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Ovarian stimulation for in-vitro fertilization/ intracytoplasmic sperm injection with gonadotrophins and gonadotrophin-releasing hormone analogues: agonists and antagonists

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The gonadotrophin-releasing hormone (GnRH) antagonists Cetrorelix and Ganirelix have been used in recent years in clinical studies to prove that these compounds reliably prevent the onset of premature luteinizing hormone (LH) surges during ovarian stimulation. Cetrorelix has been applied in single and multiple dose protocols, while Ganirelix has until now only been used in the multiple dose protocol. In the latter protocol, ovarian stimulation is started on day 2 or 3 of the spontaneous cycle with human menopausal gonadotrophin or recombinant follicle stimulating hormone. Daily administration of the GnRH antagonist at its minimum effective dose (0.25 mg/day s.c.) occurs from the sixth day of stimulation onwards until ovulation induction by human chorionic gonadotrophin. In the single dose protocol, 3 mg of the GnRH antagonist Cetrorelix was injected on day 8 of the stimulation cycle. Both protocols have been proven to be safe and effective. Fertilization rates of >60% in in-vitro fertilization and >70% in intracytoplasmic sperm injection, as well as clinical pregnancy rates of ~30% per transfer, sound most promising. The incidence of a premature LH surge is below 2%. The incidence of severe ovarian hyperstimulation syndrome seems to be lower under antagonist treatment than in the long agonistic protocol. Treatment time is significantly shortened.

Key words: GnRH antagonists/OHSS/ovarian stimulation/premature LH surge

Introduction

The use of gonadotrophin-releasing hormone (GnRH) agonists for the purposes of ovarian stimulation has influenced modern management of assisted reproduction techniques to an important extent. Premature luteinizing hormone (LH) surges were responsible for reduced effectiveness of ovarian stimulation by human menopausal gonadotrophin (HMG) in in-vitro fertilization (IVF) programmes. At the same time they negatively affected oocyte and embryo quality and

subsequent pregnancy rates (Stanger and Yovich, 1985; Loumaye, 1990). The introduction of GnRH agonist treatment has remedied most of these difficulties and drawbacks, and the cancellation rate of stimulated cycles due to premature luteinization has been reduced to ~2%. It has become possible to schedule ovulation induction so that the psychological pressure on patients and physicians has been eased. Suppression of endogenous hormone production by GnRH analogues followed by HMG stimulation has developed from second-line into first-line therapy (Macnamee *et al.*, 1989). Different treatment schedules are applied today, including the so-called long protocol, which aims at a complete pituitary suppression, and the short and ultrashort protocol, in which the initial flare-up of gonadotrophins is tried to be harvested for ovarian stimulation (Loumaye *et al.*, 1988; Smitz *et al.*, 1992). Among these protocols, the long protocol is generally the most effective and is most often used at present (Tan, 1996). In Germany for instance, >70% of all stimulated cycles performed for assisted reproduction treatment are according to the long protocol (Deutsches IVF Register 1997). Ovarian stimulation using human urinary or recombinant gonadotrophins in combination with GnRH analogues has been proven to be highly efficient for assisted reproduction techniques (Smitz *et al.*, 1992; Loumaye *et al.*, 1994). However, the long protocol has the disadvantages of a long treatment period until desensitization occurs together with relatively high costs due to an increased requirement for HMG. On the other hand, ovarian hyperstimulation with rescue rates of >30 oocytes is common, as large numbers of follicles and aspirated oocytes are regarded almost as criteria of success (Balen, 1995). It is debatable whether this is still acceptable in the presence of intracytoplasmic sperm injection (ICSI), with its high rate of fertilization independent of sperm morphology (Küpker *et al.*, 1995). Thus, the question arises of how to avoid the complexities and costs of prolonged pharmaceutically driven treatments (Edwards *et al.*, 1996). Reduced usage of gonadotrophins and lower numbers of mature oocytes (metaphase II) might be the goals to aim for, reducing both burden and risk for the patient as well as financial costs. For this, the introduction of GnRH antagonists into protocols for ovarian stimulation seems to open new avenues.

GnRH antagonists

In parallel with the development of GnRH agonists, other analogues have been synthesized which also bind with high affinity to the pituitary GnRH receptors but are not functional in inducing the release of gonadotrophins. These compounds are far more complex than GnRH agonists, with modifications in the molecular structure not only at positions 6 and 10, but also at positions 1, 2, 3 and 8. In comparison to the GnRH agonists, the pharmacological mechanism by which GnRH antagonists suppress the liberation of gonadotrophins is completely different. While the agonists act on chronic administration through down-regulation of receptors and desensitization of gonadotrophic cells, the antagonists

	1	2	3	4	5	6	7	8	9	10
GnRH	pyro-GLU	HIS	TRP	SER	TYR	GLY	LEU	ARG	PRO	Gly-NH2
Cetrorelix	Ac-D-Nal(2)	D-Phe(4Cl)	D/PAL	SER	TYR	D/CIT	LEU	ARG	PRO	D-Ala-NH2
Ganirelix	Ac-D-Nal(2)	D-Phe(4Cl)	D/PAL	SER	TYR	D-hArg(Et2)	LEU	L-hArg[Et2]	PRO	D-Ala-NH2

D-Ala: D-alanine; D/CIT; D-citrulline; D-hArg: D-homoarginine; L-hArg: L-homoarginine; D-Nal: (2-naphthyl)-D-alanine; D/PAL; (3-pyridyl)-D-alanine; D-Phe: D-phenylalanine;

Figure 1. Structure of GnRH antagonists of the third generation, Cetrorelix and Ganirelix, compared with GnRH itself. Amino acid substitutions have been made at positions 1, 2, 3, 6, 8 and 10.

bind competitively to the receptors and thereby prevent the endogenous GnRH from exerting its stimulatory effects on the pituitary cells, thus avoiding any flare-up effect. Within hours the secretion of gonadotrophins is reduced. This mechanism of action is dependent on the equilibrium between endogenous GnRH and the applied antagonist, and is therefore highly dose dependent, in contrast to the agonists (Felberbaum *et al.*, 1995).

The first generation of GnRH antagonists caused allergic side-effects due to an induced histamine release. They were also subject to gelling after administration due to their high hydrophobicity, which hampered the clinical development of these compounds. In modern GnRH antagonists such as Ganirelix (Organon, Oss, Netherlands) or Cetrorelix (ASTA-Medica, Frankfurt/M, Germany) these problems seem to have been resolved and they may thus become available medically in the near future (Reissmann *et al.*, 1995). Both compounds are third generation antagonists with substituted amino acids at positions 1, 2, 3, 6 and 10, while Ganirelix, but not Cetrorelix, also shows a modification at position 8 (Figure 1). The median terminal half-life of Cetrorelix after single dose injection ranges between 5 and 10 h, while for multiple administration a median terminal half-life of 20–80 h has been reported (Duijkers *et al.*, 1998). The elimination half-life of Ganirelix after single dose administration is ~13 h (Mannaerts *et al.*, 1999). Both compounds seem to be equipotent regarding gonadotrophin suppression, being fully effective within 4–8 h after administration (Sommer *et al.*, 1993; Mannaerts *et al.*, 1999).

GnRH antagonists within ovarian stimulation

In 1991, Dittkoff *et al.* showed that a GnRH antagonist applied for a short period is capable of suppressing the ovulation-inducing mid-cycle LH peak (Dittkoff *et al.*, 1991). They administered 50 µg of Nal-Glu [Ac-D-Nal^{1}, D-4-Cl-Phe^{2}, D-Pal^{3}, Arg^{5}, D-Glu(AA)^{6}, D-Ala^{10}] per kg body weight and day for 4 days in

the mid-cycle phase. The LH peak failed to occur, oestradiol production came to a halt and follicular growth was interrupted. After discontinuing the antagonists, gonadal function returned to normal within 8 days. A significant negative correlation was found between the oestradiol concentration measured after Nal-Glu administration and the number of days required for subsequent ovulation. Apparently, antagonists neither deplete the follicle stimulating hormone (FSH) and LH stores of gonadotrophic cells nor inhibit gonadotrophin synthesis.

Ovarian stimulation with HMG or recombinant FSH and concomitant mid-cycle GnRH antagonist treatment

The multiple dose protocol

In an attempt to transfer these results into a clinical stimulation protocol for IVF, an intermittent protocol for antagonists was proposed, using the second generation antagonist Nal-Glu (Cassidenti *et al.*, 1991; Frydman *et al.*, 1991). However, Nal-Glu could not be developed further due to its histamine release properties. Transferring the results of these pilot studies into a clinical stimulation protocol for routine use within an IVF unit, the so-called multiple dose protocol was designed, using the third generation antagonists Cetrorelix or Ganirelix (Diedrich *et al.*, 1994; Felberbaum *et al.*, 1996; the Ganirelix Dose Finding Study Group, 1998). Starting on cycle day 2, patients receiving this protocol are treated with two ampoules of urinary or recombinant gonadotrophin preparations per day. From cycle day 7, when a premature LH surge may be imminent, the GnRH antagonist is administered daily until the criteria for ovulation induction are fulfilled. On day 5, the dosage of gonadotrophins may have to be adjusted according to the ovarian response of the individual patient. This treatment is continued until induction of ovulation with 10 000 IU HCG i.m., administered when the leading follicle reaches a diameter of 18–20 mm (as measured by transvaginal ultrasound), and when oestradiol concentration indicates a satisfactory follicular response.

Figure 2 shows the LH profile of the first 47 patients treated with the GnRH antagonist Cetrorelix at the Department of Obstetrics and Gynecology at the Medical University of Lübeck for the purpose of ovarian stimulation. The patients received 3, 1, 0.5 or 0.25 mg of Cetrorelix/day. In all four groups at cycle day 8, after the first administration of Cetrorelix one day earlier, a significant decrease of LH concentration was observed. Afterwards LH concentrations were maintained at a low value, and not one patient's stimulation cycle had to be cancelled due to a premature rise of LH. In the case of FSH, the hormone profiles were quite different. Almost no suppression over the time of stimulation was observed until the day of HCG administration. This may be mainly due to the fact that exogenous FSH, which has a distinctly longer half-life in comparison to LH, had been constantly administered during ovarian stimulation. Pharmacokinetic studies have shown that, without FSH supplementation, FSH secretion under Cetrorelix

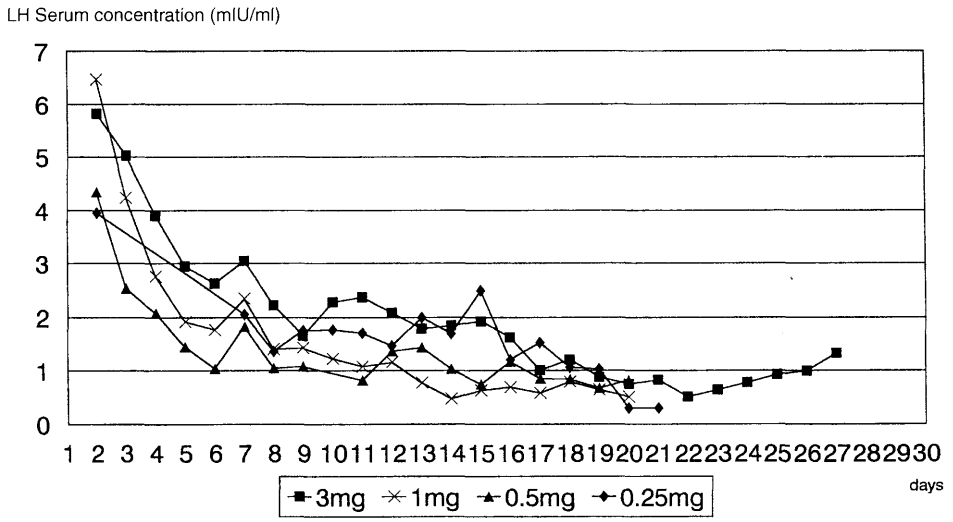

Figure 2. Mean LH serum concentration (mIU/ml) during ovarian stimulation with HMG and concomitant midcyclic GnRH antagonist treatment (Cetrorelix) at different dosages (3, 1, 0.5 and 0.25 mg/day) according to the multiple dose protocol.

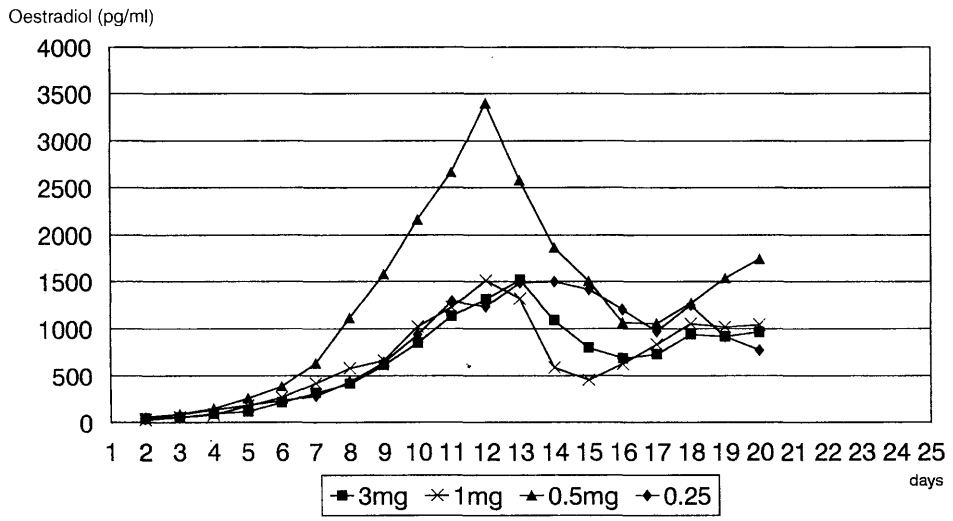

Figure 3. Mean courses of oestradiol serum concentration (pg/ml) during ovarian stimulation with HMG and concomitant midcyclic GnRH antagonist treatment (Cetrorelix) in different dosages (3, 1, 0.5 and 0.25 mg/day) according to the multiple dose protocol.

treatment is suppressed as well as LH in a dose-dependent manner, although to a lesser extent than LH values (Duijkers *et al.*, 1998).

Serum oestradiol concentrations constantly increased under GnRH antagonist treatment, reflecting ovarian follicle maturation (Figure 3). In the 0.5 mg group, a more pronounced increase of oestradiol values from day 7 onwards was observed. This led to discussions about the possibility of direct effects of the GnRH antagonist on gonadal sexual steroid production. However, upon stepping

Table I. Stimulation and intracytoplasmic sperm injection outcome in patients treated with human menopausal gonadotrophin (HMG) and concomitant midcyclic administration of gonadotrophin-releasing hormone antagonist (Cetrorelix) at 0.5 and 0.25 mg/day (Albano *et al.*, 1997)

	Cetrorelix (mg/day)	
	0.5	0.25
No. of patients	32	30
No. of HMG ampoules	35	33
Duration of HMG treatment (days)	11	10
No. of follicles >15 mm on the day of HCG administration	10	10
Oestradiol on the day of HCG (pg/ml)	2122	2491
Fertilization rate (%)	55	59
Cleavage rate (%)	78	76
Clinical pregnancy rate (%)	31	30

HCG = human chorionic gonadotrophin.

down to 0.25 mg per day, this observation was not repeated. All in-vitro studies performed so far that have examined the possible interference of the GnRH antagonist with sexual steroid secretion of the granulosa lutein cells have not provided any evidence for a direct effect of the GnRH antagonist, at least not at a dosage of 0.25 mg per day, either for Cetrorelix or for Ganirelix (Felberbaum *et al.*, 1998; Ortmann *et al.*, 1998).

Subsequent dose finding studies, using 0.5, 0.25 and 0.1 mg Cetrorelix/day proved the efficacy and safety of 0.25 mg Cetrorelix/day in avoiding premature LH surges, while at 0.1 mg Cetrorelix/day, premature LH surges were observed (Albano *et al.*, 1996, 1997). In these studies, ICSI for treatment of male subfertility was allowed, leading to fertilization rates that were within the range expected after normal oocyte maturation. It is essential to emphasize that stepping down the dosage of Cetrorelix did not have any negative impact on treatment outcome. There were no significant differences regarding two-pronuclei fertilization rates, increase in oestradiol values, cleavage rate, clinical pregnancy rate per embryo transfer and implantation rate between the group treated with 0.5 mg Cetrorelix per day and those patients treated with only 0.25 mg per day. (Table I). The clinical pregnancy rates per transfer were 30.7% in the 0.5 mg group and 29.6% in the 0.25 mg group. Interestingly, ~16% of the patients treated with 0.5 mg Cetrorelix per day and 10% of those treated with only 0.25 mg per day showed a significant rise in LH concentrations during the follicular phase, while progesterone concentrations remained low. These patients showed a significantly lower cleavage rate and no pregnancy occurred in this subgroup. As these patients showed higher oestradiol concentrations than patients who did not have a rise of LH, these findings may suggest that an earlier administration of the antagonist may be necessary in high responders to avoid the LH rise, which may compromise the quality and maturity of the oocytes recovered (Albano *et al.*, 1997).

The single dose: injection protocol

In parallel with the multiple dose administration, a different protocol for administration of GnRH antagonists within ovarian stimulation was developed, in which the compound was used as a single or dual dose of 2 or 3 mg around day 9. In this protocol the antagonist was injected at the time when oestradiol reached 150–200 pg/ml and the follicle size was >14 mm, which is usually the case on day 8 or 9 of the cycle (Olivennes *et al.*, 1994, 1995). Premature LH rises have not been observed in any of the cycles studied and published so far. As it was demonstrated that 3 mg of Cetrorelix can suppress LH values for as long as 96 h, acting like an intermediate depot preparation, the protocol was modified: 3 mg of Cetrorelix was now injected at cycle day 8 as a 'jour fixe' (i.e. fixed day). If within 96 h the criteria for ovulation induction were not met, 0.25 mg of Cetrorelix was then administered as daily injections. The injection of 3 mg Cetrorelix was capable of preventing LH surges in the patients treated, introducing a very simple treatment protocol. Clinical pregnancy rates of >30% per transfer have been reported, which sound very promising (Olivennes *et al.*, 1995).

Luteal phase support in ovarian stimulation with gonadotrophins and GnRH antagonist

After discontinuation of the mid-cycle administration of 50 μg Nal-Glu per kg body weight per day in normal cycling women, spontaneous ovulation resumes after a certain delay and a normal, non-compromised luteal phase takes place (Dittkoff *et al.*, 1991). For this reason, it seemed reasonable to question the necessity of luteal phase support in ovarian stimulation with gonadotrophins and GnRH antagonists for assisted reproduction treatment. However, using 0.5 mg Cetrorelix per day according to the multiple dose protocol without luteal phase support in five patients, the luteal phase appeared to be insufficient, with preterm bleeding in all five patients (Albano *et al.*, 1996). In all subsequent studies, luteal phase support was given to all patients, either by repeated injections of HCG or by transvaginal administration of micronized progesterone. In the absence of any firm evidence to the contrary, it seems that luteal phase support is still mandatory in the case of ovarian stimulation with gonadotrophins and concomitant GnRH antagonist treatment.

Results of phase III studies

Both protocols described above have been used in prospective, randomized open label phase III studies, comparing the results obtained in the GnRH antagonist groups with those obtained after treatment according to the long agonistic protocol.

Table II. Ovarian stimulation for ICSI with HMG and Cetrorelix (multiple dose protocol; 0.25 mg/day) versus long protocol (HMG/buserelin; nasal spray) (Felberbaum, 1999)

	Cetrorelix	Buserelin
No. of patients	188	85
Patients who reached the day of HCG administration (%)	96.3	90.6
Oocyte retrievals performed (%)	94.7	90.6
Patients in whom embryo transfer was performed (%)	83.5	78.8
Clinical pregnancy rate per transfer (%)	26	33

A total of 188 patients treated with Cetrorelix at its minimal effective dose of 0.25 mg/day according to the multiple dose protocol were compared with 85 patients treated according to the long protocol, using buserelin as nasal spray preparation for desensitization of the pituitary gland. While 84% of the patients in the antagonist (Cetrorelix) group proceeded to an embryo transfer, only 79% of those in the agonist (buserelin) group proceeded, possibly reflecting a lower cancellation rate using Cetrorelix. The clinical pregnancy rate (intrauterine pregnancies with documented heart activity of the embryo) was 26% and 33% per transfer in the antagonist and the agonist groups respectively. However, this difference was not statistically significant. The life birth rate per transferred embryo was 10% for Cetrorelix and 12% for buserelin. Concerning those couples treated with ICSI due to male infertility, there were no differences observed, either regarding oocytes in metaphase II or fertilization rates after ICSI. Interestingly, the percentage of excellent embryos transferred was clearly higher in the Cetrorelix group (45%) compared with the agonist group (27%), although it may be difficult to attribute this difference to the use of a GnRH antagonist (Table II). The distribution of follicles on the day of HCG injection for ovulation induction was almost the same in the two groups, with a tendency towards fewer small follicles in the group of patients treated with Cetrorelix. Although this tendency did not reach statistical significance, it became clear that the synchronization of follicular recruitment was not impaired by the use of a GnRH antagonist according to the multiple dose protocol. Also, the oestradiol profiles for the duration of treatment showed no significant differences between the two groups. There was a tendency towards higher oestradiol concentrations on the day of HCG administration in patients tested according to the long protocol (Figure 4). However, the incidence of ovarian hyperstimulation syndrome (OHSS II–III) was significantly different in the two groups ($P = 0.026$ according to Fisher's exact test). While in the Cetrorelix group only two cases (1.1%) were observed, both grade II, the incidence in the buserelin group was 6.5%, with one case of severe OHSS requiring hospitalization. The tendency towards higher oestradiol concentrations in the late stimulatory phase in the agonist group, as well as fewer small follicles on the day of HCG in the Cetrorelix group, may be of causal significance for this lower incidence after the antagonist treatment. While the mean amount of gonadotrophins used per cycle was not significantly different for Cetrorelix and buserelin (23.6 and 25.1 ampoules respectively), the

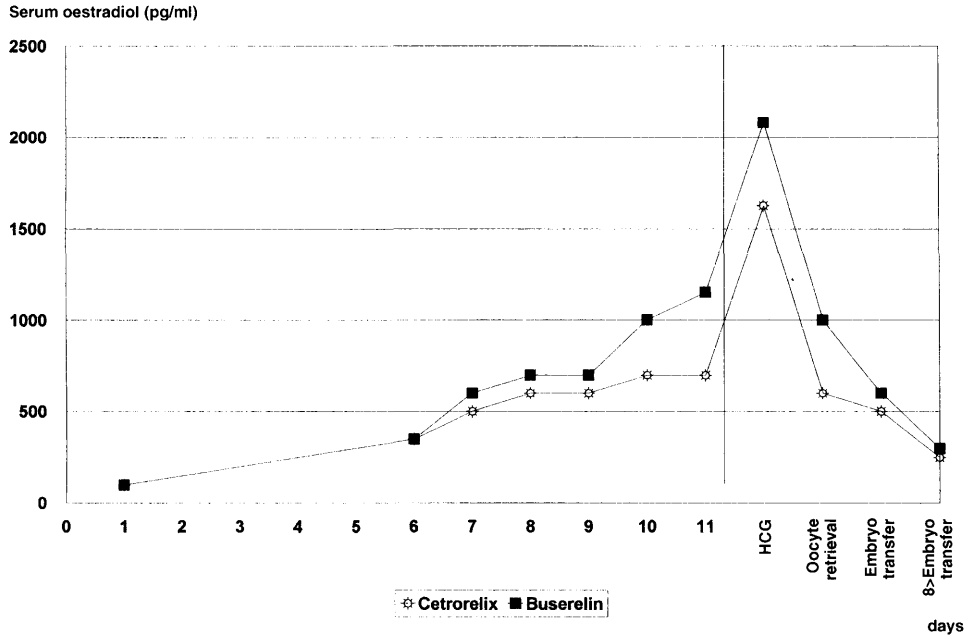

Figure 4. Mean courses of oestradiol serum concentration (pg/ml) during ovarian stimulation with HMG and concomitant midcyclic GnRH antagonist treatment (Cetrorelix) in its minimal effective dose (0.25 mg/day) according to the multiple dose protocol, compared with oestradiol serum concentrations during ovarian stimulation according to the long protocol (HMG/buserelin).

actual treatment time was always shorter by 14 days in the antagonist protocol as there was no waiting for desensitization of the pituitary gland to occur (Felberbaum, 1999).

A total of 115 patients were treated according to the single dose protocol using 3 mg of Cetrorelix injected on day 8 of the cycle. Their results were compared with those for 36 patients treated with triptorelin as a depot preparation according to the long protocol. There were no significant statistical differences observed between the two groups regarding length of stimulation (9.4 and 10.7 days respectively), oestradiol concentrations on the day of HCG (1786 and 2549 pg/ml respectively), fertilization rate (50.5 and 54.7% respectively), and implantation rate (13.8 and 17.8% respectively). As with the multiple dose protocol, the pregnancy rate was slightly higher (though not significantly) in the agonist group (27.3% per embryo transfer) than in the Cetrorelix group (21.2% per embryo transfer). As in the multiple dose protocol, the incidence of OHSS (II-III) was remarkably lower in patients who had been treated with Cetrorelix (3.5%) than in those who had been stimulated according to the long protocol using triptorelin (11.1%) (Table III). Interestingly, in those patients who had shown a rise in LH beginning at day 8, when Cetrorelix was to be administered, the LH rise was reduced by administration of the antagonist in all cases. None of these cycles had to be cancelled and pregnancies also occurred. No LH surge was observed after Cetrorelix administration. The amount of gonadotrophins used per cycle was only 24.3 ampoules in the antagonist group compared to 35.6

Table III. Ovarian stimulation for IVF/ICSI with HMG and Cetrorelix single-shot administration (3 mg at day 8) versus long protocol (HMG/triptorelin depot) (Olivennes *et al.*, 1999)

	Cetrorelix	Buserelin
Fertilization rate (%)	50.5	54.7
Mean no. of embryos obtained per patient	5.4	7.6
Implantation rate (%)	13.8	17.8
Clinical pregnancies/oocyte retrieval (%)	22.6	28.2
Patients with OHSS (%)	3.5	11.1

ampoules in the buserelin group (95% confidence interval for difference: 7.7–15.0) (Olivennes *et al.*, 1999).

In a prospective, open non-randomized study using 0.25 mg/day of Cetrorelix according to the multiple dose protocol, 346 patients were treated in several European centres. By replacing a mean of 2.66 embryos per cycle, a clinical ongoing pregnancy rate of 24% per transfer was obtained. The abortion rate was 17%. The median exposure time to Cetrorelix was 5 days, the mean time of stimulation with gonadotrophins was 10.4 days per cycle and the median number of ampoules of HMG used per cycle was 23. The incidence of premature luteinization in this study, up to now the largest using Cetrorelix for ovarian stimulation, was as low as 0.89% after starting antagonist administration (Felberbaum, 1999).

Preserved pituitary response

Based on the mechanism of competitive binding, it is possible to modulate the degree of hormone suppression by the dose of antagonist administered. This preservation of the pituitary response due to competitive mechanisms can be clearly demonstrated by using a GnRH test during GnRH antagonist treatment. At 3 h before injecting HCG for ovulation induction, 25 μg of GnRH was administered in patients treated with 1 or 3 mg Cetrorelix per day. Blood samples for LH measurement were taken before and 30 min after GnRH treatment. The mean increase was 10 mIU/ml for the 3 mg group, while the average maximum concentration of serum LH in the 1 mg group was ~32.5 mIU/ml. These results were highly significant (Felberbaum *et al.*, 1995). They might open new avenues of treatment for patients at higher risk of developing OHSS, as they would allow the avoidance of deleterious effects of HCG administration in some cases. Ovulation induction is possible by GnRH agonists or native GnRH itself under antagonistic treatment. This could help to lower the incidence of early onset OHSS (Olivennes *et al.*, 1996).

Ovarian stimulation with recombinant FSH and concomitant mid-cycle GnRH antagonist treatment

Cetrorelix and Ganirelix have been tested within clinical phase II and III studies in combination with recombinant FSH. In contrast to urinary compounds, these

Table IV. Ovarian stimulation for IVF/ICSI with recombinant FSH and concomitant midcyclic administration of GnRH antagonist (Ganirelix) at six different dosages (Ganirelix Dose Finding Study Group, 1998)

	Daily dose of Ganirelix (mg/day)					
	0.0625	0.125	0.25	0.5	1	2
No. of patients	31	65	69	69	65	30
Mean no. of cumulus–oocyte complexes	9	9.5	10	8.8	9.3	8.6
Mean no. of embryos per patient	5.4	5.9	5.4	4.6	5.3	4.9
Mean no. of good quality embryos	3.8	3.3	3.3	2.5	3.3	3.5
Implantation rate (%)	14.2	16.6	21.9	9	8.8	1.5
No. of clinical pregnancies	7	17	25	8	9	1
Clinical pregnancy/embryo transfer (%)	25.9	28.3	40.3	14.8	15.3	4.3
Abortion rate (%)	0	12	4	25	56	–

preparations are free of LH activity. Their effectiveness in ovarian stimulation according to the long protocol has been proven. Even after down-regulation of the pituitary gland, endogenous LH secretion seems to be sufficient for normal ovarian sexual steroid biosynthesis. However, extreme suppression of LH secretion by high doses of GnRH antagonists could cause problems according to the two-cell/two-gonadotrophin hypothesis of follicular oestrogen production (Chappel and Howles, 1991; Adashi, 1994). Causing a situation very similar to that in WHO-I infertile patients (World Health Organization, 1976), ovarian stimulation with pure FSH depleted of any LH activity could induce follicular growth in the absence of any oestrogen secretion, as has been described for such patients (Schoot *et al.*, 1994). The prospective, multicentric double-blind dose-finding phase II study with Ganirelix helped to elucidate this question. In this study, Ganirelix was administered in multiple dose fashion at six different dosages (0.0625, 0.125, 0.25, 0.5, 1 and 2 mg/day). While at the lower dosages oestradiol secretion was normal and sufficient, injecting 2 mg Ganirelix s.c. per day produced almost no increase in oestradiol secretion. In some cases, the follicles did not even grow, an observation that still awaits a scientific explanation (Fauser, 1999). In the 0.25 mg group, a clinical pregnancy rate of 40.3% per transfer was achieved. Increasing the dosage of Ganirelix led to a decrease in the pregnancy rates and an increase in the abortion rate. Thus 0.25 mg was defined as the minimal effective dose to be administered according to the multiple dose protocol (Ganirelix Dose Finding Study Group, 1998; Table IV).

Future aspects on GnRH antagonists in ovarian stimulation

Since 1992, a veritable revolution has occurred in treatment of male infertility as a consequence of the introduction of ICSI, with its excellent fertilization outcome irrespective of sperm morphology (Palermo *et al.*, 1992; Van Steirteghem, 1994). However, it seems as if the therapeutic approach has been overdone in the meantime. The financial burden and medical risks for our patients have

increased unacceptably. Excessive ovarian stimulation, with rescue rates of >30 oocytes, is not uncommon. In addition, the incidence of moderate and severe OHSS has increased from <1% to ~7%, while the therapeutic options still remain poor (Golan *et al.*, 1988; Ron-El *et al.*, 1991; Bauer and Diedrich, 1996). GnRH antagonists may even be an appropriate tool for prevention of imminent OHSS in cases of high responders (de Jong *et al.*, 1998). If the healthy single fetus pregnancy is the goal to aim for, a multiple pregnancy rate of >20% demonstrates how often we fail in our attempts.

The development of recombinant gonadotrophins and their introduction onto the market heralded an important scientific advance, while treatment costs were increased tremendously (Recombinant Human FSH Study Group, 1995). Although lowering the price of these compounds would have led to their replacing the urinary gonadotrophins almost totally, recombinant gonadotrophins remain extremely expensive. After years of intensive clinical trials, GnRH antagonists are now to be introduced onto the market. They will probably replace GnRH agonists in ovarian stimulation treatment for assisted reproduction techniques, due to the advantages of their mode of action as compared to agonistic analogues. A reduction in the amount of gonadotrophins used for ovarian stimulation seems to be feasible using GnRH antagonists. Although our knowledge regarding oocyte and embryo quality after ovarian stimulation with concomitant GnRH antagonist treatment may still be limited, the numbers of excellent quality embryos available for transfer seem to be satisfactory. The long agonist protocol was regarded as advantageous since recruitment of a larger follicle cohort could be stimulated by gonadotrophins at an early point in folliculogenesis. As in the short agonistic protocol, this is not the case in protocols for ovarian stimulation using antagonists, where stimulation starts almost immediately after having completed the recruitment of follicles in the spontaneous cycle. From a theoretical point of view, this could lead to a larger number of small and intermediate follicles at the time of ovulation induction by HCG. This would actually enhance the risk of onset of OHSS. In spite of this plausible hypothesis, all data available up to the present seem to indicate a remarkably lower incidence of moderate and severe OHSS after ovarian stimulation with gonadotrophins and concomitant GnRH antagonist treatment, which may be <2%. Overall, the most promising aspect of introducing GnRH antagonists into ovarian stimulation may be the possibility of making this treatment less aggressive and much less risky than a long agonistic protocol using old-fashioned schemes of stimulation such as clomiphene citrate alone or in combination with HMG (Felberbaum *et al.*, 1997).

Conclusions

The different pharmacological mode of action of GnRH antagonists allows us to reduce the length of a stimulation cycle significantly. The flare-up phenomenon is completely avoided. Premature LH surges are a rare event under this treatment modality, using Cetrorelix both in a daily fashion and in a single-

shot protocol. In fact, Cetrorelix and Ganirelix, used at their minimal effective dose of 0.25 mg/day according to the multiple dose protocol, allow sufficient LH to be secreted from the pituitary gland for normal oestradiol secretion to occur under stimulation with preparations of recombinant FSH that were devoid of any LH activity. As the pituitary responsiveness is maintained under GnRH antagonist treatment, it seems possible to induce ovulation by native GnRH or a GnRH agonist, avoiding the necessity of administering HCG with its (in some cases) deleterious effects leading to OHSS. Combining GnRH antagonist treatment with softer stimulation regimes such as clomiphene citrate/HMG may be the way to a cheap, safe and efficient ovarian stimulation for assisted reproduction techniques.

References

Adashi, E.Y. (1994) Endocrinology of the ovary. *Hum. Reprod.*, **9** (Suppl. 2), 36–51.

Albano, C., Smitz, J., Camus, M. *et al.* (1996) Hormonal profile during the follicular phase in cycles stimulated with a combination of human menopausal gonadotrophin and gonadotrophin-releasing hormone antagonist (Cetrorelix). *Hum. Reprod.*, **11,** 2114–2118.

Albano, C., Smitz, J., Camus, M. *et al.* (1997) Comparison of different doses of gonadotropin-releasing hormone antagonist Cetrorelix during controlled ovarian hyperstimulation. *Fertil. Steril.*, **67,** 917–922.

Balen, A. (1995) The effects of ovulation induction with gonadotrophins on the ovary and uterus and implications for assisted reproduction. *Hum. Reprod.*, **10,** 2233–2237.

Bauer, O. and Diedrich, K. (1996) Komplikationen der assistierten Reproduktion. *Gynäkologe*, **29,** 464–473.

Cassidenti, D.L., Sauer, M.V., Paulson, R.J. *et al.* (1991) Comparison of intermittent and continuous use of gonadotrophin-releasing hormone antagonist (Nal-Glu) in in vitro fertilization cycles: a preliminary report. *Am. J. Obstet. Gynecol.*, **165,** 1806–1810.

Chappel, S.C. and Howles, C. (1991) Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum. Reprod.*, **6,** 1206–1212.

de Jong, D., Macklon, N.S., Mannaerts, B.M.J.L. *et al.* (1998) Case report: high dose gonadotrophin-releasing hormone antagonist (Ganirelix) may prevent ovarian hyperstimulation syndrome caused by ovarian stimulation for in-vitro fertilization. *Hum. Reprod.*, **13,** 573–575.

Deutsches IVF Register, Jahrbuch 1997. Available from: DIR Bundesgeschäftsstelle Ärztekammer Schleswig Holstein, Bismarckallee 8–12, 23795 Bad Segeberg, Germany.

Diedrich, K., Diedrich, C., Santos, E. *et al.* (1994) Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist Cetrorelix during ovarian stimulation. *Hum. Reprod.*, **9,** 788–791.

Dittkoff, E.C., Cassidenti, D.L., Paulson, R.J. *et al.* (1991) The gonadotrophin-releasing hormone antagonist (Nal-Glu) acutely blocks the luteinizing hormone surge but allows for resumption of folliculogenesis in normal women. *Am. J. Obstet. Gynecol.*, **165,** 1811–1817.

Duijkers, I.J.M., Klipping, C., Willemsen, W.N.P. *et al.* (1998) Single and multiple dose pharmacokinetics and pharmacodynamics of the gonadotrophin-releasing hormone antagonist Cetrorelix in healthy female volunteers. *Hum. Reprod.*, **13** (Abstract book 1), 123.

Edwards, R.G., Lobo, R. and Bouchard, P. (1996) Time to revolutionize ovarian stimulation. *Hum. Reprod.*, **11,** 917–919.

Fauser, B.C.J.M. (1999) Endocrinological findings during treatment with Ganirelix (Orgalutran®). *Gynecol. Endocrinol.*, **13** (Suppl. 1) 13.

Felberbaum, R.E. (1999) Cetrorelix in controlled ovarian stimulation for ART: results of phase III, multiple dose treatment. *Gynecol. Endocrinol.*, **13** (Suppl. 1), 14.

Felberbaum, R.E., Reissmann, T., Küpker, W. *et al.* (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.

Felberbaum, R., Reissmann, T., Küpker, W. *et al.* (1996) Hormone profiles under ovarian stimulation with human menopausal gonadotrophin (HMG) and concomitant administration of the gonadotropin releasing hormone (GnRH)-antagonist Cetrorelix at different dosages. *J. Assist. Reprod. Genet.*, **13,** 216–222.

Felberbaum, R., Montzka, P., Küpker, W. *et al.* (1997) High fertilization rate after ovarian stimulation with clomiphene citrate for ICSI. *Hum. Reprod.*, **12** (Abstract book 1), 150.

Felberbaum, R.E., Ortmann, O., Eick, F. *et al.* (1998) GnRH-Antagonist (Cetrorelix) used for COH in vivo does not impair sexual steroid secretion of granulosa lutein cells in vitro. *Fertil. Steril.*, **70/3** (Suppl. 1), S109.

Frydman, R., Cornel, C. and de Ziegler, D. (1991) Prevention of premature luteinizing hormone and progesterone rise with gonadotrophin-releasing hormone antagonist, Nal-Glu, in controlled ovarian hyperstimulation. *Fertil. Steril.*, **56,** 923–927.

Ganirelix Dose-Finding Study Group (1998) A double-blind, randomized, dose finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist Ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon®). *Hum. Reprod.*, **13,** 3023–3031.

Golan, A., Ron-El, R., Herman, A. *et al.* (1988) Ovarian hyperstimulation syndrome following D-Trp-6-luteinizing hormone-releasing hormone microcapsules and menotropins for in vitro fertilization. *Fertil. Steril.*, **50,** 912–916.

Küpker, W., Al-Hasani, S., Schulze, W. *et al.* (1995) Morphology in intracytoplasmic sperm injection: preliminary results. *J. Assist. Reprod. Genet.*, **12,** 620–626.

Loumaye, E. (1990) The control of endogenous secretion of luteinizing hormone by gonadotrophin-releasing hormone agonists during ovarian hyperstimulation for in-vitro fertilization and embryo transfer. *Hum. Reprod.*, **5,** 357–376.

Loumaye, E., de Cooman, S., Anoma, M. *et al.* (1988) Short term utilization of a gonadotropin releasing hormone agonist (Buserelin) for induction of ovulation in an in-vitro fertilization program. *Ann. N.Y. Acad. Sci.*, **541,** 96–102.

Loumaye, E., Porchet, H.C., Beltrami, V. *et al.* (1994) Ovulation induction with recombinant human follicle-stimulating hormone and luteinizing hormone. In Filicori, M. and Flamigni, C. (eds), *Ovulation Induction. Basic Science and Clinical Advances*. Elsevier Science, The Netherlands, pp. 227–236.

Macnamee, M.C., Howles, C.M., Edwards, R.G. *et al.* (1989) Short term luteinizing hormone-releasing hormone agonist treatment: prospective trial of a novel ovarian stimulation regimen for in vitro fertilization. *Fertil. Steril.*, **52,** 264–269.

Mannaerts, B., Obereye, J., Peters, M. and Orlemans, E. (1999) Introduction to the GnRH antagonist Ganirelix (Orgalutran®). *Gynecol. Endocrinol.*, **13** (Suppl. 1), 12.

Olivennes, F., Fanchin, R., Bouchard, P. *et al.* (1994) The single or dual administration of the gonadotrophin-releasing hormone antagonist Cetrorelix in an in vitro fertilization–embryo transfer programme. *Fertil. Steril.*, **62,** 468–476.

Olivennes, F., Fanchin, R., Bouchard, P. *et al.* (1995) Scheduled administration of a gonadotrophin-releasing hormone antagonist (Cetrorelix) on day 8 of in-vitro fertilization cycles: a pilot study. *Hum. Reprod.*, **10,** 1382–1386.

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

Olivennes, F., Belaisch-Allart, J., Emperaire, J.C. *et al.* (1999) Comparison in a prospective multicentric randomized study in IVF-ET of a single dose of GnRH antagonist (Cetrorelix) to a GnRH agonist long protocol (Triptoreline in depot formula). *Gynecol. Endocrinol.*, **13** (Suppl. 1), 14.

Ortmann, O., Oltmanns, K., Weiss, J.M. *et al.* (1998) Effects of the GnRH antagonist Cetrorelix on steroidogenesis in human granulosa lutein cells. *Hum. Reprod.*, **13** (Abstract book 1), 254.

Ortmann, O., Weiss, J., Gürke, E.M. *et al.* (1999) Ovarian effects of GnRH antagonists. *Gynecol. Endocrinol.*, **13** (Suppl. 1), 51.

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A.C. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340,** 17–18.

Recombinant Human FSH Study Group (1995) Clinical assessment of recombinant human follicle-stimulating hormone in stimulating ovarian follicular development for in-vitro fertilization. *Fertil. Steril.*, **63,** 77–86.

Reissmann, T., Felberbaum, R., Diedrich, K. *et al.* (1995) Development and applications of luteinizing hormone-releasing hormone antagonists in the treatment of infertility: an overview. *Hum. Reprod.*, **10,** 1974–1981.

Ron-El, R., Herman, A., Golan, A. *et al.* (1991) Gonadotropins and combined gonadotropin-releasing hormone agonist-gonadotropins protocols in a randomized prospective study. *Fertil. Steril.*, **55,** 574–578.

Schoot, D.C., Harlin, J., Shoham, Z. *et al.* (1994) Recombinant human follicle-stimulating hormone and ovarian response in gonadotrophin-deficient women. *Hum. Reprod.*, **9,** 1237–1242.

Smitz, J., Ron-El, R. and Tarlatzis, B.C. (1992) The use of gonadotrophin-releasing hormone agonists for in-vitro fertilization and other assisted procreation techniques: experience from three centres. *Hum. Reprod.*, **7** (Suppl. 1), 49–66.

Sommer, L., Diedrich, K. and Klingmuller, D. (1993) Effects of the GnRH-antagonist Cetrorelix in normal women. In Lunenfeld, B. and Insler, V. (eds), *GnRH Analogues: the State of the Art 1993. Gynecol. Endocrinol.*, **7** (Suppl. 2), 20.

Stanger, J.D. and Yovich, J.L. (1985) Reduced in vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. *Br. J. Obstet. Gynaecol.*, **92,** 385–393.

Tan, S.L. (1991) Gonadotrophin-releasing hormone agonists in assisted reproductive therapy. *J. Br. Fertil. Soc.*, **1,** 137–142.

Van Steirteghem, A.C. (1994) IVF and micromanipulation techniques for male-factor infertility. *Curr. Opin. Obstet. Gynecol.*, **6,** 173–177.

WHO Scientific Group Report (1976) *Agent stimulating gonadal function in the Human.* World Health Organization. Technical Report Series No. 514. WHO consultation on the diagnosis and treatment of endocrine forms of female infertility.