27 CME REVIEW ARTICLE

Volume 56, Number 9
OBSTETRICAL AND GYNECOLOGICAL SURVEY

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Use of Third Generation Gonadotropin-Releasing Hormone Antagonists in *In Vitro* Fertilization-Embryo Transfer: A Review

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Before gonadotropin-releasing hormone agonists (GnRHa) became available, approximately 20% of stimulated cycles within an *in vitro* fertilization (IVF) program were cancelled due to premature LH surges. By using the GnRHa to prevent LH surges via gonadotrope GnRH receptor down-regulation and desensitization, this percentage decreased to about 2%, and concomitantly, the IVF and pregnancy rates per cycle initiated were increased. Several treatment schedules currently are in use, including the so-called "long protocol," in which the GnRHa is begun in the luteal phase and down-regulation occurs before the start of the gonadotropin-stimulation treatment phase. This is generally the most effective regimen and is presently the most frequently used protocol. However, it has some disadvantages, such as hypoestrogenic side effects and an increase in the number of ampules of FSH or hMG required for adequate stimulation. There is a new generation of GnRH antagonists now clinically available, that has been able to minimize the potential side effects and provide reliable antagonism at the GnRH receptor. These agents seem better suited than GnRHa for assisted reproductive technology (ART) cycles inasmuch as they can prevent LH surges without requiring complete gonadotropin suppression. We have reviewed the current literature concerning their use in IVF cycles.

Target Audience: Obstetricians & Gynecologists, Family Physicians

Learning Objectives: After completion of this article, the reader will be able to summarize the data surrounding the use of GnRH antagonists in IVF cycles, to list the potential treatment protocols for using GnRH antagonists, and to outline the side effects of GnRH antagonists.

Gonadotropin-releasing hormone (GnRH) is a peptide composed of 10 amino acids that was first isolated and characterized in 1971 (1). It is produced in the hypothalamus by neurons located predominantly in the arcuate nucleus and liberated into capillaries of the portal system of the pituitary in a pulsatile manner. In response, pulsatile release of LH and FSH occurs from the pituitary gland. In humans, the frequency of pulses is between 70 and 220 minutes. Circulating GnRH is extremely short-lived, with a plasma half-life of 2 to 5 minutes. GnRH binds to specific, transmembrane receptors on the gonadotrophic cells in the pituitary (2). This leads to increased synthesis of LH and FSH as well as the calcium-dependent release of gonadotropins. These events are mediated by second messengers including inositol phosphate, leukotrienes and protein kinase C (2). The pulsatile release of GnRH is essential to the

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The authors have disclosed no significant financial or other relationship with any commercial entity.

response of the gonadotrophs as revealed by the classic studies of Knobil (3), which indicated that continuous delivery of GnRH to the pituitary for a prolonged time, led to the inhibition of LH and FSH release.

Soon after the identification of the structure of GnRH, agonist analogs (GnRHa) were synthesized. The modifications introduced involved mainly positions 6 and 10, as these modifications slowed degradation of the peptide by endo- and exopeptidases. GnRHa have a 100 to 200 times higher binding affinity for GnRH receptors than the native molecule (4). Because of their long half-life (1–6 hours), these agents provide continual rather than pulsatile signaling, and thus, daily administration results in a biphasic response over time. GnRHa initially induce the liberation of large amounts of LH and FSH from the pituitary, and an increase in the number of GnRH receptors (up-regulation, flare-up effect). However, within a short time (1–2 weeks), daily administration leads to internalization of the agonist/receptor complex, and a decrease in the number of receptors (down-regulation). In addition, there is an uncoupling of the second messenger cascade from the few available receptors. This process has been termed "desensitization," and it renders the pituitary refractory to the stimulatory effect of GnRH (1). These phenomena represent the basis for the clinical use of the agonists, which has been efficacious in disorders in which suppression of gonadotropins and ovarian steroids is desired. The pituitary blockade is completely reversible upon cessation of therapy, and normal menstrual cyclicity is re-established within 3 to 6 weeks.

It is important to emphasize that the suppressive effects of continuous treatment with the agonists are always preceded by an initial stimulatory phase, in which LH and FSH are secreted in supraphysiological amounts (4). Within a period of about 12 hours, this "flare-up" effect leads to a 5-fold increase of FSH, a 10-fold rise in LH, and a 4-fold elevation of estradiol (E_2) (4). Postmenopausal E_2 levels are commonly reached after 21 days of treatment (4). Despite this generally undesirable "flare-up" effect, agonists are a valuable option for the treatment in conditions such as endometriosis, uterine myomas, carcinoma of the prostate, precocious puberty, and infertility.

In comparison with the GnRHa, the pharmacological mechanism by which GnRH antagonists suppress gonadotropins is different. The antagonists bind competitively to the receptors, and thereby prevent endogenous GnRH from exerting its stimulatory effects on the pituitary cells (5). The structural modifications of GnRH antagonists allow binding to occur without activation of the intracellular secondary messenger events. Within hours, the secretion of gonadotropins is reduced. Because this mechanism of action is dependent on the equilibrium between endogenous GnRH and the applied antagonist, effects of antagonists are dose dependent.

The "first generation" of these compounds was characterized mainly by modifications in positions 2 and 3 that proved to be effective in *in vitro* assay systems, but very high doses were necessary to achieve a fall in gonadotropins and sex steroids *in vivo* (4). These modifications were followed by changes in positions 1 and 6. However, allergic side effects ranging from local erythema and induration, to anaphylactoid reactions were noted. The observed side effects were attributed to the induction of histamine release (5).

Because of these problems, GnRH antagonists were not available for clinical use until recently. Currently available GnRH antagonists such as ganirelix and cetrorelix have minimized these problems (5). Both compounds have modifications at positions 1, 2, 3, 6, and 10, although ganirelix, but not cetrorelix, also shows a modification at position 8 (5). Median terminal half-lives ranging from 5 to 60 hours have been reported for single-dose administration of cetrorelix (6, 7). For multiple administration, median terminal half-lives of 20 to 80 hours have been reported (7). The elimination half-life of ganirelix after single- and multiple-dose administration is 13 to 16 hours (8, 9). It has been suggested that the half-life of the antagonists increases in relation to doses administered (10). However, there are also studies that did not support this dose-dependent change (6, 9). Both compounds can be administered subcutaneously (S.C.), and seem to be equipotent regarding gonadotropin suppression, with full suppression seen within 4 to 8 hours after administration (5).

GnRH ANTAGONISTS AND IVF

Before GnRHa became available, approximately 20% of stimulated cycles within an IVF program were cancelled due to premature LH surges. By using the GnRHa to prevent LH surges via gonadotrope GnRH receptor down-regulation and desensitization, this percentage decreased to about 2%, and concomitantly the IVF fertilization and pregnancy rates (PRs) were increased (11). Several treatment schedules are currently in use, including the so-called "long protocol," which produces pronounced pitu-

itary suppression before exogenous gonadotropin administration, by administering the GnRHa in the luteal phase of the pretreatment cycle, and the "short" and "ultra-short" protocols, in which the flare-up effect is used along with the down-regulation that later ensues. Among these protocols, the long protocol is generally the most effective and is most often used at present (5). However, it has some disadvantages, 1) an extended treatment period with the agonist is necessary before gonadotropins are suppressed; 2) because of the pronounced suppression of FSH secretion by GnRH agonists, higher doses of gonadotropins are needed to achieve follicular maturation, and this increases the cost of the procedure (12); 3) the higher dose of gonadotropins used has been postulated to increase the risk of ovarian hyperstimulation syndrome (OHSS) (13); 4) because suppression of the gonadotropin secretion is still present 10 to 12 days after agonist administration has been stopped, corpus luteum function is impaired, and therefore, luteal phase supplementation is mandatory (14); 5) GnRHa cannot be used to trigger an LH surge when the follicles are considered mature, and hCG, which is used instead, may increase the risk of OHSS (15); and finally, 6) GnRHa administration can be associated with side effects related to hormonal depletion (such as hot flashes and vaginal dryness). One important advantage in the long GnRHa protocol is the fact that cycles can be completely controlled in terms of gonadotropin stimulation starting dates, once suppression is achieved.

In 1991, Ditkoff et al. (16) reported that a GnRH antagonist (Nal-Glu) when administered for a short period is capable of suppressing the ovulation-inducing mid-cycle LH peak. This study demonstrated that a GnRH antagonist could temporarily prevent an LH surge, and this now has become the goal of using the clinically available GnRH antagonists, cetrorelix and ganirelix, in ovulation induction protocols for IVF.

GnRH Antagonist Protocols

GnRH antagonist analogs should only need to be used during ovarian stimulation when E_2 levels are rising, and the goal is to suppress LH surges with minimal interference of the exogenous and endogenous LH and FSH stimulation of the ovary. Since the early studies, there have been numerous publications on the use of the two clinically available GnRH antagonists, ganirelix and cetrorelix. Timing of the GnRH antagonist needs to be such that an LH surge cannot be generated. This requires that the GnRH antagonist be given when the follicles are maturing, and E_2 levels are rising. There are several reports that have addressed single versus multiple daily doses of GnRH antagonists.

There are two cetrorelix treatment protocols currently available: multiple and single dose. Single or dual injections in IVF have been studied extensively by Olivennes et al. (Tables 1 and 3) (17–20). In their initial study (17), a dose of 5 mg of cetrorelix was administered when plasma E_2 levels were between 150 and 200 pg/ml per follicle of $\geq$14 mm. A second injection was performed 48 hours later if the follicles were not ready for maturation by hCG. The success of this dosing schedule suggested that increasing the interval and decreasing the dose might be beneficial. In their subsequent study (18), a 3-mg dose of cetrorelix was administered on day 8 of the stimulation cycle and a second injection was administered 72 hours later, if hCG was not given in that interval. This protocol was capable of preventing LH surge in all of the 11 patients studied (18). When two different doses (3 mg and 2 mg) have been compared (19), it has been demonstrated that a single injection of 2 or 3 mg of cetrorelix successfully prevents LH surges for at least 3 days in all patients treated. However, there was a rise in LH observed 4 days after the antagonist administration in the 2-mg group, which led them to suggest the 3-mg dose as a dose that would be certain to suppress LH in a single administration protocol (19). Recently, a single dose of 3 mg of cetrorelix was administered to 115 patients by the same authors on day 7 of hMG stimulation unless their E_2 level was below 400 pg/ml, in which case the injection was delayed (20). If hCG administration was not given within 4 days of administration of cetrorelix, a daily injection of 0.25 mg was given until hCG administration. Eight percent of the women were given one additional dose of 0.25 mg of cetrorelix, and 2% received two additional doses of 0.25 mg. None of the 115 patients experienced an LH surge in this regimen. In this prospectively randomized study, they compared the results of this protocol with the results of IVF-embryo transfer (ET) with a depot formula of triptorelin, and 3% of the patients in the triptorelin group experienced an LH surge (Table 1) (20).

Recently, Rongieres-Bertrand et al. (21) added a single injection of cetrorelix to prevent premature LH surge in natural cycles. When plasma E_2 concentrations reached 100 to 150 pg/ml, with a leading follicle between 12 and 14 mm in diameter, a single injection of 0.5 mg ($N = 19$ cycles) or 1 mg ($N = 25$ cycles) of cetrorelix was administered, and repeated 72 hours later if ovulation was not triggered in the

TABLE 1 **Number of patients, cycle characteristics, and fertilization rates in studies conducted with cetrorelix**

Studies (Ref.)	*N* of patients	Starting Day	Protocol (mg/day)	*N* of pLH surges¶	Mean (± SD) *N* of amps (75 IU ea)	*N* of stim days	E_2 on the Day of hCG	or % of Mature Oocytes	FR (%)
Single or dual administration									
Olivennes et al. (17) 1994	17	E_2 level	5§	0	27.7 ± 4.2	N/A	N/A	7.1 ± 3.5	85.9
Olivennes et al. (18) 1995	11	8 (cd)	3	0	24.5 ± 5.8	N/A	2081 ± 634	7.1 ± 3.5	87.3
Olivennes et al. (19) 1998	34	8 (cd)	3§	0	25.5 ± 7.7	N/A	N/A	5.4 ± 3.2	N/A
	32	8 (cd)	2	1	30.5 ± 13.1	N/A	N/A	6.5 ± 3.9	N/A
Olivennes et al. (20)* 2000	126	7 (td)	3§	0	24.3 ± 7.4	9.4±1.4	1786 ± 808	7.2 ± 4.9	51 (ICSI)
	43	Long	Long	1 (2.8%)	35.6 ± 15.1	10.7±1.7	2549 ± 1194	10.3 ± 7.4	55 (ICSI)
Daily administration									
Diedrich et al. (22) 1994	15	7 (cd)	3	0	27 (median)	N/A	1000	N/A	61.5
	5		1						
Felberbaum et al. (23) 1996	12	7 (cd)	3	0	30	N/A	N/A	N/A	45.3
	12	7(cd)	1	0	27	N/A	N/A	N/A	53.2
	11	7 (cd)	0.5	0	26	N/A	N/A	N/A	67.7
Ubaldi et al. (24) 1996	24	7 (cd)	0.5	0	36	N/A	N/A	9.2	70
Albano et al. (25) 1996	32	7 (cd)	0.5	0	33 (median)	11 (median)	2122 ± 935	N/A	56/72 (IVF/ICSI)
Albano et al. (26) 1997	32	6 (td)	0.5	0	35.1 ± 11.8	11.4 ± 1.8	2122 ± 935	N/A	55
	30	6 (td)	0.25	0	33.4 ± 8.1	10.3 ± 1.4	2491 ± 819	N/A	58
	7	6 (td)	0.1	1	N/A	N/A	N/A	N/A	N/A
Albano et al. (28)* 2000	188	6 (td)	0.25	3 (1.6%)	23.6 ± 8.5	10.6 ± 2.3	1625 ± 836	N/A	N/A
	85	Long	Long	0	25.6 ± 7.6	11.4 ± 1.8	2082 ± 1049	N/A	N/A
Felberbaum et al. (11) 2000	346	6 (td)	0.25	3 (0.9%)	25.2 (median 23)	10.4	1544	6.4 (69%)	59
Akman et al. (29)* 2000	20	Foll. dia.‡	0.25	0‖	63.3 ± 4.1	N/A	776 ± 86	3.25 (80%)	66
	20	N/A	N/A	2‖	66.9 ± 5.7	N/A	804 ± 143	3.46 (77%)	72

N, number; E_2, estradiol; cd, cycle day; td, treatment day; pLH, premature LH; amps, ampules; stim, stimulation; FR, fertilzation rate; NA, not applicable.

* Indicates prospectively randomized studies that compare antagonists with agonists or no treatment.

† When the plasma E_2 level was between 150 and 200 pg/mL per follicle of >13 mm.

‡ When the leading follicle reached 14 mm in diameter.

§ Indicates the studies in which a dual dose of ganirelix was used. Olivennes et al. have injected a second dose 48 hours later in their 1994 study, 72 hours later in their 1995 study, and given a daily injection of 0.25 mg 4 days later in their 2000 study, if triggering of ovulation was not observed in the meantime.

‖ Number of premature LH surges observed after or during antagonist treatment.

¶ All surges observed before or after starting antagonist treatment have been included.

TABLE 2 **Number of patients, cycle characteristics, and fertilization rates in studies conducted with ganirelix**

Studies (Ref.)	*N* of patients	Starting Day	Protocol (mg/day)	*N* of pLH Surges*	Mean Gonadotropin Dose (IU)	*N* of stim Days	Median E_2 on the Day of hCG	FR (%)	Mean *N* of Embryos Obtained
The Ganirelix Dose-Finding SG (30) 1998	31	7 (cd)	0.0625	5			1475	N/A	5.4
	65	7 (cd)	0.125	6	181–204	10	1130	N/A	5.9
	69	7 (cd)	0.25	1	(Mean daily dose of gonadotropins in the 6 dose groups)	(Median for all groups)	1160	N/A	5.4
	69	7 (cd)	0.5	0			823	N/A	4.6
	65	7 (cd)	1	0			703	N/A	5.3
	30	7 (cd)	2	0			430	N/A	4.9
de Jong et al. (31)	29	7 (cd)	0.0625	N/A	1810	9.6	1714	N/A	5.8
	65	7 (cd)	0.125	N/A	1840	9.9 (Mean follicular phase length)	1625	N/A	5.9
	66	7 (cd)	0.25	N/A	1831 (total doses of recFSH)	10.0	1468	N/A	5.6
	63	7 (cd)	0.5	N/A	2068	10.1	1099	N/A	5.1
	62	7 (cd)	1	N/A	2243	10.6	913	N/A	5.4
	26	7 (cd)	2	N/A	1997	9.3	480	N/A	4.9
The European Orgalutran SG (32)* 2000	463	6 (td)	0.25	13 (2.8%)	150 (median daily doses)	9 (Median)	1190	62	6.0
	238	Long	Long	3 (1.3%)	178	10 (Median)	1700	62	7.1
The North American Ganirelix SG (33)* 2001	208	6 (td)	0.25	2 (1%)‡	1800 (median total doses)	8 (Median)	2001	62	6.9
	105	Long	Long	0‡	2025	10 (Median)	2768	62	8.2
The European and Middle East Orgalutran SG (34)* 2000	236	6(td)	0.25	11 (4.9%)‡	150 (median daily doses)	9 (Median)	1090	64	4.0
	119	Long	Long	5 (4.5%)‡	178	11 (Median)	1370	65	4.7

S.G, study group; cd, cycle day; td, treatment day; pLH, premature LH; stim, stimulation; FR, fertilization rate.

* Number of premature LH surges observed after or during antagonist treatment.

† Indicates prospectively randomized studies that compare antagonists with agonists or no treatment.

‡ All surges observed before or after starting antagonist treatment have been included.

meantime. Human menopausal gonadotropin was added daily during the cetrorelix treatment. LH surges were not detected in any of the patients (21).

Daily administration of different doses of cetrorelix was another approach tried by several groups (Tables 1 and 3) (11, 22–29). Overall, no premature LH surge was observed at any of the doses >0.25 mg/day, and the reported incidences of premature LH surges with the 0.25 mg/day dose were <2% in these studies (Table 1) (11, 26, 28, 29). Albano et al. (27) compared two different dosages of cetrorelix. Twenty-four patients received 0.5 mg of cetrorelix per day, whereas 45 patients received 0.25 mg of cetrorelix per day. The clinical PRs (38% in the 0.5-mg/day group, and 27% in the 0.25-mg/day group) were not statistically different (Table 3) (27). Thus, the minimal effective dose of cetrorelix, successful in preventing premature LH surges in IVF protocols has been suggested to be 0.25 mg/day (11, 26–28). Recently, Felberbaum et al. (11) and Albano et al. (28) have reported two large clinical trials using this dose. In their prospectively randomized study (28), 188 patients received 0.25 mg of cetrorelix (S.C.) daily, starting from day 6 of the hMG treatment, and 85 patients received daily doses of 4 × 150 μg of buserelin administered intranasally. Ultimately, 96% of patients in the cetrorelix group, and 91% in the buserelin group reached the day of hCG injection. The median serum LH concentrations were higher (not statistically significant) in the cetrorelix as compared with the buserelin group, before the antagonist administration. However, serum LH concentrations became similar in the two groups after cetrorelix administration. In the cetrorelix group, three patients (1.6%) had a premature LH rise with a concomitant progesterone rise after having started cetrorelix administration (28). In their subsequent study (11), a total of 346 women were administered cetrorelix. Ninety-six percent reached the day of hCG administration, and 94% underwent oocyte retrieval. Only three cases of elevated LH with increased progesterone secretion were observed after initiation of cetrorelix administration, reflecting an incidence of 0.9% (11).

Daily administration of different doses (0.0625–2.0 mg/day) of ganirelix, starting on day 7 of the menstrual cycle and continuing up to, and including the day of the hCG injection, have been studied (Tables 2 and 3) (30, 31). During ganirelix treatment, no premature LH surge was observed at any of the doses of >0.25 mg/day, and only one LH surge was observed in the 0.25 mg/day-dose group. The minimal effective dose of ganirelix, successful in preventing premature LH surges, has been suggested to be 0.25 mg/day (30). However, when daily ganirelix administration (0.25 mg/day) has been compared with a long GnRH protocol, the incidence of LH rises has been observed to be slightly higher in the ganirelix group (Table 2) (32–34).

In some patients, the rise of serum LH concentration was observed before the first day of antagonist administration (17, 20, 25, 26, 28, 31, 34). However, serum LH concentration decreased significantly on the day of antagonist administration in all patients. This early increase in LH has been attributed to the higher serum E_2 levels observed in these patients compared with patients who did not have an increase in LH (17, 20, 25, 26). There is some evidence that high LH concentrations during the follicular phase of ovarian stimulation cycles have a negative impact on fertilization and implantation rates (35, 36). Albano et al. (26) have suggested that these interrupted LH rises affect the quality and/or maturity of the oocytes and stressed the importance of early administration of the GnRH antagonist. However, in larger studies (20, 28, 32, 34), there has not been a demonstrable deleterious effect of small, short-lived LH elevations on IVF outcome. A recent study (37) also has suggested that administering the antagonist during the LH surge made no statistically significant difference to the results of the IVF-ET attempt.

In summary, single and daily dose administrations of cetrorelix and daily doses of ganirelix were able to reliably prevent the onset of premature LH surges. Comparing the single versus daily regimen, when the reported median duration of cetrorelix treatment of 5 days is considered, and the total dose of cetrorelix is calculated (5 days × 0.25 mg/day = 1.25 mg), it is apparent that daily administration uses less per total dose than a single administration (3 mg) (11). Moreover, with daily administration of the antagonist, it is possible to eliminate daily measurements of the serum LH concentration without a significant risk of missing an endogenous LH surge. Although administration of the antagonist as a single dose seems easier for the patient, the overall cost may be increased.

GnRH Antagonist Cycle Characteristics

Because prestimulation suppression of endogenous gonadotropins does not occur, the issue of whether less ampules of exogenous gonadotropin can be used has been investigated. For regimens using a single administration of cetrorelix, Olivennes et al. (17)

TABLE 3 **IVF-ET outcome in studies conducted with cetrorelix or ganirelix**

Studies (Ref.)	*N* of Patients	Starting Day	Protocol (mg/day)	IR (%)	CPR (/t,%)	OPR (/t,%)	OHSS rate (%)	MPR (%)
Cetrorelix studies								
Olivennes et al. (17) 1994	17	E_2 level†	5 mg_	N/A	37.5	25	N/A	N/A
Olivennes et al. (18) 1995	11	8 (cd)	3 mg_	N/A	40	30	N/A	N/A
Olivennes et al. (19) 1998	34	8 (cd)	3 mg	18.7	35	29	N/A	N/A
	32	8 (cd)	2 mg	14.3	26	22.6	N/A	N/A
Olivennes et al. (20)* 2000	126	7 (td)	3 mg_	N/A	25	21	3.5 (St.II–III)	N/A
	43	Long	Long	N/A	38	27	11 (St.II–III)	N/A
Diedrich et al. (22) 1994	15	7 (cd)	3	N/A	15	N/A	15 (Mod.)	N/A
	5		1					
Ubaldi et al. (24) 1996	24	7 (cd)	0.5	17.3	33	33	N/A	N/A
Albano et al. (26) 1997	32	6 (td)	0.5	13.9	30	N/A	N/A	N/A
	30	6 (td)	0.25	14.5	31	N/A	N/A	N/A
Albano et al. (27) 1999	24	6 (td)	0.5	17.2	37.5	29 (del/t)	12 (Gr.III)	28 (/del)
	45	6 (td)	0.25	11.7	26.6	22 (del/t)	2 (Gr.III)	30 (/del)
Mettler et al. (42) 2000	26	7 (cd)	0.25	N/A	55.6	N/A	0	N/A
Albano et al. (28)* 2000	188	6 (td)	0.25	N/A	22.3 (/c)	18 (del/c)	1 (Gr.II–III)	23 (/del)
	85	Long	Long	N/A	25.9 (/c)	23 (del/c)	6.5 (Gr.II–III)	10 (/del)
Felberbaum et al. (11) 2000	346	6 (td)	0.25	11.4	23.6	N/A	0.6 (Gr.III)	N/A
Akman et al. (29)* 2000	20	Foll.dia.‡	0.25	13.3	20	20	N/A	N/A
	20	N/A	N/A	3.4	6.25	6.25	N/A	N/A
Ganirelix studies								
The Ganirelix Dose Finding SG (30) 1998	31	7 (cd)	0.0625	14.2	26	26		N/A
	65	7 (cd)	0.125	16.6	28	25		N/A
	69	7 (cd)	0.25	21.9	40	37	2 (Gr.II–III, in the whole group)	N/A
	69	7 (cd)	0.5	9	15	13		N/A
	65	7 (cd)	1	8.8	15	15		N/A
	30	7 (cd)	2	1.5	4	0		N/A
The European Orgalutran SG (32)* 2000	463	6 (td)	0.25	15.7	25	23.3	2.4	23.4 (/op)
	238	Long	Long	21.8	32	29	5.9	29.5 (/op)
The North American Ganirelix SG (33)* 2001	208	6 (td)	0.25	21.1	35.4	30.8	6.1	N/A
	105	Long	Long	26.1	38.4	36.4	2	N/A
The European and Middle East Orgalutran SG (34)* 2000	236	6 (td)	0.25	22.9	36	31 (/c)	1.8	N/A
	119	Long	Long	22.9	42	34 (/c)	0.9	N/A

SG, study group; E_2, estradiol; cd, cycle day; td, treatment day; IR, implantation rate; CPR, clinical pregnancy rate; t, transfer; c, cycle initiated; OPR, ongoing pregnancy rate; del, delivery; St, Stage; Gr, grade; MPR, multiple pregnancy rate; op, ongoing pregnancy; NA, not applicable.

* Indicates prospectively randomized studies that compare antagonists with agonists treatment.

† When the plasma E_2 level was between 150 and 200 pg/mL per follicle of >13 mm.

‡ When the leading follicle reached 14 mm in diameter.

have reported that the mean (±SD) number of hMG ampules (27.7 ± 4.2) was lower than the mean (±SD) number of vials administered in a similar population treated with controlled ovarian hyperstimulation (COH) protocols, including GnRHa (38.3 ± 15.4). In their other single-dose studies (18, 19), the mean numbers of hMG ampules were 24.5 and 25.5 (Table 1). When administration of a single 3-mg dose of cetrorelix (126 patients) was compared with the administration of a depot of triptorelin (43 patients) (20), the days of stimulation, number of ampules administered, and serum E_2 levels on the day of hCG administration were significantly lower in the cetrorelix group (Table 1). The difference in serum E_2 levels was due to the fewer follicles ≥15 mm in the cetrorelix group (20).

In protocols using daily administration of cetrorelix, the mean numbers of hMG ampules were between 26 and 36 in different studies (Table 1) (11, 22–26, 28). In the largest of these studies (11), 0.25 mg of cetrorelix per day was administered to a total of 346 women, and the median duration of cetrorelix treatment was 5 days (mean 5.7 days), and a mean of 25.2 ampules (median 23) of hMG were given over a mean of 10.4 days. Concentrations of E_2 increased to a mean of 1544 pg/ml on the day of hCG injection. The mean number of follicles with a diameter ≥20 mm was 2.4 (11). When results of cetrorelix (0.25 mg/day, 188 patients) treatment were compared with the results of buserelin treatment (600 μg/day intranasally, 85 patients) (28), the means for the number of hMG ampules, duration of hMG treatment, and serum E_2 level on the day of hCG injection were significantly lower in the cetrorelix group (Table 1). The difference in serum E_2 levels was attributed to the fewer follicles with diameters between 11 and 14 mm in the cetrorelix group, although the mean number of follicles ≥15 mm were similar in both groups (28).

Daily ganirelix administration (0.25 mg/day) also has been compared with long protocols of buserelin and leuprolide acetate (32–34). Median total gonadotropin dose was lower in the ganirelix group in these studies (Table 2). This was mainly due to the shorter duration of gonadotropin treatment, because the median duration of stimulation was 1 to 2 days shorter in the ganirelix group in these studies (Table 2) (32–34). On the day of hCG administration, the median serum E_2 concentrations were lower in the ganirelix group (Table 2) (32–34). This difference has been attributed to fewer follicles on the day of hCG administration in the ganirelix group (32–34). However, when different doses of ganirelix were compared, mean numbers of follicles ≥11 mm, ≥15 mm, and ≥17 mm on the day of hCG administration were comparable, although serum E_2 levels progressively decreased with increasing ganirelix doses (30, 31). These low E_2 levels in the high-dose antagonist groups despite comparable number of follicles on day of hCG have been attributed to the reduced availability of androstenedione, and subsequent decrease in E_2 production (31).

In conclusion, in the five prospectively randomized studies (20, 28, 32–34), in which third generation antagonists were compared with a long protocol of GnRHa, it has been shown that antagonists decrease the total number of gonadotropin ampules, shorten the stimulation period, and decrease the E_2 levels on the day of hCG administration.

Luteal Phase Dynamics

Luteal phase deficiency frequently is observed in patients undergoing COH with a GnRHa. The prolonged pituitary suppression has been suggested to be an etiologic factor for early luteolysis (38). For this reason, luteal phase supplementation has been provided routinely (14). In contrast, because of the rapid recovery of the pituitary gonadotrophs after discontinuation of the antagonist and the early evidence showing no disturbance of the luteal phase with the use of the antagonist, Nal-Glu, it has been speculated that luteal phase supplementation is unnecessary in cycles associated with GnRH antagonists (16). However, in the studies reviewed herein, most of the authors supported the luteal phase in IVF cycles associated with cetrorelix or ganirelix (11, 17–20, 22–24, 27–30, 32–34, 39). Albano et al. (25, 26) did not support the luteal phase in their first six patients. However, because all these patients showed bleeding in the midluteal phase, luteal support was added to the protocol for all subsequent subjects. No pregnancy occurred in these six initial patients. Subsequently, they analyzed the luteal phase of these six patients, who underwent COH with hMG and cetrorelix without luteal phase supplementation (14). The length of the luteal phase was ≤12 days in three of these six patients, and one patient, whose luteal phase was >12 days had a low serum progesterone concentration (2.9 ng/ml) on day 10. It has been documented that serum LH concentrations decreased after the preovulatory hCG injection in all patients, however, a progressive increase in LH was observed after day 7, reaching normal values (14). It has been suggested that hCG administration exerts a direct effect on the pituitary, and is responsible for this

decrease in LH in the early luteal phase (14). Albano et al. (14) concluded that corpus luteum function remains impaired in cycles that are stimulated with cetrorelix, hMG, and hCG. Tavaniotou et al. (40) investigated the effects of GnRH antagonists on luteal phase by comparing patients stimulated with hMG and cetrorelix with patients stimulated with hMG only, for IVF. Luteal phase has been supported in both of these groups with hCG. Luteal phase serum LH concentrations were low but similar between groups. They concluded that suppressed LH concentrations may not be attributed solely to the GnRH antagonist administration, but hCG may be partially responsible as well (40).

De Jong et al. (41) also have attempted to eliminate exogenous luteal phase support. They used two stimulation regimens consisting of either 100 IU/day or 150 IU/day of recombinant (rec) FSH, starting on cycle day 5, combined with 0.25 mg of cetrorelix per day, from cycle day 8 onward, with 10,000 IU of hCG administered for oocyte maturation. When compared with a control group of regularly cycling women, significantly lower luteal phase LH and FSH concentrations were observed in the group of treated patients who did not conceive. However as expected, luteal phase serum E_2 and progesterone levels in these patients were elevated compared with the control group (847 vs. 449 pmol/liter and 180 vs. 44 nmol/liter, respectively; $P < .05$). The median duration of the luteal phase among the patients who did not conceive was 12 days (range; 10–20 days), which did not differ from that of the controls (12 days [range; 5–16 days]). Three pregnancies of 12 retrievals (clinical PR per transfer; 33%) were obtained, and these authors hypothesized that GnRH antagonists may not uniformly compromise subsequent corpus luteum function (41). Theoretically, it may be that substitution of hCG by recLH or a GnRH agonist will not disrupt the H-P-O axis in the same manner as a large dose of hCG does, and this may obviate the need for luteal phase supplementation in GnRH antagonist cycles.

IVF-ET Results

Clinical PRs (per transfer) of up to 55% have been reported in IVF studies, which were conducted with gonadotropins and GnRH antagonists (Table 3) (11, 17–20, 22, 24, 26–30, 32–34, 42). In patients treated with cetrorelix before IVF or ICSI, the number of mature oocytes, fertilization, and PRs seem to be comparable with those achieved after a long protocol of GnRHa (Tables 1 and 3) (20, 28). However, Olivennes et al. (20) and Albano et al. (28) have shown that the total number of retrieved oocytes was higher after a long GnRHa protocol than administration of cetrorelix (9.2 vs. 12.6 and 8.0 vs. 10.6 in studies by Olivennes et al. (20) and Albano et al. (28), respectively). In these same studies, the number of embryos also was higher in the GnRHa group (4.5 vs. 6.0 and 5.4 vs. 7.5) (20, 28). However, embryo quality may be independent of quantity, and implantation rate also needs to be considered. In fact, improved pregnancy outcome with GnRHa regimens compared with no GnRHa regimens is due to the improvement in oocyte quantity (43). Recently, Akman et al. (29) have compared the results of IVF-ET for poor responders stimulated with gonadotropin alone, and gonadotropin plus cetrorelix. Adding cetrorelix did not improve the mean oocyte number nor the proportion of mature oocytes retrieved (Table 1). However, improved pregnancy outcome with cetrorelix administration was hypothesized to be because of a small increase in the implantation rate (Table 3). In conclusion, cetrorelix has been shown to result in fewer mature oocytes and embryos than GnRHa, but this decrease did not affect pregnancies per transfer.

Although it is not common to perform IVF in unstimulated or natural cycles, the addition of cetrorelix to such cycles has been investigated. When a single injection of cetrorelix (0.5 or 1 mg) was used to prevent premature LH surges in natural cycles ($N = 44$), satisfactory clinical PRs were obtained (16% [per started cycle] and 32% [per transfer]) (21).

Daily ganirelix administration (0.25 mg/day) also has been compared with a long GnRHa protocol (32–34). In comparison with GnRHa treatment, ganirelix treatment resulted in one preovulatory follicle less and, consequently, one to three cumulus-oocyte complexes (COC) less were recovered at oocyte retrieval (32–34). Fertilization rates and the number of good quality embryos were comparable between groups (Table 2) (32–34). Implantation and PRs tended to be lower in the ganirelix group (Table 3) (32–34). Ongoing PRs of the freeze-thaw cycles were relatively higher in the ganirelix group in the European study (20.0 vs. 16.7% [per started cycle]) (32). In the dose-finding study (30), in which results of administration of different doses (0.0625–2.0 mg/day) of ganirelix were compared, the implantation rate was relatively low in the three highest dose groups, especially in the 2.0-mg group (Table 3). In addition, the number of pregnancy losses during the first 6 weeks after ET was relatively higher in the 1.0- and 2.0-mg treatment groups (0% to 3.7% in the four lowest dose groups vs. 8.5% and 13% in the 1.0-

and 2.0-mg groups, respectively) (30). The study on the outcome of freeze-thaw cycles using embryos cryopreserved in stimulation cycles during the above-mentioned dose-finding study, has suggested that there is no direct negative effect of ganirelix on the quality of oocytes and embryos, and high doses of ganirelix do not adversely affect the potential of embryos to establish clinical pregnancy in freeze-thaw cycles (44). However, a direct effect on the endometrium by relatively high doses cannot be excluded, inasmuch as human endometrial GnRH receptors have been identified recently (45, 46). These findings brought to question the role of GnRH antagonists at the cellular level in endometrium and extrapituitary tissues (47).

Nikolettos et al. (48) have compared the cryopreservation outcome of human oocytes obtained by COH with hMG and cetrorelix with the outcome of oocytes obtained by COH with hMG and triptorelin. The study population consisted of 31 infertile couples in each group. The implantation rate and pregnancy per transfer rate in the cetrorelix group compared with the rates in the GnRHa group were 3.26% versus 3.73% ($P > .05$) and 8.33% versus 10.25% ($P > .05$), respectively (48).

Patient Subpopulations

Craft et al. (49) have compared the outcome of IVF cycles with cetrorelix ($N = 24$) to that of previous cycles with GnRHa ($N = 23$) in poor responders. Although the differences were not significant, cancellation and total FSH dose per retrieved oocyte rates were lower in the cetrorelix group (29% vs. 57% and 709 IU vs. 1163 IU, respectively), although clinical pregnancy per completed cycle rate was higher (24% vs. 10%). However, the type of the analog was not the only difference in their protocols. They used FSH and clomiphene citrate (CC) with cetrorelix, whereas the previous cycles of the patients have been conducted with gonadotropins and GnRHa. They have suggested that using CC-plus-gonadotropin-plus-cetrorelix may be of value for poor responders who have not responded well to gonadotropins and GnRH agonists (49). Akman et al. (39) compared the outcome of the flare-up protocol with that of daily administration of cetrorelix in IVF for poor responders. Although the differences were not significant, the cancellation rate was higher in the cetrorelix group (25% vs. 21%), whereas clinical pregnancy per transfer rate was lower (26% vs. 22%) (39).

Craft et al. (49) have evaluated the same protocol in patients with polycystic ovary syndrome, and compared the results of these cycles ($N = 7$) with the patients' previous cycles with GnRHa ($N = 13$). Although the results were not statistically significant, the total FSH dose per retrieved oocyte and clinical pregnancy per completed cycle rates were 170 versus 189 IU and 33% versus 17% in the cetrorelix and GnRHa groups, respectively. Although the number of patients included in these preliminary studies is small, their results suggest that GnRH antagonists may be more advantageous in selected IVF patients.

Potential Benefits of GnRH Antagonists

One of the major risks of COH for IVF is that of OHSS. In prospective, randomized studies, it has been shown that both cetrorelix (20, 28, 50) and ganirelix (32, 33) cause less OHSS than the long GnRHa protocol (Table 3). The tendency toward lower E_2 concentrations and fewer follicles on the day of hCG in the antagonist group may be of importance (20, 28, 32).

It has also been speculated that GnRH antagonist use could further decrease OHSS risk if hCG were not used to provide the final maturation stimulus. Itskovitz-Eldor et al. (51) have suggested that a GnRH antagonist protocol, with a single injection of GnRHa to mimic the midcycle LH surge, prevents OHSS in high responders. Human chorionic gonadotropin was withheld in eight patients, who underwent COH with rec-FSH and concomitant ganirelix treatment, and who were considered to have an increased risk for developing OHSS. In these patients, ovulation was triggered with a single injection of 0.2 mg of triptorelin. After GnRHa injection, endogenous serum LH and FSH surges were observed with median peak values of 219 and 19 IU/liter, respectively. The mean ($\pm$SD) number of oocytes obtained was 23.4 ($\pm$15.4), of which 83% were mature. None of the patients developed any signs or symptoms of OHSS. Four clinical pregnancies (17% per transfer) were achieved from fresh and frozen ETs (51). Their preliminary results suggest that this regimen may prove useful, however their overall PR was disappointing. Because OHSS is a relatively infrequent complication, this regimen needs to be evaluated further in larger numbers of patients. Prevention of premature LH surge with GnRH antagonists could also allow us to use other options in IVF regimens, such as to inducing ovulation with parental administration of native GnRH. It remains to be investigated whether the elimination of the prolonged down-reg-

ulation of GnRH receptors will alter the OHSS propensity for patients undergoing COH for IVF.

Side Effect Profile of GnRH Antagonists

Because the histamine releasing potential and subsequent severe local and systemic allergic reactions had been a problem with the use of earlier GnRH antagonists, transdermal skin tests were performed in the preclinical studies of cetrorelix and ganirelix (52–54). However, no major systemic adverse reactions were reported in these trials (54), and therefore, in clinical use, this is not required (11, 20, 28, 32). In a recent clinical study (11) whereby a total of 346 women undergoing IVF have been treated with cetrorelix, none had to be cancelled because of allergic, anaphylactoid, local hypersensitivity, or other adverse reactions. Injection site reactions were seen in only 0.9% of women, although one case of hot flashes was reported (11). The tolerability of cetrorelix was also excellent in another large study (20); only 25% of women had a transitory reaction at the injection site. In three clinical trials (30, 32, 33), in which a total of 792 women undergoing IVF have been treated with ganirelix, none had to be cancelled because of allergic, anaphylactoid, local hypersensitivity, or other adverse reactions of the patient. Possibly drug-related adverse effects were reported for 2% to 3% of patients and included headache, asthenia, nausea, and malaise (30, 32). The percentage of patients with at least one moderate or severe local reaction (redness, swelling, bruising, pain, or itching) occurring 1 hour after ganirelix injection was 12% to 20% and $<2\%$, respectively (30, 33, 34). In recent controlled studies (32–34), lower rates of drug-related adverse reactions and injection site reactions have been reported in the ganirelix group than in the GnRHa group. In 45 healthy premenopausal volunteers, who received either 0.125, 0.25, or 0.5 mg of ganirelix per day for 7 days, the most common adverse events were headache (71%), injection site events (44%), and fatigue (24%) (9).

Recently, Ludwig et al. (55) have reported pregnancy, birth, and follow-up data (up to 2 years of age) from the world's largest cohort of children born after IVF using cetrorelix. They have analyzed 208 pregnancies after fresh ETs resulting in 163 deliveries of 209 live-born children and 23 pregnancies after frozen ETs resulting in 16 deliveries of 18 live-born children (55). In terms of pregnancy outcome, multiple pregnancies of 26% and 13% from fresh and cryopreservation cycles, respectively, were reported (55). Ectopic and heterotopic pregnancies were $<4.5\%$ in fresh cycles and $<1\%$ in cryopreservation cycles. They also have reported delivery rates per clinical pregnancy of 78% and 70% in fresh and frozen ET cycles, respectively. All these rates are in the expected range of rates observed after ART. Ludwig et al. (55) have also shown that children born from IVF cycles using cetrorelix had no increased risk of malformations. The rate of major malformations (3%) was in the range of that in the general population (55), and that among children born after ICSI (2% to 5%) (55). Normal physical and mental development of the children in that study was observed up to 2 years of age (55). They have concluded that cetrorelix has no detrimental effect on the pregnancy course of women or on the birth characteristics and developmental competence of children (55).

Once pregnancy is established, the use of GnRH antagonists could interfere with the H-P-O axis and disrupt luteal activity. When administered to pregnant rats and rabbits from day 6 to near term, both cetrorelix and ganirelix increased the incidence of very early litter resorptions and total implantation losses at doses from 0.4 times the human dose based on body surface area (56). In animals that maintained their pregnancy, there was no increase in the incidence of fetal abnormalities (56). The fetal resorption observed in animals is a logical consequence of the alteration in hormonal levels brought about by the antigonadotrophic properties of these drugs, which could result in fetal loss in humans as well. Therefore, these drugs should not be used in pregnancy, and they were classified as category X (56). In summary, both antagonists seem to be safe in doses that prevent premature LH surges in COH for IVF.

CONCLUSIONS

GnRH antagonists are now available clinically for use in ART cycles. Their advantages in this setting have been clearly elucidated by many authors. The studies reviewed herein indicate that they are an acceptable alternative to the use of GnRHa in IVF cycles. These studies have documented the doses that are both efficacious in preventing LH surges and nondetrimental to the IVF success rates. Additional use in clinical settings versus research protocols will help to refine their use in specific patient subpopulations. In addition, we anticipate that there will be refinements in the strategies for monitoring the stimulation phase of these cycles so that embryo quality is optimized and implantation rates are maximized.

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