Comparison of early versus late initiation of GnRH antagonist co-treatment for controlled ovarian stimulation in IVF: a randomized controlled trial


1Department of Reproductive Medicine and Gynecology, University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands 2Academic Unit of Human Development and Health, Princess Anne Hospital, University of Southampton, Southampton SO16 5YA, UK 3Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, 3848 CG Utrecht, The Netherlands 4Division of Reproductive Medicine, Erasmus University Medical Centre, 3015 CE Rotterdam, The Netherlands 5Department of Gynecology and Fertility, Isala Clinics, 8025 AB Zwolle, The Netherlands 6Department of Gynecology and Fertility, Maasstad Hospital, 3079 EZ Rotterdam, The Netherlands 7Department of Gynecology and Fertility, Catharina Hospital, 5623 EJ Eindhoven, The Netherlands 8Department of Gynecology and Fertility, Tergooi Hospital, 1261 AN Blaricum, The Netherlands 9Division of Reproductive Medicine, VU University Medical Centre, 1081 HZ Amsterdam, The Netherlands 10Department of Gynaecology-Obstetrics, Centre for Reproductive Medicine of the University Hospital of Ghent, 9000 Ghent, Belgium 11Department of Gynecology and Fertility, Medisch Spectrum Twente Hospital Group, 7511 JX Enschede, The Netherlands 12Department of Gynecology and Fertility, Bronovo Hospital, 2597 AX The Hague, The Netherlands 13Department of Gynecology and Fertility, Albert Schweitzer Hospital, 3331 ZZ Twizentrecht, The Netherlands 14Department of Gynecology and Fertility, Leiden University Medical Centre, 2333 ZA Leiden, The Netherlands 15Department of Gynecology and Fertility, ZGT Almelo, 7609 PP Almelo, The Netherlands 16Department of Gynecology and Fertility, St. Antonius Hospital, 3435 CM Nieuwegein, The Netherlands

*Correspondence address. Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, F05.126, Heidelberglaan 100, PO Box 85500, 3508 GA Utrecht, the Netherlands. E-mail: o.hamdine@umcutrecht.nl

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STUDY QUESTION: What is the impact of initiating GnRH antagonist co-treatment for in vitro fertilization (IVF) on cycle day (CD) 2 compared with CD 6 on live birth rate (LBR) per started cycle and on the cumulative live birth rate (CLBR)?

SUMMARY ANSWER: Early initiation of GnRH antagonist does not appear to improve clinical outcomes of IVF compared with midfollicular initiation.

WHAT IS KNOWN ALREADY: During ovarian stimulation for IVF, GnRH antagonist co-treatment is usually administered from the midfollicular phase onwards. Earlier initiation may improve the follicular phase hormonal milieu and therefore overall clinical outcomes.

STUDY DESIGN, SIZE, DURATION: This open-label, multicentre randomized controlled trial was conducted between September 2009 and July 2011. A web-based program was used for randomization and 617 IVF-intracytoplasmic sperm injection (ICSI) patients were included.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Recombinant FSH (150–225 IU) was administered daily from CD 2 onwards in both groups. The study group (CD2; n = 308) started GnRH antagonist co-treatment on CD 2, whereas the control group (CD6; n = 309) started on CD 6.
Introduction

The clinical efficacy of GnRH antagonist co-treatment for the prevention of premature luteinization during ovarian hyperstimulation in in vitro fertilization (IVF) has recently been established. GnRH antagonists have been shown to offer increased safety and reduced costs compared with GnRH agonist cycles, with no clear significant difference in ongoing pregnancy rate and live birth rate (LBR) (Al-Inany et al., 2011). Currently, there is a growing consensus to support a fixed daily injection protocol starting on day 6 or 7 of the menstrual cycle (i.e. 5–6 days after initiation of stimulation) (Albano et al., 1997; GaniRelix Dose-Finding Study Group, 1998; Al-Inany et al., 2005). However, the optimal protocol for routine clinical use has not yet been identified (Huirne et al., 2007).

Starting GnRH antagonist co-treatment in the midfollicular phase may be too late in some patients. Several studies have demonstrated the negative impact of hormonal fluctuations during the follicular phase on IVF outcomes. Previous research has indicated that prevention of high LH levels during the follicular phase may improve endometrial receptivity and hence pregnancy rates (Kolibianakis et al., 2003a,b, 2004a; Huirne et al., 2005). However, others have reported no impact on pregnancy rates (Merviel et al., 2004; Bosch et al., 2005; Doody et al., 2010; Greig- singer et al., 2011). Additionally, both high estradiol (E2) levels and high progesterone (P) levels have been associated with impaired endometrial receptivity and oocyte/embryo quality (Basir et al., 2001; Valbuena et al., 2001; Bosch et al., 2003, 2010; Kolibianakis et al., 2003b, 2012; Kyrou et al., 2011, 2012). Furthermore, an excessive ovarian response has been shown to markedly reduce implantation rates in both mild stimulation and GnRH agonist cycles (Verberg et al., 2009).

The possible benefits of starting GnRH antagonist treatment at the initiation of stimulation are indicated by two studies that showed this approach to result in more physiological levels of both LH and E2 during the follicular phase (Kolibianakis et al., 2003a; Hamdine et al., 2013). This protocol may improve the chance of achieving in-phase endometrial maturation at the time of embryo transfer, thereby improving clinical outcomes. Early initiation of the GnRH antagonist may also decrease the incidence of premature LH surges and hence premature luteinization. Moreover, early initiation of GnRH antagonist may moderate ovarian response by early suppression of endogenous FSH and a subsequently reduced endometrial exposure to E2 (Hamdine et al., 2013), with consequent beneficial effects on endometrial receptivity. Consequently, a moderate ovarian response could reduce the risk of the potentially life-threatening ovarian hyperstimulation syndrome (OHSS). For these reasons, it could be hypothesized that in many women, an early start of GnRH antagonist treatment would improve LBRs. The aim of this study was therefore to prospectively compare the effect of an early fixed start (on cycle day (CD) 2) versus a late fixed start (on CD 6) GnRH antagonist protocol on single cycle and cumulative live birth rates (CLBRs), and on adverse events such as OHSS.

Methods

Patient population

This open-label, multicentre randomized controlled trial was conducted between September 2009 and July 2011 in the Netherlands. The study was approved by the Institutional Review Board (IRB) of each participating centre, and registered on the Clinical Trial web site (www.clinicaltrials.gov, no. NCT00866034). In total 617 women undergoing IVF or intracytoplasmic sperm injection (ICSI) were recruited from the IVF outpatient clinics of 13 fertility centres. Randomization was performed according to a web-based computer-generated randomization schedule. The allocated treatment was concealed neither from the clinicians nor from the patient. Informed consent was obtained from all patients and each patient was enrolled into the study only once. The inclusion criteria were: age ≥39 years; body mass index (BMI) ≤32 kg/m²; regular cycle; regular indication for IVF or ICSI; and no more than two previous unsuccessful IVF/ICSI cycles. Patients with World Health Organization class 1 or 2 anovulation were excluded.
Ovarian stimulation

Ovarian stimulation was performed with recombinant FSH (recFSH, Gonal-F; Merck Serono, the Netherlands, or Puregon; MSD, the Netherlands). A GnRH antagonist (Cetrotide; Merck Serono, the Netherlands, or Orgalutran; MSD, the Netherlands) was used to prevent a premature LH surge. Two centres used oral contraceptive pretreatment (OCP) to avoid menstrual bleeding, whereas GnRH antagonist treatment continued to include the day of hCG administration, whereas GnRH antagonist treatment continued to include the day of hCG administration. Final oocyte maturation was induced by administering 6500 IU of hCG (Ovrelle; Merck Serono, the Netherlands), when at least one follicle of ≥18 mm in diameter and two follicles of ≥16 mm in diameter were visualized by ultrasound. Follicle growth was assessed by transvaginal ultrasound, starting from CD 6 and thereafter as often as necessary in order to ensure that hCG would be administered when the criteria had been met, with the possibility of postponing hCG triggering by maximally 1 day. Oocyte retrieval was performed 36 h after hCG administration. One or two embryos were transferred 3 or 4 days after oocyte retrieval according to local protocols. The luteal phase was supplemented with a daily dose of 600 mg of vaginally administered micronized natural progesterone (Utrogestan; Besins Healthcare, Brussels, Belgium). All patients underwent one treatment cycle as part of this protocol. An outline of the two treatment regimens is depicted in Supplementary data, Fig. S1.

Outcome measures

The primary outcomes of this study were LBR per started cycle, and cumulative live birth (CLBR) from fresh and/or cryopreserved embryos originating from, and transferred within 6 months of, the initial treatment cycle. Secondary outcomes included the duration of stimulation, total cumulative dose of recFSH consumed, number of oocytes retrieved, fertilization rate, number of suitable embryos, implantation rate and the biochemical, clinical and ongoing pregnancy rates. The occurrence of OHSS, cycle cancellation due to a risk of OHSS and poor response were evaluated as safety end-points.

Power analysis

Based on a power of 80% and an alpha of 5%, a study population of 1105 patients per arm was required to demonstrate an increase in LBR in a fresh cycle from 20 to 25% in the early fixed start group. Taking a ~9% loss of patients into account, an inclusion of 1215 patients per arm was deemed necessary. The total sample size of the study was therefore 2430 patients. An interim analysis was planned after half of the inclusions had been reached to evaluate the efficacy of both protocols and to reduce the number of patients needed or discontinue the trial for safety, ethical, compliance or efficacy reasons. However, due to a limited rate of patient inclusion during the first 2 years of execution, the interim analysis was performed after the inclusion of 617 patients. Data from 484 patients with a completed fresh IVF treatment cycle were available for the interim analysis, which was performed on ongoing pregnancy rate per started fresh cycle, because the primary end-point live birth was not yet available for all patients. There appeared to be no significant difference in ongoing pregnancy rates between the two groups, although a trend towards higher ongoing pregnancy rates was observed in the CD6 group (21.9 versus 20.8%, difference 1.1%, 95% confidence interval (CI) – 8.6 ~ 6.4). Since the effect of the different protocols was counter to that hypothesized, a new power calculation was performed. This calculation revealed that, in order to show this difference at interim analysis to be significant with an alpha of 5% and a power of 80%, a study population of 2415 instead of 1215 patients per arm would have been required. Since confirming superiority of the standard treatment over the experimental protocol was considered to be of insufficient clinical value, the study was terminated after IRB approval.

Statistical analysis

Data for continuous variables are presented as mean values and standard deviation (SD). Between-group statistical comparisons of mean values were performed with t-tests. Chi-squared tests and Fisher’s exact tests were used for count data. Logistic regression tests were used to check for differences between the centres. Differences were considered to be statistically significant if the P-value was <0.05. An intention-to-treat (ITT) analysis as well as a per-protocol (PP) analysis was performed. Both are shown in the results tables. Because no difference was observed between the two analyses, the ITT analysis will be mainly discussed.

Results

Study progression

A total of 617 patients consented to participate in the study. Figure 1 charts the flow of both groups through each stage of the trial. Prior to the start of treatment, 24 patients withdrew for personal reasons or as a result of conceiving naturally. A total of 593 patients started stimulation in one of the two treatment arms. IVF was performed in 296 (48.0%) couples and ICSI was performed in 297 (48.1%) couples. Oocyte retrieval was performed in 529 patients, of whom 481 proceeded to fresh embryo transfer. Protocol violations occurred in a total of 80 cases; however, these patients were all included in the ITT analysis. In the CD2 group, the recFSH dose was increased to 225 IU in 13 patients. The dose was reduced to 112.5 IU in one patient. In the CD6 group, the dose was increased to 225 IU in seven patients and to 300 IU in four patients. Dosage reduction to 112.5 IU occurred twice, and dose reduction to 75 IU occurred once. In each group, one patient was lost to follow-up. Subject demographics as well as fertility characteristics are shown in Table I.

Clinical outcome

The treatment characteristics are depicted in Table II. Logistic regression showed no difference in IVF outcomes between the 13 fertility centres. Additionally, pregnancy rates did not appear to be affected by OCP pretreatment. Premature ovulation occurred four times in the CD2 group (1.3%), and once in the CD6 group (0.3%). The total dose of recFSH used, as well as the total duration of stimulation, did not differ between the two groups. Furthermore, no differences were observed with regard to number of oocytes retrieved, fertilization rate, number of embryos suitable for transfer and number of embryos transferred or cryopreserved.

Table III demonstrates the clinical efficacy outcomes per started cycle. There were no significant differences in clinical outcomes between the two groups. However, the CD6 group showed a trend towards higher implantation rates and ongoing pregnancy rates (Table III). The difference between the two groups was much smaller in the freeze-thaw cycles (0.6%, P = 0.6). No differences were observed in the biochemical pregnancy or miscarriage rates (P = 0.5). Furthermore, the CD6 group showed a non-significant trend towards higher LBRs per started cycle as well as higher CLBRs, compared with the CD2 group. Figure 2 depicts a
Kaplan–Meier plot showing the CLBR in both treatment arms. There is a gradual increase in the rate of accumulation in favour of the CD6 group. The difference after the first treatment cycle is maintained during the time period in which pregnancies accumulate from the freeze-thaw cycles. Due to either late miscarriage or premature birth, live birth was not achieved in two CD2 and four CD6 patients.

Safety
The total cancellation rate per started cycle was 10.4% in the CD2 group and 7.4% in the CD6 group (P = 0.2). Cancellation due to poor ovarian response occurred in 13 CD2 patients and in 15 CD6 patients, whereas IVF treatment was converted into an intrauterine insemination in 12 and 5 cases, respectively (Fig. 1). Due to a risk of OHSS, seven patients in the
The overall incidence of OHSS was low (1.9%). Mild-to-moderate OHSS was observed in 10 patients (CD2: 3 and CD6: 7, $P = 0.3$), whereas one case of severe OHSS was observed in each group.

### Discussion

To our knowledge, the current study represents the largest randomized controlled trial investigating the impact of early initiation of a GnRH antagonist start.
The expected benefit of early initiation of GnRH antagonist treatment on LBRs after IVF/ICSI treatment. Early initiation of GnRH antagonist treatment showed no beneficial effect on CLBRs. Indeed, a trend towards better outcomes was observed when using the currently established, midfollicular phase fixed start regimen.

The non-physiological endocrine milieu associated with ovarian stimulation is thought to be the cause for suboptimal endometrial receptivity (Macklon et al., 2006). Moreover, endometrial advancement of more than 3 days on the day of oocyte retrieval has been associated with a decreased chance of pregnancy, especially in cases with high LH levels at initiation of stimulation and with a prolonged duration of stimulation before starting GnRH antagonist treatment (Kolibianakis et al., 2002). Additionally, a high exposure of the endometrium to untimely elevated E2 as well as elevated P levels during the follicular phase has been associated with a reduced chance of pregnancy, possibly due to endometrial advancement (Bosch et al., 2003, 2010; Kolibianakis et al., 2003b, 2012; Papanikolaou et al., 2009, 2012; Kyrou et al., 2011, 2012).

Compared with midfollicular initiation of GnRH antagonist treatment, early initiation has resulted in more profoundly suppressed LH and E2 levels on day 6 of the cycle (Kolibianakis et al., 2003a; Hamdine et al., 2013). Furthermore, in a single centre nested study of subjects recruited to this RCT, a lower oocyte yield was observed in the CD2 group compared with the CD6 group (P = 0.048), indicating a possible benefit for this approach in overcoming overexposure of the endometrium to estradiol (Hamdine et al., 2013). The lack of confirmation of this result in the present study might indicate a type 1 error or reflect subtle differences in application of the protocol in the centre that performed the nested study. The present findings are however consistent with those of a small pilot study where the number and classes of growing follicles appeared comparable between the two study groups (Kolibianakis et al., 2003a).

Conflicting data exist regarding the impact of low endogenous LH levels on reproductive outcomes in GnRH antagonist cycles (Filicori et al., 1999; Kolibianakis et al., 2004a; Merviel et al., 2004). However, it has been shown that the addition of LH to recFSH does not affect LBRs (Kolibianakis et al., 2007). Additionally, the present study shows a similar fertilization rate as well as number of embryos obtained, indicating that
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In this study, the overall incidence of premature ovulation was 0.8%, which is lower than what has been reported in the literature to date. A number of studies have demonstrated that LH rises may occur in 1.4–35% of GnRH antagonist stimulation cycles (Ganirelix Dose-Finding Study Group, 1998; Huirne et al., 2004; Messinis et al., 2005; Kolibianakis et al., 2011). Since the GnRH antagonist is a competitive GnRH receptor blocker, trigger signals such as a fast rising E2 may lead to endogenous GnRH surges that may overcome the competitive blockade. From the earlier endocrine studies, the positive effect in terms of a tighter control of LH, specifically in the first phase of the stimulation cycle, has been demonstrated (Kolibianakis et al., 2003a,b, 2004a; Huirne et al., 2005). In our study, however, a benefit in terms of improved clinical outcomes with an early start to GnRH antagonist treatment could not be claimed, and a late GnRH antagonist start regimen still offers the best clinical profile.

Finally, the role for elevated follicular phase P levels in affecting IVF outcome has become apparent from two studies (Kolibianakis et al., 2004b; Blockeel et al., 2011). Early initiation of the GnRH antagonist could aid in swift suppression of any residual corpus luteum function, by reducing LH exposure. Although the mechanism of action of elevated P in jeopardizing clinical outcomes has not been elucidated, normalization of P levels may be beneficial. So far, studies have not confirmed that early GnRH antagonist exposure will completely neutralize the negative effects of elevated early follicular phase P levels (Blockeel et al., 2011). Although the incidence of elevated early follicular phase P levels in GnRH antagonist cycles is low (4.9–6.2%, Kolibianakis et al., 2004b; Blockeel et al., 2011), we assume that a small percentage of our study population has elevated P levels. If the effect of early GnRH antagonist initiation were highly relevant in this regard, a possible trend towards higher pregnancy rates in the early start arm might be expected, but this was not observed. Moreover, in the nested endocrine study, no differences in P levels between the two arms could be identified, neither on Day 6 of the cycle nor on the day of hCG (Hamdine et al., 2013).

Contrary to expectations, this study, which involved a large patient population, showed a trend towards higher pregnancy rates in favour of the CD6 group. This may mean that a more stable endocrine profile does not necessarily result in improved endometrial receptivity and hence higher pregnancy rates. While a possible negative effect of GnRH antagonists on oocyte/embryo quality has been suggested in the past (Ganirelix Dose-Finding Study Group, 1998), this has not since been substantiated. Moreover, no adverse effect was observed on the performance of the freeze-thaw embryos in subsequent replacement cycles (Kol et al., 1999). Previously, Raga et al. have detected GnRH receptors in the endometrium (Raga et al., 1998). However, gene expression studies so far do not support a detrimental effect of GnRH antagonists on endometrial receptivity (Haouzi et al., 2010). Our data support the contention that embryo quality is not negatively affected by GnRH antagonist exposure, since the ongoing pregnancy rates in the freeze-thaw cycles were similar, in spite of different exposure time in the two arms.

The strength of this study is the multicentre, randomized controlled design. The heterogeneous patient population and ITT analysis reflect daily practice and make extrapolation of the results to the general IVF population possible. Moreover, using live birth as an end-point in clinical trials in reproductive medicine is well recognized and leads to a better understanding of their implication for clinical practice (Kolibianakis et al., 2006).

The study was terminated prematurely because no significant difference was observed in clinical outcomes after 617 inclusions. A much larger population would be needed to detect a small difference in favour of either study arm, which raises the question whether this would be relevant for clinical practice. A further limitation of the study is the lack of endocrine data to confirm our previous findings with regard to the endocrine profile in the follicular phase (Hamdine et al., 2013). The reasons we decided not to perform hormonal measurements in all subjects were related to the logistic challenges associated with the multicentre design, and possible discrepancies between the participating centres in, for example, the assays used.

The study protocol required that the dose of recFSH remain fixed throughout the entire stimulation period. However, the dose was adjusted according to ovarian response in a small number of patients (n = 28). These patients were not excluded from the ITT analysis, as variations in usage of recFSH were deemed not to influence the clinical outcomes. Moreover, it has been demonstrated that FSH dosages over 150 IU daily will not alter the stimulation or response level of the ovaries (Jayaprakasan et al., 2010; Sterrenburg et al., 2011). The PP data analysis revealed that the differences observed between the two groups were similar to those in the ITT analysis.

The observations in this study may have relevance for the optimization of GnRH antagonist stimulation cycles for IVF. The PP analysis revealed that the observed non-significant differences with regard to live birth and CLBR were slightly smaller compared with the ITT analysis. Because the PP analysis demonstrated the direct effect of the different treatment protocols, the clinical importance of this finding is probably negligible. Additionally, increasing the number of daily injections by

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**Figure 2** Kaplan–Meier plot showing the cumulative rate of pregnancies leading to live birth in both treatment arms.
four may increase patient discomfort and imposes the risk losing some of the benefits of a GnRH antagonist protocol. Furthermore, adding four extra injections will slightly increase treatment costs by € 159.38 per patient per cycle. This study shows clearly that the additional treatment burden and costs are not justified as early initiation of GnRH antagonists does not improve LBRs. In the efforts to improve GnRH antagonist cycles, the focus may be put on individualized recFSH regimens based on ovarian reserve tests such as antral follicle count (AFC) and anti-Mullerian hormone (AMH). Such an approach could aid in improving IVF outcome, as well as in reducing the risk of OHSS or cancellation due to poor response. Previous systematic reviews by our group have shown that both AFC and AMH can predict ovarian response, and hence allow for individualization of recFSH dosage (Broer et al., 2009, 2011). Whether response prediction and individualized dosing will improve clinical outcome needs to be elucidated once accurate response prediction has become established for GnRH antagonist cycles (Andersen et al., 2011; Polyzos et al., 2012).

In conclusion, this large study has demonstrated that early initiation of GnRH antagonist treatment does not improve clinical outcomes of IVF treatment compared with midfollicular initiation of GnRH antagonist. The currently used GnRH antagonist protocol starting on CD 6 remains the best choice at present.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

**Authors’ roles**

N.S.M., F.J.B. and B.C.J.M.F. were responsible for the study design. O.H., B.J.C., A.V., P.A.v.D., R.E.B., C.B.L., G.J.E.O., C.A.G.H., G.C.v.d.D.M., H.J.V., P.F.M.v.d.H., A.B. and J.S.E.L. were responsible for data collection. O.H. and M.J.C.E. performed the analyses. O.H., M.J.C.E., F.J.B., B.C.J.M.F. and N.S.M. interpreted the results and wrote the article. All the authors were responsible for revision of the article.

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**Conflict of interest**


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