

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 ± 5.8 versus 12.1 ± 6.7), mature oocytes (8.4 ± 4.4 versus 8.9 ± 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 Llata *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/m², 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 ≥ 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 μ 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

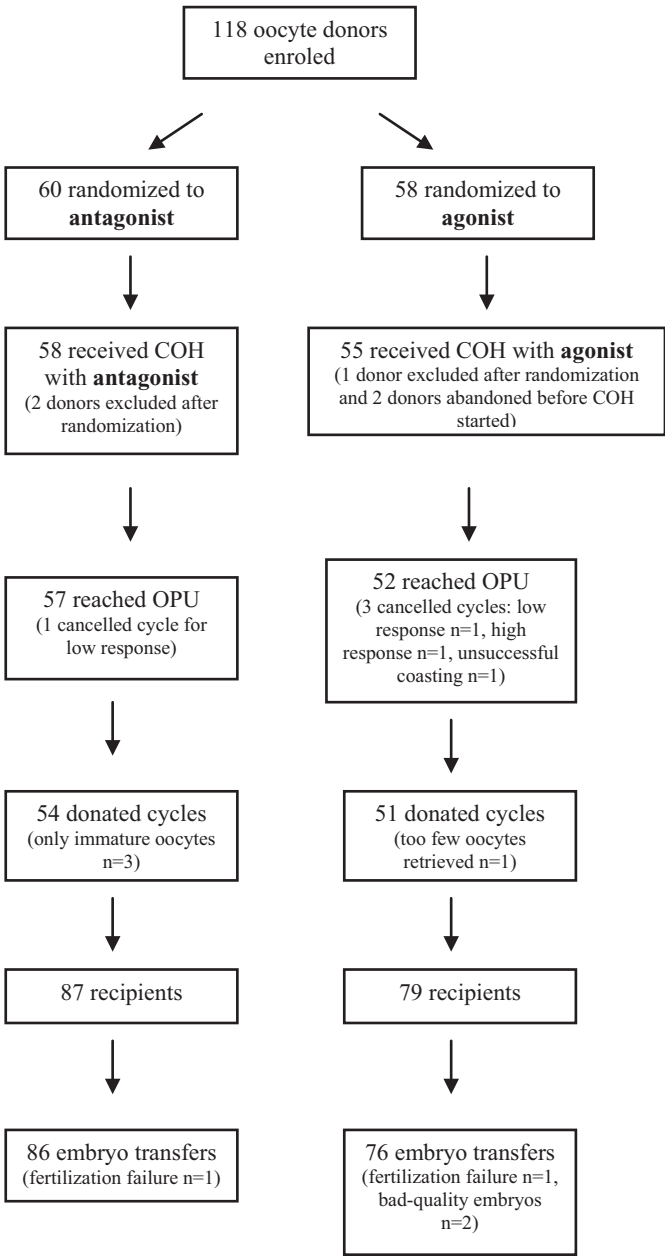


Figure 1. Flow chart of patients treated in the study.

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	P
Number	58	55	–
Age (years)	25.9 ± 3.3	24.7 ± 3.4	0.065 ^a
BMI	22.6 ± 2.8	22.6 ± 3.0	0.92 ^a
Basal FSH (IU/ml)	6.8 ± 1.5	7.3 ± 1.5	0.062 ^a
Days of stimulation ^b	9.9 ± 1.6	10.2 ± 2.1	0.32 ^a
Total rFSH (IU) used ^b	2179 ± 367	1828 ± 459	<0.0001 ^a
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 ^a
COC retrieved ^c	11.6 ± 5.8	12.1 ± 6.7	0.69 ^a
Mature oocytes retrieved ^c	8.4 ± 4.4	8.9 ± 5.3	0.52 ^a
Proportion of mature oocytes ^b (%)	70.8 ± 23.8	75.7 ± 14	0.20 ^a

COC, cumulus–oocyte–complex.
Values are mean ± SD.
^aStudent's *t*-test.
^bAnalysis performed in donors who reached the day of HCG.
^cPer started cycle (including patients with cancelled cycles).

Table II. Description of the recipients

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

OD, oocyte donation.
^aValues are mean ± SD.
^bStudent's *t*-test.
^cChi-square test.
^dOligo-, 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 ^a
Quality of transferred embryos			
Grade 1 [n (%)]	54/165 (32.7)	49/146 (33.6)	0.252 ^b
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 ^{c,d} [n (%)]	35/87 (40.2) (30.5–50.7)	36/79 (45.6) (35.0–56.5)	0.66 ^b
Clinical pregnancy rate ^{e,d} [n (%)]	35/58 (60.3) (47.4–71.9)	36/55 (65.4) (52.2–76.6)	0.78 ^b
Implantation rate ^d [n (%)]	43/165 (26.1) (19.9–33.2)	44/146 (30.1) (23.2–38.0)	0.54 ^b
Miscarriage rate ^d [n (%)]	7/35 (20) (10.0–35.9)	6/36 (16.7) (7.9–31.9)	0.76 ^b
Ongoing pregnancy rate ^{c,d} [n (%)]	28/87 (32.2) (23.3–42.6)	30/79 (37.9) (28.1–49.0)	0.58 ^b
Ongoing pregnancy rate ^{e,d} [n (%)]	28/58 (48.3) (35.9–60.8)	30/55 (54.5) (41.5–66.9)	0.70 ^b
Twins ^d [n (%)]	8/35 (22.9) (12.1–39.0)	8/36 (22.2) (11.7–38.1)	0.95 ^b
Triplets	0	0	—

^aStudent's *t*-test.^bChi-square test.^cPer recipient cycle (including recipient failures without embryo transfer).^dWith 95% CI.^ePer started donor cycle (including patients with cancelled cycles).

cancelled cycles where no oocytes were retrieved). Similar proportion of mature oocytes (70.8 ± 23.8 versus $75.7 \pm 14\%$) and similar fertilization rates (65.5 ± 22.8 versus $67.2 \pm 20.8\%$) after ICSI were observed. The mean number of cleavage-stage (3.01 ± 1.34 versus 3.38 ± 1.46) and transferred embryos (1.92 ± 0.38 versus 1.92 ± 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 ± 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 ± 1903 pg/ml) in the agonist short protocol compared with the antagonist protocol (2428 ± 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 E₂ 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 an 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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