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A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists

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BACKGROUND: Eliciting an endogenous LH surge by GnRH-agonist for the induction of final oocyte maturation may be more physiological compared with the administration of HCG. However, the efficacy of this intervention in patients treated for IVF with GnRH antagonists remains to be assessed. METHODS: 106 patients were randomized to receive either 10 000 IU urinary HCG or 0.2 mg Triptorelin for triggering final oocyte maturation. Ovarian stimulation for IVF was performed with a fixed dose of 200 IU recombinant FSH and GnRH antagonist was started on stimulation day 6. Luteal phase was supported with micronized vaginal progesterone and oral estradiol. The study was monitored continuously for safety and stopping rules were established. RESULTS: No significant differences were present in the number of cumulus-oocyte complexes retrieved, in the proportion of metaphase II oocytes, in fertilization rates or in the number and quality of the embryos transferred between the two groups. However, a significantly lower probability of ongoing pregnancy in the GnRH agonist arm prompted discontinuation of the trial, according to the stopping rules established (odds ratio 0.11; 95% confidence interval 0.02–0.52). CONCLUSIONS: Lower probability of ongoing pregnancy can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing ovarian stimulation for IVF with GnRH antagonists.

Key words: HCG versus GnRH agonist/IVF-GnRH antagonist cycles/ongoing pregnancy rates/oocyte maturation/RCT

Introduction

A bolus of GnRH agonist can stimulate the release of LH from the pituitary, triggering final oocyte maturation in the midcycle (Gonen *et al*., 1990; Imoedemhe *et al*., 1991; Itskovitz *et al*., 1991; Segal and Casper, 1992). This is not possible when agonists have already been used for pituitary down-regulation. Since inhibition of premature LH surge with GnRH agonists has been the established way of performing ovarian stimulation for several years in IVF (Hughes *et al*., 1992), an agonist induced LH surge has remained largely of theoretical interest.

The clinical availability of GnRH antagonists in recent years has made possible the replacement of urinary/recombinant HCG or recombinant LH for triggering final oocyte maturation with GnRH agonist. This is due to the competitive blockage of GnRH receptors by GnRH antagonist, which still allows the stimulation of hypophysis with a GnRH agonist and the subsequent secretion of endogenous gonadotrophins (Felberbaum *et al*., 1995; Olivennes *et al*., 1996; Itskovitz-Eldor *et al*., 2000; Fauser *et al*., 2002; Beckers *et al*., 2003).

The use of GnRH agonist to induce final oocyte maturation has been suggested to result in prevention of clinically significant ovarian stimulation syndrome by inducing quick and irreversible luteolysis (Kol *et al*., 2004). Although this approach might increase the safety of IVF, it has so far only been examined in a small randomized control trial (RCT), which did not allow solid conclusions to be drawn (Fauser *et al*., 2002). A further RCT comparing agonist and HCG triggering (Beckers *et al*., 2003) did not use luteal support after administration of HCG or GnRH agonist and its conclusions cannot be applied to GnRH antagonist cycles in which luteal supplementation is used.

The purpose of this RCT was to compare fertilization rates after triggering of final oocyte maturation with GnRH agonist or HCG in a larger series of patients.

Materials and methods

Patient population

The present study was a two centre, randomized controlled clinical trial. Forty-two patients were recruited at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University, Belgium

(Centre 1) and 64 patients at the Department of Gynaecological Endocrinology and Reproductive Medicine, University Clinic of Schleswig-Holstein, Campus Lübeck, Germany (Centre 2) from December 2003 until October 2004. The flowchart for the study is shown in Fig. 1.

Inclusion criteria were age ≤39 years, normal day-3 serum FSH levels, ≤3 previous assisted reproduction treatment (ART) attempts, normal body mass index (18–29 kg/m), regular menstrual cycles, no polycystic ovaries or previous poor response to ovarian stimulation, both ovaries present, use of fresh ejaculated sperm, and no embryo biopsy. Patients could enter the study only once. All patients participating in the study gave informed consent. The study was approved by the local ethics committee of both Centers.

Ovarian stimulation and IVF procedure

Stimulation was performed with recombinant FSH (rFSH) (Puregon®, N.V. Organon, Oss, The Netherlands) starting in the afternoon of day 2 of a spontaneous cycle at 200 IU. The dose of rFSH remained unchanged during stimulation. Daily GnRH antagonist 0.25 mg (Orgalutran; N.V. Organon) was used to inhibit premature LH surge and was always started on the morning of day 6 of stimulation. Final oocyte maturation was achieved by administration of 10 000 IU of HCG (Pregnyl, N.V. Organon) or 0.2 mg Triptorelin (Decapeptyl®, Ferring Pharmaceuticals, Copenhagen, Denmark) as soon as ≥3 follicles of ≥17 mm were present in ultrasound, according to a computer-generated randomization list. The sequence of randomization was not concealed and the study was not blind. Steroid levels were measured but were not taken into consideration for administration of HCG, which was based exclusively on follicular development.

Oocyte retrieval was carried out 36 h after triggering of final oocyte maturation by agonist or HCG by transvaginal ultrasound-guided puncture of follicles. Intracytoplasmic sperm injection (ICSI) was performed in the majority of the couples included (n = 97), while conventional IVF was carried out in nine couples. ICSI and IVF procedures were as described previously in detail by Van Steirteghem *et al*. (1993) and Devroey *et al*. (1995).

As a matter of principle, 1–2 embryos were transferred on day 3 or day 5 after oocyte retrieval in Centre 1 and 2–3 embryos on day 2 of *in vitro* culture in Centre 2. In Centre 1, embryos were classified as top quality (score 1), medium quality (score 2) and low quality (score 3) as described previously (Staessen *et al*., 1992; Gardner and Schoolcraft, 1999). The mean score of the embryos transferred to each patient was used for the calculation of the mean quality score of all embryos transferred. In Centre 2, embryos were classified as top quality, medium quality or low quality, and the cumulative embryo score in a modified version was calculated as described previously (Ludwig *et al*., 2000).

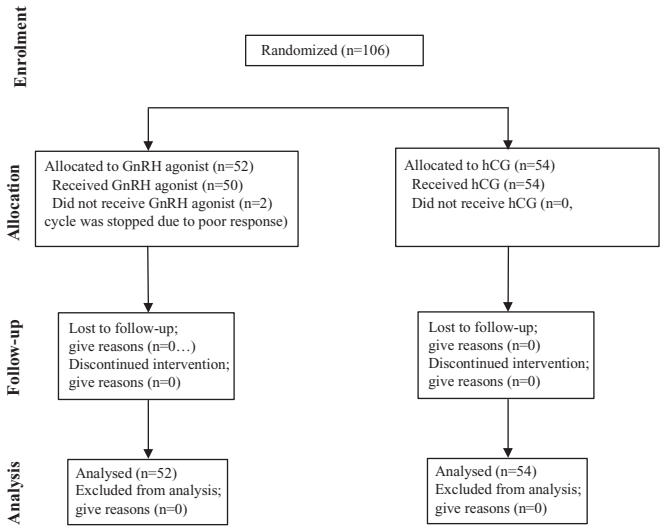

Figure 1. Flowchart of patients in the study.

Luteal supplementation

The luteal phase was supplemented with vaginal administration of 600 mg natural micronized progesterone in three separate doses (Utrogestan®; Besins, Brussels, Belgium), and daily 2 × 2 mg oral estradiol (E_2) (Progynova® Progynova; Schering, Berline, Germany), starting one day after oocyte retrieval and continued until 7 weeks of gestation in the presence of a positive HCG test. In Centre 2, vaginal and intramuscular progesterone only was administered, if conception occurred, until 7 weeks of pregnancy.

Hormonal measurements and ultrasound assessment

Hormonal assessment was performed at initiation of stimulation, on day 6, on day 8 of rFSH stimulation and on the day of triggering of final oocyte maturation by agonist or HCG. Additional blood samples were taken as necessary between antagonist initiation and HCG administration. Serum LH, FSH, HCG, E_2 and progesterone levels were measured locally as described previously (Centre 1: Kolibianakis *et al*., 2004; Centre 2: Griesinger *et al*., 2005). Ultrasound was performed on day 6 of stimulation and thereafter as necessary in order to ensure that triggering of final oocyte maturation was performed on the first day that the patient had ≥3 follicles of ≥17 mm.

Outcome measures

Fertilization rate was the primary outcome measure of the study and was calculated by dividing the number of 2-pronucleate (2PN) oocytes with the number of cumulus-oocyte complexes (COCs) retrieved. Pregnancies progressing beyond the 12th week of gestation were considered ongoing. Ongoing implantation rate was calculated by dividing the number of gestational sacs with fetal heartbeat present at 12 weeks of gestation by the number of embryos transferred.

Statistical analysis

Power analysis

Previously published data (Fauser *et al*., 2002) suggested that the use of GnRH agonist could result in a 6% higher fertilization rate compared with HCG administration. It was calculated that 223 patients reaching oocyte retrieval will be required in each arm to detect a difference of at least 6% (a = 0.05, b = 0.2) by performing four sequential tests at equally spaced time intervals.

Stopping rules

Regarding the probability of ongoing pregnancy, inclusion of 42 patients in each group achieves 82% power to detect a difference of 0.30 between the group proportions of 0.36 and 0.06 at a significance level (α) of 0.05 using a two-sided z-test with continuity correction. Such a difference in pregnancy rates in favour of HCG was reported recently by Humaidan *et al*. (2005). These results assume that four sequential tests are made using the Pocock spending function to determine the test boundaries. If a difference in pregnancy rates was detected at a probability level of 0.03 at the second interim analysis, the study should be stopped due to ethical reasons.

Statistical tests

Data were analysed stratified by centre and results are presented as combined standardized differences of the mean and combined odds

ratios (random effects model), as appropriate, with 95% confidence intervals (CIs).

Results

Table I shows the baseline characteristics of the patients included in the trial. No differences were observed between patients randomized to receive agonist or HCG for triggering of final oocyte maturation.

Diagnosis of the patients randomized did not differ between the groups compared. The majority of the patients were treated for male factor infertility (agonist: 69.2%; HCG: 74.1%). Other indications included tubal factor infertility (agonist: 7.7%; HCG: 11.1%), idiopathic infertility (agonist: 9.6%; HCG: 5.6%) or combined indications (agonist: 13.5%; HCG: 9.3%).

Table II shows the characteristics of ovarian stimulation and its outcome in the two arms of the study. Overall, patients who received agonist or HCG had a similar duration of stimulation and developed similar number of follicles.

Similar numbers of metaphase II (MII) oocytes were retrieved (in ICSI cases), similar fertilization rates were achieved and similar numbers of 2PN oocytes were available (Table II) following triggering of final oocyte maturation.

As shown in Table II, there was no difference in the number of embryos transferred in the agonist and in the HCG arm. Moreover, the embryo score of the embryos transferred was similar (P = 0.79) between the agonist and the HCG arm in both centres (Centre 1: 1.56 versus. 1.55; Centre 2: 14.8 versus 15.3, respectively).

Hormonal data on the day of triggering of final oocyte maturation appear in Table III.

Table IV shows the number of patients included in the study, their course through the IVF cycle and their cycle outcome. A significantly lower ongoing pregnancy rate (P = 0.012) was observed in Centre 1 at the second interim analysis. This prompted discontinuation of the study according to the stopping rules established. At that point, a substantial difference (although not statistically significant) in ongoing pregnancy rate in favour of HCG was also present at Centre 2. Overall, a significantly lower ongoing pregnancy rate was present in the agonist arm of the trial (odds ratio 0.11; 95% CI = 0.02–0.52; P = 0.005). Implantation rate was also significantly higher after HCG compared with agonist triggering (22.6 ± 5.3 versus 6.8 ± 3.3, respectively; P = 0.035). No cases of ovarian hyperstimulation syndrome (OHSS) were observed in the current study.

Discussion

This study has shown that, when final oocyte maturation is triggered by GnRH agonist instead of HCG, a significantly

Table I. Baseline characteristics in patients randomized to receive agonist or HCG for triggering final oocyte maturation

	Agonist	HCG	Standardized difference	95% CI	*P*
Female age (years)	32.4 ±0.6	32.3 ±0.5	-0.01	-0.4 to +0.3	0.9
Body mass index	22.9 ±0.5	23.7 ±0.5	0.30	-0.3 to +0.9	0.3
Number of previous IVF trials	0.9 ±0.3	0.5 ±0.1	-0.30	-0.7 to +0.1	0.1
Basal FSH (IU/L)	8.2 ±0.4	8.1 ±0.4	-0.05	-0.5 to +0.4	0.8

Table II. Stimulation characteristics and outcome in patients randomized to receive agonist or HCG for triggering final oocyte maturation

	Agonist	HCG	Standardized difference	95% CI	*P*
Days of stimulation	9.1 ±0.2	9.2 ±0.2	0.05	-0.3 to +0.4	0.8
Total units of rFSH	1778 ±40	1835 ±56	0.19	-0.2 to +0.6	0.3
Number of COCs	10.2 ±7.0	10.6 ±6.3	0.06	0.3 to +0.4	0.7
Follicles of ≥11 mm on the day of triggering	11.1 ±0.9	11.3 ±0.6	0.11	-0.5 to +0.7	0.7
Follicles of ≥17 mm on the day of triggering	4.4 ±0.4	4.5 ±0.3	0.10	-0.3 to +0.5	0.6
Proportion of MII oocytes %	73.5 ±4.5	78.7 ±3.3	0.15	-0.3 to +0.6	0.4
Fertilization rate (%)	55.6 ±3.8	58.0 ±3.2	0.04	-0.4 to +0.4	0.8
Number of 2PN oocytes	5.1 ±0.6	5.8 ±0.5	0.12	-0.3 to +0.5	0.6
Number of embryos transferred	1.9 ±0.1	1.8 ±0.1	-0.07	-0.6 to +0.5	0.8

Table III. Hormonal data in the agonist and the HCG group on the day of triggering final oocyte maturation

	Agonist	HCG	Standardized difference	95% CI	*P*
E_2 (pg/ml)	1910 ±139	1980 ±155	0.06	–0.3 to +0.5	0.76
Progesterone (ng/l)	1.1 ±0.1	1.0 ±0.1	–0.11	–0.8 to +0.6	0.76
LH (IU/l)	2.3 ±0.4	2.2 ±0.4	–0.15	–0.6 to +0.3	0.47
FSH (IU/l)	16.4 ±0.6	15.0 ±0.7	0.36	–0.9 to +0.2	0.17

Table IV. Cycle outcome after agonist and HCG triggering of final oocyte maturation

	Centre 1		Centre 2		
	Agonist	HCG	Agonist	HCG	
Patients who started stimulation	18	24	34	30	
Patients who reached oocyte retrieval	18	24	32	30	
Patients who reached embryo transfer	15	20	29	28	
Positive HCG per started cycle	16.7% (3/18)	45.8% (11/24)	17.6% (6/34)	20.0% (6/30)	Odds ratio (95% CI) 0.56 (0.22–1.46)
Ongoing pregnancy rate	5.6%(1/18)	41.7%(10/24)[a]	2.9%(1/34)	16.7% (5/30)[b]	Odds ratio (95% CI) 0.11 (0.02–0.52)[c]
Early pregnancy loss	66.7% (2/3)	9.1% (1/11)	83.3% (5/6)	16.7% (1/6)	Odds ratio (95% CI) 22.46 (2.5–200.6)

[a]*P* level at discontinuation of the study = 0.012. [b]*P* level at discontinuation of the study = 0.09. [c]*P* level at discontinuation of the study = 0.005.

lower ongoing pregnancy rate is to be expected in cycles stimulated with GnRH antagonists and recombinant FSH for IVF.

The use of an agonist as an alternative to HCG for triggering final oocyte maturation was evaluated recently in 122 patients (Humaidan *et al*., 2005). The results of that RCT, which were presented while the current study was ongoing, also showed a significantly lower ongoing pregnancy rate in the agonist arm. The lower pregnancy rate in the agonist arm in the study by Humaidan *et al*. (2005) might be associated with discontinuation of luteal support in the presence of a positive pregnancy test. However, the results of the present study do not support this claim, as luteal support was continued in all patients with a positive pregnancy test until 7 weeks of gestation. The present study and that by Humaidan *et al*. (2005) used different GnRH agonists to trigger final oocyte maturation (0.2 mg Triptorelin versus 0.5 mg buserelin s.c., respectively) and both showed a decreased probability of pregnancy in the agonist arm. It is thus possible that the adverse effect of agonist triggering is independent of the type of the GnRH agonist used and that this is mainly due to replacement of the HCG triggering signal.

In contrast to the present study, Humaidan *et al*. (2005) showed that the use of agonist for triggering final oocyte maturation was associated with a higher percentage of MII oocytes compared with HCG. It is not clear if this is due to differences in the stimulation protocols between the two studies. Besides triggering final oocyte maturation with a different agonist, Humaidan *et al*. (2005) administered GnRH antagonist in a flexible scheme—in contrast to a fixed GnRH antagonist protocol used in the current study. Moreover, the criteria used to administer HCG or GnRH agonist were different between the two studies (presence of at least three follicles of 17 mm versus as soon as ≥3 follicles of ≥17mm, respectively). The same was true for the time interval from injecting agonist or HCG until oocyte retrieval (35 h versus 36 h, respectively). It should be noted, however, that neither Humaidan *et al*. (2005) nor the current study randomized only ICSI patients to receive agonist or HCG for triggering final oocyte maturation. Thus, the comparison in the proportion of MII oocytes is based on a part of the patients randomized in each study (those who performed ICSI). Thus, although interesting and perhaps requiring further evaluation, the difference observed in the proportion of MII oocytes does not originate from a randomized controlled trial performed to answer this specific question.

Several hypotheses might explain the significantly lower probability of pregnancy after GnRH agonist triggering of final oocyte maturation in the current study. It might be due to a negative influence of agonist triggering on oocyte quality or it might be associated with a negative effect of GnRH agonist triggering on endometrial receptivity. The latter might be due to inadequately developed corpora lutea, insufficient stimulation of the ensuing corpora lutea or inadequate luteal support with E_2 and progesterone. Finally, a combination of the above factors cannot be excluded.

Effect of agonist triggering on oocyte quality

HCG has been used as a means for triggering final oocyte maturation for many years. The LH activity it conveys lasts for several days compared with the naturally occurring LH surge, the duration of which is about 48 h (Fauser *et al*., 2002). GnRH agonist, on the other hand, mimics more closely the natural LH surge, though the duration of the agonist-induced surge appears to be shorter (Fauser *et al*., 2002). It is possible that the lower ongoing pregnancy rate observed in the agonist arm might be associated with the shorter duration of the agonist induced LH surge, which results in decreased oocyte quality and compromised embryo development. However, this is unlikely to be true since similar numbers of MII oocytes, similar fertilization rates and numbers of 2PN oocytes were present in the two arms of the current study. This was also true for the numbers and quality of the embryos transferred.

Formation of non-functional corpora lutea or inadequate stimulation of corpora lutea after agonist triggering

It cannot be excluded that the shorter duration of the agonist-induced LH surge might not transform the existing follicles efficiently to corpora lutea capable of supporting implantation. Studies in primates have shown that corpus luteum cannot be supported or induced by LH surges with duration of <48 h (Chandrasekher *et al*., 1994). Inefficient luteinization and/or corpora lutea function might also be due to the suppression of LH, induced by pituitary agonist down-regulation.

Although both the above events are likely to take place after agonist triggering, they do not offer a clear explanation for the decreased ongoing pregnancy rates in the agonist arm. This is due to the fact that low luteal LH levels are normal after ovarian stimulation that aims at multifollicular development,

regardless of whether antagonist suppression of premature LH surge is used or not (Tavaniotou *et al*., 2001). Moreover, it is accepted that the luteal phase is defective after ovarian stimulation with gonadotrophins, HCG and GnRH analogues (Kolibianakis and Devroey, 2002). For that reason, luteal phase supplementation in the form of progesterone administration alone or in combination with E_2 (Pritts and Atwood, 2002) is compulsory in either GnRH agonist (Soliman *et al*., 1994) or GnRH antagonists cycles (Albano *et al*., 1998; Beckers *et al*., 2003; Kolibianakis *et al*., 2003) and improves pregnancy rates.

Inadequate dosing of E_2 and progesterone for luteal support after agonist triggering

The efficacy of progesterone and/or E_2 as a means for luteal support in IVF has always been examined after final oocyte maturation has been triggered with HCG. It is thus likely, that in addition to progesterone and or E_2, the luteal phase is also supported partially by the HCG which, although it is administered to mature the oocyte cohort, also stimulates the ensuing corpora lutea. Due to its long half-life, HCG will sustain LH activity for a period of ~10 days during which implantation takes place (Fauser *et al*., 2002). LH activity, however, is not sustained after agonist triggering, as the endogenous LH levels will remain low after the initial surge (Fauser *et al*., 2002). Thus, existing corpora lutea are deprived from both endogenous LH and exogenous LH activity offered by HCG during the implantation period. It has been previously shown in primates that withdrawal of LH results in luteolysis (Collins *et al*., 1986; Duffy *et al*., 1999). It is likely that, after an agonist triggering, the luteal phase support might depend entirely on exogenous progesterone and E_2 administration, which may not be efficient to sustain implantation. Why luteal supplementation in the form of progesterone and E_2 alone does not sustain pregnancy rate is not clear, although it may be that the doses of progesterone and E_2 used are low. If this is the case, increased doses such as those used in patients with primary ovarian insufficiency (Devroey *et al*., 1988) might improve pregnancy rates. However, the beneficial effect of E_2 and progesterone in high doses after ovarian stimulation for IVF might not be the same as that in patients performing a frozen cycle. Luteal transformation after ovarian stimulation for IVF starts in all cycles in the presence of a an endometrium which has already entered luteal phase (Ubaldi *et al*., 1997; Kolibianakis *et al*., 2002). The use of HCG to further stimulate corpora lutea after GnRH agonist triggering, although interesting as a concept, might not work if the shorter duration of agonist-induced LH surge does not transform follicles to functional corpora lutea amenable to HCG stimulation. More importantly, it negates the main advantage of agonist, which is a reduced risk of OHSS due to the absence of HCG stimulation in the presence of multiple follicular development.

The results of the current study put in serious doubt the feasibility of using GnRH agonist to induce final oocyte maturation in IVF when GnRH antagonists are used for premature LH surge inhibition. At present, HCG appears to be the most reliable approach for triggering final oocyte maturation both in antagonist and in agonist cycles. It was recently suggested that replacement of HCG by recombinant LH in agonist cycles results in a significantly lower pregnancy rate compared with HCG (Aboulghar and Al Inany, 2005). GnRH agonist triggering might be still be useful in oocyte donation cycles where the quality of the luteal phase is not important. For that purpose, the outcome of the frozen thawed cycles after agonist triggering needs to be examined in a future study. If the low pregnancy rates reported in a previous very small uncontrolled study (Itskovitz *et al*., 2000) in frozen embryo transfer cycles are not confirmed, then GnRH agonist triggering might still have a place in ART.

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