Comparison Between Depot Leuprorelin and Daily Buserelin in IVF*

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Objectives: *To compare the effects of depot and daily forms of GnRH analogs in IVF programs.*
Methods: *One hundred seventeen patients undergoing IVF, with no severe male factor, were randomized between two treatment groups. Pituitary desensitization was obtained in group 1 (60 patients) with a single IM injection of leuprorelin (3.75 mg), and in group 2 (57 patients) with buserelin (0.3 mg SC twice daily). In a subgroup of 10 patients (5 for the depot form and 5 for the daily form) several GnRH tests were performed to investigate pituitary desensitization.*
Results: *No differences were found in the time to reach desensitization. Resumption of pituitary activity occurred in 7 days with the daily form and in about 2 months with the depot form. No significant differences were found in the stimulation pattern, oocyte quality, percentage of fertilization. The pregnancy rate per transfer was slightly, but not significantly, better in the depot group (29.4% vs 25.9%). Implantation rate (11.9% vs 12.3%) and the percentage of miscarriages (26.6% vs 28.5%) were similar.*
Conclusion: *Depot and daily forms of GnRH analogs are equally effective in superovulation induction for IVF. Considering improved patient compliance and preference, depot forms are advantageous.*

KEY WORDS: GnRH analogs; IVF; superovulation induction; depot GnRH analogs; daily GnRH analogs.

INTRODUCTION

Nowadays, pituitary suppression with GnRH analogs (GnRH-a) before induction of multiple follicular growth is widespread in almost all IVF centers due to their well-known benefits. Further advantages in terms of compliance and management for both clinicians and patients arise from the recent use of long-acting GnRH-a administered in a single IM injection prior to the stimulation cycle. The concern raised by the persistence of unnecessary and potentially unfavorable effects during the luteal phase and, possibly, the early pregnancy (1–3), led our group to investigate the safety of these compounds. In a previous trial we showed that comparable results in IVF outcome can be obtained with both long- and short-acting triptorelin (4).

In the present study, two groups of patients undergoing assisted reproduction were treated with two different GnRH analogs: a long-acting GnRH-a (leuprorelin) and a standard direct release GnRH-a (buserelin), respectively. The aim was to compare the effect of these two GnRH-a forms on the length of pituitary down regulation, on the quality of ovarian stimulation, on the quality and performance of oocytes *in vitro*, and on the pregnancy rate and pregnancy outcome.

MATERIALS AND METHODS

One hundred and seventeen patients undergoing IVF, with no severe male factor, were randomized between two treatment groups. In group 1 (60 patients, 65 cycles), pituitary desensitization was obtained with a single IM injection of leuprorelin (Enantone Depot, Takeda Italia Farmaceutici S.p.a., Rome, Italy) in the midluteal phase of the cycle preceding treatment. In group 2 (57 patients, 64 cycles), the protocol involved the daily SC administration of buserelin (Suprefact, Hoechst, Mi-

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lan, Italy); 0.3 mg of buserelin were administered twice a day, starting in midluteal phase of the cycle preceding treatment, until the day of hCG administration.

Stimulation with human FSH (hFSH) started 10 to 20 days after GnRH administration, after the absence of ovarian activity had been confirmed by ultrasound (US) scanning and E_2 levels <30 pg/ml (110 pmol/l). These ampules (225 IU) of hFSH (Metrodin, Serono, Milan, Italy) were administered once a day for 5 days; then the dose was adjusted according to the individual response as estimated by daily E_2 assays and US scanning.

Human chorionic gonadotropin (Profasi, Serono, Milan, Italy) at a dose of 10,000 IU was administered when at least two follicles of 18 mm or more in diameter were observed, with E_2 levels corresponding to the number of follicles.

The oocyte retrieval was performed 34 to 35 h after hCG injection by US-guided transvaginal puncture. Oocytes were incubated in CO_2 atmosphere at 37°C and inseminated 4–6 h after the pickup, depending on maturation stage. About 150,000 sperm per oocyte were added to the coculture medium (human tubal fluid [HTF] Irvine Scientific, Irvine, CA) and incubated for 20 h.

No more than three embryos were replaced in patients who were <35 years, while a maximum of four embryos were transferred in patients who were >35 years.

The luteal phase was sustained with natural progesteron in oil (Gestone, AMSA, Firenze, Italy), 50 mg IM daily at least until the first pregnancy test.

Study of Onset and Duration of Desensitization

A subgroup of 10 patients (A, 5 patients from group 1; and B, 5 patients from group 2) volunteered for this study during the cycle preceding that of IVF. A GnRH test was performed between the third and fifth days of the cycle: two baseline blood samples were taken at 8:00 A.M.; then 100 μg of GnRH was administered IV. Blood samples were drawn after +20, + 40, + 60, + 80, + 100, + 150, + 200, + 250 min for gonadotropin assay. In subgroup A the test was repeated 7 days after a single IM injection of leuprorelin and then every 7 days until pituitary activity resumption was confirmed. In subgroup B the test was carried out after 7 and 14 days of buserlin daily SC administration to assess pituitary desensitization. Then GnRH-a administration was discontinued, and the test was repeated after 7 and 14 days to confirm pituitary activity resumption. In all these 10 patients a daily hormonal profile of FSH, LH, and E_2 was performed.

Hormone Assay

Plasma LH and FSH samples were analyzed by an immunoenzymatic system (IEMA, Sorin Biomedica, Saluggia, Italy), characterized by a sandwich-like structure of monoclonal antibodies. Plasma P was measured by RIA using a double-antibody kit (DIRIA PROG K, Sorin Biomedica, Saluggia, Italy). Plasma 17β-E_2 was determined using a RIA double antibody coupled to magnetic particles kit (ESTRADIOL Maia, Biodata, Rome, Italy). Plasma βhCG was determined by an immunoenzymatic system (MEIA) (IMX total βhCG; Abbot, Weisbaden, West Germany). Units used were: IU/l for LH, FSH, and βhCG, ng/ml for P (conversion factor to SI unit, 3.180), and pg/ml for 17β-E_2 (conversion factor to SI unit, 3.671).

Statistical Analysis

The results are presented as mean and SE of the mean. Statistical analyses were carried out using Student's *t* test or the chi-square test as appropriate. Analysis of variance (ANOVA) for repeated measures was used to analyze the data of each patient under several conditions in the GnRH tests. Significant differences among each issue were identified by Student's *t* test.

RESULTS

The two groups were comparable as far as age (34.1 ± 4.2 vs 34.2 ± 3.8 years) and number of years of infertility were concerned (Table I). No significant differences were seen in the seminal parameters before insemination.

The time necessary to reach pituitary desensitization was not different in the two groups (12.0 ± 2.4 vs 11.4 ± 2.8 days) (Table I).

Complete resumption of pituitary activity, as detected by GnRH tests, took place 8 weeks after leuprorelin administration in all patients of subgroup A ($P < 0.05$) and 1 week after discontinuing buserelin administration in all patients of subgroup B ($P < 0.05$) (Fig. 1).

The incidence of cysts formation after GnRH-a administration was low in both protocols (4.6% in

Table I. Clinical Features and Pattern of the Stimulation of the Two Investigated Groups (M ± SE)

	Leuprorelin	Buserelin
No. patients	60	57
Age	34.1 ± 0.5	34.2 ± 0.5
Infertility years	6.7 ± 0.5	6.6 ± 0.5
Semen no. 10^6/ml	83.5 ± 10.4	61.2 ± 3.5
Semen motility %	40.6 ± 1.7	40.3 ± 1.7
Desensitization (no. of days)	12.0 ± 0.3	11.4 ± 0.4
Stimulation days	11.6 ± 0.2	10.9 ± 0.2
No. FSH ampoules	35.6 ± 1.5	33.5 ± 1.0
E2 on hCG day		
(pg/ml)	1057 ± 81	944 ± 70.9
(pmol/l)	3880 ± 297.4	3465 ± 260.3
No. follicles		
total	14.6 ± 1.0	12.3 ± 0.8
>17 mm	4.1 ± 0.4	3.7 ± 0.3
No. oocytes	9.6 ± 0.7	9.2 ± 0.8

group 1 vs 3.1% in group 2). Their diameter ranged between 2 and 3.5 cm and did not increase during gonadotropin treatment. These cysts did not hinder follicular maturation and were usually aspirated during oocyte retrieval.

The response to gonadotropin treatment was comparable in the two groups (Table I). A similar number of FSH ampoules were administered, with no significant difference in the number of days needed to reach follicular maturation (Table I).

The E_2 levels obtained on hCG administration day were also comparable in the two groups (Table I). No significant differences in the number of developed follicles and oocyte collected were found. Oocyte quality, percentage of fertilization, and cleavage were similar in the two protocols.

The pregnancy rate per transfer was slightly, but not significantly, better in the depot leuprorelin group (29.4% vs 25.9%). The implantation rate (11.9% vs 12.3%) and the percentage of miscarriages (26.6% vs 28.5%) were similar in the two protocols (Table II).

DISCUSSION

A substantial improvement of IVF organization and outcome has been achieved with the use of GnRH analogs. A number of GnRH analogs, with different structure, different potency, different route, and protocol of administration have been used in the past few years. Despite data from various investigators are not uniform, no major advantages, in terms of IVF outcome, may be ascribed to any specific analog or protocol.

A further step to increase the advantages of GnRH analogs in assisted reproduction would be to improve patients' compliance and clinicians' convenience by making the treatment easier.

The recent development of sustained release formulation of GnRH analogs seems to meet these requirements. However, the long-lasting action of these compounds could be potentially harmful to the luteal phase, the implantation, and the development of the embryo (1–3).

This study documents that both the long acting leuprorelin and the standard form buserelin give rise to similar results in patients undergoing assisted reproduction. Indeed, the number of stimulation days, the amount of gonadotropins administered, the number of follicles detected and oocytes collected, and the E_2 levels as well as pregnancy and abortion rates are comparable in the two protocols.

The incidence of ovarian cysts is not considerable in our experience and is not significantly different in the two groups.

In about 2 weeks all patients in both protocols reached pituitary desensitization. However, the duration of desensitization is considerably different. The pituitary is still insensitive to GnRH stimulation for several weeks after depot leuprorelin administration (Fig. 1), confirming previous results of our team in different types of long-acting GnRH analogs (4,5). The pituitary response after 7 days of standard release buserelin administration is similar to the one obtained with the long-acting analog after the same time. On the other hand, a resumption of pituitary response can be observed seven days after the discontinuation of administration (Fig. 1). Despite several studies documenting the long-lasting pituitary desensitization induced by depot analogs, a recent case report describing the occurrence of a pregnancy during such a type of treatment (6) has surprisingly pointed out a possible escape from pharmacological desensitization.

Nevertheless, our data show that the effects of the long-acting leuprorelin are likely to be present in the luteal phase and in the early phases of pregnancy, at least in the majority of patients. The luteal phase is known to be impaired after combined GnRH-gonadotropins stimulation leading to premature luteolysis (7,8). We previously demonstrated that a similar degree of pituitary desensitization is present during the luteal phase of both groups of patients treated with the short- and long-acting an-

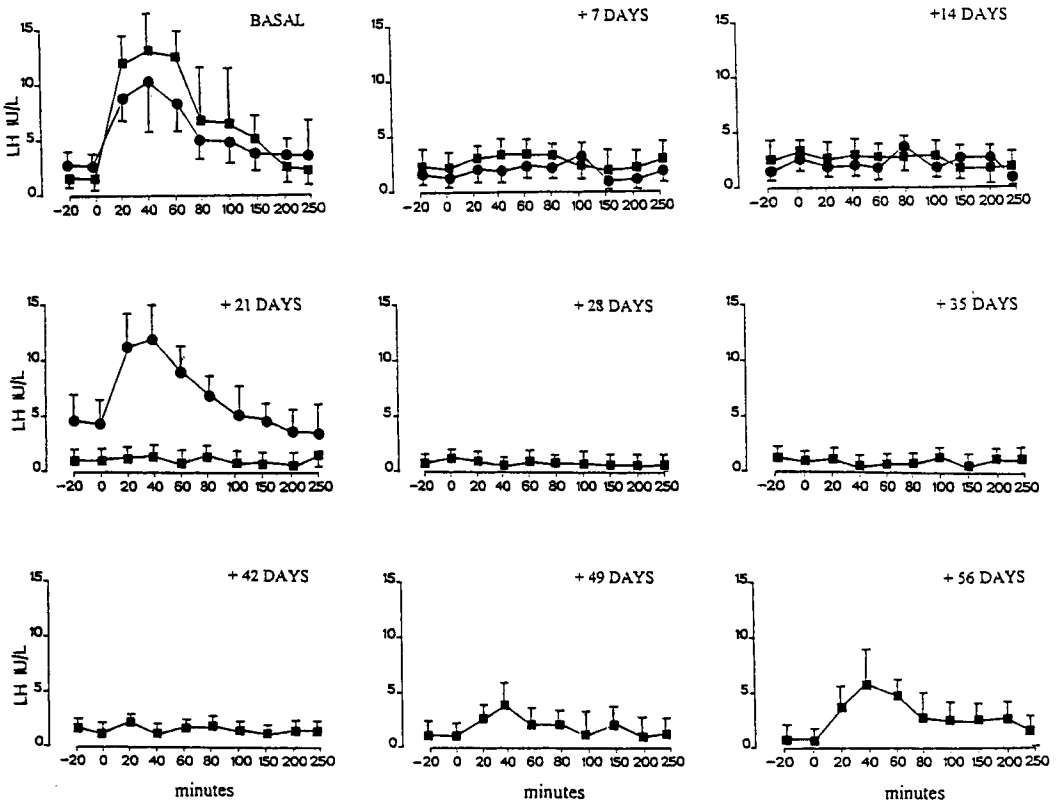

Fig. 1. Study of onset and duration of desensitization. ■ GnRH test before and after depot leuprorelin i.m. administration in group A (5 patients). ● GnRH test before (basal), during (+7 and +14) and 7 days after discontinuation (+21) of daily buserelin SC administration in group B (5 patients).

alogs (4). Provided LH is really necessary in maintaining the corpus luteum, the luteal support is equally impaired by both types of analogs.

Direct effects of GnRH analogs on ovarian steroidogenesis have been hypothesized, and a possible inhibitory action has been proposed (9–11). The existence of specific receptors for GnRH in the ovary has been demonstrated (12). However, the real direct action of GnRH on ovarian steroidogenesis is still unclear, and different investigations gave rise to conflicting results (1,9,10,12,13).

Table II. Clinical Results: Comparison Between the Two Groups of Treatment After IVF

	Leuprorelin		Buserelin	
No. cycles	65		64	
Canceled cycles	9	(13.8%)	6	(9.4%)
No. cysts	3	(4.6%)	2	(3.1%)
No. retrievals	56		58	
No. transfers	51		54	
Pregnancies/cycle	23.1%	(15/65)	21.8%	(14/64)
Pregnancies/retrieval	26.8%	(15/56)	24.1%	(14/58)
Pregnancies/transfer	29.4%	(15/51)	25.9%	(14/54)
Implantation rate	11.9%		12.3%	
Miscarriages	26.6%	(4/15)	28.5%	(4/14)

A direct inhibition of luteal steroidogenesis should be ruled out in standard-form analogs since they virtually disappear from the general circulation shortly after the suspension of treatment at the time of hCG injection. On the other hand, the long-acting analog does not seem to interfere with the percentage of pregnancies and miscarriages in the present study (Table II). We previously published the luteal profiles of patients treated with long- and short-acting analogs, and no clear-cut difference arose in the luteal steroids levels of both protocols (4).

The ability of GnRH analogs to cross the placenta and cause a reduction of testicular weight in male Rhesus monkeys (2) raised some concern about possible theratogenetic effects of these compounds in human offspring.

However, some cases of inadvertent administration of long-acting analogs during pregnancies which ended in normal live births (6,14) are reassuring. In addition the IVF outcome and perinatal

results obtained in different infertility centers (4,6,14–16) are encouraging.

The present investigation gives further support to the safety of these compounds even though more research is needed to increase the statistical consistency of these data.

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