

Article

GnRH antagonists and endometrial receptivity in oocyte recipients: a prospective randomized trial



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Abstract

The effect that gonadotrophin-releasing hormone (GnRH) antagonists exert on endometrial receptivity has not yet been elucidated. GnRH antagonists might directly affect oocytes, the embryo and/or the endometrium. The aim of this study was to investigate the direct effect of GnRH antagonists on the endometrium in oocyte donation cycles. In an oocyte donation programme, oocytes from each donor ($n = 49$), stimulated with gonadotrophins and a GnRH antagonist, were equally shared between two different matched recipients. Recipients were randomly allocated to either receive a GnRH antagonist concomitant to donor during their endometrial priming with oestradiol (group I, $n = 49$) or to solely continue with their endometrial preparation (group II, $n = 49$). Pregnancy rate was 55.1% in group I and 59.1% in group II. Implantation rate was 26.1% in group I and 24.4% in group II. Endometrial thickness was also similar between the two groups on the day of human chorionic gonadotrophin injection to the donor. In conclusion, GnRH antagonist administration during the proliferative phase at a dose of 0.25 mg per day does not appear to adversely affect endometrial receptivity in oocyte recipients.

Keywords: endometrium, implantation rate, IVF, oocyte donation, pregnancy rate

Introduction

It has been almost a decade since gonadotrophin-releasing hormone (GnRH) antagonists were introduced in clinical practice (Albano *et al.*, 1997). Although GnRH antagonist treatment has a number of advantages as compared with GnRH agonists, their uptake has so far been lower than expected, resulting in a debate in the literature (Fauser and Devroey, 2005). The most likely reason that GnRH antagonist use has not yet gained wide acceptance is that pregnancy rates have been consistently lower in IVF cycles using GnRH antagonists, as compared with GnRH agonists, which has triggered further discussions (Al-Inany and Aboulghar, 2002; Hohmann *et al.*, 2003). Published meta-analyses suggested that this slight difference in pregnancy rates might not be solely attributed to chance (Al-Inany and Aboulghar, 2002; Tarlatzis *et al.*, 2006).

Clinical efficacy of GnRH antagonists has been confirmed in large multi-centre phase III clinical trials (The Ganirelix Dose-Finding Study Group, 1998). However, a dose-dependent decrease in pregnancy rates and ongoing pregnancy rates was also indicated. For example, implantation rates varied from 1–20% when 2 or 0.25 mg were administered, respectively (The Ganirelix Dose-Finding Study Group, 1998).

A possible reason for the observed implantation failure might be a direct effect of the GnRH antagonist on the granulosa cells, endometrial cells or the embryo, as, it has been reported, that they all harbour GnRH receptors (Hernandez, 2000). Other investigators have postulated that antagonists might exert an adverse effect on oocyte quality due to the profound suppression

of endogenous LH secretion that may disrupt the co-ordination between theca and granulosa cells (Filicori *et al.*, 2001, 2002). A dose-dependent effect of the antagonist on the endometrium has also been suggested as pregnancy rates were extremely low when a higher dose of the antagonist was used (The Ganirelix Dose-Finding Study Group, 1998). However, pregnancy rates were similar in frozen–thawed cycles irrespective of the dose of the antagonist, indicating a direct, dose-dependent effect of the antagonist on the endometrium (Kol *et al.*, 1999).

Oocyte donation provides a unique model to eliminate confounding variables that typically occur when comparing groups of patients undergoing IVF. In order to assist in clarifying the direct action of GnRH antagonists on endometrial receptivity, pregnancy and implantation rates, a prospective randomized study was designed in oocyte donation cycles. In this model, sibling oocytes originating from one donor were shared between two recipients, one of them randomly assigned to receive GnRH antagonist treatment, concomitant to endometrial priming with oestradiol.

Materials and methods

Two groups of subjects were compared in this prospective randomized double-blinded trial. Subjects were infertile menopausal women participating in an oocyte donation programme in IAKENTRO IVF fertility centre from May 2004 to May 2006. For every donor, a pair of opaque envelopes were prepared, corresponding to the two matched recipients. Every pair of envelopes contained either a '0' (no GnRH antagonist administration) or a '1' (GnRH antagonist administration). Thereafter, every pair was closed and randomly numbered by a computer-generated program with an 'a' or 'b', corresponding to the sequence that envelopes should be opened. On the day that the oocyte donor started GnRH antagonist injection, the first corresponding envelope was opened by a research nurse who co-ordinated the randomization process and distribution of medication throughout the treatment cycles. Thereafter, she informed the recipient to start the GnRH antagonist injection according to envelope's content. Doctors and embryologists were blinded to the treatment allocation. The study was approved by the Internal Institutional Review Board and written informed consent was obtained from all women.

A total of 55 volunteer oocyte donors were stimulated with the association of Menogon (Ferring, Kiel, Germany) and Orgalutran 0.25 mg (NV Organon, Oss, The Netherlands) as previously described in detail (Prapas *et al.*, 2005). Oocyte donors had previously participated in an oocyte donation cycle and were known to be good responders. Oocyte donors were fertile volunteers <32 years of age with a body mass index <30 kg/m², regular menstrual cycles, normal ovaries, no endometriosis, no gynaecological or medical disorders and agreed to donate their oocytes anonymously and altruistically (Prapas *et al.*, 2005). All donors were karyotyped and screened for hepatitis B and C, human immunodeficiency virus types 1 and 2, syphilis, *Toxoplasma*, rubella, *Listeria*, cytomegalovirus and β -thalassaemia. None of the donors were heavy smokers.

Stimulation of the donors started on the 2nd day of their menstrual cycle with three ampoules of Menogon 75 IU. Before the initiation of gonadotrophin treatment, a baseline

ultrasound was performed to exclude the presence of ovarian cysts. Cycles were monitored by means of vaginal ultrasound and oestradiol measurements starting on the 4th day after Menogon administration. The daily dose of gonadotrophin was adjusted individually according to vaginal ultrasound and oestradiol measurements. When a leading follicle was ≥ 15 mm, the antagonist Orgalutran 0.25 mg was also daily co-administered late in the evening until the day before human chorionic gonadotrophin (HCG) injection. 10,000 IU of HCG (Pregnyl; NV Organon) were administered when at least three follicles with a mean diameter >17 mm were present on ultrasound scan and serum oestradiol concentrations was at least 1500 pg/ml. No antagonist was injected on the day of HCG administration. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h after HCG injection under local anaesthesia and i.v. sedation.

Metaphase II oocytes from one donor were equally divided and donated to two different recipients. The donor cycle was offered by IAKENTRO Fertility Centre for free to the recipients who were allocated to receive the GnRH antagonist. A total of 110 recipients agreed to participate in the study. Two recipients were matched with one oocyte donor before the initiation of ovarian stimulation of the donor. Recipients were infertile menopausal women <50 years of age with FSH values >35 IU/l and no menstrual period for at least 1 year. Exclusion criteria were a previous history of more than three failed oocyte donation cycles, the presence of hydrosalpinx or uterine malformation as detected with hysterosalpingography or hysteroscopy, and current smoking. Recipients underwent blood screening similar to the donors. A trial transfer was performed in all subjects and the cervix was dilated, if recommended, to ensure an uncomplicated and easy embryo transfer (Prapas *et al.*, 2004).

The day that the donor announced the onset of her period, the recipients were contacted to start endometrial preparation with daily oral oestradiol administration. The dose of oral oestradiol was demonstrated in a previous mock cycle with the aim of obtaining an endometrium ≥ 8 mm on the 10th day of oestradiol administration. As recipients were menopausal women, no GnRH agonist was administered to suppress ovarian function.

Two recipients were matched with one oocyte donor. The day that an oocyte donor started GnRH antagonist injections, one of the two matched recipients also started daily GnRH antagonist injections (Orgalutran 0.25 mg), in addition to oestradiol administration, until the day before HCG injection of the donor (Prapas *et al.*, 2005). The second matched recipient received no GnRH antagonist injection and continued only with endometrial preparation.

After oocyte collection, intracytoplasmic sperm injection was performed in all subjects. Embryo transfer took place 3 days after oocyte retrieval. At most, three good quality embryos were replaced by the same practitioner under ultrasound guidance as previously described (Prapas *et al.*, 1995). Luteal phase of recipients was supported with vaginal progesterone (Utrogestan; Laboratoires Besins, Iscovesco, France) 600 mg per day, in three divided doses concomitant to oral oestradiol treatment. Pregnancy was defined by a rising HCG value >20 IU/l 14 days after the embryo transfer. Clinical pregnancy was defined as the presence of a gestational sac visualised on an ultrasound scan at 7 weeks. Data were analysed by means of Wilcoxon rank sum test for numerical variables and Fisher's exact test for comparisons of

proportions. The MedCalc software statistical program (MedCalc Software; Mariakerke, Belgium) was used for statistical analysis. Values are expressed as mean \pm SD. Statistical significance was defined as a *P*-value of less than 0.05.

Results

Data were prospectively collected from 110 recipient cycles from May 2004 to May 2006, participating in the oocyte donation programme of IAKENTRO Fertility Centre. A flowchart of inclusion, randomization and drop-out of oocyte donation cycles is shown in **Figure 1**.

A total of 55 donors and 110 recipients were recruited in the study protocol. Arbitrarily, a minimum of six oocytes per recipient was considered as an acceptable number with the aim of obtaining at least two good quality embryos to transfer. As a result, only donors with a minimum number of 12 metaphase II oocytes were finally included in the study. Two donors and the four matched recipients were cancelled due to the presence of ovarian cyst in the donor and subsequent cycle cancellation. Four donors and the eight matched recipients were also excluded from the study due to the retrieval of less than 12 oocytes from the donor and the subsequent cycle cancellation of one of the two matched recipients.

In total, 49 donors and the 98 matched recipients participated and completed the study protocol. Cycle characteristics are presented in **Table 1**. Mean age of donors was 28 ± 2.1 years. Age of the recipients was similar between group I (recipients with daily GnRH antagonist injection in the proliferative phase) and group II (recipients with no GnRH antagonist injections). Days of GnRH antagonist administration were equal among the donor and the corresponding GnRH antagonist recipients (2.2 ± 0.8). Selected oocytes were equally divided among two different recipients. A mean of 14.3 ± 2.2 oocytes were collected from every donor. Mean number of good quality embryos transferred (grade A or B) was similar between the two groups (2.6 ± 0.5 in group I and 2.7 ± 0.4 in group II) (**Table 1**). Endometrial thickness was also similar between the two groups on the day GnRH antagonist administration commenced (8.1 ± 1.1 mm in group I and 7.8 ± 1.3 mm in group II), as well as on the day of HCG injection to the donor (9.5 ± 1.1 mm in group I and 9.3 ± 1 mm in group II) (**Table 2**). Also no difference was detected between the two groups with regard to the percentage of multilayered endometrial pattern on the evening before the oocyte retrieval, when recipients commenced progesterone support. No difference was found between group I and group II as regards total pregnancy rate, clinical pregnancy rate and implantation rate (**Table 3**).

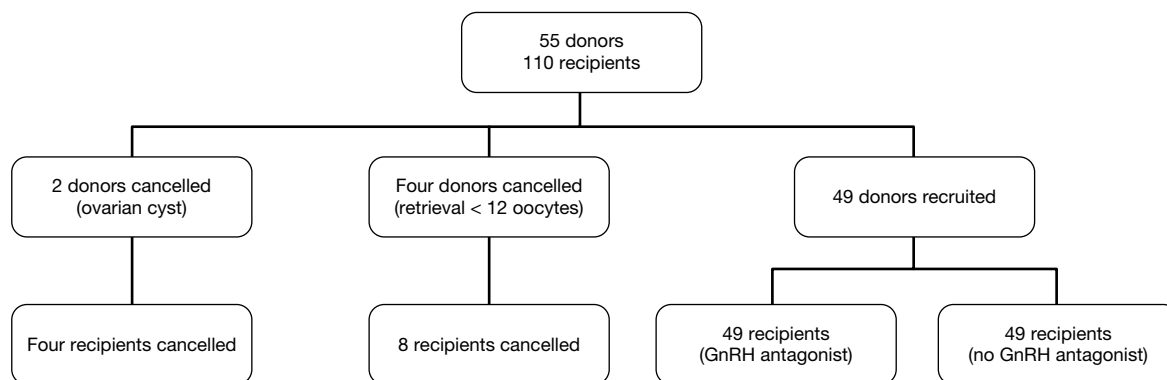


Figure 1. Flow chart of randomization and drop-out in oocyte donation cycles.

Table 1. Cycle characteristics of the two groups of oocyte recipients.

Characteristic	GnRH antagonist (Group I)	No antagonist (Group II)
Age (years)	41.8 ± 2.3	41.7 ± 2.3
Previous failed cycles	0.76 ± 0.9	0.7 ± 0.8
Days of stimulation	9.7 ± 1.2	9.5 ± 1.3
Oestradiol on day of HCG (pg/ml)	2289 ± 327	2141 ± 448
Oocytes injected	7.1 ± 1.2	7.2 ± 1.1
Oocytes fertilized	5.5 ± 1.2	5.7 ± 1.1
Fertilization rate (%)	273/349 (78.2)	282/356 (79.2)
Total number of embryos	273	282
Embryos transferred	2.6 ± 0.5	2.7 ± 0.4

Values are mean \pm SD unless otherwise stated.

HCG = human chorionic gonadotrophin.

There were no statistically significant differences between the two groups.

Table 2. Endometrial pattern in the two oocyte recipient groups.

Characteristic	GnRH antagonist (Group I)	No antagonist (Group II)
Endometrial thickness (mm)		
On day of antagonist injection	8.1 ± 1.1	7.8 ± 1.3
On day of HCG injection	9.5 ± 1.1	9.3 ± 1.0
Multilayered endometrial pattern (%)	34/49 (69.3)	31/49(63.2)

Values are mean ± SD unless otherwise stated.

GnRH = gonadotrophin-releasing hormone; HCG = human chorionic gonadotrophin.

There were no statistically significant differences between the two groups.

Table 3. Pregnancy outcome in the two groups of oocyte recipients.

Outcome	GnRH antagonist (Group I)	No antagonist (Group II)
Total pregnancy rate (%)	27/49 (55.1)	29/49 (59.2)
Implantation rate (%)	34/130 (26.1)	33/135 (24.4)
Biochemical pregnancies	1	1
Clinical pregnancy rate (%)	26/49 (53.1)	28/49 (57.1)
First trimester abortions	4	7
Twins	6	5
Triplets	1	0

GnRH = gonadotrophin-releasing hormone.

There were no statistically significant differences between the two groups.

Discussion

Despite a number of advantages in GnRH-antagonist IVF cycles, their acceptance is still lower than expected, although they were introduced into clinical practice almost a decade ago (Fauser and Devroey, 2005). Advantages of GnRH antagonist administration compared with GnRH agonists include reduced gonadotrophin injection, shortened ovarian stimulation, no hot flushes, no cyst formation as well as a lower incidence of ovarian hyperstimulation syndrome (Tarlatzis *et al.*, 2006). Yet, a minor reduction in pregnancy rates has been reported in all comparative studies, resulting in more debate (Al-Inany and Aboulghar, 2002; Kolibianakis *et al.*, 2006).

This reduction in pregnancy rates might be attributed to multiple reasons. Firstly, it could be postulated that it might be due to a direct effect that GnRH antagonists exert on oocytes and, as a result, on embryo quality. Although not undisputable, it has been suggested that GnRH antagonists might affect ovarian steroidogenesis and granulosa cell function (Minaretzis *et al.*, 1995; Albano *et al.*, 2000; Ortmann *et al.*, 2001) or directly affect oocytes, or embryonic development (Dekel *et al.*, 1988; Kane *et al.*, 1997; Raga *et al.*, 1999). However, oocyte and embryo quality resulting from donors treated with a GnRH antagonist was similar for donors treated with a GnRH agonist, indicating that treatment with GnRH antagonists did not affect oocyte or embryo quality (Sauer *et al.* 1997; Prapas *et al.*, 2005; Vlahos *et al.*, 2005). Similar conclusions were reported in phase III studies where good-quality embryos were obtained

whether GnRH agonists or antagonists were used (Albano *et al.*, 2000; Borm and Mannaerts, 2000).

As antagonists inhibit LH secretion, differences in LH serum concentrations in GnRH antagonist cycles have also been suggested as being responsible for an adverse effect of the antagonist on embryonic implantation. As such, high follicular LH concentration was found to be correlated with reduced pregnancy rates (Tavaniotou *et al.*, 2003; Kolibianakis *et al.*, 2004). However, this has not been demonstrated in other trials (Bosch *et al.*, 2003).

The possible impact of GnRH antagonist on endometrial receptivity has not yet been fully elucidated. A direct effect of GnRH antagonists on endometrial development was suggested as pregnancy rates were extremely low when a higher dose of the antagonist was used, although pregnancy rates were similar in frozen-thawed cycles irrespective of the initial dose of antagonist (The Ganirelix dose-finding study group, 1998; Kol *et al.*, 1999). Altered endometrial histology was also detected in the luteal phase of non-supplemented cycles in oocyte donors stimulated with gonadotrophins and GnRH antagonists as well as an earlier expression of progesterone receptors in the follicular phase of the cycle, compared with natural cycles (Kolibianakis *et al.*, 2003; Papanikolaou *et al.*, 2005). However, similar endometrial development and gene expression was found in supplemented cycles of oocyte donors comparing GnRH agonists, GnRH antagonists and natural cycles (Simon *et al.*, 2005). GnRH antagonists might directly affect endometrial proliferation as GnRH antagonists inhibited the development

of human endometrial cancer cell lines although data on the presence of GnRH receptors on the endometrium are rather conflicting (Ikeda *et al.*, 1997; Raga *et al.*, 1998; Takeuchi *et al.*, 1998; Gründker *et al.*, 2004).

GnRH antagonist is administered at a point in the follicular phase of the cycle, when the endometrial cells are obliged to perform synchronous waves of mitosis to form a ripe endometrium. The presence of GnRH mRNA in the endometrium, as well as the differential expression of the GnRH gene, provided physiological evidence that human GnRH may play a paracrine/autocrine function in the human uterus (Dong *et al.*, 1998; Raga *et al.*, 1998). As a result, the possibility exists that the GnRH antagonists may disrupt an autocrine/paracrine loop that is essential for the mitotic programme of the endometrial cells and this might be manifested by a decrease in pregnancy rates (Hernandez, 2000). Recently, it has been postulated that changes in the expression of sex-steroid receptors and metabolizing enzymes may lead to alterations in the activity and intracellular availability of oestrogens, progestogens and androgens in the endometrium of women treated with Cetrorelix and recombinant FSH (Vani *et al.*, 2007).

The clinical assessment of endometrial function in terms of receptivity in IVF cycles is controversial. In oocyte donation cycles, endometrial pregnancy rates and endometrial receptivity was found to be affected by serum oestradiol concentrations and HCG administration (Tesarik *et al.*, 2003; Cobo *et al.*, 2007). In daily practice, the physician deals with the echographic pattern of the endometrium with the growing endometrium and the three-layer pattern indicating adequate proliferation. Some studies have shown that endometrial thickness might be a predictive parameter for IVF outcome whereas other groups could not reproduce these findings (Check *et al.*, 1991; Noyes *et al.*, 1995; Rinaldi *et al.*, 1996; Yuval *et al.*, 1999; De Geyter *et al.*, 2000; Bassil, 2001). Polak de Fried *et al.* (2004) demonstrated that there was no difference in endometrial growth rates between IVF patients treated with a single 3 mg Cetrorelix injection and patients who were desensitized with GnRH agonists. In this study, no relevant difference in endometrial thickness and pattern was observed between the two groups on the day of HCG injection to the donor.

As GnRH antagonist treatment might exert its effect at different levels, a possible direct effect of GnRH antagonists on the endometrium was investigated. As far as is known, this is the first prospective randomized trial to investigate the effect of GnRH antagonist on endometrial receptivity, implantation and pregnancy rates in oocyte recipient cycles. With the oocyte donation model, it was possible to circumvent any possible effect of the GnRH antagonist on oocytes or embryo quality, as oocytes from the same donor were equally divided between two different recipients. Recipients were also menopausal women who received no GnRH agonist to exclude any effect of the analogue on the endometrium. In addition, there was no effect of supraphysiological steroid serum concentrations on endometrial development including thickness and histological growth as subjects received oral oestradiol only for endometrial priming.

In this study, pregnancy rates were exactly the same between the two protocols indicating that GnRH antagonist administration during the proliferative phase does not adversely affect

endometrial development, implantation and pregnancy rates in oocyte recipients. These results appear to be reassuring as regards a direct effect of GnRH antagonist on implantation; however, it should be taken into account that the power of this study is low due to the limited number of patients. As a result, a possible direct effect of the antagonist on oocytes as well as endometrial histology should be further investigated. In addition, higher GnRH antagonist doses might exert a different effect on the endometrial compartment.

In conclusion, GnRH antagonist administration in the proliferative phase, in the dose of 0.25 mg does not appear to reduce pregnancy and implantation rates in oocyte recipients. Thus, it might be postulated that the dose of 0.25 mg does not adversely and directly affect endometrial receptivity in these cycles. However, these results should be interpreted with caution as supraphysiological steroid serum concentrations might alter the endometrial environment in stimulated cycles.

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