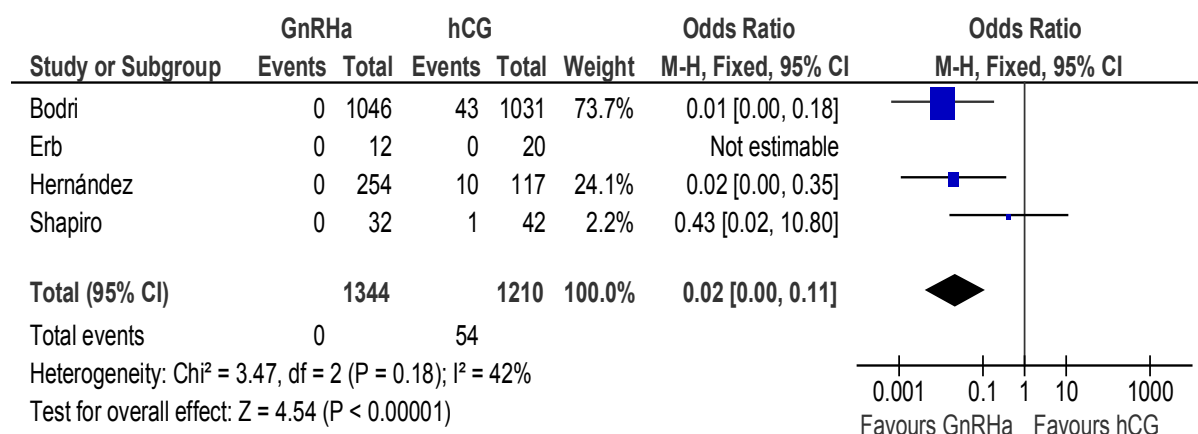
cycles because it can largely simplify the treatment, reduce the inconvenience caused by multiple injections and increase donor satisfaction. It could potentially also increase patient compliance if the single shot is given in the clinic at the time of a scheduled ultrasound control. This could be achieved with delayed introduction of the GnRH antagonist beyond the 6<sup>th</sup> stimulation day and/or prolonging the “cover-period” of the depot GnRH antagonist up to 5 days. This hypothesis however still remains to be verified by a randomized clinical trial. In our retrospective series in the SD group with the delayed introduction of the GnRH antagonist the proportion of cycles with a single antagonist injection has risen proportionally, whereas outcome remained comparable.

Although the cost-effectiveness of the SD protocol has not been formally evaluated in our study, we have estimated that in our study group the cost of the GnRH antagonist was an average 85€ higher compared to the MD regimen (the cost of other drugs used such as gonadotropins was not taken into account). Patient-friendliness or patient compliance was not evaluated in our study however we could assume that fewer injections are less cumbersome to donors. The main limitation of our study include its retrospective nature and the fact the choice between the two stimulation regimens was done in an uncontrolled manner depending on the physician’s choice. Due to the absence of an initial experience with the SD protocol this choice was apparently biased to donor cycles with a higher initial response. Although this fact had a major influence on our findings the present cohort still represents the largest published clinical experience with the use of the SD protocol in oocyte donors and suggests that in the context of oocyte donation the SD GnRH antagonist protocol could be as successfully used as the MD protocol. Modifying the SD protocol with delayed introduction in the late follicular phase may further decrease the number of required GnRH antagonist injections thus making this regimen even more donor-friendly. A prospective randomized

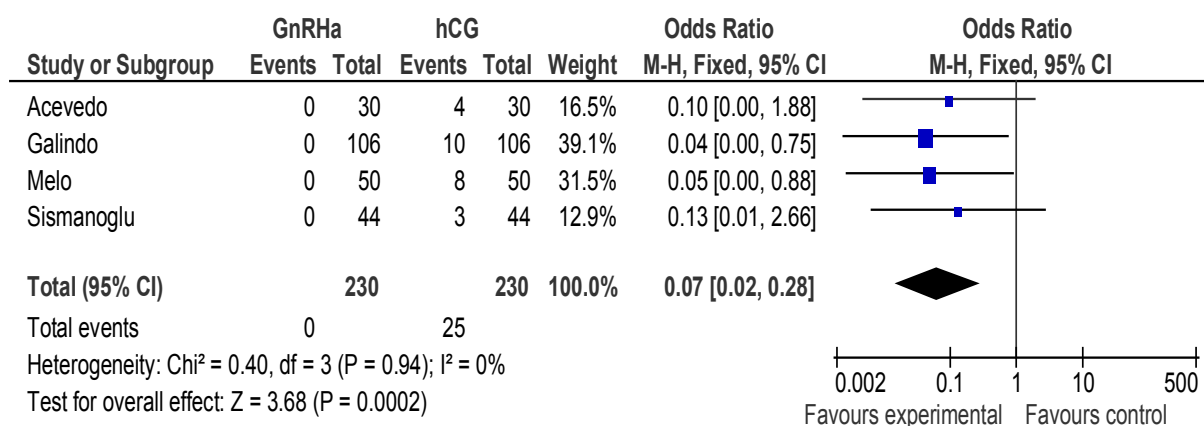
study is warranted to evaluate more precisely the differences between the single-dose and multiple-dose GnRH antagonist protocols.

#### **5.4. GnRH agonist triggering in oocyte donation cycles**

Several retrospective cohort studies (Erb et al.; Shapiro et al. 2007; Bodri et al. 2009; Hernandez et al. 2009) evaluated the outcome of oocyte donation cycles after the newly available agonist trigger. Although they varied largely in size (ranging from 32 to 2077 cycles) all of them were concordant in finding no significant differences in the key outcome variables such as the proportion of mature oocytes, fertilization rates and subsequent recipient implantation and pregnancy rates. More importantly no moderate/severe OHSS cases at all were detected after agonist trigger whereas in the hCG group its incidence reached as high as 5.7%. Pooled data from retrospective series showed (Figure 5.) that in a total of over 2.500 oocyte donation cycles the incidence of OHSS (all degrees) was considerably reduced by 98% (OR: 0.02, 95%CI: 0.00-0.11). An observational follow-study (Article N° 2.) included in the present thesis has even suggested that it is completely eliminated after GnRH agonist triggering. The previously mentioned retrospective series had an inherent bias between the examined treatment groups because agonist triggering was preferentially applied to cycles with a much higher ovarian response.

**Figure 5.** OHSS incidence in oocyte donors: retrospective cohort studies

A number of methodologically more appropriate randomized clinical trials (Acevedo et al. 2006; Galindo et al. 2009; Melo et al. 2009; Sismanoglu et al. 2009) have evaluated the same variables as retrospective series. They included a total number of oocyte donors ranging from 60 to 212 per study. No significant difference was observed in the number of retrieved oocytes (total and mature), fertilization rates, embryo quality and pregnancy rates in corresponding recipients. OHSS cases only occurred in only the hCG arm (between 9-17%). Pooled data from RCTs series showed (Figure 6.) that in a total of 460 oocyte donation cycles the incidence of OHSS (all degrees) was considerably reduced by 93% (OR: 0.07, 95%CI: 0.02-0.28). Hence data from both retrospective cohort studies and controlled clinical trials suggests that GnRH agonist triggering does not adversely affect the quality of retrieved oocytes or the implantation potential of resulting embryos and considerably reduces the incidence of OHSS compared to hCG.

**Figure 6.** OHSS incidence in oocyte donors: randomized clinical trials

#### ***5.4.1. Triggering with human chorionic gonadotropin or a GnRH agonist in GnRH antagonist treated oocyte donor cycles: findings of a large retrospective, cohort study***

The development of a simple and safe treatment protocol is of paramount importance in the setting of oocyte donation where treatment risks for the donor should be minimized as much as possible. In recent years the clinical practice of our oocyte donation program has gradually changed in favor of the use of the GnRH antagonist protocol for donor stimulation (Bodri et al. 2006). This enabled us to introduce the GnRH agonist triggering especially in high-risk patients. The aim of our retrospective study was to compare the proportion of mature and fertilized oocytes per donor cycle; to compare the clinical, ongoing pregnancy and implantation rates in recipients and to determine the incidence of moderate/severe OHSS on a large cohort of oocyte donation cycles. All consecutive oocyte donation cycles performed in a private fertility center in which donors were treated with a GnRH antagonist stimulation protocol and reached oocyte retrieval were analyzed retrospectively. The study period was between November 2003 and March 2007. In our center during the study period the GnRH antagonist protocol was used in

the majority of oocyte donor cycles (72%). Triggering was performed when at least three follicles of  $\geq 18$  mm were present, either with 250  $\mu$  gr. recombinant human choriogonadotrophin (rhCG) (Ovitrelle, Serono, Madrid, Spain) or with 0.2 mg of triptorelin s.c. (Decapeptyl, Ipsen Pharma, Barcelona, Spain). The triggering agent was chosen by the physician who performed the last control taking into account the total number of follicles and/or the final serum estradiol level. Primary outcome measures were the proportion mature (MII) and fertilized oocytes per donor cycle as well as clinical and ongoing pregnancy rate, implantation and miscarriage rate in recipients. Secondary outcome measure was the incidence of moderate/severe ovarian hyperstimulation syndrome (OHSS) in oocyte donors.

A total number of 2077 stimulation cycles that reached oocyte retrieval performed on 1171 donors were analyzed retrospectively. For the statistical analysis only the first cycle of every donor were included (1171 cycles). In 624 stimulation cycles the triggering agent was rhCG, whereas in 547 cycles a GnRH agonist was used to induce final oocyte maturation. Final estradiol levels, the follicular count at the last ultrasound, the number of retrieved oocytes (COC and mature) were higher in the group triggered with a GnRH agonist. The proportion of mature (MII) oocytes was not significantly different. The difference in fertilization rates after ICSI reached statistical significance ( $p=0.003$ ), whereas fertilization failure occurred at a comparable rate in the two groups. Mature oocytes were attributed to 763 and 878 recipients, respectively. Embryo replacement ensued in 718 and 840 cycles, respectively. No significant differences were observed in the number and the quality of transferred embryos or clinical and ongoing pregnancy rates between the two groups. Implantation and miscarriage rates were also comparable. There were no differences observed in twinning and triplet rates between the two groups. Moderate/severe ovarian hyperstimulation syndrome was diagnosed in 13 donors. OHSS occurred only in cases where rhCG was used as a

triggering agent. Ultrasound and/or clinical evidence of ascites were found in all patients. Hemoconcentration ( $Hct \geq 45$ ) was found in six patients, whereas elevated liver enzymes were found in five cases. Six patients required hospitalization; four of them were severe OHSS cases. Consequently, the incidence of moderate/severe OHSS in the group triggered with rhCG was found to be 1.26% (13/1031) (95%CI: 0.74-2.15), whereas no cases (0/1046) (95%CI: 0.00-0.37), were observed in the group triggered with the GnRH agonist.

In our retrospective, cohort study when comparing two large groups of GnRH antagonist- treated donor cycles triggered with two different pharmacological agents we have found no significant difference in the proportion of mature oocytes per donor cycle, whereas the difference in fertilization rates reached statistical significance. Furthermore, measures characterizing recipient outcome (clinical and ongoing pregnancy rates and implantation and miscarriage rates) were statistically also not different. We did however observe a significantly lower incidence of moderate/severe OHSS in donor cycles triggered with the GnRH agonist. Although in our study the differences in fertilization rates between the two groups reached statistical significance (65% vs. 69%,  $p=0.003$ ) this is probably a chance finding due to the large sample size and seems not to have any clinical significance by not really affecting recipient outcome. The above findings suggest that oocyte and subsequent embryo quality is not altered by GnRH agonist triggering and that pregnancy rates are similar when replacing embryos to a recipient's endometrium unaffected by the pronounced corpus luteum insufficiency observed in IVF patients. The current study has several limitations mainly due to its retrospective nature. At the beginning of the study period no clinical experience was yet available as to the preventive effect of GnRH agonist triggering on OHSS, therefore the distinction between low- and high-risk groups was made on a somewhat arbitrary basis. The choice of the triggering agent could also be influenced by the

individual physician who scheduled the oocyte pick-up procedure depending on his/her personal experience. Furthermore, as doctors were not blinded for the triggering agent used when attending donors who presented for check-up after OPU, it could influence their readiness to diagnose OHSS. The calculated incidence of OHSS could also be influenced by co-interventions such as coasting, although this was used in only a very small proportion of cases. In our opinion even if the data of current study were obtained from the everyday clinical practice and not from a thoroughly designed randomized clinical trial, the retrospective evaluation of so large a cohort is still justified.

In conclusion the findings of our retrospective study from a cohort which is to our knowledge is the largest published in the literature so far, contribute to the growing body of evidence which supports the hypothesis that oocyte developmental potential and embryo quality is not affected by using the GnRH agonist for final oocyte maturation. Due to the observed significantly lower occurrence of moderate/severe OHSS in egg donors this particular stimulation protocol could be preferentially used in high-responder donors, thus minimizing treatment risks. Prospective randomized studies of a larger-size are still warranted to evaluate more precisely the effectiveness of GnRH agonist triggering in a more homogeneous population of donor cycles, which include normal responders.

#### ***5.4.2. Triggering with HCG or GnRH agonist in GnRH antagonist treated oocyte donation cycles: a randomized clinical trial***

A prospective, randomized, open-label study was performed between September 2005 and March 2007 in a private fertility centre. Pregnancy follow-up was conducted until November 2007. The oocyte donors were between 18 and 35 years old and had a BMI less than 30 kg/m<sup>2</sup> and regular (26-35 days) menstrual cycles. Donors with a previous history of low response to ovarian stimulation, with

polycystic ovaries or using oral contraceptive pills were excluded. In the antagonist protocol the ovarian stimulation began with 225 IU of recombinant FSH (Gonal-F, Serono, Madrid, Spain) from day 2 of the menstrual cycle, and the GnRH antagonist (Cetrotide, Serono, Madrid, Spain) (0.25 mg/day) was introduced at the presence of a 14mm leading follicle and/or estradiol level  $\geq 400$  pg/ml according to a multiple-dose, flexible protocol. Triggering was performed when at least three follicles of  $\geq 18$  mm were present, either with 250  $\mu$ gr. recombinant human choriogonadotrophin (rhCG) (Ovitrelle, Serono, Madrid, Spain) or with 0.2 mg of triptorelin s.c. (Decapeptyl, Ipsen Pharma, Barcelona, Spain) according to the randomization procedure. The randomization was performed at the time of the last ultrasound control by a study nurse with sealed, opaque envelopes. Donors with a final estradiol level  $\geq 4.500$  pg/ml and/or  $\geq 20$  follicles  $\geq 14$  mm at their last control were excluded from randomization and triggered with the GnRH agonist because of a high OHSS risk. Donors who needed coasting were also excluded from randomization. Donors were followed-up for two weeks after oocyte retrieval.

Out of 257 oocyte donors that fulfilled eligibility criteria, 212 were prospectively randomized at the last follicular control to receive rhCG or a GnRH agonist for the induction of final oocyte maturation. Forty-five patients were excluded from randomization because of a high OHSS risk according to criteria included in the study design (n=33), coasting (n=5), low ovarian response (n=6) or any mistake during treatment (n=1). All 33 patients who were excluded for high OHSS risk were subsequently triggered with a GnRH agonist. No cases of OHSS were observed in this group. The donor groups were comparable regarding their age and BMI. No differences were observed in stimulation days, total FSH dose, final estradiol levels, the follicular count at the last ultrasound and the number of retrieved oocytes (COCs and mature) between the groups. The proportion of mature (MII) oocytes and fertilization rates after ICSI were comparable, whereas



fertilization failure occurred once in each group. The rate of non-attributed donor cycles (less than three mature oocytes retrieved) was similar. Nine donors had mild and one donor a severe OHSS in the rhCG group. No OHSS cases occurred when the GnRH agonist was used as a triggering agent.

Mature oocytes were attributed to 142 and 132 recipients, respectively. In a total of 72 donor cycles (37%) oocytes were distributed to more than one recipient. Recipient groups were comparable regarding age and indications for oocyte donation. Embryo replacement ensued in 140 and 129 cycles, respectively. Data on recipient outcome according to the triggering agent used in the donor cycle are shown in Table II. No significant differences were observed in the number and the quality of transferred embryos or clinical pregnancy, ongoing pregnancy and live birth rates between the two groups. Implantation and miscarriage rates were also comparable. Although significantly more twins were observed in the GnRH agonist group at the first ultrasound scan ( $p=0.047$ ), this difference was not any more significant at delivery ( $p=0.18$ ).

In the present clinical trial following randomization to triggering with hCG or GnRH agonist no significant differences were observed in donor cycle outcome measures such as the number of retrieved oocytes (COCs and MII), proportion of mature oocytes, fertilization rate and fertilization failure. Moreover recipients' ongoing pregnancy and live birth rates as well as implantation and miscarriage rates were comparable between the two treatment arms. Although the difference in the twinning rate reached low statistical significance (0.047) in favour of the GnRH agonist group, it was probably a chance finding related to sample size. Beside comparable donor and recipient outcomes no OHSS cases were observed in the GnRH agonist group. As a limitation of our study it could be mentioned that mature oocytes were distributed to more than one recipient in approximately one third of

donor cycles. Although this could be considered as a methodological error, the comparison between recipients groups did not show any significant difference. This egg-sharing policy is widely practiced in many large oocyte donation programmes, a fact which is also reflected in most studies on oocyte donation.

In summary, the findings of our randomized clinical trial confirm that the GnRH agonist is as effective as hCG in inducing final oocyte maturation in oocyte donor cycles. Although currently there is no benefit in applying GnRH agonist trigger to normoresponder IVF patients, in the context of oocyte donation, this strategy could still be universally applied. Future ovarian stimulation protocols and current coasting and cancellation criteria for egg donors might be substantially influenced by this fact. Further research could still be conducted on different GnRH agonists as triggering agents or on exploring the safety and the limits of OHSS prevention in egg donors.

### **5.5. Complications and risks related to oocyte donors**

Although serious short term complications are reported to occur at a relatively low rate (<1%) (Sauer 2001; Maxwell et al. 2008), it is universally recognized that ovarian stimulation and oocyte retrieval might involve significant inconvenience, discomfort as well as risk for the donor. Oocyte donors are only exposed to early-onset OHSS only due to the absence of a subsequent pregnancy (Sauer et al. 1996). This risk however should not be underestimated because donors are typically young women selected to have a good ovarian reserve and they frequently yield a large number of oocytes. It was estimated that donors who develop >20 follicles are exposed to a 15% OHSS risk with the possibility subsequent hospital admission (Jayaprakasan et al. 2007).

There is an increasing awareness of possible short term complications (Merlet and Senemaud) as well as for the need of long-term follow-up of oocyte donors. Currently there are only few well defined guidelines which are specifically related to oocyte donors. The American Society for Reproductive Medicine issued a guideline (2008) focused on repetitive oocyte donation in order to limit health risks to the oocyte donor. They suggested that limiting the donor's participation to six stimulated cycles would be reasonable. In fact a similar opinion was also issued by the Catalonian Health Authority in Spain which fixed the same limit of oocyte retrievals per donor. The inclusion of new fertility drugs that appear on the market and the modification of existing ovarian stimulation protocols should lead to the development of safer and simpler donor treatments that also maintain the expected good pregnancy outcome for the recipient. By taking into account the specifics of donor stimulation the findings could also be extrapolated to the general IVF population.

#### ***5.5.1. Complications related to ovarian stimulation and oocyte retrieval in 4052 oocyte donor cycles***

All consecutive oocyte donation cycles performed in a private fertility centre in which donors reached oocyte retrieval were analyzed retrospectively. The study period was between January 2001 and October 2007. Data was obtained from an exhaustive review of all medical charts related to oocyte donors. In cases of hospitalization a medical report was obtained from the centre where the patient was treated. Ovarian stimulation protocols were described previously (Bodri, 2006). In the first half of the study period (2001-2003) donor stimulation was performed with the use of GnRH agonists predominantly with the short "flare-up" protocol. In a few cases the "classical" luteal-phase long agonist protocol was also used. From the second half of the study period (2004-2007) the flexible GnRH

antagonist protocol was used increasingly. In the GnRH agonist protocols triggering was performed with 250  $\mu$ g. recombinant human chorionic gonadotrophin (rhCG) (Ovitrelle, Serono, Madrid, Spain). In the GnRH antagonist protocols the triggering agent was chosen by the physician who performed the last control taking into account the total number of follicles and/or the final serum oestradiol level, either recombinant hCG or the GnRH agonist in the form of 0.2 mg of triptorelin s.c. (Decapeptyl, Ipsen Pharma, Barcelona, Spain). Donors were observed in a post-op recovery unit for 2 hours following the procedure and were systematically contacted by phone in the evening following oocyte retrieval. In the case of acute complications donors were admitted to a tertiary university-affiliated centre. After oocyte retrieval, all donors were informed about the possible symptoms of intra-abdominal bleeding or infection as well as about OHSS and its preventive measures. They were also counselled to avoid sexual intercourse until their next menstrual period. From the day of the oocyte retrieval, systematic follow-up was performed by telephone, and if necessary, donors were managed on an outpatient basis until two weeks after the procedure. Primary outcome measures were the incidence of serious complications (intra-abdominal bleeding, ovarian torsion, infection, injury or severe pain) related to the oocyte retrieval requiring hospitalization or outpatient management. The secondary outcome measure was the incidence of moderate/severe ovarian hyperstimulation syndrome (OHSS) in oocyte donors requiring hospitalization or outpatient management.

A total of 4052 stimulation cycles that reached oocyte retrieval performed on 1917 donors were analyzed retrospectively. A total of 1238 cycles (30.6%) were stimulated with the GnRH agonist protocol, whereas in 2814 cycles (69.4%) the GnRH antagonist protocol was used. In the GnRH antagonist treated cycles the

triggering agent was hCG in 1295 cycles, whereas in 1519 cycles a GnRH agonist was used to induce final oocyte maturation.

Fourteen patients (0.35%) were hospitalized due to different complications related to oocyte retrieval, the diagnoses and the received treatments are summarized in Table 1. The mean duration of hospital stay was 2.6 days (range: 0.5-7 days). Intra-abdominal bleeding was diagnosed in a total of fourteen donors (0.35%), five of them required surgery (0.12%), four patients by laparoscopy and one patient by laparotomy. One patient who had surgery received a transfusion. In the remaining nine donors (six of them hospitalized and three followed with outpatient management alone) the bleeding showed to be self-limiting during the observation period and no intervention was necessary. One case of unilateral ovarian torsion (0.024%) occurred and the patient was successfully operated on by laparoscopy keeping her ovary intact. Two patients were hospitalized for pain treatment following oocyte retrieval. No cases of pelvic infection or injury of pelvic anatomical structures were observed in our series. No anaesthesiological complication occurred in the study group. Early-onset moderate/severe OHSS was diagnosed in 22 donors during the first week following oocyte retrieval. OHSS occurred only in cases where rhCG was used as a triggering agent (0.87%). Eight donors were stimulated with the GnRH agonist, while 14 with the GnRH antagonist protocol. Ultrasound and/or clinical evidence of ascites were found in all patients. Haemoconcentration ( $Hct \geq 45$ ) was found in eight patients, whereas elevated liver enzymes were found in nine cases. Eleven patients required hospitalization; six of them were severe OHSS cases. The mean duration of hospital stay was 5.2 days (range: 1-9 days). Ascites puncture was performed by culdocentesis in one patient. All donors recovered completely within two weeks of oocyte retrieval; with outpatient management alone or after hospitalization.

Our retrospective study performed on a large cohort of oocyte donor cycles showed, that a low rate of serious complications can be expected following oocyte retrieval. The most frequent complication was intra-abdominal bleeding which in approximately in one third of the cases required surgical intervention. No cases of pelvic infection or injury of anatomic structures were diagnosed in our study group. Moderate/severe OHSS also occurred at an overall low rate as expected in oocyte donors. Moreover, its pathogenesis was directly related to the type of stimulation protocol used. The substitution of hCG with the GnRH agonist as a triggering agent virtually eliminated the risk of clinically significant OHSS. Our retrospective series showed a clear advantage of GnRH agonist triggering with no cases of moderate/severe OHSS observed at all as compared to donors treated with the hCG triggered stimulation protocols. Moreover, even in the hCG triggered group the rate of OHSS remained relatively low (0.87%). Interestingly, although it was not the aim of the present study a trend towards a higher rate of OHSS was observed with the use of GnRH antagonists as compared to GnRH agonists (although this was not statistically significant). One of the main limitations of the present study is related to its retrospective nature which has an inherent possibility for bias. However, due to the strict donor follow-up in our centre and to the thorough checking of all medical records during data retrieval we are quite confident that all clinically relevant complications related to donor cycles were registered.

In conclusion, the findings of our retrospective study from a cohort, which is to our knowledge is the largest published to date regarding oocyte donor cycles, confirm that egg-donation is a safe procedure with a low rate of serious complications related to oocyte retrieval. The short-term risks of ovarian stimulation can be further reduced or even completely eliminated using innovative stimulation protocols that are specifically tailored for oocyte donors. Nonetheless

prospective oocyte donors should be thoroughly informed about existing short-term as well as potential long-term risks of the procedure.

### **5.6. Practical consequences of using GnRH antagonist based protocols**

Apart from a significantly reduced OHSS risk additional benefits include the shorter duration of the unsupported luteal phase (4-6 days), reduced ovarian volume (Engmann et al. 2008) and diminished abdominal distension which altogether might substantially decrease the burden of treatment for oocyte donors (Cerrillo et al. 2009). A recent observational follow-up study (Bodri et al.) performed in 102 high OHSS risk oocyte donors examining biochemical and ultrasound signs of early-onset OHSS has even suggested the complete elimination of the syndrome (absence of hemoconcentration or ascites) after agonist triggering. This finding also suggests that currently GnRH agonist triggering is one of the most efficient and powerful ways of preventing OHSS. However caution must be exercised because excessive stimulation of oocyte donors is still not encouraged.

In the context of oocyte donation the application of GnRH agonist triggering has also important practical consequences. Given the fact that OHSS risk is greatly diminished or even eliminated cycle monitoring could become less stringent (i.e. less need for repetitive E2 assays or ultrasound monitoring) and probably could be simplified even further in the future. In addition, under GnRH agonist triggering cycle cancellation or interventions reducing OHSS risk (such as coasting) are entirely unnecessary (Hernandez et al. 2009). All these positive effects would allow a reduction in the workload of medical teams and greatly simplify the everyday management of oocyte donation cycles. It is reasonable to assume that in any

oocyte donation programme the rate of short term complications experienced by oocyte donors (particularly OHSS) would strictly correlate with the proportion of GnRH antagonist stimulated / GnRH agonist triggered cycles used (Bodri et al. 2008). From the point of view of good clinical practice the fact that a protocol exists which can significantly increase the safety and well being of oocyte donors is of great importance.

In summary extensive recent evidence suggests that in the context of oocyte donation the suggested new protocol practically eliminates the risk of any clinically significant OHSS and at the same time maintains good recipient outcome therefore it should be preferentially used for donor stimulation.



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## **6. CONCLUSIONS**

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1. Pooled evidence from available randomized clinical trials showed that in the context of oocyte donation **no differences were observed in the number of retrieved oocytes and recipient pregnancy rates** following donor stimulation with GnRH agonist or antagonist protocols.
  
2. GnRH antagonist protocols allow us the use of a GnRH agonist instead of hCG for the induction of final oocyte maturation. The use of the agonist is associated to a **significant decrease in the incidence of moderate-severe OHSS** in oocyte donors; therefore **OHSS can be practically eliminated** from oocyte donation programmes.



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## **7. FUTURE DIRECTIONS**

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The further simplification of stimulation protocols for oocyte donors is achievable by developing new long-acting drug compounds or exploring new routes (mainly oral) of drug administration.

### **7.1. Corifollitropin alpha**

The development of recombinant DNA technologies recently has permitted the creation of a new recombinant molecule - corifollitropin alpha – the combination of a  $\alpha$ -subunit common to many glycopeptid hormones (FSH, LH, hCG, TSH) and a chimeric FSH  $\beta$ -subunit which was specifically modified to incorporate the carboxy terminal peptide of the hCG  $\beta$ -subunit. Due to this modification this drug has a prolonged half-life compared to wild-type FSH and was found to be able to induce and maintain multifollicular development in phase II and III studies (Fauser, 2009 #36).

Recently a multicentre randomized clinical trial (Devroey et al. 2009) was conducted in a large number (1.506) of 18-36 year old potential normo-responder infertile patients requiring IVF. This impressive double blind, double-dummy, active-controlled, non-inferiority trial has shown that ovarian response was slightly higher (13.7 versus 12.5 COC,  $p=0.001$ ) whereas ongoing pregnancy rates per started cycle were not significantly different (38.9% versus 38.1%,  $p=0.71$ ) between the corifollitropin alfa and recombinant FSH groups. No differences were observed in the duration of stimulation (median 9 days) or the incidence of moderate/severe OHSS (4.1% versus 2.7%,  $p=0.15$ ). The authors concluded that the new corifollitropin-alfa regimen provides similar success rate compared with the current care using the GnRH antagonist protocol. The incidence of OHSS was higher (although not significant) in the corifollitropin-alpha group which could be

related to a higher number of recruited follicles and to the fact that dose-adjustments were not possible during the first 7 days of treatment. In this context it could be hypothesized that if this drug was to be used for donor stimulation the risk of any clinically significant OHSS due to an eventual hyper-response could be simply eliminated by the powerful protective effect of GnRH agonist triggering.

## **7.2. Clomiphene-based stimulation protocols**

Clomiphene citrate (CC) was used for ovulation induction purposes for more than five decades since 1960s (Dickey and Holtkamp 1996). Until the introduction of GnRH agonists CC alone or in combination with gonadotropins was also used for controlled ovarian stimulation for IVF, later however it was rapidly replaced by the more efficient gonadotropin/GnRH analogue-based regimens.

Recently new studies showed that an extended (administration until the day of triggering) CC regimen might be beneficial by effectively suppressing LH surges which occur approximately in 25% non-suppressed gonadotropin-only cycles. In fact it was hypothesised that CC - which is racemic mixture of two isomers with different respective half-lives - might also have differential effects on hypothalamic level. Enclomiphene which has the shorter half-life <24 hours contributes with its anti-oestrogenic activity to the blockage of hypothalamic negative feedback and to a 50% increase in endogenous FSH levels. Furthermore a blockage of positive feedback was also observed with CC administration especially if it was given until the day of triggering.

In the context of minimal stimulation a Japanese group used efficiently this “new” CC-based protocol on a very large scale (Teramoto and Kato 2007) and demonstrated that LH surges occur in <5% of the cycles significantly less compared



to natural IVF cycles. A more recent randomized clinical trial (Al-Inany et al.) performed in IUI patients reached similar conclusions by showing that the incidence of premature LH surge was significantly lower (5.45% versus 15.89%) in the CC group.

Unpublished data from an initial pilot study performed in our centre confirmed the feasibility of a gonadotropin/CC/GnRH agonist triggering protocol for the purpose of a more vigorous stimulation in oocyte donors. The “rediscovering” of CC and its combination with GnRH agonist triggering might provide the base for GnRH analogue-free, simple and cost-effective stimulation protocol in oocyte donors.

### **7.3. Minimal stimulation / natural cycle IVF protocols**

The “ultimate” simplification of donor stimulation could be achieved by substantially reducing or even eliminating the dose of stimulatory gonadotropins by applying minimal stimulation or natural cycle IVF protocols to the donor population. A recent case-report (Kadoch et al. 2009) investigated this option in a healthy 35-years old donor who had undergone a modified natural cycle IVF protocol with the concomitant administration of a daily GnRH antagonist, 150 IU recombinant FSH and oral indomethacin from the day when a leading follicle reached 14 mm. After triggering with hCG a single mature oocyte was obtained from the donor and a a day-2 embryo was transferred to the hormonally prepared recipient resulting in the subsequent live birth of a healthy male infant. Although this treatment option is undoubtedly the least invasive for the oocyte donor it also has inherent drawbacks such as the high cancellation rate of this approach and the need for specialized centres with high motivation and extensive everyday experience in the application natural cycle IVF protocols.



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## **8. ANNEXES**

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**8.1. *Published article:*** Comparison between a GnRH antagonist and a GnRH agonist flare-up protocol in oocyte donors: a randomized clinical trial. *Human reproduction (Oxford, England) 2006;21: 2246-51.*



# Comparison between a GnRH antagonist and a GnRH agonist flare-up protocol in oocyte donors: a randomized clinical trial

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**BACKGROUND:** Little information is available on the outcome of controlled ovarian hyperstimulation (COH) using GnRH antagonist in oocyte donation cycles especially in comparison with the short GnRH agonist protocol. This study was aimed at comparing the two stimulation protocols in oocyte donation (OD) cycles. **METHODS:** A total of 113 donors randomly received COH using either GnRH antagonist or GnRH agonist. The primary endpoint was the mean number of mature oocytes retrieved per started donor cycle. Secondary endpoints were the mean number of cumulus–oocyte–complexes (COCs) retrieved, the mean proportion of mature oocytes, pregnancy and implantation rates in recipients. **RESULTS:** Oocytes were distributed to 166 recipients. The mean number ( $\pm$  SD) of COC ( $11.6 \pm 5.8$  versus  $12.1 \pm 6.7$ ), mature oocytes ( $8.4 \pm 4.4$  versus  $8.9 \pm 5.3$ ) and the proportion of mature oocytes (70.8 versus 75.7%) retrieved per started donor cycle were similar in the antagonist and agonist groups, respectively. The implantation rate (26.1 versus 30.1%), clinical (40.2 versus 45.6%) and ongoing pregnancy rate per recipient cycle (32.2 versus 37.9%) were comparable in antagonist and agonist protocols, respectively. **CONCLUSIONS:** Similar mean number of mature oocytes and comparable pregnancy rates are achieved after OD in which donors received COH using GnRH antagonist or short GnRH agonist protocols.

*Key words:* GnRH agonist/GnRH antagonist/IVF/oocyte donation/ovarian stimulation

## Introduction

The achievement of a simple, safe and cost-effective treatment protocol in controlled ovarian hyperstimulation (COH) is of paramount importance to improve the quality of care in assisted reproduction. It is particularly important in the case of oocyte donors who are giving their oocytes in a voluntary and an altruistic way; and therefore, all efforts should be made to minimize their exposure to unnecessary treatment risks.

GnRH antagonists were introduced into clinical practice in the late nineties and are nowadays widely used (Felberbaum and Diedrich, 2002). The GnRH antagonist protocol, compared with the classical agonist long protocol, is more convenient for the patient because fewer injections are required; the stimulation period can be shortened requiring lower amounts of gonadotrophins with fewer side effects (The European Orgalutran Study Group, 2001). However, some studies have reported a slight decrease in pregnancy and implantation rates in GnRH antagonist cycles (Al-Inany and Aboulghar, 2002).

In oocyte donation (OD) cycles, few small-scale studies are available evaluating the clinical utility of GnRH antagonists in COH (Sauer *et al.*, 1997; Lindheim and Morales, 2003; Ricciarelli *et al.*, 2003; Thong *et al.*, 2003; Vlahos *et al.*, 2005). One study showed a tendency to a lower pregnancy rate in recipients

receiving oocytes from a donor stimulated with a GnRH antagonist protocol compared with a long agonist protocol (Ricciarelli *et al.*, 2003). However, others found no significant difference in pregnancy rate between these two groups (Saucedo de la Lata *et al.*, 2004; Vuong, 2004). The only prospective randomized study available also showed that the clinical pregnancy rate, the implantation rate and the first-trimester abortion rate were similar in the long agonist protocol and antagonist protocol (Prapas *et al.*, 2005).

Owing to the shorter duration of treatment in comparison to long protocols and the lower rate of side effects, antagonist protocols could be the treatment of choice. In our clinical practice, convenience for the oocyte donors has always been a priority; and therefore, agonist short protocols were preferred to the long ones. As antagonist protocols are patient convenient too, we wanted to compare the clinical outcomes of both short protocols as to our knowledge, no information on this subject is available in the literature.

## Materials and methods

### Oocyte donors

A randomized clinical trial, approved by the local Ethics committee, was performed between August 2004 and May 2005 in a private fertility

centre. Inclusion criteria were as follows: the age of oocyte donors were between 18 and 30 years, had a BMI less than 30 kg/m<sup>2</sup>, had regular menstrual cycles (26–36 days) and had normal basal FSH and LH levels. Donors with a previous history of low response to ovarian stimulation or with polycystic ovaries were excluded. OD was performed according to the Spanish Act on Reproduction in a voluntary, anonymous and altruistic manner. A conventional clinical and psychological workup was performed, including karyotype. One hundred and eighteen oocyte donors who fulfilled eligibility criteria and gave their informed consent were prospectively randomized to receive COH with a GnRH antagonist or short GnRH agonist protocol. Allocation was performed by sealed opaque envelopes with consecutive numbers and the use of a computer-generated randomization table. Randomization was performed, by a study nurse, as soon as preliminary testing was completed during the cycle preceding the COH. Donors were included only once in the study.

### Ovarian stimulation protocols

In the antagonist protocol, the ovarian stimulation began with 225 IU of recombinant FSH (Gonal-F, Serono, Madrid, Spain) from day 2 of the menstrual cycle, and the GnRH antagonist (Cetrotide, Serono) (0.25 mg/day) was introduced on the sixth day of the stimulation according to a multiple-dose, fixed protocol. The agonist stimulation protocol consisted of the administration of the GnRH agonist (Decapeptyl, Ipsen Pharma, Barcelona, Spain) (0.1 mg/day) from day 2 of the menstrual cycle followed by 187.5 IU recombinant FSH from day 4 of the cycle. This lower initial dose was chosen to avoid hyperstimulation because of the known initial flare-up effect in this type of protocol. In both groups, the first control (ultrasonography and serum estradiol) was performed after 5 days of stimulation, and the daily dose of FSH was adjusted individually according to the ovarian response. Recombinant HCG (Ovitrelle, Serono) was administered when at least three follicles of  $\geq 18$  mm were present. No previous treatment with oral contraceptives was used for the donors.

Cycles were cancelled for low response if less than four follicles were observed during the ultrasound scans and/or estradiol levels were way below the expected range. Coasting was initiated if estradiol levels  $>6000$  pg/ml and  $>20$  large follicles were observed before HCG administration. Cycles were also cancelled if coasting procedure lasted more than 4 days or if extremely high estradiol levels were observed during stimulation. From the day after the oocyte retrieval, follow-up was performed by telephone, and the donors were seen on an outpatient basis 14 days after the procedure.

### Recipients

A total of 166 recipient cycles were included in the study (some donors gave for more than one recipient). All recipients were  $<50$  years old. Recipients were only included once in the study. Recipient couples in which the male partner had azoospermia were excluded from the study. All recipients who had ovarian function were down-regulated with the administration of a long-acting GnRH agonist (Decapeptyl, i.m. 3.75 mg, Ipsen Pharma) on the twenty-first day of their cycle. Thereafter, oral estradiol valerate (Progynova, Schering, Spain) was used in a constant dose regime for endometrial preparation. Recipients on standby received up to 6 mg a day, and the duration of the treatment varied in accordance with the availability of the oocytes. From the day of the oocyte retrieval, 800 mg of micronized vaginal progesterone daily (Utrogestan, Laboratorio Seid, Barcelona, Spain) were added.

### ICSI procedure and embryo assessment

Cumulus–oocyte–complexes (COC) were recovered 36 h after the administration of recombinant HCG. After the surrounding cumulus

and corona cells were removed, the nuclear maturation of the oocytes was assessed under an inverted microscope. Mature oocyte was defined as an oocyte that expelled the first polar body and remained in the metaphase II stage (MII) of the second meiotic division. Only MII oocytes were injected with motile spermatozoon into the ooplasm. These procedures have been described previously (Van Steirteghem *et al.*, 1993; Joris *et al.*, 1998). Further culture of injected oocytes was performed in 25  $\mu$ l micro drops of culture medium under lightweight paraffin oil. Fertilization was confirmed after 16–18 h by the observation of two distinct pronuclei under an inverted microscope. Developing embryos were classified according to their morphological appearance (Veeck, 1998). Cleaving embryos with less than 50% of their volume filled with nucleate fragments were considered eligible for transfer (Grade 1–4). Cleaving embryos were transferred into the uterine cavity 2 or 3 days after the ICSI procedure.

### Outcome measures

The primary outcome measure was the mean number of mature oocytes retrieved per started donor cycle. The secondary endpoints were the mean number of COC retrieved, the mean proportion of MII oocytes and pregnancy and implantation rates in recipients.

A rise in serum HCG levels on two consecutive occasions from 14 days after transfer indicated pregnancy. Each pregnancy with at least one intrauterine sac revealed by ultrasonography approximately 5 weeks after transfer was considered as a clinical pregnancy. The implantation rate was defined as the ratio of gestational sacs to the number of embryos transferred. Ongoing pregnancy was defined as a viable pregnancy confirmed on an ultrasound scan performed at 12 weeks.

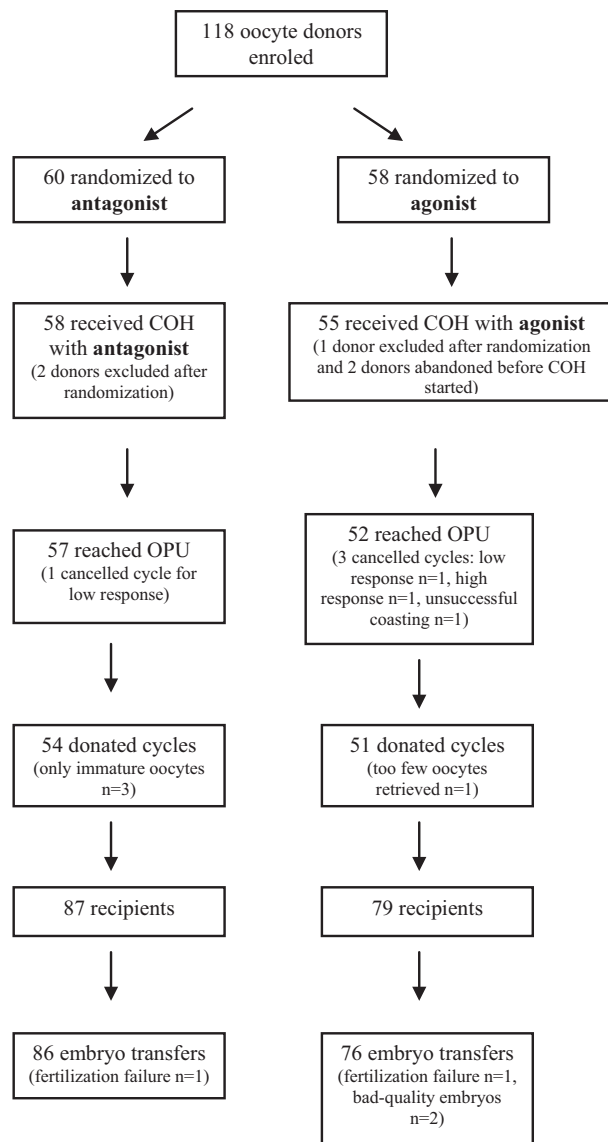
### Statistical analysis

Sample size calculations were based on the assumption that a difference of two mature oocytes in the primary outcome measure would mean a clinically significant difference that could influence the outcome in recipients. Consequently, to achieve this difference, approximately 36 OD cycles would be needed in each treatment arm (with a significance level of 5% and power of 80%, assuming a standard deviation of three oocytes for each group). Power calculations performed after the closure of the trial showed that with the actual standard deviation of approximately five oocytes per group, the study had in fact a 56% power to detect a difference of two oocytes. Values are expressed as mean  $\pm$  SD. The Student's *t*-test and chi-square test were used when appropriate.  $P < 0.05$  was considered statistically significant.

### Results

Of the 118 randomized donors, 113 received the allocated treatment and 109 reached oocyte retrieval (Figure 1). Two donors were excluded after randomization in the antagonist group (one for an altered karyotype and one for an unexpected early pregnancy diagnosed before the start of COH). Three donors were excluded after randomization in the agonist group (one for altered karyotype and two donors abandoned for personal reasons). Details on the age, BMI and basal FSH level of the donors who started COH are summarized in Table I. Of 113 donors who received COH, 24 and 18 had a previous delivery in the antagonist and agonist group, respectively. Only two donors in the agonist group had one previous OD cycle. Of the 113 oocyte donors, 58 received GnRH antagonists and 55 the GnRH agonists. In the antagonist group, one cycle was cancelled for low response, whereas in the agonist group, three





**Figure 1.** Flow chart of patients treated in the study.

cycles were cancelled (one for low response, one for high response and one for unsuccessful coasting procedure that lasted more than 4 days). No recipients could be attributed in three antagonist donor cycles for immaturity of all oocytes and in one agonist donor cycle for insufficient number of oocytes retrieved. In each treatment group, 93% of the started cycles resulted in OD. In 27 antagonist (50%) and in 23 agonist (45%) cycles, oocytes could be donated to more than one recipient. In four antagonist cycles, oocytes were distributed to three recipients, and in one up to four patients. In three agonist cycles, oocytes were donated to three, and in one cycle to four recipients. One donor presented a moderate ovarian hyperstimulation syndrome (OHSS) in the antagonist group. Details on the stimulation of the oocyte donors are summarized in Table I.

Oocytes were distributed to 166 recipients; 87 in the antagonist group and 79 in the agonist group. Details on the recipients'

**Table I.** Description of donors and donor cycle outcomes

	GnRH antagonist	GnRH agonist	<i>P</i>
Number	58	55	–
Age (years)	25.9 ± 3.3	24.7 ± 3.4	0.065 <sup>a</sup>
BMI	22.6 ± 2.8	22.6 ± 3.0	0.92 <sup>a</sup>
Basal FSH (IU/ml)	6.8 ± 1.5	7.3 ± 1.5	0.062 <sup>a</sup>
Days of stimulation <sup>b</sup>	9.9 ± 1.6	10.2 ± 2.1	0.32 <sup>a</sup>
Total rFSH (IU) used <sup>b</sup>	2179 ± 367	1828 ± 459	<0.0001 <sup>a</sup>
Days of antagonist/agonist administration	5.6 ± 1.7	13.3 ± 2.1	–
Estradiol on the day of HCG (pg/ml)	2428 ± 1318	4634 ± 1903	<0.0001 <sup>a</sup>
COC retrieved <sup>c</sup>	11.6 ± 5.8	12.1 ± 6.7	0.69 <sup>a</sup>
Mature oocytes retrieved <sup>c</sup>	8.4 ± 4.4	8.9 ± 5.3	0.52 <sup>a</sup>
Proportion of mature oocytes <sup>b</sup> (%)	70.8 ± 23.8	75.7 ± 14	0.20 <sup>a</sup>

COC, cumulus–oocyte–complex.

Values are mean ± SD.

<sup>a</sup>Student's *t*-test.

<sup>b</sup>Analysis performed in donors who reached the day of HCG.

<sup>c</sup>Per started cycle (including patients with cancelled cycles).

**Table II.** Description of the recipients

	GnRH antagonist	GnRH agonist	<i>P</i>
Number of allocated recipients	87	79	–
Age (years) <sup>a</sup>	39.4 ± 5.4	39.7 ± 5.5	0.78 <sup>b</sup>
BMI <sup>a</sup>	22.5 ± 3.9	22.5 ± 3.9	0.96 <sup>b</sup>
Indication of oocyte donation			
Menopause [ <i>n</i> (%)]	25 (29)	24 (30)	0.099 <sup>c</sup>
Previous failed IVF cycles [ <i>n</i> (%)]	20 (23)	29 (37)	
Reduced ovarian reserve [ <i>n</i> (%)]	40 (46)	26 (33)	
Others [ <i>n</i> (%)]	2 (2)	0 (0)	
Sperm parameters			
Normozoospermia (%)	29 (33)	25 (32)	0.81 <sup>c</sup>
Abnormal sperm parameters <sup>d</sup> (%)	58 (67)	54 (68)	

OD, oocyte donation.

<sup>a</sup>Values are mean ± SD.

<sup>b</sup>Student's *t*-test.

<sup>c</sup>Chi-square test.

<sup>d</sup>Oligo-, astheno-, teratospermia or combination of these anomalies (according to WHO, 1999 Guidelines).

age, BMI, indication for OD and the partner's sperm parameters are summarized in Table II. Twenty recipients (23%) had one to three previous OD attempts in the antagonist group, and sixteen recipients (20%) between one and five previous attempts in the agonist group (*P* = 0.44). Four and five recipients had previous uterine surgery in the two groups, respectively. In the antagonist group, one recipient did not reach embryo transfer because of fertilization failure. In the agonist group, three recipients did not reach embryo transfer; one because of fertilization failure and two because of the absence of good-quality embryos. Embryo transfer was performed on day 2 in 53 of the 86 recipients (62%) in the antagonist group and in 50 of the 76 recipients (66%) in the agonist group. In the rest of the recipients reaching embryo transfer, the procedure was performed on day 3.

There was no significant difference observed in the antagonist and agonist groups, in the mean number (±SD) of COC (11.6 ± 5.8 versus 12.1 ± 6.7), and mature oocytes retrieved (8.4 ± 4.4 versus 8.9 ± 5.3) per started donor cycle (including

**Table III.** Recipient outcome

	GnRH antagonist	GnRH agonist	P
Embryo replacements (n)	86	76	–
Transferred embryos per recipient mean $\pm$ SD	1.92 $\pm$ 0.38	1.92 $\pm$ 0.39	0.97 <sup>a</sup>
Quality of transferred embryos			
Grade 1 [n (%)]	54/165 (32.7)	49/146 (33.6)	0.252 <sup>b</sup>
Grade 2 [n (%)]	37/165 (22.4)	39/146 (26.7)	
Grade 3 [n (%)]	18/165 (10.9)	22/146 (15.1)	
Grade 4 [n (%)]	50/165 (30.3)	31/146 (21.2)	
Clinical pregnancy rate <sup>c,d</sup> [n (%)]	35/87 (40.2) (30.5–50.7)	36/79 (45.6) (35.0–56.5)	0.66 <sup>b</sup>
Clinical pregnancy rate <sup>c,d</sup> [n (%)]	35/58 (60.3) (47.4–71.9)	36/55 (65.4) (52.2–76.6)	0.78 <sup>b</sup>
Implantation rate <sup>d</sup> [n (%)]	43/165 (26.1) (19.9–33.2)	44/146 (30.1) (23.2–38.0)	0.54 <sup>b</sup>
Miscarriage rate <sup>d</sup> [n (%)]	7/35 (20) (10.0–35.9)	6/36 (16.7) (7.9–31.9)	0.76 <sup>b</sup>
Ongoing pregnancy rate <sup>c,d</sup> [n (%)]	28/87 (32.2) (23.3–42.6)	30/79 (37.9) (28.1–49.0)	0.58 <sup>b</sup>
Ongoing pregnancy rate <sup>c,d</sup> [n (%)]	28/58 (48.3) (35.9–60.8)	30/55 (54.5) (41.5–66.9)	0.70 <sup>b</sup>
Twins <sup>d</sup> [n (%)]	8/35 (22.9) (12.1–39.0)	8/36 (22.2) (11.7–38.1)	0.95 <sup>b</sup>
Triplets	0	0	–

<sup>a</sup>Student's *t*-test.<sup>b</sup>Chi-square test.<sup>c</sup>Per recipient cycle (including recipient failures without embryo transfer).<sup>d</sup>With 95% CI.<sup>e</sup>Per started donor cycle (including patients with cancelled cycles).

cancelled cycles where no oocytes were retrieved). Similar proportion of mature oocytes (70.8  $\pm$  23.8 versus 75.7  $\pm$  14%) and similar fertilization rates (65.5  $\pm$  22.8 versus 67.2  $\pm$  20.8%) after ICSI were observed. The mean number of cleavage-stage (3.01  $\pm$  1.34 versus 3.38  $\pm$  1.46) and transferred embryos (1.92  $\pm$  0.38 versus 1.92  $\pm$  0.39), as well as the quality of transferred embryos were almost identical (Table III). The clinical and ongoing pregnancy rates expressed per attributed recipient (including recipients without transfer for fertilization failure or bad-quality embryos), as well as per started donor cycle (including cancelled donor cycles) were comparable in both study groups. There was also no significant difference observed in implantation or miscarriage rates between the two groups (Table III).

## Discussion

This may be the first study on the comparison of two short COH protocols among oocyte donors; the short GnRH agonist versus the antagonist protocol. This study suggests that ovarian response, embryo development, pregnancy and implantation rates are comparable among short GnRH agonist and antagonist protocols in OD programmes. The only difference found between the two study groups was the significantly higher serum estradiol levels in the agonist group.

Two previous small-scale studies showed a negative impact of GnRH antagonist in oocyte donor cycles. One study showed that a decline in serum estradiol level after the antagonist administration resulted in an adverse clinical outcome (Lindheim and Morales, 2003). Ricciarelli *et al.* (2003) compared the antagonist to the long GnRH agonist protocol and reported a non-significant

decrease in pregnancy rate but a significant decrease in implantation rate after the use of a GnRH antagonist. They concluded that their results may suggest that the small decrease in the pregnancy rates seen in the GnRH antagonist donor cycles could be of oocyte or embryonal origin. In contrast, several other studies did not corroborate this negative impact of the GnRH antagonist in donor cycles (Saucedo de la Llata *et al.*, 2004; Vuong, 2004; Prapas *et al.*, 2005). So far, only the work of Prapas *et al.* (2005) was a prospectively randomized study performed on a larger number of patients. The starting dose (300 UI/day) and the mean total FSH used were similar in the antagonist compared with the long agonist group. There was no statistically significant difference in the achieved estradiol levels and the mean number of oocytes retrieved. As a fixed protocol was used with a relatively late introduction of the GnRH antagonist on the eighth day of the stimulation, the authors suggest that the equal reproductive results were because of the short antagonist exposure (1.86  $\pm$  0.73 days), thus avoiding LH over-suppression before its introduction.

Our prospective, randomized study based on a large number of patients was also unable to detect a significant difference between the use of the GnRH agonist or antagonist. Nevertheless, our study fundamentally differs from the previous studies, because the comparison was made between the antagonist and the short agonist protocol. The chosen starting dose difference between the study groups compensated well for the flare-up effect of the short agonist protocol; and consequently, no difference was observed in the ovarian response with similar number of COC retrieved in the antagonist and agonist groups. The high implantation rates observed between the compared groups are in line with findings in the recent studies (Saucedo de la Llata *et al.*, 2004; Vuong, 2004; Prapas *et al.*, 2005).

In this study, the serum estradiol levels reached significantly higher levels (4634  $\pm$  1903 pg/ml) in the agonist short protocol compared with the antagonist protocol (2428  $\pm$  1318 pg/ml), although a lower stimulation dose of gonadotrophins was used ( $P < 0.0001$ ). The negative influence of high estradiol levels on embryo implantation has been investigated in several studies. It has been proposed that high E<sub>2</sub> levels after COH in IVF patients only impair endometrial receptivity, because oocyte quality, fertilization rate and embryo cleavage were normal with high response (Simón *et al.*, 1995), and the quality of embryos and the implantation rate seemed normal in subsequent frozen-thawed embryo transfer cycles (Yu Ng *et al.*, 2000). A retrospective study performed in 330 OD cycles found that sustained supraphysiological estradiol levels do not adversely affect the quality of developing oocytes or embryos; on the contrary, a significantly higher implantation rate was observed in the subgroup with the highest estradiol levels (>3000 pg/ml) (Pena *et al.*, 2002). Our results are in line with these observations as the number of MII oocytes, embryo quality, and embryo implantation is comparable in both protocols, although estradiol levels on the day of HCG are significantly higher in the agonist protocol.

The lower estradiol levels observed in the antagonist protocol can be explained by the fact that GnRH antagonists induce a different pattern of follicular growth compared with the long agonist protocol (Albano *et al.*, 2000; The European

Orgalutran Study Group, 2001). The initial follicular growth is faster, but the final cohort of growing follicles is smaller and produces less estradiol, which is explained by the different endocrine status of the patients at the beginning of the stimulation. The smaller cohort of follicles and the lower estradiol concentrations on the day of HCG are in agreement with a lower incidence of OHSS in the antagonist group (Ludwig *et al.*, 2001).

Previous publications reported a low incidence of severe OHSS in oocyte donors and a lack of requirement of hospitalization. Sauer *et al.* (1996), in a retrospective study of 400 consecutive donors cycles treated with a long agonist protocol, reported a low incidence (1.5%) of severe OHSS despite the fact that 10% of the stimulations resulted in an estradiol level over 5000 pg/ml. Because donors do not undergo embryo transfer and thus avoid pregnancy, it is believed that they were largely spared from further clinical deterioration of a hyperstimulated state. The overall low incidence of OHSS in our donor population is in line with the previously mentioned finding. The number of patients in our study, however, is too small to draw conclusions on this subject.

In non-donation IVF patients, a trend towards a lower pregnancy rate with GnRH antagonists compared with agonists has been observed in almost all randomized controlled studies (Al-Inany and Aboulghar, 2002). The exact cause of the reduced pregnancy rate in the antagonist protocol compared with the long GnRH agonist protocol is still unknown. So far, no studies have found an adverse effect of GnRH antagonists on the developing human follicle (Tarlantzis and Kolibianakis, 2002; Engel *et al.*, 2005). Moreover, Kol *et al.* (1999) showed that the implantation potential of frozen embryos from cycles stimulated with GnRH antagonists is not dependent on the dose of antagonist used, indicating that the adverse effect of the high doses of GnRH antagonists is not exerted on the oocyte but possibly on the endometrium. Furthermore, the study by Kolibianakis *et al.* (2003) also suggests that the adverse effect of the antagonist is based on the endometrium level. OD is an ideal model to help answer this question as the embryos are transferred into an endometrial cavity of recipients undisturbed by the antagonist. Our study shows no difference in the total number of mature COC, the fertilization rate, the number and the quality of transferred embryos. The pregnancy and implantation rates are also comparable in both groups. The results of this study contribute to the growing body of evidence supporting the absence of a negative antagonist influence at an ovarian or embryonic level and support the idea that the lower pregnancy rates observed in non-donation IVF cycles must be a result of the adverse effect of the antagonist on the endometrium.

This study has two limitations. One is a direct consequence of the methodological complexity of randomized clinical trials in the field of OD, because the outcome is measured in a person other than the one who was randomized. In this trial, it is made even more complex, because many of the donors provided oocytes to more than one recipient in the same cycle. The second limitation is that it was insufficiently powered to compare one of the secondary outcome measures i.e. the pregnancy rates in recipients (approximately 900 oocyte donors would be needed in each treatment arm to demonstrate a 5%

difference in recipients' clinical pregnancy rates). Consequently, we preferred to choose the mean number of mature oocytes retrieved per started donor cycle as a primary outcome measure.

In conclusion, this study shows that in OD cycles, both the short GnRH agonist and antagonist protocols appear to be similar in ovarian response and embryo quality and comparable in terms of recipients' pregnancy and implantation rates. The GnRH antagonist protocol could be the protocol of choice for ovarian stimulation in OD cycles, especially if the risk of OHSS could be reduced by the triggering of ovulation with a GnRH agonist. Further studies on this subject are still needed.

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#### Antagonist versus agonist protocol in oocyte donors

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**8.2. *Published article:*** Triggering with chorionic gonadotropin or a gonadotropin-releasing hormone agonist in gonadotropin-releasing hormone antagonist-treated oocyte donor cycles: findings of a large retrospective cohort study. *Fertility and sterility* 2009;91:365-71.



## Triggering with human chorionic gonadotropin or a gonadotropin-releasing hormone agonist in gonadotropin-releasing hormone antagonist-treated oocyte donor cycles: findings of a large retrospective cohort study

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**Objective:** To compare pregnancy rates and the incidence of ovarian hyperstimulation syndrome (OHSS) in donor stimulation cycles where final maturation of oocytes was induced with recombinant hCG or GnRH agonist.

**Design:** Retrospective, cohort study.

**Setting:** Private infertility clinic.

**Patient(s):** A total of 1171 egg donors performing 2077 stimulation cycles.

**Intervention(s):** Controlled ovarian hyperstimulation of egg donors with GnRH antagonist protocol triggered with recombinant hCG (rhCG; 250  $\mu$ g) or GnRH agonist (triptorelin 0.2 mg) based on the physician's decision.

**Main Outcome Measure(s):** Proportion of mature and fertilized oocytes per donor cycle; clinical, ongoing pregnancy and implantation rate in recipients; and incidence of moderate/severe OHSS in oocyte donors.

**Result(s):** The proportion of mature oocytes was comparable, whereas the difference in the fertilization rate reached statistical significance (65% vs. 69%). No significant differences were observed in the implantation rate or clinical and ongoing pregnancy rates per ET. The incidence of moderate/severe OHSS was 1.26% (13/1031; 95% confidence interval [CI], 0.74–2.15) and 0% (0/1046; 95% CI, 0.00–0.37) in the rhCG and GnRH agonist groups, respectively.

**Conclusion(s):** Recipient outcome was not significantly different when using oocytes from GnRH antagonist-treated donor cycles triggered with hCG or GnRH agonist. However, GnRH agonist triggering was associated with a lower incidence of moderate/severe OHSS in egg donors. (Fertil Steril® 2009;91:365–71. ©2009 by American Society for Reproductive Medicine.)

**Key Words:** Oocyte donation, GnRH antagonist, GnRH agonist triggering, OHSS

The substitution of hCG with a GnRH agonist as a triggering agent of final oocyte maturation has been described in a clinical setting from the early nineties (1–5); nevertheless, its widespread use was hampered by the fact that it could be applied only to ovulation induction or non-down-regulated gonadotropin-only IVF cycles. With the advent and increasing use of GnRH antagonist protocols, a renewed interest in GnRH agonist triggering has emerged. Uncontrolled, observational trials described a beneficial effect virtually eliminating ovarian hyperstimulation syndrome (OHSS) in high-risk patients (6, 7). On the other hand, randomized clinical trials (8, 9) performed in normal-responder IVF patients have shown disappointing results in terms of pregnancy rates,

probably due to a pronounced luteal phase insufficiency. Controversy still exists as to the possibility of a modified luteal phase support regimen that could counterbalance the negative effect of GnRH agonist triggering (10–12).

The favorable outcome observed in frozen-thawed embryo replacement cycles supports the hypothesis (13, 14) that oocyte developmental potential and embryo quality remain unaffected after GnRH agonist triggering. This finding is further supported by analyzing the outcome in oocyte donation cycles. To date, only small-scale studies are available (15, 16). These found comparable pregnancy rates but could not evaluate the effect of GnRH agonist triggering on OHSS incidence due to their small sample size.

The development of a simple and safe treatment protocol is of paramount importance in the setting of oocyte donation, in which treatment risks for the donor should be minimized as much as possible. In recent years, the clinical practice of

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our oocyte donation program has gradually changed in favor of the use of the GnRH antagonist protocol for donor stimulation (17). This enabled us to introduce the GnRH agonist triggering especially in high-risk patients. The aim of our retrospective study was to compare the proportion of mature and fertilized oocytes per donor cycle; to compare the clinical, ongoing pregnancy and implantation rates in recipients; and to determine the incidence of moderate/severe OHSS on a large cohort of oocyte donation cycles.

## MATERIALS AND METHODS

### Donors

All consecutive oocyte donation cycles performed in a private fertility center in which donors were treated with a GnRH antagonist stimulation protocol and reached oocyte retrieval were analyzed retrospectively. The study period was between November 2003 and March 2007. In our center, during the study period the GnRH antagonist protocol was used in the majority of oocyte donor cycles (72%). Oocyte donation was performed according to the Spanish Act on Assisted Reproduction. Donation was anonymous and altruistic, and donors were required to be between 18 and 35 years of age. A conventional clinical and psychological workup was performed, including karyotype. Institutional Review Board approval was not required for the present study because of its retrospective nature and because of the fact that study data were constantly managed in a way that excluded the identification of subjects.

### Ovarian Stimulation

Donors were stimulated with recombinant FSH (Gonal-F, Serono; or Puregon, Organon, Madrid, Spain) or purified hMG (Menopur, Madrid, Spain; Ferring, Madrid, Spain) from day 2 of their menstrual cycle with an average starting dose of 225 IU. The first control (ultrasound and serum  $E_2$ ) was normally performed after 5 days of stimulation, and the daily gonadotropin dose was adjusted individually according to the ovarian response. The GnRH antagonist (Cetrotide; Serono, Madrid, Spain) was introduced according to a multiple-dose protocol (0.25 mg/day) when a leading follicle of 14 mm and/or an  $E_2$  level of 400 pg/mL were present. Triggering was performed when at least three follicles  $\geq 18$  mm were present, either with 250  $\mu$ g recombinant hCG (rhCG; Ovitrelle; Serono, Madrid, Spain) or with 0.2 mg of triptorelin SC (Decapeptyl, Ipsen Pharma, Barcelona, Spain). The triggering agent was chosen by the physician who performed the last control, taking into account the total number of follicles and/or the final serum  $E_2$  level. Coasting was initiated when  $E_2$  levels exceeded 6000 pg/mL. Altogether, 33 cycles (2.8%) were coasted; all but two of them were triggered with the GnRH agonist. The outcome of these cycles was not significantly different from the other noncoasted cycles (data not shown). The oocyte retrieval was scheduled 36 hours after triggering. The last dose of the GnRH antagonist was injected  $\leq 24$  hours before triggering. A secular trend was observed in the use of the GnRH agonist triggering with an increasing use during the study period.

Nonetheless, because in our program no significant differences were observed in fertilization, pregnancy, and implantation rates during the examined period (data not shown), the above fact probably did not influence the final results.

### Donor Follow-up and Management

After oocyte retrieval, all donors were informed about the possible symptoms of OHSS and about preventive measures. They were also counseled to avoid sexual intercourse until their next menstrual period. From the day of the oocyte retrieval, systematic follow-up was performed by telephone, and, if necessary, donors were managed on an outpatient basis until 2 weeks after the procedure. Every donor was encouraged to present for check-up if experiencing any symptoms. On consultation, a physical examination, vaginal and abdominal ultrasound scan, and blood analysis (blood count including leukocytes and hematocrit, liver and renal function, ionogram, and coagulation tests) were performed. The outpatient management was bed rest, fluid balance monitoring, and symptomatic therapy for relief of pain and discomfort. OHSS was classified according to criteria described by Navot et al. (18). Donors diagnosed with mild/moderate OHSS were followed up every 48 hours until the complete resolution of the syndrome had been achieved. Donors were hospitalized when there was a high risk of developing severe OHSS or if there was no clinical improvement during outpatient management. Inpatient treatment was conducted according to a previously published conservative medical approach (19).

### Recipients

Before treatment, careful clinical assessment was carried out in the oocyte recipient candidates including a general physical and gynecological examination, blood tests (hematology, biochemistry, and serology), a cervical smear, and a pelvic ultrasound scan. The uterine cavity was assessed by either a hysterosalpingography or hysteroscopy. Oral estradiol valerate (Progynova, Schering Spain, Madrid, Spain) was used in a constant-dose regimen for the endometrial preparation. Patients on standby received up to 6 mg a day, and the duration of the treatment varied in accordance with the availability of the oocytes. From the day of the oocyte retrieval, 800 mg of micronized vaginal P (Utrogestan, Laboratorio Seid, Barcelona, Spain) was added.

### Intracytoplasmic Sperm Injection (ICSI) Procedure and Embryo Assessment

The ICSI procedure was performed routinely to avoid immature oocytes being given to the recipients and to increase fertilization rate. Less than three mature (metaphase II [MII]) oocytes were not attributed to recipients.

Developing embryos were classified according to their morphological appearance using a modification of the combined embryo score described by Coroleu et al. (20), taking into account the number of blastomeres, their form, and the



percentage of cytoplasmic fragmentation, with an optimal quality embryo scoring maximum of 10. Embryos were transferred into the uterine cavity almost exclusively on day 2 or 3 after the ICSI procedure. The ET procedure was performed using abdominal ultrasound guidance.

In case of pregnancy, hormone therapy was continued during the 100 days after the embryo replacement. Each pregnancy with at least one intrauterine sac revealed by ultrasound approximately 5 weeks after transfer was considered to be a clinical pregnancy. The implantation rate was defined as the ratio of gestational sacs to the number of embryos transferred. Miscarriage was defined as a first-trimester pregnancy loss occurring after the first ultrasound scan (sixth to seventh week of pregnancy) but before the confirmation of an ongoing pregnancy. Only pregnancy losses documented earlier by the presence of gestational sac(s) were considered as first-trimester miscarriages, otherwise they were considered as biochemical pregnancies. Ongoing pregnancy was defined as a viable pregnancy confirmed on an ultrasound scan performed at 12 weeks.

### Outcome Measures

Primary outcome measures were the proportion of mature (MII) and fertilized oocytes per donor cycle as well as clinical and ongoing pregnancy rates and implantation and miscarriage rates in recipients. The secondary outcome measure was the incidence of moderate/severe OHSS in oocyte donors.

### Statistical Analysis

For the statistical analysis concerning the primary outcome measures, only the first cycle of every donor was included. For the calculation of OHSS incidence, all donor cycles were taken into account. Values are expressed as mean  $\pm$  SD. Metric variables were analyzed by the independent *t*-test, and nominal variables by the  $\chi^2$ -test. *P* < .05 was considered statistically significant. Although currently no data are available about the exact risk reduction in OHSS related to GnRH agonist triggering (21), it is reasonable to suppose that such an effect would be detectable only in large cohorts. One must take into account the low occurrence of moderate/severe OHSS if clinically not significant mild cases are excluded. The study of Papanikolaou et al. found a 1.2% rate of early-onset moderate/severe OHSS in GnRH-antagonist treated IVF patients triggered with hCG (22). Power calculations performed with data from the present study showed that it was sufficiently powered (at a statistical power of 78% and an alpha-error of 5%) to detect a four-fold difference in the occurrence of clinically significant OHSS.

### RESULTS

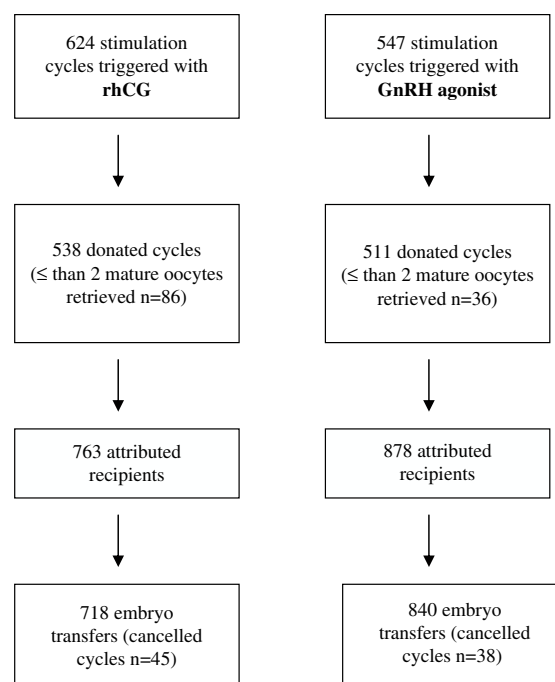
A total number of 2077 stimulation cycles that reached oocyte retrieval performed on 1171 donors were analyzed retrospectively. For the statistical analysis, only the first cycle of every donor was included (1171 cycles).

In 624 stimulation cycles, the triggering agent was rhCG, whereas in 547 cycles, a GnRH agonist was used to induce final oocyte maturation. A flow chart of patient events in the study is depicted in Figure 1. Stimulation data and other measures of donor outcome according to the triggering agent used are shown in Table 1. No differences were observed in stimulation days and total FSH dose between the two groups, whereas the mean donor age was significantly lower in the GnRH agonist group. Final E<sub>2</sub> levels, the follicular count at the last ultrasound, and the number of retrieved oocytes (cumulus-oocyte complexes and mature) were higher in the group triggered with a GnRH agonist. The rate of nonattributed donor cycles (less than three mature oocytes retrieved) was significantly higher in the hCG group. The proportion of mature (MII) oocytes was not significantly different. The difference in fertilization rates after ICSI reached statistical significance (*P* = .003), whereas fertilization failure occurred at a comparable rate in the two groups.

Mature oocytes were attributed to 763 and 878 recipients, respectively. Embryo replacement ensued in 718 and 840 cycles, respectively. Data on recipient outcome according to the triggering agent used in the donor cycle are shown in Table 2. No significant differences were observed in the number and the quality of transferred embryos or clinical and ongoing pregnancy rates between the two groups. Implantation and miscarriage rates were also comparable. There were no

**FIGURE 1**

Flow-chart of study events.



Bodri. GnRHa versus hCG trigger in donor cycles. Fertil Steril 2009.

**TABLE 1****Description of donor cycles according to the triggering agent.**

Triggering agent	rhCG	GnRH agonist	P
No. of cycles	624	547	—
Mean donor age	26.5 ± 4.1	25.5 ± 4.1	<.0001 <sup>a</sup>
Days of stimulation	10 ± 1.68	9.9 ± 1.5	.61 <sup>a</sup>
Total FSH used, IU	2256 ± 536	2175 ± 529	.1 <sup>a</sup>
Final E <sub>2</sub> level, pg/mL	2241 ± 1099	3128 ± 1520	<.0001 <sup>a</sup>
No. of follicles ≥ 18 mm	3.1 ± 1.8	4.1 ± 2.6	<.0001 <sup>a</sup>
No. of follicles ≥ 16 mm	6.0 ± 2.5	8.5 ± 3.6	<.0001 <sup>a</sup>
No. of follicles ≥ 14 mm	9.2 ± 3.8	13.5 ± 4.9	<.0001 <sup>a</sup>
No. of follicles ≥ 10 mm	14.5 ± 6.1	21.5 ± 7.4	<.0001 <sup>a</sup>
No oocytes retrieved, n (%)	3 (0.48%)	6 (1.09%)	.23 <sup>b</sup>
No attributed recipient (≤2 MII oocytes) n, (%)	86 (13.7%)	36 (6.5%)	.0002 <sup>b</sup>
Retrieved oocytes (COC)	9.8 ± 5.8	13.6 ± 7.3	<.0001 <sup>a</sup>
Mature (MII) oocytes	6.9 ± 4.3	9.2 ± 4.8	<.0001 <sup>a</sup>
Proportion of MII oocytes (%)	69.6 ± 24.1	68.9 ± 18	.56 <sup>a</sup>
Fertilized (2PN) oocytes	5.1 ± 3.2	6.8 ± 3.8	<.0001 <sup>a</sup>
Fertilization rate (%)	65 ± 24	69 ± 20.1	.003 <sup>a</sup>
Fertilization failure, n (%)	9 (1.67%)	5 (0.97%)	.33 <sup>b</sup>

Note: Values are mean ± SD.

<sup>a</sup> Independent *t*-test.

<sup>b</sup>  $\chi^2$ -square test.

Bodri. GnRHa versus hCG trigger in donor cycles. *Fertil Steril* 2009.

differences observed in twinning and triplet rates between the two groups.

Moderate/severe OHSS was diagnosed in 13 donors. OHSS occurred only in cases in which rhCG was used as a triggering agent. Ultrasound and/or clinical evidence of ascites were found in all patients. Hemoconcentration (hematocrit ≥45) was found in six patients, whereas elevated liver enzymes were found in five cases. Six patients required hospitalization, and four of them were severe OHSS cases. The mean duration of hospital stay was 7.0 days (range, 4–9 days). Ascites puncture was performed by culdocentesis in one patient. All donors recovered completely within 2 weeks of oocyte retrieval, with outpatient management alone or after hospitalization. Consequently, the incidence of moderate/severe OHSS in the group triggered with rhCG was found to be 1.26% (13/1031; 95% CI, 0.74–2.15), whereas no cases (0/1046; 95% CI, 0.00–0.37) were observed in the group triggered with the GnRH agonist.

## DISCUSSION

In our retrospective, cohort study, when comparing two large groups of GnRH antagonist-treated donor cycles triggered with two different pharmacological agents, we found no significant difference in the proportion of mature oocytes per donor cycle, whereas the difference in fertilization rates reached statistical significance. Furthermore, measures characterizing

recipient outcome (clinical and ongoing pregnancy rates and implantation and miscarriage rates) were also not statistically different. We did, however, observe a significantly lower incidence of moderate/severe OHSS in donor cycles triggered with the GnRH agonist. The rate of donor cycles in which no recipient could be attributed due to the low number of retrieved mature oocytes (less than three) was significantly higher in the hCG group. This could be ascribed to the inherent bias in the follicular count between the two groups as hCG was systematically used in cycles with fewer follicles. Although in our study the differences in fertilization rates between the two groups reached statistical significance (65% vs. 69%; *P*=.003), this is probably a chance finding due to the large sample size and seems not to have any clinical significance because it does not really affect recipient outcome. The above findings suggest that oocyte and subsequent embryo quality is not altered by GnRH agonist triggering and that pregnancy rates are similar when replacing embryos to a recipient's endometrium unaffected by the pronounced corpus luteum insufficiency observed in IVF patients.

GnRH agonists administered in the midcycle are capable of eliciting the gonadotropin surge from the hypophysary, which is necessary to provoke final oocyte maturation (2, 23, 24). The GnRH agonist-induced surge, however, has a shorter duration and a different profile compared with the natural surge or especially compared with hCG administration (2). This difference has also been elegantly demonstrated

**TABLE 2**

<b>Recipient outcome.</b>			
	<b>rhCG</b>	<b>GnRH agonist</b>	<b>P</b>
Number of allocated recipients, n	763	878	—
Cancelled cycles, n	45	38	.16 <sup>a</sup>
Embryo replacements, n	718	840	—
Transferred embryos per recipient, mean $\pm$ SD	1.93 $\pm$ 0.4	1.93 $\pm$ 0.32	.87 <sup>b</sup>
Mean embryo score, mean $\pm$ SD <sup>c</sup>	8.5 $\pm$ 1.2	8.6 $\pm$ 1.1	.3 <sup>b</sup>
Clinical pregnancy rate, <sup>d</sup> n (%) (95% CI)	305/718 (42.4) (38.9–46.1)	326/840 (38.8) (35.5–42.1)	.33 <sup>a</sup>
Implantation rate, <sup>e</sup> n (%) (95% CI)	404/1386 (29.1) (26.8–31.6)	421/1624 (25.9) (23.8–28.1)	0.13 <sup>a</sup>
Miscarriage rate n (%) (95% CI)	55/305 (18) (14.1–22.7)	56/326 (17.1) (13.4–21.6)	.81 <sup>a</sup>
Ongoing pregnancy rate, <sup>d</sup> n (%) (95% CI)	250/718 (34.8) (31.4–38.3)	270/840 (32.1) (29–35.3)	.43 <sup>a</sup>
Twins, <sup>e</sup> n (%) (95% CI)	93/305 (30.4) (25.5–35.8)	93/326 (28.5) (23.9–33.6)	.69 <sup>a</sup>
Triplets, <sup>e</sup> n (%) (95% CI)	3/305 (0.98) (0.34–2.85)	1/326 (0.31) (0.05–1.72)	.28 <sup>a</sup>

<sup>a</sup>  $\chi^2$ -square test.  
<sup>b</sup> Independent *t*-test.  
<sup>c</sup> To calculate the mean embryo score, only cleavage-stage embryos (day 2–3) were taken into account (out of 3010 embryos, 43 compacted embryos and four blastocysts could not be scored with the combined embryo score).  
<sup>d</sup> Per ET.  
<sup>e</sup> According to gestational sacs observed at the sixth – seventh gestational week's ultrasound scan.

*Bodri. GnRHa versus hCG trigger in donor cycles. Fertil Steril 2009.*

at the follicular level by studying follicular fluid obtained during oocyte retrieval (25). The study of luteal phase endocrine profiles also showed significantly lower steroid levels after GnRH agonist triggering as compared with triggering with hCG (26–28). This is due to a quick, irreversible luteolysis that could be mediated by pituitary down-regulation and/or by some other as yet unknown mechanism (15, 29). This fact is of particular interest in the prevention of OHSS as it is universally acknowledged that the syndrome is closely related to the presence of multiple overstimulated corpora lutea. The concept of GnRH agonist triggering has gained wide popularity after demonstrating complete prevention of clinically significant OHSS in high-risk patient groups (6, 7).

Nonetheless, subsequent randomized clinical trials that evaluated pregnancy outcome in IVF cycles dampened the initial enthusiasm (8, 9). Griesinger et al. (21) in their meta-analysis analyzing the three available randomized clinical trials at the time of writing concluded that GnRH agonist triggering yields a comparable number of oocytes capable of undergoing fertilization and subsequent embryonic cleavage as compared with hCG. The proportion of mature oocytes, fertilization rates, and mean embryo score were also comparable. This hypothesis was further supported in a follow-up study of the previously mentioned randomized clinical trials that evaluated live-birth rates after frozen-thawed embryo

replacement cycles (14). Moreover, in a recent proof-of-concept study, a favorable outcome was observed after GnRH agonist triggering and elective cryopreservation in high-risk IVF patients (13). The above-mentioned meta-analysis could not draw any conclusions regarding OHSS incidence from the available data. On the other hand, the prospective study of Babayof et al. detected a significant reduction in OHSS incidence in a high-risk group that was characterized by a majority of patients with polycystic ovarian morphology and high oocyte yield (26).

Until methods are found that prove to be unequivocally efficient in improving outcome in IVF patients after GnRH agonist triggering, as stated in the above-mentioned meta-analysis (21), this approach could still be successfully applied in oocyte donation cycles insofar as it represents a safe treatment concept for the oocyte donor and provides a good outcome for the recipient. So far only small-scale studies have evaluated the outcome of GnRH agonist triggering in oocyte donation cycles (15, 16). The only randomized clinical trial published to date (15) found no significant difference in the number of obtained oocytes, fertilization rates, or recipients' clinical pregnancy and implantation rates. Shapiro et al. (16) found similar results in their retrospective study of comparable size. Although in line with our findings, these studies were insufficiently powered to detect any difference in

clinically significant OHSS. Acevedo et al. (15) with 30 donors in each treatment arm detected a significant difference in favor of the agonist group (4/30 vs. 0/30). Nevertheless, all of their OHSS cases were of a mild degree. Shapiro et al. (16) found only one case of OHSS in the hCG group requiring culdocentesis.

The current study has several limitations mainly due to its retrospective nature. At the beginning of the study period, no clinical experience was yet available as to the preventive effect of GnRH agonist triggering on OHSS; therefore the distinction between low- and high-risk groups was made on a somewhat arbitrary basis. The choice of the triggering agent could also be influenced by the individual physician who scheduled the oocyte pick-up procedure depending on his/her personal experience. Furthermore, as doctors were not blinded to the triggering agent used when attending donors who presented for check-up after oocyte pick-up, it could influence their readiness to diagnose OHSS. The calculated incidence of OHSS could also be influenced by co-interventions such as coasting, although this was used in only a very small proportion of cases. In our opinion, even if the data of current study were obtained from the everyday clinical practice and not from a thoroughly designed randomized clinical trial, the retrospective evaluation of so large a cohort is still justified.

In conclusion, the findings of our retrospective study from a cohort that is, to our knowledge, the largest published in the literature so far contribute to the growing body of evidence that supports the hypothesis that oocyte developmental potential and embryo quality are not affected by using the GnRH agonist for final oocyte maturation. Because of the observed significantly lower occurrence of moderate/severe OHSS in egg donors, this particular stimulation protocol could be preferentially used in high-responder donors, thus minimizing treatment risks. Prospective randomized studies of a larger size are still warranted to evaluate more precisely the effectiveness of GnRH agonist triggering in a more homogeneous population of donor cycles that includes normal responders.

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**8.3. *Published article:* Complications related to ovarian stimulation and oocyte retrieval in 4052 oocyte donor cycles. *Reproductive biomedicine online 2008;17:237-43.***





## Article

# Complications related to ovarian stimulation and oocyte retrieval in 4052 oocyte donor cycles



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## Abstract

A retrospective study was conducted in a private infertility centre to evaluate the rate of complications in a large oocyte donation programme. A total of 4052 oocyte retrievals were performed between January 2001 and October 2007. Altogether, 1238 cycles (30.6%) were stimulated with the use of gonadotrophin-releasing hormone (GnRH) agonists and in 2814 cycles (69.4%) the GnRH antagonist protocol was used. The GnRH antagonist treated cycles were triggered with human chorionic gonadotrophin (HCG) or a GnRH agonist in 1295 and 1519 cycles, respectively. Complications related to oocyte retrieval occurred in 17 patients (0.42%) (intra-abdominal bleeding:  $n = 14$ , severe pain:  $n = 2$ , ovarian torsion:  $n = 1$ ). Fourteen of these were hospitalized (0.35%) and six donors (0.15%) required surgical intervention. Pelvic infections, injury to pelvic structures or anaesthesiological complications were not observed in this series. Moderate/severe ovarian hyperstimulation syndrome (OHSS) occurred in 22 donors; 11 required hospital admission and 11 were managed on an outpatient basis. All cases were related to HCG triggering (0.87%). Serious complications related to oocyte retrieval occurred at a low rate in healthy young donors. The risk of OHSS can be substantially reduced by specific stimulation protocols, which include GnRH agonist triggering. Prospective oocyte donors should be adequately counselled about the risks related to egg donation.

**Keywords:** OHSS, oocyte donation, transvaginal ultrasound-guided oocyte retrieval

## Introduction

Since the advent and worldwide spread of oocyte donation (Lutjen *et al.*, 1984), the indications for this particular treatment option grow continually, with a concomitant increased demand for egg donors. The regulation of egg donation and the availability of egg donors vary considerably between countries. In some, there is a complete prohibition of gamete donation, others allow egg sharing in IVF patients, while others allow the recruitment of altruistic or paid voluntary donors (Sauer and Kavac, 2006). In any case, special effort should be made to minimize the burden of treatment and potential treatment risks for oocyte donors.

Since the description of transvaginal ultrasound-guided oocyte retrieval (Wikland *et al.*, 1985), several observational

studies have evaluated the rate of complications related to this procedure (Bennet *et al.*, 1993; Dicker *et al.*, 1993; Tureck *et al.*, 1993; Roest *et al.*, 1996; Govaerts *et al.*, 1998). These studies have shown that the procedure can be considered as safe, with rates of serious complications varying between 0.02 and 0.3% for intra-abdominal bleeding, 0.01 and 0.6% for pelvic infection and 0.08 and 0.13% for ovarian torsion. Cases of bowel, ureter and pelvic vessel injuries have been described as case reports (Van Hoorde *et al.*, 1992; Bennet *et al.*, 1993; Akman *et al.*, 1995; Roest *et al.*, 1996; Coroleu *et al.*, 1997; Miller *et al.*, 2001; Fiori *et al.*, 2006; Ludwig *et al.*, 2006).

Information is very scarce regarding complication rates in oocyte donors. Sauer (2001), in a retrospective series of 1000

cases, observed seven serious complications, including severe ovarian hyperstimulation syndrome (OHSS), adverse reaction to anaesthetics, intra-abdominal bleeding and bladder atony with haematuria. It is universally acknowledged that despite their young age and potentially high ovarian response, donors have low probabilities of developing OHSS due to the absence of subsequent pregnancy (Sauer *et al.*, 1996). Nevertheless cases involving oocyte donors with severe early-onset OHSS have been reported in the literature (Halme *et al.*, 1995; Sauer, 2000).

With the advent and increasing use of gonadotrophin-releasing hormone (GnRH) antagonist protocols the substitution of human chorionic gonadotrophin (HCG) with a GnRH agonist as a triggering agent of final oocyte maturation became possible. Uncontrolled, observational trials described a beneficial effect, virtually eliminating OHSS in high-risk IVF patients (Itskovitz-Eldor *et al.*, 2000; Kol and Muchtar, 2005). To date, only a few small-scale studies (Acevedo *et al.*, 2006; Shapiro *et al.*, 2007) have evaluated the effect of GnRH agonist triggering in oocyte donation cycles. These were, however, insufficiently powered to detect any difference in clinically significant OHSS. The concept of GnRH antagonist stimulation protocol, combined with GnRH agonist triggering, is of great interest in this patient group (Griesinger *et al.*, 2006), as it represents a safe OHSS-free treatment option for the egg donor and a favourable outcome for the recipient.

In recent years, the clinical practice of oocyte donation programme has gradually changed in favour of the use of the GnRH antagonist protocol for donor stimulation (Bodri *et al.*, 2006), with a concomitant ever increasing proportion of GnRH agonist-triggered cycles. The aim of the present retrospective study was to evaluate the rate of complications related to oocyte retrieval and ovarian stimulation in a large cohort of oocyte donation cycles in order to be able to give precise information concerning risks related to egg donation to prospective oocyte donors.

## Materials and methods

### Oocyte donors

All consecutive oocyte donation cycles that were performed in a private fertility centre in which donors reached oocyte retrieval were analysed retrospectively. The study period was between January 2001 and October 2007. Data were obtained from an exhaustive review of all medical charts related to oocyte donors. In cases of hospitalization, a medical report was obtained from the centre where the patient was treated. Oocyte donation was performed according to the Spanish Act on Assisted Reproduction. Donation was anonymous and altruistic, and donors were required to be between 18 and 35 years of age. A conventional clinical (including coagulation screening tests) and psychological workup was performed, including karyotype. Although it was not the primary aim of the study, a control group was created by taking into account all IVF (not oocyte donor) cycles reaching oocyte retrieval performed during the same period.

### Ovarian stimulation

Ovarian stimulation protocols were described previously (Bodri *et al.*, 2006). In the first half of the study period (2001–2003), donor stimulation was performed with the use of GnRH agonists predominantly with the short ‘flare-up’ protocol. In a few cases, the ‘classical’ luteal phase long agonist protocol was also used. From the second half of the study period (2004–2007) the flexible GnRH antagonist protocol was used increasingly. In the GnRH agonist protocols, triggering was performed with 250 µg recombinant human chorionic gonadotrophin (rHCG) (Ovitrelle; Serono, Madrid, Spain). In the GnRH antagonist protocols, the triggering agent was chosen by the physician, taking into account the total number of follicles and/or the final serum oestradiol concentration, either recombinant HCG or the GnRH agonist in the form of 0.2 mg of triptorelin s.c. (Decapeptyl; Ipsen Pharma, Barcelona, Spain).

### Oocyte retrieval

A single-dose of 1 g of azythromycine was administered as a prophylactic antibiotic on the evening before the intervention (Zitromax; Pfizer, Madrid, Spain). Oocyte retrieval was performed with the transvaginal ultrasound-guided approach using a 17G ovum-aspiration needle (Cook Ireland Ltd, Limerick, Ireland). The ultrasound probe was thoroughly disinfected before and after use and covered with a sterile plastic sheet. The vagina was disinfected with chlorhexidine and thereafter abundantly rinsed with isotonic saline solution. The patient was covered with sterile surgical sheets and the gynaecologist performing the procedure wore sterile surgical gloves. The intervention was performed under general anaesthesia by an anaesthesiologist using intravenous alfentanil (Limifen; Janssen-Cilag, Barcelona, Spain) and propofol (Recofol; Schering, Madrid, Spain) as well as assisted mask ventilation with oxygen and an inhalatory anaesthetic (Sevorane; Abbot, Madrid, Spain). At the end of the procedure, the vagina was thoroughly examined with a speculum and if necessary local compression was applied to allow haemostasis. Donors were observed in a post-operative recovery unit for 2 h following the procedure, and were systematically contacted by telephone in the evening following oocyte retrieval. In the case of acute complications, donors were admitted to a tertiary university-affiliated centre.

### Donor follow-up and management

After oocyte retrieval, all donors were informed about the possible symptoms of intra-abdominal bleeding or infection as well as about OHSS and its preventive measures. They were also counselled to avoid sexual intercourse until their next menstrual period. From the day of the oocyte retrieval, systematic follow-up was performed by telephone, and if necessary, donors were managed on an outpatient basis until 2 weeks after the procedure. Every donor was encouraged to present for check-up if experiencing any symptoms. On consultation, physical examination, vaginal and abdominal ultrasound scan and blood analysis were performed. In the case of OHSS, the outpatient management comprised bed rest, fluid balance monitoring and symptomatic therapy to relieve pain and discomfort. OHSS was classified according

to criteria described by Navot *et al.* (1992). Donors diagnosed with mild/moderate OHSS were followed up every 48 h until a complete resolution of the syndrome had been achieved. Donors were hospitalized where there was a high risk of developing severe OHSS or if there was no clinical improvement during outpatient management. Inpatient treatment was conducted according to a previously published conservative medical approach (Balasch *et al.*, 1996).

## Outcome measures

Primary outcome measures were the incidence of serious complications (intra-abdominal bleeding, ovarian torsion, infection, injury or severe pain) related to oocyte retrieval requiring hospitalization or outpatient management. The secondary outcome measure was the incidence of moderate/severe ovarian hyperstimulation syndrome (OHSS) in oocyte donors requiring hospitalization or outpatient management.

## Statistical analysis

Nominal variables were analysed by the chi-squared test.  $P < 0.05$  was considered statistically significant.

## Results

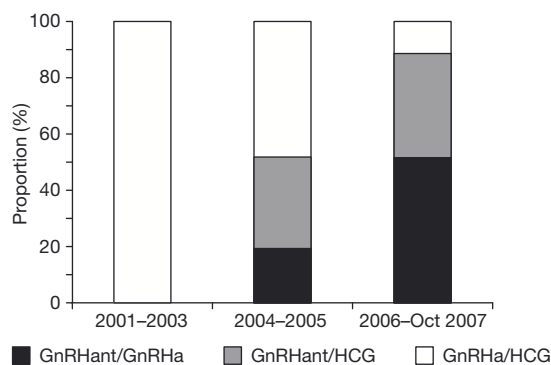
A total of 4052 stimulation cycles that reached oocyte retrieval performed on 1917 donors was analysed retrospectively. A total of 1238 cycles (30.6%) was stimulated with the GnRH agonist protocol, whereas in 2814 cycles (69.4%) the GnRH antagonist protocol was used. In the GnRH antagonist treated cycles the triggering agent was HCG in 1295 cycles, whereas in 1519 cycles a GnRH agonist was used to induce final oocyte maturation. Trends in different stimulation protocols and triggering agents used during the study period are shown in **Figure 1**.

Fourteen patients (0.35%) were hospitalized due to complications related to oocyte retrieval; the diagnoses and the received treatments are summarized in **Table 1**. The mean duration of hospital stay was 2.6 days (range: 0.5–7

days). Intra-abdominal bleeding was diagnosed in a total of 14 donors (0.35%); five of them required surgery (0.12%), four patients by laparoscopy and one patient by laparotomy. One patient who had surgery received a transfusion. In the remaining nine donors (six of them hospitalized and three followed with outpatient management alone), the bleeding showed to be self-limiting during the observation period and no intervention was necessary. One case of unilateral ovarian torsion (0.024%) occurred and the patient was successfully operated on by laparoscopy keeping her ovary intact. Two patients were hospitalized for pain treatment following oocyte retrieval. No cases of pelvic infection or injury of pelvic anatomical structures were observed in this series. No anaesthesiological complication occurred in the study group.

Early-onset moderate/severe OHSS was diagnosed in 22 donors during the first week following oocyte retrieval. OHSS occurred only in cases where rHCG was used as a triggering agent (0.87%). Eight donors were stimulated with the GnRH agonist, while 14 with the GnRH antagonist protocol. Ultrasound and/or clinical evidence of ascites were found in all patients. Haemoconcentration ( $Hct \geq 45$ ) was found in eight patients, whereas elevated liver enzymes were found in nine cases. Eleven patients required hospitalization; six of them were severe OHSS cases. The mean duration of hospital stay was 5.2 days (range: 1–9 days). Ascites puncture was performed by culdocentesis in one patient. All donors recovered completely within 2 weeks of oocyte retrieval, with outpatient management alone or after hospitalization. The incidence of moderate/severe OHSS according to the stimulation protocol and triggering agent used is summarized in **Table 2**.

In the control group, a total of 1047 oocyte retrievals were performed on 736 IVF patients (age range: 21–46 years). Intra-abdominal bleeding was diagnosed in two patients (0.27% of patients, 0.19% of cycles); one patient was operated on by laparoscopy and the other one remained in observation. Moderate/severe OHSS occurred in 15 patients (2.04% of patients, 1.43% of cycles), nine of whom were hospitalized. Eight of the above-mentioned cases were early-onset related to HCG triggering, whereas the late-onset form related to embryonic implantation occurred in seven cases (**Table 3**).



**Figure 1.** Trends in different stimulation protocols and triggering agents used during the study period. GnRH<sub>a</sub> = gonadotrophin-releasing hormone agonist; GnRH<sub>ant</sub> = GnRH antagonist; HCG = human chorionic gonadotrophin.

**Table 1.** Description of donors hospitalized following oocyte retrieval.

<i>Donor</i>	<i>Number of retrieved oocytes</i>	<i>Diagnosis</i>	<i>Treatment</i>	<i>Hospital stay (days)</i>
1	18	Intra-abdominal bleeding	Laparoscopy	3
2	20	Intra-abdominal bleeding	Laparoscopy	3
3	23	Intra-abdominal bleeding	Laparoscopy	6
4	8	Intra-abdominal bleeding	Laparoscopy + transfusion	2
5	16	Intra-abdominal bleeding, acute abdomen	Laparotomy	5
6	19	Intra-abdominal bleeding	Observation	1
7	19	Intra-abdominal bleeding	Observation	1
8	5	Intra-abdominal bleeding	Observation	1
9	4	Intra-abdominal bleeding	Observation	2
10	23	Intra-abdominal bleeding	Observation	3
11	10	Intra-abdominal bleeding	Observation	7
12	30	Ovarian torsion	Laparoscopy	1.5
13	13	Pain	Observation	1
14	7	Pain	Observation	0.5

**Table 2.** The incidence of moderate/severe ovarian hyperstimulation syndrome (OHSS) according to the stimulation protocol and triggering agent used in donor oocyte cycles.

<i>Stimulation protocol/triggering agent</i>	<i>No. of cycles</i>	<i>Moderate/severe OHSS (n)</i>	<i>Incidence % (95% CI)</i>
GnRH agonist/HCG	1238	8	0.65 <sup>a</sup> (0.33–1.27)
GnRH antagonist/HCG	1295	14	1.08 <sup>a</sup> (0.64–1.80)
GnRH antagonist/GnRH agonist	1519	0	0 (0–0.25)

<sup>a</sup>Chi-squared test (not statistically significant).

CI = confidence interval; GnRH = gonadotrophin-releasing hormone; HCG = human chorionic gonadotrophin.

**Table 3.** Complications in the IVF control group (1047 cycles).

<i>Type of complication</i>	<i>No. of cases</i>	<i>Incidence % (95% CI)</i>
Intra-abdominal bleeding	2	0.19 (0.05–0.68)
Early-onset moderate/severe OHSS	8	0.76 (0.39–1.49)
Late-onset moderate/severe OHSS	7	0.66 (0.33–1.37)
Moderate/severe OHSS (all cases)	15	1.43 (0.87–2.34)

CI = confidence interval; OHSS = ovarian hyperstimulation syndrome.

## Discussion

This retrospective study, performed on a large cohort of oocyte donor cycles, showed that a low rate of serious complications can be expected following oocyte retrieval. The most frequent complication was intra-abdominal bleeding, which in approximately in one-third of the cases required surgical intervention. No cases of pelvic infection or injury of anatomical structures were diagnosed in the study group. Moderate/severe OHSS also occurred at an overall low rate, as expected in oocyte donors. Moreover, its pathogenesis was directly related to the type of stimulation protocol used. The substitution of HCG with the GnRH agonist as a triggering agent virtually eliminated the risk of clinically significant OHSS.

Minor vaginal bleeding that stops spontaneously or after local compression can frequently occur following oocyte retrieval in up to 8.6% of cases (Bennett *et al.*, 1993). It rarely requires other measures than local compression for <1 min, although exceptionally vaginal tamponade or suture is applied (Ludwig *et al.*, 2006). On the other hand, intra-abdominal bleeding is a more serious complication that is usually caused by trauma of vessels of the ovarian capsule or bleeding from ruptured follicles. According to the estimation of Dessole *et al.* (2001), an average 230 ml blood loss can be considered normal during the first 24 h after non-complicated transvaginal oocyte retrieval. Retrospective studies observed a rate of intra-peritoneal bleeding or haemoperitoneum varying between 0.02 and 0.3% (Bergh and Lundkvist, 1992; Bennett *et al.*, 1993; Dicker *et al.*, 1993; Tureck *et al.*, 1993; Govaerts *et al.*, 1998), whereas no cases were observed in the prospective study of Ludwig *et al.* (2006). This complication was also described in case reports as a consequence of coagulation disorders such as essential thrombocythemia (El-Shawarby *et al.*, 2004a,b) or factor XI deficiency (Battaglia *et al.*, 2001). A case report by Moayeri *et al.* (2007) described a patient with von Willebrand disease initially undetected by routine coagulation screening tests who had recurrent haemorrhage after oocyte retrieval. The present study included cases not requiring surgical intervention (9 of 14 donors) due to the minor degree of intra-peritoneal blood loss managed by hospital observation or outpatient care alone.

Whereas in the literature the rate of pelvic infections following oocyte retrieval in IVF patients was reported to be between 0.01 and 0.6% (Dicker *et al.*, 1993; Bennet *et al.*, 1993; Tureck *et al.*, 1993; Roest *et al.*, 1996; Ludwig *et al.*, 2006), in the study group no cases were observed. This can probably be explained at least in part by the absence of significant risk factors such as the history of pelvic inflammatory disease or the presence of hydrosalpinges and severe endometriosis among oocyte donors (Moini *et al.*, 2005; El-Toukhy and Hanna, 2006). The benefit of preventive antibiotics seems to be controversial (El-Shawarby *et al.*, 2004a,b), and in the current protocol they were given on an empirical basis.

Adnexal torsion is a very rare but serious complication related to ovarian stimulation. The presence of enlarged and at the same time mobile ovaries is recognized as a predisposing factor. Retrospective series report its incidence between 0.08 and 0.13% (Roest *et al.*, 1996; Govaerts *et al.*, 1998), in some cases involving the loss of the patient's ovary.

Trauma to pelvic structures (bladder, ureter, bowel, large vessels, nerves) caused by the ovum aspiration needle is a potentially severe complication of ultrasound-guided oocyte retrieval. To date, only case reports have been published in the literature. Cases of perforated appendix have been described by several authors (Van Hoorde *et al.*, 1992; Akman *et al.*, 1995; Roest *et al.*, 1996). Several cases of ureter injury have been reported (Coroleu *et al.*, 1997; Miller *et al.*, 2001; Fiori *et al.*, 2006; Ludwig *et al.*, 2006), which often can represent a diagnostic challenge. The much-feared case of a large vessel injury was described in the series of Bennet *et al.* (1993), with favourable resolution by conservative management. In comparison, a massive retroperitoneal haematoma following injury of a sacral vein and requiring surgical intervention was described by Azem *et al.* (2000).

To date, the incidence of OHSS in the context of oocyte donation has only been addressed by a few studies. Sauer *et al.* (1996) reported a 1.5% incidence of severe OHSS in a series of 400 long agonist donor cycles. Despite high peak oestradiol concentrations, no severe complications occurred and hospitalization was not required. This relatively favourable course is due to the absence of a subsequent pregnancy; compared with IVF patients, donors are exposed only to HCG-induced early OHSS. Similarly, Morris *et al.* (1995), in a series of 139 high-risk patients with high oestradiol concentration and oocyte yield, concluded an absence of severe OHSS in the donor group (72 patients) compared with around 10% (6/61) in the IVF patient group. In comparison, in IVF patients the incidence of OHSS is generally estimated to be 3–6% for the moderate and 0.1–2% for the severe form, but there is considerable variation according to different studies and due to the different classifications used (Delvigne and Rozenberg, 2002).

With the advent and the increasing use of GnRH antagonist protocols, considerable interest has emerged in GnRH triggering. Uncontrolled, observational trials described a beneficial effect, virtually eliminating OHSS in high-risk patients (Itskovitz-Eldor, 2000; Kol and Muchtar, 2005). This fact was due to a complete, quick and irreversible luteolysis that followed the flare-up effect of the GnRH agonist (Kol, 2004). To date, only small-scale studies have evaluated the outcome of GnRH agonist triggering in oocyte donation cycles. Due to their sample size, they were insufficiently powered to detect any difference in clinically significant OHSS. Acevedo *et al.* (2006), in a prospective randomized trial with 30 donors in each treatment arm, detected a significant difference in favour of the agonist group (5/30 versus 0/30). Nevertheless, all of their OHSS cases were of a mild degree. Shapiro *et al.* (2007), in a retrospective study of similar size, found only one case of OHSS in the HCG group requiring culdocentesis.

This retrospective series showed a clear advantage of GnRH agonist triggering with no cases of moderate/severe OHSS observed at all, as compared with donors treated with the HCG-triggered stimulation protocols. Moreover, even in the HCG-triggered group the rate of OHSS remained relatively low (0.87%). Interestingly, although it was not the aim of the present study, a trend towards a higher rate of OHSS was observed with the use of GnRH antagonists as compared with GnRH agonists (although this was not statistically significant). Published meta-analyses (Kolibianakis *et al.*, 2006; Al-Inany *et al.*, 2007) do not support the above finding; however, one must take into



account the retrospective nature of the data in comparison with those obtained from randomized clinical trials.

One of the main limitations of the present study is related to its retrospective nature, which has an inherent possibility for bias. However, due to strict donor follow-up and to thorough checking of all medical records during data retrieval, all clinically relevant complications related to donor cycles should have been registered.

In conclusion, the findings of this retrospective study from a cohort, which, as far as is known, is the largest published to date regarding oocyte donor cycles, confirm that egg donation is a safe procedure with a low rate of serious complications related to oocyte retrieval. The short-term risks of ovarian stimulation can be further reduced or even completely eliminated using innovative stimulation protocols that are specifically tailored for oocyte donors. Nonetheless, prospective oocyte donors should be thoroughly informed about existing short-term as well as potential long-term risks of the procedure.

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**8.4. *Published article:*** Triggering with HCG or GnRH agonist in GnRH antagonist treated oocyte donation cycles: a randomised clinical trial. *Gynecol Endocrinol 2009;25:60-6.*



## HCG

# Triggering with HCG or GnRH agonist in GnRH antagonist treated oocyte donation cycles: a randomised clinical trial

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### Abstract

**Aim.** To compare donor and recipient outcome after inducing the final oocyte maturation with hCG or GnRH agonist in GnRH-antagonist treated oocyte donation (OD) cycles.

**Methods.** Two-hundred fifty-seven oocyte donors were enrolled to participate in a clinical trial in a private fertility centre. After stimulation with 225 IU rFSH and Cetrorelix 0.25 mg/day, 212 oocyte donors were randomised with sealed envelopes for triggering with recombinant hCG (Ovitrelle 250 µg,  $n = 106$ ) or a GnRH agonist (triptorelin 0.2 mg,  $n = 106$ ).

**Results.** The number of retrieved COCs ( $12 \pm 6.3$  vs  $11.4 \pm 6.4$ ), mature oocytes ( $8 \pm 4.6$  vs  $7.5 \pm 4.1$ ), the proportion of mature oocytes ( $67.2 \pm 20.4\%$  vs  $67.1 \pm 20.9\%$ ) and fertilisation rates ( $67.8 \pm 23.5\%$  vs  $71.1 \pm 22.1\%$ ) were comparable. Clinical, ongoing pregnancy and live birth rates were not statistically different in the corresponding recipient groups. Nine cases of mild and one case of severe OHSS occurred in hCG group, whereas no cases were detected in GnRH agonist group.

**Conclusions.** The findings of our RCT suggest that donor and recipient outcome are comparable in OD cycles triggered with hCG or a GnRH agonist. Furthermore, the risk of OHSS seems to be reduced considerably, therefore the combination of a GnRH antagonist protocol with GnRH agonist triggering constitutes a safe treatment option for egg-donors.

**Keywords:** In-vitro fertilisation, oocyte donation, GnRH antagonist, GnRH agonist, OHSS

### Introduction

Since the advent and the worldwide spread of oocyte donation (OD) [1], the indications of this particular treatment option grow continually with a concomitant increased demand for egg-donors. The regulation of egg donation and the availability of egg-donors require information of risks related to egg donation to prospective oocyte donors. The safety of these potential donors is one of the goals of all egg-donation programmes. One of the most feared risks of a controlled ovarian stimulation (COS) either in IVF patients or egg donors is the ovarian hyperstimulation syndrome (OHSS). The classical protocols for a COS have been using the GnRH agonists to avoid the spontaneous LH surges and the consequent cancellation of the cycle. In these cases, the final follicle maturation and ovulation triggering have been induced using hCG either purified from pregnant woman urine or the latest recombinant version

available in the market. The latter doesn't seem to represent a substantial change, other than its purity in terms of the final objective, the ovulation [2].

The current availability of drugs like GnRH antagonists with their different action mechanism compared with GnRH agonist (mostly its quick suppression on the endogenous gonadotrophins and its reversibility), allows us to think of other possible options to trigger the final follicle maturation and ovulation, such as inducing a flare-up effect with GnRH agonist. From a theoretical point of view, the ovulation induced by the flare-up effect with the agonist would be a more physiological way (FSH and concomitant LH peak, also the duration of LH surge) compared with the hCG effect [3,4]. This fact could diminish some of the possible complications after triggering of the ovulation. One of these complications, already mentioned, is the OHSS in its different grades (mild, moderate or severe), especially in its early presentation, usually associated with the ovarian

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stimulation, other than the late presentation, usually related with pregnancy [5].

Current evidence seems to show that in the context of GnRH antagonist protocol for COS for IVF patients, the ovulation triggering with GnRH agonist have comparable results in terms of embryological laboratory results but a possible lack in the patient's luteal phase that ends in poorer pregnancy rates, compared with hCG [6–8]. In the context of an egg donation programme, where patients are not going to get pregnant, GnRH agonist triggering seems to provide a simple, safe and effective treatment protocol. To date only retrospective cohort studies [9] or small-scale randomised clinical trials [10] have been published on the effect of GnRH agonist triggering in OD cycles. Our study has been designed to provide more evidence on this subject.

## Materials and methods

### Patients

A prospective, randomised, open-label study was performed between September 2005 and March 2007 in a private fertility centre. Pregnancy follow-up was conducted until November 2007. The oocyte donors were between 18 and 35 years old and had a BMI less than 30 kg/m<sup>2</sup> and regular (26–35 days) menstrual cycles. Donors with a previous history of low response to ovarian stimulation, with polycystic ovaries or using oral contraceptive pills were excluded. OD was performed according to the Spanish Act on Reproduction in a voluntary, anonymous and altruistic manner. In the antagonist protocol, the ovarian stimulation began with 225 IU of recombinant FSH (Gonal-F, Serono, Madrid, Spain) from day 2 of the menstrual cycle, and the GnRH antagonist (Cetrotide, Serono, Madrid, Spain) (0.25 mg/day) was introduced at the presence of a 14 mm leading follicle and/or estradiol level  $\geq 400$  pg/mL according to a multiple-dose, flexible protocol. The first control (ultrasonography and serum estradiol) was performed after 5 days of stimulation and the daily dose of rFSH was adjusted individually according to the ovarian response. Triggering was performed when at least three follicles of  $\geq 18$  mm were present, either with 250  $\mu$ g recombinant human chorionadotrophin (rhCG) (Ovitrelle, Serono, Madrid, Spain) or with 0.2 mg of triptorelin s.c. (Decapeptyl, Ipsen Pharma, Barcelona, Spain) according to the randomisation procedure. The oocyte retrieval was scheduled 36 h after triggering. The last dose of the daily GnRH antagonist was injected  $\leq 24$  h before triggering. Donors could be included only once in the study. The randomisation was performed at the time of the last ultrasound control by a study nurse with sealed, opaque envelopes. Donors with a final estradiol level  $\geq 4.500$  pg/mL

and/or  $\geq 20$  follicles  $\geq 14$  mm at their last control were excluded from randomisation and triggered with the GnRH agonist because of a high OHSS risk. Donors who needed coasting were also excluded from randomisation. Donors were followed-up for 2 weeks after oocyte retrieval. OHSS was classified according to criteria described by Navot et al. [11].

A total of 274 recipients cycles were included in the study (in some cases donor oocytes were given to more than one recipient). All recipients were  $\leq 50$  years old and the main indications of egg donation were premature ovarian failure, reduced ovarian reserve or a history of previous failed IVF cycles. All recipients who had ovarian function were down regulated with the administration of a long-acting GnRH agonist (Decapeptyl, i.m. 3.75 mg, Ipsen Pharma, Barcelona, Spain) in the mid-luteal phase of their cycle. Thereafter oral estradiol valerate (Progynova, Schering, Spain) was used in an initial progressive dosage to a constant dose regimen for the endometrial preparation. Patients on standby received up to 6 mg a day and the duration of the treatment varied in accordance with the availability of the oocytes. From the day of the donor's oocyte retrieval, 800 mg of micronised vaginal progesterone daily (Utrogestan, Laboratorio Seid, Spain) was added.

### ICSI procedure and embryo assessment

The ICSI procedure was performed routinely in order to avoid immature oocytes being given to the recipients and to increase fertilisation rate. Less than three mature (MII) oocytes were not attributed to recipients. Developing embryos were classified according to their morphological appearance using a modification of the combined embryo score described by Coroleu et al. [12], taking into account the number of blastomeres, their shape and the percentage of cytoplasmic fragmentation, with an optimal quality embryo scoring maximum 10. Embryos were transferred into the uterine cavity almost exclusively on day 2 or 3 after the ICSI procedure. The embryo transfer procedure was performed using abdominal ultrasound guidance. In case of pregnancy, hormonal replacement therapy was continued during 100 days following the embryo replacement. Each pregnancy with at least one intrauterine sac revealed by ultrasonography approximately 5 weeks after transfer was considered to be a clinical pregnancy. The implantation rate was defined as the ratio of gestational sacs to the number of embryos transferred. Miscarriage was defined as a first-trimester pregnancy loss occurring after the first ultrasound scan (6–7th week of pregnancy). Ongoing pregnancy was defined as a viable pregnancy confirmed on an ultrasound scan performed at 12 weeks. Live birth was defined as the delivery of a viable infant after the 24th pregnancy week. The study was approved by the

Ethical Committee of our institution and informed consent was obtained from all participants.

#### Outcome measures

Donor outcome measures were the duration of stimulation, total FSH dose, final E2 levels and follicular count at the last ultrasound control, the number of retrieved oocytes (COC and MII) and fertilisation rate. The incidence of mild and moderate/severe OHSS was also registered. Outcome measures in recipients were clinical, ongoing and live birth rates as well as implantation and miscarriage and twinning rates.

#### Statistical analysis and sample size calculations

Values are expressed as mean  $\pm$  SD. Metric variables were analysed by the independent *t*-test or the Mann-Whitney U-test according to their distribution. Nominal variables were analysed by the Chi-square or the Fisher's exact test as appropriate.  $P < 0.05$  was considered statistically significant. According to Daya S [13] when comparing different ovarian stimulation protocols the difference of two mature (MII) oocytes between the groups is usually considered clinically significant. Using data obtained in a previous clinical trial of our group [14] ( $8.4 \pm 4.4$  MII oocytes were obtained in the GnRH antagonist group) sample calculations showed that a total of 212 oocyte donors (106 per treatment arm) would be needed to detect the above-mentioned difference (at a power of 90% and significance level of 5% with a two-tailed test).

#### Results

Out of 257 oocyte donors that fulfilled eligibility criteria, 212 were prospectively randomised at the last follicular control to receive rhCG or a GnRH agonist for the induction of final oocyte maturation. Forty-five patients were excluded from randomisation because of a high OHSS risk according to criteria included in the study design ( $n=33$ ), coasting ( $n=5$ ), low ovarian response ( $n=6$ ) or any mistake during treatment ( $n=1$ ). A flow chart of patient events is depicted in Figure 1. All 33 patients who were excluded for high OHSS risk were subsequently triggered with a GnRH agonist. No cases of OHSS were observed in this group. Stimulation data and other measures of donor outcome according to the triggering agent used are shown in Table I. The donor groups were comparable regarding to their age and BMI. No differences were observed in stimulation days, total FSH dose, final estradiol levels, the follicular count at the last ultrasound and the number of retrieved oocytes (COCs and mature) between the groups. The proportion of mature (MII) oocytes and fertilisation rates after ICSI were comparable,

whereas fertilisation failure occurred once in each group. The rate of non-attributed donor cycles (less than three mature oocytes retrieved) was similar. Nine donors had mild and one donor a severe OHSS in the rhCG group. This patient was diagnosed as having ascitis and elevated liver enzymes and was successfully managed on an outpatient basis. No OHSS cases occurred when the GnRH agonist was used as a triggering agent.

Mature oocytes were attributed to 142 and 132 recipients, respectively. In a total of 72 donor cycles (37%) oocytes were distributed to more than one recipient. Recipient groups were comparable regarding age and indications for OD. Embryo replacement ensued in 140 and 129 cycles, respectively. Data on recipient outcome according to the triggering agent used in the donor cycle are shown in Table II. No significant differences were observed in the number and the quality of transferred embryos or clinical pregnancy, ongoing pregnancy and live birth rates between the two groups. Implantation and miscarriage rates were also comparable. Although significantly more twins were observed in the GnRH agonist group at the first ultrasound scan ( $P=0.047$ ), this difference was not any more significant at delivery ( $P=0.18$ ).

#### Discussion

In the present clinical trial following randomisation to triggering with hCG or GnRH agonist no significant differences were observed in donor cycle outcome measures such as the number of retrieved oocytes (COCs and MII), proportion of mature oocytes, fertilisation rate and fertilisation failure. Moreover recipients' ongoing pregnancy and live birth rates as well as implantation and miscarriage rates were comparable between the two treatment arms. Although the difference in the twinning rate reached low statistical significance (0.047) in favour of the GnRH agonist group, it was probably a chance finding related to sample size. Beside comparable donor and recipient outcomes no OHSS cases were observed in the GnRH agonist group.

The final maturation of oocytes during ovarian stimulation is usually induced with hCG, which is potent surrogate to natural LH. Alternatives such as triggering with LH, native GnRH hormone or the GnRH agonist were proposed to overcome the side-effects of hCG [15]. Since the introduction of GnRH antagonist-based stimulation protocols in the clinical practice a renewed interest emerged in GnRH triggering. It was found that the surge evoked by the agonist has a shorter duration and a different profile, as compared with the natural surge or especially to hCG administration [4]. The study of luteal phase endocrine profiles also showed significantly lower steroid levels after GnRH agonist

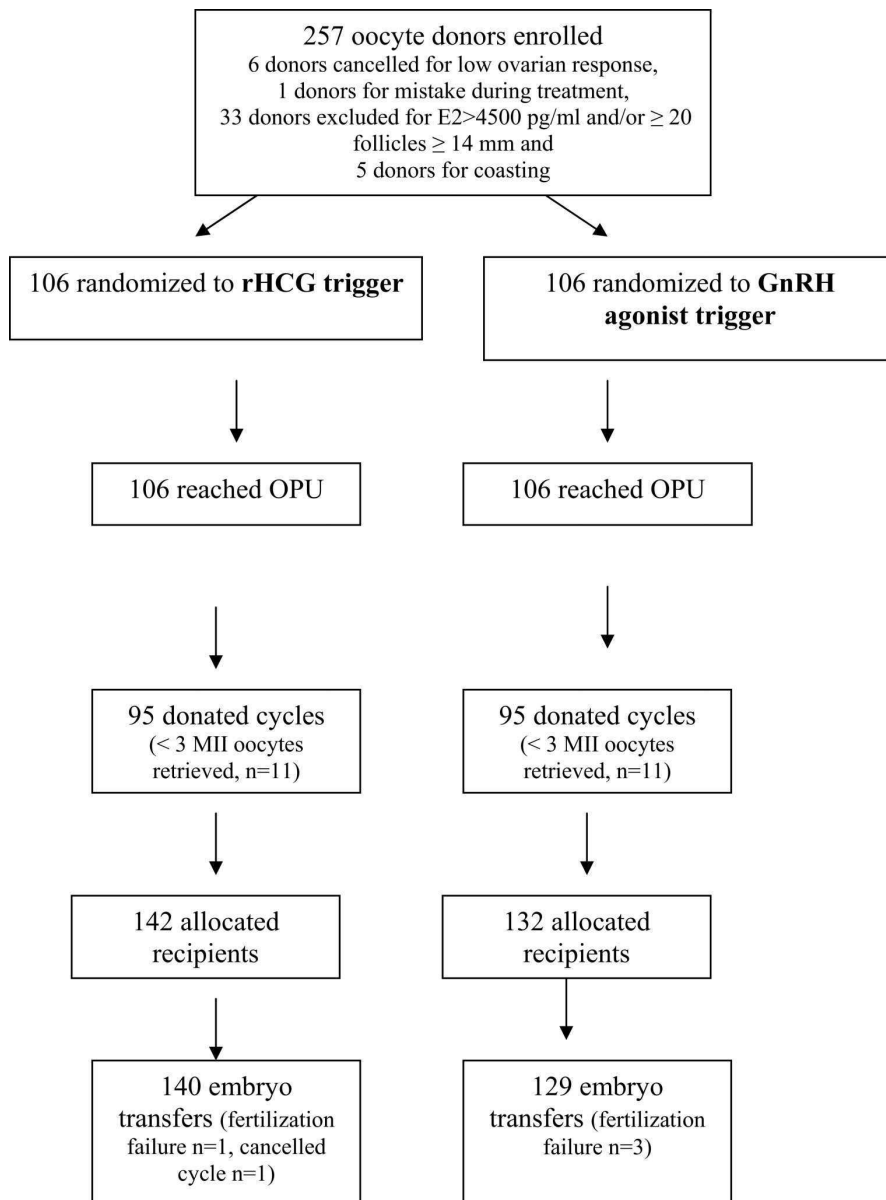


Figure 1. Flow-chart of patients treated in the study.

triggering as compared with hCG [3,16,17]. The absence of over-stimulated corpora lutea is the key to OHSS prevention [18].

Observational, uncontrolled reports on the use of the GnRH agonist to induce final oocyte maturation were published since the early nineties [4,19,20]. Later on a number of randomised clinical trials were performed in such different settings like IVF patients at high OHSS risk, normoresponder IVF patients or egg donors. Clinical trials performed in high risk patients with polycystic ovaries [16,21] showed that whereas in the control group the rate of moderate/severe OHSS cases reached 15–31%, in turn after

GnRH agonist triggering no cases occurred. Complete OHSS prevention (at least that of clinically significant moderate/severe cases) was already demonstrated since the earliest studies on GnRH agonist triggering [22]. The body of evidence accumulated to date leaves no place to doubt regarding the benefits of GnRH agonist triggering, to the point that any further prospective trial comparing hCG with the GnRH agonist in high OHSS risk patients could be considered as unethical [23].

Randomised clinical trials conducted in normoresponder IVF patients [7,8] confirmed that after GnRH agonist triggering the number of obtained

Table I. Description of donors and donor cycle outcomes.

Triggering agent	rhCG	GnRH agonist	P
Number of randomised patients	106	106	–
Age	26.6 ± 3.6	25.8 ± 3.9	0.13 <sup>a</sup>
BMI	22.8 ± 2.9	22.9 ± 2.9	0.62 <sup>a</sup>
Days of stimulation	10 ± 1.5	9.9 ± 1.4	0.57 <sup>b</sup>
Total rFSH (IU) used	2237 ± 435	2188 ± 357	0.37 <sup>a</sup>
Oestradiol on triggering day (pg/mL)	2233 ± 870	2038 ± 852	0.1 <sup>a</sup>
Number of follicles ≥18 mm	3.4 ± 1.7	3.7 ± 1.9	0.07 <sup>b</sup>
Number of follicles ≥16 mm	6.7 ± 2.6	7 ± 2.7	0.28 <sup>b</sup>
Number of follicles ≥14 mm	10.4 ± 3.6	10.6 ± 3.6	0.32 <sup>b</sup>
Number of follicles ≥10 mm	16.4 ± 5.8	16.7 ± 6.1	0.53 <sup>b</sup>
COC retrieved	12 ± 6.3	11.4 ± 6.4	0.12 <sup>b</sup>
Mature oocytes retrieved	8 ± 4.6	7.5 ± 4.1	0.38 <sup>b</sup>
Proportion of mature oocytes (%)	67.2 ± 20.4	67.1 ± 20.9	0.79 <sup>b</sup>
No attributed recipient (<3 MII oocytes retrieved) <i>n</i> (%)	11 (10.3)	11 (10.3)	1 <sup>c</sup>
Fertilisation rate (%)	67.8 ± 23.5	70.1 ± 22.1	0.14 <sup>b</sup>
Fertilised oocytes (2PN)	5.9 ± 3.4	5.8 ± 3	0.83 <sup>b</sup>
Fertilisation failure <i>n</i> (%)	1 (0.94)	1 (0.94)	1 <sup>d</sup>

Values are mean ± SD.

<sup>a</sup>Independent *t*-test.

<sup>b</sup>Mann–Whitney U-test.

<sup>c</sup>Chi-square test.

<sup>d</sup>Fisher's exact-test.

oocytes, the proportion of mature oocytes, fertilisation rates and embryo quality were comparable with the hCG group. Ongoing pregnancy rates were, however, disappointingly low in these studies despite enhanced luteal support. Further research is still ongoing on finding an effective luteal support after GnRH agonist triggering [24,25] or on such concepts as “dual trigger” [26]. A subsequent follow-up study of one of the above mentioned trials [27] showed that the developmental potential of frozen-thawed embryos is similar to that of the control group. This was proved to be the basis for an effective OHSS prevention strategy in IVF patients at high OHSS risk [28].

In the setting of OD a small scale retrospective study [9] showed favourable results with comparable donor and recipient outcome. Similar findings were confirmed by a recent retrospective report of our group [29], however on a much larger sample of egg donors. Given that the incidence of clinically significant moderate/severe OHSS in oocyte donors is relatively low (1.2%), only large size trials could detect any significant difference. A Spanish group [10] was the first to publish the results of a randomised clinical trial comparing the two different triggering agents in oocyte donor cycles treated with the GnRH antagonist protocol. Using a sample of 30 patients per treatment arm they found no significant difference in the number of retrieved and fertilised oocytes or pregnancy and implantation rates

Table II. Description of recipients and recipient outcome.

Triggering agent	rhCG	GnRH agonist	P
Number of allocated recipients	142	132	–
Age (mean ± SD)	40.6 ± 4.6	40.6 ± 4.8	0.96 <sup>e</sup>
Indication of oocyte donation			0.26 <sup>e</sup>
Menopause <i>n</i> (%)	30 (21%)	21 (16%)	
Previous failed IVF cycles <i>n</i> (%)	40 (28%)	30 (23%)	
Reduced ovarian reserve <i>n</i> (%)	71 (50%)	78 (59%)	
Others <i>n</i> (%)	1 (1%)	3 (2%)	
Embryo replacements	140	129	–
Transferred embryos per recipient, mean ± SD	1.92 ± 0.4	1.93 ± 0.4	0.86 <sup>d</sup>
Mean embryo score, mean ± SD <sup>a</sup>	8.5 ± 1.1	8.7 ± 1.1	0.09 <sup>d</sup>
Clinical pregnancy rate <sup>b</sup> (%)	54/142 (38)	53/132 (40.1)	0.81 <sup>e</sup>
Implantation rate (%)	62/269 (23)	69/249 (27.7)	0.34 <sup>e</sup>
Miscarriage rate (%)	12/54 (22.2)	12/53 (22.6)	0.96 <sup>e</sup>
Ongoing pregnancy rate <sup>b</sup> (%)	42/142 (29.5)	41/132 (31)	0.84 <sup>e</sup>
Twins at 6–7th pregnancy week (%)	6/54 (11.1)	16/53 (30.1)	0.047 <sup>e</sup>
Triplets at 6–7th pregnancy week (%)	1/54 (1.8)	0/53 (0)	0.32 <sup>f</sup>
Live-birth rate <sup>b</sup> (%)	42/142 (29.5)	40/132 (30.3)*	0.92 <sup>e</sup>
Twins at birth (%)	6/42 (14.2)**	12/41 (29.2)***	0.18 <sup>e</sup>

<sup>a</sup>To calculate the mean embryo score only cleavage-stage embryos (day 2–3) were taken into account (out of 518 embryos, 8 compacted embryos and one blastocyst could not be scored with the combined embryo score).

<sup>b</sup>per recipient cycle (including recipient failures without embryo transfer).

<sup>c</sup>Independent *t*-test.

<sup>d</sup>Mann–Whitney U-test.

<sup>e</sup>Chi-square test.

<sup>f</sup>Fisher's exact-test.

\*One patient delivered a stillborn child at the 35th pregnancy week.

\*\*Two cases of spontaneous embryo reduction occurred (one case of twin to singleton and another case of a triplet to a twin).

\*\*\*Four cases of spontaneous embryo reduction occurred (twin to a singleton).

in recipients. Another RCT of comparable size [30] – published in an abstract form – reported similar findings among oocyte donors with high ovarian response. In both previous reports OHSS occurred only in the group triggered with hCG in approximately 17% of the donors. These cases were, however, of a mild degree and therefore of less clinical significance. Although in line with the findings of the present clinical trial the previous ones were still insufficiently powered, therefore a larger, adequately powered RCT remained warranted in the context of OD.

As a limitation of our study it could be mentioned that mature oocytes were distributed to more than one recipient in approximately one third of donor



cycles. Although this could be considered as a methodological error, the comparison between recipients groups did not show any significant difference. This egg-sharing policy is widely practiced in many large OD programmes, a fact which is also reflected in most studies on OD.

In summary, the findings of our randomised clinical trial confirm that the GnRH agonist is as effective as hCG in inducing final oocyte maturation in oocyte donor cycles. Although currently there is no benefit in applying GnRH agonist trigger to normoresponder IVF patients, in the context of OD, this strategy could still be universally applied. Future ovarian stimulation protocols and current coasting and cancellation criteria for egg donors might be substantially influenced by this fact. Further research could still be conducted on different GnRH agonists as triggering agents or on exploring the safety and the limits of OHSS prevention in egg donors.

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