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The use of GnRH antagonists in ovarian stimulation

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GnRH antagonists induce a rapid decrease in LH and FSH, preventing and interrupting LH surges. Their properties do not require a desensitization period, and this allows their use in the late follicular phase. GnRH antagonists could replace GnRH agonists in controlled ovarian stimulation without their side-effects and their long desensitization period. Two protocols for assisted reproduction technology (ART) cycles were designed: the single-dose protocol allies simplicity and efficacy, while the multiple-dose protocol is efficient and could reduce monitoring of the cycle, though compliance is mandatory. A review of the available literature on GnRH antagonists in ART cycles is presented, focusing on phase III controlled trials and ART results. Both protocols using GnRH antagonists were associated with the need for a smaller dose of gonadotrophin, a shorter stimulation period and a lower incidence of ovarian hyperstimulation syndrome (OHSS), albeit with statistically comparable pregnancy rates. A trend is observed in all studies showing a lower pregnancy rates in GnRH antagonist cycles as compared with GnRH agonist cycles. The role of the lower number of embryos, and the potential adverse effects of GnRH antagonists on endometrium or follicle must be studied. More cycles using GnRH antagonists are necessary to confirm their equivalent pregnancy rates. There is room for improvement in both protocols with regard to scheduling, antagonist dose level and the timing of its administration. Until further studies have been conducted, luteal support seems to remain mandatory. Perinatal outcome appears similar to that with other stimulation regimens. Triggering of ovulation can be obtained with GnRH agonist for patients at risk of OHSS. With regard to GnRH antagonists, questions remain regarding pregnancy rates, the indications of their use in patients with polycystic ovary syndrome or poor responders, and in ovarian stimulation outside IVF.

Keywords: assisted reproduction/GnRH antagonists/ovarian hyperstimulation syndrome/ovarian stimulation

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Introduction

The use of ovarian stimulation has the objective of increasing the success rate in IVF, allowing the development of multiple follicles, and consequently several oocytes and embryos.

The combination of GnRH agonist and gonadotrophins is nowadays the most successful treatment regimen, and therefore the treatment most currently prescribed. GnRH agonists have increased pregnancy rates, though mainly by increasing the number of oocytes and embryos obtained rather than by improving embryo quality (Liu *et al*., 1992). The main advantage of a GnRH agonist is to prevent the premature LH surge that is associated with cycle cancellation. By using the long protocol, and starting the GnRH agonist during either the follicular or luteal phase, desensitization will occur after a period of flare-up. This regimen allows the start of the treatment cycles to be scheduled, and for the activities of a large IVF centre to be organized.

In France, GnRH agonists are used in over 90% of IVF cycles (Bachelot *et al*., 1998). The most commonly prescribed therapeutic regimen is the long protocol, in which case the use of GnRH agonist increases the duration of the treatment period (as 2–3 weeks are usually needed to obtain desensitization), the amount of gonadotrophin needed, and the risk of ovarian hyperstimulation syndrome (OHSS). The desensitization period is associated with side-effects (hot flushes, headaches, bleeding

and vaginal dryness) (Ben Rafael *et al*., 1991; Rizk and Smitz, 1992).

The new generation of GnRH antagonists, which are devoid of the anaphylactoid reactions described with previous such compounds (Karten *et al*., 1990), are now available for clinical use. Due to their competitive receptor properties, GnRH antagonists induce an immediate and rapid inhibition in LH and FSH secretion without 'flare-up'. If administered during the follicular phase, GnRH antagonists can either prevent or interrupt LH surges (Dubourdieu *et al*., 1994). In addition, their use has been proposed in IVF cycles in order to obtain similar results to those obtained with GnRH agonist, though by using a simpler protocol and producing fewer side-effects (Olivennes *et al*., 2000).

Two different compounds are available: cetrorelix (Cetrotide®; formerly ASTA Medica, now Serono), and ganirelix (Antagon® or Orgalutran®; Organon). Two different protocols of administration (Figure 1) have been proposed in the literature for the use of GnRH antagonist in controlled ovarian stimulation. In the multiple-dose protocol, small doses (0.25 mg) of GnRH antagonist are injected in the mid-follicular phase (Diedrich *et al*., 1994; Albano *et al*., 1997; The Ganirelix Dose-Finding Study Group, 1998). A single-dose protocol has been designed in which a higher dose (3 mg) is injected during the late follicular phase, when the LH surge is most feared (Olivennes *et al*., 1994, 1995).

The protocols of GnRH antagonist administration (phase II and phase III controlled and uncontrolled studies) are well defined, though there is room for improvement. However, some important questions remain unanswered with regard to their use in clinical practice, and doubt persists as to how IVF results with GnRH antagonists compare with results obtained with GnRH agonists.

The aim of this review was to summarize the available literature on the efficacy and safety of GnRH antagonists, and to compare GnRH agonist and GnRH antagonist protocols with regard to IVF results. In addition, some different paradigms for ovarian stimulation are discussed.

Mechanism of action

GnRH, a 10 amino acid peptide, is secreted by the hypothalamus in a pulsatile pattern. GnRH binds to a specific receptor in the pituitary cells to regulate the secretion and synthesis of LH and FSH. After binding with the receptor, the GnRH–receptor complex elicits several (calcium-dependent) reactions to release the pituitary hormones (LH and FSH). In addition, the number of GnRH receptors changes during certain physiological states such as lactation and old age (Clayton and Catt, 1981).

Recently available GnRH antagonists are GnRH molecules with amino acid modifications at positions 1, 2, 3, 6 and 10. These compounds are not associated with the histamine-releasing effects seen with previous such compounds; rather, they immediately block the GnRH receptor in a competitive fashion and hence reduce LH and FSH secretion within a period of 8 h. The inhibition of LH secretion is more pronounced than that of FSH, this being most likely due to the different forms of gonadotrophin regulation, the prolonged FSH half-life or the immunoactive and bioactive forms of FSH (Matikainen *et al*., 1992; Bouchard *et al*., 1994).

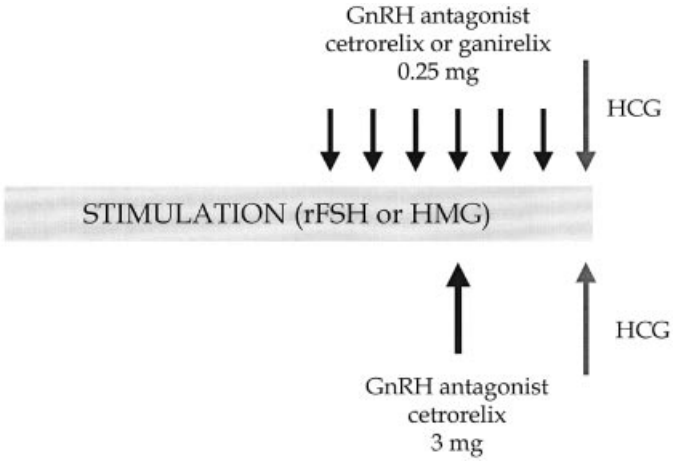

Figure 1. GnRH antagonist multiple- and single-dose protocols.

Unlike GnRH antagonists, GnRH agonists exert their effect by binding to the transmembrane receptor and, following a period of flare-up, produce a down-regulation phenomenon (Reissmann *et al*., 1995). The main disadvantages of these compounds are their need for chronic administration, and their induction of side-effects due either to the flare-up phenomenon (ovarian cysts) or desensitization (ovarian deprivation syndrome). The inhibitory effect of GnRH antagonists is more dose-dependent, and is associated with the equilibrium between endogenous GnRH and antagonist concentration (Reissmann *et al*., 1995).

Phase II dose-finding studies

Single-dose protocol

In the first investigation with cetrorelix, the previously published Nal-Glu protocol consisting of two 5 mg injections given 48 h apart during the late follicular phase was reproduced (Frydman *et al*., 1991, 1992), with the first injection being given on stimulation day 7. It was noted that a second injection was often unnecessary as HCG was given on the same day. It was concluded that the 5 mg dose induced deep suppression of LH, and that a lower dose should be investigated (Olivennes *et al*., 1994). A single-dose protocol was designed whereby a single (3 mg) injection of GnRH antagonist was carried out on stimulation day 7 (Olivennes *et al*., 1995).

In order to determine the minimal effective dose, a dose-finding study was conducted. The use of 2 and 3 mg was compared to investigate the 'protection period'—the time after antagonist administration during which a LH surge is prevented. The IVF results were strictly comparable between the two doses, with the 2 mg dose preventing LH surges for 3 days in all patients. However, it was noted that the suppression of LH tended to be reduced 3 days after injection of the 2 mg dose (Figure 2), while a LH surge was observed 4 days after 2 mg cetrorelix administration. No differences were observed between the different doses in terms of IVF results. The 3 mg dose was therefore selected as a safer choice, as a 'protection period' of at least 4 days can be obtained (Olivennes *et al*., 1998). As shown in Figure 2, the plasma LH levels were rapidly decreased by antagonist administration, and no LH surge was observed in all patients treated with the 3 mg dose. In some patients, a plasma LH rise (to >10

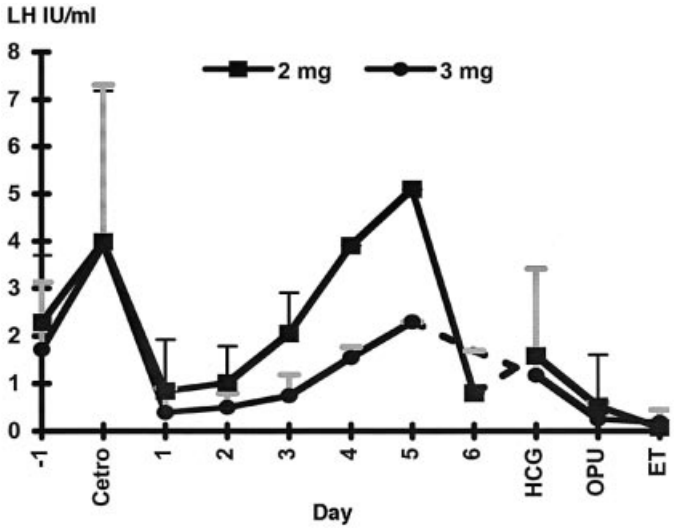

Figure 2. Serum levels of LH in patients before and after treatment with 2 or 3 mg cetrorelix.

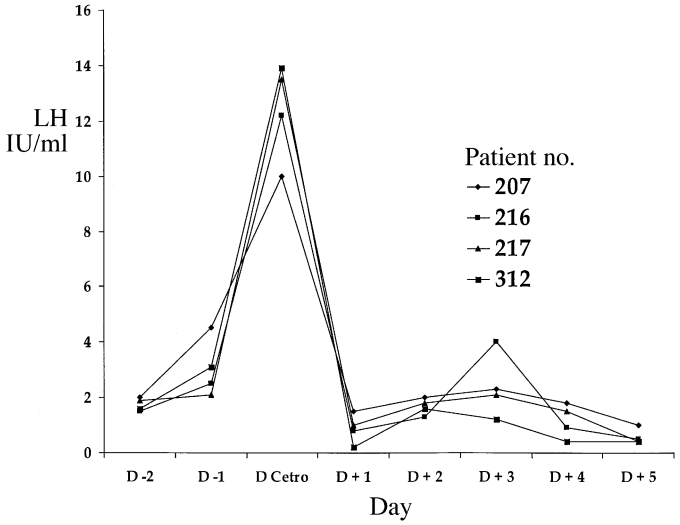

Figure 3. Interruption of LH surges by the GnRH antagonist administration (cetrorelix 3 mg).

IU/l) was observed on the day of antagonist administration. Cetrorelix was able to prevent any further rise in LH, and immediately lowered the LH levels such that no surge was observed in these patients (Figure 3). The interruption of LH rises does not appear to have any deleterious effect on IVF results (Christin-Maitre *et al*., 2000). The consumption of HMG was also clearly reduced (24–30 HMG ampoules) when compared with the use of GnRH agonist in the long protocol using a depot preparation (Olivennes *et al*., 1995). Tolerance towards cetrorelix was excellent, with transient erythema being seen at the injection site in only 15% of the patients.

Multiple-dose protocol

The two GnRH antagonists (cetrorelix or ganirelix) were studied in order to determine the optimum dose to block the premature LH rise but not oversuppress the pituitary. An initial study (Sommer *et al*., 1994) described the suppression of gonadotrophin and estradiol secretion by 3 mg cetrorelix given daily to normal-cycling women. Subsequently, several dose-finding studies (Diedrich *et al*., 1994; Felberbaum *et al*., 1996; Albano *et al*., 1997; The Ganirelix dose-finding study group, 1998) generally initiated gonadotrophin (recombinant or urinary) treatment on day 2 of the menstrual cycle, while daily antagonist administration was initiated on stimulation day 6. The decision to start antagonist administration was based on the concept of preventing the premature LH rise without causing any harmful effect on ovarian stimulation. The risk of premature LH surge is greater after the sixth day of ovarian stimulation, and this day was chosen to initiate antagonist injections (Diedrich *et al*., 1994). The authors of various studies compared different doses of cetrorelix or ganirelix in order to determine the best dose with respect to the most appropriate assisted reproduction technology (ART) results.

When comparing cetrorelix administration of 3 and 1 mg or 0.5 and 0.25 mg after day 6 of the stimulation protocol, all patients had a decline in LH serum levels. Patients receiving 0.5 and 0.25 mg/day showed the best ART results in terms of pregnancy and implantation rates without the risk of pituitary oversuppression that occurred with 1 and 3 mg doses (Diedrich *et al*., 1994; Felberbaum *et al*., 1996). In another study (Albano *et al*., 1997), it was shown that patients receiving cetrorelix starting doses of 0.5 or 0.25 mg/day during the follicular phase did not show any premature LH surges, as evidenced by lower LH serum levels. However, one in seven patients receiving 0.1 mg/day showed a premature LH rise with progesterone elevation, and hence the 0.1 mg dose was abandoned. Results were similar in patients receiving 0.25 or 0.5 mg/day in terms of clinical pregnancy and implantation rates. These investigators concluded that the minimal effective cetrorelix dose to prevent premature LH surge was 0.25 mg/day.

The minimal safe/effective dose of ganirelix required to achieve good IVF results was also investigated (The Ganirelix dose-finding study group, 1998). A multiple-dose protocol with ganirelix showed the minimal effective dose to be 0.25 mg/day; this inhibited premature LH secretion without compromising IVF results in stimulated cycles with recombinant FSH. Patients receiving 0.25 mg/day ganirelix had the highest vital pregnancy rate per transfer (40.3%) compared with other phase II studies (doses of 0.0625 to 2 mg) (see Table I). Based on an analysis of the database from the ganirelix dose-finding study which examined the effect of GnRH antagonist in freeze–thaw cycles, it was concluded that high doses (1.0 and 2.0 mg/day) of ganirelix did not affect the biological potential of embryos to develop clinical pregnancy (Kol *et al*., 1999).

Phase III randomized controlled trials and open studies

Single-dose protocol

The single-dose protocol was compared with the GnRH agonist long protocol using a depot formula of triptorelin in a prospective randomized study (Olivennes *et al*., 2000). A 3:1 randomization was selected, including 115 patients in the cetrorelix group and 36 in the agonist long protocol group. No difference was observed between the GnRH agonist and antagonist groups in terms of demographic and baseline data.

Table I. Results of phase II studies regarding GnRH antagonist administration in assisted reproduction technology

Parameter	Diedrich *et al.* (1994)	Felberbaum *et al.* (1996)		Albano *et al.* (1997[a])			The Ganirelix dose-finding study group (1998)							Olivennes *et al.* (1994)	Olivennes *et al.* (1995)	Olivennes *et al.* (1998)
Study design	CT	CT			CT		Randomized CT							CT	CT	CT
Medication	Cetrorelix	Cetrorelix			Cetrorelix		Ganirelix multiple dose (mg)							Cetrorelix	Cetrorelix	Cetrorelix
No. of patients	20	11	12	12	30	32	31	65	69	69	65	30	17	11	31	34
Protocol studied	1 & 3 mg/day	0.50 mg/day	1.0 mg/day	3.0 mg/day	0.25 mg/day	0.50 mg/day	0.0625	0.125	0.25	0.5	1.0	2.0	5 mg SD	3 mg SD	2 mg SD	3 mg SD
Gonadotrophin (ampoules/IU)	27	26	27	30	33	35	NA	NA	NA	NA	NA	NA	27.7	24.5	30.5	25.5
Serum E_2 on HCG day (pg/ml)[b]	NA	2164 ± 2102	1022 ± 602	852 ± 325	2491	2122	1475	1130	1160	823	703	430	NA	2081	NA	NA
No. of oocytes	8.1	12.7	7.8	8.8	16.2	12.4	9.0 ± 5.7	9.5 ± 5.5	10.0 ± 5.4	8.8 ± 6.6	9.3 ± 6.0	8.6 ± 4.4	7.1 ± 3.5	7.1± 3.5	8.4± 4.2	6.5 ± 3.4
Fertilization rate (%)	61.5	67.7	53.1	45.3	58	55	–	–	–	–	–	–	85.9	87.3	NA	NA
Implantation rate (%)	NA	NA	NA	NA	11.8	12.5	14.2	16.6	21.9	9.0	8.8	1.5	35.3	NA	14.3	18.7
Clinical pregnancy rate (%)[c]	NA	NA	NA	NA	30	28	23.3	26.2	36.8	11.4	14.1	3.8	35.3	36.3	25.8	32.4
Premature LH rise (%)	None	None	None	None	None	None	16.1	9.2	1.4	None	None	None	None	None	3.0	None

[a]A premature LH surge with a concomitant P rise occurred in one of the seven patients treated with 0.1 mg; hence, the authors abandoned this study arm.
[b]Values are mean ± SD.
[c]Per attempt.
CT = clinical trial; E_2 = estradiol; NA = not available; SD = single dose.

Among the 115 cetrorelix patients, 104 (90.4%) received only one 3 mg dose. If the criteria for triggering of ovulation were not reached within 4 days (the protection period), additional doses of cetrorelix (0.25 mg) was administered. Only nine (7.8%) of the patients received one additional dose on the morning of the HCG, and two patients (1.7%) received two additional doses of 0.25 mg.

Only 18 patients in the cetrorelix group (15.6%) presented a LH rise (to >10 IU/l) on the day of cetrorelix injection, as the administration of cetrorelix inhibited LH secretion. Four of these patients (22.2%) became pregnant, and these interrupted LH rises appeared to have no measurable deleterious effect in this study. Only one patient in the triptorelin group (2.8%) experienced a LH surge. None of the 115 patients in the cetrorelix group experienced a LH surge after cetrorelix administration. In addition, no LH surge has yet been reported within the 4 days following single administration of 3 mg cetrorelix.

IVF results of the single-dose protocol are presented in Table II. The mean duration of stimulation was significantly lower in the cetrorelix group, while the mean number of gonadotrophin ampoules used was significantly higher in the triptorelin group. Estradiol levels on the day of HCG were significantly lower in the cetrorelix than in the triptorelin group. The mean number of follicles of 18 mm diameter was comparable between the two groups on the day of HCG. The total number of follicles $\geqslant$15 mm and $\leqslant$ 17 mm was higher in the triptorelin group [5.0 $\pm$ 3.9 versus 3.4 $\pm$ 2.6; confidence interval (CI) 0.5–2.8)]. The long GnRH agonist protocol resulted in more oocytes and more embryos, as has already been demonstrated in comparison with other stimulation regimens (Liu *et al*., 1992). However, the percentage of mature oocytes, fertilization rate, clinical and ongoing pregnancy rates and miscarriage rates were not statistically different between the two groups. The incidence of OHSS was lower in the GnRH antagonist group; this difference did not reach statistical significance, but some patients in the GnRH agonist group were cancelled as they were at risk of OHSS. Adding these patients into the statistical analysis rendered the difference statistically significant.

In conclusion, this study (Olivennes *et al*., 2000) confirmed the efficacy of a single 3 mg dose of cetrorelix, administered during the late follicular phase, in preventing premature ovulation as indicated by LH surges. The single-dose protocol is easy to use and assures patient compliance; moreover, the 3mg dose was tolerated well by patients in this study, with only mild and transitory reactions at the injection site. This protocol provides a shorter duration of treatment, uses less HMG and has a lower incidence of OHSS. In addition, the IVF results compared favourably with the long protocol using a depot formula of triptorelin. The study results strongly suggest that the single-dose antagonist protocol offers a valid and interesting alternative treatment regimen for IVF.

Results with the use of recombinant FSH (rFSH) as a source of gonadotrophins are preliminary, as the studies conducted with the single-dose protocol used HMG. In a small prospective study, two groups of 30 patients using either HMG or rFSH were compared, but no differences were observed in the IVF results (unpublished data). A large multicentre study is ongoing with the use of the single-dose protocol and rFSH.

In some patients treated with rFSH, a decrease in the estradiol level was observed after cetrorelix injection. This was also observed in an initial study using a higher dose (5 mg) with HMG (Olivennes *et al*., 1995). An increase in the HMG dose on the day of antagonist administration suppressed the estradiol decreases that were probably related to LH suppression, though not exclusively so (De Jong *et al.*, 2001a). However, no differences were observed in IVF results in patients with or without an estradiol decrease following cetrorelix administration (unpublished results).

Multiple-dose protocol

In all studies presented, the multiple-dose protocol used 0.25 mg/day of either cetrorelix or ganirelix. In order to compare the antagonist multiple-dose protocol (0.25 mg/day) with the GnRH agonist in IVF cycles, the European Cetrorelix Study Group (Albano *et al*., 2000) published the results of an open randomized trial (Table II). In total, 188 patients were treated with cetrorelix, and 85 with the long (buserelin) agonist protocol; all patients received HMG. Embryos were transferred in 83.5% of the cetrorelix group, and 79% of the buserelin group. The clinical pregnancy rates were 22.3 and 25.9% per started cycle in these groups respectively (P = not significant). The duration of treatment with gonadotrophins, as well as serum estradiol levels on the day of HCG were lower in the antagonist (cetrorelix) group. The incidence of OHSS (II and III) was higher in patients using agonist (buserelin) treatment.

A controlled, multicentre, randomized trial was also carried out to compare two treatment regimens for ovarian stimulation (multiple-dose antagonist versus long-agonist) in women receiving recombinant FSH (The European Orgalutran Study Group, 2000). A total of 672 patients was investigated and randomized (Table II). The total dose of FSH administered was higher in the buserelin group (1500 IU and 1800 IU), while patients receiving antagonist also had a shorter duration of stimulation than the agonist group. Serum estradiol levels on the day of HCG administration were higher in patients using buserelin than ganirelix, and the incidence of OHSS was higher in the buserelin group (2.9 versus 1.0%). Otherwise, the number of good quality embryos, fertilization rate (62.1% in both groups) and replaced embryos were similar between the two treatment schemes. The implantation rate was lower in the ganirelix group (15.7%) than in buserelin group (21.8%), though the clinical pregnancy rates per attempt were not statistically significant.

Another study was conducted to evaluate the efficacy and safety of ganirelix (multiple-dose protocol) versus leuprolide (long protocol) in IVF patients (The North American Ganirelix Study Group, 2001). This multicentre (USA and Canada) trial showed that the mean number of retrieved oocytes was similar between the groups (11.6 in antagonist group versus 14.1 in agonist group). Moreover, the fertilization rates (62.4 and 61.9%) and implantation rates (21.1 and 26.1%) were also similar in both groups. The ongoing pregnancy rates per attempt were 30.8% in the ganirelix group and 36.4% in the leuprolide group; however, the antagonist group showed fewer local site reactions after injection administration (12.5%) than the leuprolide group (25.5%). The authors proved the effectiveness and safety of the antagonist multiple-dose protocol with its shorter stimulation period and fewer side-effects compared with the long agonist (leuprolide) protocol.

Table II. Results of prospective randomized phase III studies comparing GnRH antagonist multiple and single dose protocols with GnRH agonists

Parameter	Cetrorelix single dose study (Olivennes *et al.*, 2000)		Ganirelix multiple dose study (The European Orgalutran Study Group, 2000)		Cetrorelix multiple dose study (Albano *et al.*, 2000)		Ganirelix multiple dose study (The North American Ganirelix Study Group, 2001)		Ganirelix multiple dose study (The European and Middle East Orgalutran Study Group, 2001)	
Medication	Cetrorelix (n = 113)	Buserelin (n = 35)	Ganirelix (n = 448)	Buserelin (n = 224)	Cetrorelix (n = 188)	Buserelin (n = 85)	Ganirelix (n = 208)	Leuprolide (n = 105)	Ganirelix (n = 226)	Triptorelin (n = 111)
Duration of stimulation (days)	9.4 ± 1.4	10.7 ± 1.7	9 (6–18)	10 (6–19)	10.6 ± 2.3	11.4 ± 1.8	Median: 8	Median: 10	9 (6–14)	11 (7–16)
LH surge (%)	0	2.8	2.8	1.3	1.5	0	1.0	0	3	0
Gonadotrophin total dose (IU/or 75 IU ampoules)	24.3 ± 7.4	35.6 ± 15.1	1500 (900–5400)	1800 (900–6450)	23.6 ± 8.5	25.6 ± 7.6	1800	2025	1350 (900–3900)	1800 (1050–4050)
Serum E_2 on HCG day (pg/ml)	1786 ± 808	2549 ± 1194	1190 (373–3105)	1700 (527–4070)	1625 ± 836	2082 ± 1049	2001	2768	1090 (385–2790)	1370 (255–3930)
No. of oocytes										
Before IVF	9.2 ± 5.1[a]	12.6 ± 7.4[a]	9.6 ± 5.7	10.3 ± 5.7	8.0 ± 4.9[a]	10.6±6.6[a]	11.6[a]	14.1[a]	7.9±5.1[a]	9.6±6.8[a]
Before ICSI	–	–	8.6 ± 4.8	10.3 ± 5.9	–	–	–	–	–	–
Total embryos (n)[b]	5.4 ± 3.5	7.5 ± 4.9	6.0 ± 4.5	7.1 ± 5.2	4.5 ± 3.3	6.0 ± 4.1	6.9	8.2	4 ± 3	4.7 ± 3
Implantation rate (%)	14.3	19.7	15.7	21.8	12.2	14.2	21.1	26.1	22.9	22.9
Miscarriage rate (%)	15.4	27.3	12.0	13.9	16.6	9.0	NA	NA	10.3	11.4
Clinical pregnancy per attempt (%)	22.6	28.2	21.8	28.2	22.3	25.9	35.4	38.4	35.8	41.7
Ongoing pregnancy per attempt (%)	18.3	23.1	20.3	25.7	19.1	25.9	30.8	36.4	31	33.9
Ongoing pregnancy per transfer (%)	21.2	27.3	23.3	29.0	21.6	29.8	NA	NA	34	38.5
OHSS (%)	1.7	5.6	1.0	2.9	1.1	6.5	6.1	2.0	1.8	0.9

[a]Oocyte numbers in both IVF and ICSI cycles (separate numbers not available).
[b]Values are mean ± SD.
E_2 = estradiol; NA = not available; OHSS = ovarian hyperstimulation syndrome.

Recently, another multicentre European trial comparing two treatment schemes (ganirelix and triptorelin) in 337 women showed that the median dose of rFSH was lower in the antagonist protocol (The European and Middle East Orgalutran Study Group, 2001). Serum estradiol levels were also shown to be lower in the ganirelix group on the day of HCG. Fertilization rates (64% for ganirelix, 64.9% for triptorelin), mean number of good quality embryos (2.7 and 2.9 respectively), implantation rate (22.9% for both treatments) and, finally, the ongoing pregnancy rate per attempt were similar between the two treatments (31 and 33.9% respectively).

The multiple-dose protocol, when compared with the long-agonist regimen, offers a simple, safe and efficient option, and with comparable IVF results. The risk of OHSS is reduced (Ludwig *et al.*, 2000), while the total dose of gonadotrophin needed to stimulate ovulation and the stimulation period is also less than in the long protocol. Patients receiving antagonist treatment had lower serum estradiol levels at the time of HCG administration, most likely because of the lower number of follicles. The impact of this finding in implantation rates is both disputed and unknown, however.

In the multiple-dose protocol, there was a very low incidence of LH surge (between 1 and 2.5%). These surges were often associated with a lack of compliance, mainly when patients forgot to take one antagonist treatment. Consequently, the importance of this point should be stressed to patients. More recently, some centres have observed a higher incidence of LH surge in poor responders using the multiple-dose protocol (unpublished data), and these protocol observations should be confirmed and documented. In addition, the 0.25 mg dose might not be always sufficient, and might have to be adapted to the patient's body weight.

Recently, a prospective uncontrolled phase III study was carried out to evaluate the effectiveness on ovarian stimulation of using HMG and cetrorelix at a dose of 0.25 mg/day (Felberbaum *et al.*, 2000). In total, 346 women aged 18–39 years and with regular menstrual cycles were included for classic IVF or ICSI as the infertility treatment. Only three women showed a premature progesterone and LH rise (0.9%), while the fertilization rate was 59.2% and the implantation rate 11.4%. The incidence of severe OHSS was very low (0.6%) and the overall ongoing clinical pregnancy rate was 23.6% per transfer. This report confirmed later results relating to the efficacy and safety of cetrorelix in a large number of patients.

Follicular development was also studied in a randomized controlled multicentre study, in patients using ganirelix at different doses (0.0625 to 2.0 mg/day). Patients received rFSH after day 2 of the menstrual cycle, and ganirelix was administered daily after day 6 of the ovarian stimulation protocol (de Jong *et al.*, 2001b). Overall, 311 patients were studied and compared in terms of number of follicles, total follicular surface area, and serum gonadotrophin and steroid hormone levels. Increasing GnRH antagonist doses demonstrated an additional suppressive action on estradiol and androstenedione serum levels, most likely by an important inhibition of LH secretion, which may have exerted a harmful effect. The follicular growing pattern was not affected by the dose of GnRH antagonist. The reduction in the secretion of androstenedione and estradiol were not totally explained by the LH inhibition. Other(s) mechanism(s) might be involved in GnRH antagonist action and influence the cycles stimulated with this regimen protocol.

Two starting doses of rFSH (150 versus 225 IU) were also evaluated in a prospective randomized trial, with the multiple-dose cetrorelix protocol (Wikland *et al.*, 2001). The aim was to increase follicle, oocyte and embryos numbers in order to raise pregnancy rates. Despite a higher number of recovered oocytes in patients receiving 225 IU rFSH, pregnancy and implantation rates were similar.

The effect of GnRH antagonists on oocyte and embryo quality has been measured by studying implantation and pregnancy rates after cryopreservation of pronuclear oocytes or embryos. In one such study (Nikolettos *et al.*, 2000), 62 patients were allocated to two groups; one group received the multiple-dose GnRH antagonist protocol (group I), and the other group the conventional GnRH long protocol (group II). Implantation and pregnancy rates, after freeze–thawing procedures at the pronuclear embryo stage, were similar between groups (3.26 and 8.33% for group I; 3.73 and 10.25% for group II).

GnRH antagonists in minimal stimulation

The objective of this regimen is to combine the possible prevention of a LH surge by administration of a GnRH antagonist with the simplicity of the natural cycle and minimal stimulation (Figure 4). The administration of cetrorelix in the late follicular phase of minimally stimulated cycle in women of good prognosis was investigated (Rongières-Bertrand *et al.*, 1999). The patients were aged 26–36 (mean 34.1 ± 1.4) years, and had normal menstrual cycles, day 3 FSH <8 UI/l, day 3 estradiol <50 pg/ml, fewer than three previous IVF procedures, and male factor infertility requiring IVF and ICSI. In order to assess the minimal effective dose, a single subcutaneous injection of cetrorelix (1 or 0.5 mg) was administered when plasma estradiol levels reached 100–150 pg/ml, and a lead follicle was 12–14 mm diameter. As previous studies with Nal-Glu (Kettel *et al.*, 1991) and cetrorelix (Leroy *et al.*, 1994) had shown that estradiol secretion can be reduced after GnRH antagonist administration, daily administrations of 150 IU of HMG were carried out at the time of the first cetrorelix injection and repeated thereafter until HCG administration. This treatment scheme was not a complete natural cycle, as low gonadotrophin support was associated (minimal stimulation). Triggering of ovulation (5000 IU HCG) was initiated when the lead follicle reached 16–20 mm and serum estradiol values were >200 pg/ml. Oocyte retrieval was carried out 36–40 h later, without anaesthesia (Ramsewak *et al.*, 1990).

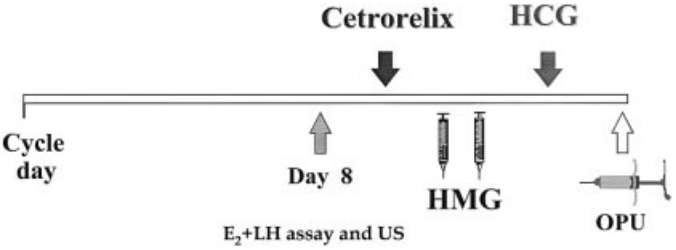

Figure 4. Cetrorelix single dose in minimal ovarian stimulation protocol. OPU = oocyte pick-up; US = ultrasonography.

A total of 33 patients (44 cycles) was included. The mean number of HMG ampoules was 4.7 ± 1.4 and the mean time between cetrorelix and HCG administrations was 2.0 ± 0.7 days. Four cycles were cancelled (9.0%). Follicular growth and estradiol secretion were not affected by cetrorelix administration. A total of 40 oocytes retrievals leading to 22 transfers (55%) was performed; no oocyte was obtained in 10 cycles. Fertilization failure occurred in six cycles, and in two patients the transfer was not performed because of developmental arrest of the embryo at the 2 pronuclear stage. The fertilization rate was 80% (24 embryos from 30 oocytes). A total of five clinical pregnancies was obtained (32.0% per transfer, 17.5% per retrieval) of which four are ongoing. The number of patients in whom the cycle was cancelled for premature LH surge was very low (9.0%) as compared with previous reports on natural cycles, thus confirming the efficacy of the antagonist administration. In addition, the pregnancy rate seems interesting, even though this must be confirmed in larger series.

The high burden and drawbacks of a 'heavy' ovarian stimulation protocol (side-effects, multiple pregnancies, potential serious health complications) make a clear demand for 'softer' protocols (Edwards *et al*., 1996; Fauser *et al*, 1999) and 'friendly IVF' (Olivennes and Frydman, 1998). IVF with spontaneous cycles or minimal stimulation protocols have been rapidly replaced by stimulated cycles with gonadotrophins in order to increase oocyte and embryo numbers. Nowadays, spontaneous cycles are rarely used because of the supposed low pregnancy rates and the cancellation rate that is mainly related to frequent spontaneous LH surges (Claman *et al*., 1993).

If these preliminary results with spontaneous cycle and HMG support are confirmed with larger patient numbers, then repetition of two or three of these cycles could lead to acceptable cumulative pregnancy rates without the potential adverse effects of ovarian stimulation (Lenton *et al*., 1992; Ingerslev *et al.*, 2001; Nargund *et al.*, 2001) and also be more cost effective (Daya *et al.*, 1995).

Recent questions and paradigms

Pregnancy rates

There is a trend in most controlled studies using GnRH antagonist (with both compounds and protocols) to find slightly lower pregnancy rates as compared with the GnRH agonist long protocol, and this had led to a questioning of the IVF results achieved with GnRH antagonists. Care should be taken in drawing conclusions based on these observations, as some population factors were not equivalent in the groups despite randomization. In addition, the learning curve—which is inherent to the use of any new treatment scheme—influences the study outcome. The trend towards higher pregnancy rates in the GnRH agonist group may be associated with the relative higher number of obtained embryos due to the higher number of oocytes. This hypothesis was not confirmed by one study however (Wikland *et al*., 2001). The difference could be related to the absence of desensitization of the previous luteal phase, and in fact a difference of the same magnitude in the pregnancy rates was found between the short and long protocols (Hughes *et al*., 1992), though reasons for this difference remain unclear. The potential deleterious effect of GnRH antagonist on the endometrium, or even on the fertilization process, has been presented (Hernandez, 2000), though no clinical data exist which confirm this hypothesis in humans (Mannaerts and Gordon, 2000; Ortman *et al.*, 2001). Therefore, a careful analysis is needed before drawing conclusions based on pregnancy rate. Indeed, a comparative study designed to assess a 5% difference for pregnancy rate in the region of 20% would require over 1200 patients in each treatment group.

Indications

Polycystic ovary syndrome (PCOS) patients

The use of GnRH antagonists in large series of PCOS patients has not yet been published. One of the most important hormonal aspects of PCOS patients is the increased LH tone secretion. This group of patients is characterized by anovulation, and the induction of ovarian ovulation is usually carried out using clomiphene citrate and FSH associated (or not) with GnRH agonists. The rationale for using GnRH antagonist in PCOS patients is that the LH/FSH ratio will be reduced; this occurs because LH secretion is affected more by antagonist administration than by FSH secretion (Reissmann *et al*., 1995). In IVF, another clear advantage is the reduced incidence of OHSS with the use of GnRH antagonist. Using a GnRH antagonist protocol also allows induction of oocyte final maturation with a GnRH agonist; this elicits an endogenous LH surge and, subsequently, decreases the risk of OHSS (Olivennes *et al*., 1996). The conduction of a large prospective trial is necessary however to confirm these physiological hypotheses. Recently, two case reports were made of PCOS patients treated with GnRH antagonist before treatment with GnRH agonist to induce ovulation (Lubin *et al*., 1998). The patients showed a normalization of serum LH and testosterone levels, although the authors failed to induce an appropriate ovarian response.

Larger studies are needed to evaluate further the potential benefits of the association of GnRH antagonist in PCOS patients.

Poor responders

The definition of poor responders, together with the heterogeneity of this group of patients, has caused an important bias in published series. The rationale for using ovarian stimulation protocols with GnRH antagonists in poor responders is that GnRH antagonists do not require desentisitization and do not depress gonadotrophin secretion during stimulation.

A total of 42 poor-responder patients were divided into two groups for ICSI treatment (long GnRH agonist or cetrorelix multiple-dose protocols) (Nikolettos *et al*., 2001). The stimulation protocol also included, in some patients, clomiphene citrate associated with gonadotrophins. Age, number of oocytes retrieved, number of fertilized oocytes, transferred embryos, embryo quality score and clinical pregnancy were not significantly different between the groups. A trend was observed in the pregnancy rates (14.28% for cetrorelix versus 9.52% for GnRH agonist treatment), but the difference was not significant. Although the authors discussed the sample size utilized, it is most likely that with an adequate power calculation this difference in terms of pregnancy rate would be statistically important.

With the same objective of the above-mentioned paper, others (Akman *et al*., 2001) presented a randomized trial comparing the microdose flare-up GnRH agonist protocol versus the antagonist multiple-dose protocol. In total, 48 patients were allocated to two regimen protocols. The implantation rates (15.07% for flare-up, 11.36% for cetrorelix) and the ongoing pregnancy rates per transfer (21.05 and 16.6% for flare-up and cetrorelix respectively) were similar between the two groups. Although the clinical outcomes were very similar in these two studies, larger randomized trials with appropriate power calculations should be carried out in order to assess the utility of antagonist regimens (single- and multiple-dose protocols) in poor responder patients.

Luteal phase

LH secretion is fundamental for the development of a normal luteal phase and also for progesterone secretion. The luteal phase defect induced by agonist administration is well known and studied, and is caused mainly by profound pituitary suppression (Tavaniotou *et al*., 2001). The antagonists exert a transitory LH inhibition and, hypothetically, the luteal phase is less disturbed. Some authors (Albano *et al.*, 1999; Tavaniotou *et al*., 2001), by comparing serum LH levels in the early and mid-luteal phase of HMG-treated cycles with or without antagonist (cetrorelix multiple dose), concluded that there is a decrease in serum LH levels in the cetrorelix group. However, the implications of this phenomenon were not studied. In a small group of patients treated with cetrorelix multiple doses without luteal support, no pregnancy was obtained (Albano *et al.*, 1998). Others (Lin *et al*., 1999) showed that, when comparing antagonist and agonist treatments, the granulosa cells cultured *in vitro* from IVF patients were less impaired, in terms of progesterone secretion, in the antagonist group. This result was confirmed by others (Ragni *et al*., 2001), who showed that, in IUI cycles, the use of GnRH antagonist was safe and did not affect either luteal phase duration or progesterone secretion.

Until full scientific data and controlled studies are available, it seems preferable to maintain luteal support of GnRH antagonist-treated cycles.

Triggering ovulation with GnRH agonist in GnRH antagonist cycles

The use of GnRH agonist to induce an endogenous LH surge during an ovarian stimulation cycle with GnRH antagonist has been described (Olivennes *et al*., 1996). All patients showed an appropriate LH and progesterone rise after GnRH agonist administration, confirming that this approach can be used to induce LH secretion during the final stage of ovarian stimulation.

Others (Gonen *et al.*, 1990; Emperaire and Ruffie, 1991; Itskovitz *et al*., 1991; Lewit *et al*., 1996) have proposed this strategy previously to reduce the risk of OHSS, as endogenous LH has a lower plasma half-life than HCG. However, this approach is not suitable in patients previously down-regulated with GnRH agonist.

A recent study comparing HCG, leuprolide acetate (0.2 mg) and triptorelin (0.1 mg) to trigger ovulation in IVF patients treated with ganirelix, found similar IVF results between the three groups of patients (Fauser *et al*., 2002). A small group of high responders were treated with a combination of GnRH antagonist and agonists, and no OHSS was observed in this preliminary report (Itskovitz *et al.*, 2000).

Patients with an increased risk of OHSS (PCOS, previous OHSS, young age and low body weight) could be managed with this simple and effective measure during their ovarian stimulation with a combination of GnRH agonist and antagonist.

IUI

Although no reports have yet been made on the use and potential benefits of a GnRH antagonist protocol in IUI cycles, some of the advantages shown in IVF cycles might be applicable to IUI. In case of premature LH surge when criteria of optimal follicular maturation are not obtained, GnRH antagonists could be proposed to prevent and postpone ovulation. The luteal phase of stimulated cycles in IUI cycles has recently been studied (Ragni *et al*., 2001).

Regulation of the timing of IUI could also be achieved with GnRH antagonist. Clearly, this is not a medical indication, and IUI can be advanced if a LH surge is detected, though this is not always possible. It remains to be shown that to postpone the triggering of ovulation with GnRH antagonist when adequate follicular size and serum estradiol levels are reached does not adversely affect the results.

Perinatal outcome of pregnancy after GnRH antagonist for ovulation induction

Recently, two reports have been made on the perinatal outcome of IVF pregnancies obtained with GnRH antagonist. One of these followed 67 pregnant patients after ovarian induction with a ganirelix multiple-dose protocol (Olivennes *et al*., 2001). The miscarriage rate was 9%, and full data on perinatal outcome were obtained from 61 patients. The mean gestational age was 39.4 weeks for singleton pregnancies and 36.6 weeks for multiple pregnancies. A birth weight <2500 g was present in 8.7% of patients, while one baby had a major congenital malformation and seven minor malformations were reported in five infants. These results were found not to differ from data available on IVF pregnancies.

Another study addressed the same objective with the use of cetrorelix (multiple- and single-dose protocols). Pregnancies resulting from phase II and III trials were followed in order to investigate the safety of GnRH antagonist (Ludwig *et al*., 2001). In total, 227 newborn children were evaluated in terms of outcome of pregnancy, delivery, birth weight; subsequently, at 1 and 2 years of age the children were examined for any developmental disorders. The incidence of major congenital malformation was 3.1%, and minor malformations occurred in 2.6% of the cases. The clinical abortion rate was 16.8% and the ectopic pregnancy rate was 3.4%. The follow-up data on physical development did not show any significant abnormality.

The authors of both studies concluded that the use of GnRH antagonist in ovarian stimulation protocols did not cause any harmful or detrimental effect on the pregnancy course or perinatal outcome of those patients. However, these studies involved too few cases to discuss malformation rates.

Conclusions

In ovarian stimulation, several different studies have confirmed the efficacy of a single dose (3 mg) of cetrorelix in preventing

premature LH surges when administered during the late follicular phase. The single-dose protocol is easy to use, and also assures patient compliance. When compared with the long protocol using a depot formula of triptorelin, the IVF results showed a shorter duration of treatment, a lesser quantity of HMG used, and a lower occurrence of OHSS among patients treated with cetrorelix. Moreover, clinical trials have shown that the multiple-dose protocol using either cetrorelix or ganirelix is both effective and safe. A shorter duration of treatment, lower amount of gonadotrophins, and a lower occurrence of OHSS was observed in patients treated with cetrorelix or ganirelix. Single- and multiple-dose protocols have not yet been compared prospectively, however. The single-dose approach is simple, but requires monitoring of the cycle. In contrast, although the multiple-dose approach may reduce the need for hormone assessments, patient compliance is mandatory. When compared with the long GnRH agonist regimen, patients treated with both GnRH antagonist protocols reported a better quality of life, though this aspect was not evaluated scientifically.

Pregnancy rates were not statistically different from those after GnRH agonist treatment. Because of the trend towards lower pregnancy rates in most GnRH antagonist groups in controlled studies, further data are needed on this point. Nonetheless, there is room for optimization of the antagonist protocol, as administration can either be proposed on a fixed day of stimulation or be based on monitoring with a flexible approach. Although this could in time reduce the amount of antagonist required, the optimal timing of a flexible approach of GnRH antagonist administration is difficult as it is not easy to predict the LH surge. The optimal dose could be also further studied, especially with regard to the body weight of the patients. The regulation of treatment by manipulating the luteal phase with progestative compounds and/or estrogen must be evaluated. Likewise, the need for luteal phase supplementation must be properly evaluated.

The use of a GnRH antagonist in a mild stimulation regimen (clomiphene citrate/gonadotrophins or natural cycle with HMG support) allows a reduction to be made in the rate of premature LH surges, and therefore also in the cancellation rate. Stimulation can be minimal, and pregnancy rates in some preliminary reports have been satisfactory. Moreover, if more extensive studies confirm these results, then mild stimulation protocols associated with GnRH antagonist single-dose administration could represent an interesting first-choice IVF treatment regimen for selected indications. These protocols could also reduce the complications and risks of ovarian stimulation protocols. In addition, the possible reduction in cost that would be achieved by offering oocyte retrieval on an outpatient basis would also be of major interest. Successive cycles with an acceptable success rate could result in interesting cumulative pregnancy rates.

Tolerance of the antagonist injections (0.25 or 3 mg) was excellent in all patients treated, with only mild and transitory reactions occurring at the injection site.

Currently, new GnRH antagonists are available for clinical use in most countries, and this will undoubtedly lead to changes being made in the existing protocols of ovarian stimulation. If similar pregnancy rates were to be confirmed, then the main advantage of these compounds would be a reduction in the adverse side-effects and complications that occur with existing stimulation protocols, and these would offer clear benefits to the patients. In addition, such newer agents would also allow the design of 'softer' stimulation protocols.

More studies are needed to clarify certain important clinical questions regarding the utilization of GnRH antagonists in PCOS patients and in IUI cycles.

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