Human Reproduction Vol.20, No.5 pp. 1213–1220, 2005 doi:10.1093/humrep/deh765
Advance Access publication March 10, 2005

GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study

P.Humaidan[1,5], H.Ejdrup Bredkjær[2], L.Bungum[1], M.Bungum[1], M.L.Grøndahl[2], L.Westergaard[3] and C.Yding Andersen[4]

[1]The Fertility Clinic, Viborg Hospital (Skive), DK 7800 Skive, [2]The Fertility Clinic, Hvidovre Hospital, DK 2650, Hvidovre, [3]The Fertility Clinic Trianglen, Lundevangsvej 12, DK 2900 Hellerup and [4]Laboratory of Reproductive Biology, Section 5712, University Hospital of Copenhagen, DK 2100 Copenhagen, Denmark

[5]To whom correspondence should be addressed. E-mail: peter.humaidan@sygehusviborg.dk

BACKGROUND: We aimed to determine the efficacy of ovarian hyperstimulation protocols employing a GnRH antagonist to prevent a premature LH rise allowing final oocyte maturation and ovulation to be induced by a single bolus of either a GnRH agonist or hCG. METHODS: A total of 122 normogonadotrophic patients following a flexible antagonist protocol was stimulated with recombinant human FSH and prospectively randomized (sealed envelopes) to ovulation induction with a single bolus of either 0.5 mg buserelin s.c. ($n = 55$) or 10 000 IU of hCG ($n = 67$). A maximum of two embryos was transferred. Luteal support consisted of micronized progesterone vaginally, 90 mg a day, and estradiol, 4 mg a day *per os*. RESULTS: Ovulation was induced with GnRH agonist in 55 patients and hCG in 67 patients. Significantly more metaphase II (MII) oocytes were retrieved in the GnRH agonist group ($P < 0.02$). Significantly higher levels of LH and FSH ($P < 0.001$) and significantly lower levels of progesterone and estradiol ($P < 0.001$) were seen in the GnRH agonist group during the luteal phase. The implantation rate, 33/97 versus 3/89 ($P < 0.001$), clinical pregnancy rate, 36 versus 6% ($P = 0.002$), and rate of early pregnancy loss, 4% versus 79% ($P = 0.005$), were significantly in favour of hCG. CONCLUSIONS: Ovulation induction with a GnRH agonist resulted in significantly more MII oocytes. However, a significantly lower implantation rate and clinical pregnancy rate in addition to a significantly higher rate of early pregnancy loss was seen in the GnRH agonist group, most probably due to a luteal phase deficiency.

Key words: agonist/antagonist/IVF/ovulation induction

Introduction

hCG has been used for decades to achieve final oocyte maturation and, thereby a correct timing of oocyte retrieval in connection with ovarian hyperstimulation protocols. The administration of hCG in cycles with multiple follicular development results in the formation of multiple corpora lutea with sustained luteotrophic effect and supraphysiological levels of estradiol and progesterone. Despite the widespread use of hCG, some studies have suggested a negative impact on endometrial receptivity (Forman *et al.*, 1988; Simon *et al.*, 1998) and embryo quality (Valbuena *et al.*, 2001). In addition, the sustained luteotrophic effect may facilitate and deteriorate the development of ovarian hyperstimulation syndrome (OHSS).

As an alternative to hCG, a GnRH agonist has been used to trigger the endogenous release of LH (and FSH) in a fashion resembling the mid-cycle surge of gonadotrophins (Nakano *et al.*, 1973). GnRH agonist has been shown to be as effective as hCG for induction of ovulation (Gonen *et al.*, 1990; Itskovitz *et al.*, 1991; Imoedemhe *et al.*, 1991; Segal and Casper, 1992). Sharing the same α subunit and 85% of the amino acid residues of their β subunit, hCG and LH bind to the same LH/hCG receptor (Kessler *et al.*, 1979). The half-life of hCG, however, is >24 h (Damewood *et al.*, 1989) whereas that of LH is ~60 min (Yen *et al.*, 1968).

The LH surge in the natural, non-stimulated cycle is characterized by three phases: a rapidly ascending phase lasting for 14 h, a plateau of 14 h and a descending phase of 20 h (Hoff *et al.*, 1983). By contrast, the LH surge induced by injection of a bolus of GnRH agonist consists of only two phases: a short ascending limb (>4 h) and a long descending limb (>20 h), in total ~24–36 h (Itskovitz *et al.*, 1991). Despite the significantly longer duration of the natural LH surge, the GnRH agonist-induced surge effectively stimulates ovulation and final oocyte maturation (Gonen *et al.*, 1990; Itskovitz *et al.*, 1991). Another aspect of the GnRH agonist-induced

ovulation is the induction of an FSH surge comparable to the surge of the natural cycle, in addition to the LH surge. The exact role of the mid-cycle FSH surge in the natural cycle is not fully clear, but one function of FSH is to induce LH receptor formation in the luteinizing granulosa cells, thus optimizing the function of the corpus luteum. Moreover FSH specifically seems to promote nuclear maturation, i.e. resumption of meiosis (Zelinski-Wooten *et al.*, 1995; Yding Andersen *et al.*, 1999) and cumulus expansion (Stickland and Beers, 1976; Eppig, 1979).

The use of GnRH agonist for ovulation induction gained some interest in the late 1980s and in the early 1990s (Itskovitz *et al.*, 1988; Gonen *et al.*, 1990; Imoedehme *et al.*, 1991; Emperaire *et al.*, 1992). The simultaneous use, however, of GnRH agonist for pituitary down-regulation and ovulation induction is not possible. With the recent introduction of GnRH antagonist protocols for the prevention of a premature LH surge (Albano *et al.*, 1997; Itskovitz-Eldor *et al.*, 1998; European Orgalutran Study Group, 2000), it has now again become an option to induce ovulation with GnRH agonist. This concept is based on the fact that the suppressive effect of the GnRH antagonist can be reversed by the administration of GnRH agonist. The GnRH agonist is capable of displacing the antagonist from the receptor and inducing an initial activation (flare-up) prior to down-regulation of the receptor, leading to a concomitant LH and FSH surge. Whereas the ovulatory feasibility of using GnRH agonist in connection with antagonist protocols has been demonstrated (Felberbaum *et al.*, 1995; Olivennes *et al.*, 1996; Itskovitz *et al.*, 2000; Fauser *et al.*, 2002; Beckers *et al.*, 2003), the clinical efficacy of combining the antagonist protocol with GnRH agonist-induced ovulation (Itskovitz *et al.*, 2000; Bracero *et al.*, 2001; Fauser *et al.*, 2002; Beckers *et al.*, 2003) still needs to be confirmed by larger studies.

Studies performed prior to the introduction of the antagonist protocol have compared the ovulatory effectiveness of hCG versus GnRH agonist. In the largest study, Romeu *et al.* (1997) compared the results of 345 intrauterine insemination (IUI) cycles induced with leuprolide acetate with 416 cycles in which hCG had been used to induce ovulation. No difference was found in ovulation rates (99 versus 99%), but a significantly higher pregnancy rate (27.3 versus 17.3%) was found in cycles induced with leuprolide acetate compared to hCG ($P = 0.0007$).

Some studies, however, have found that triggering ovulation with GnRH agonist leads to a suboptimal luteal phase as reported in both controlled (Segal and Casper, 1992; Balasch *et al.*, 1995) and uncontrolled studies (Balasch *et al.*, 1994), whereas others failed to find any difference in the luteal phases of ovulations triggered by hCG or GnRH agonist (Shalev *et al.*, 1995; Romeu *et al.*, 1997).

The primary endpoint of the present study was to compare pregnancy outcome in patients undergoing IVF/ICSI following a flexible GnRH antagonist protocol, randomized to ovulation induction with a single bolus of either hCG or GnRH agonist. Secondary endpoints were oocyte maturation and endocrine parameters.

Materials and methods

Patients and hormonal treatment

A total of 122 normogonadotrophic women undergoing IVF or ICSI treatment from August 2003 until February 2004 were included in this open label, prospective, randomized, two-centre study. Each patient contributed with only one cycle. A signed written informed consent was obtained from all participants and patients fulfilling the following inclusion criteria were prospectively enrolled in a consecutive manner: (i) female age >25 and <40 years; (ii) baseline FSH and LH <12 IU/l; (iii) menstrual cycles between 25 and 34 days; (iv) body mass index (BMI) >18 and <30 kg/m^2; (v) both ovaries present; (vi) absence of uterine abnormalities. Ovarian stimulation was initiated with recombinant human (r)FSH (Puregon; Organon, Denmark) from cycle day 2 and continued until the day of inducing ovulation. A fixed dose of rFSH was used, either 150 or 200 IU per day for the first 6 days, according to age, BMI, basal FSH, antral follicle count and ovarian volume. After 6 days, doses were adjusted according to ovarian response. Once the leading follicle had reached a size of 15 mm, co-treatment with the GnRH antagonist ganirelix (Orgalutran; Organon) 0.25 mg was initiated and continued up to and including the day of ovulation induction. When at least three follicles had reached a size of 17 mm, patients were randomized to ovulation induction with either a single bolus of 0.5 mg buserelin s.c. (Suprefact; Hoechst, Denmark), or 10 000 IU of hCG s.c. (Pregnyl; Organon). Oocyte retrieval was performed 35 h later, followed by either IVF or ICSI. The randomization was performed by a study nurse, using computer-generated random numbers in sealed, unlabelled envelopes, each containing a unique study number. A maximum of two embryos was transferred on day 2 or 3 after retrieval. All patients received luteal phase support in the form of micronized progesterone vaginally, 90 mg a day (Crinone; Serono Nordic, Denmark) and estradiol 4 mg a day *per os* (Estrofem; Novo Nordisk, Denmark) commencing from the day following the oocyte retrieval and continuing until the day of the pregnancy test, i.e. day 12 after embryo transfer. Calculations of the percentage of MII oocytes, normal fertilization (2PN) and embryos per oocyte were performed using the patient (cycle) as a unit of analysis. A biochemical pregnancy was defined by a plasma β-hCG concentration >10 IU/l on day 12 after embryo transfer. A clinical pregnancy was defined as an intrauterine gestational sac with a heartbeat 3 weeks after a positive hCG test. The implantation rate was defined as the number of gestational sacs per patient (i.e. either 0, 50 or 100%) divided by the number of embryos transferred. The study was approved by the Ethics Committee of Viborg County.

Blood samples and hormone assays

Blood sampling was performed on six occasions: on stimulation day 1 (S1), day 6 (S6), on the day of ovulation induction, on the aspiration day and 7 and 14 days after oocyte aspiration. Sera were analysed immediately locally for estradiol using a Vidas kit (BioMérieux, France) with a detection limit of 33 pmol/l. Aliquots were frozen at −20 °C for subsequent analysis of LH, FSH and progesterone. LH and FSH were measured by time-resolved immunofluorometric assay, the AutoDelfia spec.kit (Wallac Oy, Finland). The assays were performed at the Department of Clinical Biochemistry, Odense University Hospital, Denmark. The detection limit of the LH assay as given by the manufacturer is 0.05 IU/l and Department of Clinical Biochemistry, Odense found the intra- and inter-assay variation of samples containing 0.1–0.2 IU/l to be <3% (Westergaard *et al.*, 2000). Progesterone was measured according to manufacturer's instructions using a commercially available

radioimmunoassay kit intended for measurements in serum (DSL-4200; Diagnostic System Laboratories, USA).

Statistical methods

Statistical differences were evaluated using Student's *t*-test or Fisher's exact test as appropriate. $P < 0.05$ was considered to be statistically significantly different. For calculation of the implantation rate, Wilcoxon rank sum test for two variables was used (i.e. the implantation rate was either 0, 50 or 100% depending on whether zero, one or two embryos implanted). Assuming a significance level of 0.05 and a power of 0.80 and a difference in pregnancy rates between the two treatment arms of 12% in favour of the agonist ovulation group, there was a total number of 296 treated patients. However, the study had to be terminated earlier due to differences obtained in clinical outcome between the groups compared.

Results

Cancellations

A total of 122 patients was randomized, 55 to ovulation induction with GnRH agonist and 67 to ovulation induction with hCG. Embryo transfer was cancelled in seven patients in the GnRH agonist group and in 10 patients in the hCG group due to either total fertilization failure or poor embryo development.

Patient characteristics

There were no significant differences regarding demographic data between groups. The two treatment groups were comparable regarding age and BMI. The mean ± SD age of the GnRH agonist group ($n = 55$) was 33.4 ± 3.9 years, and the mean BMI 23.6 ± 3.1 kg/m^2, whereas in the hCG group ($n = 67$) the mean age was 32.3 ± 3.8 years and the mean BMI 23.5 ± 3.0 kg/m^2. The vast majority of participants (98%) were Caucasians.

The mean ± SD baseline FSH and LH was 6.8 ± 2.4 and 5.6 ± 2.6 IU/l respectively in the GnRH agonist group compared to 6.7 ± 2.0 and 5.7 ± 1.5 IU/l respectively in the hCG group (not significant). There were no differences between groups regarding infertility diagnosis and number of previous IVF/ICSI attempts.

The total amount of exogenous FSH required (1831 ± 535 and 1754 ± 475 IU), the total dose of ganirelix administered (1.0 ± 0.4 versus 0.9 ± 0.3 mg), the duration of FSH stimulation (13.1 ± 2.1 versus 12.6 ± 2.3 days) for the GnRH agonist and hCG groups, respectively, did not differ significantly.

The mean stimulation day on which ganirelix co-treatment was initiated did not differ between the two groups (hCG: day 8.5 ± 1.2 versus GnRH agonist: day 8.3 ± 0.9), neither did the mean size of the largest follicle on the day of ganirelix initiation (hCG: 15.1 ± 1.1 mm versus GnRH agonist: 15.1 ± 0.8 mm). The mean follicle size of the largest follicle at ovulation induction did not differ (hCG: 18.1 ± 1.3 mm versus GnRH agonist: 18.5 ± 1.1 mm) (data not shown).

The distribution of IVF and ICSI cycles was 27 and 28 versus 36 and 31 in the GnRH agonist and hCG groups respectively (Table III). Comparable numbers of day 2 and day 3 transfers were performed in both groups. In the GnRH agonist group, 42 day 2 and six day 3 transfers were performed. In the hCG group, 46 day 2 transfers and 10 day 3 transfers were performed. No cases of OHSS were reported in either group.

Table I. Follicular phase serum estradiol, LH, FSH and progesterone

	Buserelin	hCG	P^a
Patients (*n*)	55	67	
Estradiol, S1 (nmol/l)	0.1 ± 0.1	0.2 ± 0.2	NS
Estradiol, S6 (nmol/l)	3.4 ± 2.0	3.1 ± 1.8	NS
Estradiol, day of ovulation induction (nmol/l)	7.1 ± 4	6.4 ± 3	NS
LH, S1 (IU/l)	4.3 ± 1.5	4.1 ± 1.5	NS
LH, S6 (IU/l)	4.6 ± 6.4	3.2 ± 2.7	NS
LH, day of ovulation induction (IU/l)	1.3 ± 1.1	1.3 ± 1.8	NS
FSH, S1 (IU/l)	7.4 ± 2.9	6.6 ± 1.9	NS
FSH, S6 (IU/l)	11.7 ± 4.5	12.2 ± 5.6	NS
FSH, day of ovulation induction (IU/l)	12.2 ± 4.4	12.4 ± 4.3	NS
Progesterone, day of ovulation induction (nmol/l)	5.5 ± 5.8	5.0 ± 3.0	NS

Values are mean ± SD.
[a]Student's *t*-test.
NS = not significant.

Serum hormone levels

Serum estradiol levels, LH levels and FSH levels during the follicular phase—S1, S6 and day of ovulation induction—did not differ significantly between groups (Table I). In contrast LH levels on day of oocyte retrieval (DOR), DOR + 7 and DOR + 14 were significantly higher in the GnRH agonist (buserelin) group compared to the hCG group (3.0 ± 1.8 versus 1.1 ± 1.1, 1.5 ± 1.0 versus 0.2 ± 0.4, 1.6 ± 1.2 versus 1.0 ± 1.0 IU/l) respectively ($P < 0.001$) (Table II). FSH levels on day of DOR, DOR + 7 and DOR + 14 were also significantly higher in the GnRH agonist group compared to the hCG group (9.6 ± 3.0 versus 6.2 ± 2.9, 1.9 ± 1.6 versus 0.4 ± 0.2, 2.6 ± 1.6 versus 1.4 ± 1.6 IU/l) respectively ($P < 0.001$). Estradiol levels on day DOR + 7 and day DOR + 14 were significantly lower in the GnRH agonist group compared to the hCG group (2.9 ± 2 versus 7.1 ± 4, 2.7 ± 2 versus 5.6 ± 5 nmol/l respectively) ($P < 0.001$) (Table II). Progesterone levels on the day of DOR and DOR + 7 were also significantly lower in the GnRH agonist group compared to the hCG group (28 ± 18 versus 49 ± 33, 39 ± 30 versus 283 ± 205 nmol/l respectively) ($P < 0.001$) (Table II).

Clinical outcome

All oocytes exposed to ICSI were assessed for nuclear maturity. When calculating per oocyte per patient, the proportion of MII oocytes was significantly higher in the GnRH agonist group compared to the hCG group, 84 ± 18 versus 68 ± 22% ($P < 0.02$) (log-transformed). No differences were found regarding fertilization rates, 60 ± 30 versus 54 ± 25% and cleavage rates 56 ± 30 versus 51 ± 28% (Table III). The mean number of embryos transferred (1.71 versus 1.64) and transfer rate (87 versus 85%) did not differ between

Table II. Luteal phase serum FSH and LH (IU/l) and estradiol and progesterone (nmol/l) in GnRH agonist versus hCG group

	FSH		LH		Estradiol		Progesterone	
	Buserelin	hCG	Buserelin	hCG	Buserelin	hCG	Buserelin	hCG
DOR	9.6 ± 3.0[a]	6.2 ± 2.9[a]	3.0 ± 1.8[d]	1.1 ± 1.1[d]	4.2 ± 2.0	4.4 ± 2.0	28 ± 18[i]	49 ± 33[i]
DOR +7 days	1.9 ± 1.6[b]	0.4 ± 0.2[b]	1.5 ± 1.0[e]	0.2 ± 0.4[e]	2.9 ± 2.0[g]	7.1 ± 4.0[g]	39 ± 30[j]	283 ± 205[j]
DOR +14 days	2.6 ± 1.6[c]	1.4 ± 1.6[c]	1.6 ± 1.2[f]	1.0 ± 1.0[f]	2.7 ± 2.0[h]	5.6 ± 5.0[h]		

Values are mean ± SD.
Numbers with same letters show a significant difference, $P < 0.001$ (Student's *t*-test) in all instances.
DOR = day of oocyte retrieval.

Table III. Oocyte maturation, fertilization and cleavage in GnRH agonist versus hCG group

	Buserelin	hCG	P[a]
IVF (*n*)	27	36	
ICSI (*n*)	28	31	
Oocytes [*n* (mean per DOR)]	442 (8.4)	651 (9.7)	>0.10
MII, ICSI (%, mean ± SD per patient)	84 ± 18	68 ± 22	<0.02
2PN oocytes (total %, mean ± SD per patient)	60 ± 30	54 ± 25	NS
Embryos (%) (mean ± SD per patient)	56 ± 30	51 ± 28	NS

[a]Fisher's exact test.
DOR = day of oocyte retrieval; MII = metaphase II; 2PN = two-pronuclear; NS = not significant.

Table IV. Pregnancy outcome in GnRH agonist versus hCG group

	Buserelin	hCG	P[a]
Patients (*n*)	55	67	
Rate of embryo transfer (ET) [*n* (%)]	48 (87)	57 (85)	NS
No. of embryos transferred [mean (range)]	1.71 (1–2)	1.64 (1–2)	NS
Positive hCG per ET [*n* (%)]	14 (29)	25 (44)	>0.10[a]
Clinical pregnancy [*n* (% per cycle)]	3 (6)	24 (36)	0.002[a]
Implantation rate (*n*)	3/89	33/97	<0.001[b]
Early pregnancy loss [*n* (%)]	11 (79)	1 (4)	0.005[a]

[a]Fisher's exact test.
[b]Wilcoxon rank sum test for two independent variables using the implantation rate per patient (i.e. 0, 50 or 100% depending on whether 0, 1 or 2 embryos implanted).

the GnRH agonist and hCG groups respectively (Table IV). No significant difference was seen regarding numbers of positive pregnancy tests per embryo transfer (29% versus 44%) in the GnRH agonist and hCG group respectively. In contrast, implantation rates (3/89 versus 33/97), clinical pregnancy rates (6 versus 36%) and the rate of early pregnancy loss (79 versus 4%) for the GnRH agonist and hCG groups respectively differed significantly in favour of hCG ($P < 0.001$, $P = 0.002$, $P = 0.005$) (Table IV).

Discussion

This study demonstrates that ovulation induction with a GnRH agonist following pre-treatment with an antagonist results in a significantly higher proportion of mature oocytes as compared to ovulation induction with hCG. The result was based on the assessment for maturity of all ICSI-exposed oocytes, denuded for evaluation of nuclear status. Despite the fact that more oocytes resumed meiosis in the GnRH agonist group, the implantation rate and the clinical pregnancy rate was significantly lower and the rate of early pregnancy loss significantly higher in the GnRH agonist group. This was seen even though patients were luteal phase-supported with progesterone and estradiol in a way similar to that normally applied to patients following a standard long protocol. Primarily the study was planned to include 150 cycles in each arm, i.e. a total of 300 cycles. However, when a total of 110 patients, i.e. 55 patients in each group, had completed the study, the study was opened due to a suspicion of extremely low clinical pregnancy rates in one of the two arms. At that time a total of 122 patients had been included. As it seemed unethical to proceed with the GnRH agonist group after the opening of the study, all patients had ovulation induction with hCG, thus creating an unequal number of patients in the two groups (55 versus 67 patients).

Until now, only small-scale studies exist focusing on the ovulatory capacity of GnRH agonist following treatment with a GnRH antagonist (Felberbaum *et al.*, 1995; Olivennes *et al.*, 1996; Itskovitz *et al.*, 2000; Fauser *et al.*, 2002; Beckers *et al.*, 2003), all of them showing an effective LH surge elicited by a GnRH agonist following treatment with a GnRH antagonist.

The uncontrolled study by Itskovitz *et al.* (2000) included only eight patients considered to have an increased risk of developing OHSS. One out of seven fresh embryo transfers resulted in a pregnancy. Four pregnancies, three of which ended in early abortions, resulted from a total of 17 transfers of frozen–thawed embryos. Despite luteal support including both progesterone and estradiol, pregnancy rates were quite low for the fresh transfers, and the rate of early pregnancy loss in the frozen–thawed cycles was high, resembling the rates of early pregnancy loss in fresh transfers of the current study (79%). The high pregnancy loss in frozen–thawed cycles, which bypasses possible negative effects directly or indirectly on the endometrium by GnRH agonist, may indicate a direct negative impact of the GnRH agonist surge on the developmental capacity of the oocyte/embryo itself.

In a retrospective cohort study with a set-up comparable to that of Itskovitz *et al.* (2000), eight patients of a total of 19, at risk of developing OHSS, also showed a poor implantation rate of only 8% despite luteal support with micronized progesterone vaginally (Bracero *et al.*, 2001).

In the prospective randomized three-arm study by Fauser *et al.* (2002) including a total of 47 ICSI cycles, two different agonists were used (0.2 mg triptorelin and 0.5 mg leuprorelin) in comparison with hCG for the triggering of ovulation after stimulation with recombinant FSH and GnRH antagonist (ganirelix) administration from stimulation day 6. The percentage of MII oocytes, fertilization rates, as well as embryo morphology did not differ between the three groups. Intramuscular progesterone was administered as luteal support to all patients. Although relatively low, no significant differences were seen regarding implantation rates (15, 18 and 7%) and pregnancy rates (18, 20 and 13%) when comparing the triptorelin and leuprorelin groups versus the hCG group. Pregnancy loss within the first 12 weeks was high: three of six (50%) and two of five (40%) for the triptorelin and leuprorelin groups respectively.

Finally, Beckers *et al.* (2003) found low pregnancy rates in women treated with either recombinant hCG, 250 μg ($n = 11$), recombinant LH, 1 mg ($n = 13$) or triptorelin 0.2 mg ($n = 15$) for ovulation induction after stimulation with recombinant FSH combined with a GnRH antagonist (Antide) during the late follicular phase. The luteal phase, however, was not supplemented for any group and the authors concluded that the luteal phase should be supplemented when using a GnRH antagonist for IVF.

Taken together, although numbers are small, all available studies in which patients have been stimulated with exogenous gonadotrophins and co-treated with a GnRH antagonist reported low implantation and pregnancy rates when a GnRH agonist was used to trigger ovulation. Furthermore, the rate of early pregnancy loss was high in two studies (Itskovitz *et al.*, 2000; Fauser *et al.*, 2002). These results are further confirmed and extended by the present study, suggesting that the luteal phase is severely compromised by the use of a GnRH agonist for ovulation induction.

A plausible explanation behind the deleterious impact on the clinical outcome parameters in the GnRH agonist group of the current study seems to be an insufficient luteal function. Due to pituitary suppression by the GnRH agonist, the levels of circulating endogenous LH, after the initial gonadotrophin 'flare up', were too low in the GnRH agonist group to sustain a normal function of the corpus luteum, leading to a subsequent luteal phase insufficiency. In the hCG group the luteal phase levels of LH were even lower than those of the GnRH agonist group, but the hCG group had high circulating levels of hCG to maintain a normal luteal function. Luteal phase support with both progesterone and estradiol, as used in the present study, apparently did not overcome the luteal insufficiency of the GnRH agonist group. Earlier animal and human studies have confirmed that withdrawal of LH induces the initiation of luteolysis (Collins *et al.*, 1986; Duffy *et al.*, 1999), but the corpus luteum can survive the lack of support for a limited number of days (Weissman *et al.*, 1996). The question is for what period of time does the suppressive effect of 0.5 mg of buserelin last on the pituitary? Injection of only 0.05 mg of D-trp, Pro9 NET in the mid-luteal phase resulted in complete refractoriness of LH and FSH secretion to a second dose 24 h later (Casper, 1996). Moreover, studies in primates showed that LH surges with duration of <48 h are insufficient to support, or even induce, the corpus luteum (Chandrasekher *et al.*, 1994). Secondly, are the first 24–48 h of crucial importance for a normal function of the corpus luteum? And will an exposure of too low LH levels during this period cause corpus luteum demise?

In contrast, significantly more metaphase II oocytes were retrieved in the current study when GnRH agonist was used to trigger ovulation ($P < 0.001$). This is actually the first time that a possible beneficial effect of the mid-cycle FSH surge on oocyte maturity has been observed in a clinical trial and the result contrasts with those obtained by Fauser *et al.* (2002). Thus, the result of this study supports a number of earlier *in vitro* studies demonstrating a pronounced positive effect of FSH on oocyte maturation (Yding Andersen *et al.*, 1999; Yding Andersen, 2002). Furthermore it suggests that an optimal oocyte maturation may be achieved by a simultaneous and coordinated effect by both FSH and LH.

The hormonal profiles of the two arms of the study were comparable until the day of oocyte retrieval. On this day the GnRH agonist group had significantly higher FSH and LH levels indicative of a surge, which was still visible 35 h after triggering of ovulation. This is consistent with the findings of other authors (Itskovitz *et al.*, 2000; Bracero *et al.*, 2001; Fauser *et al.*, 2002; Beckers *et al.*, 2003). Moreover in the luteal phase, FSH and LH levels were significantly lower in the hCG group due to negative feedback on the pituitary by high levels of estradiol in this group. Finally progesterone levels were comparable between the two groups until and including the day of ovulation induction, after which levels were significantly lower in the GnRH agonist group, indicative of a corpus luteum insufficiency.

Extragonadal LH/hCG receptors have been located in the human endometrium and uterus (Rao, 2001). Though receptor levels are low, $<10\%$ receptor occupancy leads to a maximal biological response (Rao, 2004), and it may be speculated that the low clinical outcome of the GnRH agonist group reflects low levels of circulating endogenous LH which cause an insufficient stimulation of the uterus (Tesarik *et al.*, 2003). Apart from this indirect negative impact of the mid-cycle GnRH agonist administration on the corpus luteum and the endometrium, there is also a possible direct negative impact on the same structures, as GnRH receptors have been described in both compartments (Tavaniotou *et al.*, 2001).

In this trial a flexible rather than a fixed antagonist protocol was used. Several authors investigated the fixed versus the flexible protocol and did not find any significant difference in pregnancy rates (Ludwig *et al.*, 2002; Mansour *et al.*, 2003; Escudero *et al.*, 2004; Klipstein *et al.*, 2004). Moreover, using a flexible protocol might reduce the treatment costs due to a decreased total antagonist consumption. We are, however, aware that others (Kolibianakis *et al.*, 2003) found a significantly lower implantation rate in patients following a flexible

protocol, when the antagonist was applied beyond the sixth day of stimulation as compared with a fixed protocol, starting on day 6 of stimulation. The present study using a flexible protocol, however, revealed no differences between the two groups regarding the mean day from which the antagonist was administered (day 8.3 versus day 8.5), the mean follicle size at the first day of antagonist administration (15.1 versus 15.1 mm) and the mean size of the largest follicle when ovulation was triggered (18.5 versus 18.1 mm) for the GnRH agonist and hCG groups respectively.

Controversy exists whether serum progesterone levels >1 ng/ml on the day of hCG may have a negative impact on the clinical outcome in IVF/ICSI cycles. Several authors failed to find any adverse effects of a subtle progesterone rise on oocyte and embryo quality (Ubaldi *et al.*, 1995) and clinical outcome (Givens *et al.*, 1994; Ubaldi *et al.*, 1995), while others have reported decreased implantation and pregnancy rates, presumably due to alterations of endometrial receptivity (Fanchin *et al.*, 1993; Harada *et al.*, 1995). In the present study, serum progesterone levels on the day of triggering ovulation were comparable and thus unlikely to represent an explanation of the observed results.

As previously shown (Beckers *et al.*, 2003), the non-supplemented luteal phase in antagonist cycles, although insufficient, was less disturbed in patients receiving hCG for ovulation induction compared to patients who received a single bolus of an agonist. In the current study, all patients were luteal-supported with a combination of vaginal micronized progesterone gel, 90 mg/day (Crinone 8%) and orally administered estradiol. Two previous trials (Saucedo *et al.*, 2000; Schoolcraft *et al.*, 2000) found no difference in pregnancy rates, when comparing patients treated with vaginal progesterone gel or intramuscular progesterone. In our clinics, we have used Crinone gel for luteal support for the past 3 years and have found that this compound provides an effective luteal support in agonist- as well as antagonist-treated patients. On the other hand there appear to have been some periodic problems with the manufacture and quality of Crinone in the last few years, and therefore we cannot completely exclude the possibility that some of the results could be due to a reduced bioactivity in the Crinone used in the trial. Nevertheless, during the study period we did not experience a lower pregnancy rate or a higher rate of early pregnancy loss in non-study patients, who were all luteal phase-supported with this compound.

The length of the luteal support with vaginal progesterone as well as orally administered estradiol was 12 days after embryo transfer. A previous study (Nyboe Andersen *et al.*, 2002) reported no advantage regarding clinical pregnancy rates by extending the luteal phase support beyond day 12–14 after embryo transfer when using a long agonist down-regulation protocol. Accordingly our policy for several years has been to stop luteal support at the day of the pregnancy test in agonist as well as antagonist protocols. This policy did not reduce our overall clinical pregnancy rates. Moreover, in the present study the mean plasma β-hCG concentration on the day of the pregnancy test was already significantly lower in the GnRH agonist group as compared to the hCG group (data not shown), indicative of an early malfunction of the trophoblast irrespective of the luteal phase support given. In earlier studies, intramuscular progesterone injections (Fauser *et al.*, 2002), micronized progesterone vaginally (Bracero *et al.*, 2001), intramuscular progesterone injections and estradiol orally (Itskovitz *et al.*, 2000) was used for luteal support. Penarrubia *et al.* (1998) showed that luteal hCG support can overcome luteal phase insufficiency after triggering of ovulation with GnRH agonist in gonadotrophin-stimulated cycles, a concept which is applicable in patients at low risk of OHSS. An alternative to this model could be a single bolus of hCG, 1500 IU s.c., following the oocyte retrieval. In IUI cycles in which ovulation was triggered with 0.1 mg triptorelin, Emperaire *et al.* (2004) showed that the luteal phase was completely restored after a single injection of 1500 IU hCG given 12 h after triggering of ovulation. A study is currently being undertaken in our clinic focusing on optimizing the luteal phase support.

In summary, the present data suggest for the first time a significant beneficial effect of the mid-cycle FSH surge on oocyte maturation. The use of a GnRH agonist for triggering of ovulation in FSH/GnRH antagonist IVF/ICSI cycles, however, disclosed a detrimental effect on clinical outcome parameters, presumably due to a luteal phase inadequacy, despite luteal support. Future studies are needed to explore the type of luteal phase support needed before this model is clinically acceptable.

References

Albano C, Smitz J, Camus M, Riethmuller-Winzen H, Van Steirteghem A and Devroey P (1997) Comparison of different doses of gonadotropin-releasing hormone antagonist Cetrorelix during controlled ovarian hyperstimulation. Fertil Steril 67,917–922.

Balasch J, Tur R, Creus M, Buxaderas R, Fabregues F, Ballesca JL, Barri PN and Vanrell JA (1994) Triggering of ovulation by a gonadotropin releasing hormone agonist in gonadotropin-stimulated cycles for prevention of ovarian hyperstimulation syndrome and multiple pregnancy. Gynecol Endocrinol 8,7–12.

Balasch J, Fabregues F, Tur R, Creus M, Casamitjana R, Penarrubia J, Barri PN and Vanrell JA (1995) Further characterization of the luteal phase inadequacy after gonadotrophin-releasing hormone agonist-induced ovulation in gonadotrophin-stimulated cycles. Hum Reprod 10, 1377–1381.

Beckers NG, Macklon NS, Eijkemans MJ, Ludwig M, Felberbaum RE, Diedrich K, Bustion S, Loumaye E and Fauser BC (2003) Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in in vitro fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. J Clin Endocrinol Metab 88,4186–4192.

Bracero NJ, Jurema MW, Posada MN, Whelan JG, Garcia JE and Vlahos NP (2001) Triggering ovulation with leuprolide acetate (LA) instead of human chorionic gonadotropin (hCG) after the use of ganirelix for in vitro fertilization–embryo transfer (IVF-ET) does not compromise cycle outcome and may prevent ovarian hyperstimulation syndrome. Fertil Steril 76, O–245.

Casper RF (1996) Ovarian hyperstimulation: effects of GnRH analogues. Does triggering ovulation with gonadotrophin-releasing hormone analogue prevent severe ovarian hyperstimulation syndrome? Hum Reprod 11, 1144–1146.

Chandrasekher YA, Hutchison JS, Zelinski-Wooten MB, Hess DL, Wolf DP and Stouffer RL (1994) Initiation of periovulatory events in primate follicles using recombinant and native human luteinizing hormone to mimic the midcycle gonadotropin surge. J Clin Endocrinol Metab 79,298–306.

Collins RL, Sopelak VM, Williams RF and Hodgen GD (1986) Prevention of gonadotropin-releasing hormone antagonist induced luteal regression by concurrent exogenous pulsatile gonadotropin administration in monkeys. Fertil Steril 46,945–953.

Damewood MD, Shen W, Zacur HA, Schlaff WD, Rock JA and Wallach EE (1989) Disappearance of exogenously administered human chorionic gonadotropin. Fertil Steril 52,398–400.

Duffy DM, Stewart DR and Stouffer RL (1999) Titrating luteinizing hormone replacement to sustain the structure and function of the corpus luteum after gonadotropin-releasing hormone antagonist treatment in rhesus monkeys. J Clin Endocrinol Metab 84,342–349.

Emperaire JC, Ruffie A and Audebert AJM (1992) Déclenchement de l'ovulation par la LH endogène libérée par l'administration d'un agoniste de la GnRH après stimulation folliculaire pour FIV. J Gynecol Obstet Biol Reprod 21,489–494.

Emperaire JC, Parneix I and Ruffie A (2004) Luteal phase defects following agonist-triggered ovulation: a patient-dependent response. Reprod Biomed Online 9,22–27.

Eppig J (1979) FSH stimulates hyaluronic acid synthesis by oocyte-cumulus complexes from mouse preovulatory follicle. Nature 281,483–486.

Escudero E, Bosch E, Crespo J, Simon C, Remohi J and Pellicer A (2004) Comparison of two different starting multiple dose gonadotropin releasing hormone antagonist protocols in a selected group of in vitro fertilization transfer patients. Fertil Steril 81,562–566.

European Orgalutran® Study Group, Borm G and Mannaerts B (2000) Treatment with the gonadotropin-releasing hormone antagonist ganirelix in women undergoing controlled ovarian hyperstimulation with recombinant follicle stimulating hormone is effective, safe and convenient: results of controlled, randomized, multicentre trial. Hum Reprod 15,1490–1498.

Fanchin R, de Ziegler D, Taieb J, Hazout A and Frydman R (1993) Premature elevation of plasma progesterone alters pregnancy rates of in vitro fertilization and embryo transfer. Fertil Steril 59,1090–1094.

Fauser BC, de Jong D, Olivennes F, Wramsby H, Tay C, Itskovitz-Eldor J and van Hooren HG (2002) Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization. J Clin Endocrinol Metab 87,709–715.

Felberbaum RE, Reissmann T, Kupker W, Bauer O, al Hasani S, Diedrich C and Diedrich K (1995) Preserved pituitary response under ovarian stimulation with HMG and GnRH antagonists (Cetrorelix) in women with tubal infertility. Eur J Obstet Gynecol Reprod Biol 61,151–155.

Forman R, Fries N, Testart J, Belaisch-Allart J, Hazout A and Frydman R (1988) Evidence for an adverse effect of elevated serum estradiol concentrations on embryo implantation. Fertil Steril 49,118–122.

Givens CR, Schriock ED, Dandekar PV and Martin MC (1994) Elevated serum progesterone levels on the day of human chorionic gonadotropin administration do not predict outcome in assisted reproduction cycles. Fertil Steril 62,1011–1017.

Gonen Y, Balakier H, Powell W and Casper RF (1990) Use of GnRH agonist to trigger follicular maturation for in vitro fertilization. J Clin Endocrinol Metab 71,918–922.

Harada T, Yoshida S, Katagiri C, Takao N, Ikenari T, Toda T, Mio Y and Terakawa N (1995) Reduced implantation rate associated with a subtle rise in serum progesterone concentration during the follicular phase of cycles stimulated with a combination of a gonadotrophin-releasing hormone agonist and gonadotrophin. Hum Reprod 10,1060–1064.

Hoff JD, Quigley ME and Yen SS (1983) Hormonal dynamics at midcycle: a revaluation. J Clin Endocrinol Metab 57,792–796.

Imoedemhe DA, Sigue AB, Pacpaco EL and Olazo AB (1991) Stimulation of endogenous surge of luteinizing hormone with gonadotropin-releasing hormone analogoue after ovarian stimulation for in vitro fertilization. Fertil Steril 55,328–332.

Itskovitz J, Boldes R, Barlev A, Erlik Y, Kahana L and Brandes JM (1988) The induction of LH surge and oocyte maturation by GnRH analogue (buserelin) in women undergoing ovarian stimulation for in vitro fertilisation. Gynecol Endocrinol 2 (Suppl 2),165.

Itskovitz J, Boldes R, Levron J, Erlik Y, Kahana L and Brandes JM (1991) Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil Steril 56,213–220.

Itskovitz-Eldor J, Kol S, Mannaerts B and Coelingh Bennink H (1998) Case report: first established pregnancy after controlled ovarian hyperstimulation with recombinant follicle stimulating hormone and gonadotrophin-releasing hormone antagonist ganirelix (Org 37462). Hum Reprod 13, 295–295.

Itskovitz-Eldor J, Kol S and Mannaerts B (2000) Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: preliminary report. Hum Reprod 9,1965–1968.

Kessler MJ, Reddy MS, Shah RH and Bahl OP (1979) Structures of N-glycosidic carbohydrate units of human chorionic gonadotropin. J Biol Chem 254,7901–7908.

Klipstein S, Reindollar RH, Regan MM and Alper MM (2004) Initiation of the gonadotropin-releasing hormone antagonist ganirelix for fertilization cycles in which the lead follicle is >14 mm. Fertil Steril 81,714–715.

Kolibianakis E, Albano C, Kahn J, Camus M, Tournaye H, Steirteghem AC and Devroey P (2003) Exposure to high levels of luteinizing hormone and estradiol in the early follicular phase of gonadotropin-releasing hormone antagonist cycles is associated with a reduced chance of pregnancy. Fertil Steril 79,873–880.

Ludwig M, Katalinic A, Banz C, Schroder AK, Loning M, Weiss JM and Diedrich K (2002) Tailoring the GnRH antagonist cetrorelix acetate to individual patients' needs in ovarian stimulation for IVF: results of a prospective, randomized study. Hum Reprod 17,2842–2845.

Mansour RT, Aboulghar MA, Serour GI, Al-Inany HG, Amin YM and Abou-Setta AM (2003) The use of gonadotropin-releasing hormone antagonist in a flexible pro pilot study. Am J Obstet Gynecol 189,444–446.

Nakano R, Mizuno T, Kotsuji F, Katayama K, Wshio M and Tojo S (1973) "Triggering" of ovulation after infusion of synthetic luteinizing hormone releasing factor (LRF). Acta Obstet Gynecol Scand 52,269–272.

Nyboe Andersen A, Popovic-Todorovic B, Schmidt KT, Loft A, Lindhard A, Hojgaard A, Ziebe S, Hald F, Hauge B and Toft B (2002) Progesterone supplementation during early gestations after IVF or ICSI has no effect on the delivery rates: a randomized controlled trial. Hum Reprod 17, 357–361.

Olivennes F, Fanchin R, Bouchard P, Taieb J and Frydman R (1996) Triggering of ovulation by a gonadotropin-releasing hormone (GnRH) agonist in patients pretreated with a GnRH antagonist. Fertil Steril 66,151–153.

Penarrubia J, Balasch J, Fabregues F, Creus M, Casamitjana R, Ballesca JL, Puerto B and Vanrell JA (1998) Human chorionic gonadotrophin luteal support overcomes luteal phase inadequacy after gonadotrophin-releasing hormone agonist-induced ovulation in gonadotrophin-stimulated cycles. Hum Reprod 13,3315–3318.

Rao CV (2001) Multiple novel roles of luteinizing hormone. Fertil Steril 76,1097–1100.

Rao CV (2004) Exploring the role of LH/hCG in human reproduction. Abstract, pp 22–23.

Romeu A, Monzo A, Peiro T, Diez E, Peinado JA and Quintero LA (1997) Endogenous LH surge versus hCG as ovulation trigger after low-dose highly purified FSH in IUI: a comparison of 761 cycles. J Assist Reprod Genet 14,518–524.

Saucedo LLE, Galache VP, Hernández A, Santos H, Arenus M and Patrizio P (2000) Randomized trial of three different forms of progesterone supplementation in ART: preliminary results. Fertil Steril 74 (S150),P-175.

Schoolcraft WB, Hesla JS and Gee MJ (2000) Experience with progesterone gel for luteal support in a highly successful IVF programme. Hum Reprod 15,1284–1288.

Segal S and Casper RF (1992) Gonadotropin-releasing hormone agonist versus human chorionic gonadotropin for triggering follicular maturation in in vitro fertilization. Fertil Steril 57,1254–1258.

Shalev E, Geslevich Y, Matilsky M and Ben-Ami M (1995) Gonadotrophin-releasing hormone agonist compared with human chorionic gonadotrophin for ovulation induction after clomiphene citrate treatment. Hum Reprod 10,2541–2544.

Simon C, Garcia Velasco JJ, Valbuena D, Peinado JA, Moreno C, Remohi J and Pellicer A (1998) Increasing uterine receptivity by decreasing estradiol levels during the preimplantation period in high responders with the use of a follicle-stimulating hormone step-down regimen. Fertil Steril 70, 234–239.

Stickland S and Beers W (1976) Studies on the role of plasminogen activator in ovulation. In vitro response of granulosa cells to gonadotrophins, cyclic nucleotides and prostaglandins. J Biol Chem 251, 5694–5699.

Tavaniotou A, Smitz J, Bourgain C and Devroey P (2001) Ovulation induction disrupts luteal phase function. Ann NY Acad Sci 943,55–63.

Tesarik J, Hazout A and Mendoza C (2003) Luteinizing hormone affects uterine receptivity independently of ovarian function. Reprod Biomed Online 7,59–64.

Ubaldi F, Smitz J, Wisanto A, Joris H, Schiettecatte J, Derde MP, Borkham E, Van Steirteghem A and Devroey P (1995) Oocyte and embryo quality as well as pregnancy rate in intracytoplasmic sperm injection are not affected by high follicular phase serum progesterone. Hum Reprod 10,3091–3096.

Valbuena D, Martin J, de Pablo JL, Remohi J, Pellicer A and Simon C (2001) Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. Fertil Steril 76,962–968.

Weissman A, Loumaye E and Shoham Z (1996) Recovery of corpus luteum function after prolonged deprivation from gonadotrophin stimulation. Hum Reprod 11,943–949.

Westergaard LG, Laursen SB and Yding Anderson C (2000) Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in IVF treated normogonadotrophic women. Hum Reprod 15,1003–1008.

Yding Andersen C (2002) Effect of FSH and its different isoforms on maturation of oocytes from pre-ovulatory follicles. Reprod Biomed Online 5,232–239.

Yding Andersen C, Leonardsen L, Ulloa-Aguirre A, Barrios-De-Tomasi J, Moore L and Byskov AG (1999) FSH-induced resumption of meiosis in mouse oocytes: effect of different isoforms. Mol Hum Reprod 5,726–731.

Yen SS, Llerena O, Little B and Pearson OH (1968) Disappearance rates of endogenous luteinizing hormone and chorionic gonadotropin in man. J Clin Endocrinol Metab 28,1763–1767.

Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP and Stouffer RL (1995) Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. Hum Reprod 10,1658–1666.

Submitted on September 7, 2004; resubmitted on December 22, 2004; accepted on January 7, 2005