Prospective Randomized Comparison of Ovarian Blockade With Nafarelin Versus Leuprolide During Ovarian Stimulation With Recombinant FSH in an ICSI Program

JOSÉ G. FRANCO JR,[1,2,3] RICARDO L. R. BARUFFI,[1] ANA L. MAURI,[1] CLAUDIA G. PETERSEN,[1] JOSÉ E. CHUFALLO,[2] VALÉRIA FELIPE,[1] and ERIKA GARBELLINI[1]

Submitted: January 22, 2001
Accepted: June 31, 2001

Purpose: *A prospective study was conducted to compare the efficiency of ovarian blockade with nafarelin versus leuprolide in a population whose indication for assisted reproduction was the male factor.*

Methods: *A total of 238 patients were assigned at random to two types of treatment: Group I (119 aspirations), nafarelin (Synarel, Searle), 200 μg by the nasal route twice a day, and Group II (119 aspirations), leuprolide acetate (Lupron, Abbott), 0.5 mg by the subcutaneous route once a day. Both types of blockade were started during the 2nd phase (21st day of the menstrual cycle) and were continued until the day of HCG (5,000–10,000 IU). All patients received a fixed dose of recombinant FSH for 7 days and on the 8th day of stimulation the doses started to be adapted according to ovarian response.*

Results: *Patients' age did not differ (*$p = 0.93$*) between Group I (34.1 ± 3.79) and Group II (34 ± 4.64). The number of oocytes retrieved from Group I (10.5 ± 5.93) was also similar (*$p = 0.57$*) to that retrieved from Group II (10.2 ± 6.36). In addition, there was no difference (*$p = 0.58$*) in the number of oocytes retrieved at Metaphase II between Group I (8.2 ± 4.61) and Group II (7.9 ± 5.2). Fertilization rates and embryo scores were similar (*$p = 0.81$ *and* $p = 0.25$*, respectively) for Group I (67.5% ± 21.3 and 34.4% ± 14.4) and Group II (68.1% ± 23 and 32.2% ± 14.7, respectively). In addition, pregnancy rates per puncture and per embryo transfer and implantation rates were similar for Group I (30.2, 31.3, and 16.2%, respectively) then compared with Group II (24.4, 25.2, and 12.6%, respectively) (*$p = 0.38$*,* $p = 0.37$*, and* $p = 0.22$*, respectively).*

Conclusions: *Implantation and pregnancy rates per puncture and per embryo transfer are not statistically significant in the nafarelin group when compared with leuprolide group.*

KEY WORDS: ICSI; implantation; leuprolide; nafarelin; ovarian stimulation; pregnancy; recombinant FSH.

INTRODUCTION

The agonists of gonadotropin releasing hormone (GnRH-a) are modifications of the decapeptide GnRH and their action is based on reversible blockade of pituitary gonadotropin release, which results in the suppression of ovarian function. Over the last decade, most in vitro fertilization (IVF) centers have used GnRH-a for pituitary suppression before gonadotropin administration. Pretreatment with GnRH-a increases the number of developing follicles, prevents the luteinizing hormone (LH) peak, and reduces the number of cancelled cycles.

Several types of GnRH-a have been developed, with different biological potentials, plasma half-lives and administration routes. Because of the inconvenience of daily injections, intranasal GnRH-a could be an alternative of simple and noninvasive administration.

Recently, recombinant FSH (r-FSH) has also enjoyed widespread use in ovarian stimulation program with considerable success. The pure FSH preparations, in contrast to the classical human menopausal gonadotropins, are devoid of LH activity, and GnRH-a more or less prevents pituitary release of endogenous LH. These low concentrations of LH

[1] Center for Human Reproduction, Sinhá Junqueira Maternity Foundation, Ribeirão Preto, SP, Brazil.
[2] Department of Obstetrics and Gynecology, University of Ribeirão Preto (UNAERP), Ribeirão Preto, SP, Brazil.
[3] To whom correspondence should be addressed at Center for Human Reproduction, Sinhá Junqueira Maternity Foundation, Rua D. Alberto Gonçalves, 1500-14085-100, Ribeirão Preto, SP, Brazil; e-mail: crh@highnet.com.br.

1058-0468/01/1100-0593$19.50/0

lead to a reduced thecal production of androgen precursors, and consequently to a reduction of ovarian estradiol biosynthesis (1). On the other hand, it has been suggested that nafarelin, particularly at the 200 μg twice-daily dose, does not achieve as rapid or profound a level of pituitary suppression as some of the other agonists (2). Thus, nafarelin plus r-FSH could be a perfect combination.

The objective of the present study was to compare nafarelin (Synarel, Searle) to leuprolide (Lupron, Abbott) used as adjuvant in ovulation induction with r-FSH in patients submitted to a program of intracytoplasmic sperm injection (ICSI).

MATERIALS AND METHODS

A total of 250 patients without specific ovulatory dysfunction were submitted to ovarian stimulation for ICSI program and divided prospectively and at random (drawing lots, using a randomization table previously elaborated for the study) into two groups: Group I: 200 μg nafarelin acetate by the nasal route twice a day, and Group II: 0.5 mg leuprolide acetate by the subcutaneous route once a day. The cancellation rate for ovarian stimulation was similar in the Groups I and II (4.8%). Both blockades were started during the 2nd phase (21st day of the menstrual cycle) and continued until the day for human chorionic gonadotropin (hCG) administration (5,000–10,000 IU).

Habitually, 14 days after the use of the analogue, with establishment of the blockade (menstruation), the administration of recombinant FSH was started at a fixed dose of 150–225 IU for a period of 7 days (3). On the 8th day of ovarian stimulation, follicular development started to be monitored by vaginal ultrasound only, with the doses of pure FSH being adapted according to ovarian response. Then, when a minimum of 3 follicles measuring >17 mm in diameter were observed, hCG was administered at the dose of 5,000–10,000 IU (4).

Oocytes were retrieved from the follicles by ultrasound-guided transvaginal puncture 34–36 h after hCG administration. After identification in follicular fluid, the oocytes were classified in terms of maturity. The cumulus-corona complex was removed by exposure to a type IV hyaluronidase solution (H-4272, Sigma, USA) at the concentration of 80 IU/mL and the denuded oocytes were incubated in IVF-50 medium (Scandinavian IVF Science AB, Sweden) until the time for ICSI. Discontinuous gradients of Sperm-Prep-100TM (Scandinavian IVF Science AB, Sweden) were used to separate the spermatozoa from seminal fluid in the 40–90% fractions. ICSI was performed by an established method (5).

On the day of embryo transfer (ET) the embryos were scored as follows: Grade 4—regular and symmetrical blastomeres with the absence of fragmentation; Grade 3—irregular blastomeres or less than 10% fragmentation; Grade 2—10–50% blastomere fragmentation; Grade I—more than 50% fragmentation, or embryo in the pronucleus stage. The morphological grade of the embryo was then multiplied by the number of blastomeres in order to obtain a qualitative score for each embryo. The scores of all embryos transferred per patient were summed to obtain the embryo score for each patient. ET is routinely performed after 48 h in culture and supernumerary embryos are cryopreserved at the end of the 2nd day.

Data were analyzed statistically by the Student's *t* test and by the Mann–Whitney and Fisher exact tests.

RESULTS

The age of Group I patients (34.1 ± 3.79) was similar ($p = 0.93$) to that of Group II patients (34 ± 4.64). The number of oocytes collected from Group I (10.5 ± 5.93) was also identical ($p = 0.57$) to the number of oocytes collected from Group II (10.2 ± 6.36). In addition, the number of oocytes in Metaphase II did not differ significantly ($p = 0.58$) between Group I (8.2 ± 4.61) and Group II (7.9 ± 5.2). The mean number of embryos transferred from Group I (2.97 ± 0.96) was similar ($p = 0.65$) to the mean number of embryos transferred from Group II (2.92 ± 1). Fertilization rate and embryo scores were similar ($p = 0.81$ and $p = 0.25$, respectively) for Group I ($67.5 \pm 21.3\%$ and 34.4 ± 14.4, respectively) and Group II ($68.1 \pm 23\%$ and 32.2 ± 14.7, respectively). The pregnancy rate per puncture, pregnancy rate per transfer, and the implantation rate were not statistically significant for Group I (30.2, 31.3, and 16.2%, respectively) compared with Group II (24.4, 25.2, and 12.6%, respectively) ($p = 0.38$, $p = 0.37$, and $p = 0.22$, respectively). The abortion rate was 8.3% for Group I and 6.9% for Group II, and the rate of ectopic pregnancy was 2.7% for Group I and 3.4% for Group II (Table I).

DISCUSSION

GnRH-a has been successfully used for ovulation induction in assisted reproduction programs in order to prevent a premature LH peak and for a better

Table I. Clinical and Laboratory Results

	Group I (Nafarelin)	Group II (Leuprolide)	*p*
Punctures	119	119	
Age	34.1 ± 3.79	34 ± 4.64	0.93
Retrieved oocytes	10.5 ± 5.93	10.2 ± 6.36	0.57
Oocytes in Metaphase II	8.2 ± 4.61	7.9 ± 5.2	0.58
Fertilization rate	67.5 ± 21.3%	68.1 ± 23%	0.81
Embryo score	34.4 ± 14.4	32.2 ± 14.7	0.25
No. of embryos transferred	2.97 ± 0.96	2.92 ± 1	0.66
Implantation rate	16.2%	12.6%	0.22
Pregnancy rate/puncture	30.2%	24.4%	0.38
Pregnancy rate/transfer	31.3%	25.2%	0.37
Abortion rate	8.3%	6.9%	
Ectopic pregnancy	2.7%	3.4%	

control of follicular development and maturation. The present study was conducted to compare two different types of GnRH-a, nafarelin and leuprolide acetate, administered by two different routes, and was the first with ovarian stimulation with recombinant FSH.

Yuzpe *et al.* (6) reported on 200 patients receiving intranasal nafarelin acetate at two different dosages (400 μg/day vs. 600 μg/day) in a long second-phase protocol and compared these cycles to a historical control group of 179 patients receiving leuprolide, 0.5 mg/day. Serum estradiol levels on day 12 of GnRH agonist administration were lower than 43 pg/mL in 82–87% of the patients in the groups treated with 400–600 μg nafarelin, respectively. In comparison, in the leuprolide control group, serum estradiol levels were higher than 43 pg/mL on day 12 of treatment (34.5% of patients). There were no differences in the number of gonadotropin ampoules required between the two doses of nafarelin and no data were reported for the leuprolide controls. Mean estradiol levels on the day hCG administration were highest for the lower dose nafarelin group and lowest for the leuprolide controls. The number of oocytes recovered was 9.7 in the 400 μg group and 8.9 in the 600 μg group (statistical differences not reported). There were no LH surges in either nafarelin group, as determined by the lack of any significant rises in daily serum LH levels. The clinical pregnancy rate was 21.7% per ET for the 400 μg nafarelin group and 30.1% for the 600 μg group. The pregnancy rate for the leuprolide controls was 17.5% per ET, but this difference must be disregarded since this was a historical control group. As the primary purpose of this study was to compare the two doses of nafarelin, no strict conclusions can be drawn regarding the comparisons to leuprolide. The data suggest that the lower dose of nafarelin was adequate for suppression, and a trend toward higher estradiol levels and a greater number of oocytes suggests less profound suppression during stimulation. In the present study, we opted for the use of 200 μg administered twice a day.

The first randomized trial was published in 1992 by Penzias *et al.* (7) who compared outcomes in only 21 patients treated with nafarelin at a dose of 400 μg twice a day to an equal number of patients down-regulated with leuprolide at a dose of 0.5 mg daily. Patients began the agonists on day 1 of the cycle preceding gonadotropin treatment. Both dosages were decreased by half with the demonstration of pituitary suppression and the start of gonadotropins administration. The authors reported no differences between the groups in the number of days of drug administration required to achieve pituitary suppression and no differences in serum FSH, LH, or estradiol values at suppression. However, they did report a decrease in the average gonadotropin dose required for the patients treated with nafarelin compared with the leuprolide patients (29.3 vs. 33 ampoules, $p = 0.01$). The authors also reported no differences in the numbers of oocytes recovered or fertilization or embryos cleavage rates, but did report a statistically significant increase in the number of embryos available for cryopreservation in the nafarelin group (4.4 vs. 1.3, $p = 0.04$).

Goldman *et al.* (8) compared nafarelin acetate with buserelin acetate, both in the form of a nasal spray, in patients submitted to IVF. In this population there was no significant difference in patient response measured in terms of cancellation of cycles, number of oocytes retrieved, fertilization rate or pregnancy rate. In another study comparing nafarelin and buserelin, Lockwood *et al.* (9) also observed no differences in pregnancy rates between the two GnRH-a preparations.

In addition, Dantas *et al.* (10), in a smaller but randomized study compared a total of 24 women submitted to IVF and receiving 200 μg of nafarelin twice a day versus 1 mg/day leuprolide acetate, beginning in the preceding luteal phase. An interesting finding in that study, which must be considered in light of the fact that the leuprolide was injecting 1.0 mg daily throughout treatment, is the difference in the serum estradiol in the two patients groups on the day 2 following menses. The mean estradiol level in the leuprolide acetate group was 33.7 pg/mL compared to 75.2 pg/mL in the nafarelin group ($p = 0.01$), suggesting that administration of leuprolide at this dose during the luteal phase induces a more rapid and profound suppression of endogenous gonadotropin levels than nafarelin at

the dose of 200 μg twice a day. The protocol in this study indicated that gonadotropins were initiated on cycle 2–4, and there was no indication of any threshold level of estradiol required prior to initiation of gonadotropins. There were no differences in estradiol levels on the day of hCG administration, also observed no difference in number of follicles, retrieved oocytes, fertilization rate, number of embryos transferred, number of frozen embryos, or pregnancy rate between groups.

However, Martin *et al.* (11), in a retrospective study of 510 consecutive IVF cycles in women who had received nafarelin (284 patients) and leuprolide (226), found no differences in cycle cancellation rates, peak estradiol levels, duration of gonadotropin stimulation, total number of gonadotropin ampoules required, total and mature oocytes, fertilization rates, embryo cleavage rates, or number of embryos transferred. The delivery rate per oocyte collection was 34% in the nafarelin group and 24% in the leuprolide group ($p = 0.03$). When only first IVF cycles were evaluated, the delivery rate was 34% per retrieval for nafarelin cycles and 20% per retrieval for leuprolide cycles, a difference that did not quite reach statistical significance ($p = 0.052$).

Another randomized prospective study comparing nafarelin to leuprolide was a double-blind study by Corson *et al.* (12) in which 22 patients receiving leuprolide acetate at a dose of 0.5 mg twice daily were compared to 17 patients receiving 200 μg three times a day beginning in the preceding luteal phase. Leuprolide was decreased to 0.5 mg once a day and nafarelin was decreased 200 μg twice daily when pituitary suppression was achieved. No statistically significant differences were noted between the two groups with regard to the number of FSH ampoules, days of gonadotropin stimulation, estradiol levels on the day of hCG, number of oocytes and mature oocytes recovered, fertilization rates, or embryos cleavage. Implantation and pregnancy rates were not reported due to the small sample size.

Recently, Dada *et al.* (13) concluded that leuprolide, nafarelin, and buserelin are equally effective when pituitary suppression, ovarian stimulation, and IVF outcome are compared.

Evaluation of present results did not show a difference between patients receiving nafarelin and leuprolide in terms of number of retrieved oocytes in Metaphase II, fertilization rate, embryo score, pregnancy rate per puncture, pregnancy rate per transfer, or implantation rate. At present, the data do not demonstrate statistically significant differences in ovarian blockade with nafarelin versus leuprolide during ovarian stimulation with r-FSH in patients whose indication for assisted reproduction was the male factor.

However, when patients are provided a choice of route of administration of GnRH agonist (intranasal twice daily vs. subcutaneous injection once daily), with counseling that no significant clinical differences exist with regard to cycle outcome, 60% of patients will choose the intranasal route. Reducing the number of injections in an IVF cycle appears to be the primary motivation for patients choosing nafarelin over leuprolide (14).

Prospective and randomized studies comparing leuprolide acetate to nafarelin are scarce in the literature (only 4) and involve a reduced number of cases a fact that impairs the evaluation of the variables commonly analyzed, that is, implantation rate, pregnancy rate per puncture or transfer, and deliveries. Although these drugs are extensively used in assisted reproduction programs, there is a lack of comparative studies about the advantages or disadvantages of these medications.

In conclusion, these results indicate that pregnancy rates per puncture and per ET are not statistically significant in the nafarelin group when compared with leuprolide group for IIVF/ICSI ovarian stimulation with gonadotropins.

ACKNOWLEDGMENT

The authors wish to thank Mrs Elettra Greene for revising the English text.

REFERENCES

1. Westergaard LG, Laursen SB, Andersen CY: Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. Hum Reprod 2000;15:1003–1008
2. Simberg N, Tulppala M, Husa LM, Tiitinen A: Comparison of buserelin and nafarelin in IVF cycles and in subsequent frozen-thawed embryo transfer cycles. Acta Obstet Gynecol Scand 1998;77:854–859
3. Franco JG, Jr, Baruffi RLR, Coelho J, Mauri AL, Petersen CG, Garbellini E: A prospective and randomized study of ovarian stimulation for ICSI with recombinant FSH versus highly purified urinary FSH. Gynecol Endocrinol 2000;14: 5–10
4. Oliveira JBA, Baruffi RLR, Mauri AL, Petersen CG, Borges MC, Franco JG, Jr: Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme after

ovarian stimulation and gonadotrophin-releasing hormone and gonadotrophins. Hum Reprod 1997;12:2515–2518
5. Svalander P, Forsberg AS, Jakobsson AH, Wikland M: Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility. Fertil Steril 1995;63:828–837
6. Yuzpe AA, Nisker JA, Kaplan BR, Tummon IS, Auckland J: Nafarelin acetate for pituitary down-regulation in in vitro fertilization. Comparison of two dosages. J Reprod Med 1995;40:83–88
7. Penzias AS, Shamma FN, Gutmann JN, Jones EE, DecHerney AH, Lavy G: Nafarelin versus leuprolide in ovulation induction for in vitro fertilization: A randomized clinical trial. Obstet Gynecol 1992;79:739–742
8. Goldman JA, Dicker D, Feldberg D, Ashenazi J, Voliowich I: A prospective randomized comparison of two gonadotropin-releasing hormone agonists, nafarelin acetate and buserelin acetate in in-vitro fertilization-embryo transfer. Hum Reprod 1994;9:226–228
9. Lockwood GM, Pinkerton SM, Barlow DH: A prospective randomized single-blind comparative trial of nafarelin acetate with buserelin in long-protocol gonadotropin releasing hormone analogue controlled in-vitro fertilization cycles. Hum Reprod 1995;10:293–298
10. Dantas ZN, Vicino M, Balmaceda J, Asch RH, Stone SC: Comparison between nafarelin and leuprolide acetate for in vitro fertilization: Preliminary clinical study. Fertil Steril 1994;61:705–708
11. Martin MC, Givens CR, Schriock ED, Glass RH, Dandekar PV: The choice of a gonadotropin-releasing hormone analog influences outcome of in vitro fertilization treatment. Am J Obstet Gynecol 1994;170:1629–1632
12. Corson SL, Guttmann JN, Batzer FR, Gocial B: A double-blind comparison of nafarelin and leuprolide acetate for down-regulation in IVF cycles. Int J Fertil Menopausal Stud 1996;41:446–449
13. Dada T, Salha O, Baillie HS, Sharma V: A comparison of three gonadotropin-releasing hormone analogues in an in-vitro fertilization programme: A prospective randomized study. Hum Reprod 1999;14:288–293
14. Givens CR: Nafarelin acetate for pituitary suppression for in vitro fertilization: Results from a decade of use. Assisted Reprod 1999;9:192–198