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ARTICLE

Follicular and endocrine profiles associated with different GnRH-antagonist regimens: a randomized controlled trial

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Abstract This trial assessed the impact of early initiation of gonadotrophin-releasing hormone (GnRH) antagonist on follicular and endocrine profiles compared with the fixed GnRH-antagonist protocol. Eighty-five oocyte donors were randomized to GnRH antagonist starting in the mid-luteal phase of the prestimulation cycle (degarelix-ML group), on stimulation day 1 (early follicular phase, degarelix-EF group) or day 6 (fixed protocol) (mid-follicular phase, ganirelix-MF group). Subjects in the degarelix-EF and ganirelix-MF groups received placebo in the prestimulation cycle. At start of stimulation, serum concentrations of FSH (4.6 ± 2.3 versus 6.0 ± 1.8 IU/l), LH (2.7 ± 1.4 versus 4.7 ± 1.9 IU/l) and oestradiol (87 ± 35 versus 129 ± 50 pmol/l) were markedly lower ($P < 0.001$) in the degarelix-ML group than in the placebo group. The coefficients of variation of follicle size (36.7 ± 5.5% versus 39.2 ± 9.4%) were not significantly different. No differences in endometrial histology, embryo quality and pregnancy rates in recipient cycles were observed between the regimens. In conclusion, early administration of GnRH antagonist altered the endocrine profile without modifying the follicular synchrony for the majority of subjects. Whether patients with a more heterogeneous follicle size at start of stimulation may benefit from an earlier intervention remains to be proven.

KEYWORDS: degarelix, endocrinology, follicular development, GnRH antagonist, ovarian stimulation

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Introduction

The introduction of gonadotrophin-releasing hormone (GnRH) antagonist protocols in patients undergoing IVF/intracytoplasmic sperm injection has been associated with a lower number of retrieved oocytes compared with the long GnRH-agonist protocol (Albano et al., 2000; Al-Inany et al., 2007; Borm and Mannaerts, 2000; Fluker et al., 2001; Olivennes et al., 2000; Roulier et al., 2003; The European Middle East Orgalutran study group, 2001). GnRH agonists suppress endogenous LH and FSH and the co-ordinated follicle recruitment is a direct response to exogenous gonadotrophin stimulation alone (Fauser and van Heusden, 1997). The standard fixed GnRH-antagonist protocol, on the other hand, in which the antagonist is administered from stimulation day 5 or 6 is associated with a two-stage follicular recruitment, since growth of a few leading follicles will be initiated by endogenous FSH before exogenous gonadotrophin is administered. As a result, a more heterogeneous follicle cohort in terms of size (asynchrony) will be achieved with the standard GnRH-antagonist protocol compared with the long GnRH-agonist protocol, potentially leading to reduced number of oocytes retrieved (Fleming, 2002; Huirne et al., 2007).

To improve ovarian-stimulation outcome in GnRH-antagonist cycles, it has been suggested to induce pituitary suppression of endogenous FSH and LH during the transitional luteal—follicular phase to allow optimal synchronization of the follicular cohort before exogenous gonadotrophin stimulation. This suppression can be achieved by administration of oestrogen in high doses or combined oestrogen/progestogen preparations in the transitional phase. Thus, pretreatment with oestradiol (Fanchin et al., 2003) or the oral contraceptive pill (Cedrin-Durnerin et al., 2007; Huirne et al., 2006; Rombauts et al., 2006) has been suggested to improve synchronization in GnRH-antagonist cycles through its FSH-suppressive effects. However, oestrogen preparations and oral contraceptives may have an impact on follicle/oocyte development parameters and the endometrial profile. Actually, oral-contraceptive pretreatment in GnRH-antagonist cycles may lower implantation rates (Rombauts et al., 2006) and increase pregnancy-loss rates (Griesinger et al., 2010; Kolibianakis et al., 2006).

A different approach to achieve a more synchronized follicular growth and lower exposures to FSH, LH, oestradiol and progesterone (endocrine synchrony) in the follicular phase of the stimulation cycle in GnRH-antagonist regimens is to initiate the GnRH-antagonist treatment earlier than in the mid-follicular phase. In particular, a mid-luteal start of GnRH-antagonist treatment may mimic more closely the follicular growth and endocrine profile of the standard long GnRH-agonist protocol than the fixed GnRH-antagonist protocol. Furthermore, due to the immediate onset of action of GnRH antagonists on pituitary suppression and subsequent decrease of ovarian steroid concentrations and menstrual shedding, gonadotrophin stimulation could start just a few days after administration of the GnRH antagonist. In line with this approach, a study performed in healthy female volunteers suggested that GnRH-antagonist administration during the last days of the luteal phase reduces the serum concentration of FSH and the mean follicular size as well as attenuates follicular-size discrepancies during the subsequent early follicular phase (Fanchin et al., 2004).

The purpose of the present phase-II prospective randomized trial was to assess if earlier initiation of GnRH antagonist than in the standard fixed GnRH-antagonist protocol could improve endocrine and follicular synchrony at the start of stimulation. Two GnRH-antagonist compounds with different profiles were used for this investigation: (i) the investigational degarelix depot formulation (2.5 mg); and (ii) the commercially available ganirelix daily formulation (0.25 mg). Clinical data from healthy female volunteers have shown that degarelix administration at a dose of 2.5 mg causes an immediately profound suppression of LH and FSH and that it takes approximately 7 days for LH to return to the baseline concentration. In contrast, dosing with ganirelix 0.25 mg causes only temporary drops in LH and FSH concentrations before returning to baseline within 24 h (Oberyé et al., 1999). Given that regimens with early administration of GnRH antagonist would require dosing for more than 15 days, a 7-day administration of degarelix 2.5 mg (i.e. one injection/week) would be more convenient as it minimizes the number of injections for the study subjects. The reference arm was chosen to be ganirelix 0.25 mg daily starting on day 6 of stimulation as the efficacy and safety of this regimen have been established in comparative clinical trials (Borm and Mannaerts, 2000; The European and Middle East Orgalutran study group, 2001). Since degarelix 2.5 mg starting on day 6 of stimulation would be expected to be associated with a different endocrine response compared with daily administration of ganirelix 0.25 mg, given the stronger suppression of LH and FSH with the depot regimen, it would not represent the most appropriate reference arm for this investigation on early initiation of GnRH antagonist.

Materials and methods

Study population

Subjects eligible for this trial were oocyte donors undergoing ovarian stimulation for assisted reproduction technology and recipient couples receiving oocytes from these donors.

Oocyte donors

Main inclusion criteria were: age 18—35 years; body mass index (BMI) 18—29 kg/m^2; regular menstrual cycle of 26—35 days, presumed to be ovulatory; early follicular-phase serum concentration of FSH within normal limits (1—12 IU/l). Main exclusion criteria were: abnormal karyotype; polycystic ovarian syndrome, endometriosis stage III/IV; history of being a 'poor responder', defined as >20 days of gonadotrophin in a previous stimulation cycle, or any previous cancellation of a stimulation cycle due to limited follicular response, or development of less than 4 follicles ≥15 mm in a previous stimulation cycle; history of recurrent miscarriage; severe OHSS in a previous stimulation cycle or any contraindication for the use of gonadotrophins.

Recipient couples

Main inclusion criteria were: woman's age between 18 and 49 years; woman's BMI between 18 and 29 kg/m^2; and consent for transfer of one or two embryos. Main exclusion criteria were: woman with a history of recurrent miscarriage; man with known abnormal karyotype or severe male factor (unless donor sperm was used).

Ethical approval

The protocol, the subject information sheet and the consent form were reviewed and approved by the independent ethics committees (Committee for Medical Ethics, Brussels; Ethics Committee of the Institute for Clinical and Experimental Medicine and Faculty Thomayer Hospital, Prague and Ethics Committee for Multi-Centric Clinical Trials of the University Hospital Motol, Prague; Ethics Committee of Hospital Universitario la Paz, Madrid; and Ethics Committee of IVI Valencia, Valencia; reference number FE200486 CS24) and the regulatory authorities in Belgium, Czech Republic and Spain (EudraCT number 2006-004684-58) prior to trial initiation. Written informed consent was obtained from all trial subjects. The trial was conducted in accordance with the Declaration of Helsinki.

Study design

This was a randomized, multicentre, multinational, exploratory, proof-of-concept trial (ClinicalTrials.gov NCT00434 122) conducted at four fertility centres distributed in Belgium (UZ Brussel), Czech Republic (ISCARE IVF a.s., Prague) and Spain (IVI-Madrid and IVI-Valencia).

A double-blinded, placebo-controlled design was applied for assessment of variables prior to start of gonadotrophin stimulation and an assessor-blinded, active-controlled design was applied for the assessments from day 6 of stimulation. For the standard fixed GnRH-antagonist cycle, ganirelix (Orgalutran; Organon, The Netherlands) was used and daily injections were administered from day 6 of stimulation. In order to reduce the number of injections for the two early starts of GnRH-antagonist regimen, degarelix (Firmagon; Ferring Pharmaceuticals, Denmark) was used, which is a new selective GnRH antagonist with self-depot-forming properties leading to a long-acting profile (Broqua et al., 2002). The use of degarelix implied that only two injections were needed for the whole treatment period as compared with multiple daily injections if ganirelix would have been used.

The oocyte donors underwent screening procedures to evaluate eligibility for participation in the trial, including an early follicular (natural cycle day 3 ± 1) transvaginal ultrasound (TVU) of the ovaries and blood sampling for hormonal evaluation, as well as daily assessments of serum concentration of LH starting when ≥1 follicle of ≥14 mm was documented by TVU to establish the LH peak. Seven days after the LH peak (day LH+7), the oocyte donors completed the screening procedures and were randomized in a ratio of 2:1:1, based on a computer-generated randomization list prepared by a statistician at Ferring Pharmaceuticals not involved in the trial, to one of the following three GnRH-antagonist regimens: (i) GnRH antagonist starting in the mid-luteal phase (degarelix-ML group): degarelix (2.5 mg in 1 ml) s.c. injection on day LH+7 in the menstrual cycle prior to ovarian stimulation, placebo (5% mannitol) s.c. injection on stimulation day 1 and degarelix (2.5 mg) s.c. injection on stimulation day 6; (ii) placebo treatment in the mid-luteal phase and GnRH antagonist starting in the early follicular phase (degarelix-EF group): placebo s.c. injection on day LH+7 in the cycle prior to ovarian stimulation, degarelix (2.5 mg) s.c. injection on stimulation day 1 and degarelix (2.5 mg) s.c. injection on stimulation day 6; and (iii) placebo-treatment in the mid-luteal phase and GnRH antagonist starting in the mid-follicular phase (ganirelix-MF group): placebo s.c. injection day LH+7 in the cycle prior to ovarian stimulation and on stimulation day 1, ganirelix (0.25 mg in 0.5 ml) s.c. injection once daily from stimulation day 6 until the end of stimulation. The placebo control group consisted of all subjects in the degarelix-EF and ganirelix-MF groups from randomization to day 1 of stimulation (prior to dosing), as these subjects received only a placebo injection during the prestimulation period. A schematic overview of the treatment regimens for the oocyte donors is shown in **Figure 1**.

For all treatment groups, the gonadotrophin used for ovarian stimulation was highly purified human menopausal gonadotrophin (HP-HMG; Menopur; Ferring Pharmaceuticals). Stimulation was started on cycle day 2 in the subsequent cycle after the natural-cycle assessments (day 3 and day LH+7). The daily dose was fixed to 225 IU throughout stimulation. When ≥3 follicles of ≥17 mm were observed, 250 μg of human chorionic gonadotrophin (HCG; Ovitrelle; Serono, Switzerland) was injected s.c. to induce final follicular maturation. Oocyte retrieval took place 36 ± 2 h after the HCG injection. Luteal support with vaginal progesterone 200 mg, twice daily (Utrogestan; Seid, Spain) was provided from the day after oocyte retrieval until 7 days after the HCG injection (day HCG+7).

The safety profile of degarelix was documented prior to the start of this trial, including exposure to healthy female volunteers of single doses up to 10 mg. The 2.5 mg dose of degarelix selected for this trial was based on pharmacokinetic/pharmacodynamic models from data obtained in female volunteers. It was estimated that 80% of the subjects would have an LH concentration at least 20% below their individual baseline concentration 7 days after two 2.5-mg s.c. doses of degarelix and that this degarelix dose regimen would be able to prevent premature LH surges throughout the stimulation.

Assessments

Transvaginal ultrasound of ovaries

A Voluson *i* ultrasound system (GE Healthcare) with default pre-settings was provided to the participating centres by Ferring Pharmaceuticals. TVU of the ovaries (right and left) was performed to obtain two-dimensional (2D) and three-dimensional (3D) images (B-mode and power Doppler) in the natural cycle (day 3 ± 1) and in the treatment cycle on stimulation day 1 (before start of stimulation), day 6 of stimulation and last stimulation day. The 2D images were evaluated locally by the investigators at the trial sites, while the digitally stored 3D images were evaluated by a

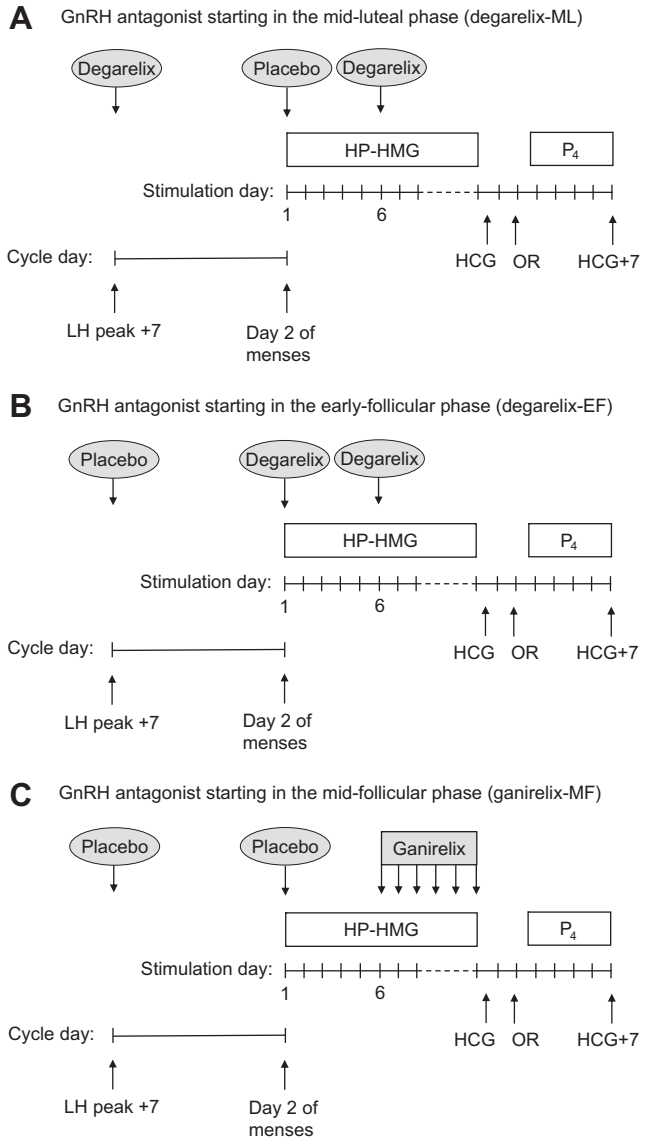

Figure 1 Overview of the gonadotrophin-releasing hormone (GnRH) antagonist treatment regimens for oocyte donors. (**A**) Degarelix starting in the mid-luteal phase. (**B**) Degarelix starting in the early follicular phase. (**C**) Ganirelix starting in the mid-follicular phase. HCG = human chorionic gonadotrophin; HP-HMG = highly purified human menopausal gonadotrophin; OR = oocyte retrieval; P_4 = progesterone.

central independent assessor (SK) for number and size of follicles.

Blood samples

Blood samples were taken on day 3 ± 1 in the natural cycle, on stimulation day 1 (prior to stimulation), stimulation day 6 and last day of stimulation (prior to HCG administration). Serum was analysed for endocrine parameters by a central laboratory (Laboratorium für Klinische Forschung, Germany) using electrochemiluminescence immunoassays (FSH, LH, oestradiol and progesterone; Roche Diagnostics) and a radioimmunoassay (androstenedione; Beckmann-Coulter). The lower detection limit (and total imprecision, CV) of the validated analytical methods were as follows: FSH 0.10 IU/l (<4%), LH 0.10 IU/l (<3%), oestradiol 18.4 pmol/l (<6%), progesterone 0.095 nmol/l (<6%) and androstenedione 0.10 nmol/l (<10%).

Clinical parameters in oocyte donors and recipients

Clinical parameters assessed in oocyte donors were frequency of premature LH surge (defined as LH concentration ≥10 IU/l and a concomitant rise in progesterone concentration above 1 ng/ml from stimulation day 6 to the last stimulation day), number of cumulus–oocyte–complexes retrieved, duration of gonadotrophin treatment and total gonadotrophin dose administered. TVU of the endometrium of oocyte donors was performed on day HCG+7 and digitally stored 3D images were evaluated by a central independent assessor (SK) for volume, thickness, triple-layer structure and dating. Endometrial biopsy specimens were taken from the pars functionalis of the uterine fundus on day HCG+7 and were assessed according to the criteria described by Noyes et al. (1950) by a central independent assessor (CB). Data on fertilization rates, embryo quality, biochemical pregnancy (defined as a positive βHCG test), clinical pregnancy (defined as a transvaginal ultrasound showing at least one intrauterine gestational sac with fetal heart beat 5–6 weeks after embryo transfer) and ongoing pregnancy (defined as a transvaginal ultrasound showing at least one intrauterine viable fetus 10–11 weeks after embryo transfer) rates as well as pregnancy outcome were collected for recipients. In addition, pregnancy and outcome data were collected from the frozen embryo-transfer cycles conducted within 6 months after freezing of the embryos.

Blinding

Code envelopes were used to conceal treatment allocation. A double-blind design was applied from screening to the assessments on day 6 of stimulation. From that point until the end of trial, the investigator remained as assessor blinded but the donors were not blinded any longer due to the different administration regimens of degarelix and ganirelix. In order for the investigators to remain blinded to treatment allocation throughout the trial, other persons prepared the syringes and dispensed/administered the investigational compounds. All histological and ultrasound parameters were analysed by assessors blinded to treatment allocation. Laboratory personnel analysing endocrine and routine safety laboratory parameters at the central laboratory were also blinded to treatment allocation.

Study outcome measures

The primary objective of the trial was to assess the effect of starting the GnRH antagonist in the mid-luteal phase of the menstrual cycle prior to ovarian stimulation on follicular synchrony. Secondary objectives were to assess the hormone profile at start of gonadotrophin stimulation compared with placebo treatment and the overall clinical profile at end of stimulation and the luteal phase of three different GnRH-antagonist regimens. As part of the safety follow-up, treatment-outcome parameters in the recipient transfer cycles were collected.

The primary outcome measure of the study was coefficient of variation (CV) of follicular sizes on stimulation day 1 in the oocyte donors. Secondary outcome measures in the oocyte donors included: CV of follicular sizes on natural cycle day 3 ± 1 and stimulation day 6; total number of follicles, follicle size and SD of follicle size on natural cycle day 3 ± 1, stimulation days 1 and 6; the number of cumulus—oocyte—complexes retrieved; endocrine profile in serum on natural cycle day 3 ± 1, stimulation days 1, 6 and last day; endometrial dating, endometrial thickness and endometrial volume on day HCG+7; circulating concentrations of routine safety laboratory parameters; and frequency and intensity of adverse events. Follow-up information in recipient couples included: fertilized oocytes; total number of embryos suitable for transfer/freezing; number of top-quality embryos; positive βHCG rate; clinical pregnancy rate; ongoing pregnancy rate; and live-birth rate. The procedures related to managing the oocyte recipients followed local practise and regulations.

Sample size and statistical methods

The CV (SD × 100 ÷ mean) of follicular sizes on stimulation day 1 was chosen as primary outcome measure of antral follicular synchrony. The rationale for the choice of CV rather than SD as a measure of follicular synchrony was that the variation in follicular size increases when the follicle cohort grows, i.e. SD increases when the mean follicular size increases. Using CV provides a more valid comparison between treatment groups since it adjusts for a potential difference between treatment groups in mean follicle size. Furthermore, CV has previously been used to measure follicle synchronicity (Fanchin et al., 2004). A sample size of 72 (36 subjects in the degarelix-ML group and 36 subjects in the placebo group) had a power of 90% to detect a difference of 35% in CV using a two-sided *t*-test at the 5% level on the log-transformed values assuming a variation in CV of 40%. All follicles ≥2 mm were included when calculating CV. Follicular parameters were evaluated using the Wilcoxon test. Treatment differences of endocrine parameters were evaluated using one-way ANOVA on log-transformed values. The safety analysis set included all randomized and exposed subjects. The intention-to-treat (ITT) analysis set included all randomized and exposed subjects not withdrawn before day 1 of gonadotrophin stimulation.

Results

Study populations

A total of 98 oocyte donors undergoing ovarian stimulation for assisted reproduction technology were screened for participation between March and November 2007. Of these, 13 were ineligible and 85 were randomized, and 78 provided data for the primary endpoint on stimulation day 1: 39 in the degarelix-ML group and 39 in the placebo group (**Figure 2**). There were no significant differences between the treatment groups regarding demographics, menstrual and obstetric history and previous oocyte-donation experience (**Table 1**). Each donor only donated to one matched recipient couple. The number of oocyte recipient couples found eligible for inclusion was 78, but the final recipient population consisted of 76 couples due to one recipient couple being matched to an oocyte donor who did not have any oocytes retrieved and one oocyte recipient who withdrew her consent. The median age of the recipient women was 38 years in all treatment groups (range 26—48 years). The duration of infertility among the recipients was comparable between treatment groups, with an average of 5.5 years. The most common primary reasons for infertility were anovulatory infertility groups I (21%) and II (17%) (World Health Organization, 1973) and unexplained infertility (18%).

Hormone measurements in oocyte donors

The results of the hormone measurements in the oocyte donors are presented in **Table 1**. The endocrine profiles were comparable between the degarelix-ML and placebo groups at screening on day 3 of the menstrual cycle prior to ovarian stimulation. The number of days between day LH+7 and stimulation day 1 was significantly fewer in the donors treated with GnRH antagonist compared with those given placebo (4.5 ± 2.5 versus 8.9 ± 4.3 days; $P < 0.001$).

Prior to start of gonadotrophin administration, the mean serum concentrations of FSH, LH and oestradiol were significantly lower in the degarelix-ML group compared with the placebo group ($P < 0.001$ for all three hormones). At this time point, the serum concentrations of androstenedione and progesterone were not significantly different between the groups, but less heterogeneous progesterone concentrations were observed in the degarelix-ML group compared with the placebo group (2.8 ± 1.7 nmol/l versus 3.8 ± 4.8 nmol/l, respectively).

On stimulation day 6, the serum concentrations of LH and oestradiol were significantly lower in the degarelix-ML group ($P < 0.01$) and the degarelix-EF group ($P < 0.001$), compared with the ganirelix-MF group (prior to start of ganirelix treatment). The concentrations of FSH, progesterone and androstenedione were comparable among the three treatment groups. The LH concentration remained significantly ($P < 0.01$) lower in the degarelix-EF group compared with the ganirelix-MF group on the last stimulation day, with no other significant differences in the hormonal concentrations between the groups. No premature LH surges were reported in any of the groups during the stimulation period.

Follicular measurements in oocyte donors

Both the 2D and 3D TVU assessments of the follicles for each treatment group are shown in **Table 1**. At screening in the natural cycle, the mean antral follicular size and the CV and SD of the follicular size as well as the number of follicles were similar between the degarelix-ML and placebo groups. On stimulation day 1, the mean ± SD of total number of follicles and follicle size of all donors were 20.1 ± 7.6 and 3.2 ± 0.6 mm versus 16.7 ± 6.0 and 4.7 ± 1.1 mm ($P < 0.001$) for 3D and 2D imaging, respectively. The higher resolution of the 3D images compared with the 2D images resulted in detection of more small follicles with the 3D assessments, which in its turn resulted in significantly higher mean number and smaller mean size of the follicles with the 3D technique. Administration of degarelix in the mid-luteal

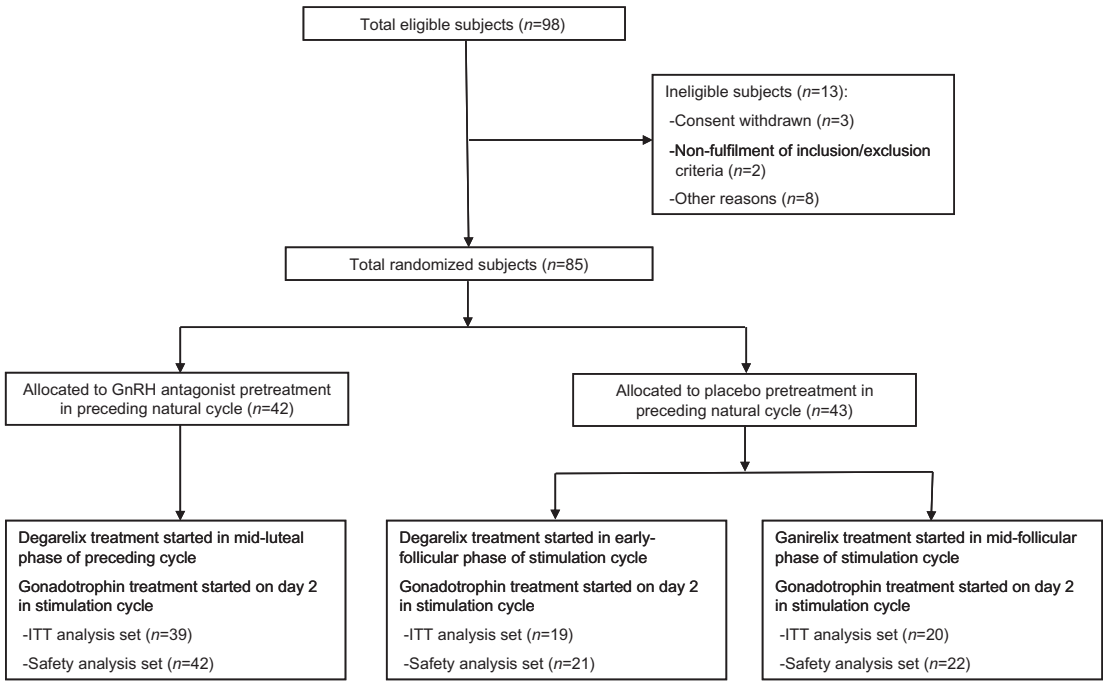

Figure 2 Study flow chart of the oocyte donors. GnRH = gonadotrophin-releasing hormone; ITT = intention to treat.

phase did not result in reduced size discrepancies of early antral follicles at the start of gonadotrophin stimulation. The CV of follicular size (3D) was not statistically significantly different between the GnRH-antagonist and placebo groups (primary endpoint), neither were the numbers of follicles, mean follicular size or SD of follicular size significantly different between the degarelix-ML and placebo groups at stimulation day 1.

Follicular growth during the early follicular phase in response to gonadotrophin stimulation appeared faster with the standard fixed GnRH-antagonist regimen (ganirelix-MF group) compared with the degarelix-ML and degarelix-EF groups, as indicated by a significantly higher mean size of the three largest follicles ($P = 0.013$), higher mean number of follicles $\geq$12 mm ($P = 0.025$) and a higher proportion of subjects with at least one follicle of $\geq$12 mm on stimulation day 6 ($P = 0.032$) (**Table 1**).

Last stimulation day was defined as the day when $\geq$3 follicles of $\geq$17 mm were observed and, consequently, the differences in follicular size of the three largest follicles were small and non-significant at end of stimulation between the three regimens (17.3 ± 2.1, 17.2 ± 3.4 and 17.8 ± 1.7 for the degarelix-ML, degarelix-EF and ganirelix-MF groups, respectively). The number of treatment days with gonadotrophin as well as the total dose of gonadotrophin in the degarelix-ML and degarelix-EF groups were not significantly different when compared with the ganirelix-MF group (9.1 ± 1.4 and 8.9 ± 1.9 versus 7.9 ± 2.3 days, and 2042 ± 324 and 2001 ± 430 versus 1778 ± 510 IU, respectively).

As shown in **Table 1**, the evaluation of individual responses in CV of follicular size did not suggest any effect of early administration of GnRH antagonist for the majority of subjects. When stratifying the whole trial population into $\leq$75th ($\leq$47.1%) and >75th CV percentiles at screening, the subjects in the >75th percentile were equally distributed between the degarelix-ML and placebo groups ($n = 9$ and 10, respectively). However, there was a trend towards fewer GnRH-antagonist pretreated subjects with CV >47.1% at start of stimulation compared with placebo-treated subjects (one versus seven subjects); however, this did not reach statistical significance (**Figure 3**). Moreover, the group of placebo-treated subjects with CV >47.1% at start of stimulation had a significantly higher mean serum FSH concentration at this time point, (7.5 ± 2.1 versus 5.7 ± 1.6 IU/l; $P = 0.012$), less follicles at end of stimulation (15.7 ± 5.1 versus 24.4 ± 10.5; $P = 0.042$) and fewer oocytes retrieved (6.3 ± 5.1 versus 11.5 ± 5.5; $P = 0.029$) than the placebo-treated subjects with CV $\leq$47.1%.

Clinical measurements in oocyte donors

There were no statistically significant differences between the treatment groups with respect to the number of cumulus–oocyte–complexes retrieved. The mean numbers retrieved per oocyte donor were 11 ± 6, 12 ± 6 and 11 ± 5 for the degarelix-ML, degarelix-EF and ganirelix-MF groups, respectively.

On day HCG+7, the endometrial dating was within 1 day (in-phase endometrium) in 97% (31/32) of the subjects in the degarelix-ML group, 79% (11/14) in the degarelix-EF group and 88% (14/16) in the ganirelix-MF group. The mean endometrial thickness (10.2 ± 2.7, 11.0 ± 3.4, 10.9 ± 3.1 mm) and endometrial volume (3.9 ± 1.7, 4.9 ± 2.8, 4.3 ± 1.9 ml) on day HCG+7 were not significantly different between the degarelix-ML, degarelix-EF and ganirelix-MF groups.

The frequency of adverse events was comparable between subjects exposed to degarelix (25%, 16/63) or ganirelix (18%, 4/22), respectively. Five injection-site reactions were reported after injection of degarelix, one after injec-

Table 1 Comparison of characteristics between the different gonadotrophin-releasing hormone antagonist regimens and placebo.

Variable	*Degarelix-ML (n = 39)*	*Placebo (n = 39)*[a]	*Degarelix-EF (n = 19)*	*Ganirelix-MF (n = 20)*	P-*value*
Demographics					
Age (years)	28.9 ± 4.1	28.0 ± 3.9	28.1 ± 3.9	27.9 ± 4.1	NS[c]
BMI (kg/m^2)	22.9 ± 2.9	22.6 ± 3.0	22.4 ± 2.8	22.8 ± 3.2	NS[c]
Menstrual history					
Duration of cycle (days)	28.3 ± 1.0	28.5 ± 1.1	28.2 ± 1.1	28.8 ± 1.2	NS[c]
Obstetric history					
Pregnancy	77	69	74	65	NS[d]
Oocyte donor history					
Oocyte donation	56	46	42	50	NS[d]
Donation cycles	2.9 ± 2.5	3.2 ± 2.6	3.0 ± 1.8	3.3 ± 3.1	NS[c]
Natural cycle (day 3 ± 1)					
FSH (IU/l)	6.7 ± 1.8	6.9 ± 1.6	–	–	NS[e]
LH (IU/l)	5.2 ± 2.5	5.3 ± 2.6	–	–	NS[e]
Oestradiol (pmol/l)	146 ± 94	121 ± 63	–	–	NS[e]
Progesterone (nmol/l)	2.2 ± 0.9	2.1 ± 1.1	–	–	NS[e]
Androstendione (nmol/l)	4.9 ± 2.3	4.3 ± 2.1	–	–	NS[e]
Follicles (3D)	19.3 ± 7.5	18.4 ± 12.0	–	–	NS[f]
Follicles (2D)	17.1 ± 6.3	16.8 ± 6.6	–	–	NS[f]
Follicle size (3D; mm)	3.44 ± 0.66	3.31 ± 0.63	–	–	NS[f]
Follicle size (2D; mm)	5.04 ± 1.18	4.97 ± 1.26	–	–	NS[f]
SD of follicle size (3D)	1.44 ± 0.66	1.44 ± 0.56	–	–	NS[f]
SD of follicle size (2D)	0.88 ± 0.46	0.98 ± 0.41	–	–	NS[f]
CV of follicle size (3D)	40.3 ± 11.2	42.6 ± 12.0	–	–	NS[f]
CV of follicle size (2D)	18.8 ± 10.0	21.5 ± 10.4	–	–	NS[f]
Stimulation day 1[b]					
FSH (IU/l)	4.6 ± 2.3	6.0 ± 1.8	–	–	<0.001[e]
LH (IU/l)	2.7 ± 1.4	4.7 ± 1.9	–	–	<0.001[e]
Oestradiol (pmol/l)	87 ± 35	129 ± 50	–	–	<0.001[e]
Progesterone (nmol/l)	2.8 ± 1.7	3.8 ± 4.8	–	–	NS[e]
Androstendione (nmol/l)	4.5 ± 2.0	4.4 ± 2.2	–	–	NS[e]
Follicles (3D)	20.6 ± 7.5	19.6 ± 7.8	–	–	NS[f]
Follicles (2D)	17.2 ± 6.1	16.2 ± 6.0	–	–	NS[f]
Follicle size (3D; mm)	3.15 ± 0.45	3.33 ± 0.63	–	–	NS[f]
Follicle size (2D; mm)	4.56 ± 1.12	4.75 ± 1.09	–	–	NS[f]
SD of follicle size (3D)	1.17 ± 0.30	1.35 ± 0.57	–	–	NS[f]
SD of follicle size (2D)	0.85 ± 0.48	1.03 ± 0.60	–	–	NS[f]
CV of follicle size (3D)	36.7 ± 5.5	39.2 ± 9.4	–	–	NS[f]
CV of follicle size (2D)	20.6 ± 12.1	22.9 ± 12.6	–	–	NS[f]
Stimulation day 6					
FSH (IU/l)	13.0 ± 3.0	–	14.7 ± 3.5	14.5 ± 3.6[h]	NS[e]
LH (IU/l)	0.9 ± 0.6[**]	–	0.7 ± 0.6[***]	1.5 ± 0.8[h]	<0.001[e]
Oestradiol (pmol/l)	1230 ± 808[*]	–	1351 ± 1174[*]	1943 ± 1209[h]	NS[e]
Progesterone (nmol/l)	2.2 ± 0.9	–	2.0 ± 0.6	2.2 ± 1.1[h]	NS[e]
Androstendione (nmol/l)	5.8 ± 2.2	–	5.1 ± 1.6	5.2 ± 2.3[h]	NS[e]
Follicle size (3D; mm)	5.44 ± 1.21[*]	–	5.44 ± 1.60[*]	6.34 ± 1.41[h]	NS[g]
Three largest follicles (3D; mm)	9.62 ± 1.64[*]	–	8.95 ± 2.26[*]	11.0 ± 2.17[h]	0.013[g]
Follicles ≥12 mm (3D)	0.54 ± 1.19[**]	–	0.58 ± 1.07	1.32 ± 1.45[h]	0.025[g]
Subjects with ≥1 follicle ≥12 mm (3D)	27[*]	–	32	63[h]	0.032[g]

(*continued on next page*)

Table 1 (continued)

Variable	*Degarelix-ML (n = 39)*	*Placebo (n = 39)*[a]	*Degarelix-EF (n = 19)*	*Ganirelix-MF (n = 20)*	*P-value*
Last stimulation day					
FSH (IU/l)	13.9 ± 3.1	—	14.8 ± 3.6	15.9 ± 4.3	NS[e]
LH (IU/l)	1.0 ± 0.9	—	0.7 ± 0.8**	1.5 ± 1.3	0.006[e]
Oestradiol (pmol/l)	6126 ± 3512	—	5726 ± 3094	6365 ± 3289	NS[e]
Progesterone (nmol/l)	2.6 ± 1.0	—	2.6 ± 1.5	2.9 ± 1.3	NS[e]
Androstendione (nmol/l)	7.6 ± 3.3	—	7.1 ± 2.9	7.2 ± 3.1	NS[e]
Three largest follicles (3D; mm)	17.3 ± 2.1	—	17.2 ± 3.4	17.8 ± 1.7	NS[g]

Values are mean ± SD or %. Degarelix-ML = GnRH antagonist starting in the mid-luteal phase; degarelix-EF = GnRH antagonist starting in the early follicular phase; Ganirelix-MF = GnRH antagonist starting in the mid-follicular phase. 2D = two-dimensional transvaginal ultrasound; 3D = three-dimensional transvaginal ultrasound; BMI = body mass index; CV = coefficient of variance; NS = not statistically significant; SD = standard deviation.

[a]Combined degarelix-EF and ganirelix-MF groups, which were given placebo treatment prior to start of stimulation on cycle day 2.

[b]Prior to stimulation.

[c]One-way ANOVA.

[d]Chi-squared test.

[e]One-way ANOVA on log-transformed values for overall comparisons (on stimulation day 6 and last stimulation day).

[f]Wilcoxon test.

[g]Kruskal—Wallis test for overall comparisons.

[h]Prior to administration of ganirelix.*$P < 0.05$, versus the ganirelix-MF group (*t*-test).**$P < 0.01$, versus the ganirelix-MF group (*t*-test).
***$P < 0.001$, versus the ganirelix-MF group (*t*-test).

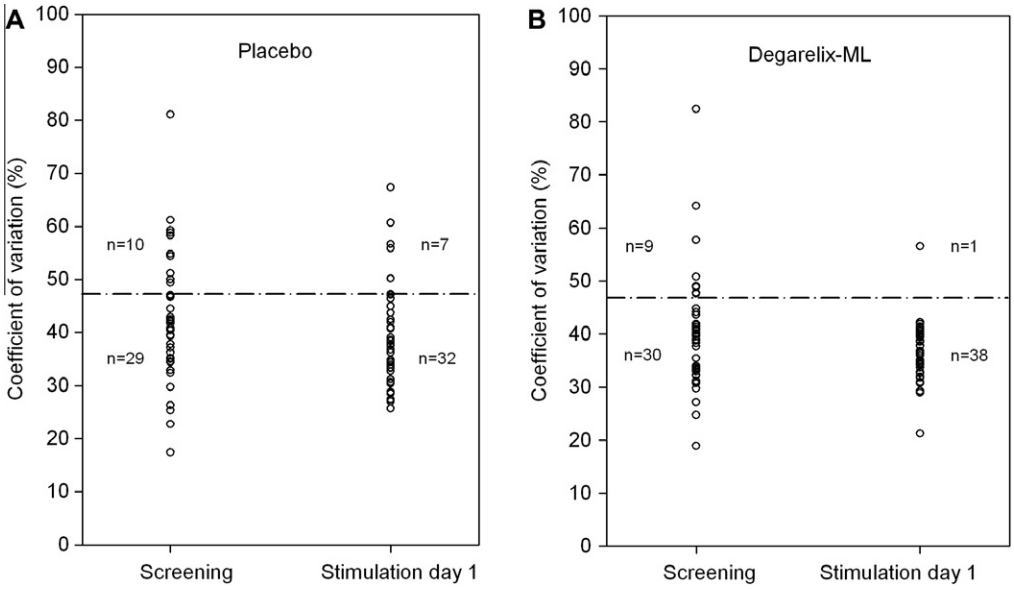

Figure 3 Individual coefficients of variation of follicular size at screening on day 3 ± 1 of the natural cycle and on stimulation day 1 in oocyte donors treated with placebo (*n* = 39) (**A**) or GnRH antagonist (*n* = 39) (**B**) in the mid-luteal phase of the natural cycle (the degarelix-ML group). The horizontal dashed line displays the 75th percentile (47.1%) of the whole trial population at screening.

tion of gonadotrophin and none for ganirelix. No clinically significant values for routine biochemistry or haematology laboratory parameters were reported after the two injections of degarelix.

Treatment outcome in recipients

The mean number of oocytes with two pronuclei at 20 h was comparable between treatment groups: 5.4 ± 4.0 in the degarelix-ML group, 5.6 ± 3.2 in the degarelix-EF group and 6.0 ± 2.9 in the ganirelix-MF group. The mean number of embryos suitable for transfer/freezing (3.2 ± 2.6, 3.6 ± 2.5 and 3.3 ± 1.8) and mean number of top-quality embryos (2.0 ± 2.5, 2.2 ± 2.2 and 1.6 ± 1.8) per recipient were not significantly different between the degarelix-ML, degarelix-EF and ganirelix-MF groups, respectively. In total, 89 embryo transfers were performed: 41 in the degarelix-ML group (36 and 5 in fresh and frozen cycles, respectively), 24 in the degarelix-EF group (18 and 6 in fresh and frozen cycles, respectively) and 24 in the ganirelix-MF group (20 and 4 in fresh and frozen cycles, respectively). The mean numbers of embryos transferred per cycle was 1.7—1.9 in all groups. The cumulative clinical outcomes (% of number of pregnancies and live births in fresh + frozen embryo-transfer cycles

per recipient) according to the treatment groups from which the oocytes originated were (degarelix-ML, degarelix-EF and ganirelix-MF groups, respectively): positive βHCG rate (56%, 38% and 42%), clinical pregnancy rate (34%, 25% and 29%), ongoing pregnancy rate (34%, 25% and 29%) and live-birth rate (32%, 21% and 29%). The neonatal birthweight, length and Apgar score of the infants were similar across the treatment groups.

Discussion

In the present trial, pretreatment with a GnRH antagonist in the mid-luteal phase of the menstrual cycle prior to the stimulation cycle was found to markedly reduce FSH and LH concentrations at start of ovarian stimulation. However, this earlier start of administration, as compared with the fixed GnRH-antagonist protocol, did not result in reduced size discrepancies of antral follicles, even though the reductions in serum FSH concentrations were similar to those observed after pretreatment in the luteal phase with oestradiol (Fanchin et al., 2003) and oral contraceptive pill or synthetic progestogen (Cedrin-Durnerin et al., 2007), regimens which have been associated with a reduction in size discrepancies of early antral follicles during the subsequent follicular phase. The absent impact on follicular synchronization of GnRH-antagonist pretreatment in the present trial is in marked contrast to a study in healthy female volunteers by Fanchin et al. (2004), who found a reduction in CV of about 50% in GnRH-antagonist pretreated cycles as compared with baseline cycles. Younger age and narrower age span of the population in the present trial may have contributed to the difference in results. Furthermore, the antral follicle count of the women included in Fanchin et al. (2004) was much lower compared with that of the women in the present trial (9 versus 17 with 2D TVU assessments). This difference suggests that a favourable effect of early initiation of GnRH antagonist in terms of increased size homogeneity may be predominantly seen in women with a lower number of antral follicles.

The data obtained in the present trial indicate that the general IVF/intracytoplasmic sperm injection population will not benefit from an early intervention with GnRH antagonist in terms of more oocytes retrieved and higher pregnancy rates. However, pretreatment with GnRH antagonist in patients with a high degree of follicle asynchrony may reduce discrepancies in follicular size during stimulation and thereby increase the number of oocytes retrieved. Given that the population in the present study had a relatively homogeneous follicle size at start of stimulation, it would be of interest to further explore in prospective studies if older women or women with low ovarian reserve or poor response in a previous cycle, possibly due to an apparent more heterogeneous follicle size at the start of stimulation, may benefit from an earlier start of GnRH-antagonist treatment as compared with the current standard antagonist regimen.

Advanced endometrial development has been reported in women undergoing ovarian stimulation with the long GnRH-agonist protocol (Ubaldi et al., 1997) or with a protocol starting the GnRH antagonist in the mid-follicular phase (Kolibianakis et al., 2002). The endometrial receptivity appears to correlate with the endocrine profile in the follicular phase, and elevated concentrations of progesterone in the follicular phase have been proposed to have a negative impact on treatment outcome by affecting endometrial receptivity (Bosch et al., 2010; Fleming and Jenkins, 2010; Huirne et al., 2007; Kolibianakis et al., 2004). In the present trial, no premature LH surge was observed in any of the groups and there were no differences in progesterone concentration at any time point during the stimulation cycle across the three GnRH-antagonist regimens. The histological data indicated that there were no detrimental effects of early introduction of GnRH antagonist on the endometrium as compared with the standard ganirelix regimen.

Injection-site reactions to the GnRH-antagonist administration were reported only in subjects treated with degarelix, which potentially could be attributed to the higher dose and volume used of this compound as compared with those of ganirelix (i.e. 2.5 mg degarelix in 1.0 ml versus 0.25 mg ganirelix in 0.5 ml). Moreover, it has been previously shown that degarelix has low histamine-releasing properties *in vitro* and a very low effect on vascular permeability *in vivo*, less than that caused by other GnRH antagonists (Broqua et al., 2002).

The present trial followed a complicated design and has some limitations. As from start of stimulation, the trial consisted of three arms and differences between the variables measured from end of stimulation onwards could reflect not only the effect of the different timing of GnRH-antagonist initiation but also the effect of the different antagonists used. As mentioned above, degarelix was chosen as the GnRH antagonist in the two early regimens due to the more long-acting profile of this compound compared with ganirelix to minimize the number of injections for the study subjects. Since a depot formulation of GnRH antagonist would induce a more sustained LH suppression compared with daily administration of a low dose of GnRH antagonist (i.e. ganirelix 0.25 mg), a mid-follicular start of degarelix 2.5 mg would not have been the most appropriate reference arm to evaluate the clinical impact of early initiation of a GnRH antagonist. Thus, the interpretation of the clinical data obtained with early GnRH-antagonist start is performed in relation to the current standard GnRH-antagonist protocol, in terms of dose (i.e. 0.25 mg), daily regimen and starting date (i.e. from day 6 of stimulation).

In summary, administration of GnRH antagonist in the mid-luteal phase of the pretreatment cycle reduces the concentrations of FSH, LH and oestradiol at start of stimulation, but with no significant impact on the synchrony of the follicle cohort compared with placebo treatment for the majority of subjects. The timing of administration and/or the long-acting profile of the GnRH antagonist degarelix appears to modify the dynamics of the follicular growth during the early follicular phase. The early start of GnRH antagonist did not cause any apparent detrimental effects on the endometrium, embryo quality or the ability of resultant embryos to implant in fresh or frozen recipient cycles compared with the standard GnRH-antagonist regimen. The efficacy and safety of early administration of a GnRH antagonist in IVF patients undergoing embryo transfer needs to be established, and the profile of patients who would benefit most from this new regimen should be prospectively validated in further clinical trials.

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